AN AUTOMATED ATTENTIONAL SET-SHIFTING TASK IN HAP, LAP, AND ALCOHOL-EXPOSED CHAP MICE

by

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For my family, whose love and support encouraged me to always pursue my goals.

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ABSTRACT

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Alcoholics often experience difficulties ceasing drinking, potentially related to excessive behavioral inflexibility that either precedes or results from high alcohol consumption. Components of the Wisconsin Card-Sorting Task (WCST) a type of Attentional Set-Shifting (AttSS) task measuring impairments in behavioral flexibility have been modified to measure similar constructs within animals. Previous work has shown impaired AttSS in abstinent alcoholics and nonalcoholic individuals with a family history of alcoholism, as well as in mice exposed to chronic-intermittent alcohol vapor (Gierski et al., 2013; Hu et al., 2015; Oscar-Berman et al., 2009). The aim of the current study was to assess whether selectively-bred High- vs. Low- Alcohol Preferring (HAP vs LAP) mice display behavioral inflexibility as measured by an operant AttSS task, and furthermore, whether a history of voluntary drinking in cross-bred HAP (cHAP) mice further increases inflexibility. Impairments in the AttSS task are assessed by evaluating the number of trials to reach criterion, as well as the number and types of errors committed during the second experimental phase. In Experiment 1, male and female HAP and LAP mice first learned to press one of two levers signaled by a visual cue, but random with respect to spatial orientation, for a 0.1% saccharin solution reward. The following experimental phase consisted of an egocentric discrimination, such that side (left or right) now signaled correct reinforcement and the location of the visual cue was irrelevant. In Experiment 2, prior to identical operant procedures as Experiment 1, male and female

cHAP mice were given free-choice access to 10% alcohol or water for seven weeks. Ethanol-exposed animals drank an average of 29.6 g/kg/day.

CHAPTER 1. INTRODUCTION

1.1 Attentional Set-Shifting & Alcoholism

The DSM-5 emphasizes drinking in spite of negative consequences as one of the critical aspects of problem drinking associated with alcohol use disorder (American Psychiatric Association, 2013). Compulsive drinking (i. e., the inability to cease drinking) may potentially be related to cognitive and behavioral inflexibility, and help explain why many individuals are unable to stop drinking. More research is needed to determine the role of behavioral inflexibility in the context of alcohol use disorder (AUD), as it is currently unknown whether drinking exacerbates neural mechanisms related to behavioral inflexibility, or if behavioral inflexibility may be a precursor to problem drinking.

While the exact mechanisms related to compulsive drinking are unknown, research has indicated several brain regions and neurotransmitters that may play crucial roles in both behavioral inflexibility and this type of alcohol consumption. The prefrontal cortex (PFC) is one brain region that is primarily responsible for a variety of executive functions (EF) that may be involved in mediating both behavioral inflexibility and compulsive drinking. Specifically, the medial PFC (mPFC) has been implicated in both impulsive and compulsive behaviors as well as behavioral flexibility (Floresco, Block, and Maric, 2008; Kroener et al., 2012). It is important to use behavioral research to further examine what is happening within the brain. Attentional set-shifting paradigms measure behavioral flexibility, as well as permit the study of numerous brain regions. Research has identified that specific aspects of these AttSS tasks recruit different brain regions. For example, reversal learning utilizes both the orbital frontal cortex and dorsal striatum, whereas set-shifting employs the mPFC, and dorsomedial striatum (DMS) (Birrell and Brown, 2000).

Additionally, research has demonstrated that different strategies utilize different circuitry. Dopaminergic input-output from the mPFC is imperative for the suppression of a previously acquired strategy as a reversal or shift is being initiated; whereas, after perseveration has ceased, maintenance of a novel strategy requires the DMS (Ragozzino, 2007). Bearing in mind these brain regions can also be important for behaviors related to substance use disorders, it is important to utilize animal models in behavioral tasks such as AttSS which recruit these regions (Hu, Morris, Carrasco, and Kroener, 2015). Doing so enables researchers to examine the potential relationship of these brain regions to behaviors associated with problematic alcohol use, with control and manipulations not feasible in human populations.

