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# INVESTIGATING REACTIVITY TO INCENTIVE DOWNSHIFT AS A CORRELATED RESPONSE TO SELECTION FOR HIGH ALCOHOL PREFERENCE AND A DETERMINANT OF RASH ACTION AND ALCOHOL CONSUMPTION

A Dissertation

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by

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This dissertation is dedicated to my family and friends. I'd especially like to dedicate this to my love, Mahim Jain and to my sisters, the people I can always count on for love and support. Also, to my parents and who have always encouraged me to meet my goals and to do what I am passionate about.

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# ABSTRACT

Matson, Liana M. Ph.D., Purdue University, May 2014. Investigating Reactivity to Incentive Downshift as a Correlated Response to Selection for High Alcohol Preference and a Determinant of Rash Action and Alcohol Consumption. Major Professor: Nicholas Grahame.

Losing a job or a significant other are examples of incentive shifts that result in negative emotional reactions. The occurrence of negative life events is associated with increased drinking, and alleviation of negative emotions has been cited as a drinking motive for individuals with problematic drinking patterns (Keyes et al., 2011; Adams et al., 2012). Further, there is evidence that certain genotypes drink alcohol in response to stressful negative life events (Blomeyer et al., 2008; Covault et al., 2007). It is possible that shared genetic factors contribute to both alcohol drinking and emotional reactivity, but there is a critical need for this relationship to be understood. The first aim of this proposal will use an incentive downshift paradigm to address whether emotional reactivity is elevated in mice predisposed to drink alcohol. The second aim of this proposal will address if reactivity to an incentive shift can result in rash action using a differential reinforcement of low rates of responding task, and whether this response is also associated with a predisposition for high drinking. The third aim of this proposal will investigate if experimenter administered ethanol reduces contrast effects, and if an incentive shift increases ethanol consumption in a high drinking line. The overall goal of this proposal is to investigate whether reactivity to incentive shift is an important mechanism underlying alcohol drinking in these mice, and the role an incentive shift may play in producing rash action and influencing ethanol consumption.

# CHAPTER 1. INTRODUCTION

## 1.1 Reward Loss and Alcohol Consumption

Reward loss is a universal human experience that occurs in many forms. Losing a job, a significant other, or property are examples of incentive changes that result in negative emotional reactions. Checklists of life events are often used in epidemiological and clinical studies, and arguably, a majority of the negative stressful events include some type of negative incentive change (1). Further, there is evidence that the occurrence of negative life events, such as job loss or divorce, results in increased alcohol drinking or problematic drinking (2). Alcohol consumption to alleviate a negative emotional state has also been consistently cited as a drinking motive for individuals with problematic drinking patterns (3, 4). Additionally, recent studies have related certain genotypes with increased alcohol consumption in the face of stressful negative life events (5, 6). Drinking in response to stress, rather than using a more socially acceptable coping response, can be conceived as both emotionally reactive and impulsive behavior. Taking these observations into account, it is possible that a predisposition for emotional reactivity is associated with a propensity to drink alcohol.

All of the before-mentioned studies relied on self-report, which can be subject to response bias and memory alterations. In addition, studies on drug or alcohol use can be confounded by the fact that the drug use itself may create or exacerbate a phenotype

of interest. Additionally, many human studies fail to examine detailed temporal patterns of behavior following negative life events. Therefore, while human self-report studies are both useful and informative, research using animal models and human laboratory tasks are needed to study the relationship between negative emotional reactivity and a propensity to drink alcohol. Additionally, use of animal models allows for experimental determination of a treatment's effects and for use of prospective studies. Therefore, the overall goal of this dissertation is to investigate reactivity to reward shift as a correlated response in mice selectively bred for high alcohol preference, and as a determinant of rash action and alcohol consumption.

## 1.2 Measuring Emotional Reactivity

It is difficult to obtain a quantitative measure of positive or negative emotion in animals (or in humans, aside from self-report), therefore, indirect indicators of affect must be used to infer emotional reactions in animals. Incentive or reward downshift tasks have been used to study negative emotionality since the early 20$^{th}$ century. Tinklepaugh (7) gave monkeys access to banana pieces following correct choices on a response learning task. When the less desired lettuce was substituted for the more desired banana, he noted the monkey would not touch the lettuce, stating "on occasions she has turned toward observers present in the room and shrieked at them in apparent anger." Although a variety of other emotional states may be used to describe negative emotional reactivity, the proposed studies will focus on the response to an incentive downshift as a measure of emotional reactivity. Termed successive negative contrast (SNC), reward downshift, or incentive downshift, these procedures have been widely used to model emotional reactivity in rats and other species (8, 9). The general procedure consists of a training, or pre-shift period, followed by a testing, or post-shift period. During the pre-shift

training sessions, unshifted animals have access to a low reward and shifted animals have access to a high reward. During post-shift sessions, all of the animals have access to the low reward, and the shifted group can be compared to the consistently rewarded control group. Decreased responding or intake in the experimental group below the level of the control group is called a negative contrast effect, and has been used widely to model an emotional reaction in rodents (10).

A large body of available behavioral, pharmacological, and neuroanatomical data on incentive downshift suggests that contrast behavior is affectively motivated (10, 11). Incentive and aversive learning processes likely interact during contrast behavior, as recovery from SNC has been hypothesized to be a conflict between approach and avoidance behaviors (12). Incentive downshift procedures also have high face, predictive, and construct validity for modeling emotional reactivity in rodents (13). Contrast effects have been demonstrated using human lab tasks, making SNC a translational procedure (14, 15). Therefore, it is possible to experimentally assess whether emotional reactivity, in the form of reactivity to incentive downshift, is associated with a predisposition for excessive alcohol use in rodent populations. Considering contrast effects have also been demonstrated in humans, it might also be possible to extend rodent findings to human populations.

Amsel's theory of frustration states that primary frustration occurs following unexpected non-reward or un-reinforced responses, and this response is subject to Pavlovian conditioning (12, 16, 17). With repeated exposure to frustrative events, secondary or conditioned frustration develops. In the case of SNC, the theory holds that associations are formed between environmental stimuli (for example, the sipper tube) and impending reward, resulting in reward expectation when an animal is exposed to conditioned stimuli in the environment. When the reward is reduced, primary frustration

results, which in rats usually occurs during the first post-shift day. New associations are created with the stimuli that had previously signaled reward, resulting in the development of conditioned frustration. As this new associative process occurs, it competes with associations that had previously developed to signal reward availability, resulting in a conflict between approach and avoidance behavior.

In *Incentive Relativity*, Flaherty (10) proposed a multi-stage theory of contrast. The primary frustration response is generally characterized by behavioral invigoration, appetitive or “search” behaviors. In the earliest studies of contrast, rats or monkeys that were shifted from a preferred to a non-preferred reward seemed to search for the missing reward (7, 18). Early studies investigated open field behavior during a consummatory SNC study, and found an increase in ambulation and rearing in rats shifted from 32 to 4% sucrose (19). Similarly, Pecoraro and colleagues (20) approached this issue from a behavior-systems point of view, with the hypothesis that an incentive downshift would switch animals from a consummatory to an appetitive motivational state (21 for review of behavior systems theory). In this study, rats had access to 32% sucrose at fixed or variable locations in an open field. When switched from 32% to 4% sucrose, the switched groups elicited search and sampling behaviors following the downshift, providing support for the hypothesis that rats switched from a consummatory to appetitive behavior state. In another study, Flaherty (10) investigated the activity of animals in a radial arm maze following a 32 to 4% sucrose shift, and observed a contrast effect in lick behavior, accompanied by an increase in open arm entries by the shifted group. In addition, Grigson, Spector, and Norgren (22) performed a microstructural analysis of licking behavior during a reward downshift, and observed an increase in lick bursts but fewer licks per burst compared to unshifted animals. This type of behavior may indicate increased sampling, but rejection of the post-shift reward.

In addition, Flaherty and colleagues (23) attempted to find associations between negative contrast and three measures of emotionality, the plus maze, open field emergence, and context-shock fear conditioning. Interestingly, contrast loaded as a separate factor from any of these measures, and its relationship to the other measures changed throughout four post-shift days. On the first post-shift day, contrast behavior was unrelated to any of the other measures of emotionality. Taken together, these results support Flaherty's hypothesis that post-shift day 1 involves detection of the post-shift reward, search for the pre-shift reward, and rejection of the post-shift reward. Future post-shift days (or periods of time past this initial process) involve recovery from contrast, which may be conceptualized as a conflict between approach and avoidance behaviors as described by Amsel. Flaherty proposed that recovery from contrast likely relies on GABAergic signaling, as benzodiazapenes and ethanol are effective at attenuating contrast on the second post-shift day (24, 25). Additionally, Flaherty and colleagues (23) found that contrast was related to context-shock fear conditioning and open field emergence on the second and third post-shift days, while it was only related to context-shock fear conditioning on the fourth post-shift day. Therefore, there is evidence that the initial reaction to an incentive downshift results in search or appetitive behaviors. Amsel (1992) theorized that the initial reaction to an incentive downshift is dictated primary frustration. Once this response subsides, there is support for the idea that a negative affective response is likely driving behavior. This negative affect may be thought of as conditioned frustration or anxiety-like, and is hypothesized to be driven by a conflict between approach and avoidance behaviors.

Although reward omission studies likely observe similar behavioral processes that rely on parallel neurological systems, contrast studies provide the advantage of being able to study relative reward effects. Using animal models of reward omission, it is

difficult to study continued appetitive processes following reward or reinforcement omission, as extinction eventually occurs. I would also argue that contrast studies are a more valid way to study reward or reinforcement loss in humans. Because humans exist in complex social environments, they are likely to have alternate rewards or reinforcement available following loss, and therefore can direct appetitive behaviors toward these stimuli. For example, at face value, losing a significant other is an example of reward or reinforcement omission. But breaking up with one's significant other may result in negative affect, which can be compared to the affect of individuals who have not broken up with a significant other but rely on other forms of attachment such as friends or family. Although friends and family are arguably not a "low" form of reward or reinforcement, they may be considered as such compared to the significant other that is no longer available. For example, Terhell and colleagues (26) observed different patterns of social network changes in individuals following a divorce. One group declined its level of contact with all friends, acquaintances, colleagues, in-laws and neighbors up to 12 years following divorce. The other groups either observed a short-lived reduction in social contact, a short-lived increase in social contact, or a long-lived increase in social contact following divorce. These observations suggest there is individual variability in the response to incentive loss, but that incentive loss (at least in the form of divorce) has the ability to influence the reward value of other relationships. Therefore, I would argue that incentive downshift studies are more translational than reward omission studies because human losses are not true losses of all available rewards or sources of reinforcement in their environment.

Emotional reactivity to an incentive shift can present in one of three plausible ways; with increased likelihood of exhibiting an emotional reaction to an incentive downshift (threshold), by exhibiting a large initial reaction to an incentive downshift

(magnitude), or by having an increased recovery period following an incentive downshift (duration). There are no examples of threshold differences from the preclinical incentive downshift literature, but there are examples of individual differences in the magnitude and duration of the shift. For example, in a selected high contrast line as well as in Lewis rats, an increased magnitude and longer duration of recovery were observed compared to a selected low contrast line and Fisher rats, respectively (13, 27). Thus, it appears the mechanisms that result in a large initial reaction to the shift can be related to the mechanisms involved in recovery from the shift. Further, the magnitude of contrast varies normally in outbred Sprague Dawley rats, and is capable of being selected for through bi-directional selection pressure for low and high contrast (13). Therefore, it is apparent that individual differences in contrast behavior exist, and these differences can be genetically determined, which is supported by the finding that selection for high and low contrast are both heritable responses.

### 1.3 Rash Action and Emotion

As discussed, the initial response to an incentive downshift results in an increase in appetitive behaviors, which has been theorized to consist of a “search” for the missing reward (10, 20). This “search” reaction is particularly interesting, because it is similar to the behavior that can lead to certain types of impulsive decision-making. Though the “search” response may not necessarily constitute rash action, the immediate reaction to an incentive downshift could theoretically lead to rash action in humans. Appetitive behaviors allow individuals to come in contact with rewards, so increased search behavior may allow individuals to not only search for the “missing” reward, but to come in contact with additional rewards. Although increasing appetitive behaviors in response to incentive loss or downshift has probably been a highly adaptive evolutionary response

to reward downshift, and arguably still is in some situations, it may be counterproductive if long-term goals or other environmental factors are not taken into account. For the purpose of the following experiments, rash or impulsive action will be considered as behavior that is maladaptive or counterproductive to an individual or subject.

Craving or other strong emotion states have been related to rash decision-making related to drug use, including excessive alcohol intake (28-31). Negative urgency is the predisposition to act rashly in response to negative emotions (32, 33). According to the theory of urgency, strong emotion provides a signal for action that causes one to focus on the immediate situation and not necessarily attend to long-term goals, thereby resulting in rash action (34). Strong relationships between urgency and problems related to alcohol use have been identified in humans (35-37). An animal model would be useful for establishing a temporal pattern between the occurrence of a stressor, urgent behavior, and problematic drinking. It would also be useful to study the neurological mechanisms underlying urgency, as well as to understand shared mechanisms underlying urgency and drinking. Differential Reinforcement of Low Rates of Responding (DRL) has been used extensively to model motor impulsivity, or the inability to withhold responding (38-40). DRL is an appetitive response inhibition task during which rodents withhold a response during a specified time interval (each time interval is a trial) in order to receive reinforcement. If the rodent fails to withhold responding during the time interval, then a new trial begins, thus negatively reinforcing lower rates of responding. Therefore, rodents that learn to inhibit responding gain more reinforcers, and are considered less impulsive, while rodents that do not inhibit responding gain a lower number of reinforcers, and are considered more impulsive. The DRL might be an effective procedure for modeling urgency, because in theory, an increase in responding (or search-like) behavior occurs immediately following incentive downshift. Therefore, if

an animal increases its level of responding in the face of negative affect (due to an incentive downshift), it gains fewer reinforcers, thereby demonstrating a greater level of motor impulsivity on the task.

## 1.4 Bi-Directional Selection for Alcohol Preference and Correlated Responses

High Alcohol Preferring (HAP) and Low Alcohol Preferring (LAP) mice were bi-directionally selected for alcohol preference during 4 weeks of 24-hour, free-choice access to 10% ethanol and water (41, 42). While all of the HAP lines drink considerable quantities of alcohol, the highest intakes are seen in the crossed HAP (cHAP) line, generated by a cross and subsequent selection from HAP replicate 1 (HAP1) X HAP replicate 2 (HAP2). The other HAP lines drink less, with the HAP1 line drinking is most likely because it is farther along in the selection process, followed by the HAP2 and HAP3 lines, respectively (42).

Following 3 weeks of ethanol access, drinking rhythms were assessed during the dark portion of a 12:12 light-dark cycle in the HAP lines during 24-hour access to both 10% ethanol and water (43). We observed that all HAP lines drink above the rate of their alcohol metabolism during the dark portion of the light-dark cycle. Further, all HAP lines reach pharmacologically relevant blood ethanol concentrations (BEC), with HAP1 and cHAP mice reaching average BECs of 218 and 262 mg/dl, levels that are reminiscent of those reached by chronic alcoholics (44). Further, cHAP mice show signs of intoxication during early ethanol access, and develop both metabolic and functional tolerance during chronic, free-choice ethanol access (45, 46). Therefore, cHAP mice meet several criteria required to be a good rodent model of alcoholism and exhibit several behaviors characteristic of alcohol-dependent individuals (47).

As discussed, HAP mice are an effective model of alcoholism, and can be used to study drinking behavior and its neurobiological and genetic substrates. Additionally, selection for high and low alcohol preference produces a heritable and replicable response, due to fixation of alleles that influence alcohol preference. It is possible that some of the alleles that affect alcohol preference also determine the phenotype of another trait, which exemplifies the concept of pleiotropy, one heritable cause for many seemingly unrelated traits. Pleiotropic relationships are responsible for producing correlated responses in populations of genetically selected animals (48, 49). For example, HAP mice are more impulsive on a delayed discounting task than LAP mice, demonstrating that high cognitive impulsivity is a correlated response to selection in HAP mice (50). In the following studies, reactivity to incentive downshift will be examined as a potential correlated response to selection in HAP mice. I will also try to replicate the unpublished observation that HAP mice are more impulsive than LAP mice using a motor impulsivity task, Differential Reinforcement of Low Rates of Responding (DRL). Finally, I will also assess whether HAP and LAP mice demonstrate urgent-like behavior following an incentive downshift, using the DRL task.

## 1.5 Hypothesis and Specific Aims

As discussed, it is possible that shared genetic factors contribute to both alcohol drinking and emotional reactivity, but there is a critical need for this relationship to be understood. The *first aim* of this dissertation will use an incentive downshift paradigm to address whether emotional reactivity is elevated in HAP mice predisposed to drink alcohol. The *second aim* of this dissertation will address if reactivity to an incentive shift can result in rash action, and whether this response is also associated with a predisposition for high drinking. The *third aim* of this dissertation will investigate whether an incentive shift

increases ethanol consumption in a high drinking line. The overall goal of this dissertation is to investigate if and how reactivity to incentive shifts is a mechanism underlying alcohol drinking in HAP mice.

*Specific Aim 1* will determine if emotional reactivity is genetically correlated with alcohol preference in bi-directionally selected High Alcohol Preferring (HAP) and Low Alcohol Preferring (LAP) mice. Negative contrast procedures have been widely utilized to model emotional reactivity, and involve shifting a high reward group (e.g., 32% sucrose access for 10 days) to a low reward (e.g., 4% sucrose access), and comparing the shifted group to a consistently rewarded control group. Contrast is defined as intake or responding of the shifted group below the level of the consistently rewarded group, and has been used to model an emotional reaction in rodents (10). I hypothesize that the HAP lines will exhibit larger and longer-lasting contrast effects than in their corresponding replicate LAP lines.

*Specific Aim 2* will assess whether incentive downshift can produce rash action, and whether this urgent-like behavior is related to a predisposition for high ethanol consumption. HAP mice have previously been shown to be more impulsive than LAP mice using both Delayed Discounting (DD) and Differential Reinforcement of Low Response (DRL) tasks. As part of this aim, we will evaluate if the HAP line's predisposition for rash action can be modulated by emotional status using a DRL task. Specifically, I hypothesize that evoking an emotional reaction in HAP mice by using an incentive downshift will result in increased rash action compared to mice not experiencing a negative emotional reaction. Further, I will test both HAP2 and LAP2 lines to determine if HAP2 mice show an increased predisposition for rash action in the face of emotion than LAP2 mice. I hypothesize that incorporating incentive downshift in a

DRL procedure will increase impulsive choice, and that this type of rash action will positively correlate with a predisposition for high alcohol intake.

*Specific Aim 3* will determine the degree to which emotional reactivity to an incentive shift increases ethanol consumption. It is plausible that high emotional reactivity is associated with alcohol abuse and dependence, because at least during early use, alcohol acts to reduce the magnitude of an emotional reaction. Indeed, ethanol reduces the degree of negative contrast (51, 52); however, effects of negative contrast on alcohol consumption have never been evaluated. Therefore, as part of this aim, I will first attempt to replicate the finding that administered ethanol attenuates contrast, which has never been observed in mice. Secondly, I will assess if incentive downshift increases ethanol consumption in HAP mice. I hypothesize that administered ethanol will reduce contrast, and that recently experiencing an incentive downshift will increase ethanol intake.

# CHAPTER 2. RESEARCH DESIGN

## 2.1 Subjects

*Specific Aim 1* included 24 HAP2 (12 m, 12 f) and 22 LAP2 (10 m, 12 f) mice from the 46th generation, and 24 HAP3 (12 m, 12 f) and 24 LAP3 (12 m, 12 f) mice from the 20th generation of selection. Mice were aged 73-89 days at the beginning of training. *Specific Aim 2* included 23 HAP2 (11 m, 12 f) and 24 LAP2 (12 m, 12 f) from the 47th generation, and 24 HAP3 (12 m, 12 f) and 24 LAP3 (12 m, 12 f) mice from the 22nd generation of selection. Mice were aged 67-79 days at the beginning of training. In *Specific Aims 1* and *2*, subjects were counterbalanced by Line, Sex, and Family to assign the shifted 32-4 and unshifted 4-4 conditions. *Specific Aim 3* consists of two experiments using cHAP mice; the first experiment included 48 (24 m, 24 f) cHAP mice from the 24th generation, while the second included 32 (16 m, 16 f) cHAP mice from the 25th generation. Mice were aged 67-78 days at the beginning of the experiments. For all of the specific aims, squads were assigned by distributing mice by Group, Line, Sex, (and Family if possible). All mice were single-housed using standard Plexiglas cages with pine bedding the week prior to experimentation, and were maintained on a 12:12 reverse-light dark cycle with ad libitum access to water, except during testing.

## 2.2 Apparatus

Twelve operant boxes were used in all of the experiments (Med-Associates). In all experiments, a retractable sipper tube with a 10-ml graduated pipette was used to

provide and measure sucrose intakes, and lick-o-meters were used to count licks and lick latencies, and the nosepoke light was used as a houselight. In *Specific Aim 2*, two levers were also used. During operant testing, Cellsorb bedding was placed under wire grid flooring and was changed bi-weekly. For bi-hourly drinking procedures, 10 ml pipette tubes readable to .05 ml were used.

## 2.3 Food Restriction

We adapted a similar food restriction protocol to those used by Heyser and colleagues (53) and Mahoney and colleagues (54), which involves reducing mice to 85% of their initial body weight to increase general motivation to obtain a reward or reinforcer. Baseline body weight was measured after the mice were 60 days old (adulthood), in order not to interfere with developmental changes in weight gain. Weight reduction was accomplished by following the procedure described by Mahoney and colleagues (54). *Ad libitum* food intake was measured for one week prior to beginning food restriction. Following this period, about 30% restriction of the average daily intake was given, and adjusted each day (± 5%) to achieve a final body weight reduction of approximately 15% (± 5%). As noted by the authors, weight was chosen as an endpoint for restriction rather than intake because body weight is a more stable variable than food intake. Animals were weighed within 30 minutes of each daily session, and then were given their daily food allotment. Animals were maintained under conditions of food restriction for *Specific Aims 1* and *2* and for the injected ethanol experiment within Specific Aim 3**.** As the drinking experiment for *Specific Aim 3* involved using a 2-bottle, free-choice drinking paradigm, food restriction was not used, as findings of the study would be confounded by the fact that ethanol might be consumed for its caloric content rather than its pharmacologic properties.

## 2.4 Successive Negative Contrast

*Specific Aims 1* and *3* used this basic procedure, with slight variations described for each experiment. *Specific Aim 2* incorporated a modified SNC procedure. Half of the mice were assigned to the shifted 32% to 4% sucrose (32-4) group, with the additional mice assigned to the unshifted 4% sucrose to 4% sucrose (4-4) group. The day prior to testing in operant boxes, mice will receive 1 ml of their assigned training concentration of sucrose using a 10 ml tube, which was used to ensure all mice had an equal level of experience with their assigned concentration prior to training. Tubes were removed at the time of feeding, 2 hours later, and it was noted whether the 1 ml was consumed. On days 1-10 of training, mice were placed in operant boxes with the sipper tube available, and a 5-minute access period took place as soon as each mouse licks twice. On days 11-14, all mice will receive the 4% sucrose solution. On all days, licking behavior and intake were recorded. Sucrose solutions were made fresh for each 14-day period, were refrigerated between sessions, and were placed in room temperature while the mice were habituating each day to allow them to warm.

The ratio between post-shift and pre-shift responding can be used to describe the magnitude of a contrast effect, with a small ratio indicating a large contrast effect. This measure can be useful, but does not necessarily take into account a low lick or intake baseline in the unshifted group. If the unshifted group has a particularly low baseline, the shift ratio may be misrepresentative of a contrast effect, as a small ratio may be driven by the value of the low reward. Further, it is possible to have a very low shift ratio (indicator of large contrast), without actually obtaining between subjects contrast (a comparison of post-shift behavior between the unshifted 4-4 and shifted 32-4 groups). Thus, although shift ratios were used in a study using the cHAP line, shift ratios were not

appropriate to use in comparing HAP and LAP mice, because there were baseline differences in pre-shift intake.

### 2.5 Differential Reinforcement of Low Rates of Responding Task

Differential Reinforcement of Low Response rate (DRL) is a task used to maximize the number of available reinforcers to rodents capable of inhibiting responses. Mice were assigned a correct and incorrect lever before training, which remained the correct lever throughout the duration of the experiment. Lever assignment was balanced across Line (HAP or LAP), Sex (Female or Male), Group (32-4 or 4-4), and Parents if possible. During trials, mice were required to withhold responding until 32 seconds elapsed. Responses occurring within the inter-trial interval (ITI) will cause the interval clock to be reset, resulting in no reinforcement availability during that trial. Reinforcement was .32% saccharin (v/v in DI water). Sessions were 30 minutes long and occurred daily, except during the SNC followed by DRL test day, which was a 10-minute session.

Initially, mice were shaped for one day, during which a reward was available every 60 seconds for a 30 minute session. Correct lever presses during shaping were also reinforced. Following shaping, mice were trained to respond on a Fixed Ratio 1 (FR1) schedule, during which reinforcement was available following a single lever press. The reinforcer was available for 10 seconds, and access time was decreased to 2.5 seconds when criterion was met. Mice were next trained on a Fixed Interval (FI) schedule, during trials consisted of 2.5 seconds of reinforcement availability after 32 seconds elapsed (FI-32), but responses during the ITI did not affect reinforcer availability for that trial. After mice were trained on an FI-32 schedule, they were trained on a DRL-32 second schedule (DRL-32) until all mice reached criterion, as well as an asymptote in the level of responding for each Line and Group. Mice were moved to each schedule of

reinforcement once they reached a criterion of obtaining 10 reinforcers and drinking at least .1 ml in a daily operant session.

# CHAPTER 3. EXAMINING EMOTIONAL REACTIVITY TO INCENTIVE DOWNSHIFT AS A CORRELATED RESPONSE IN SELECTIVELY BRED HAP AND LAP MICE

## 3.1 Introduction

Reactivity to incentive shifts is a relatively universal phenomenon that is thought to have evolved in support of behavioral processes responsible for foraging, and may be considered a major source of affective reactions in humans and other species (10, 55). Many have argued that foraging behavior is adaptive and has been conserved, as it is crucial to obtaining food, but the underlying neurological processes also likely contribute to obtaining other rewards in the environment: food, sex, drugs, etc. (56). Further, variation in foraging behavior has been shown to have a strong genetic component in a variety of animal models (for review see 57). Allelic variation in the *For* gene determines high or low foraging phenotype in flies, and the same gene encodes for a protein that affects foraging behavior in a number of species (58). Interestingly, in the presence of a food source, rover flies, homozygous for one allele, seek out other food sources while sitter flies, homozygous for the other allele, will spend more time at an available food source (59). Therefore, genetic variation can influence foraging behavior. It follows that the reaction to an incentive downshift, because it is a highly conserved pattern of behavior, may be present in many human reactions to negative life events. Further, because genetic variation influences foraging behavior, it is possible that genetic variation could also influence the reaction to incentive downshift.

Of course, there are other factors to consider (e.g. social, cultural) that make human behavior more complex than fly or other animal behaviors, but it is possible that certain individuals are predisposed to being less likely to overcome reward downshift events. This type of phenotype in humans could explain why certain individuals are more susceptible to experiencing affective reactions, as they may be more likely to react to, react more strongly to, and/or be less likely to recover from incentive downshift events. Reactivity to incentive shifts may arise from a primary frustrative process that elicits search behavior for the missing reward (10, 20). A strong initial response to incentive shift may be indicative of a particularly strong memory of the pre-shift reward, which could interfere with approach behavior for the less desirable, available reward initially and during recovery. Thus, rejection of the available reward allows the individual to search for the missing reward, or possibly even a better alternative. This is supported by evidence that the initial reaction to incentive downshift has been shown to elicit “search” behaviors for the missing reward (20). It is my general hypothesis that emotional reactivity to incentive shifts is related to an individual’s sensitivity for or level of seeking rewards.

As discussed, there is significant evidence that drinking increases following stressful negative life events (2). Drinking to cope is a commonly reported drinking motive among individuals with alcohol use problems (3, 4). The tension-reduction hypothesis of alcoholism states that individuals drink to relieve the tension associated with stressful life events, although this theory has received mixed support because of the complexity of alcohol expectancies, genetics, environmental and gender factors that may influence results (60-62). Recently, Baars and colleagues (63) found that the number of relapses in a population of abstinent alcoholics was correlated with frustration sensitivity for social non-reward. This supports the idea that individuals turn to drinking in response

to incentive downshift or loss in their lives. In this study a higher sensitivity to frustration was associated with a greater number of relapses, suggesting high reactivity to incentive downshift in humans is associated with drinking. Because this study is correlational in nature, it is difficult to definitively make this conclusion. It is also unclear if drinking in response to frustration developed following alcoholism or whether high frustration was a predisposing factor for the development of alcoholism. Other recent studies have related certain genotypes with increased alcohol consumption in the face of stressful negative life events (5, 6), suggesting that an underlying predisposition for stress reactivity influences drinking. It is possible that in individuals with a heightened sensitivity for frustration, drinking reduces the frustration associated with incentive loss or downshift. It is also possible that drinking does not alleviate the frustration, but is an emotionally reactive (and potentially impulsive) response to frustration that is chosen instead of another healthy coping mechanism (such as seeking counseling, social support, exercise, etc.).

My specific hypothesis is that high reactivity to an incentive shift is genetically correlated with high alcohol preference. Although there is a paucity of evidence to support this hypothesis, one example exists in the literature of reactivity to incentive downshift being related to a reward-seeking phenotype. Lewis and Fisher rats have been characterized on a number of behavioral tasks, and generally Lewis rats are thought to exemplify a reward-seeking or addictive phenotype compared to Fisher rats (64). A significant amount of research has been performed investigating the divergent response to drugs of abuse in Lewis and Fisher rats, and Lewis rats have been shown to self-administer a greater level of alcohol, cocaine, and morphine (65-68). Three studies have compared the drinking behavior of Lewis rats and Fisher rats, finding greater alcohol consumption in Lewis rats. In addition, Lewis rats have higher serotonin and

glutamate efflux within the nucleus accumbens and prefrontal cortex than Fisher rats in response to an acute low dose of alcohol (69). Therefore, there is strong evidence for a differing behavior towards and neurological response to ethanol between the strains. Inbred Fisher/344 and Lewis rats also demonstrate a divergent SNC phenotype (70). In this experiment, rats were switched from 1.0 M (34.2%) to .1 M (3.4%) sucrose. During post-shift testing, contrast occurred in both strains, but the magnitude of shift was larger in Lewis rats. Recovery was also slower in shifted Lewis rats, which had not recovered post-shift day 3, while Fisher rats recovered by post-shift day 3. This experiment provides strong initial evidence for a relationship between reactivity to incentive downshift and reward-seeking phenotypes. Further research should attempt to elucidate this relationship, as little attention has been paid to it thus far. More importantly, in order to establish a true phenotypic or genotypic correlation, 8 inbred strains or outbred selected lines should be compared (49). Therefore, the results from Fisher and Lewis rats are somewhat limited because they are a comparison of 2 inbred strains. As inbred strains are isogenic across animals, results from the comparison of 2 inbred strains cannot necessarily be generalized to a genetically diverse population.

It would be useful to compare animals selectively bred for high and low alcohol preference, or for other drugs of abuse, on contrast behavior to elucidate whether there is a phenotypic or genetic correlation between alcohol preference and emotional reactivity to incentive downshift. *Specific Aim 1* will examine whether reactivity to an incentive downshift is correlated with selection for alcohol preference. As discussed, selective breeding for alcohol preference fixes alleles relevant for the phenotype of interest. It is possible that alleles fixed by selection pressure for alcohol preference also influence negative emotional reactivity to incentive shift, making it a potential correlated

response to selection. My specific hypothesis is that high reactivity to an incentive downshift will be correlated with high alcohol preference.

### 3.2 Specific Research Design

All mice were first food restricted, and underwent the SNC procedure as described in Chapter 2. Repeated measures ANOVAs using the variables Sex (female and male), Line (HAP and LAP), Group (32-4 and 4-4), and Replicate (2 or 3) were used to analyze the baseline weights, pre-shift data, and post-shift data. Intake, licks and latencies were analyzed as dependent variables for the pre-shift and post-shift analyses. Each replicate was tested as a separate cohort, with both LAP and HAP lines represented. The replicates were also analyzed separately using repeated measures ANOVAs performed on both pre-shift and post-shift intake (ml and ml/kg) and licks. The data are presented in Table 2.

There were several incorrect lick data, either because the mice made constant contact or because the lick-o-meters were not working correctly. Because we were using sucrose, which is relatively viscous, this tended to happen somewhat frequently. I developed a criterion to remove incorrect lick data using the following information. Upon looking at histograms of the lick volumes for the lick data (which was calculated by dividing intake/licks *1000 to obtain the data in microliters), it was clear that values above 4 microliters/lick were on the tail of the normal distribution for lick volume. Davis and Smith (71) demonstrated that <250 ms between lick onsets is considered continuous licking, and that this behavior is stereotyped across mice. Therefore, taking this number into account, the average lick rate of mice is about 4 licks/second. Johnson and colleagues (72) also found that the inter-lick interval during continuous licking for c57, 129 and an F1 cross of the two inbred strains were 129, 108, and 105 ms,

respectively. Therefore, these inbred strains are able to perform > 4 licks/second. To compare these data with my data, I calculated mean intake rates for HAP mice and the max 5 minute average intake rate was for unshifted 4-4 HAP mice, which drank 1.38 ± .08 ml. Using the 250 ms ILI or 4 licks/second determined by Davis and Smith (71), the number of licks should be about or above 1200 in a 5 minute period, which would also be a lick volume of 1.15 microliters/lick. This lick volume (1.15 microliters/lick) is the same as the one obtained by Dotson and Spector (73) for 4 inbred strains using the same size tubes as were used in the following studies. Based on this information, I created a very conservative lick volume of 1 lick/second, which for the max intake average would be a lick volume of 4.6 microliters/lick. Therefore any licks with a lick volume greater than or equal to 4.6 microliters/lick were removed. To impute these values, I analyzed the linear regression of intake and licks (with all incorrect or missing data removed). The correlation was significant, $r = .729$, $p < .001$. Therefore, I was able to use the formula for the linear regression to impute lick values from intake values, as this is a previously verified method for imputation (74). The following formula was used to impute lick values, Licks = (527.68 * Intake) + 79.36, where 527.68 is the slope and 79.36 is the y-intercept.

## 3.3 Results

### 3.3.1 Baseline Weights

A Univariate ANOVA was performed on the average baseline weights, which was calculated from the last 5 days of weight measured before food restriction began. Replicate, Sex, Group, and Line were included as between-subjects variables. There

was one LAP2 4-4 male mouse that never responded for sucrose during the pre-shift period, and therefore was removed from all analyses.

Weight was different between the sexes, $F(1, 76) = 90.4$, $p < .001$, with males weighing more than females, 27.2 $\pm$ .3 g and 23.0 $\pm$ .3 g, respectively. There was also an interaction of Line x Replicate, $F(1, 76) = 12.7$, $p < .005$, which was driven by a difference in weights between HAP2 and LAP2 mice, $F(1, 43) = 4.9$, $p < .05$, with HAP2 mice weighing more than LAP2 mice. HAP3 and LAP3 mice did not differ in weight, $F(1, 45) = 2.4$, $p > .05$ (*Table 1*).

### 3.3.2 Pre-shift Behavior

Two repeated measures ANOVAs were performed on intake (1) total intake in ml and 2) intake corrected for weight in ml/kg using Pre-shift Days as a within-subjects variable, and Sex, Group, Line, and Replicate as between-subjects variables. Mauchley's test for sphericity was significant for all ANOVAs, therefore the Greenhouse-Geisser correction was used. Day 2 data were not included in any of the analyses because there was a loss of intake data on that day. For missing intakes, the mean of that animal's behavior from the day before and after were used to impute a value (2 intake values), and these were compared with the lick values, which both produced lick volumes under the established criterion of 4.6 microliters/lick.

During the pre-shift period, LAP 4-4 mice drank and licked less than LAP 32-4 mice, but the HAP 32-4 and 4-4 groups did not differ. Where Replicate interactions were not obtained, Line data are presented collapsed across Replicate 2 and 3 mice. For intake (in ml), there was a main effect of Day $F(4.7, 357.6) = 43.3$, $p < .001$, as well as interactions of Day x Group, $F(4.7, 357.6) = 3.4$, $p < .01$, Day x Sex, $F(4.7, 357.6) = 2.4$, $p < .05$, and Day x Line $F(4.7, 357.6) = 2.5$, $p < .05$, but there were no other interactions

with Day (all Fs $< 2.3$, *p*s $> .05$). A follow-up trend analysis indicated that intake changed in a linear, quadratic, and cubic pattern across Day (*p*s $< .005$). Follow-up trend analyses indicated that both the 4-4 and 32-4 Group intakes had significant linear, quadratic, and cubic trends (*p*s $< .05$), but that the 4-4 group had a trend for an order 5 polynomial pattern ($p = .07$), while the 32-4 group had a trend for an order 4 polynomial pattern ($p = .08$). An a priori comparison indicated that 32-4 intake was significantly higher than 4-4 intake on the last day of the pre-shift period ($p < .001$), with intakes of 1.25 $\pm$ .05 and .88 $\pm$ .08 ml, respectively. Follow-up trend analyses for the Day x Sex interaction indicated that female pre-shift intake had significant linear, quadratic, and cubic trends (*p*s $< .005$), while males had a different pattern of pre-shift intake, as the data had linear, quadratic, and order 4 polynomial trends (*p*s $< .01$). An *a priori* comparison indicated that intake did not differ between the sexes by the last day of the pre-shift period ($p > .05$). Finally, trend analyses for the Day x Line interaction indicated that both HAP and LAP intake had linear, quadratic, and cubic trends (*p*s $< .05$), but there was also a trend for HAP intake to have an order 5 polynomial pattern ($p = .07$). An a priori comparison on the last day of pre-shift intake indicated that sucrose intake was higher in HAP mice than LAP mice ($p < .005$), with intakes of 1.28 $\pm$ .06 and .84 $\pm$ .08 ml, respectively.

There was a main effect of Group, $F(1, 76) = 26.9$, $p < .001$, with 32-4 mice drinking more sucrose (1.13 $\pm$ .04 ml) than 4-4 mice (.82 $\pm$ .04 ml) throughout the preshift period. There was also a main effect of Line $F(1, 76) = 54.0$, $p < .001$, with HAP mice drinking more than LAP mice during the pre-shift period, 1.20 $\pm$ .04 ml and .76 $\pm$ .04 ml, respectively. There was an interaction of Group x Line, $F(1, 76) = 20.8$, $p < .001$, which was driven by a difference in the LAP groups (*Figure 1A, 1B*). LAP 32-4 had higher intake than LAP 4-4 mice, $F(1, 43) = 33.9$, $p < .001$, with overall intakes of 1.05 $\pm$ .06 and .46 $\pm$ .06 ml, respectively. The HAP 32-4 and 4-4 groups did not differ in

their intakes, $F(1,45) = .25$, $p > .05$, with overall intakes of 1.21 $\pm$ .06 and 1.18 $\pm$ .06 ml, respectively. There was also a highly significant difference in sucrose intake between HAP 4-4 and LAP 4-4 groups, $F(1, 44) = 53.1$, $p < .001$, while there was only a nominally significant difference in sucrose intake between HAP 32-4 and LAP 32-4 groups, $F(1, 44) = 4.8$, $p < .05$. There was a Group x Replicate interaction, $F(1, 76) = 7.4$, $p < .01$, which was driven by a difference in intake between Replicate 2 groups, $F(1,43) = 13.2$, $p < .001$ (*Figure 3A*). Replicate 2 32-4 mice consumed more sucrose than 4-4 mice, with respective intakes of 1.18 $\pm$ .06 and .71 $\pm$ .06 ml. Replicate 3 group 32-4 and 4-4 intakes did not significantly differ, $F(1,45) = 1.7$, $p > .05$, with intakes of 1.08 $\pm$ .06 and .93 $\pm$ .06 ml, respectively. Neither Replicate differed for in their consumption of 4% or 32% sucrose ($F$s < 1.9, $p$s > .05). Finally, there was a Line x Replicate interaction, $F(1, 76) = 4.2$, $p < .05$. Although with both Replicates there were significant Line differences in intake, the effect was more significant for Replicate 2 mice, $F(1, 43) = 25.89$, $p < .001$, with higher overall intakes in HAP2 mice compared to LAP2 mice (*Table 1*). The effect was not as significant for Replicate 3 mice, $F(1, 45) = 10.00$, $p < .005$, but HAP3 mice still consumed more sucrose than LAP3 mice. Neither HAPs nor LAPs differed in sucrose consumption by Replicate ($F$s < 2.0, $p$s > .05). There were no other main effects or interactions (all $F$s < 3.6, $p$s > .05).

For intake (in ml/kg), there was a main effect of Days $F(4.7, 359.1) = 33.8$, $p < .001$. A follow-up trend analysis demonstrated that intake had linear, quadratic, cubic, and order 8 polynomial trends (ps < .05). There was also an interaction of Day x Group, $F(4.7, 359.1) = 3.3$, $p < .01$. A follow-up trend analysis showed that 4-4 intake increased in a linear, quadratic, cubic and order 5 polynomial trend ($p$s < .05), while 32-4 intake increased in a linear and quadratic pattern ($p$s < .05). An *a priori* comparison on the last day of pre-shift intake indicated that 32-4 intake (in ml/kg) was higher than 4-4 intake (p

$< .001$), with intakes of $56.46 \pm 2.45$ and $43.33 \pm 4.49$ ml/kg, respectively. There were no other interactions with Day (all $Fs < 2.2$, $ps > .05$) There were main effects of Group, $F(1, 76) = 23.2$, $p < .001$, with 32-4 mice drinking more sucrose than 4-4 mice, $51.62 \pm 1.86$ ml/kg and $38.92 \pm 1.86$ ml/kg, respectively. There was also a main effect of Line $F(1, 76) = 56.6$, $p < .001$, with HAP mice drinking more than LAP mice, $55.18 \pm 1.84$ ml/kg and $35.36 \pm 1.89$ ml/kg, respectively. Lastly, there was a main effect of Replicate $F(1,76) = 4.1$, $p < .05$, with Line 3 mice drinking more sucrose than Line 2 mice overall, $47.95 \pm 1.84$ ml/kg and $42.60 \pm 1.89$ ml/kg, respectively. There was also an interaction of Group x Line, $F(1, 76) = 56.6$, $p < .001$, which was driven by a difference between LAP 32-4 and 4-4 groups, $F(1,43) = 30.3$, $p < .001$ (*Figure 1C, D*). LAP 32-4 mice had higher overall intake than LAP 4-4 overall intake, with intakes of $48.33 \pm 2.63$ and $22.39 \pm 2.70$ ml/kg, respectively. HAP 32-4 and 4-4 groups did not differ, $F(1, 45) = 0.1$, $p > .05$, with intakes of $54.91 \pm 2.63$ and $55.46 \pm 2.57$, respectively. HAP 4-4 mice and LAP 4-4 mice differed in their sucrose intake, $F(1, 44) = 53.6$, $p < .001$, while HAP 32-4 and LAP 32-4 did not differ in their level of intake $F(1, 44) = 3.4$, $p > .05$. Finally, there was an interaction of Group x Replicate, $F(1, 76) = 9.2$, $p < .005$, which was driven by a difference in intake between the Line 2 32-4 and 4-4 groups, $F(1,43) = 15.1$, $p < .001$, with intakes of $52.95 \pm 2.63$ and $32.25 \pm 2.70$ ml/kg, respectively (*Figure 3B*). Neither Replicate 3 nor Replicate 2 had differences in intake of 32% or 4% sucrose ($Fs < 3.4$, $ps > .05$).

For licks, there was a main within-subject effect of Days, $F(5.4, 414.1) = 22.4$, $p < .001$, and a follow-up trend analysis indicated there were linear, quadratic, cubic, and order 6 polynomial trends in licks ($ps < .05$). Females and males had a different pattern of licks across days, as there was an interaction of Days x Sex, $F(2.8, 414.1) = 2.8$, $p < .05$. Female licks had linear, quadratic, cubic, and order 6 polynomial trends ($ps < .05$),

while male licks had linear, cubic, and order 8 polynomial trends ($p$s $< .05$). An *a priori* comparison on the last day of intake revealed there was no difference between males and females by the end of the pre-shift period ($p > .05$). There was also an interaction of Days x Sex x Replicate, $F(5.4, 414.1) = 2.4$, $p < .05$, which was driven by the Replicate 3 mice, $F(5.3, 239.5) = 3.5$, $p < .005$. There was no interaction of Days x Sex for Replicate 2 mice, $F(5.0, 215.8) = 1.3$, $p > .05$. There was a change in licks across Days for Replicate 3 males, $F(4.7, 109.1) = 9.7$, $p < .001$ with lick data that had both linear and quadratic trends ($p$s $< .05$). The Replicate 3 females lick data also changed across Days, $F(8, 176) = 4.8$, $p < .001$, and had linear, quadratic, and cubic trends ($p$s $< .05$). There were no other interactions with Day (all $F$s $< 2.5$, $p$s $> .05$). There were between-subjects main effects of Group, $F(1, 76) = 27.2$, $p < .001$, with 32-4 mice having a greater number of licks than 4-4 mice during the pre-shift period, $726.7 \pm 35.1$ and $467.6 \pm 35.1$ licks, respectively. There was also a main effect of Line, $F(1, 76) = 11.8$, $p < .001$, with HAPs having a greater number of licks than LAP mice, $682.5 \pm 34.7$ and $511.8 \pm 35.5$ licks, respectively. There was an interaction of Line x Group, $F(1, 76) = 10.7$, $p < .005$, which was driven by a difference in licks between the LAP groups (*Figure 2 A,B*). LAP 32-4 mice licked more than LAP 4-4 mice, $F(1, 43) = 27.0$, $p < .001$, with $722.6 \pm 49.7$ and $301.0 \pm 50.8$ licks, respectively. There was no difference between HAP 32-4 and 4-4 groups, $F(1, 45) = 2.6$, $p > .05$, which had $730.8 \pm 49.7$ and $634.2 \pm 48.5$ licks, respectively. HAP 4-4 mice licked more than LAP 4-4 mice, $F(1, 44) = 26.3$, $p < .001$. On the other hand, there was no difference in lick behavior between HAP 32-4 and LAP 32-4, $F(1, 44) = 0.0$, $p > .05$. There was also a Group x Replicate interaction, $F(1, 76) = 8.3$, $p < .01$, which was due to a difference between 32-4 and 4-4 Replicate 2 groups, $F(1, 43) = 27.0$, $p < .001$ (*Figure 3C*). Replicate 2 32-4 mice licked more than 4-4 mice, $783.7 \pm 49.7$ and $381.2 \pm 50.1$ licks, respectively. On the other hand, there was no difference

in the number of licks between the Line 3 groups, $F(1, 45) = 2.3$, $p > .05$, as Replicate 3 32-4 mice had $669.7 \pm 49.7$ licks and 4-4 mice had $554.0 \pm 48.5$ licks. There were no other main effects or interactions, (All $Fs < 3.5$, $ps > .05$). Replicate 2 and 3 mice differed in their consumption of 4% sucrose, $F(1, 44) = 4.2$, $p < .05$, but did not differ in their consumption of 32% sucrose, $F(1, 44) = 2.0$, $p > .05$.

Pre-shift behavior was also analyzed separately for each replicate, and showed a very similar pattern to the overall analyses. Repeated measures ANOVAs were performed for pre-shift days, using Group and Line as between-subjects measures. I report here if there were significant Group x Line interactions for each Replicate, and report the other effects if an interaction was not present. For pre-shift intake (ml) in Replicate 2 mice, there was a significant interaction of Group x Line, $F(1, 41) = 13.4$, $p < .005$. There was also significant interaction of Group x Line for pre-shift intake (in ml/kg), $F(1, 41) = 20.9$, $p < .001$. Finally, there was a trend for a Group x Line interaction for licks, $F(1, 41) = 3.7$, $p = .06$. There were main effects of Group and Line for licks, $Fs > 18.9$, $ps < .001$. For Replicate 3 mice, there was a Group x Line interaction for intake (ml), $F(1, 43) = 8.8$, $p < .01$. There was also an interaction of Group x Line for intake (ml/kg), $F(1, 43) = 8.8$, $p < .01$. Finally, there was a Group x Line interaction for pre-shift licks, $F(1, 43) = 7.6$, $p < .01$. Follow-up comparisons were performed for each Line and Group, regardless of if an interaction was present and are reported in Table 2.

### 3.3.3 Post-shift Behavior

Three repeated measures ANOVAs were performed on intake 1) total volume in ml, 2) volume corrected for weight in ml/kg), and 3) licks using post-shift Days as a within-subjects variable, and Sex, Group, Line, and Replicate as between-subjects variables.

Mauchley's test for sphericity was significant for most of the ANOVAs, and in those cases, the Greenhouse-Geisser correction was used.

Contrast effects were obtained, as was indicated by lower intake (ml and ml/kg) in the 32-4 shifted group compared to the 4-4 unshifted control group. As expected, HAP shifted 32-4 mice experienced a larger contrast effect than LAP shifted 32-4 mice. A repeated measures ANOVA for intake (in ml) indicated that intake changed across Days, $F(2.5, 193.3) = 3.1$, $p < .05$. A follow-up trend analysis indicated that intake changed in a linear fashion across Days ($p < .05$). There was no interaction of Days and any other variable (all Fs < 2.1, *p*s > .05). There were between-subjects main effects of Group, $F(1, 76) = 36.2$, $p < .001$ with the 32-4 shifted group drinking less than the unshifted 4-4 group, .57 $\pm$ .05 and .95 $\pm$ .05 ml, respectively. There was also a main effect of Line, $F(1, 76) = 73.3$, $p < .001$, with HAP mice drinking more sucrose overall compared to LAP mice, 1.04 $\pm$ .05 and .48 $\pm$ .05 ml, respectively. There was also a main effect of Replicate, $F(1,76) = 10.3$, $p < .005$, with Replicate 3 mice drinking more sucrose than Replicate 2 mice, .86 $\pm$ .05 and .66 $\pm$ .05 ml, respectively. There was a significant interaction of Group x Line, $F(1, 76) = 5.7$, $p < .05$, which was driven by a larger contrast effect in HAP mice than in LAP mice (*Figure 4A, B*). HAP 32-4 shifted mice consumed less than HAP 4-4 mice, $F(1, 45) = 34.9$, $p < .001$, with intakes of .76 $\pm$ .06 and 1.31 $\pm$ .06 ml, respectively. LAP 32-4 mice also consumed less sucrose than LAP 4-4 mice, but the significance was smaller than in HAP mice, $F(1, 43) = 6.0$, $p < .05$, with intakes of .37 $\pm$ .06 and .60 $\pm$ .07 ml, respectively. The effect size for contrast was also much larger in HAP mice than in LAP mice, with partial eta squares of .44 and .12, respectively. HAP 4-4 mice drank more sucrose than LAP 4-4 mice, $F(1, 44) = 32.6$, $p < .001$, and HAP 32-4 mice also drank more sucrose than LAP 32-4 mice, $F(1, 44) = 35.2$, $p < .001$. Finally, there was an interaction of Line x Replicate, $F(1,76) = 6.8$, $p$

$< .01$, which was driven by a large difference in post-shift intake between HAP2 and LAP2 mice, $F(1, 43) = 55.4$, $p < .001$ (*Table 1*). HAP3 mice also consumed more sucrose than LAP3 mice, $F(1, 45) = 11.0$, $p < .005$. The LAP replicates also differed in post-shift intake, $F(1, 44) = 17.3$, $p < .001$, while the HAP replicates did not, $F(1, 44) = 0.2$, $p > .05$. There were no other main effects or interactions, (all Fs $< 1.5$, *p*s $> .05$).

A repeated measures ANOVA for intake (in ml/kg) indicated that intake changed across Days, $F(2.5, 193.3) = 3.5$, $p < .05$, and a follow-up trend analysis indicated that post-shift intake (in ml/kg) changed in a linear pattern ($p < .05$). Days did not interact with any other variables (all Fs $< 2.3$, *p*s $> .05$). The 32-4 group drank less than the 4-4 group, $F(1, 76) = 29.7$, $p < .001$, with intakes of $27.62 \pm 2.18$ and $44.41 \pm 2.18$ ml/kg, respectively. HAP mice drank more sucrose than LAP mice overall, $F(1, 76) = 73.6$, $p < .001$, with intakes of $49.22 \pm 2.15$ and $22.81 \pm 2.20$ ml/kg, respectively. Replicate 3 mice drank more sucrose than Replicate 2 mice, $F(1, 76) = 16.4$, $p < .001$, with intakes of $42.25 \pm 2.15$ and $29.78 \pm 2.20$ ml/kg, respectively. There was also an interaction of Group x Line, $F(1, 76) = 4.3$, $p < .05$, which was due to the occurrence of a larger contrast effect in the HAP groups, $F(1, 45) = 26.6$, $p < .001$, 32-4 and 4-4 mice had intakes of $37.64 \pm 3.08$ and $60.80 \pm 3.00$ ml/kg, respectively (*Figure 4C,D*). LAP 32-4 and 4-4 groups also had different intakes, indicating contrast occurred, $F(1, 43) = 5.5$, $p < .05$, with $17.60 \pm 3.08$ and $28.01 \pm 3.15$ ml/kg, respectively. The effect size for HAP contrast was also larger than the LAP effect size, with partial eta squares of .37 and .11, respectively. HAP 4-4 mice also consumed more sucrose than LAP 4-4 mice, $F(1, 44) = 31.5$, $p < .001$; HAP 32-4 mice consumed more sucrose than LAP 32-4 mice as well, $F(1, 44) = 34.0$, $p < .001$. There were no other main effects or interactions (all Fs $< 2.2$, *p*s $> .05$).

A repeated measures ANOVA for licks was performed, and Mauchley's test was not significant, therefore we proceeded with the assumption of sphericity. Licks changed across Days during the post-shift period, $F(3, 228) = 7.0$, $p < .001$, and a follow-up trend analysis demonstrated that this change was in a linear and cubic fashion ($p$s $< .05$). There was also an interaction of Days x Group x Replicate, $F(3, 228) = 3.0$, $p < .05$. In Replicate 2 mice, there was a Group x Days interaction, $F(3, 129) = 2.7$, $p < .05$, which was driven by a change in licks by the 32-4 group across days, $F(3,66) = 4.6$, $p < .01$. The Replicate 2 32-4 group increased in licks in a linear fashion across days ($p < .05$), while the 4-4 group did not change, $F(2.2, 46.1) = 2.3$, $p > .05$. On the other hand, there was no Group x Days interaction for Replicate 3 mice, $F(2.5, 114.4) = 1.6$, $p > .05$. There were no other interactions with Day (all Fs $< 2.0$, $p$s $> .05$). The 4-4 group licked more during the post-shift period than the 32-4 group, $F(1, 76) = 23.5$, $p < .001$, with $546.1 \pm 26.4$ and $364.7 \pm 26.4$ licks, respectively. Males licks more than females, $F(1, 76) = 4.6$, $p < .05$, with $495.6 \pm 26.4$ and $415.3 \pm 26.4$, respectively. HAP mice licked more than LAP mice, $F(1,76) = 47.7$, $p < .001$, with $584.6 \pm 26.1$ and $326.3 \pm 26.8$ licks, respectively. Replicate 3 mice licked more than Replicate 2 mice, $F(1, 76) = 4.3$, $p < .05$, with $494.2 \pm 26.1$ and $416.7 \pm 26.8$ licks, respectively. There was an interaction of Line x Replicate, $F(1,76) = 10.3$, $p < .005$, which was driven by HAP2 having a much greater number of licks than LAP2 mice, $F(1, 43) = 54.1$, $p < .001$ (*Table 1*). HAP3 mice also had a larger number of licks than LAP3 mice, but the effect was not as significant as for Replicate 2 mice, $F(1, 45) = 4.5$, $p < .05$. LAP3 mice licked more during the post-shift period than LAP2 mice, $F(1, 44) = 12.5$, $p < .005$, but HAP licking behavior was not different between the replicates, $F(1, 44) = 0.3$, $p > .05$. There was also a trend for a Line x Group interaction, $F(1, 76) = 2.8$, $p = .099$. Because it was an a priori hypothesis of ours that a Line x Group interaction would exist, a follow-up ANOVA was performed

for each Line, which tested for differences between the 32-4 and 4-4 groups (*Figure 5A, B*). This analysis indicated that there was a significant difference between HAP 32-4 and 4-4 groups, F(1, 45) = 18.6, *p* < .001, which had 462.7 $\pm$ 37.4 and 706.6 $\pm$ 36.5 licks, respectively. There was a marginally significant difference between the LAP 32-4 and 4-4 groups, F(1, 43) = 4.2, *p* < .05 (*p* =.046), with 385.7 $\pm$ 38.3 and 266.8 $\pm$ 37.4 licks, respectively. The effect size was also higher for HAP contrast than for LAP contrast, with partial eta squares of .29 and .09, respectively. HAP 4-4 mice consumed more sucrose than LAP 4-4 mice, F(1, 44) = 21.1, *p* < .001; HAP 32-4 mice also consumed more sucrose than LAP 32-4 mice, F(1, 44) = 17.4, *p* < .001.

Post-shift behavior was also analyzed separately for each replicate. Repeated measures ANOVAs were performed for pre-shift days, using Group and Line as between-subjects measures. I report here if there were significant Group x Line interactions for each Replicate, and report the other effects if an interaction was not present. In Replicate 2 mice, there was an interaction of Group x Line for intake (ml), F(1, 41) = 6.7, *p* < .05. There was also a Group x Line interaction for intake (ml/kg), F(1, 41) = 5.9, *p* < .05. There was no interaction of Group x Line for licks, F(1, 41) = 0.3, *p* > .05. There were main effects of Group and Line, Fs > 6.4, *p*s < .05. In Replicate 3 mice, there was no Group x Line interaction for intake (ml), F(1, 43) = 0.6, p > .05. There were main effects of Group and Line, Fs > 14.0, *p*s < .001. There was also no interaction of Group x Line for intake (ml/kg), F(1, 43) = 1.1, *p* > .05. There were main effects of Group and Line, Fs > 13.4, *p*s < .005. Finally, there was no interaction of Group x Line for licks, F(1, 43) = 2.5, p > .05. There were main effects of Line and Group, Fs > 5.6, *p*s < .05. Follow-up comparisons were performed for each Line and Group, regardless of whether an interaction was present or not, and are reported in Table 2.

## 3.4 Discussion

This study provides the first evidence of a genetic correlation between reactivity to incentive shift and alcohol preference. Specifically, a high level of reactivity to an incentive downshift, or a high level of frustrative behavior, is correlated with high alcohol preference. This is supported by the observation that HAP2 and HAP3 mice shifted mice experience larger contrast effects than their respective LAP2 and LAP3 replicate lines. These data may provide a preclinical model for treatment development to decrease aberrant emotional reactivity in predisposed populations. Further, it is possible to use the HAP and LAP lines to study the shared genetic and neurological mechanisms underlying both frustrative emotional reactivity and high alcohol preference. Looking at reactivity to incentive downshift is also a novel way to study stress in relation to alcoholism. This might be particularly important for humans, because negative life events often involve some type of reward downshift or loss. Predisposed individuals that react more strongly to reward loss or downshift may be at a greater risk for problematic drinking behavior. This could be because they either drink to reduce frustration or tension associated with a negative occurrence (61), or because drinking itself may be conceived as an emotionally reactive response to stress.

Analysis of baseline weights uncovered a difference in weight between HAP2 and LAP2 mice, but not a difference in weight between HAP3 and LAP3 mice. Lower weight in LAP2 mice could have potentially been responsible for the Line x Replicate interactions that were observed in intake and licks during the pre-shift and post-shift periods (*Table 1*). This notion is supported by the fact that there were not Line x Replicate effects for intake when sucrose was measured in ml/kg, which controls for differences in weight between the lines. Fortunately, as contrast is measured in a

between-subjects manner, and compares two groups of the same line (HAP 32-4 versus HAP 4-4 and LAP 32-4 versus LAP 4-4), the differences in weight between the HAP2 and LAP2 mice should not have affected the main comparison of interest.

During the pre-shift period, HAP and LAP mice differed in their consumption of 4% sucrose and 32% sucrose, though the effect was much more pronounced for 4% sucrose, as a line difference was present for intake (in ml and ml/kg) and in licks, but was only present for intake (in ml) for the 32-4 groups. This very significant line difference was also present in the 4-4 HAP and LAP control groups during the post-shift period, and became both significant and evident for all measures once HAP 32-4 and LAP 32-4 groups were downshifted. Interpreting a line difference in the downshifted groups is somewhat more complicated, though, because of their previous experience with 32% sucrose. Interestingly, it has been shown that variation in the *tas1r3* gene contributes to strain differences in sucrose and other sweet solutions at low concentrations, but not at high concentrations (75, 76). The *tas1r3* gene encodes for the T1R3 receptor, which is one of two main types of peripheral taste receptors involved in the initial detection of sweet substances (77). Inoue and colleagues (75) used 2 129.B6-*Tas1r3* segregating congenic strains that only vary at the *tas1r3* locus, and demonstrated that the gene contributes to variations in sucrose consumption at low concentrations. The authors note that other genes, post-ingestive factors, and motivational factors probably contribute to strain differences at higher concentrations. Glenndinning and colleagues (76) used 8 inbred strains known to vary in their *tas1r3* alleles to assess lick behavior and short-term access to sucrose, in hope of evaluating the role of the alleles without the influence of post-ingestive or motivational influences present with long-term access. Four of the inbred strains had the taster allele and 4 had the non-taster allele; the taster allele conveys increased sensitivity for low

concentrations of sweet taste (78, 79). At low sucrose concentrations, the strains varied in their consumption of sucrose, but did not vary at high concentrations of sucrose. Additionally, these authors observed variation in consummatory responding in strains with taster allele, suggesting that strain differences in consumption at high concentrations of sucrose are due to additional genetic or post-ingestive factors. The lack of a difference in HAP groups and a difference between LAP groups, as well as a Line difference in 4% sucrose consumption, but not as strong and for all measures of 32% sucrose consumption, may be explained by variation at the tas1r3 locus or other genetic loci that regulate taste differences. Specifically, the HAP lines may have a higher frequency of taster alleles, while LAP mice may have a higher frequency of non-taster alleles for the gene. Additionally, that the lick data in the 4-4 groups suggest that the line differences are due to more immediate taste processes, which would be supportive of the lines differing in *tas1r3* allele frequency, although repeated exposure to sucrose may influence motivation. Future studies could assess whether HAP and LAP mice differ in allele frequencies of the *tas1r3* gene. That being said, as HAP and LAP mice did differ in their pre-shift intake of 32% sucrose, as well, which suggests that there are other genes, post-ingestive, or motivational factors that contribute to higher sucrose intake in HAP mice. Altogether, the observation of differing consummatory behavior between HAP and LAP mice suggests that the lines perceive rewards differently, which is one observation that supports the idea that HAP and LAP mice differ in reward sensitivity.

Sweet preference has been investigated and confirmed by several investigators as being related to high alcohol preference in rodents. An association between sweet preference and alcohol preference has also been observed in humans (80), but this can be a complicated relationship or is not always the case (81-83). Oberlin and colleagues (2011) demonstrated that saccharin preference is a correlated response to selection in

HAP1, 2, 3 and LAP 2, 3 mice, with all HAP lines drinking greater levels of saccharin than the HS/Ibg progenitor line, while the LAP lines either drank less or the same amount as the HS/Ibg mice. There was also a very strong, positive correlation between each line's 10% free-choice alcohol intake and .32% saccharin intake (the most preferred concentration by all lines). Observing this correlated response in saccharin preference confirmed an earlier finding by Grahame and colleagues (1999) that the HAP1 mice prefer saccharin compared to LAP1 mice. Others have found associations between ethanol intake and sweet preference, both in rat high and low alcohol preferring lines and inbred strain panels (84-88). On the other hand, Agabio and colleagues (89) found no evidence for a correlated response in saccharin preference between Sardinian Alcohol Preferring and Non-preferring rats. Although there are disassociations between sweet preference and alcohol preference in rats and mice, the majority of findings support an association. The overall analyses and separate analyses of pre-shift behavior in each replicate confirm sucrose preference, particularly at low concentrations of sucrose, as a correlated response to selection. There was a greater effect in the Line 2 mice, which are further along in selection, and therefore a greater number of trait-relevant alleles may have been fixed, potentially influencing the strength of a correlated response. Although it was not a finding we had anticipated, the pre-shift 32-4 and 4-4 data, as well as the post-shift 4-4 data, provide support for the idea that sucrose preference is associated with high alcohol preference, an effect that was particularly evident at the low concentration of sucrose.

Line differences in consummatory behavior may be considered a limitation of this experiment because HAP and LAP mice differ in baseline behavior. On the other hand, I hypothesized that differences in reward sensitivity are responsible for differences in contrast behavior. Therefore, baseline differences in pre-shift intake might be a common

occurrence if one is looking for individual differences in contrast. Freet and colleagues (27) observed a similar pattern of pre-shift behavior in Lewis and Fisher rats, where Lewis rats had much higher intake of both 32% and 4% sucrose than Fisher rats and also exhibited a larger contrast effect than Fisher rats during the post-shift period. Interestingly, selection for high and low contrast also resulted in pre-shift intake line differences (13). The Large Contrast line had a higher level of licks during pre-shift compared to the Small Contrast line, an effect that was present across several generations of selection. On the other hand, no pre-shift difference existed for Maudsley Reactive and Maudsley Non-Reactive rats, even though Maudsley Non-Reactive rats exhibited a larger contrast effect (90). Therefore, it is not always the case that individual differences in contrast occur in conjunction with individual differences in pre-shift intake. While piloting contrast in HAP and LAP mice, I attempted to match intake by trying different concentrations of saccharin and sucrose, but was unable to find sucrose concentrations that both produced contrast and did not result in higher intake of the control solution, which would make interpreting the data difficult. Therefore, although LAP mice have a low level of 4% sucrose intake, we reasoned that contrast was possible, because intake of 4% sucrose was statistically different from zero. Therefore as long as contrast is detectable in both lines, this may not be a major flaw in the experimental design. Line differences in sucrose intake may be considered a strength, because they support the idea that HAP and LAP mice differ in reward sensitivity.

Contrast effects occurred in both HAP and LAP lines following an incentive downshift, as was evidenced by suppressed intake in the 32-4 shifted mice compared to the 4-4 unshifted controls. This study is the second reported occurrence of contrast effects occuring in mice. Mustaca and colleagues (91) gave BALB/c mice access to 4 or 32% sucrose during 1-hour and 3-hour home-cage sessions. This procedure is different

from the traditional SNC procedure that has been used in rats, which normally uses food restricted rats and 5-minute sessions in operant boxes. Therefore, although the study by Mustaca and colleagues is useful because it demonstrates that contrast occurs in mice, the results from this study and procedure could not be easily compared to the rest of the incentive downshift literature. Additionally, having short pre-shift and post-shift session ensures that the contrast effects are present throughout several daily post-shift sessions, as contrast does not dissipate in the 5-minute period. On the other hand, contrast only occurred for 1 day when both 1-hour and 3-hour sessions were used, making it possible that the effect dissipated with longer access times. Using operant boxes equipped with lick-o-meters allows for the quantification of lick behavior. Use of operant boxes instead of home cages also creates an environment in which the rodent expects sucrose by allowing specific associations to develop between the operant box and sucrose availability (24). Therefore, the present study is the first example of contrast in mice that uses the same procedure as has traditionally been used in rats, making it easier to compare results from mice to the rest of the contrast literature.