In humans, the Wisconsin Card Sorting Task (WCST) is utilized to measure impairments in EF related to behavioral flexibility. Deficits in this task are observed in a variety of disorders such as schizophrenia, depression, and addiction. Impairments in AttSS tasks such as the WCST are assessed by examining the number of trials to criterion as well as the number and types of errors committed when individuals are required to learn a new strategy. In general, impairments are indicated when individuals require more trials to reach criterion than those to whom they are being compared. Additionally, there are three specific types of errors that can be committed during the shift. Perseverative errors are committed when the individual utilizes the previously correct strategy instead of the new required strategy. Once the new strategy is being used at least 50% of the time, any perseverative error made is considered a regressive error. Never reinforced errors occur when an individual makes a choice that is incorrect according to the new strategy, and would also be incorrect if they were still following the old strategy. Thus, AttSS tasks allow researchers to differentiate between multiple types of impairments, as these tasks require the ability to

recognize a previously acquired strategy as being inappropriate, while simultaneously requiring the formation and maintenance of a new strategy (Floresco et al., 2008).

Research indicates that abstinent individuals with alcohol dependence (AD) show impairments on neuropsychological tasks geared towards attentional set-shifting processes including the WCST (Oscar-Berman et al., 2009). However, as alcohol toxicity is thought to negatively affect EF (Gierski et al., 2013), it is important to tease apart whether impairments are present prior to AD, or if AD induces these deficits in EF. Interestingly, research has revealed that adolescents with high family history of AD performed worse on the WCST compared to adolescents with a low family history of AD (Corral, Holguin, and Cadaveria, 2003). Considering the frontal cortex and EF continues to develop into early adulthood, research assessing adults with family histories of alcoholism for impairments in set shifting has also been conducted. Gierski et al. (2013) found that nonalcoholic adults with a family history (FH+) of AD performed worse on the WCST compared to nonalcoholic adults with a negative family history (FH-) of AD. Together these findings implicate a genetic component to performance on WCST, in that a family history of AD predicts poorer performance on an attentional set-shifting task.

1.2 Problems in the Attentional Set-Shifting Literature

There are a number of paradigms that have been developed to investigate behavioral inflexibility in animals, which require the intra- and extradimensional shifts necessary for reversal and attentional set-shift learning. Intradimensional shifts are often referred to as reversals, as the new strategy is still dependent on the stimulus necessary for the original attentional set. For example, if animals are required to first press the left lever following a nose poke, the intradimensional shift would require animals to then learn that the right lever is correct, whereas, extradimensional shifts frequently incorporate new stimuli. With the previous example, an

extradimensional shift following the lever dependent attentional set could be a stimulus light indicating the correct lever choice rather than a specific egocentric lever. AttSS tasks use a variety of procedures including digging media, cross-mazes, and operant chambers (Floresco et al., 2008). Operant versions of the different attentional set-shifting paradigms, while relatively new, are ideal. This is because the non-operant tasks often only allow testing of relatively small animal cohorts due to the labor-intensive nature of the tasks and require novel complex stimuli during the extradimensional shift (Floresco et al., 2008). Conversely, light and lever stimuli in the operant procedure remain constant across the task, similar to WCST, and allow for an increased number of subjects to be tested at one time (Floresco et al., 2008). Furthermore, operant versions enable reversal or shift testing without requiring physical apparatus alterations.

Alcoholism is often characterized by chronic, excessive alcohol intake, which is difficult to model in a translational manner in rodents (Matson and Grahame, 2014). This type of alcohol consumption is challenging to reproduce in rodents as they do not typically sustain pharmacologically relevant voluntary alcohol intakes across the diurnal cycles (Matson and Grahame, 2014). For this reason, most of the current research on attentional set-shifting in both rats and mice examining the effect of alcohol has utilized chronic intermittent EtOH (CIE) vapor administration instead of voluntarily consumption which does not result in relevant pharmacological blood ethanol concentrations (BECs) (Hu et al., 2015; Kroener et al., 2012). CIE paradigms often incorporate an alcohol history ranging anywhere from several weeks to several months (Gilpin, Richardson, Lumeng, and Koob, 2008; Hu et al., 2015; Kroener et al., 2012; Gass et al., 2014). Following CIE, these paradigms can also include limited intermittent oral access. BECs achieved in the vapor portion of CIE typically surpass those reached during the intermittent oral access, although BECs for the voluntary consumption are not typically reported. While alcohol

models incorporating intermittent alcohol access such as drinking in the dark (DID) have been developed to control for the variation observed in 24-hour free choice access paradigms, this type of alcohol consumption better simulates human binge drinking rather than chronic alcohol intake (Matson and Grahame, 2013). Although some animal strains such as C57BL/6J (B6) drink pharmacologically relevant amounts of alcohol in DID, other selectively bred lines exist which display intakes and BEC's similar to chronic alcoholics throughout the active (dark) portion of their light cycle given 24-hour free alcohol access (Matson and Grahame, 2013). Previous research within this lab observed quinine resistant drinking, a model of compulsive drinking (Houck, in preparation; Hopf, F. W., and Lesscher, H. M. B., 2014), following as little as two weeks of 2BC. However, an extended alcohol history of seven weeks was employed in Experiment 2 in cHAPs in order to make contact with the longer alcohol histories utilized in CIE (e.g. Hu et al., 2015) in our highest drinking animals. Additionally, it is important to assess a paradigm incorporating self-administration that more accurately models chronic human alcohol consumption such as 2BC as opposed to CIE vapor or DID.

1.3 Selectively Bred Lines in the Attentional Set-Shifting Literature

Relatively little animal research has assessed genetic contributions to AttSS tasks and experiments that have used genetic approaches have not also examined alcohol in this context. The majority of research on AttSS in mice has focused on male B6 inbred strain (Bissonette, Powell, and Roesch 2013; Colacicco, Welzl, Lipp, and Wurbell 2002; Hu et al., 2015). There is a lack of research examining alcohol administration in combination with a genetic predisposition to drink. Due to this missing element in animal research, human complexities that may contribute to poorer performance on these tasks may not have been accurately modeled. To date, no AttSS research has utilized selectively bred lines such as the high alcohol preferring (HAP) and low alcohol preferring

(LAP) mice. The HAP, LAP, and the crossed HAP1 and HAP2 replicate (cHAP) selectively bred lines employed in this study were bred from heterogeneous HS/Ibg stock using bidirectional selection for differences in consumption of 10% v/v EtOH and water in a two-bottle choice (2BC) design (Grahame, 1999). Repeated selection of the progenitor line has resulted in lines that are consistent with respect to this selection phenotype as well as an increased propensity for impulsive behaviors in the HAPs (Matson and Grahame, 2013).

Although the HAP1 line no longer exists, each of the following replicate lines is drinking more than the preceding line (Grahame personal communication). Bidirectional selection has created high drinking lines such as the HAP3s, as well as the counterpart replicates line (i.e., LAP3s), both of which are readily available in our lab. This enables research examining genetic differences in relation to high or low alcohol preference to be examined. While the HAP2 and LAP2 replicate lines are also available, LAP2s have been notoriously difficult to motivate and thus, LAP3s are preferable for research that a requires more effort. The continued selective breeding of the crossed replicate lines has culminated within the extremely high, 25-g/kg/day average intake in the cHAP mice. Considering this, inbred lines such as the B6 have comparatively modest intakes and also lack the genetic variability present in the HAP and LAP lines. HAP and LAP mice are uniquely capable of examining questions surround FH+ and FH- behaviors. Moreover, these selectively bred strains typically reach peak BECs far greater than inbred strains such as B6s with cHAPs drinking considerable quantities which surpass their metabolic rate (Matson and Grahame, 2013). Furthermore, while most animal models show stable intakes over time, that does not reflect the long-term pattern of excessive intake achieved in alcoholics; cHAP mice mimic human alcohol consumption as they develop a behavioral tolerance to the ataxic effects of alcohol that is paralleled by an escalation in consumption (Matson and Grahame, 2014).

This tolerance bolsters the cHAP line as a rodent model for alcoholism as tolerance defined by either a need for an increased amount of a substance or a diminished effect of a drug with a consistent amount of a substance is part of the DSM-5 AUD criteria (American Psychiatric Association, 2013). Considering these phenotypes, selectively bred lines such as the HAP3, LAP3 and cHAP are ideal animal models of alcoholism for examining traits that may be associated with a genetic predisposition to drink or lack thereof, in addition to the possible effects of voluntary heavy alcohol consumption.

This study is the first of its kind attempting to assess whether genetic differences in alcohol intake are related to differences in attentional set-shifting. Research using HAPs and LAPs are essential to the AttSS literature, considering they provide a model of the family history of AUD that affects performance on WCST in humans. It is important to determine whether there are innate genetic differences between these selectively bred lines on a task that utilizes the PFC, as this may further explain a neurobiological susceptibility to compulsive drinking. The following experiments, utilizing a procedure adapted from Floresco et al. (2008), aim to address the missing components in the attentional set-shifting literature to better understand genetic influences and the effect a voluntary drinking history may have on performance in tasks exploring EF and its relationship to addiction.

1.4 Thesis Hypotheses

1. HAP3 relative to LAP3, mice will display impaired attentional set-shifting, indicated by a greater number of trials and errors committed to reaching criteria following the shift.

2. cHAPs exposed to an extended alcohol history will display impaired attentional set-shifting, indicated by a greater number of trials and errors committed to reaching criteria following the shift compared to alcohol-naive cHAPs.