Most importantly, this study shows that HAP mice experience larger contrast effects than LAP mice. This assertion is supported by the interaction of Line x Group, driven by a more significant effect in the HAP lines compared to LAP lines. There were also Group x Line interactions for the Line 2 mice for both measures of intake, which supports the idea that HAP2 mice experienced larger contrast effects than LAP2 mice. There were more significant contrast effects in HAP2 mice compared to LAP2 mice on both measures of intake, and there was a trend ($p = .06$) for a contrast effect in HAP2 licks. Though there were not Line x Group interactions for post-shift intake in Replicate 3 mice, the pairwise comparisons in Table 2 support the occurrence of larger contrast in HAP3 mice compared to LAP3 mice. There was no evidence for an overall contrast

effect in licks for LAP3 mice, and there was a trend ($p$ = .06) for a contrast effect in intake (ml/kg). On the other hand, HAP3 mice demonstrated significant contrast effects for all measures. The effect size for HAP contrast was also higher than LAP lines for all measures and for both HAP replicates, which further supports the idea that the magnitude of the effect was stronger in HAP mice than in LAP mice. Considering the follow-up comparisons and effect sizes for contrast in each Line and Replicate, there is moderate to strong evidence for a genetic correlation between alcohol preference and emotional reactivity to an incentive shift. To my knowledge, no animal studies have assessed whether high emotional reactivity as a result of incentive downshift is associated with a genetic predisposition for high alcohol preference. As discussed, Lewis rats experience larger contrast effects than Fisher rats (27). Lewis rats and Fisher rats also vary in their behavior and neurological response to alcohol, as Lewis rats readily self-administer alcohol and other drugs of abuse, while Fisher rats do not (65-68). Freet and colleagues provided initial evidence that a drug-seeking phenotype might be associated with reactivity to incentive downshift, but these results are limited because Lewis and Fisher rats are inbred strains. Because the strains are isogenic, the results cannot be inferred to be representative of the general population. Our study supports this idea, but by using selectively bred high and low alcohol preferring mice, which are outbred, and therefore more representative of the general population. Therefore, to our knowledge, this is the first demonstration of a genetic correlation between high alcohol preference and emotional reactivity to incentive downshift.

Finding larger negative contrast in HAP mice compared to LAP mice provides initial evidence that this type of emotional reactivity to incentive downshift is a potential behavioral endophenotype for alcoholism. This type of emotional reactivity is relevant because it involves a stressor that is similar to the types of stressors humans normally

encounter, in that human negative life events often involve some type of incentive downshift or loss. Therefore, studying incentive downshift and frustration are relevant to studying human stress in relation to drinking. An incentive downshift task could also easily be translated into a human task, as contrast effects have been assessed in humans (14). Translating the findings from this project into humans would be an important next step in determining the degree to which emotional reactivity contributes to addictive behaviors as well as to emotion-driven rash action. Further, learning about predisposing factors for alcoholism is important for developing both preventative and tailored treatments for both aberrant emotional reactivity and the disorder. For example, if we demonstrate that emotional reactivity associates with drinking, one could target reactivity in humans with cognitive behavioral therapy (92) or pharmacological treatment (93) with the aim of preventing reactivity in predisposed populations or reducing drinking problems. Lastly, the increased emotional reactivity seen in HAP mice may allow for exploration into the shared genetic and neurological mechanisms underlying emotional reactivity and high alcohol preference.

# CHAPTER 4. DOES INCENTIVE DOWNSHIFT PRODUCE RASH ACTION, AND IS THIS URGENT-LIKE BEHAVIOR RELATED TO A PREDISPOSITION FOR HIGH ALCOHOL PREFERENCE

## 4.1 Introduction

As discussed, the initial response to an incentive downshift results in an increase in appetitive behaviors, which has been theorized to consist of a "search" for the missing reward (10, 20). This "search" reaction is particularly interesting, because it is similar to the behavior that can lead to certain types of impulsive decision-making. Though the "search" response may not necessarily constitute rash action, the immediate reaction to an incentive downshift could theoretically lead to rash action in humans. Appetitive behaviors allow individuals to come in contact with rewards, so increased search behavior may allow individuals to not only search for the "missing" reward, but to come in contact with additional rewards. Although increasing appetitive behaviors in response to incentive loss or downshift has probably been a highly adaptive evolutionary response to reward downshift, and arguably still is in some situations, it may be counterproductive if long-term goals or other environmental factors are not taken into account.

Craving or other strong emotion states have been related to rash decision-making related to drug use, including excessive alcohol intake (28-31). Negative urgency is the predisposition to act rashly in response to negative emotions (32, 33). According to the theory of urgency, strong emotion provides a signal for action that causes one to

focus on the immediate situation and not necessarily attend to long-term goals, thereby resulting in rash action (34). In a highly urgent individual, loss of a job or significant other may result in a strong negative emotional state, causing the person to heavily consume ethanol and engage in other risky behaviors. Strong relationships between urgency and problems related to alcohol use have been identified (35-37). Interestingly, Stojek and Fisher (94) found that women who endorsed high levels of “drinking to cope” and had high negative urgency also had the highest increase in symptoms of alcohol dependence across a three-month time period. This finding suggests that the co-occurrence of both drinking to relieve emotional distress and rash action in the face of strong emotion predict worse drinking outcomes. It is plausible that having an increased initial reaction to incentive downshift events is related to negative urgency if the resulting affective reaction reduces an individual’s ability to maintain sight of other goals compared to individuals that are less likely to experience or experience smaller reactions to incentive downshift.

There is a significant amount of evidence that urgent-like behavior is associated with higher rates of problematic drinking, but there is no animal model that fully models emotion-driven rash action. An animal model could be used to address if predisposition plays a role in high urgency. It could also be useful for establishing a temporal pattern between the occurrence of a stressor, urgent behavior, and problematic drinking. Finally, it would be useful to study the neurological mechanisms underlying urgency, as well as to understand shared mechanisms underlying urgency and drinking. Gipson and colleagues (95) attempted to design a laboratory human and animal model of urgency by looking at operant responding immediately following a reward omission. In the animal model, animals were trained on a Pavlovian component, which used a cue light (CS±) followed by pellet delivery. They also used an operant component, and trained rats on a

FR-10 schedule of reinforcement. In this task, on test days, the pellet was omitted immediately following the CS± on some trials and not others, resulting in an increase in operant responding immediately following reward omission trials. This was similar to the human task, but small amounts of money were used as reinforcement instead of sucrose pellets. In the human task, individuals also increased responding on an operant portion immediately following reward omission trials. Although the results are interesting because they show an increase in appetitive behavior following reward loss, the design itself does not actually address urgency. This is because both the animals and humans simply increased in responding, but did not demonstrate rash action. It is not necessarily a rash action to increase one's rate of responding, although perseveration is considered by some individuals to be an index of impulsivity (96). It could be considered rash action if the increase in rate of responding was counter-productive to the individual. For example, an increase in responding following reward omission is actually probably considered adaptive behavior under most circumstances, because it promotes finding the "missing" reward. But if an increase in responding occurred under a contingency where it is maladaptive, then the behavior would be considered maladaptive. On the other hand, Gipson and colleagues also found that an increase in responding in humans, indicative of frustration-like behavior, was correlated with negative urgency scores. Therefore, the study provides some support for the idea that frustration and negative urgency are related. In a similar study by Burokas and colleagues (97), mice were trained on a fixed ratio of responding, followed by a day of progressive ratio of responding. Immediately following, there was a 10 minute extinction session. Three groups were tested, including animals that had undergone unexpected reward omission in the presence of a conditioned stimulus, and animals received a reward in the presence of a conditioned stimulus, and animals that experienced no reward and no

conditioned stimulus. Animals that had experienced the unexpected reward omission increased their responding compared to the two other groups, and also exhibited an increased level of aggression on a resident intruder task when tested following the reward omission session. The increases in perseveration and aggression are not fully demonstrative of rash action, but both behaviors may be related to emotion-driven rash action. Incorporating an accepted model of rash action would be useful, because it could be used effectively to model urgency in rodents.

Differential Reinforcement of Low Rates of Responding (DRL) has been used extensively to model motor impulsivity, or the inability to withhold responding (38-40). DRL is an appetitive response inhibition task during which rodents withhold a response during a specified time interval (each time interval is a trial) in order to receive reinforcement. The DRL might be an effective procedure for modeling urgency, because in theory, an increase in responding (or search-like) behavior occurs immediately following incentive downshift. Therefore, if an animal increases its level of responding in the face of negative affect (due to incentive downshift), it gains fewer reinforcers, thereby demonstrating a greater level of motor impulsivity on the task. We have preliminary evidence that HAP2 mice are more impulsive than LAP2 mice during a DRL procedure. Therefore, HAP mice behave more impulsively on the task, and we can modulate an already high level of impulsivity. On the other hand, using the DRL procedure may be disadvantageous because HAP and LAP mice have baseline differences in DRL and SNC, which makes it difficult to directly compare the lines. Thus, although both HAP and LAP mice are included in the following experiment, their behavior during the test of incentive downshift effects on DRL performance will be analyzed separately.

HAP mice have previously been shown to be more impulsive on a than LAP mice using both Delayed Discounting and DRL tasks. As part of this aim, I will try to replicate

the correlated response for higher impulsivity on a DRL task in HAP mice, and also evaluate if the predisposition for rash action can be modulated by emotional status using a DRL task. Specifically, I hypothesize that evoking an emotional reaction in HAP mice by using an incentive downshift will result in increased rash action compared to mice not experiencing a negative emotional reaction. Further, HAP2, HAP3 and LAP2, LAP3 lines will be tested to determine if HAP mice show an increased predisposition for rash action in the face of emotion. Specifically, I hypothesize that an incentive downshift will result in an increased likelihood for rash action in the face of negative affect, as will be inferred by the presence of contrast behavior, and the magnitude of this effect will be correlated with high alcohol preference.

## 4.2 Specific Research Design

All mice underwent the food restriction protocol as described, and were trained on Fixed Interval (FI) followed by the DRL procedure as discussed in Chapter 2. When mice reached a stable level of responding on the DRL procedure, SNC training commenced using 3-minute training sessions. The session length of SNC was shortened in order to avoid satiation and to try to capture the very early response to an incentive downshift. In Replicate 3 mice, pre-shift sessions were run for 10 days, but in Replicate 2 mice, pre-shift sessions took place for 7 days. 7 days of pre-shift was necessary due to scheduling, but should not have affected post-shift behavior greatly, because mice normally reach a behavioral asymptote with the pre-shift concentration prior to 7 pre-shift days. Levers were also taken out of boxes and no nosepoke light was used to create a different context for SNC training. On post-shift day 1 (day 11), all mice received 4% sucrose for 3 minutes. Immediately following SNC, they were placed in another operant box with levers, lever lights, and nose-poke illuminated and underwent the DRL

procedure for 10 minutes. Lever presses were time-stamped throughout pre-shift and post-shift sessions.

Univariate ANOVAs were performed on baseline weights, days to criterion for the FR1-10s and FR1-2.5s phases. They were also used to analyze correct responding, incorrect responding, intake, reinforcers, and efficiency on the last day of FR-2.5 and FI-32 training for each mouse. Efficiency was calculated by dividing rewards by correct responses. Repeated measures ANOVAs using the variables Sex (Female and Male), Line (HAP and LAP), Group (32-4 and 4-4), and Replicate (2 or 3) were used to analyze the FI-32 correct responding data across time-bins and all days of the DRL-32 data. On post-shift day 1, univariate ANOVAs were used to analyze intake and licks. The test day was analyzed because it was necessary to confirm the occurrence of contrast, and the variable durations of pre-shift exposure made it difficult to analyze both replicates. Lick-o-meter functions were analyzed and imputed using the same criterion (a lick volume of less than 4.6 microliters/lick) and procedure described in Chapter 3. Univariate ANOVAs were used to analyze incorrect responding, percent correct responding, intake, efficiency, and reinforcers on the 10-minute DRL test session. A repeated measures ANOVA was used to analyze correct responding across the 10-minute DRL session. Each replicate was tested as a separate cohort, with both LAP and HAP lines represented. The before mentioned analyses were also performed for each replicate (2 and 3) separately, and are reported in Table 3. Because the lines were not directly compared for testing the effect of an incentive downshift on DRL, these data are not included in Table 3.

## 4.3 Results

### 4.3.1 Baseline Weights

One LAP3 male became sick during training and had to be sacrificed; therefore it was removed from all analyses. Although Group was not included as a variable prior to SNC training, it was included in the analyses to ensure the groups did not differ during any phase of the experiment prior to SNC training. Sex differences in weight were present in all mice, LAP2 mice also had low body weights compared to LAP3 and HAP2 mice. A Sex (female, male) x Line (HAP, LAP) x Replicate (2, 3) x Group (4-4, 32-4) ANOVA for baseline weight indicated there was a significant effect of Replicate, $F(1, 78) = 5.9$, $p < .05$, driven by Replicate 2 mice weighing less than Replicate 3 mice, $23.5 \pm 0.3$ and $24.3 \pm 0.3$ g, respectively. Males weighed more than females, $F(1, 78) = 89.2$, $p < .001$, with weights of $25.6 \pm 0.3$ and $22.2 \pm 0.3$ g, respectively. There was also a Line x Replicate interaction, $F(1, 78) = 11.1$, $p < .001$ (*Figure 6*). HAP2 mice weighed more than LAP2 mice, $F(1, 45) = 5.9$, $p < .05$, with weights of $24.3 \pm 0.4$ and $22.6 \pm 0.4$ g, respectively. On the other hand, HAP3 and LAP3 mice did not differ in weight, $F(1, 45) = 0.7$, $p > .05$, weighing $24.0 \pm 0.4$ and $24.7 \pm 0.4$, respectively. LAP 2 weighed less than LAP3 mice, $F(1, 45) = 8.5$, $p < .01$, while HAP2 and HAP3 mice did not differ in weight, $F(1, 45) = 0.1$, $p > .05$. There were no other main effects or interactions for baseline weight (Fs < 2.7, *p*s > .05).

### 4.3.2 Operant Training

LAP2 mice took the longest to acquire lever pressing during the FR1-10s phase, compared to both LAP3 mice and HAP2 mice. For FR1-10s training, a ANOVA with Sex, Replicate, Group, and Line as independent variables was performed on days to criterion,

and there was a Line effect, $F(1, 78) = 9.7$, $p < .005$, with HAPs taking 1.7 ± 0.2 days and LAPs taking 2.4 ± 0.2 days to reach criterion. Replicate 2 mice took longer to reach criterion than Replicate 3 mice, $F(1, 78) = 8.1$, $p < .01$, taking 2.4 ± 0.2 and 1.7 ± 0.2 days, respectively. There was also a Line x Replicate interaction, $F(1, 78) = 13.8$, $p < .001$ (*Figure 7A*). This effect was driven by LAP2 mice taking longer to reach criterion than HAP2 mice, $F(1, 45) = 22.1$, $p < .001$, with HAP2 mice taking 1.6 ± 0.2 days and LAP2 mice taking 3.3 ± 0.2 days to reach criterion. LAP3 and HAP3 mice did not differ in days to criterion, $F(1, 45) = 0.2$, $p > .05$, taking 1.6 ± 0.2 and 1.8 ± 0.2 days, respectively. LAP2 mice also took longer to meet criterion than LAP3 mice, $F(1, 45) = 16.9$, $p < .001$, while HAP2 and HAP3 mice did not differ in days to reach criterion, $F(1, 45) = 0.6$, $p > .05$. There was also a trend for a Line x Group interaction, $F(1, 78) = 4.0$, $p = .05$, but since it did not fully reach significance, I did not follow up on the interaction. There were no other main effects or interactions, $F$s $< .3.6$, $p$s $> .05$.

HAP lines consumed and responded more than LAP mice during the FR1-2.5s phase of training, although this effect varied depending on Replicate. During FR1-2.5 s training, a univariate ANOVA for Sex, Line, Replicate, and Group indicated there was a trend for the Lines to differ in the number of days to reach criterion, $F(1, 78) = 3.7$, $p = .06$, with LAPs taking slightly longer to reach criterion than HAPs, 1.1 ± 0.4 and 1.0 ± 0.4 days, respectively (Figure 7B). There were no other main effects or interactions, $F$s $< 1.4$, $p$s $> .05$. Another ANOVA was performed to assess for differences in intake on the last day of FR1-2.5 training for all mice, which indicated there was a main effect of Line, $F(1, 78) = 31.7$, $p < .001$, with HAPs consuming more saccharin than LAPs, 2.4 ± 0.1 and 1.6 ± 0.1 ml, respectively. There was also a main effect of Replicate, $F(1, 78) = 6.1$, $p < .05$, with Replicate 2 mice consuming more than Replicate 3 mice, with intakes of 2.2 ± 0.1 and 1.8 ± 0.1 ml, respectively. There was also an interaction of Line and Replicate,

F(1, 78) = 6.1, $p < .05$, driven by a significant difference between LAP2 and LAP3 intake, F(1, 45) = 10.6, $p < .005$, with intakes of 2.0 ± 0.1 and 1.2 ± 0.2 ml, respectively (*Figure 7C*) . HAP2 and HAP3 mice did not differ in their intake of saccharin, F(1, 45) = 0.0, $p > .05$, consuming 2.4 ± 0.2 and 2.4 ± 0.1 ml, respectively. There was also a more significant line difference between HAP3 and LAP3 mice, F(1, 45) = 40.7, $p < .001$. The HAP2 and LAP2 mice differed in intake, but the effect was less significant, F(1, 45) = 4.7, $p < .05$. Another ANOVA was performed for correct responding on the last day of FR1-2.5 s training for each mouse. This analysis indicated there was a difference between the Lines, F(1, 78) = 26.1, $p < .001$, with HAPs responding more than LAPs, 111.4 ± 4.4 and 79.4 ± 4.4 correct responses, respectively. There was also a Line x Replicate interaction, F(1, 78) = 8.6, $p < .01$, driven by a difference between LAP replicates, F(1, 45) = 7.4, $p < .01$, with LAP2 mice having 93.0 ± 6.2 correct responses and LAP3 mice having 65.8 ± 6.3 correct responses (*Figure 7D*). HAP2 and HAP3 mice did not differ in their level of responding, F(1, 45) = 1.8, $p > .05$, with 106.7 ± 6.3 and 116.0 ± 6.2 correct responses, respectively. HAP2 and LAP2 mice also did not differ from each other in level of correct responses, F(1, 45) = 2.1, $p > .05$, while HAP3 mice had a greater number of correct responses than LAP3 mice, F(1, 45) = 34.9, $p < .001$.

HAP mice responded more during the FI-32 phase and had lower efficiency, but tended to obtain a greater number of reinforcers than LAP mice. There were no differences between of the variables in meeting criterion for the FI-32 phase, Fs < 2.9, *p*s > .05. A repeated measure was performed for correct responses across time-bins for the last FI-32s session. Sex, Replicate, Line, and Group were also included as between-subjects variables. There was a line difference in correct responding, F(1, 78) = 21.1, $p < .001$, with HAPs responding more than LAP mice, 308.3 ± 19.1 and 181.4 ± 19.1 correct responses, respectively (*Figure 8A*). Replicate 2 mice also responded more than

Replicate 3 mice, $F(1, 78) = 26.5$, $p < .001$, with 315.8 $\pm$ 19.1 and 173.9 $\pm$ 19.1 responses, respectively. There were no other between-subjects main effects or interactions for correct responding, all Fs < 3.3, *p*s > .05. Mauchley's test for sphericity was significant for all within-subjects analyses, therefore the Greenhouse-Geisser correction was used. There was a main effect of Bin, $F(1.5, 114.5) = 97.8$, $p < .001$, a follow-up trend analysis indicated that correct responses changed in linear, quadratic, cubic, and order 4 trends (*p*s < .01). There was an interaction of Bin and Line, $F(1.5, 114.5) = 10.0$, $p < .001$ (*Figure 8B*). Follow-up trend analyses indicated HAPs had linear, cubic, and order 4 polynomial patterns of correct responses (*p*s < .05), while LAP correct responses had linear, quadratic, and cubic trends (*p*s < .05). Bonferroni post hoc comparisons were used to compare the lines at each time bin, and alpha was corrected to (.05/7 = .007). HAP and LAP mice did not differ for bin 5, t(920 = 0.7, $p > .007$, but differed for bins 10-30, ts(92) > 3.2, *p*s < .007. For bin 32, there was a strong trend for HAPs to have a higher number of responses than LAPs, $t(92) = 2.7$, $p = .009$. A Bin x Replicate interaction was also present, $F(1.5, 114.5) = 42.9$, $p < .001$. Follow-up trend analyses indicated that the Replicate 2 correct responses had linear, quadratic, and cubic trends (*p*s < .05), while Replicate 3 correct responding had linear, quadratic, cubic, and order 6 polynomial trends (*p*s < .05). There was also a Bin x Line x Group interaction, $F(1.5, 114.5) = 5.7$, $p < .05$, which was driven by a different pattern of responding between HAP and LAP 4-4 mice, $F(1.3, 55.4) = 8.5$, $p < .005$. HAP 4-4 mice had linear and cubic trends in their correct responding data (*p*s < .05), while LAP 4-4 mice had linear, quadratic, and cubic trends in their correct responding data. (*p*s < .05). Finally, there was a Bin x Line x Sex x Replicate, $F(1.5, 114.5) = 4.1$, $p < .05$. A follow-up analysis revealed that females had a Bin x Line x Replicate interaction, $F(1.3, 58.3) = 4.5$, $p < .05$, while males did not, $F(1.7, 72.5) = 0.9$, $p > .05$. Replicate 3 females had a

Bin x Line interaction for correct responding, $F(1.3, 28.6) = 10.4$, $p < .005$, while Replicate 2 mice did not, $F(1.3, 28.1) = 0.5$, $p > .05$. Replicate 3 female HAP mice correct responding changed across bins, $F(1.2, 13.1) = 10.2$, $p < .01$, in linear, quadratic, and cubic trends (*p*s < .05), but Replicate 2 female LAP mice correct responding did not change across bins, $F(2.6, 28.2) = 1.8$, $p > .05$. There were no other interactions with Bin, Fs < 2.2, *p*s > .05.

Univariate ANOVAs were performed on incorrect responding, intake, reinforcers, and efficiency on the last day of FI-32 training for each mouse. An ANOVA for intake (in ml) indicated there was a main effect of Line, $F(1, 78) = 14.5$, $p < .001$, with HAP mice consuming more saccharin than LAP mice, $1.5 \pm 0.1$ and $1.2 \pm 0.1$ ml, respectively (*Figure 8C*). Replicate 2 mice also consumed more saccharin than Replicate 3 mice, $F(1, 78) = 20.8$, $p < .001$, with intakes of $1.6 \pm 0.1$ and $1.1 \pm 0.1$ ml, respectively. There were no other main effects or interactions for intake, all Fs < 1.9, *p*s > .05. Another ANOVA for reinforcers indicated there was a main effect of Line, $F(1, 78) = 28.3$, $p < .001$, with HAPs gaining more reinforcers than LAPs, $39.4 \pm 0.7$ and $33.8 \pm 0.7$, respectively. Replicate 2 mice also gained a greater number of reinforcers than Replicate 3 mice, $F(1, 78) = 31.7$, $p < .001$, with $39.5 \pm 0.7$ and $33.6 \pm 0.7$ reinforcers, respectively. There was also an interaction of Line x Replicate, $F(1, 78) = 8.3$, $p < .01$, due to a difference in number of reinforcers gained by HAP3 and LAP3 mice, $F(1, 45) = 38.2$, $p < .001$, with $37.9 \pm 1.0$ and $29.3 \pm 1.1$ reinforcers, respectively (*Figure 8D*). HAP2 and LAP2 did not differ in the number of reinforcers gained, although there was a trend for this to be the case, $F(1, 45) = 3.0$, $p = .09$, with $40.8 \pm 1.1$ and $38.3 \pm 1.0$ reinforcers, respectively. HAP2 gained a greater number of reinforcers than HAP3 mice, $F(1, 45) = 5.9$, $p < .05$, but the effect was not as strong as in the LAP mice, $F(1, 45) = 30.5$, $p < .001$. There were no other main effects or interactions for reinforcers, all Fs < 0.8, all

*p*s > .05. An ANOVA for efficiency indicated there was a Line difference in efficiency, $F(1, 78) = 20.9$, $p < .001$, with LAP mice being more efficient than HAP mice, 0.24 ± 0.01 and 0.17 ± 0.01, respectively (*Figure 8E*). Replicate 2 mice were also less efficient than Replicate 3 mice, $F(1, 78) = 22.2$, $p < .001$, with efficiencies of 0.16 ± 0.01 and 0.24 ± 0.01, respectively. There were no other main effects or interactions, all Fs < 3.1, all *p*s > .05. Another ANOVA for incorrect responding indicated there was a Sex difference, $F(1, 78) = 8.8$, $p < .005$, with males having a higher number of incorrect responses than females, with 24.8 ± 2.0 and 16.5 ± 2.0 responses, respectively. There was also a Group difference in incorrect responding, $F(1, 78) = 6.1$, $p < .05$, with the 32-4 group having 24.1 ± 2.0 incorrect responses and the 4-4 group having 17.2 ± 2.0 incorrect responses. There was also a Line x Replicate difference in incorrect responding, $F(1, 78) = 7.6$, $p < .01$ (*Figure 8F*). This interaction was driven by a difference in incorrect responding between HAP2 and LAP2 mice, $F(1, 45) = 8.7$, $p < .01$, with responses of 24.4 ± 2.8 and 11.8 ± 2.8, respectively, There was no difference in responding between HAP3 and LAP3 mice, $F(1, 45) = 0.4$, $p > .05$, with each line having 21.8 ± 2.8 and 24.7 ± 2.8 incorrect responses, respectively. HAP2 and HAP3 mice did not differ from each other in the number of incorrect responses, $F(1, 45) = 0.3$, $p > .05$, while LAP2 mice had a lower number of incorrect responses than LAP3 mice, $F(1, 45) = 21.0$, $p < .001$. A Sex x Group interaction was also present, $F(1, 78) = 15.6$, $p < .001$, driven by, which was driven by high responding in male 32-4 mice compared to 4-4 mice, $F(1, 45) = 14.0$, $p < .005$, with 33.8 ± 2.8 and 15.9 ± 2.9 incorrect responses, respectively. The females groups did not differ in their level of incorrect responding, $F(1, 45) = 1.5$, $p >. 05$, the 32-4 group had 14.5 ± 2.8 incorrect responses and the 4-4 group had 18.6 ± 2.8 incorrect responses. Finally, there was a Line x Sex x Group interaction for incorrect responding, $F(1, 78) = 5.7$, $p < .05$. This was driven by HAP males in the 32-4 group, $F(1, 43) = 13.2$, $p < .005$,

which had 40.3 $\pm$ 3.9 incorrect responses which was higher than HAP male 4-4 mice, $F(1, 21) = 10.5$, $p < .005$, which had 13.9 $\pm$ 4.1 incorrect responses, and higher than HAP 32-4 females, $F(1, 22) = 10.9$, $p < .005$, which had 14.7 $\pm$ 3.9 incorrect responses. There was no Sex x Group interaction for LAPs, $F(1, 43) = 2.3$, $p > .05$. There were no other main effects or interactions for incorrect responding, all Fs < 3.4, *p*s > .05.

### 4.3.3 DRL-32 Training

During the DRL testing, one of the squad's intakes is missing for Day 1, so that day was not included in the intake analysis. On another day during testing, 6 intake values are missing. There was an equipment malfunction on one day for 2 of the mice, resulting in loss of data for those mice. Lastly, one mouse started DRL one day later than the other mice because it was slow to learn during training For all missing values, mean of each line and sex combination was imputed so the data could be included in with-in subjects analysis. The imputed means constituted less than 1% of all DRL data. Repeated measures ANOVAs for intake, correct, incorrect, percent correct, rewards, and efficiency across DRL were performed, using Days as the repeated measure and Sex, Line, Replicate, and Group as between-subjects measures. Mauchley's test for sphericity was significant for a majority of the dependent variables, and if this was the case, the Greenhouse-Geisser correction was used.

HAPs had lower intakes than LAPs during DRL-32, which was driven by low intake in HAP male mice. An ANOVA for intake indicated there was a between-subjects effect of Line, $F(1, 78) = 21.5$, $p < .001$, with LAP mice consuming more saccharin than HAP mice, 0.34 $\pm$ 0.02 and 0.25 $\pm$ 0.02 ml, respectively (*Figure 9A*). There was also an interaction of Line x Sex, $F(1, 78) = 5.9$, $p < .05$, which was driven by a difference between HAP males and LAP males, $F(1, 44) = 29.8$, $p < .001$, with intakes of 0.23 $\pm$

0.02 and 0.38 $\pm$ 0.02 ml, respectively (*Figure 10A*). On the other hand, HAP and LAP female intake did not differ, $F(1, 46) = 2.1$, $p > .05$, with intakes of 0.27 $\pm$ 0.02 and 0.32 $\pm$ 0.02 ml, respectively. HAP female and males did not differ in intake, nor did LAP female and males, Fs < 3.3, *p*s > .05. There were no other main effects or interactions, Fs < 3.3, *p*s > .05. There was a main effect of Days, $F(3.2, 248.9) = 6.1$, $p < .001$, which had linear, quadratic, and cubic trends (*p*s < .05). There was also a significant interaction of Day and Replicate, $F(3.2, 248.9) = 2.6$, $p < .05$. Intake changed in both Replicate 2, $F(5.9, 270.6) = 2.4$, $p < .05$ and Replicate 3 mice, $F(2.1, 95.6) = 5.1$, $p < .01$, but the trends were different for each replicate. Follow-up trend analyses indicated that Replicate 2 intake changed in both linear and order 7 polynomial trends across days (*p*s < .05), while Replicate 3 intake changed in linear, quadratic, and order 8 polynomial trends across days (*p*s < .05). There were no other interactions with Day, Fs < 2.5, *p*s > .05.

HAP mice responded more than LAP mice during DRL-32, but had lower efficiencies and gained fewer reinforcers. Another repeated measures ANOVA for correct responding uncovered a between subjects main effect of Line, $F(1, 78) = 34.2$, $p < .001$, with higher levels of correct responding in HAPs compared to LAPs, 182.7 $\pm$ 6.3 and 130.3 $\pm$ 6.3 correct responses, respectively. Replicate 2 mice responded more than Replicate 3 mice, $F(1, 78) = 5.6$, $p < .05$, with 167.1 $\pm$ 6.3 and 145.9 $\pm$ 6.3 correct responses, respectively. Finally, there was a Sex x Line x Replicate effect, $F(1, 78) = 6.5$, $p < .05$ (*Figure 10C*). In males, there was a Line x Replicate interaction, $F(1, 42) = 9.5$, $p < .005$, but this was not the case for females, $F(1, 44) = 0.5$, $p > .05$. A follow-up on this interaction indicated HAP2 and LAP2 males differed in their level of correct responding, $F(1, 21) = 22.5$, $p < .001$, with 222.1 $\pm$ 13.1 and 131.2 $\pm$ 12.5 responses, respectively. HAP3 and LAP3 male mice did not differ in their number of correct

responses, $F(1, 21) = 1.5$, $p > .05$, $152.7 \pm 12.5$ and $135.5 \pm 13.1$ correct responses, respectively. Additionally, HAP2 and HAP3 males differed from each other, $F(1, 21) = 14.8$, $p < .005$, but LAP2 and LAP3 males did not, $F(1, 21) = 0.1$, $p > .05$. In HAPs, there was also a Replicate x Sex interaction, but this was not the case for LAPs. In both replicate 2 and 3, HAP male and females did not differ in number of correct responses, $Fs < 3.6$, $ps > .05$, the Sex x Replicate effect was driven by the difference in correct responding between HAP2 and HAP3 males. Neither Replicate had a Sex x Line interaction, $Fs < 3.6$, $ps > .05$. There were no other main effects or interactions for correct responding $Fs < 2.4$, $ps > .05$. There was a main effect of Days, $F(3.2, 251.8) = 40.7$, $p < .001$, with correct responding changing in linear, quadratic, cubic, order 4 polynomial, and order 5 polynomial trends across time ($ps < .05$). There was an interaction of Days x Replicate, $F(3.2, 251.8) = 13.6$, $p < .001$. Correct responding changed across days in both replicates. In Replicate 2 mice, $F(2.6, 118.6) = 44.7$, $p < .001$, correct responding changed in linear, quadratic, cubic, order 4, order 5, order 7, order 8, and order 9 polynomial trends across days ($ps < .05$), while correct responding in Replicate 3 mice, $F(3.7, 168.4) = 4.9$, $p < .005$, changed in linear, quadratic, order 4, and order 8 polynomial trends across days ($ps < .05$). There was an interaction of Days x Line, $F(3.2, 251.8) = 4.8$, $p < .005$. Both HAP and LAP mice changed in correct responding across Days, $F(3.0, 138.6) = 26.7$, $p < .001$ and $F(2.6, 118.8) = 10.0$, $p < .001$, respectively. Thus, the interaction was driven by different patterns of correct responding by HAPs and LAPs, with HAP responding changing in linear, quadratic, cubic, order 4, and order 5 polynomial trends across days ($ps < .05$), while LAP responding changed in linear, quadratic, cubic, order 4, and order 7 polynomial trends across days ($ps < .05$). Because this was the main interaction of interest, we also performed Bonferroni post-hoc comparisons between the lines on each day, resulting in

an alpha level of (.05/11 = .005). HAPs had a higher level of correct responding than LAPS on all DRL-32 days, $ts > 4.1$, $ps < .001$ (*Figure 9B*). There were no other interactions with Day, $Fs < 2.4$, $ps > .05$.

Another repeated measures ANOVA for incorrect responding revealed a main effect of Line, $F(1, 78) = 5.4$, $p < .05$, with HAPs having more incorrect lever presses than LAPs, $30.1 \pm 2.6$ and $21.4 \pm 2.6$ responses, respectively. There was also a main effect of sex, $F(1, 78) = 8.4$, $p < .01$, with males having a greater number of incorrect lever presses than females, $31.1 \pm 2.7$ and $20.4 \pm 2.6$ responses, respectively. Finally, there was a Line x Replicate interaction, $F(1, 78) = 5.1$, $p < .05$, which was driven by a difference between HAP2 and LAP2 mice, $F(1, 45) = 8.3$, $p < .01$, each line had $30.9 \pm 3.8$ and $13.9 \pm 3.7$ incorrect responses, respectively (*Figure 9C*). HAP3 and LAP3 mice did not differ in number of incorrect lever presses, $F(1, 45) = 0.0$, $p > .05$, with $29.2 \pm 3.7$ and $28.9 \pm 3.8$ incorrect responses, respectively. LAP2 mice had fewer incorrect responses than LAP3 mice, $F(1, 45) = 15.7$, $p < .001$, while HAP2 and LAP2 mice did not differ in incorrect responses, $F(1, 45) = 0.1$, $p > .05$. There was no main effect of Days or interaction with Days, all $Fs < 2.0$, $ps > .05$.

The lines did not differ in percent correct responding. A repeated measures ANOVA for percent correct responding revealed a main effect of Sex, $F(1, 78) = 6.8$, $p < .05$, with females having a higher percent correct responding compared to males, $0.88 \pm 0.01$ and $0.84 \pm 0.01$, respectively. Replicate 2 also had a higher percent correct responding than Replicate 3 mice, $F(1, 78) = 12.0$, $p < .005$, with $0.89 \pm 0.01$ and $0.83 \pm 0.01$, respectively. There were no other between-subjects main effects or interactions, all other $Fs < 3.0$, $ps > .05$. There was a main effect of Days, $F(5.6, 435.4) = 3.1$, $p < .01$, which was due to linear and quadratic changes across time ($ps < .05$). There was an interaction of Day and Line, $F(5.6, 435.4) = 2.6$, $p < .05$ (*Figure 9D*). Interestingly, there

was an effect of Day in HAPs, $F(4.5, 207.9) = 4.4$, $p < .005$, but there was no effect of Day in LAPs, $F(5.3, 244.2) = 1.1$, $p > .05$. HAP percent correct changed in linear and quadratic trends across time ($p$s < .01). Bonferroni post-hoc comparisons were performed to assess for line differences across Days, corrected to an alpha level of (.05/11 = .005). There were no line differences for percent correct on any day during DRL testing ts < $\pm$ .3, $p$s > .005. There were no other interactions with Day, all Fs < 2.1, $p$s > .05.

A repeated measures ANOVA indicated that HAP mice gained fewer reinforcers than LAP mice overall, $F(1, 78) = 49.6$, $p < .001$, the lines obtained 8.3 $\pm$ 0.5 and 13.8 $\pm$ 0.5 reinforcers, respectively. There were no other between-subjects main effects or interactions, all Fs < 2.5, $p$s > .05. There was a main effect of Day, $F(7.5, 584.4) = 5.5$, $p < .001$. There was also an interaction of Day x Line, $F(7.5, 584.4) = 2.0$, $p < .05$. Again, there was a change in reinforcers gained across Days for HAPs, $F(6.8, 313.2) = 5.1$, $p < .001$, and although there was a trend for LAPs, it did not meet significance, $F(6.9, 316.1) = 2.0$, $p = .06$ (*Figure 9F*). A follow-up trend analysis for HAP reinforcers indicated the data changed in linear and order 4 polynomial trends across days ($p$s < .05). Bonferroni post-hoc comparisons were used to compare reinforcers gained between the lines, with an alpha level of (.05/11 = .005). LAPs gained more reinforcers than HAPs on all DRL testing days, ts > $\pm$ 4.8, $p$s < .001. There was also a Days x Line x Sex interaction, $F(7.5, 584.4) = 2.8$, $p < .01$, which was driven by a Days x Line interaction in the males, $F(7.4, 324.4) = 3.0$, $p < .005$, but not in the females, $F(6.8, 313.2) = 0.6$, $p > .05$ (*Figure 10B*). Both HAP and LAP male reinforcers changed across Days, $F(5.0, 110.3) = 3.6$, $p < .01$ and $F(10, 220) = 1.9$, $p < .05$, respectively. HAP male reinforcers changed in a linear pattern across days ($p < .001$), while LAP male reinforcers changed in order 4 and order 8 polynomial trends across days ($p$s < .05). There was a strong

trend for a Days x Line x Sex x Replicate interaction ($p = .05$), but since this did not meet significance, no follow-up trend analyses were performed. There were no other interactions with Day, Fs < 2.0, *p*s > .05.

Finally, a repeated measures ANOVA for efficiency indicated there was a main effect of Line, F(1, 78) = 33.2, $p < .001$, with HAPs having a lower efficiency than LAPs, 0.06 $\pm$ .01 and 0.13 $\pm$ 0.01, respectively (*Figure 9E*). There was also a Line x Replicate x Sex interaction, F(1, 78) = 4.1, $p < .05$ (*Figure 10D*). There was a Line x Sex effect in Replicate 2 mice, F(1, 43) = 6.8, $p < .05$, but not in Replicate 3 mice, F(1, 43) = 0.6, $p > .05$. This was driven by a difference in efficiency between HAP2 and LAP2 males, F(1, 21) = 25.9, $p < .001$, 0.04 $\pm$ 0.02 and 0.13 $\pm$ .02, respectively. No difference in efficiencies existed between the female HAP2 and LAP2 mice, F(1, 22) = 2.8, $p >.05$, which had efficiencies of 0.07 $\pm$ 0.02 and 0.09 $\pm$ 0.02, respectively. Neither LAP2 nor HAP2 mice had sex differences in efficiency, Fs < 3.9, ps > .05. There was no Line x Replicate interaction for both sexes, Fs < 3.2, *p*s > .05. Neither Line had Sex x Replicate interactions, Fs < 2.5, *p*s > .05.There was an interaction of Days and Replicate, F(5.4, 418.0) = 4.8, $p < .001$, which was due to a change in efficiency across Days in Replicate 2 mice, F(4.6, 213.5) = 5.9, $p < .001$, but not in Replicate 3 mice, F( 4.1, 189.1) = 1.7, $p > .05$. Efficiency in Replicate 2 mice changed in linear, quadratic, cubic, order 4, order 7, and order 8 polynomial trends across days (*p*s < .05). There was another interaction of Days x Line x Replicate, F(5.4, 418.0) = 3.3, $p < .01$. In Replicate 2 mice there was a Days x Line effect, F(4.7, 212.5) = 2.3, $p < .05$, but this was not the case for Replicate 3 mice, F(4.2, 189.1) = 2.0, $p > .05$. HAP2 efficiency did not change across Days, F(3.2, 70.4) = 1.7, $p > .05$, but LAP2 efficiency increased across Days, F(4.1, 94.4) = 4.8, $p < .005$, in linear, quadratic, order 4, order 7, and order 8 polynomial trends (*p*s < .05). There was also a Days x Replicate Effect in LAP mice, F(6.1, 274.2) = 5.7, $p < .001$, but

not in HAP mice, F(2.4, 108.1) = 1.2, $p > .05$, and this interaction was driven by the change in LAP2 efficiency across Days. There were no other main effects or interactions.

#### 4.3.4 SNC Post-shift Day 1

One LAP2 male never responded during the pre-shift period, so was removed from all SNC and DRL test day analyses. The line difference in contrast was present during the 3-minute post-shift session. On post-shift day 1, univariate ANOVAs were performed on intake (1) total volume in ml and 2) total volume corrected for weight in ml/kg and licks to confirm the occurrence of contrast effects. An ANOVA for intake (ml) indicated there was a main effect of Group, F(1, 77) = 30.4, $p < .001$, with the unshifted controls (4-4 group) consuming more sucrose than the shifted group (32-4 group), 0.65 $\pm$ 0.04 and 0.38 $\pm$ 0.04 ml, respectively. HAP mice consumed more sucrose than LAP mice, F(1, 77) = 69.5, $p < .001$, with intakes of 0.73 $\pm$ 0.04 and 0.31 $\pm$ 0.04, respectively. There was also an interaction of Group x Line, F(1, 77) = 8.8, $p < .005$ (*Figure 11A*). There was a very significant difference between shifted and unshifted HAP mice, F(1, 45) = 25.6, p < .001, with HAP 32-4 mice consuming 0.51 $\pm$ 0.05 and 0.94 $\pm$ 0.05 ml, respectively. LAP 32-4 and 4-4 mice did not differ, though there was a trend for a group difference, F(1, 44) = 3.6, $p = .06$, with intakes each group consuming 0.24 $\pm$ 0.05 and 0.37 $\pm$ 0.05 ml, respectively. Both HAP 32-4 and 4-4 groups consumed more sucrose than their respective LAP 32-4 and 4-4 groups. There was an interaction of Line x Replicate, F(1, 77) = 8.8, $p < .01$. HAP2 and HAP3 mice did not differ in intake, F(1, 45) = 0.4, $p > .05$, with intakes of 0.76 $\pm$ 0.05 and 0.69 $\pm$ 0.05 ml, respectively. LAP3 mice consumed more sucrose than LAP2 mice, F(1, 44) = 11.6, $p < .001$, with intakes of 0.41 $\pm$ 0.05 and 0.20 $\pm$ 0.05 ml, respectively. There were significant line differences for both replicate 2 and 3

mice, $F(1, 44) = 45.\ 6$, $p < .001$ and $F(1, 45) = 10.3$, $p < .005$. There were no other main effects or interactions, $Fs < 3.2$, $ps > .05$.

Another univariate ANOVA for intake (ml/kg) indicated that 4-4 mice consumed more sucrose than 32-4 mice, $F(1, 77) = 24.1$, $p < .001$, with intakes of $31.1 \pm 1.9$ and $18.2 \pm 1.8$ ml/kg, respectively. HAP mice also consumed more sucrose than LAP mice, $F(1, 77) = 55.6$, $p < .001$, with intakes of $34.5 \pm 1.8$ and $14.9 \pm 1.9$ ml/kg, respectively. There was also a main effect of Replicate, $F(1, 77) = 4.0$, $p < .05$, with Replicate 2 mice consuming $22.0 \pm 1.9$ ml/kg and Replicate 3 mice consuming $27.3 \pm 1.8$ ml/kg of sucrose. There was a significant Group x Line interaction, $F(1, 77) = 6.3$, $p < .05$. HAP 4-4 mice consumed more sucrose than HAP 32-4 mice, $F(1, 45) = 18.7$, $p < .001$, with intakes of $44.2 \pm 2.6$ and $24.7 \pm 2.6$ ml/kg, respectively (*Figure 11B*). LAP 32-4 and 4-4 groups did not differ, though there was a trend for this to be the case, $F(1, 44) = 3.9$, $p = .06$, with intakes of $18.1 \pm 2.7$ and $11.7 \pm 2.6$ ml/kg, respectively. Both HAP 32-4 and 4-4 groups consumed more sucrose than their respective LAP groups, $F(1,46) = 33.2$, $p < .001$ and $F(1, 43) = 26.3$, $p < .001$. There was also a significant Group x Line x Sex effect, $F(1, 77) = 7.0$, $p < .05$ (*Figure 12A*). In females, there was a significant Line x Group effect, $F(1, 44) = 12.5$, $p < .001$, but there was not a Line x Group effect in males, $F(1, 41) = 0.0$, $p > .05$. In female HAP mice, there was a contrast effect, evidenced by higher intake in 4-4 females compared to 32-4 females, $F(1, 22) = 22.8$, $p < .001$, with intakes of $52.7 \pm 3.6$ and $21.2 \pm 3.6$ ml/kg, respectively. There was no evidence of contrast in LAP females, $F(1, 22) = 1.3$, $p > .05$, with intakes of $15.7 \pm 3.6$ and $11.3 \pm 3.6$, respectively. Both of the female HAP groups also consumed more sucrose than their respective LAP 4-4 and 32-4 groups, $F(1, 22) = 26.7$, $p < .001$ and $F(1, 22) = 13.0$, $p < .005$. There was also a Sex x Group interaction in HAP mice, $F(1, 43) = 8.2$, $p < .01$, but not in LAP mice, $F(1, 42) = 0.4$, $p > .05$. There was a group difference for female

HAPs, $F(1, 22) = 22.8$, $p < .001$, but not for male HAPs, $F(1, 21) = 1.9$, $p > .05$. In 4-4 mice, there was a Sex x Line interaction, $F(1, 41) = 4.8$, $p < .05$, but there was no Sex x Line interaction in 32-4 mice, $F(1, 44) = 2.0$, $p > .05$. There were line differences in intake in both females and males, $F(1, 22) = 26.7$, $p < .001$ and $F(1, 19) = 5.1$, $p < .05$, respectively. In HAP 4-4 mice there was a sex difference, with females consuming more sucrose than males, $F(1, 21) = 4.7$, $p < .05$, but there was no sex difference in LAP 4-4 mice, $F(1, 20) = 0.6$, $p > .05$. There were no other main effects or interactions.

Lastly, a univariate ANOVA for licks indicated that a contrast effect in licks occurred, with 4-4 mice licking more than 32-4 mice, $F(1, 77) = 14.0$, $p < .001$, with $347.7 \pm 23.5$ and $225.4 \pm 22.7$ licks, respectively. HAP mice licked more than LAP mice, $F(1, 77) = 26.7$, $p < .001$, with $370.9 \pm 23.0$ and $202.1 \pm 23.3$ licks, respectively. Replicate 3 mice licked more than Replicate 2 mice, $F(1, 77) = 11.8$, $p < .001$, with $342.6 \pm 23.0$ and $230.4 \pm 23.3$ licks, respectively. There was a Line x Group interaction, $F(1, 77) = 4.4$, $p < .05$. HAP 4-4 mice licked more than HAP 32-4 mice, $F(1, 45) = 15.8$, $p < .001$, with $466.5 \pm 32.9$ and $275.4 \pm 32.1$ licks, respectively (*Figure 11C*). LAP 4-4 and LAP 32-4 groups did not differ in number of licks, $F(1, 44) = 1.2$, $p > .05$, with $228.8 \pm 33.7$ and $175.3 \pm 32.1$ licks, respectively. HAP 4-4 and 32-4 mice licked more than their respective LAP 4-4 and 32-4 groups, $F(1, 43) = 15.7$, $p < .001$ and $F(1, 46) = 10.3$, $p < .005$. There was also a Line x Group x Sex interaction, $F(1, 77) = 4.1$, $p < .05$ (*Figure 12B*). Again, there was no Line x Group interaction in males, $F(1, 41) = 0.0$, $p > .05$ but there was a Line x Group interaction in females, $F(1, 44) = 6.8$, $p < .05$. HAP 4-4 females licked more than HAP 32-4 mice, $F(1, 22) = 15.7$, $p < .001$, $520.4 \pm 45.4$ and $219.3 \pm 45.4$ licks, respectively. LAP females did not differ, $F(1, 22) = 0.2$, $p > .05$; LAP 4-4 females had $209.3 \pm 45.4$ licks and 32-4 females had $178.2 \pm 45.4$ licks. Female HAP and LAP 4-4 groups differed, $F(1, 22) = 10.4$, $p < .005$, but HAP and LAP 32-4 groups

did not differ, $F(1, 22) = 1.2$, $p > .05$. HAPs had a Sex x Group interaction for licks, $F(1, 43) = 5.0$, $p < .05$, while LAPs did not, $F(1, 42) = 0.2$, $p > .05$. In female HAPs, 4-4 mice licked more than 32-4 mice, $F(1, 22) = 15.7$, $p < .001$, but this was not the case for male HAPs, $F(1, 21) = 2.4$, $p > .05$. In HAP 4-4 mice there was no sex difference, $F(1, 21) = 1.4$, $p > .05$, but HAP 32-4 males licked more than HAP 32-4 females, $F(1, 22) = 6.5$, $p < .05$. There was no Sex x Line interaction in either the 32-4 or 4-4 group, Fs < 1.3, *p*s > .05.

Post-shift behavior was also analyzed separately for each replicate. I report here if there were significant Group x Line interactions for each Replicate, and report the other effects if an interaction was not present. In Replicate 2 mice, there was an interaction of Group x Line for intake (ml), $F(1, 42) = 15.2$, $p < .001$. There was also a Group x Line interaction for intake (ml/kg), $F(1, 42) = 9.4$, $p < .005$. Lastly, there was an interaction of Group x Line for licks, $F(1, 42) = 7.0$, $p < .05$. In Replicate 3 mice, there was no Group x Line interaction for intake (ml), $F(1, 43) = 0.6$, $p > .05$. There were main effects of Group and Line, Fs > 11.1, *p*s < .005. There was also no interaction of Group x Line for intake (ml/kg), $F(1, 43) = .61$, $p > .05$. There were main effects of Group and Line, Fs > 10.1, *p*s < .005. Finally, there was no interaction of Group x Line for licks, $F(1, 43) = .78$, $p > .05$. There were main effects of Line and Group, Fs > 7.1, *p*s < .05. Follow-up comparisons were performed for each Line and Group, regardless of whether an interaction was present or not, and are reported in Table 3.

### 4.3.5 SNC Effects on DRL

Because the LAP mice did not achieve a significant contrast effect during the reward downshift, it becomes difficult to interpret any effects of contrast on DRL. Because there was no interaction of Line x Group for males intakes (ml/kg), but there was a Group

effect, there is some evidence that LAP males demonstrated contrast effects. Therefore, in the following analysis, only LAP male DRL data will be analyzed. Also, as explained, because of the line differences in both baseline DRL and SNC behavior, it is necessary to analyze HAP and LAP data separately. Therefore, rather than looking for a correlated response, I am looking for the occurrence of urgent-like behavior in both lines. Because I was looking for a time-dependent effect, correct responding was analyzed in one-minute bins across the 10-minute session. Intake, incorrect responding, percent correct, and efficiency were all analyzed.

#### 4.3.5.1 HAP DRL Behavior

A repeated measures ANOVA for HAP correct responding indicated there was a main effect of Sex, $F(1, 39) = 4.2$, $p < .05$, with males having more correct responses than females, $6.2 \pm 0.4$ and $5.0 \pm 0.4$ responses, respectively. There was a significant main effect of Bins across time, $F(6.2, 246.4) = 7.0$, $p < .001$, with correct responding changing in linear, quadratic, and order 4 polynomial trends across the 10-minute session (*p*s < .05). There was also a Bin x Replicate x Sex interaction, $F(6.2, 246.4) = 2.2$, $p < .05$. Neither of the Replicates had a Sex x Bin interaction, Fs < 2.0, *p*s > .05. Prior to the Greenhouse-Geisser correction, Replicate 3 mice had a significant Bin x Sex interaction, which was likely driving the 3-way interaction, but since Mauchley's test for sphericity was significant, the Greenhouse-Geisser correction had to be utilized. Neither Sex had a Bin x Replicate interaction, either, Fs < 1.8, *p*s > .05. Repeated measures ANOVAs were performed on correct responding for each replicate, and indicated there were no Group effects or Group x Minute interactions for either HAP2 or HAP3 mice, Fs < 3.2, *p*s > .05.

There was no effect of contrast on DRL responding, but there was evidence for suppressed intake in HAP3 mice during the 10 minute DRL-32 session. A univariate ANOVA was performed for HAP intake, which indicated that the 32-4 shifted HAPs consumed less saccharin than the 4-4 HAPs, $F(1, 39) = 4.4$, $p < .05$, with intakes of 0.10 $\pm$ 0.01 and 0.06 $\pm$ 0.01 ml, respectively. There was an interaction of Group x Replicate, $F(1, 39) = 4.4$, $p < .05$. The interaction was driven by a group difference in HAP3 mice, $F(1, 22) = 5.7$, $p < .05$, with 32-4 HAP3 mice consuming 0.03 $\pm$ 0.02 ml and 4-4 HAP3 mice consuming 0.11 $\pm$ 0.02 ml. HAP2 32-4 and 4-4 groups did not differ, $F(1, 21) = 0.0$, $p > .05$, with intakes of 0.09 $\pm$ 0.2 and 0.09 $\pm$ 0.02, respectively. Lastly, there was also an interaction of Sex x Replicate, $F(1, 39) = 4.4$, $p < .05$, which was driven by a sex difference in HAP2 intake, $F(1, 21) = 6.4$, $p < .05$, with females consuming 0.11 $\pm$ 0.02 ml and males consuming 0.07 $\pm$ 0.02 ml. There was no sex difference in HAP3 intake, $F(1, 22) = 1.2$, $p > .05$, with females consuming 0.05 $\pm$ 0.02 ml and males consuming 0.09 $\pm$ 0.02 ml. Female HAP2 and HAP3 mice intakes differed, $F(1, 22) = 8.6$, $p < .01$, while male HAP2 and HAP3 intakes did not differ, $F(1, 23) = 0.3$, $p > .05$. Another univariate ANOVA for HAP incorrect responding indicated there was a main effect of Sex, $F(1, 39) = 5.5$, $p < .02$, with males having more incorrect responses than females, 18.9 $\pm$ 2.5 and 10.8 $\pm$ 2.4 incorrect responses, respectively. There were no other main effects or interactions, all $Fs < 3.6$, $ps > .05$. Another univariate ANOVA for percent correct indicated there were no main effects or interactions, all $Fs < 2.3$, $ps > .05$. An ANOVA for rewards indicated there was no significant difference in rewards achieved for any of the groups, all $Fs < 3.3$, $ps > .05$. Lastly, an ANOVA for efficiency indicated there was a main effect of sex, $F(1, 39) = 7.4$, $p < .01$, due to the fact that HAP males were less efficient than HAP females, 0.04 $\pm$ 0.01 and 0.08 $\pm$ 0.01, respectively.

#### 4.3.5.2 LAP Male DRL Behavior

LAP male 32-4 mice increased correct responding early in the DRL-32 test session. A repeated measures ANOVA for LAP male responding indicated there was a significant main effect of Replicate, $F(1, 18) = 5.5$, $p < .05$, with LAP3 males responding more than LAP2 males, $4.7 \pm 0.4$ and $3.4 \pm 0.4$ correct responses, respectively. There was also a significant main effect of Bin, $F(9, 162) = 4.4$, $p < .001$, which was driven by linear and quadratic trends in correct responding across the 10 minute session ($p$s $< .05$). There was also an interaction of Bin x Group, $F(9, 162) = 2.5$, $p < .05$. LAP 4-4 male correct responding changed across time, $F(9, 81) = 2.0$, $p < .05$, in a quadratic trend ($p < .05$). LAP 32-4 male correct responding also changed across time, $F(9, 99) = 4.9$, $p < .001$, in quadratic and cubic trends ($p$s $< .05$). Because we expected any group effect to be somewhat immediate, *a priori* comparisons were performed on LAP 32-4 and 4-4 males responding during the first 5 minutes, resulting in a significant increase in responding by 32-4 LAP males at minute 4, $t(20) = -2.7$, $p < .05$, but not at the other time-points, ts $< \pm 1.6$, $p$s $> .05$ (*Figure 13B*). When SNC was analyzed separately for the replicates, there was evidence that LAP3 mice experienced contrast using one measure of intake (ml/kg). Therefore, correct responding was analyzed in LAP3 mice, and revealed no effect of Group or a Group x Minute interaction, Fs $< 1.6$, $p$s $> .05$. Therefore the increase in responding in 32-4 mice appeared to be driven by an effect in both LAP male replicates.

There were no other effects of SNC on DRL responding in LAP male mice. A univariate ANOVA for intake in LAP males indicated there were no differences in intake between Groups or Replicates, Fs $< 2.8$, $p$s $> .05$. An ANOVA for incorrect responding revealed there was a main effect of Replicate, $F(1, 18) = 9.6$, $p < .01$, with LAP3 males pressing incorrectly more than LAP2 males, with $16.2 \pm 2.2$ and $6.6 \pm 2.2$ lever presses,

respectively. There was no main effect of Group or interaction with Group, Fs $< 0.4$, *p*s $> .05$. An ANOVA for percent correct responding revealed that LAP2 males had a higher percent correct than LAP3 males, $F(1, 18) = 4.7$, $p < .05$, with $0.85 \pm 0.04$ and $0.74 \pm 0.04$, respectively. For LAP male rewards, there were no main effects or interactions, Fs $< 1.8$, *p*s $> .05$. There were no differences in efficiency, all Fs $< 2.4$, *p*s $> .05$.

## 4.4 Discussion

In this study, line differences in both contrast and impulsive behavior were replicated. HAP mice responded more than LAPs during FI-32 and DRL-32, which tended to benefit them in FI-32 but was disadvantageous during DRL-32. Thus, there is evidence that HAP mice exhibit higher motor impulsivity on a DRL task than LAP mice. HAP mice also experienced contrast during a 3-minute post-shift session, while LAP mice did not experience contrast effects. On one measure (intake in ml/kg), LAP males experienced contrast effects. Thus, this study also replicated the finding that HAP mice are more reactive to an incentive downshift than LAP mice. These findings also support using HAP lines to study the shared genetic and neurobiological mechanisms underlying motor impulsivity and affective reactivity. I tested if eliciting an incentive downshift produces rash action, and an early increase in shifted LAP males provides initial evidence that this is the case for LAP males, which was an unexpected finding. There is some evidence that suppressed intake occurs during DRL following SNC in shifted HAP3 mice, suggesting that the affective portion of the task transferred to DRL. The divergence in behavior between HAP and LAP mice in DRL behavior following SNC is unexpected. Modeling urgency, or emotion-driven rash action, is important because it provides another avenue by which to study the phenomenon, which has typically been characterized using self-report or laboratory tasks in humans (35). Having a rodent

model of urgency would afford the opportunity for future studies to explore the neurobiological and genetic determinants of the trait. The differences in behavior suggest that there may be a different time-course to the behavioral processes governing contrast behavior in the lines. It is also possible that the incentive downshift acts to suppress other behaviors in HAPs, while it activates behaviors in LAPs. Future inquiry is needed to confirm these initial findings.

Analysis of baseline weights uncovered weight differences between HAP2 and LAP2 mice, but not a difference in weight between HAP3 and LAP3 mice (*Figure 6*). Lower weights in LAP2 mice did not really seem to affect behavior on any of the operant tasks, with the exception of intake during the FR1-2.5 phase of operant training. The other behaviors are not intake-dependent. Further, intake was limited in the FI-32 and DRL-32 tasks, due to lower reinforcer availability inherent in these two schedules of reinforcement, and thus was possibly not limited by weight. Therefore, although the main behavior of interest (correct responding and rewards) compared HAP and LAP groups on these tasks, these behaviors were not intake dependent, and therefore should not have been as influenced by line differences in weight as intake-dependent measures.

During FR1-10s training, LAP2 mice took longer to acquire lever pressing than HAP2 and LAP3 mice, and there was a trend for LAPs to take longer than HAPs to meet criterion during the FR1-2.5s phase. LAP mice also consumed less saccharin than HAPs during FR1-2.5s phase, and LAP3 mice had fewer lever presses than HAP3 mice (*Figure 7 A-D*). Separate analyses of the replicates supported these observations. Individual differences in the acquisition of instrumental responding have been recognized, and there are hypotheses that these individual differences may influence behaviors underlying addiction (98). It is unclear what drives faster learning of instrumental responding in HAP mice, whether it is an increased general level of

motivation, learning differences, attribution of salience to the levers or some other environmental stimuli, increased habit-learning, or a combination of factors. Future experiments could attempt to understand what underlies faster acquisition of lever pressing in HAPs. Further, a line difference in saccharin intake replicates the previous finding that HAPs consume more saccharin than LAP mice (41, 42). Though somewhat inconsistent across the replicates, the observations from the FR1 training data suggest that HAP and LAP mice differ in unreinforced (intake) and instrumental (lever pressing) reinforcer-related behavior.

During FI-32, HAP and LAP mice both increased their overall level of responding from the FR1 phases, and both lines learned the fixed interval, as was indicated by increases in lever-pressing across trial time (*Figure 8A, B*). The lines differed on all behavioral measures on the FI-32 task. As observed in the FR1 phase, HAP mice also consumed more saccharin than LAP mice. A separate analysis of the replicates revealed this effect was largely driven by a difference between HAP3 and LAP3 mice. It is unclear why LAP2 mice exhibited a lower number of incorrect responses than the other lines, but it could have been due to a lower level of behavior in general. In the overall analyses, the lines differed in correct responses, reinforcers achieved, and efficiency (reinforcers/correct responses). In analyses for each replicate, there was a line difference in correct responses for both replicates, but only HAP2 and LAP2 mice differed in reinforcers achieved and efficiency. Behavior on fixed interval tasks has been used to study perseveration, or inappropriate repetition of behavior (40, 96, 99). That LAP efficiency was higher suggests the HAPs are more perseverative on this task, due to the fact that HAP mice are emitting more responses per reward than LAP mice. On the other hand, HAP3 mice gained more reinforcers than LAP3 mice, and HAP2 and LAP2 mice gained an equal number of reinforcers. Additionally, in the separate analysis

of the replicates, the HAP2 and LAP2 mice did not differ in efficiency. So although their response rate was much higher and efficiency lower, it is difficult to say that the HAPs were inappropriately responding on this task compared to LAPs. Rather, in the HAP3 mice, an increased response rate resulted in a greater number of rewards, and thus may be considered a more advantageous behavioral strategy. This observation illustrates that simply showing an increase in response rate or perseveration (95, 97) is not a suitable way to model impulsivity, in that, perseveration can be an adaptive behavioral strategy depending upon what response contingencies are in place. Therefore, although responding in HAP mice may be perseverative in comparison to LAP mice, it is not necessarily “impulsive” on this task, because the HAP mice are not losing the opportunity to gain reinforcers because of their behavior (exhibiting an increased level of correct responding).

HAP mice also had an elevated level of responding during the DRL-32 phase of testing, but this behavioral strategy proved to be disadvantageous. During the DRL-32 task, HAP mice had more correct lever presses than LAPs across days, resulting in a lower number of reinforcers achieved across days (*Figure 9 B, F*). HAP intake and efficiency were also lower than LAP mice, although the males likely drove this line difference, as will be discussed. These observations support the idea that HAP mice gain fewer reinforcers per lever press, resulting in less sipper tube availability and lower overall intake (*Figure 9 A, E*). HAP and LAP mice do not differ in percent correct responding, and both lines had a high level of percent correct responding. In DRL, failure to withhold responding during a defined time interval (32 seconds in this case) results a new trial, and a lower rate of responding is reinforced. Thus, in the case where an effective behavioral strategy is to withhold responding to obtain a greater number of rewards, HAP mice are unable to adapt their behavior. HAP mice fail to inhibit

responding and gain a lower number of reinforcers, and therefore can be considered more impulsive on the DRL task than LAP mice. The separate analyses of the replicates also support this assertion, considering both HAP replicates had higher correct responding, but a lower number of reinforcers and efficiency than their respective LAP replicates (Table 3). Therefore, the analyses of the replicates also provide good evidence for a genetic correlation between alcohol preference and motor impulsivity during DRL.

Although our lab has evidence that suggests HAP mice are more impulsive than LAPs on a DRL task, this has yet to be replicated or published. Therefore, this study confirms the finding that HAPs are more impulsive on a response inhibition, or motor impulsivity, task. Others have found that response inhibition deficits are genetically associated with high alcohol preference. Steinmetz and colleagues (100) found that alcohol preferring (P) rats were more impulsive on a DRL task than non-alcohol preferring (NP) rats. Wilhelm and colleagues (101) compared short-term selected lines for high alcohol preference, and found that the short-term high alcohol preferring line (STDRHI2) had lower response inhibition on a Go-No Go task than the short-term low alcohol preferring line (STDRLO2). In a panel of inbred strains, higher response inhibition was negatively correlated with ethanol intake (102).. In addition, different types of impulsivity are not always found in a particular selected line, for example, STDRHI2 mice were more impulsive on Go-No Go task, but did not differ from STDRLO2 mice on a delayed discounting task. This is not surprising because different types of impulsive behavior are governed by different neurobiological mechanisms (40, 103, 104). Recently, Oberlin and Grahame (50) found that HAP mice were more impulsive on a delayed discounting task, which is a measure of cognitive impulsivity. Together, the findings that HAP mice are more impulsive than LAP mice on delayed discounting task

and DRL tasks suggest that HAP mice exhibit deficits in two types of impulsivity, which is a multi-faceted construct (32, 105).

In human studies, several types of impulsivity have been associated with alcohol use disorders, but these associations are subject to prior alcohol use exacerbating levels of observed impulsivity (103, 106). Longitudinal studies using children of individuals with substance disorders have confirmed that higher levels of impulsivity are associated with earlier alcohol use, as well as alcohol use problems, but often these children have other co-morbid disorders, making it difficult to concretely determine what leads to alcohol use problems (107-109). Nigg and colleagues (2006) found that poor response inhibition predicted future alcohol related problems in a population of high-risk adolescents that had a family history of alcohol use disorders. This study provides some evidence that poor response inhibition is a predisposing factor for the development of alcohol use problems. To complement the human studies, selectively bred animals or panels of inbred strains can provide important information about impulsivity as predisposing factor for substance use disorders in alcohol-naïve animals, and also remove the influence of poly-substance abuse or comorbid disorders that commonly occur in human substance abusers.

Several of the sex differences during DRL-32 were not present during the FR1 or FI-32 phases. In particular, a line difference in consumption between the males apparently drove the reduced overall intake between HAP and LAP mice, as there were no differences between Female HAP and LAP mice. (*Figure 10A*). HAP correct responses were generally higher in both sexes, with the exception of HAP3 male mice, which were not different from LAP3 male mice. On the other hand, HAP2 male mice had extremely high responding, as it was increased compared to LAP2 males and HAP3 males (*Figure 10C*). Since Line 2 mice are further along in selection, it is possible that

more alleles for high alcohol preference have been fixed, and therefore more alleles for any correlated responses (in this case low response inhibition) have also been fixed. It is interesting that this would occur differently in males and females, though, because both female HAP replicates had higher responding than their respective LAP replicates. Overall, HAPs gained lower numbers of reinforcers, and there was a tendency for LAP males to have an increased number of reinforcers (*Figure 10B*). Although the pattern of sex differences was somewhat complicated, it seems that there is a pattern for HAP males (especially HAP2 males) to be deficient in response inhibition, although line differences exist for both female replicates as well. There is also some evidence that LAP males are more proficient in response inhibition (based on their trajectory for an increased number of reinforcers). In the preclinical literature, males tend to be more impulsive on response inhibition tasks (110), therefore HAP2 male behavior may be more in line with other preclinical findings.