CHAPTER 2. METHODS

2.1 Subjects

Experiment 1. A total of 18 HAP3 and 18 LAP3 mice (9 males and 9 females per line) were individually housed in standard Plexiglas cages with pine bedding and accommodated to a 12-hour reverse light cycle with lights on from 1900- 0700 daily for at least 7 days prior to the beginning of the operant training. To encourage operant responding and to increase motivation for the liquid reinforcer, mice were water restricted and received approximately one hour of water access each day immediately following their experimental session. Water restriction is necessary as LAPs do not display motivation to work for reinforcement unless heavily water deprived.

Experiment 2. A total of 48 cHAP mice (24 males and 24 females) were individually housed in standard Plexiglas cages with pine bedding and accommodated to a 12-hour reverse light cycle with lights on from 1900- 0700 daily for at least 7 days prior to the beginning of the alcohol history. A 2BC procedure was used in order to expose the animals to ethanol history. Animals in the ethanol group (EtOH) were given 24-hour access to one bottle of 10% EtOH (in a 50-mL graduated cylinder) and one bottle of water (25-mL graduated cylinder) for seven weeks. Naive animals were given two bottles of water (one in a 50-mL tube and another in a 25-mL tube) for the same period of time. Intakes were measured on the cage without disturbing the bottles every Monday, Wednesday, and Friday in order to determine the amount of ethanol and water consumed. On these days, after intakes were recorded the sides of the bottles were switched in order to deter animals from forming a side preference. All animals were weighed weekly. After cessation of 2BC mice were water restricted and received approximately one hour of water access each day

immediately following their experimental session, to encourage operant responding and to increase motivation for the liquid reinforcer as with the previous experiment.

2.2 Apparatus

Twelve operant chambers (Med Associates, St. Albans, VT) were used for the operant testing in these experiments. Each chamber measured 21.6 x 19.7 x 12.7 cm and was housed in a light- and sound-attenuating box. The operant boxes were equipped with green lights positioned above levers located to the left and right side of the sipper-tube opening. Additionally, a nose-poke hole and accompanying green light was positioned above the sipper tube. The 10mL sipper-tube containing 0.1% saccharin solution descended into the chamber's opening upon a correct response. Intake for each animal was measured on the sipper tubes before and after the session. Session duration, trials, nose-pokes, reinforcers, omissions, and correct and incorrect lever presses were recorded using MED-PC IV software (Med Associates, St. Albans, VT).

2.3 Attentional Set-Shifting Pre-Training – Experiments 1 & 2

Prior to attentional set-shift testing, mice went through five stages of operant pre-training responding for 0.1% saccharin solution. Stage 1 was a single training session that consisted of all center nose-pokes being reinforced on a fixed ratio 1 (FR1) schedule with 20 seconds of sipper access. A non-response-contingent reinforcement was also presented every 120 seconds. The center nose-poke light was on for the entire session. Both levers and lever lights were removed and were replaced with metal plates. For all the following stages, criteria to move to the next stage was the completion of 20 trials in a 45-minute session with a minimum of 0.2 mL reinforcer consumed. Stage 2 required a center nose-poke for reinforcement on an FR1 schedule resulting in sipper access for 10 seconds. The center nose-poke light was on for the entire session. Stage 3 was

the same as stage 2; however, following a nose poke, the nose-poke light turned off and after the 10 seconds of reinforcement access, a 4-second inter-trial-interval (ITI) was applied. In stage 4, as in the previous stage following the center nose-poke, the center light extinguished. In this stage following a nose-poke, the light above either the left or right lever was illuminated to signify that the lever was active. During stages 4 and 5, only one lever was present at a time. Reinforcement access was 8 and 5 seconds, respectively, during these sessions. Lever-side was counterbalanced between the left lever and the right lever across animals. For stage 5 the switch from previously available lever (i.e. if the right lever was previously available in stage 4 it was removed, and now only the left lever was made available) was made and the implementation of a 10 second nose-poke latency was added, wherein if the animal failed to nose-poke in within 10 seconds the nose-poke light turned off and the ITI was initiated prior to the nose-poke light was re-illuminated. During this time, the nose-poke was inactive and any nose-pokes made would fail to activate the lever light.