In this study, we used shortened (3 minute) pre-shift and post-shift sessions in order to try to capture DRL behavior very early during the reaction to incentive downshift. This study replicates the finding from Chapter 3 that HAPs react more strongly to incentive downshift, which is strong support for the idea that reactivity to incentive downshift is a correlated response to selection for high alcohol preference. Although there was a trend for LAPs to show a contrast effect in intake (in ml), there were no significant contrast effects in consumption (ml or ml/kg) or licks, as would be indicated by differences between LAP 32-4 and 4-4 groups. Because LAP contrast is a smaller effect, perhaps it takes more time to become apparent, considering LAP contrast was evident during 5-minute post-shift sessions. Again, separate analyses of contrast in the replicates revealed support for line differences in contrast (Table 3). A Line x Group interaction in the Line 2 mice was driven by a highly significant contrast effect in HAP2

mice, but no evidence of contrast in LAP2 mice. Although there was no interaction for Line 3 mice, follow-up comparisons indicated contrast occurred in contrast occurred in HAP3 mice for all the measures, but there was only a marginally significant contrast effect in LAP3 mice for intake (ml/kg). Additionally, both HAP replicates had larger effect sizes for contrast than the LAP replicates. Therefore, this study also provides moderate to strong evidence of a genetic correlation between alcohol preference and emotional reactivity to incentive shift.

In addition, there were sex differences in contrast using a 3-minute session. These became apparent when subject weight was included in calculating intake and in lick behavior, which should not be a weight-dependent measure. Female HAPs experienced strong contrast effects at the 3-minute time-point, while there was no evidence that LAP females experienced contrast. On the other hand, an overall Group effect in the males, but lack of Group x Line interaction in intake (ml/kg) suggested that contrast occurred in LAP and HAP males, but it was smaller for both lines than in female HAPs. A contrast effect was not evident for males in lick behavior, but there was a contrast effect for HAP females. Therefore, these data provide some evidence that the time-course of contrast or the strength of contrast may be slightly different for males and females, at least in HAP mice. Sex differences in contrast have not traditionally been observed (13, 111); the differences observed in this study in comparison to previous observations may be explained by different experimental design. First, most of the contrast literature use only males; it is difficult to make a conclusion about the occurrence of sex differences when a paucity of information on the topic exists. Second, most of the contrast literature has used 5-10 minute sessions, possibly obscuring earlier, stronger effects in females. Third, a bulk of the contrast literature also reports intake in ml, rather than in ml/kg, possibly missing weight dependent effects. A more systematic

evaluation is necessary to make a conclusion regarding the occurrence and time-course of sex differences in contrast. It would also be interesting for future studies to take estrous status into account, because collapsing across the estrous cycle can mask sex effects.

Lastly, I attempted to assess if an incentive downshift would produce rash action in HAP and LAP mice. There is some evidence that shifted LAP 32-4 male mice exhibited an increase in correct responding compared to unshifted LAP 4-4 male mice early during the test session, supporting the idea that “urgent’-like behavior occurs in male LAP mice. I analyzed only male LAP behavior, because there was no evidence that female LAP mice experienced contrast effects during the 3-minute post-shift session, while there was some evidence that LAP males experienced contrast. The study design did not differentiate between a lack of satiation inherent in shifted mice (compared to unshifted controls), which may have influenced responding. This is because an increase in responding could have been due to thirst issues, rather than to affect. It would be useful to have a yoked-control 4-4 group that receives the same amount of liquid as the 32-4 mice to try to differentiate between satiation and affect causing an increase in responding.

There is strong evidence that HAP mice experienced contrast, based on the intake (ml and ml/kg) and lick data, and there was no evidence of an increase in responding in HAP mice. If satiation were truly driving behavior, I would expect behavior to increase in both lines. Actually, there was some evidence that HAP3 shifted mice suppressed their saccharin intake, which can be interpreted as an increased negative affective state that was carried over from the recent incentive downshift. It is possible that there was a different time-course of contrast in HAPs, such that the appetitive drive had already occurred prior to the DRL session, thus any increase in responding the

HAPs exhibited was earlier in time. It is also possible that responding on the DRL task is so high in HAP mice that it was difficult to observe an increase in shifted mice due to a ceiling in responding behavior, although this seems unlikely because during FI, the rates of correct responding were higher, therefore it is possible for correct lever-pressing to increase.

There is also a literature on consummatory contrast not producing changes in instrumental behavior. Papini and Pellegrini (112) hypothesize that during consummatory SNC, an activated memory of the pre-shift reward is compared to the sensory trace of the post-shift reward, which is referred to as recognition relativity. On the other hand, cue-recall memory is necessary for the production of instrumental SNC, as animals must recall information about the downshift prior to coming into contact with the reward (113). This may explain why instrumental contrast is not observed when sucrose solutions are used as the reinforcers. In these studies, consummatory contrast was observed, but instrumental contrast (decrease in run-speed on a runway) is not observed (114, 115). Therefore, HAP3 behavior during DRL might be interpreted in the same manner. The contrast effect from the consummatory SNC session might be carried over to the consummatory portion of DRL. It is unclear why this would not also occur in LAPs, though. It might be possible to see an effect on lever-pressing in both lines if the manipulation was somehow incorporated into the operant procedure. For example, it might be possible to increase the length of DRL and see if responding increases compared to a group that has undergone training at a longer DRL interval. It is possible that an appetitive drive was activated in the LAPs but contrast did not “carry over” in the same way it did in HAP3 mice. HAP3 behavior during the DRL test might suggest impulsive inaction. Smith and colleagues (2013) showed in early adolescents that urgency predicted future depressive symptoms, which supports the idea that urgency

can lead to both rash action and ill-advised inaction (116). Its possible that the normal pattern of behavior in HAP mice is rash action, as would be supported by the findings that HAPs are more impulsive on delayed discounting and DRL tasks, but in the face of strong emotion, HAPs become frustrated and shift to impulsive inaction. Their "inaction" might be thought of as impulsive, because HAP mice are acting to their disadvantage by failing to consume an available reinforcer. This idea is limited by the fact that HAP2 shifted mice did not also suppress their saccharin intake. Although the same argument might be applied to contrast behavior, in that HAP mice fail to consume available 4% sucrose, therefore they are working to their disadvantage, and engaging in impulsive inaction.

In conclusion, line differences in both contrast and impulsive behavior were replicated. These findings support using HAP lines to study the shared genetic and neurobiological mechanisms underlying motor impulsivity and affective reactivity. I also tested if eliciting an emotional reaction results in rash action, and an early increase in shifted LAP males provides initial evidence that this may be the case. There is some evidence that suppressed intake occurs during DRL following SNC in shifted HAP3 mice, suggesting that the affective portion of the task transferred to DRL. The divergence in behavior between HAP and LAP mice in DRL behavior following SNC is unexpected, but HAP mice may engage in impulsive inaction in the face of frustration. Future inquiry is needed to confirm these initial findings. Modeling urgency, or emotion-driven rash action, is important because it provides another avenue by which to study the phenomenon, which has typically been characterized using self-report or laboratory tasks in humans (35). A rodent model of urgency would afford the opportunity for future studies to explore the neurobiological and genetic determinants of the trait.

# CHAPTER 5. DETERMINE THE DEGREE TO WHICH EMOTIONAL REACTIVITY TO AN INCENTIVE DOWNSHIFT INCREASES ETHANOL CONSUMPTION

## 5.1 Introduction

A couple of studies have investigated the effects of ethanol on contrast. Preclinical consummatory contrast data suggest that ethanol administration during the initial reaction to incentive downshift does not decrease contrast (117). Rather, it may be after the relatively transient initial reaction to an incentive downshift that ethanol has its effects, as preclinical evidence suggests that alcohol may have its effects during the recovery period (52, 117). Two instrumental contrast studies by Cox (118, 119) suggest that ethanol has effects during all days of contrast. In his first study, Cox (118) observed that consuming alcohol immediately prior to an incentive downshift increased runspeeds on a runway, but did not eliminate contrast. In addition, alcohol consumption prior to incentive downshift caused the contrast effect to occur for a longer duration of time than non-consuming rats. Therefore, alcohol acted to prolong recovery from an incentive downshift. Another study by Cox and colleagues (119) investigated activity levels immediately following contrast in animals that had consumed alcohol. Alcohol increased activity levels in both shifted and unshifted animals. Interestingly, the shifted group had lower activity levels than the control group in animals that had consumed alcohol, while the shifted group that had consumed alcohol had higher activity levels than the unshifted group that had consumed alcohol. This observation suggests that alcohol acted to reduce the suppressive effects of contrast on locomotion. The ethanol consumption data

may be limited by the fact that the rats were both food-restricted and fluid deprived prior to consuming alcohol, though the main focus of the studies was to investigate the pharmacological effects on various aspects of contrast behavior. Having a predisposition for high reactivity to or failure to recover from incentive downshifts provides a putative role for alleviation of these states by alcohol, particularly during recovery. As mentioned, ethanol attenuates the reaction to an incentive downshift (52, 117). Therefore, theoretically, during early periods of human consumption, alcohol may serve to temporarily decrease emotional reactions by providing an alternative and highly incentive reward. Alternatively, alcohol has anxiolytic properties, and may act to reduce the conditioned frustration resulting from an incentive downshift. Finally, ethanol may act to inhibit a negative affective state, by making the individual feel better. This idea is similar to the “tension reduction hypothesis”, which maintains that individuals consume alcohol to alleviate anxiety (61, 120). There is also evidence from animal models that a predisposition for alcohol consumption is related to increased anxiety behaviors as well as an increased anxiolytic response to alcohol (121, 122). Interestingly, alcohol has temporary effects on contrast, and actually tends to prolong the duration of contrast, suggesting it interferes with the recovery process (119). When it was administered on post-shift day 2, contrast was attenuated, but it returned in shifted animals on post-shift day 3 (117). Therefore, ethanol may temporarily act to reduce feelings of tension or provide an alternative reward following incentive downshift, though it may also prolong the duration of negative affect associated with incentive downshift.

Amsel (12) theorized a role for conditioned frustration in dispositional learning. It is possible that some individuals learn to respond in a maladaptive way to all frustrating non-reward events. Thus, rather than learning to overcome them, conditioned frustration persists due to any of the previously discussed reasons. As conditioned frustration is

thought to develop in response to both interoceptive and exteroceptive stimuli associated with downshift events, it is possible for internal physical states to produce feelings of conditioned frustration without another incentive downshift event occurring. This is a similar notion to Bouton, Mineka, and Barlow's (123) theory regarding the etiology of panic disorder. In short, a panic attack is associated with a variety of interoceptive stimuli as well as external stimuli that act as conditioned stimuli to produce conditioned anxiety which is thought to develop as a response to primary fear or pain. In a similar respect, conditioned frustration might be more likely to develop and persist in some individuals in trait-like anticipatory manner for unpredictable non-reward or frustrating situations, resulting in an increased likelihood for drinking once one has learned that it alleviates the negative emotional state (conditioned frustration) associated with incentive downshift. Alternatively, drinking could be used as a maladaptive reaction to feelings of frustration.

If alcohol acts to reduce contrast during recovery from incentive downshift, it is possible that individuals predisposed to reactivity to an incentive downshift might also be predisposed to excessive alcohol use. *Specific Aim 3* will explore that idea that alcohol can reduce the affective reaction to SNC using an animal model of excessive alcohol consumption, the crossed HAP (cHAP) line. The cHAP line is a cross of the HAP1 and HAP2 replicate lines, which was selectively bred with the idea that a cross of the parent HAP lines would fix a higher number of alleles relevant for alcohol preference. As discussed in the general introduction, the cHAP line drinks higher than either parent line, achieving mean intakes in excess of 25 g/kg/day and blood ethanol concentrations (BEC) greater than 250 mg/dl (42, 43). Therefore, the cHAP line is an excellent genetic model of excessive alcohol consumption. I plan to test if injected ethanol attenuates SNC in ethanol-naïve cHAP mice, as this effect has only been observed in rats. I will

also measure ethanol consumption in cHAP mice immediately following an incentive shift. It is my *specific hypothesis* that experimenter administered ethanol will reduce contrast, and further that cHAP mice having recently experienced a negative incentive shift will consume more ethanol than unshifted animals.

### 5.2 Specific Research Design

To test whether ethanol attenuates contrast in cHAP mice, all mice were food restricted, and then trained and tested using the SNC procedure described. Ten minutes prior to post-shift day 2, 32-4% and 4-4% mice were injected with a 0, 1, and 1.5 g/kg injection of 20% ethanol/ saline (v/v). The 1 and 1.5 g/kg injections were used to avoid causing a depression of lick behavior in the unshifted control mice. A 1 g/kg injection of 20% ethanol was effective in attenuating contrast in rats, but 1.5 g/kg and 2 g/kg injections depressed behavior of unshifted rats (117). As mice are generally less sensitive to the behavioral effects of ethanol than rats, we administered both 1 g/kg and 1.5 g/kg doses of ethanol. Because the mice seemed very stimulated 10 minutes following the injection, and we saw no effect of ethanol, we conducted 3 more days of training with the pre-shift concentration and then shifted again. Twenty minutes prior to post-shift day 2, mice were again injected with 0, 1.5, or 2 g/kg of 20% ethanol/saline. The same groups were used as ethanol test day 1, therefore the saline group remained the saline group for both days, the 1 g/kg group received the 1.5 g/kg dose, and the 1.5 g/kg group received the 2.0 g/kg dose.

For experiment 2, all mice were given 12 hours of free-choice access to 10% ethanol and water, and bihourly readings were performed to confirm cHAPs reached intoxicating levels. We have previously shown 12 hours of free-choice ethanol is sufficient to produce evidence of intoxication and mean BECs of 100 mg/dl in a majority

of cHAP mice (46). The following day, mice received 1 hour of ethanol access (1 bottle) in the operant boxes, with 15-minute, 30-minute, and 60-minute readings taken during the access period, and were assigned to groups by Sex, g/kg intake in the 1-hour session, and family if possible. Then all mice underwent the SNC task as described. Immediately following post-shift days 1 and 2, all mice received 1 hour of ethanol access with readings taken at the same intervals.

For experiments 1 and 2, repeated measures ANOVAs were used to analyze both pre-shift and post-shift sucrose intake and licks using Days as the repeated measure, and Sex (M, F), Group (4-4, 32-4), and if applicable, Dose (0, medium (1 or 1.5 g/kg), and high (1.5 or 2.0 g/kg) doses) as between-subjects measures. For experiment 2, repeated measures ANOVAs were also used to analyze ethanol intake across each test day, across hours for the first 12-hour session, and minutes across the 1-hour sessions, with Group and Sex as between subjects measures. Mauchley's test of sphericity was performed for all repeated measures ANOVAs, and in the case it was significant, the Greenhouse-Geisser correction was used. The 4.6 microliters/lick criterion was also used to remove lick malfunctions, and a new linear regression was calculated for cHAP mice, since a new line was used in the following studies. The following formula was used to impute lick values, Licks = (499.71 * Intake) + 52.66, where 499.71 is the slope and 52.66 is the y-intercept. For experiment 2, shift ratios were calculated (post-shift intake/pre-shift intake), and correlated with ethanol intake on each corresponding 1-hour test day.

## 5.3 Results

### 5.3.1 Effect of Injected Ethanol on Contrast

#### 5.3.1.1 Pre-shift Behavior

The 32-4 and 4-4 groups did not tend to differ during the pre-shift period in cHAP mice. A repeated measures ANOVA for intake (in ml) across pre-shift Days was run, using Sex and Group as repeated measures. There was a main effect of Day, $F(4.9, 216.1) = 12.5$, $p < .001$. A follow-up trend analysis indicated that intake changed in linear and order 7 polynomial trends across days, ($p$s < .05). There was also an interaction of Day x Group, $F(4.9, 216.1) = 3.2$, $p < .01$, with both 4-4 and 32-4 intakes changing across days, $F(3.4, 77.7) = 6.2$, $p < .001$ and $F(5.3, 121.5) = 11.0$, $p < .001$, respectively (*Figure 14A*). Each group's intake changed in different patterns across days; 4-4 intake changed in linear, quadratic, order 4, and order 7 polynomial trends ($p$s < .05), while 32-4 intake increased in a linear trend ($p < .001$). *A priori* comparisons on the first and last days of pre-shift intake indicated 4-4 and 32-4 groups did not differ at the beginning and end of the pre-shift period, ts < 0.95, $p$s > .05. There was also a main effect of Sex, $F(1, 44) = 9.1$, $p < .005$, with females consuming more sucrose than males, $.88 \pm .04$ and $.70 \pm .04$, respectively. Another repeated measures ANOVA for intake (in ml/kg) revealed a main effect of Days, $F(4.8, 211.0) = 10.4$, $p < .001$, which was due to linear, order 4, and order 7 polynomial trends ($p$s < .05). There was also an interaction of Group x Days, $F(4.8, 211.0) = 3.0$, $p < .05$ (*Figure 14B*). Intake in the 4-4 and 32-4 groups changed across days, $F(3.4, 79.0) = 5.0$, $p < .005$ and $F(4.5, 104.5) = 10.4$, $p < .001$, although there were different trends for each group. Intake in the 4-4 group changed in linear, order 4, and order 7 polynomial trends ($p$s < .05), while intake in the 32-4 group increased in a linear

pattern ($p < .001$). Females consumed more sucrose than males, $F(1, 44) = 27.7$, $p < .001$, with intakes of $49.7 \pm 2.0$ and $34.9 \pm 2.0$, respectively. Finally, a repeated measures ANOVA for licks during pre-shift days indicated there was a main effect of Days, $F(6.3, 277.1) = 17.2$, $p < .001$. A follow-up trend analysis indicated licks changed in linear, cubic, and order 7 polynomial trends across days ($p$s < .05). There was also an interaction of Days x Group, $F(6.3, 277.1) = 3.4$, $p < .005$ (*Figure 14C*). Both 4-4 and 32-4 groups licking behavior changed across days, $F(4.8, 109.8) = 6.0$, $p < .001$ and $F(5.0, 115.8) = 14.1$, $p < .001$, respectively, but 4-4 licks changed in linear, order 4, and order 7 polynomial trends across days ($p$s < .05), while 32-4 licks changed in linear and cubic trends across days ($p$s < .05). *A priori* comparisons on the first and last days of pre-shift licks indicated that the 4-4 group had a greater number of licks on pre-shift day 1, $t(46) = 2.2$, $p < .05$, but the groups did not differ on the last day of the pre-shift period, $t(46) = 0.6$, $p > .05$. Females also licked more than males overall, $F(1, 44) = 7.5$, $p < .01$, with $509.9 \pm 26.2$ and $408.5 \pm 26.2$ licks, respectively.

A repeated measures ANOVA for intake (ml) was run on the pre-shift days from the second shift cycle, and there were no main effects or interactions, all $F$s < 2.2, $p$s > .05 (*Figure 15A*). Another repeated measures ANOVA for intake (ml/kg) indicated there was a main effect of sex, $F(1, 44) = 9.9$, $p < .005$, but no other main effects or interactions, $F$s < 1.9, $p$s > .05 (*Figure 15B*). Females consumed more sucrose than males, with $56.3 \pm 2.6$ ml/kg and $44.7 \pm 2.6$ ml/kg, respectively. Lastly, a repeated measures ANOVA for licks during the pre-shift period indicated there was a main effect of Group, $F(1,44) = 4.4$, $p < .05$, but there were no other main effects or interactions, $F$s < 2.3, $p$s > .05 (*Figure 15C*). 32-4 mice licked more than 4-4 mice, with $662.3 \pm 40.0$ and $543.6 \pm 40.0$ licks, respectively.

#### 5.3.1.2 Post-shift Behavior

Contrast occurred in cHAP mice, and there were no effects of any ethanol doses. A repeated measures ANOVA for intake was run for the two post-shift intake days, including Group, Sex, and Dose as between-subjects measures. Contrast occurred, as was indicated by a main effect of Group, $F(1, 36) = 14.1$, $p < .005$, 4-4 mice had higher intake than 32-4 mice, 0.95 $\pm$ 0.07 and 0.60 $\pm$ 0.07 ml, respectively (*Figure 14 A,D*). There was also a Group x Day effect, $F(1, 36) = 4.4$, $p < .05$. Bonferroni post-hoc comparisons were performed on both days, resulting in an alpha level of (.05/2 = .025). There was a contrast effect on post-shift day 1, $t(46) = 5.5$, $p < .001$, but only a trend for contrast on post-shift day 2, the day of ethanol injections, $t(46) = 2.1$, $p = .04$. There were no other main effects or interactions, $Fs < 1.1$, $p$s > .05. Another repeated measures ANOVA for intake (ml/kg) was run, which revealed that a contrast effect occurred, as there was a main effect of Group, $F(1, 36) = 15.3$, $p < .001$ (*Figure 14 B,E*). The 4-4 mice consumed 50.8 $\pm$ 3.4 ml/kg, more than the 32-4 mice, which consumed 32.1 $\pm$ 3.4 ml/kg. Lastly, a repeated measures ANOVA for licks indicated that a contrast effect occurred in lick behavior, $F(1, 36) = 18.3$, $p < .001$ (*Figure 14 C,F*). The 4-4 mice had 540.6 $\pm$ 33.2 licks, while the 32-4 mice had 339.7 $\pm$ 33.2 licks. There was also a Days x Sex effect, $F(1, 36) = 8.2$, $p < .01$, which was driven by a decrease in licks by females, $F(1, 23) = 7.3$, $p < .05$, but no change in licks by males, $F(1, 23) = 2.1$, $p > .05$.

A repeated measures ANOVA was performed on intake (ml) from the second shift cycle, again using Group, Sex, and Dose as between-subjects factors. A contrast effect occurred in intake, $F(1, 36) = 9.0$, $p < .01$, with intakes of 1.05 $\pm$ 0.09 ml by 4-4 mice and 0.66 $\pm$ 0.09 ml by 32-4 mice (*Figure 15 A,D*). There was also a main effect of Days, $F(1, 36) = 11.2$, $p < .005$, intake decreased between post-shift day 1 and 2. An

interaction was found for Days x Dose, $F(1, 36) = 3.7$, $p < .05$. On post-shift days 1 and 2, there was no effect of Dose, Fs < 0.7, *p*s > .05. The interaction was driven by intake changes between post-shift days 1 and 2 in the saline and 1.5 g/kg dose groups, $F(1, 15) = 15.7$, $p < .005$ and $F(1, 15) = 16.8$, $p < .005$, but no change in the 2.0 g/kg dose group, $F(1, 15) = 0.0$, $p > .05$. Lastly, there was an interaction of Days x Sex x Dose, $F(1, 36) = 3.6$, $p < .05$. This was driven by a Days x Dose effect in female cHAPs, $F(2, 21) = 5.2$, $p < .05$, but not in male cHAPs, $F(2, 21) = 1.4$, $p > .05$. There was an increase in female intake from post-shift day 1 to postshift day 2 in saline mice, $F(1, 8) = 33.2$, $p < .001$, but not in the other dose groups, Fs < 4.1, *p*s > .05. Another repeated measures ANOVA for intake (ml/kg) during the second shift also demonstrated a contrast effect in intake (ml/kg), $F(1, 36) = 9.4$, $p < .005$, with the 4-4 group consuming $57.2 \pm 4.9$ ml/kg and the 32-4 group consuming $36.0 \pm 4.9$ ml/kg (*Figure 15 B,E*). There was a main effect of Days, $F(1, 36) =14.5$, $p < .005$, due to an increase in intake between post-shift day 1 and 2 ($p < .005$). There was also an interaction of Days x Dose, $F(1, 36) = 3.6$, $p < .05$, although none of the dose groups differed on either post-shift day, $F(2, 45) = 0.4$ and $F(2, 45) = 0.3$, *p*s > .05. Intake increased in the saline and 1.5 g/kg dose groups from post-shift day 1 to post-shift day 2, $F(1,15) = 18.8$, $p < .005$ and $F(1, 15) = 19.2$, $p < .005$, but did not change for the 2 g/kg dose group, $F(1, 15) = 0.2$, $p > .05$. Finally, there was an interaction of Days x Dose x Sex, $F(1, 36) = 3.6$, $p < .05$, which was again driven by a change in intake by the females, $F(2, 21) = 5$, $p < .05$, but not the males, $F(2, 21) = 1.3$, $p > .05$. Female intake increased from post-shift day 1 to post-shift day 2 in the saline group, $F(1, 8) = 30.7$, $p < .005$, and there was a trend for an increase in the 1.5 g/kg dose group, $F(1, 8) = 5.3$, $p = .05$, but no change in the 2.0 g/kg dose groups, $F(1, 5) = 0.7$, $p > .05$. Lastly, a repeated measures ANOVA was performed on licks from the two post-shift days, and there were no significant main effects or interactions,

although there was a trend for a main effect of Group, $F(1, 36) = 4.0$, $p = .05$, with 548.6 ± 47.3 licks in the 4-4 group and 415.3 licks in the 32-4 group (*Figure 15 C,F*).

### 5.3.2 Effect of SNC on Ethanol Consumption

#### 5.3.2.1 Pre-shift Behavior

Intake and licks increased across pre-shift days, and the groups did not differ during this time. A repeated measures ANOVA was performed to analyze intake (ml) across the 10 pre-shift days, using Sex and Group as between-subjects measures. For pre-shift intake, one squad's intakes are missing from day 4, therefore, that day was not included in any of the pre-shift analyses. There was a main effect of Days, $F(8, 224) = 6.5$, $p < .001$, and intake increased in a linear pattern across days ($p < .001$) (*Figure 16A*). There were no other main effects or interactions, Fs < 1.5, *p*s > .05. Another repeated measure ANOVA for intake (ml/kg) found the same pattern of results, there was a main effect of Day, $F(8, 224) = 5.8$, $p < .001$, and intake increased in a linear fashion across days ($p < .001$) (*Figure 16B*). There were no other main effects or interactions, Fs < 3.5, *p*s > .05. Lastly, a repeated measures ANOVA for licks indicated there was a main effect of Day, $F(8, 224) = 5.1$, $p < .001$, and there was a linear increase in licks across days ($p < .001$) (*Figure 16C*).

#### 5.3.2.2 Post-shift Behavior

Contrast effects occurred for all measures during post-shift days. Repeated measures ANOVAs were performed on the 2 post-shift days to analyze intake (ml and ml/kg) and licks, using Group and Sex as between-subjects measures. A repeated measures ANOVA on intake (ml) indicated contrast occurred on both post-shift days, $F(1, 28) = 20$, $p < .001$, because 4-4 mice consumed more sucrose than 32-4 mice, 0.40 ± 0.04 and

0.17 ± 0.04, respectively (*Figure 16A*). There were no other main effects or interactions, Fs < 1.1, *p*s > .05. Another ANOVA for intake (ml/kg) also indicated there was a main effect of Group, F(1, 28) = 19.1, $p < .001$, but there were no other main effects or interactions, Fs < 1.7, *p*s > .05 (*Figure 16B*). 4-4 mice consumed more sucrose than 32-4 mice, 18.1 ± 1.7 and 7.9 ± 1.7 ml/kg, respectively. Lastly, an ANOVA for licks indicated there was a contrast effect in licks, F(1, 28) = 9.3, $p < .01$, the 4-4 group had 181.0 ± 16.8 licks and the 32-4 group had 108 ± 16.8 licks (*Figure 16C*).

5.3.2.3 Ethanol Intake

Ethanol intake rate was higher in females than males during the first 12 hours of access. The first 12 hours of ethanol access, taken in bi-hourly measurement (g/kg/h), were analyzed using a repeated measures ANOVA, with Hours as a repeated measure, and Sex and Group (future group) as between-subjects variables. There was a main effect of Hours, F(1, 28) = 19.8, $p < .001$ (*Figure 17A*). A follow-up trend analysis indicated that ethanol intake changed in linear and quadratic trends across hours (*p*s < .05). There was also a main effect of Sex, F(1, 28) = 8.4, $p < .01$. Females consumed ethanol at a higher rate than males overall, 1.12 ± 0.03 and 1.00 ± 0.03 g/kg/h, during the first 12 hours of ethanol access. Another repeated measures ANOVA was used analyze total ethanol intake across the 3 one-hour sessions. There was a main effect of Sex, F(1,28 ) = 10.1, $p < .005$, with females drinking more than males across the sessions, 2.33 ± 0.13 and 1.76 ± 0.13 g/kg. There were no changes in total ethanol intake across the three test days, or any other effects, Fs < 2.4, *p*s > .05 (*Figure 17B*).

The rates of intake differed between 32-4 and 4-4 groups during the ethanol test session, and there was trend for overall intake to be different. A repeated measures ANOVA was performed on cumulative ethanol intake immediately following the first post-

shift day. It included Sex and Group as between-subjects measures and cumulative intake per time-point as a within-subjects measure. There was a main effect of Sex, with females consuming more than males per time-point, with average intakes of 1.62 $\pm$ 0.12 and 1.10 $\pm$ 0.12 g/kg. There was a main effect of Time, $F(1.4, 39.6) = 108.4$, $p < .001$, with intake increasing in linear and quadratic trends across the hour of access (*p*s < .05). There was also a Time x Group interaction, $F(1.4, 39.6) = 11.2$, $p < .005$ (*Figure 18A*). Both 4-4 and 32-4 group intakes increased across time, $F(1.4, 20.6) = 25.5$, $p < .001$ and $F(2, 30) = 100.3$, $p < .001$, respectively. Intake by the 4-4 group increased linearly across the session ($p < .001$), while intake by the 32-4 group increased in linear and quadratic trends (*p*s < .05). Bonferroni post-hoc comparisons between the groups were performed for each of the time-point, resulting in an alpha level of (.05/3 = .017). The groups differed during the first 15 minutes, $t(30) = 2.9$, $p < .01$, but did not differ at 30 minutes, $t(30) = 0.6$, $p > .017$, or at 60 minutes, although there was a trend for a group difference, $t(30) = -2.0$, $p = .06$. A repeated measures ANOVA for intake rate (g/kg/15 min) during each time-point revealed a main effect of Sex, $F(1, 28) = 6.8$, $p < .05$, with females consuming 0.67 $\pm$ 0.05 and males consuming 0.49 $\pm$ 0.05 g/kg/15 min. There was also an interaction of Time x Group, $F(2, 56) = 11.0$, $p < .001$. (*Figure 18B*). Both groups had intake rate changes across time, in the 4-4 group, $F(2, 30) = 6.5$, $p < .01$, intake changed in a linear pattern across time ($p < .01$). In the 32-4 group, $F(2, 30) = 5.9$, $p < .01$, intake changed in a quadratic pattern across the hour of access ($p < .05$). Bonferroni post-hoc comparisons were also performed to compare the groups at each time-point, resulting in an alpha level of (.05/3 = .017). The groups differed during the first 15 minutes, $t(30) = 2.9$, $p < .01$ and the last 30 minutes, $t(30) = -3.1$, $p < .01$, of the session, but not between 15 to 30 minutes, although there was a trend for a group difference, $t(30) = -2.3$, $p = .03$. There was a Time x Sex interaction, $F(2, 56) = 3.5$, *p*

$< .05$ (*Figure 18C*). Intake rates did not change across time in either sex, $Fs < 3.2$, *p*s $> .05$. Bonferroni post-hoc comparisons were performed to compare the sexes at each time-point, resulting in an alpha level of ($.05/3 = .017$). Females had higher intake than males at the first time-point, $t(30) = 2.8$, $p < .01$, but the sexes did not differ at the other 2 time-points, $ts < \pm 1.3$, *p*s $> .05$. There was a significant positive Pearson's correlation of the shift ratio for intake and ethanol intake in 32-4 mice, $r = .52$, $p < .05$; while there was no significant Pearson's correlation of the shift ratio for intake and ethanol intake for 4-4 mice, $r = -0.06$, $p > .05$ (*Figure 19 A, B*).

Rates of intake also differed for the 32-4 and 4-4 groups on post-shift day 2. A repeated measures ANOVA was performed on cumulative intake immediately following post-shift day 2. There was a main effect of Time, $F(1.5, 40.6) = 86.6$, $p < .001$, which was due to intake increasing in linear and quadratic trends across the session (*p*s $< .01$). There was a main effect of Sex, $F(1, 28) = 11.0$, $p < .005$, which was driven by higher average intake across time-points in females than males, $1.43 \pm 0.13$ and $0.85 \pm 0.13$ g/kg. There was also a main effect of Group, $F(1, 28) = 5.3$, $p < .05$, with 4-4 mice having higher average intake than 32-4 mice across time-points, $1.34 \pm 0.13$ and $0.94 \pm 0.13$ g/kg, respectively. There were no other main effects or interactions, $Fs < 2.6$, *p*s $> .05$ (*Figure 20A*). Another repeated measures ANOVA for intake rate (g/kg/15min) indicated there was a main effect of Sex, $F(1, 28) = 2.3$, $p < .01$, with a higher rate of intake in females than males, $0.62 \pm 0.06$ and $0.39 \pm 0.06$ g/kg/15min. There was also a main effect of Group, $F(1, 28) = 5.5$, $p < .05$, driven by higher ethanol intake in the 4-4 group compared to the 32-4 group, $0.60 \pm 0.06$ and $0.42 \pm 0.06$, respectively. There was also an interaction of Time and Group, $F(2, 56) = 6.2$, $p < .005$ (*Figure 20B*). Both the 4-4 and 32-4 groups had changes in intake rate across the session, $F(2, 30) = 4.8$, $p < .05$ and $F(2, 30) = 3.4$, $p < .05$, respectively. The 4-4 group's intake changed in a quadratic

trend ($p < .01$), while there was a trend for the 32-4 group's intake rate to change in a linear fashion ($p = .05$). Bonferroni post-hoc comparisons were performed to compare the groups at each time-point, and the alpha level was corrected to (.05/3 = .017). The groups did not differ in the first 15 minutes or in the last 30 minutes, ts < $\pm$ 1.2, *p*s > .017, while the 4-4 group had higher intake from 15-30 minutes, $t(30) = 3.3$, $p < .005$. There were no correlations between the shift ratio and ethanol intake for the 32-4 group or the 4-4 group, $r = 0.32$, $p > .05$ and $r = 0.01$, $p > .05$. (*Figure 20 C, D*).

## 5.4 Discussion

Contrast effects occurred in food-restricted and non-restricted cHAP mice. There was no effect of injected ethanol on contrast at the doses and pre-treatment times tested. Total ethanol intake did not change across 1-hour access sessions, and there were no group differences in overall intake. Contrast suppressed ethanol intake in the 15 minutes following the incentive downshift on post-shift day 1, and there was evidence it also suppressed ethanol intake during the first 30 minutes on post-shift day 2. Although the results were unexpected, in the face of frustration, HAP mice consume less ethanol, which is a highly preferred reward. Thus, frustration in HAP mice due to a reward downshift impacts behavior toward other available rewards.

In experiment 1, cHAP mice were downshifted and given either a 1.0 g/kg or 1.5 g/kg dose of 20% ethanol 10 minutes prior to post-shift day 2, and no effects on contrast were observed (*Figure 14*). These parameters were adapted from those described by Becker and Flaherty (52, 117), who observed a decrease in contrast when a 1.0 g/kg dose of ethanol was administered 10 minutes prior to post-shift day 2 in rats. As the only available effects of ethanol on contrast studied rats, we had to work from these parameters to design an experiment in mice. The 1.5 g/kg dose was included because

mice are generally less sensitive to the effects of ethanol than rats, and because Barrenha et al (122) observed anxiolytic effects of ethanol in HAP mice at this dose. Anecdotally, it was apparent that cHAP mice were stimulated at the point of testing, which is unsurprising, considering mice are stimulated within the first 15 minutes following low doses of ethanol. On the other hand, rats generally do not experience locomotor stimulation to ethanol (124, 125). Therefore, it is possible that ethanol-induced stimulation interfered with any possible contrast-reducing effects of ethanol.

Thus, another shift cycle was run to see if increasing the ethanol pre-treatment time and doses would affect contrast. Contrast was achieved during the second cycle, which is also the first observation of repeated shifts in mice, making it possible to perform chronic contrast experiments in mice (*Figure 15*). With the second ethanol test day, I increased the pre-treatment time to twenty minutes and used 1.5 g/kg and 2.0 g/kg doses, which were decided after looking at a variety of papers investigating the anxiolytic effects of ethanol on contrast (122, 126-130). Generally, ethanol anxiolysis is most effective with pre-treatment times of 15 to 30 minutes in mice and at doses between 1 g/kg and 2 g/kg. Therefore, the new pre-treatment and doses were chosen with these parameters in mind. Dosing on post-shift day 2 was also not effective at reducing contrast. Although this was the case, the group means in 32-4 mice injected with a 1.5 g/kg dose of ethanol increased slightly, suggesting there may have been an undetectable effect of ethanol on contrast. This might be because contrast was too small to actually detect an effect of ethanol. The magnitude of contrast in cHAP mice measured by their average shift ratio for the first day of contrast was $.86 \pm .05$. Thus, cHAP mice consumed about 86% of their preshift consumption during the first day of contrast in this experiment. In their selective breeding paper, Flaherty and colleagues (13) created a distribution of shift ratios from a large sample of outbred Sprague-Dawley

rats, and about half of the sample had shift ratios of .3 or lower. These observations suggest that contrast magnitude in mice is smaller than contrast magnitude in rats, possibly making it more difficult to statistically detect any effects of pharmacological agents on contrast. In order to detect an effect on contrast, it might be a more effective strategy to show that certain doses do not depress lick behavior, then attempt to modulate shifted intake or licks, rather than trying to compare to dosed unshifted controls. We also could have tried administering ethanol immediately prior to post-shift day 1. In order to make a firm conclusion about possible effects of ethanol on contrast in HAP mice, a complete dose effect curve should be undertaken, and mice should be dosed prior to post-shift days 1 and 2.

Experiment 2 investigated if voluntary ethanol consumption increases in mice immediately following an incentive downshift. Contrast was obtained in cHAP mice that were not food restricted, which is a novel observation in HAP mice, in that all of the other described experiments observed contrast in food-restricted mice (*Figure 16*). In this experiment, it was undesirable to food restrict mice because voluntary ethanol consumption was also used. Food restriction would have complicated interpretation of ethanol intake data, because ethanol consumption could be for caloric reasons rather than for its pharmacologic properties in food-restricted mice. We observed high levels of intake in all cHAP mice on the first day of ethanol access (*Figure 17A*). We also observed higher rates of intake and levels of intake in females overall compared to males, which is similar to patterns of previous results in HAP mice (42, 43). In particular, all of the mice reached an average intake rate of at least 1.0 g/kg/h, suggesting they encountered the pharmacological properties of ethanol during this 12-hour period. This was important for the experiment, in that, if mice encountered the pharmacologic, and presumably pleasant, effects of ethanol prior to the downshift, then possibly drinking to

counteract the negative affect associated with the downshift would occur. Instead, we observed a suppression of ethanol intake immediately following the incentive downshift on both post-shift days (*Figure 18, 20*). On post-shift day 1, ethanol intake in the shifted mice was immediately suppressed, but increased after 15 minutes of access time above the level of the 4-4 mice. There was a trend for the 32-4 mice to consume more ethanol than the 4-4 mice overall, suggesting there may have been a rebound effect in drinking. On the other hand, there was also a positive correlation between level of drinking and shift ratios, suggesting that the smaller the contrast, the larger amount of ethanol consumed (*Figure 19*). Therefore, the data suggest that contrast had suppressive effects on ethanol consumption. This interpretation is somewhat limited by the fact that the pattern of ethanol consumption changed drastically in the unshifted mice between post-shift day 1 and 2, therefore it is difficult to make comparisons to a variable control group. It is possible that prior experience with sucrose influenced the pattern of ethanol consumption in unshifted mice. Therefore, it probably would have been beneficial to include an ethanol group that only had previous water exposure. The total ethanol intakes from each test day suggest that sucrose exposure does not affect total ethanol consumption during the one-hour sessions, though, because the two test sessions did not vary from baseline, and occurred prior to sucrose exposure (*Figure 17*).

No effect of ethanol on contrast was detected, though other studies have shown effects of ethanol on contrast (52, 117, 119). Contrast in mice appears to be of smaller magnitude than in rats, possibly making it difficult to detect any effects of ethanol. A large range of possible doses and pre-treatment periods were not used in experiment 1, so this conclusion is somewhat limited. Additionally, an incentive downshift did not significantly increase ethanol consumption. There was a trend for ethanol to be increased on post-shift day 1, but this may have been more due to a rebound effect

following a suppression of ethanol intake. This assertion is supported by the fact that small shift ratios (indicative of larger contrast) were correlated with lower levels of ethanol consumption on post-shift day 1. Though this is an unexpected result, it is interesting because ethanol is a highly preferred substance by cHAPs, as they are selectively bred to drink high quantities of it. Further, cHAP mice will work for ethanol in an operant task (unpublished observation), and HAP mice find moderate doses of ethanol to be rewarding as measured by a conditioned place preference paradigm (131). Therefore, this study highlights an interesting juxtaposition that HAP mice consume high amounts of available rewards normally, but in the face of frustration, they reduce this behavior.

# CHAPTER 6. GENERAL DISCUSSION

## 6.1 Rewards and Reward Downshift in HAP and LAP Mice

As discussed, reactivity to incentive shifts is a relatively universal phenomenon that evolved in support of behavioral processes responsible for foraging, and may be a major source of affective reactions in humans and other species (10, 55). Many have argued that foraging behavior is adaptive, and has been conserved because it is crucial to obtaining food, but the underlying processes likely contribute to obtaining other rewards in the environment (56). Variation in foraging behavior also has a strong genetic component in a variety of animal models (for review see 57). Therefore, it seems likely the reaction to an incentive downshift is a highly conserved pattern of behavior that may be present in human reactions to negative life events, and is also subject to genetic variation. Human behavior is more complex than animal behavior, but it is possible that certain individuals are predisposed to being less likely to overcome reward downshift events. This type of phenotype in humans could explain why certain individuals are more susceptible to experiencing affective reactions, as they may be more likely to react, react more strongly to, and/or be less likely to recover from incentive downshift events. Reactivity to incentive shifts may arise from a primary frustrative process that elicits search behavior for the missing reward. A strong initial response to incentive shift may be indicative of a particularly strong memory of the pre-shift reward, which could then interfere with approach behavior for the available reward initially and during recovery.

It is my general hypothesis that emotional reactivity to incentive shifts is related to an individual's reward sensitivity or level of reward-seeking. This hypothesis is supported by the observations that HAP mice consume more sucrose, saccharin, and ethanol than LAP mice. HAP mice also perform more responses to obtain reinforcers than LAP mice. Therefore, behavioral data from these studies support the idea that HAP mice may have higher appetitive and consummatory drives for rewards compared to LAP mice. This conclusion would be fully supported by data that separately analyzed appetitive and consummatory behaviors in HAP and LAP mice, because purely appetitive behavior has not been investigated in HAP and LAP mice to my knowledge (132, 133). The described data also only refer to consummatory rewards, HAPs may or may not behave in a similar manner towards other non-consumed rewards (such as social, sexual, or exercise-related reinforcers). This is important, considering HAPs were selectively bred for a consummatory behavior, and selection may have preferentially influenced consumption related phenotypes. Recently, though, Trujillo and colleagues (134) observed increased home cage and wheel-running behavior in HAP2 mice compared to LAP2 mice. Wheel running is considered to be a rewarding behavior in rodents, therefore the study by Trujillo and colleagues supports the assertion that HAPs respond more for an available reward than LAPs, regardless of the reward type. Additionally, that HAP mice are more impulsive than LAPs on both delayed discounting and DRL tasks may support the assertion that HAP mice are driven by reward-seeking or reinforcement-seeking. It has been theorized that a high level of reward-seeking is associated with impulsivity, and there is support for the phenotypes to be related (135-137).

Second, the assertion that reward sensitivity or seeking is related to one's emotional reactivity to incentive shifts is supported by the observation that HAP mice experience larger contrast effects than LAP mice. Due to an unexpected reward

downshift, HAP consumption of an available reward, sucrose, is suppressed compared to shifted LAP mice. Amsel's theory of frustration states that primary frustration occurs following unexpected non-reward or un-reinforced responses, and this response is subject to Pavlovian conditioning (12, 16, 17). With repeated exposure to frustrative events, secondary or conditioned frustration develops. In the case of SNC, the theory holds that associations are formed between environmental stimuli (for example, the sipper tube) and impending reward, resulting in reward expectation when an animal is exposed to conditioned stimuli in the environment. When the reward is reduced, primary frustration results, which usually occurs during the first post-shift day. New associations are created with the stimuli that had previously signaled reward, resulting in the development of conditioned frustration. As this new associative process occurs, it competes with associations that had previously developed to signal reward availability, resulting in a conflict between approach and avoidance behavior. This process takes several days to overcome, and Amsel (12) argues this is why contrast effects last for several days. Further, primary and secondary frustration are present in four different stages of behavior that present in frustrating situations, including behavioral invigoration, suppression, persistence, and regression. Amsel (12) explains that these behavioral states are the endpoints of dispositional learning, which he describes as an associative process that is largely implicit and requires repeated experience with reward and non-reward to develop. If contrast is interpreted via Amsel's frustration theory, in the face of both primary and conditioned frustration, HAP mice reduce consummatory behavior in a more profound manner than LAP mice do. This is not only the case for sucrose during SNC, but also extends to saccharin intake during DRL and ethanol intake. Therefore, primary frustration seems to transfer to other tasks in HAPs in the form of suppressing behavior. It is unclear whether appetitive behavior is also blunted in HAPs, but there is

some evidence that the incentive downshift invigorated correct responding in LAP males. It is unclear why HAP mice did not show any evidence of behavioral invigoration, aside from the literature on consummatory contrast not transferring to instrumental behavior (114, 115). It would be interesting to compare responding in HAP and LAP mice on another task following incentive downshift to observe if there are differences in appetitive behavior following incentive downshift. Overall, it seems that when HAPs expect a reward will be available, they will readily consume it or work for it. If the reward is reduced unexpectedly, HAP consummatory behavior tends to be suppressed towards the available reward, and this behavior transfers to tasks with other rewards. Future endeavors should attempt to understand if the same is the case for appetitive behavior in HAPs.

## 6.2 Reward Prediction Error as a Potential Mechanism Underlying Contrast Behavior

A great deal of work on unexpected reward loss has focused on dopamine function. Though many other brain regions are involved in the initial reaction to and recovery from contrast (10), little attention has focused on if reward prediction error is involved in contrast behavior. This seems like an area ripe for discovery, especially with regard to addiction, because individual differences in reward prediction error have been related to addictive behavior (138). Recently, Phillips, Vacca, and Ahn (139) observed that the level of dopamine efflux is decreased immediately following an incentive downshift in shifted animals compared to unshifted animals. The authors point out that incentive value associated with a stimulus is not static, and changes depending on the internal physiological state and prior experience with the stimulus (140, 141). The change in incentive value of sucrose following an incentive downshift is a demonstration of this idea. Dopamine may be representative of the motivational state, which can be

determined by the incentive salience of a stimulus. Phillips and colleagues (139) state that their findings are in agreement with a prediction error viewpoint, which argues that phasic dopamine release provides a signal for reward or for error in predicting a reward that serves to drive learning or is stored and used for decision-making (142-145). Thus, in reaction to incentive downshift, dopamine firing may decrease to signal the value of reward is less than what was expected. On the other hand, Schultz (146) states that dopamine prediction error signals are phasic and occur within a subsecond timeframe; therefore would not be able to be detected by microdialysis as was used by Phillips and colleagues (135). There is a significant amount of evidence that tonic dopamine signaling has a permissive role on motivational processes, though (146). Therefore, decreased dopamine may not have been reflective of a prediction error signal, per say, but the finding still provides support for a role of dopamine signaling in the incentive-motivation processes underlying contrast behavior.

These considerations aside, a great deal is known about dopamine prediction error signals, which are based on how an obtained reward deviates from the mean expected (or predicted) value of a reward. Mean expected reward value takes into account both the probability and magnitude of reward (145). Therefore, reward prediction error may be important for producing contrast effects, in that, if the magnitude of reward is larger or smaller than expected, a dopamine prediction error signal may occur. Magnitude in these studies generally refers to volume of reward rather than change in concentration, but dopamine neurons have been shown to respond to the value of a reward or reward-predicting stimulus, rather than the stimulus characteristics or location. Therefore, a change in sucrose concentration would be expected to elicit a prediction error signal because the downshift involves a change in reward value. This idea would need to be supported by electrophysiological or fast scan cyclic voltammetry

experiments, which can be used to detect phasic changes in dopamine signaling. While dopamine prediction error signals may detect the incentive downshift, they likely act by informing brain networks devoted to learning and decision-making based on value. In addition, heterogenous populations of neurons in the amygdala, striatum, prefrontal cortex, parietal cortex, and orbitofrontal cortex have also been shown to provide signals about reward value, therefore these areas may also be important for encoding and updating reward value (147-149). Recently, several studies have implicated certain areas in the detection of aversive outcomes and stimuli, and provide inhibitory input to ventral tegmental area (VTA) dopamine neurons (150). Neurons in the rostromedial tegmental nucleus (RMTg) and lateral habenula are activated in response to unexpected aversive outcomes and aversive-predicting stimuli, while they are inhibited in response to unpredicted rewards and reward-predicting stimuli (151, 152). More research is needed, but reciprocal connections between the lateral habenula, the RMTg, and the VTA form a putative network for processing incentive and aversive information. Aversive prediction error signaling could also drive the learning processes following an incentive downshift, thus it is possible that the areas signaling aversive outcomes and stimuli contribute to contrast behavior. It is possible that individual differences in positive and negative prediction error signals from this network could result in behavioral differences following incentive downshift. Further, individual differences may exist in projection regions or the functional networks receiving prediction error signals, many of which have also been implicated as being involved in contrast behavior, which could result in behavioral differences during the reaction to and recovery from an incentive downshift.

## 6.3 Reactivity to Incentive Downshift and Its Relationship to Impulsive Action and Addiction

Damasio's (153, 154) somatic marker hypothesis argues that the viscera provide the brain with an updated representation of the body's interaction with the external environment, providing a physical basis for emotions. The body's response to interoceptive and exteroceptive cues creates emotions, while the perception of these states are feelings. He argues that emotions evolved to provide an automatic decision-making system that has allowed all species to take advantage of rewards in the environment, thus promoting survival. Further, there is evidence that emotions are necessary for proper decision-making capabilities in humans (155, 156). Therefore, the brain areas underlying emotions are inextricably linked to those producing conscious thought in humans. Although emotions are necessary for many behaviors, Damasio (153) asserts that emotions can also be counterproductive to situations that require deliberate, conscious decisions, and there is a significant amount of evidence for this supposition (157). Lowenstein (158) also makes a similar assertion, hypothesizing that visceral factors (or intense physiological emotion states) have a disproportionate effect on behavior by overturning other goals than those that are immediately responsible for eliciting a visceral factor. Further, craving or other strong emotion states have been related to rash decision-making related to drug use, including excessive alcohol intake (28-31).

Negative urgency is the predisposition to act rashly in response to negative emotions (32, 33). According to the theory of urgency, strong emotion provides a signal for action that causes one to focus on the immediate situation and not necessarily attend to long-term goals, thereby resulting in rash action (34). For example, in a highly urgent

individual, loss of a job or significant other may result in a strong negative emotional state, causing the person to heavily consume ethanol and engage in other risky behaviors. To this effect, strong relationships between negative urgency and problems related to alcohol use have been identified (35-37). It is plausible that having an increased initial reaction to incentive downshift events is related to the urgency trait. I originally hypothesized that incentive downshift would produce an increased level of impulsive behavior, due an increase in appetitive behaviors that occurs immediately following the downshift. It is also possible that an emotional reaction can suppress behavior in a maladaptive manner, which is referred to by Smith and colleagues (159) as ill-advised inaction. This concept might be similar to the behavioral suppression in consumption by HAP mice immediately following a reward downshift. In particular, it could be considered a maladaptive to not consume a reward under the condition of food restriction. It is also possible that the DRL experiment was not designed so that an increase in appetitive behavior resulting from the downshift could be observed. For example, HAPs could have already "searched" for the missing reward during the downshift session, or the search behavior could have not transferred to the DRL task. It has also been observed repeatedly that consummatory contrast usually does not affect instrumental behavior (114). As discussed, unexpectedly changing the response contingency might be more likely to change responding, and result in detection of impulsive behavior following an incentive downshift. On the other hand, there is some evidence for an increase in appetitive behaviors in LAP males immediately following the downshift. If HAP and LAP behaviors diverge following an incentive downshift, it could possibly be due to differences in arousal driving behavior. Although theoretical, the high level of reward-seeking normally observed in HAPs possibly suggests they are more aroused than LAP mice. Perhaps the reaction to incentive downshift increased arousal

too much in HAPs, resulting in behavioral suppression. In LAPs, it increased arousal enough to produce behavioral invigoration. This argument would be similar to an inverted U account of motor performance, which holds that behavioral performance is dependent on level of arousal and follows an inverted U shape in performance. Therefore, at a moderate level of arousal, performance is best, but if arousal is too high or low, performance suffers (160, 161). More inquiry is needed to understand how impulsivity is affected by an unexpected incentive downshift or loss in HAP and LAP mice. It is possible that in predisposed humans an incentive downshift or loss acts to temporarily suppress behavior in normally impulsive individuals. Once the primary frustration has been resolved, individuals persist and return to their normal impulsive tendencies (12). Although also theoretical, it might be possible that once suppression has taken place, the rebound from suppression results in increased impulsive behaviors, due to opponent-process effects on motivation (162). This idea might be supported by the trend for a rebound in alcohol consumption following suppressed intake that was observed in experiment 4.

I also hypothesized that having a predisposition for high reactivity or failure to recover from incentive downshifts provides a putative role for alleviation of these states by alcohol, particularly during recovery. Ethanol has been shown to attenuate successive negative contrast in rats (52, 117). Although this was not replicated in cHAP mice, it could be because contrast is of smaller magnitude in mice, and therefore it is more difficult to detect the effect of pharmacological manipulations. Other doses or pre-treatment times may also be more effective in reducing contrast. Based on the rat studies, it is possible alcohol may serve to temporarily decrease emotional reactions by providing an alternative reward. Alternatively, alcohol has anxiolytic properties, and may act to reduce the conditioned frustration resulting from an incentive downshift event.

Ethanol may also act to disinhibit a negative affective state, or make an individual feel better. This idea is similar to the “tension reduction hypothesis”, which maintains that individuals consume alcohol to alleviate anxiety (conditioned frustration in this case) (61, 120). There is evidence from animal models that an increased predisposition for alcohol consumption results in increased anxiety behavior and an increased anxiolytic response to alcohol (121, 122). Although this might be the case, Cox (119) also showed that alcohol acted to prolong recovery, suggesting it increases the duration of negative affect following incentive downshift.

Ethanol consumption did not increase following an incentive downshift. Though the described interpretations of ethanol’s role are not supported by the present studies, the rest of the preclinical literature supports a role for ethanol in reducing the effects of incentive downshift. Instead, an incentive downshift seemed to suppress ethanol consumption in cHAP mice for up to 30 minutes post-shift. On the first day, there seemed to be a bit of a rebound effect, suggesting HAP mice consumed ethanol at a very fast rate after being suppressed initially. Although this study does not support a role for ethanol reducing the negative affect associated with the downshift, it supports the idea that consumption in HAP mice is suppressed following an incentive downshift, even in the presence of ethanol, which is a highly preferred reward. It is possible that in predisposed individuals, unexpected reward loss or changes result in behavioral suppression. Rather than persevering, individuals turn to drinking as part of a maladaptive emotional reaction to incentive downshift. In a way, turning to drinking could be conceived as behavioral suppression. By failing to persevere over an incentive loss in a normal, healthy manner such as seeking social support, etc., individuals drink as an emotionally reactive coping mechanism. Cox and Klinger (163) make a similar assertion in their motivational model of alcohol use, that individuals frustrated by failures in

pursuing goals may turn to drinking as a way of coping. Therefore, although there is support in the literature that ethanol may reduce the effects of incentive downshift, drinking could also conceivably occur in humans as an emotionally reactive behavioral strategy to incentive downshift. In conclusion, it is an interesting juxtaposition that HAP mice consume high amounts of reward (in the form of sucrose and ethanol) normally and act in impulsive ways to achieve them, but in the face of frustration, their behavior is reduced. This pattern of behavior may have implications for predisposed humans, as it is possible that they also interact in a similar pattern with rewards and unexpected loss of reward.

Table 1 Line and replicate average weights and consummatory behavior

| | HAP2 | LAP2 | HAP3 | LAP3 |
|---|---|---|---|---|
| Baseline Weight (g) | 26.1 ± 0.4 | 24.4 ± .5# | 24.1 ± .4 | 25.7 ± .4 |
| Pre-shift | | | | |
| Intake (ml) | 1.22 ± .06 | .66 ± .06## | 1.17 ± .06 | .85 ± .06# |
| Intake (ml/kg) | 53.71 ± 2.57 | 31.54 ± 2.76 | 56.71 ± 2.63 | 39.18 ± 2.57 |
| Licks | 714.3 ± 48.5 | 450.6 ± 52.0 | 650.7 ± 49.7 | 573.0 ± 48.5 |
| Post-shift | | | | |
| Intake (ml) | 1.02 ± .06 | .30 ± .07## | 1.06 ± .06 | .67 ± .06#++ |
| Intake (ml/kg) | 45.27 ± 3.00 | 14.28 ± 3.22 | 53.17 ± 3.08 | 31.33 ± 3.00 |
| Licks | 604.8 ± 36.5 | 227.6 ± 39.1## | 563.4 ± 37.4 | 425.0 ± 36.5#+ |

Table 2 Pre-shift and post-shift analyses by replicate

| | HAP2 | LAP2 | HAP3 | LAP3 |
|---|---|---|---|---|
| Pre-shift | | | | |
| Intake 32-4 (ml) | 1.32 ± .07+ | 1.03 ± .08** | 1.10 ± .09 | 1.06 ± .09* |
| Intake 4-4 (ml) | 1.13 ±.07++ | .28 ± .08 | 1.23 ± .09++ | .64 ± .09 |
| Intake 32-4 (ml/kg) | 57.03 ± 2.92 | 48.63 ± 3.05** | 52.09 ± 4.50 | 47.81 ± 4.31* |
| Intake 4-4 (ml/kg) | 50.28 ± 2.92++ | 14.22 ± 3.20 | 60.63 ± 4.31++ | 30.56 ± 4.31 |
| Licks 32-4 | 855.0 ± 59.2** | 706.6 ± 61.9** | 609.0 ± 76.0 | 732.8 ± 72.8* |
| Licks 4-4 | 573.6 ± 59.2++ | 188.8 ± 64.9 | 694.8 ± 72.8+ | 413.2 ± 72.8 |
| Post-shift | | | | |
| Intake 32-4 (ml) | .74 ± .07** ++ | .21 ± .08* | .79 ± .11** | .52 ± .10* |
| Intake 4-4 (ml) | 1.29 ± .07++ | .38 ± .08 | 1.32 ± .10+ | .82 ± .10+ |
| Effect size | .49 | .21 | .39 | .17 |
| Intake 32-4 (ml/kg) | 33.88 ± 3.02** ++ | 10.46 ± 3.16* | 40.72 ± 5.30* ++ | 24.64 ± 5.08 |
| Intake 4-4 (ml/kg) | 56.66 ± 3.02++ | 18.00 ± 3.31 | 64.94 ± 5.08+ | 38.03 ± 5.08 |
| Effect size | .48 | .20 | .32 | .15 |
| Licks 32-4 | 529.5 ± 47.5 | 177.9 ± 49.6 | 400.0 ± 61.6** | 353.1 ± 58.9 |
| Licks 4-4 | 682.1 ± 47.5 | 274.7 ± 52.0 | 731.0 ± 58.9 | 496.8 ± 58.9 |
| Effect Size | .15 | .13 | .46 | .12 |

Table 3 Experiment 2 analyses separated by replicate

| | HAP2 | LAP2 | HAP3 | LAP3 |
|---|---|---|---|---|
| FR-1 | | | | |
| FR1-10s Criterion | 1.57 ± .26++ | 3.25 ± .25 | 1.79 ± .23 | 1.65 ± .23 |
| FR1-2.5s Criterion | 1.00 ± .03 | 1.04 ± .03 | 1.00 ± .07 | 1.17 ± .07 |
| FR1-2.5s Intake | 2.43 ± .15+ | 1.96 ± .15 | 2.43 ± .13++ | 1.21 ± .14 |
| FR1-2.5s Correct | 106.3 ± 6.5 | 93.0 ± 6.3 | 116.0 ± 6.0++ | 65.7 ± 6.1 |
| FI-32 | | | | |
| Correct | 374.6 ± 33.5+ | 252.0 ± 32.8 | 237.0 ± 19.1++ | 111.2 ± 19.6 |
| Incorrect | 24.9 ± 3.2+ | 11.8 ± 3.1 | 21.8 ± 3.2 | 24.7 ± 3.2 |
| Intake | 1.68 ± .09 | 1.46 ± .09 | 1.39 ± .09++ | .90 ± .1 |
| Reinforcers | 40.7 ± 1.0 | 38.3 ± 1.0 | 37.9 ± 1.0++ | 29.3 ± 1.0 |
| Efficiency | .14 ± .02 | .19 ± .02 | .19 ± .02 | .29 ± .02 |
| DRL-32 | | | | |
| Correct | 199.0 ± 9.5++ | 133.9 ± 9.3 | 165.1 ± 8.4++ | 126.3 ± 8.6 |
| Incorrect | 30.8 ± 4.2+ | 13.9 ± 4.1 | 29.2 ± 3.3 | 28.8 ± 3.4 |
| Percent Correct | .87 ± .02 | .90 ± .01 | .85 ± .02 | .82 ± .02 |
| Intake | .28 ± .02 | .33 ± .02 | .24 ± .03+ | .34 ± .03 |
| Reinforcers | 8.3 ± .7++ | 12.7 ± .6 | 8.5 ± .8++ | 14.8 ± .9 |
| Efficiency | .05 ± .01++ | .11 ± .01 | .07 ± .01++ | .14 ± .01 |

Table 3 continued

| SNC | | | | |
|---|---|---|---|---|
| Intake 32-4 (ml) | .50 ± .06** ++ | .18 ± .06 | .52 ± .08* ++ | .30 ± .08 |
| Intake 4-4 (ml) | 1.02 ± .06++ | .22 ± .06 | .85 ± .08+ | .51 ± .08 |
| Effect size | .51 | .03 | .25 | .16 |
| Intake 32-4 (ml/kg) | 22.88 ± 3.33** ++ | 8.98 ± 3.33 | 26.56 ± 4.16* ++ | 14.51 ± 4.16* |
| Intake 4-4 (ml/kg) | 45.76 ± 3.48++ | 11.03 ± 3.48 | 43.28 ± 4.16+ | 24.65 ± 4.35 |
| Effect size | .39 | .03 | .22 | .17 |
| Licks 32-4 | 228.7 ± 35.6* + | 130.0 ± 35.6 | 322.1 ± 52.7* + | 220.7 ± 52.7 |
| Licks 4-4 | 429.7 ± 37.2++ | 138.8 ± 37.2 | 510.6 ± 52.7 | 315.3 ± 55.0 |
| Effect size | .32 | .00 | .23 | .07 |

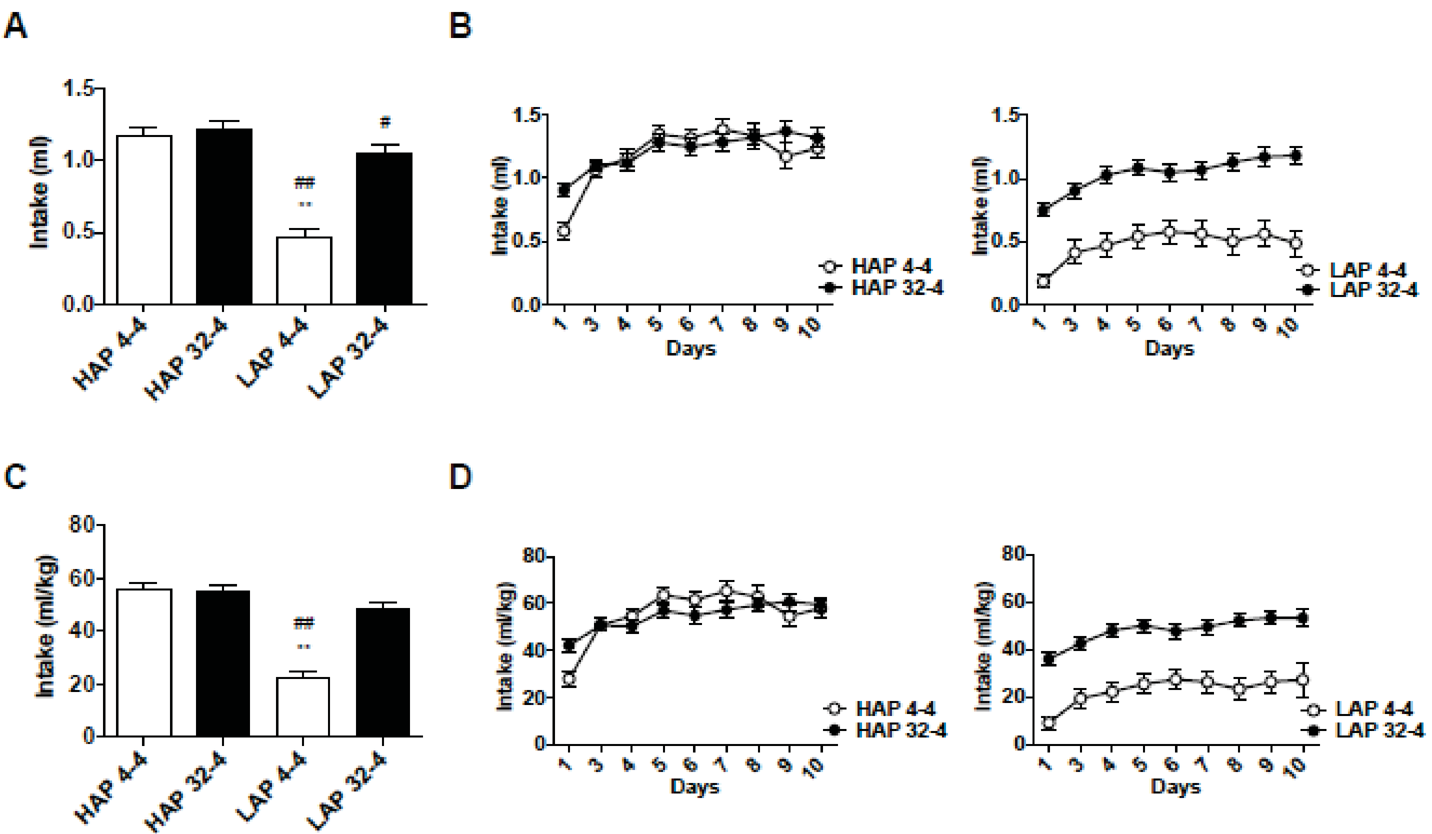


Figure 1 Preshift intake (in ml and ml/kg) in each Line (HAP, LAP) and Group (32-4, 4-4). (A) Overall average intake (in ml) during preshift days. (B) Intake (in ml) across pre-shift days. (C) Overall average intake (in ml/kg) during pre-shift days. (D) Intake (in ml/kg) across pre-shift days.