2.4 Attentional Set-Shifting Testing – Experiments 1 & 2

Attentional set-shift testing occurred in two concurrent phases with both levers present in the operant chambers. The first phase is the attentional set, wherein animals were required to always choose the active lever, indicated by the illumination of the lever stimulus light. As with pre-training, the operant chambers were dark and the illumination of the nose-poke hole indicated the availability to start a trial. If a nose poke was not made in 10 seconds, a nose-poke omission was scored and the ITI was initiated. After the mouse nose-poked to start the trial, the lever light for one lever was illuminated. Following a correct lever press, the lever light would turn off and the reinforcer was available (2s access to 0.1% saccharin), followed by an ITI before the nose-poke hole became active indicating the availability of a new trial. If the mouse responded on the

inactive lever (i.e. an incorrect response), the chamber reverted to the inter-trial state. Failure to respond on either lever within 10s resulted in the extinguishing of the lever light and the trial was recorded as an omission (omission trials were not included in the trials to criterion measure). The stimulus light was randomized so that each lever was active ~50% of the time. Criteria to move on to the next phase was 80% correct responses across the animal's session. If criterion was not met on the first day of training, subsequent sessions of visual-cue discrimination learning were administered. Once criteria for this phase was met, the set-shift took place.

For the shift to egocentric responding, mice were required to ignore the previously relevant stimulus light, which now only predicted the correct response on 50% of the trials. Instead, they needed to choose the lever opposite of their lever bias. Lever bias was determined by analysis of incorrect lever presses (i.e., pressing in the absence of the visual cue) during the attentional set (visual-cue discrimination) phase. If an animal chose the incorrect lever on 10% or more of the trials during the attentional set, that lever was considered the lever bias. For all animals that did not make incorrect lever presses or had incorrect lever presses below 10% of their trials, they were randomly assigned to either the left or right lever so that levers were counterbalanced across animals. Additionally, during the set-shift, an incorrect lever press resulted in a forced correction trial. Forced correction trials were different from free choice trials in that following a nose-poke, the light stimulus was not random and instead remained on the same side as where the light was active in the previous trial. Also, forced correction trials did not contain lever omissions. Forced correction trials continued in this manner until the correct lever was chosen, following which animals would return to free choice trials. Forced correction trials were not included in the trials to criterion measure. Criteria for the shift was met when an animal made 10 consecutive correct responses upon which testing was terminated. If animals failed to meet criteria in the first session,

subsequent sessions were administered on the following days for up to a total of three shift sessions.

2.5 Statistical Analysis

Data were analyzed using SPSS software (SPSS, Version 22, Chicago, IL) and graphed using Prism software (GraphPad Prism, v. 6.0, La Jolla, CA). Significance was set at an α–value of 0.05. To determine there were any group differences, a 2x2 factorial analysis of variance (ANOVA) was conducted. For Experiment 1, this was Line (HAP3 v. LAP3) x Sex (Male v. Female) and for Experiment 2, was Group (EtOH v. Naive) x Sex (Male v. Female). Fishers Exact Test of Independence was used to analyze whether any Line, Group, or Sex showed differential attrition, and independent sample t-test was applied to analyze potential drinking differences among the EtOH exposed animals.

2.6 Error Analysis

For the attentional set-shift, there are three types of errors that can be committed: Perseverative, Regressive, Never Reinforced. Perseverative errors were made when the mouse choose the lever under the active cue light when this lever was opposite the assigned correct egocentric lever. All consecutive trials in which a perseverative error could be committed were assessed in blocks of eight trials. After making fewer than five perseverative errors in a block of eight trials, the following errors were considered regressive errors as the mouse was following an alternative strategy at least fifty percent of the time. Never Reinforced errors were scored when a mouse selected the lever opposite the correct response when the visual-cue stimulus was active above their assigned egocentric lever.

CHAPTER 3. RESULTS

3.1 Experiment 1 Findings

Six animals (3 HAPs, 2 females; 3 LAPs, 2 females) were removed from the study prior to testing during various pre-training stages for failing to consume 0.2mL of the reinforcer or failing to make at least 20 responses within a session. Thirty animals met criteria for the visual-cue discrimination. Five animals (1 female HAP, 3 female LAPs, 1 male LAP) failed to successfully complete the shift. The performance was highly variable with animals ranging from 38-160 trials and 2-41 errors to reach criterion. According to Fishers Exact Test of Independence analyses, there was no statistically significant deviation in attrition rate for Line (p = .3295) or Sex (p = .3295). The ANOVA revealed a difference between lines for the time it took to successfully complete the shift as indicated by the number of sessions required to meet criteria (F (1, 24) = 4.31, p = 0.05; Figure 1) with LAPs requiring more sessions. However, there was no difference between lines for the number of trials in the first session of the shift. There was a significant difference between the number of trials for animals reaching criteria and those not during this first day of the shift (F (1, 24) = 3.31, p = 0.003; Figure 2). Most importantly, contrary to the hypothesis there were no significant differences between lines for number of trials to criteria or number of errors committed (F (1, 24) = 1.30, p = 0.265; F (1, 24) = 2.73, p = 0.112; Figure 3 & Figure 4 respectively). However, males made more Perseverative errors than females (F (1, 24) = 5.80, p = 0.025; Figure 5). Males also required a greater number of total trials to reach criteria (F (1, 24) = 5.05, p = 0.035; Figure 6) driven by a significant difference in the number of Forced Correction (FC) Trials (F (1, 24) = 5.05, p = 0.035).