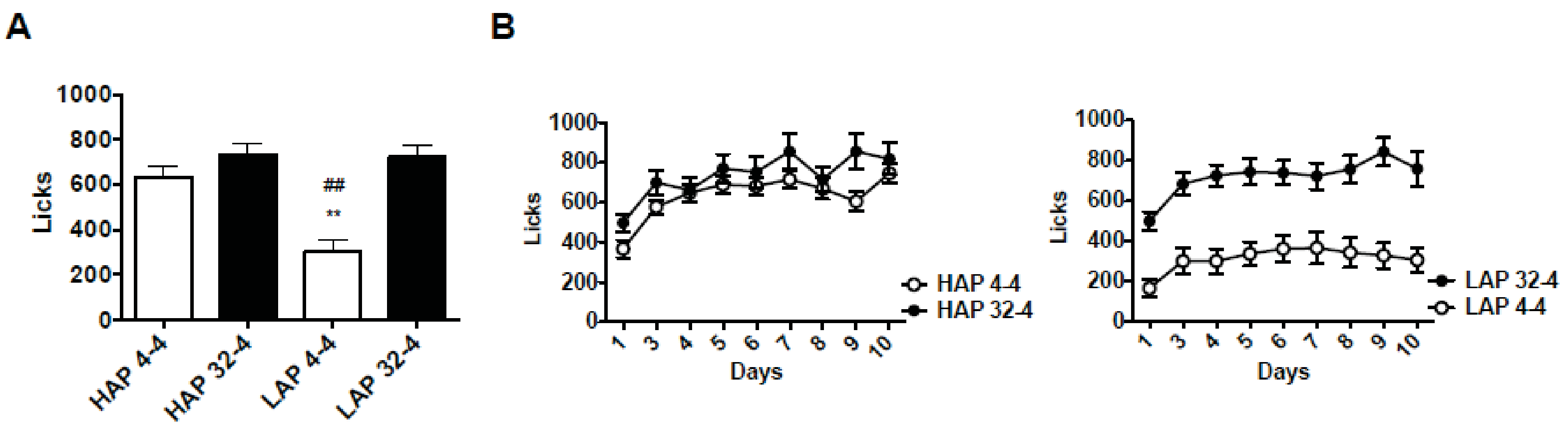


Figure 2 Preshift licks in each Line (HAP, LAP) and Group (32-4, 4-4). (A) Overall licks during pre-shift days. (B) Licks across pre-shift days.

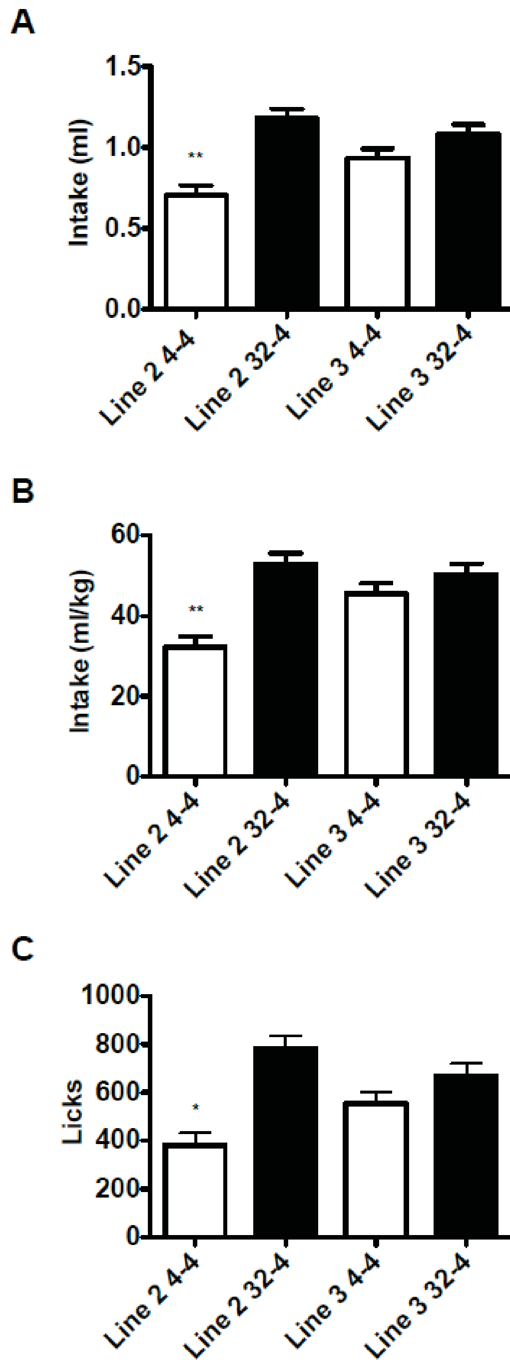


Figure 3 Preshift behavior for each Replicate (2,3) and Group (32-4, 4-4). (A) Overall average intake (in ml) during pre-shift days. (B) Overall average intake (in ml/kg) during pre-shift days. (C) Overall licks during pre-shift days

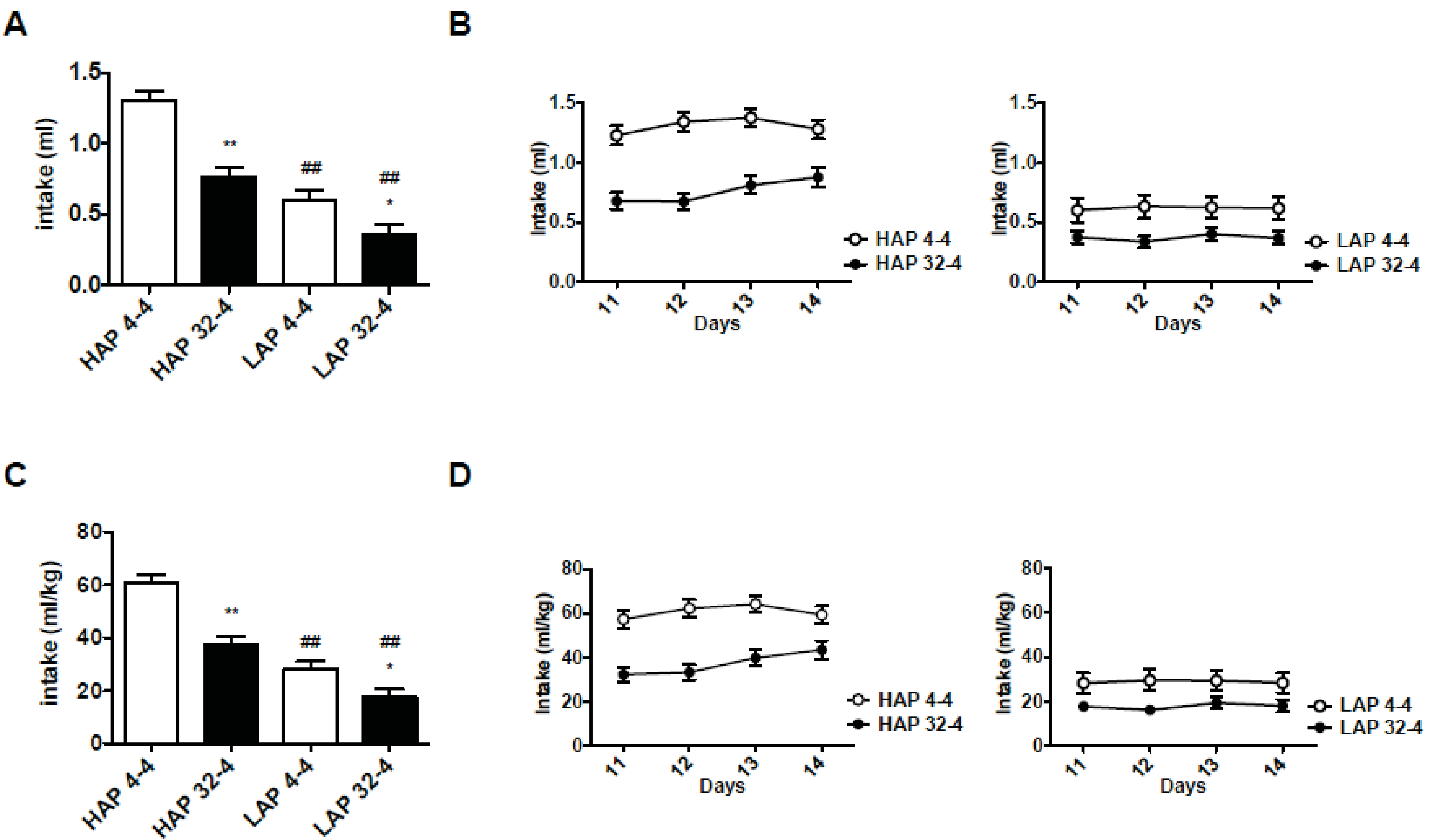


Figure 4 Post shift intake (in ml and ml/kg) for each Line (HAP, LAP) and Group (32-4, 4-4). (A) Overall intake (in ml) during post-shift days. (B) Intake (in ml) across post-shift days (C) Overall average intake (in ml/kg) during post-shift days. (D) Intake (in ml/kg) across post-shift days.

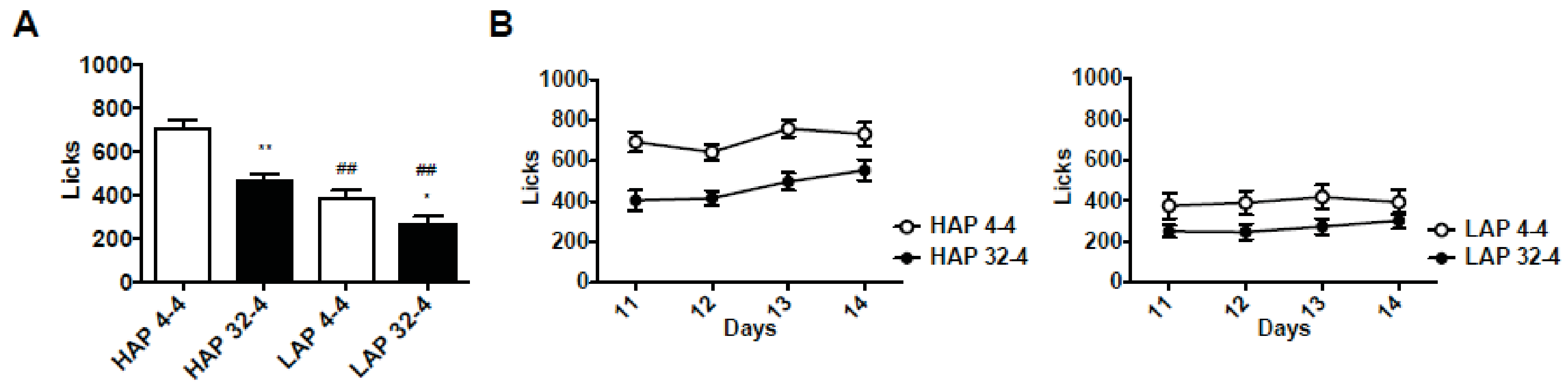


Figure 5 Post-shift licks for each Line (HAP, LAP) and Group (32-4, 4-4). (A) Overall licks during post-shift days. (B) Licks across post-shift days.

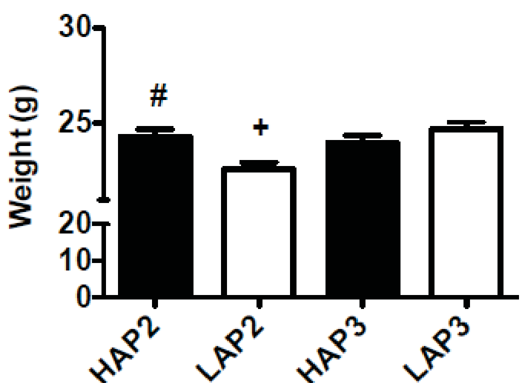


Figure 6 Baseline weights in the Lines (HAP, LAP) and Replicates (2, 3).

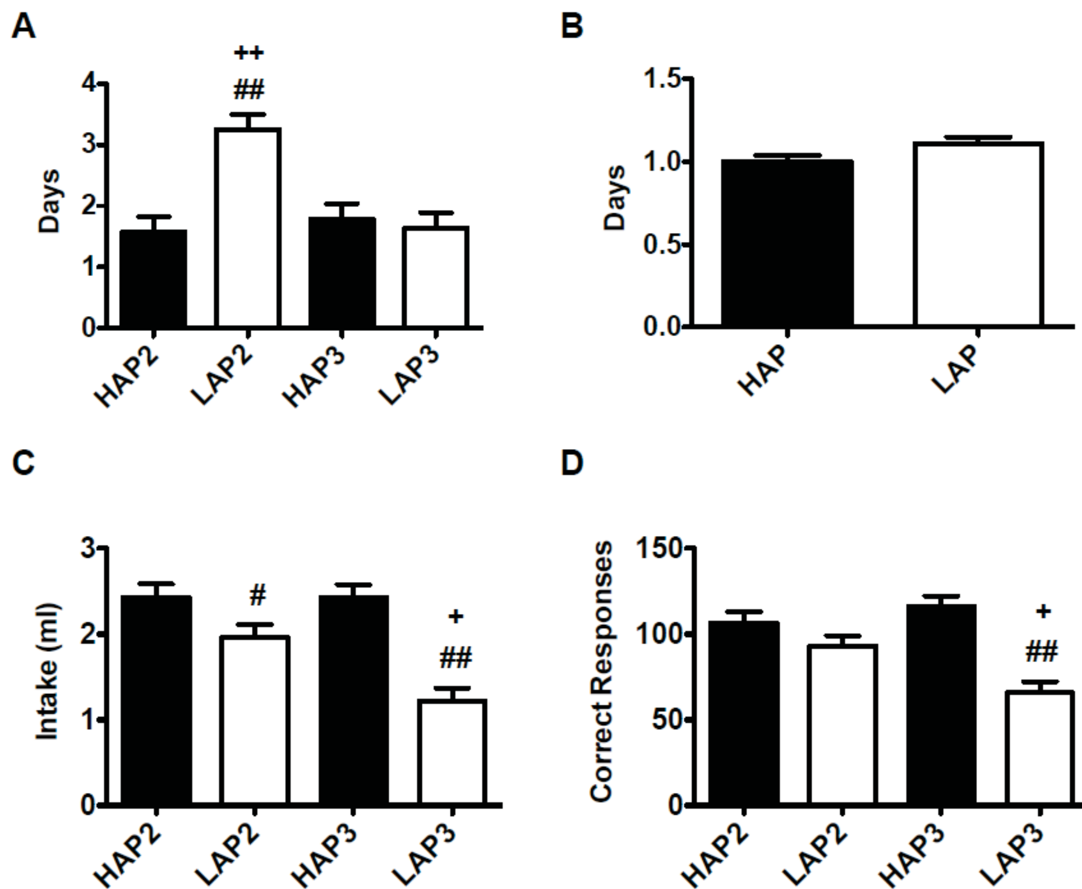


Figure 7 Behavior from the Fixed Ratio-1 (FR1) schedule of reinforcement phase in the Lines (HAP, LAP) and Replicates (2,3). (A) Days to criterion for FR1-10s schedule of reinforcement. (B) Days to criterion for the FR1-2.5 s schedule of reinforcement. There was a trend for LAPs to take longer than HAP mice to meet criterion (*p* = .06) (C) Intake (in ml) on the last day of FR1-2.5s schedule of reinforcement for each mouse. (D) Correct responses on the last day of FR1-2.5s schedule of reinforcement for each mouse.

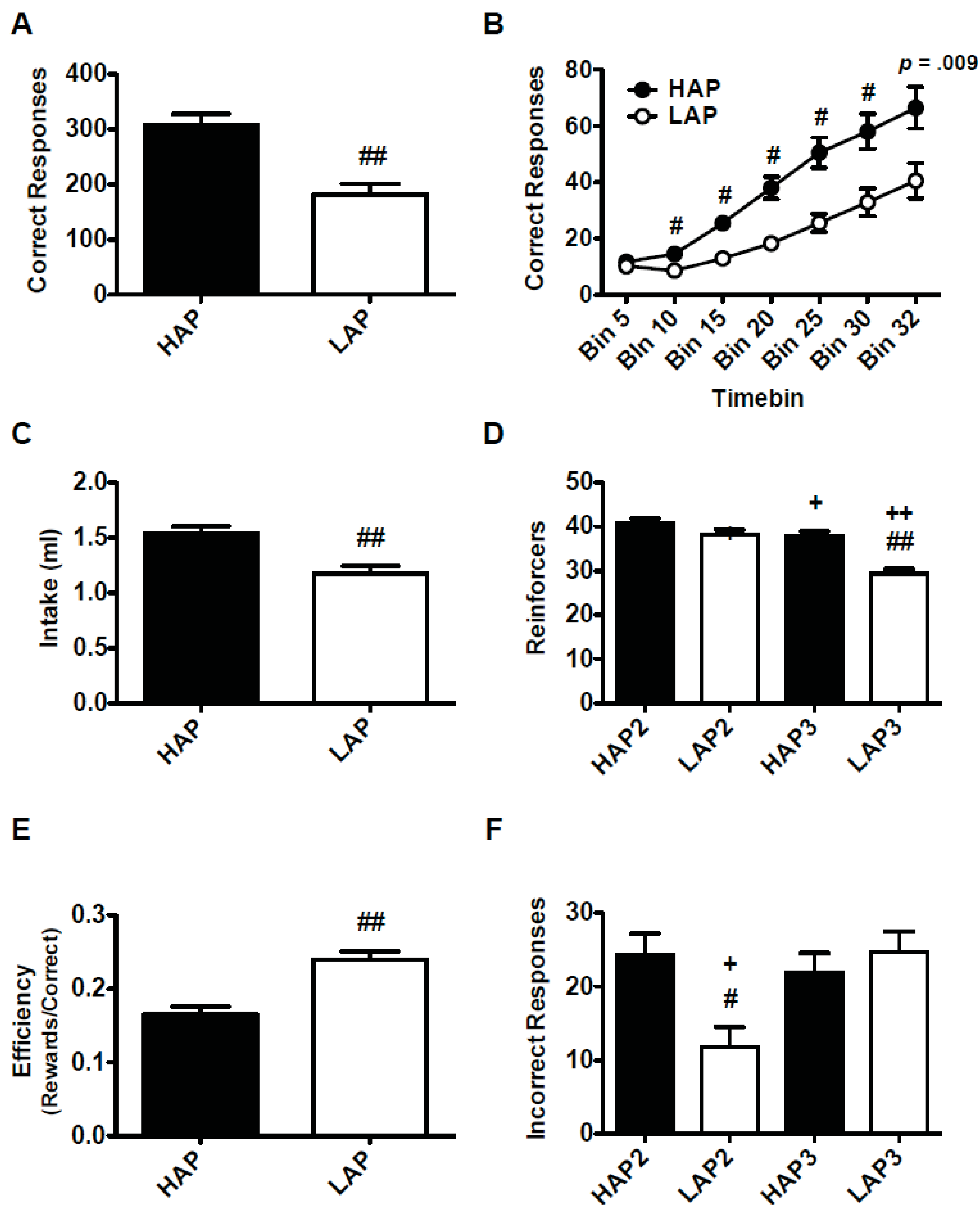


Figure 8 Behavior from the last day of the Fixed Interval phase (FI-32) by Line (HAP, LAP) and Replicate (2, 3) (A) Total correct responses in each line. (B) Correct responses per time-bin in each line. There was a trend at Bin 32 for the lines to differ ($p$ = .009). (C) Intakes in HAP and LAP mice. (D) Number of reinforcers gained in each line and replicate. There was a trend for HAP2 and LAP2 mice to differ ($p$ = .09). (E) Efficiency (rewards/correct) for HAP and LAP mice. (F) Incorrect response in each line and replicate.

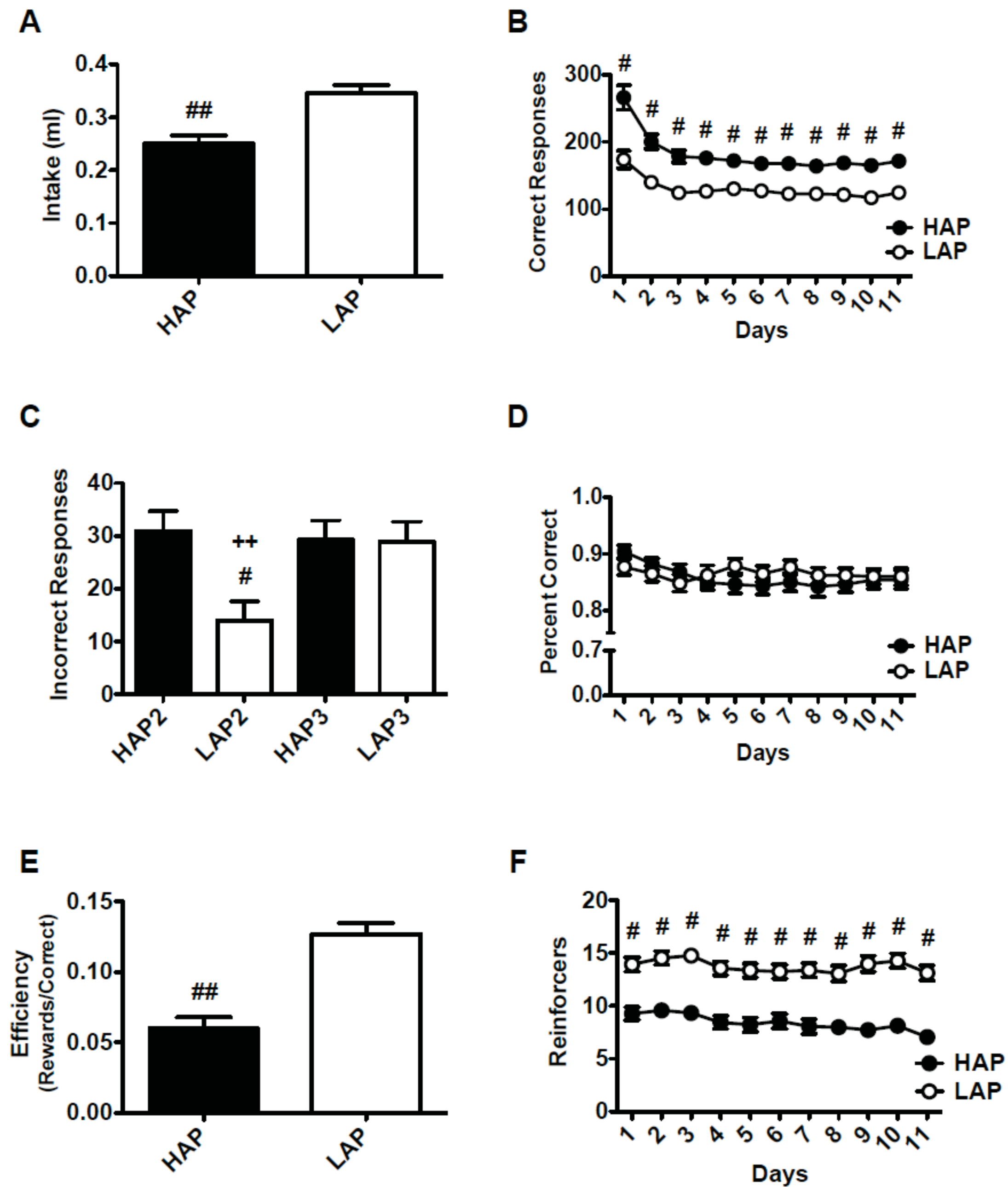


Figure 9 Behavior from DRL-32s testing. (A) Overall intake in HAP and LAP mice. (B) Correct responding across days in HAP and LAP mice. (C) Overall incorrect responses in each line and replicate. (D) Percent correct in HAP and LAP mice across days. (E) Overall efficiency in HAP and LAP mice. (F) Reinforcers gained across days in HAP and LAP mice.

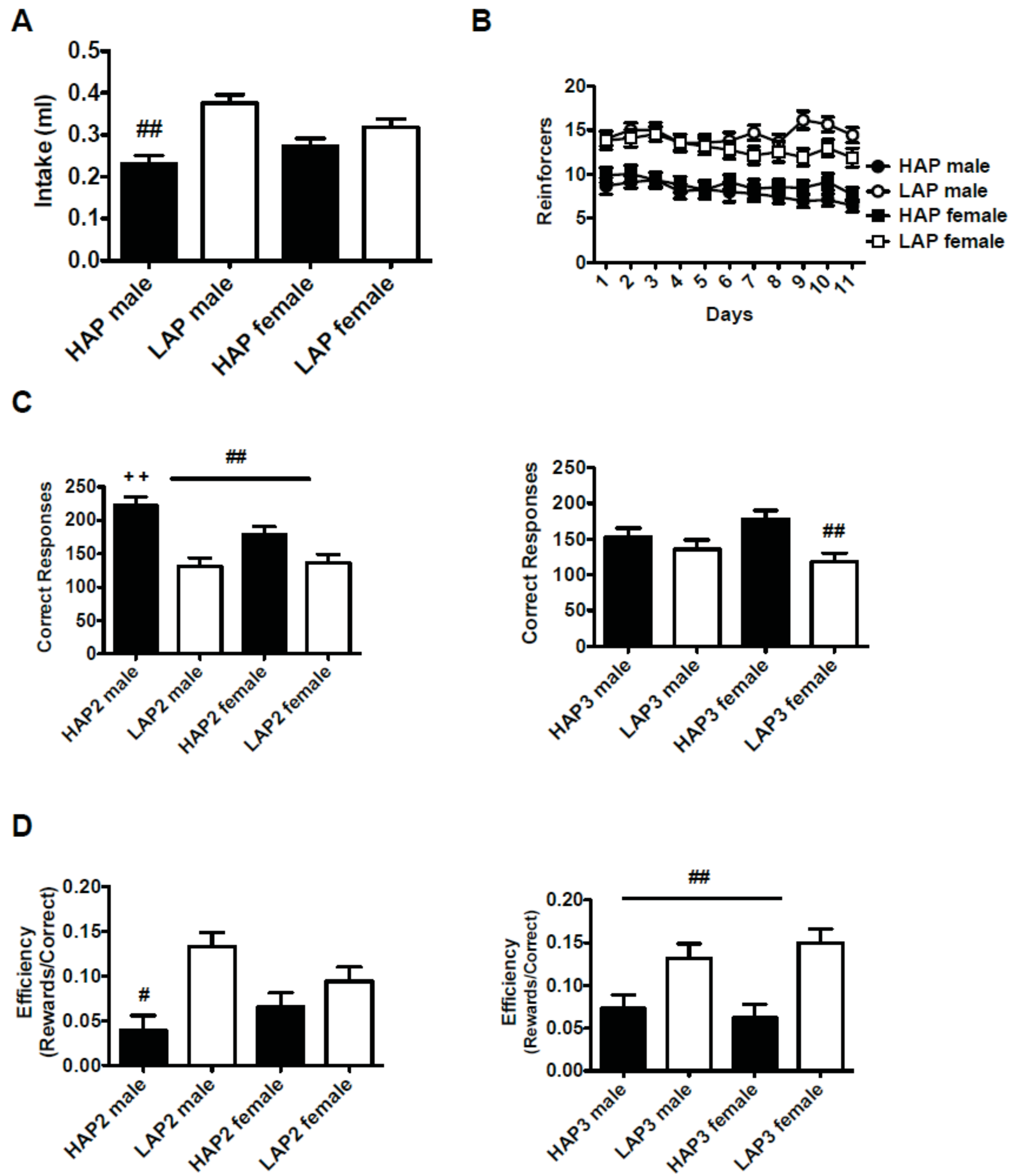


Figure 10 Sex differences during DRL-32s testing. (A) Overall intake in HAP and LAP males and females. (B) Reinforcers gained across days in HAP and LAP males and females. (C) Correct responses in each line, replicate and sex. (D) Efficiency (rewards/correct) in each line, replicate, and sex.

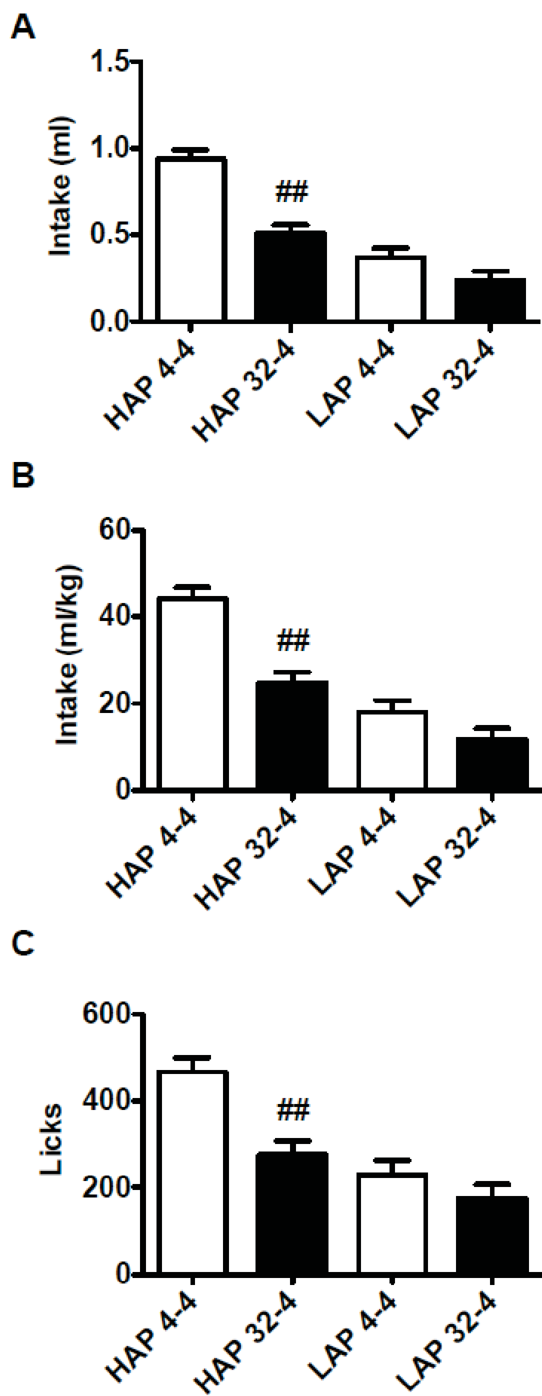


Figure 11 Post-shift day 1 intake and licks in HAP and LAP mice. (A) Intake (in ml) in HAP and LAP mice. (B) Intake (in ml/kg) in HAP and LAP mice (C) Licks in HAP and LAP mice.

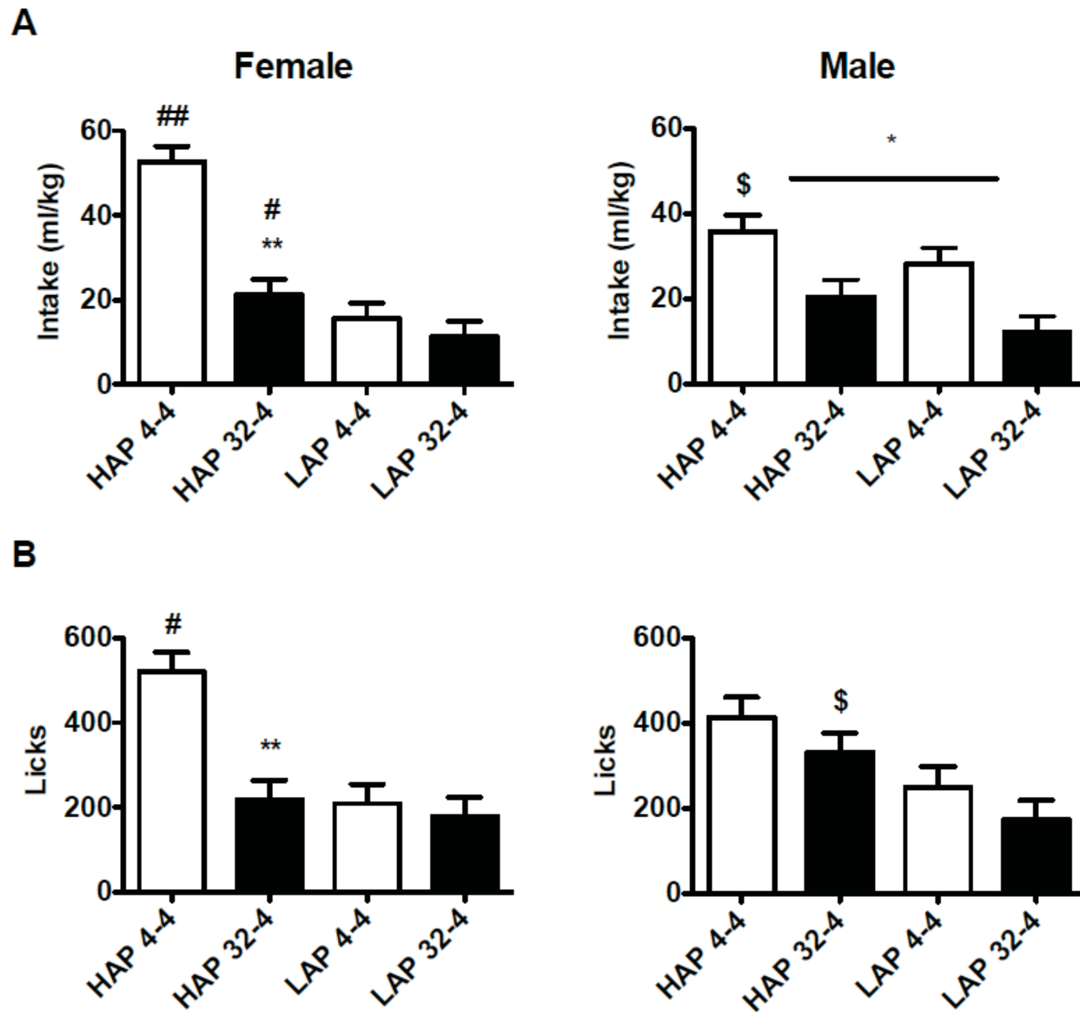


Figure 12 Sex difference in post-shift day 1 intake and licks in HAP and LAP mice. (A) Intake (ml/kg) in each line, group, and sex. (B) Licks in each line, group, and sex.