3.2 Experiment 2 Findings

EtOH animals drank an average of 29.6 g/kg/day. One animal (female EtOH) was removed from the study prior to testing due to failure to meet criteria during pre-training. Three EtOH animals (2 female) failed to complete the visual-cue discrimination phase of the task. Five female animals (2 EtOH, 3 Naive) failed to successfully complete the shift. This was statistically significant for Sex (p = .019). As with the previous experiment, performance was highly variable, with animals ranging from 18-361 trials and 2-50 errors to reach criterion. There was a significant difference in the amount males and females drank (F (1, 23) = -5.74, p = 0.000; Figure 7), as well as between the amount consumed among animals who completed the task and those who did not reach or complete the final phase of the task (F (1, 23) = -2.46, p = 0.02; Figure 8).

Contrary to the hypothesis, the EtOH animals displayed no deficits for this task based on number of trials to criteria or errors committed (F (1, 37) = 1.27, p = 0.266; F (1, 37) = .578, p = 0.425; Figure 9 & Figure 10 respectively). These animals also did not require more sessions to reach criteria during the shift. No sex differences were observed for trials to criteria or number of errors made (F (1, 37) =.476, p =0.489; F (1, 37) =.564, p =0.458; Figure 11 & Figure 12 respectively). It is possible that lack of responding is responsible for animals that were unable to complete the shift as the number of trials for those failing to reach criteria (mean=6.8) fell outside the 95% confidence interval of those completing the task (mean= 42.91, SD= 26.01).

CHAPTER 4. DISCUSSION

4.1 Discussion

As there were no differences between any experimental groups for factors such as trials to criterion, or total errors it is important to consider why expected differences were not observed. Furthermore, many of the differences observed were between the sexes, including differences in perseverative errors in Experiment 1 and in the average amount of ethanol consumed in Experiment 2.

Although these experiments failed to replicate findings prevalent in the human literature, there are a number of possibilities as to why the hypothesized genetic and alcohol-induced deficits were not observed. One potential factor of particular importance is the type of shift chosen for these experiments. The attentional set utilized here was a visual cue discrimination while the shift required an egocentric discrimination. Literature indicates that rats have an innate tendency to engage in a visual cue based strategy and thus stimulus cues may be more salient compared to spatial location; therefore, it has been suggested that attentional sets which first require an animal to ignore stimulus lights are more difficult to learn (Floresco et al., 2008). As this paradigm had not been previously used in mice or in this particular lab, the perceived "easier" shift was chosen to increase the likelihood that animals would be able to complete both phases of the task. However, it is possible that the chosen shift may not have been difficult enough to fully examine potential existing differences among these groups. Evidence supporting this conclusion comes from examining the performance of mice in these experiments and rats in the experiment from which this paradigm was adapted. Although animals in these tasks required slightly more trials to reach criterion (~96) compared to rats in Floresco et al. (2008) study (~80), mice in both Experiments 1

and 2 committed on average ten total errors (sum of perseverative, regressive, and never reinforced errors) whereas control rats committed on average 15-20 perseverative errors alone. Thus, the relatively low number of errors committed by mice in these experiments may indicate that the shift was not challenging enough to detect potential impairments in behavioral flexibility.

4.2 Genetic Influences on Attentional Set-Shifting

While Experiment 1 aimed to identify if genetic differences between selectively bred lines would demonstrate similar effects on attentional set-shifting tasks as in humans with and without a family history of alcoholism, impairments in the HAPs compared to the LAPs were not observed. HAPs and LAPs required similar numbers of trials to reach criterion with LAPs committing slightly more errors. Although not significant, LAPs committing more errors opposes the hypothesized line differences. Despite comparable overall trial numbers, the pattern in which the shift was completed varied between the lines with HAPs requiring fewer sessions to shift than LAPs. The reason for this difference is unknown as both lines completed a similar number of trials per minute during the first shift session. It is possible that the lack of differences observed here indicates that genetic differences in alcohol intake within these lines are not actually predictive of impairments in attentional set-shifting. However, as the WCST reportedly has relatively low heritability (Anokhin et al., 2010; Gierski et al., 2013), it is possible that another task which requires behavioral flexibility may be better suited to examine genetic differences in alcohol intake and the potential relationship of these differences to EF.