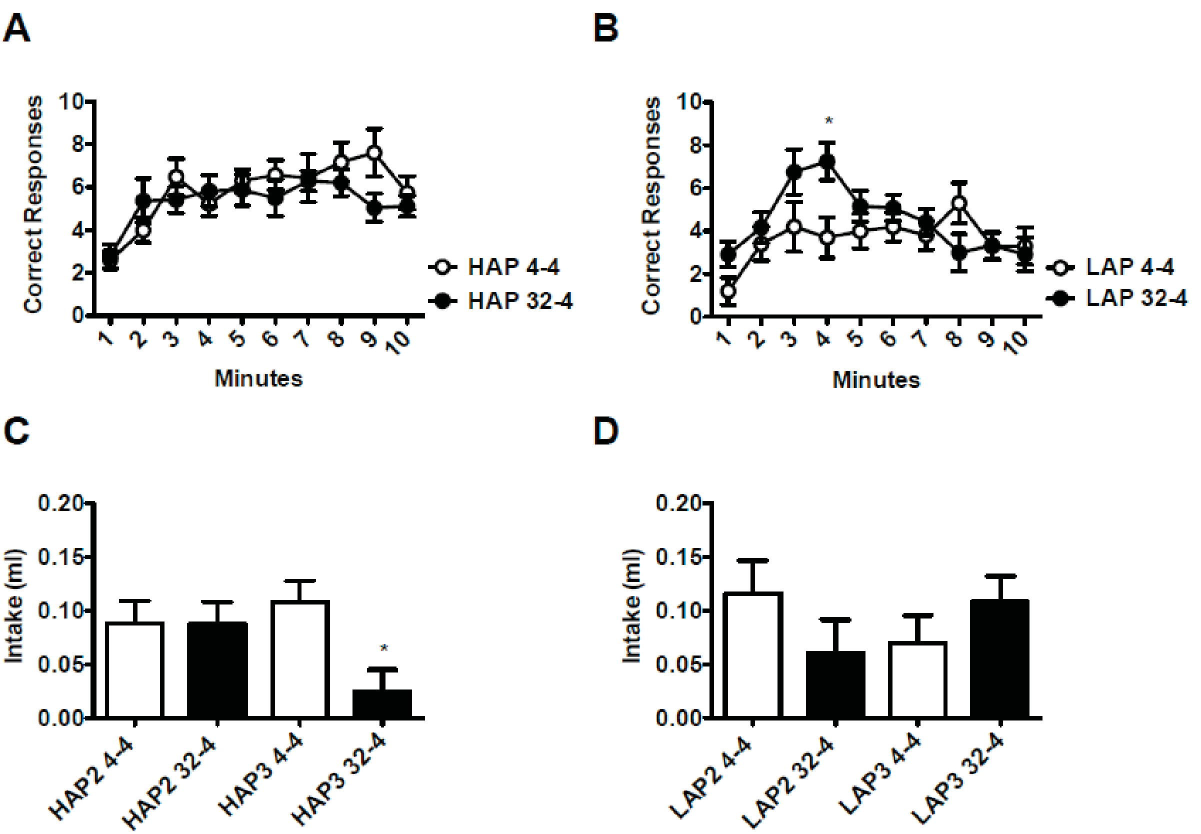


Figure 13 SNC effect on DRL test day. (A) Correct responding in HAP 32-4 and 4-4 groups. (B) Correct responding in LAP male 32-4 and 4-4 groups. (C) Intake during DRL testing by 32-4 and 4-4 groups in each HAP replicate. (D) Intake during DRL testing by 32-4 and 4-4 groups in each LAP replicate.

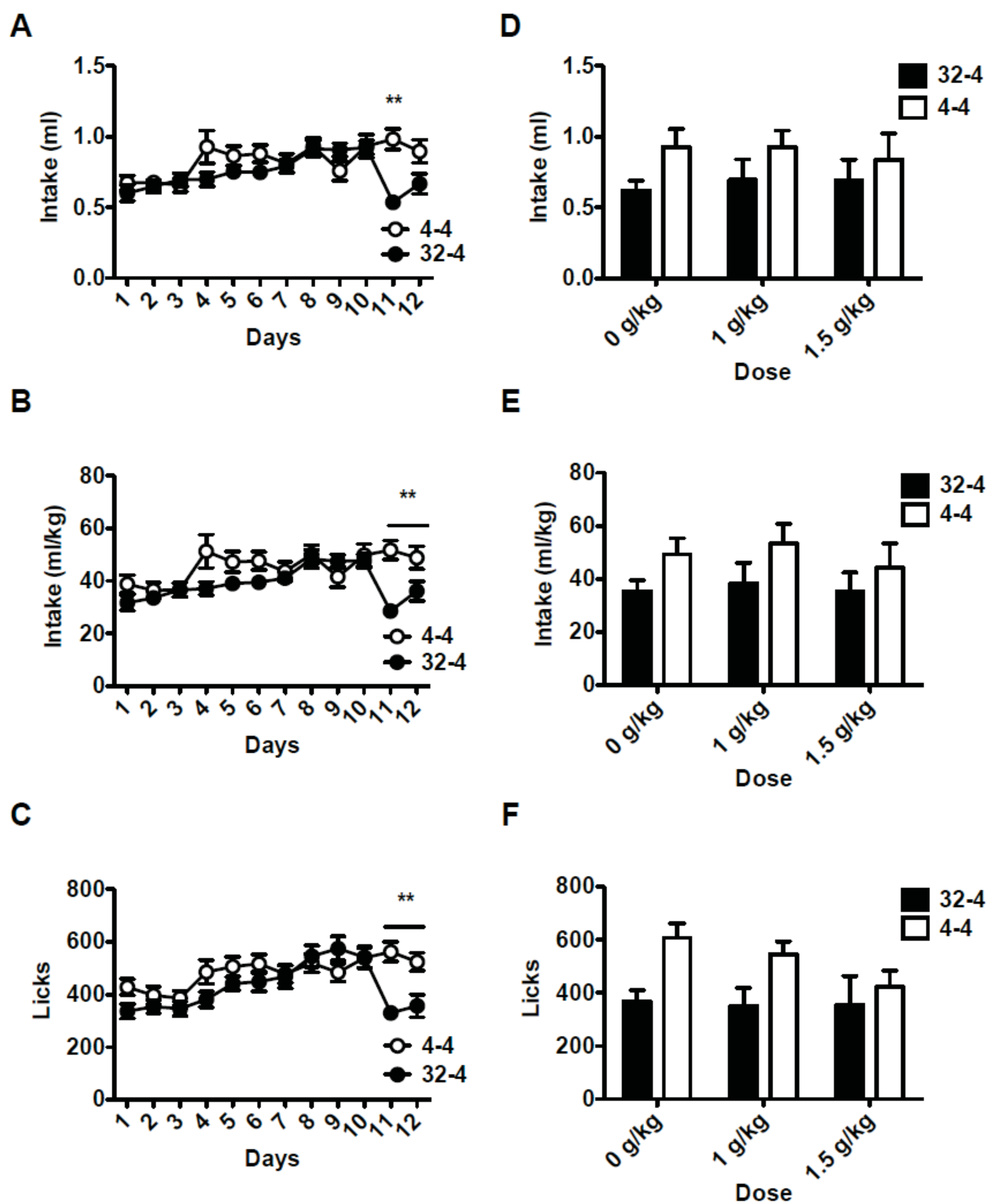


Figure 14 Pre-shift and post-shift intake and licks in cHAP mice, and ethanol test day 1 data. (A) Pre-shift and post-shift intake (ml). (B) Pre-shift and post-shift intake (ml/kg). (C) Pre-shift and post-shift licks. (D) Intake (ml) on ethanol test day 1. (E) Intake (ml/kg) on ethanol test day 1 (F) Licks on ethanol test day 1.

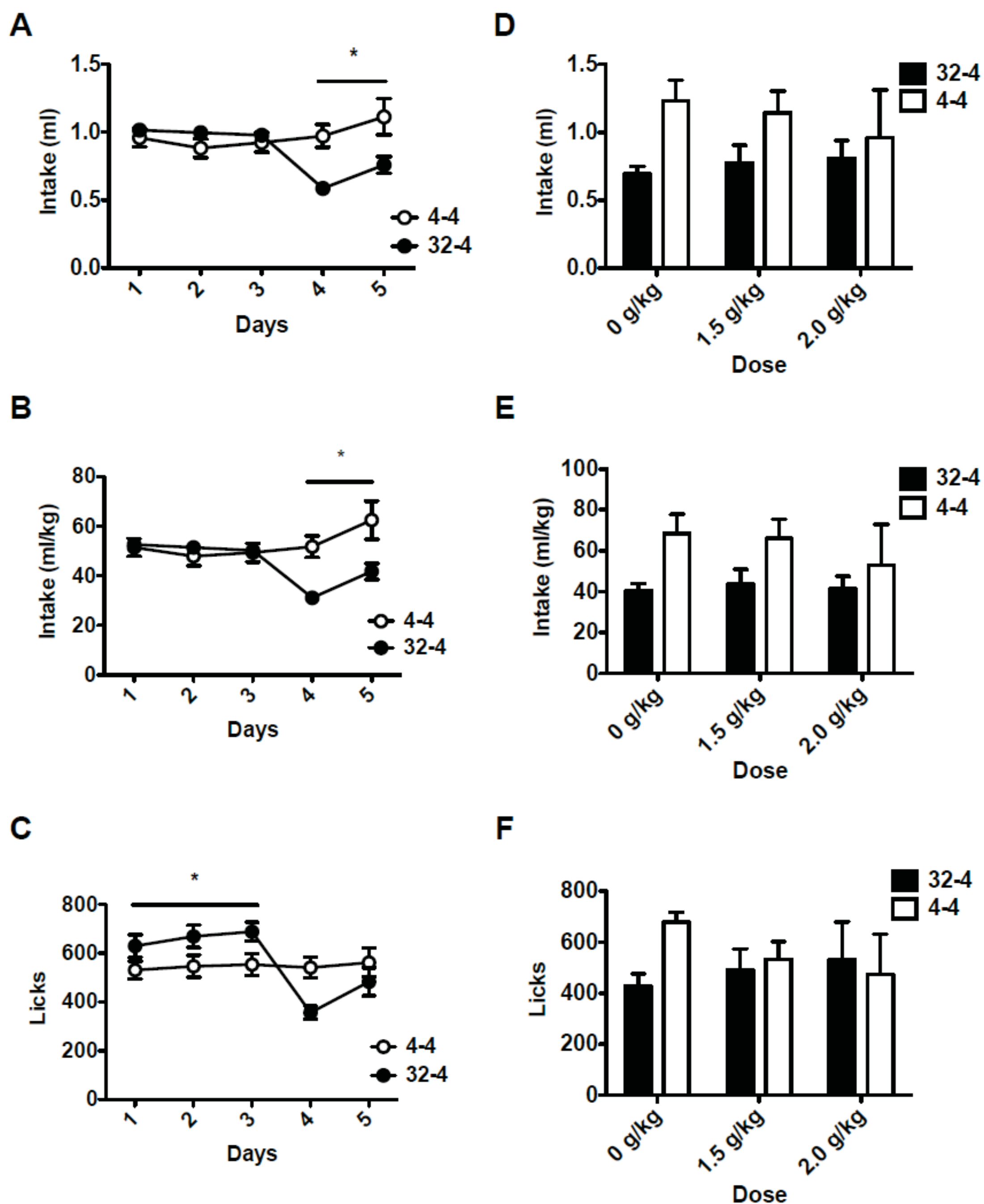


Figure 15 Pre-shift and post-shift intake and licks in cHAP mice during shift cycle 2, and ethanol test day 2 data. (A) Pre-shift and post-shift intake (ml). (B) Pre-shift and post-shift intake (ml/kg) (C) Pre-shift and post-shift licks. (D) Intake (ml) on ethanol test day 2. (E) Intake (ml/kg) on ethanol test day 2. (F) Licks on ethanol test day 2.

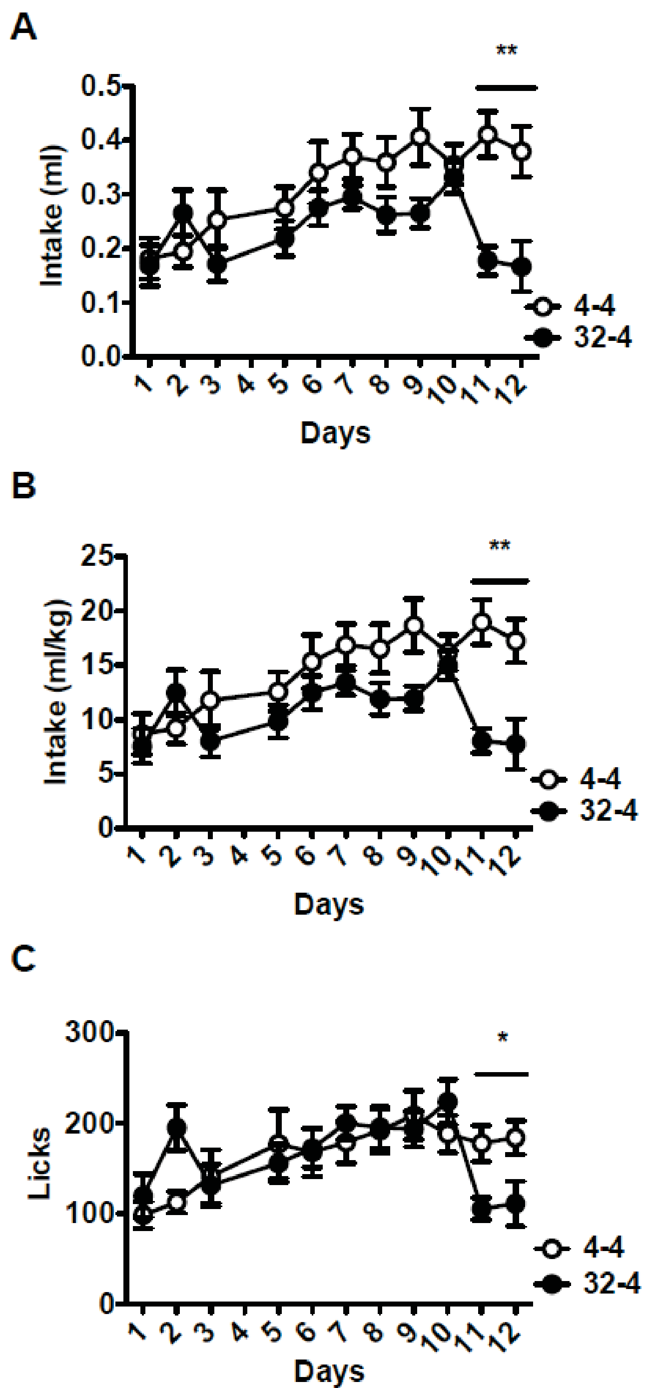


Figure 16 Pre-shift and post-shift intake in cHAP mice. (A) Pre-shift and post-shift intake (ml). (B) Pre-shift and post-shift intake (ml/kg). (C) Pre-shift and post-shift licks.

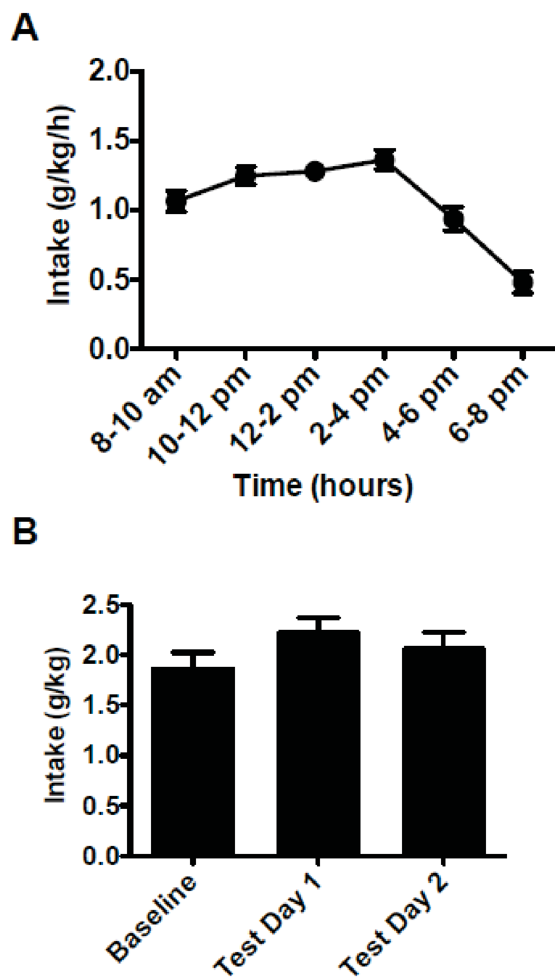


Figure 17 Ethanol intake during 12-hour acquisition and the 1-hour baseline and test sessions. (A) Ethanol intake (g/kg/hour) for the first 12 hours of ethanol access. (B) Total ethanol intake (g/kg) during the baseline and ethanol test sessions.

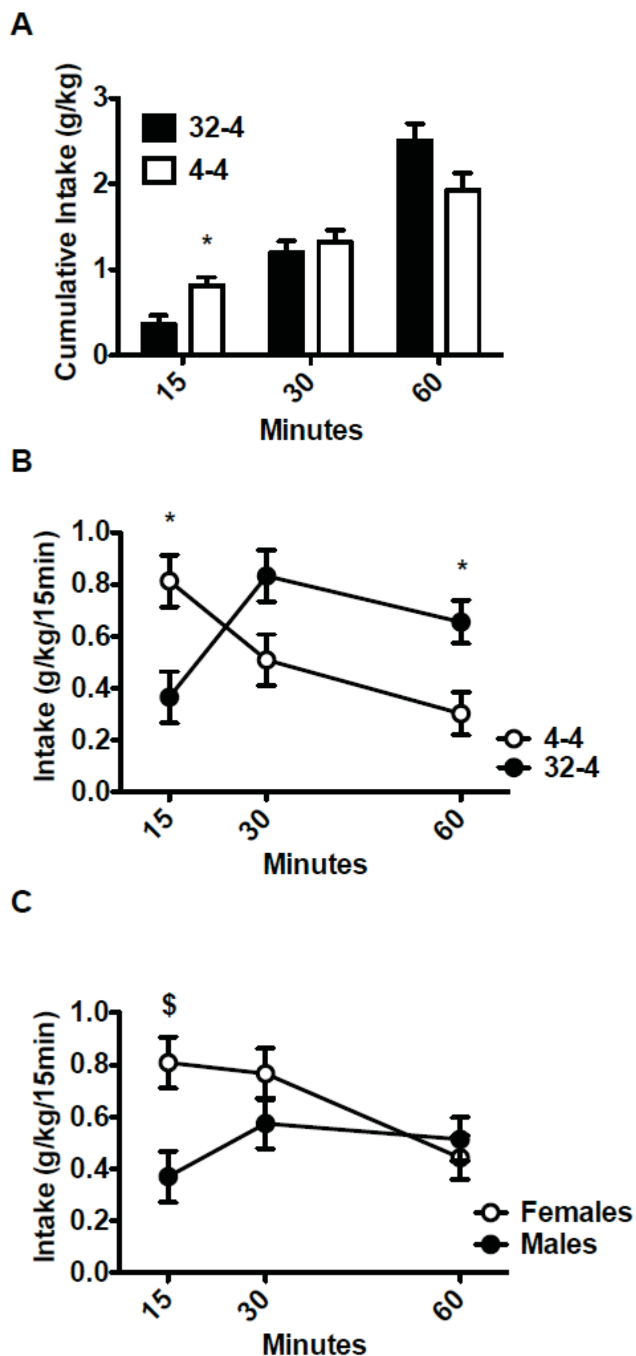


Figure 18 Ethanol intake during test session 1, which took place immediately following post-shift day 1. (A) Cumulative intake (g/kg) in the 4-4 and 32-4 groups, therefore the graph depicts total ethanol at 15, 30, and 60 minute points in each group. (B) Rate of ethanol intake (g/kg/15 min) in the 4-4 and 32-4 groups, therefore the graph depicts the rate of ethanol consumption from 0-15 minutes, 15-30 minutes, and 30-60 minutes. (C) Rate of ethanol intake (g/kg/15 min) in the sexes, therefore the graph depicts the rate of ethanol consumption from 0-15 minutes, 15-30 minutes, and 30-60 minutes.

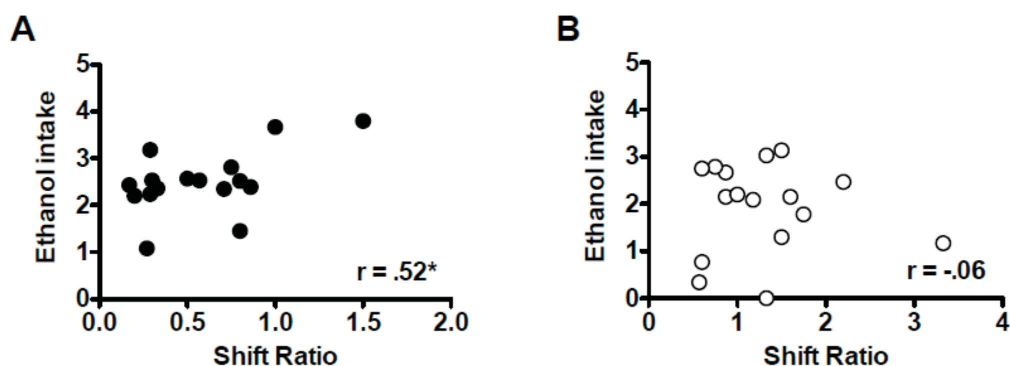


Figure 19 The correlation between shift ratios and ethanol intake (g/kg) during post-shift day 1 (A) Pearson correlation of shift ratios and ethanol intake in the 32-4 group. (B) Pearson correlation of shift ratios and ethanol intake in the 4-4 group.

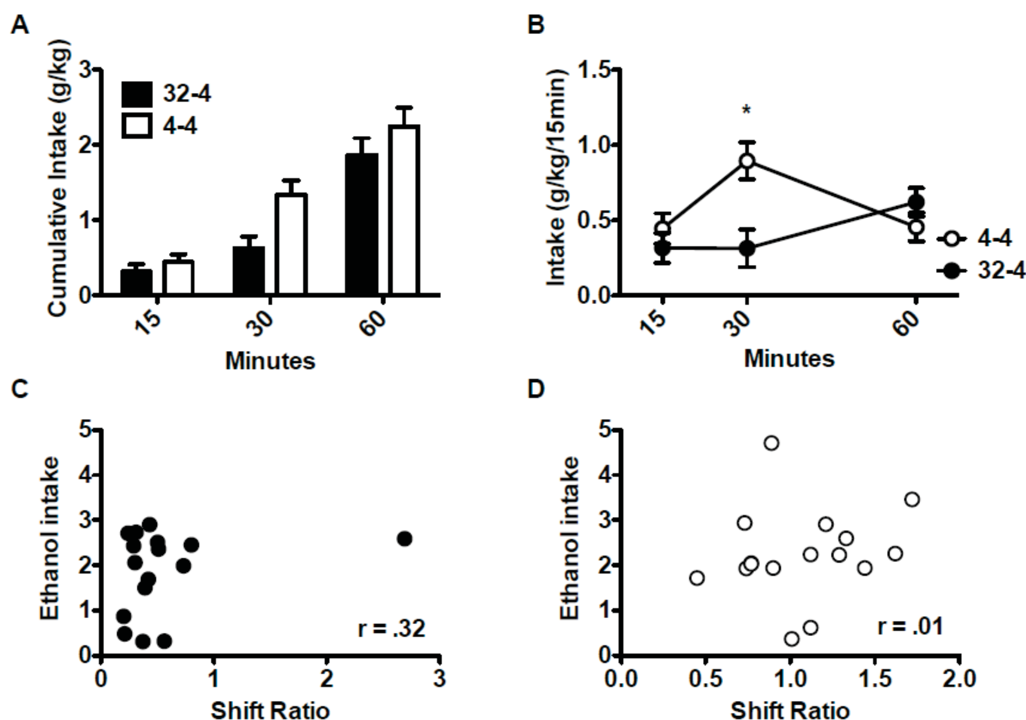


Figure 20 Ethanol intake during test session 2, which took place immediately following post-shift day 2. (A) Cumulative intake (g/kg) in the 4-4 and 32-4 groups, therefore the graph depicts total ethanol at 15, 30, and 60 minute points in each group. (B) Rate of ethanol intake (g/kg/15 min) in the 4-4 and 32-4 groups, therefore the graph depicts the rate of ethanol consumption from 0-15 minutes, 15-30 minutes, and 30-60 minutes. (C) Pearson correlation between shift ratios and ethanol intake (g/kg) in the 32-4 group. (D) Pearson correlation between shift ratios and ethanol intake (g/kg) in the 4-4 group.

# NOTES

For all figures and tables, the following notes apply. Asterisks (*) denote a Group (32-4, 4-4) differences. ** is significance at a level lower than .005 level, while * is significance at the .05 level or lower. Hashtags (#) indicate a Line (HAP, LAP) difference. # is significance at the .05 level or lower, ## is significance a level lower than .005. Plus signs (+) denote Replicate (Line 2, 3) differences. + is significance at the .05 level or lower, ++ is significance at lower than the .005 level. Dollar signs ($) indicate a Sex (M, F) difference. $ is at the .05 level or lower, $$ is lower than the .005 level. Unless indicated, all figures are collapsed across replicate.

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# REFERENCES

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# VITA

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## Education

*Doctor of Philosophy, Addiction Neuroscience*

Indiana University Purdue University Indianapolis

Graduation: May 2014

*Master of Science, Psychobiology of Addictions*

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*Bachelor of Science, Neuroscience*

Furman University, Greenville, SC

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## Research Interests

My areas of interest focus on: Circadian and consummatory rhythms in conjunction with alcoholism; adaptations following ethanol consumption; the behavioral genetics of alcoholism and related endophenotypes, including rash action and affective reactivity.

<u>Research Experience</u>

*August 2009- Present*

Graduate Research Assistant, Dr. Nicholas Grahame

Psychobiology Lab, IUPUI, IN

*June 2006 – May 2009*

Undergraduate Research Assistant, Dr. Judith Grisel

Behavioral Neuroscience Lab, Furman University, SC

*June 2007 – August 2007*

Undergraduate Research Assistant, Dr. Rainer Spanagel

Psychopharmacology Lab, The Institute of Mental Health, Mannheim, Germany

<u>Awards</u>

Paul McKinley Award, IUPUI (2014)

NIAAA Training Grant Fellowship (2011-present)

NIAAA T32 Training Directors Meeting and Trainee Workshop Travel Award (2013)

Covance outstanding poster awardee at Indianapolis Local Society for Neuroscience Meeting (2012)

Research Society for Alcoholism Student Merit Travel Award (2011, 2012)

NIAAA T32 Training Directors Meeting and Trainee Workshop Travel Award (2011)

Reserve Officer Training Corps 4-Year Scholarship (2005- 2009)

Furman Advantage Recipient (undergraduate research award) (2007)

Furman Advantage Recipient (undergraduate research award) (2006)

Professional Affiliations

Research Society on Alcoholism (2010-present)

International Neural and Behavioral Genetics Society (2012- 2013)

Manuscripts

O'Tousa, D*, Warnock, K*, ***Matson, L***, Namjoshi, O, Van Linn, M, Tiruveedhula, V, Halcomb, M, Cook, J, Grahame, N, June, H (2013) *Triple monoamine uptake inhibitors demonstrate a pharmacologic association between excessive drinking and impulsivity in high-alcohol-preferring (HAP) mice.* Addiction Biology. PMID: 24118509

***Matson, L,*** Kasten, C, Boehm, S, Grahame, N (2013) *Selectively bred crossed High Alcohol Preferring mice drink to intoxication and develop functional tolerance, but not locomotor sensitization during free-choice ethanol access.* Alcoholism Clinical and Experimental Research. PMID: 23909817, PMCID: PMC3844084

***Matson, L,*** Liangpunsakul, S, Crabb, D, Buckingham, A, Ross, R, Halcomb, M and Grahame, N (2012) *Chronic free-choice drinking in crossed HAP (cHAP) mice leads to sustained blood ethanol levels and metabolic tolerance without evidence of liver damage.* Alcoholism Clinical and Experimental Research. PMID: 22757960

O'Tousa, D, ***Matson, L***, Grahame, N (2012) *Effects of Intoxicating Free-Choice Alcohol Consumption During Adolescence on Drinking and Impulsivity During Adulthood in Selectively Bred High Alcohol Preferring Mice.* Alcoholism Clinical and Experimental Research. PMID: 22725646

***Matson, L***. and Grahame, N. (2011) *Pharmacologically relevant intake during chronic, free-choice drinking rhythms in selectively bred high alcohol preferring mice.* Addiction Biology. PMID: 22126215

Oberlin B, Best C, ***Matson L***, Henderson A, Grahame N. (2010) *Derivation and characterization of replicate High- and Low-Alcohol Preferring Lines of mice and a high-drinking Crossed HAP line*. Behavior Genetics. PMID: 20853157

Poster Presentations

***Matson, L,*** Grahame, N (June 2013) *Investigation of affective reactivity to a negative incentive shift as a correlated response to selection for high alcohol preference.* Poster presentation at Research Society on Alcoholism in Orlando, FL.

***Matson, L****,* Liangpunsakul, S, Crabb, D, Buckingham, A, Ross, R, Halcomb, M and Grahame, N (June 2012) *Chronic Free-Choice Drinking in Crossed High Alcohol Preferring Mice Leads to Metabolic Tolerance and CYP2E1 Induction Without Evidence of Liver Damage.* Poster presentation at Research Society on Alcoholism in San Francisco, CA.

***Matson, L***, Halcomb, M, June, H, Grahame, N (June 2012) Manipulation of Pharmacologically Relevant Free-Choice Drinking in HAP1 Mice Using the Triple Monoamine Re-Uptake Inhibitor Amitifadine (DOV 21,947). Poster presentation at Research Society on Alcoholism in San Francisco, CA.

O'Tousa, D, Buckingham, A, ***Matson, L***, Huffman, E, Grahame, N (June 2012) Tolerance is Proportional to the Level of Alcohol Drinking in Crossed High Alcohol Preferring Mice Following 2, But Not 5 Weeks of Free Choice Drinking. Poster Presentation at Research Society on Alcoholism in San Francisco, CA.

***Matson, L***, Buckingham, A, Tombers, E, Grahame, N (May 2012) Selectively bred crossed High Alcohol Preferring (cHAP) mice demonstrate acute intoxication and develop functional tolerance during free-choice access to ethanol. Poster presentation at International Behavioral and Neural Genetics Conference in Boulder, CO.

***Matson, L,*** Halcomb, M, O'Tousa, D, Buckingham, A, Villalta, N, Grahame, N (September 2011) Pharmacologically Relevant Intake During Chronic, Free Choice Drinking Rhythms in Selectively Bred High Alcohol Preferring Mice. Presentation at the NIAAA T32 Training Directors Meeting and Trainee Workshop, Providence, RI.

***Matson, L,*** O'Tousa, D, Heighton, M, Villalta, N, Grahame N (June 2011) Drinking Rhythms in Alcohol Preferring Mice. Presentation at the Research Society on Alcoholism Meeting, Atlanta, GA.

O'Tousa, D, Villalta, N, ***Matson, L***, Grahame, N (June 2011) Adolescent and Adult Two-Bottle Choice Ethanol Drinking and Adult Impulsivity in Genetically Selected High Alcohol Preferring Mice. Presentation at the Research Society on Alcoholism Meeting, Atlanta, GA.

***Matson, L,*** Best, C, Oberlin, B and Grahame, N. (June 2010) *Selective breeding for High and Low Alcohol Preference replicate 3 mice and assessment of the correlated response of saccharin intake.* Presentation at the Research Society for Alcoholism, San Antonio, TX.

***Matson, L***, Usala J, Blaker, W, Grisel, J. (November 2009). *Visual detection of estrous status in mice.* Presentation at Society for Neuroscience, Washington D.C.

Usala, J, ***Matson, L,*** Porth, J, Allen S, Grisel J. (October 2006) *Sex and estrous-cycle dependent effects of $\alpha$-melanocyte stimulating hormone ($\alpha$MSH) on social behavior.* Presentation at the Faculty for Undergraduate Neuroscience Conference, Atlanta, GA

Teaching Experience

*January 2011-May 2011*

Online Instructor

Introduction to Psychology as a Biological Science

*August 2010-December 2010*

Lab instructor

Introduction to Psychology as a Biological Science

*January 2010-May 2010*

Teaching Assistant, Dr. Cristine Czachowski

Introduction to Psychology as a Biological Science, IUPUI

*August 2009 – December 2009*

Teaching Assistant, Dr. Bethany Neal-Beliveau

Introduction to Psychology as a Biological Science, IUPUI

<u>Relevant Endeavors</u>

Volunteer as an Audio Visual Aide at Research Society for Alcoholism (2010-2013)

Medical Services Corps Inactive Reserve Officer, 1LT US Army (2009-present)

Participant in Professional Skills Development Workshop (2013)

Participant in Grant Writing Workshop, IUPUI (2012)

Student Representative for Psychology Department External Review, IUPUI (2012)

Founding Member and Co-chair of Psychology Graduate Student Organization, IUPUI (2010-11)

Organized Brain Awareness Week activities at Arsenal Tech High School, Indianapolis, IN (February 2011)

Volunteer as an Aide at Fronto-Temporal Dementia International Conference (2010)

Civil Affairs Officer for the ROTC Paladin Battalion, Furman University (2008-2009)

President of Collaborative Neuroscience Society, Furman University (2008-2009)

Public Relations Chair of Collaborative Neuroscience Society, Furman University (2007-2008)