Interestingly, while FH+ individuals reported more lifetime past drunkenness, they reported lower amounts of max alcohol than FH- individuals (Gierski et al, 2013). Although the authors suggest this may be due to FH- individuals not as closely monitoring their alcohol consumption as closely as FH+, one potential issue of particular importance in alcohol research is

the accuracy of drinking histories. This is not an issue with animal research as there are not issues such as response bias, and the drinking history is strictly controlled. Therefore, animals traditionally lack discrepancies between reported intake and actual intake. Although there are drinking measures which display high levels of internal and external validity, studies of alcoholics are naturalistic and thus lack the control and precision of drinking histories achieved in animal research.

Another consideration to explain the differential results observed here is that the human literature may not be accurately modeled by this paradigm. A number of factors may have contributed to the deficits in individuals with a family history of alcoholism detected by Gierski and colleagues such as higher rates of major depressive episodes and lifetime anxiety disorders. HAP mice typically display impulsive behavior which is often observed in alcoholics and individuals with a family history of alcoholism (Matson and Grahame, 2013); in fact, individuals in the Gierski (2013) study also reported higher levels of impulsivity compare to the control group. However, these selectively bred lines were not created to model anxiety or depression symptoms and it is potentially a combination of these traits with a history of alcoholism that leads to the observed human results.

A number of factors outside of genetics can contribute to the prevalence of psychological disorders in alcoholics. Environmental factors associated with having alcoholic family members must be considered. Variables such as poor childhood nutrition and potentially volatile home environments are not present in the animal model used here. Although animal models allow for high levels of internal validity due to experimental manipulations, external validity can actually be reduced as a result of experimental rigor. Individuals with a family history of alcoholism were most likely exposed to different environments than individuals with no family history of

alcoholism. This differs from animals utilized here which regardless of family history were exposed to the same conditions as adolescents. These animals were not exposed to parents actively consuming alcohol, nor were they subjected to any stressful or traumatic experiences as adolescents. Environmental factors may be a key factor in the results observed in by Gierski et al., 2013 as the number of AD relatives had a significant negative effect on executive function performance, as did the increased likelihood of having a psychiatric disorder. Thus, incorporating manipulations attempting to cause depressive or anxious behaviors within these selectively bred lines or varying the environment between experimental groups may better imitate the population used in this particular human study. Alternatively, replication of findings reported by Gierski and colleagues with individuals who did not meet criteria for AUD or any other psychological disorders may help to clarify the disparity seen here between animal and human research.

4.3 The Alcohol Effect – Implications for Attentional Set-Shifting & Addiction

One possibility as to why this study failed to see deficits in the animals exposed to an extended alcohol history is the route of chronic alcohol administration that was used. Research has demonstrated that the schedule of chronic ethanol treatment is important for the following structural and neurodegenerative alterations that occur, where higher blood alcohol concentrations are deterministic of harm (Fadda and Rossetti 1998). Consequently, the time in which changes are observed is relative to how the drug was administered, with rapid changes appearing just four days later in paradigms using intra-gastric administration of high doses of ethanol at short intervals versus liquid diets which may require anywhere from three-six months of administration to see changes (Fadda and Rossetti 1998). CIE models typically produce BECs ranging from 175-225 mg/dl for the length of exposure (Hu et al., 2015; Kroner et al., 20213), comparable to BECs achieved by the 2BC administration in the selectively bred lines utilized here. Specifically,

previous research has shown that cHAPs drinking ~24g/kg/day reached between 250-275 mg/dl, meaning that peak BECs from this model were higher than those typically achieved in CIE models (Matson and Grahame, 2013). Considering higher blood alcohol concentrations appear to be critical for producing neurodegenerative changes it is likely that following a seven-week history with an average alcohol consumption of 29.6 g/kg/day structural changes could have occurred. Bearing in mind the high BECs achieved by this model, it is unlikely that differences in BEC would explain why this model compared to models using CIE failed to see the hypothesized deficits in the attentional set-shifting task.

Another consideration is that withdrawal reactions have also been associated with structural brain changes (Fadda and Rossetti 1998). As CIE utilizes multiple withdrawal periods it is possible that these events resulted in an additive effect to produce even greater structural changes than those that may occur with alcohol administration alone. Additionally, CIE may be more stressful and generally unpleasant compared to models such as 2BC. This stress could further exacerbate structural and behavioral changes resulting from alcohol administration and repeated withdrawal.

Furthermore, the way in which deficits on attentional set-shifting tasks are traditionally measured may not accurately evaluate deficits caused by high alcohol consumption in this model. The highest drinking animals may have been too impaired to complete the task, as the animals which did not reach or complete the final phase of the attentional set-shifting task drank significantly more than animals which successfully completed the task. Animals that fail to make it to, and through, the final phase of the task are not incorporated into the group analysis and could explain why there were no significant group differences observed between ethanol-drinking and naive animals.

Downstream effects on learning and memory may be the result of alcoholic neurodegeneration or alcohol inhibition of neurogenesis (Nixon, 2006). Cell proliferation in the hippocampus following binge alcohol exposure has been found to return to normal levels as soon as twenty-four hours after the last dose of alcohol was administered, hence compensatory or regenerative brain responses may be associated with both the return of cognitive function and different effects observed between various models and studies (Nixon, 2006). Here, the length of time between alcohol exposure to the final phase of this task ranged 38-43 days; it is important to consider then that potential recovery mechanisms could have affected performance. Kroner et al. 2008, observed synaptic plasticity changes in the ratio of NMDA/AMPA receptors following CIE exposure and while behavioral deficits on a maze attentional set-shifting task persisted up to a week after alcohol administration, the synaptic changes were no longer present. Performance for some animals, especially those minimally impaired, may improve as more time passes between the alcohol exposure and crucial phases of the task. There is evidence for rapid cognitive recovery (little as 5 weeks) for some neuropsychological deficits in male humans (Mann, Gunther, Stetter, and Ackermann, 1999), as well as recovery of cognitive flexibility in long-term abstinent alcoholics (Fein, Torres, Price, and Di Sclafani, 2006). More research is needed to examine the length of time that behavioral inflexibility and cognitive deficits resulting from alcohol exposure may persist in rodents, as well as what type of recovery mechanism may exist and the length of time surrounding their occurrence. Future research utilizing this operant procedure should examine alcohol exposure following pre-training and the initial attentional set. This manipulation could reduce potential recovery mechanisms if they exist by minimizing the time between the alcohol history and attentional set-shift.

4.4 Sex Differences – Implications for Attentional Set-Shifting & Addiction

Whilst the hypothesized group differences were not observed in either experiment, there were a number of apparent sex effects. In the HAP and LAP mice, males required more trials and committed more perseverative errors compared to females. This could be the result of greater accuracy or may be related to reduced activity in females. Research on sex differences in attentional set-shifting tasks in both rodents and humans is limited and in what research is available, there are conflicting results. In animals, it is possible that contradictory findings stem from the use of different animal lines (Bissonette et al., 2012). The integration of both sexes may also lead to differential findings as until recently the majority of animal research did not use females. Rat studies have primarily used male Long Evans or Lister hooded rats (Birrell and Brown, 2000; Floresco et al., 2008; Gass et al., 2014) and mouse studies have utilized males from the C57BL/6 strain (Colacicco et al., 2002; Hu et al., 2015; Kroener et al., 2012). Subsequently, relatively few studies are available to examine potential sex differences on many tasks, including attentional set-shifting paradigms.

Females in Experiment 2 drank significantly more than males. Furthermore, 5/6 of the animals failing to complete the entire task were female. Potentially, females may suffer more impairment as a result of an extended alcohol history compared to males. However, as they also drank more, it is also possible that the higher BECs achieved in females resulted in greater impairment for a task that required behavioral flexibility.

Within human populations, there are a number of factors that may contribute to divergent results when examining sex as a factor for performance on a task such as the WCST. As with animal studies, human studies of alcoholics historically neglected to incorporate female participants. In tasks which require cognitive control differences between sexes at the behavioral

level are often not observed, this may be attributed to a lack of sensitivity of the measures being used to examine these differences (Plas, Crone, Wildenberg, Tranel, and Bechara, 2009). Therefore, when alcohol-dependent individuals have been examined using the WCST, it may not be surprising that sex differences have not been observed (Plas et al., 2009). However, instances in which sex differences in the WCST have been significant are restricted to highly specific populations such as adolescents and schizophrenics, with male schizophrenics being more impaired than females (Seidmann et al., 1997). While some research in adolescents has shown female impairment compare to male, changing biological factors are likely to be responsible. Research shows deficits associated with certain aspects of prefrontal cortical development and as females and males differ in rate of brain maturation the observed sex differences in performance are potentially more related to age rather than reflecting enduring sex differences (Anokhin et al., 2010). Animals used in these experiments were not exposed to conditions that are used model schizophrenia. Furthermore, animals began experimental conditions at an age that is considered adulthood making it unlikely that the sex differences observed were related to either schizophrenic phenotypes or maturation differences.

4.5 Conclusions & Future Directions

Future experiments utilizing a more difficult shift may elucidate potential differences between experimental conditions that the present research was not sensitive enough to detect. There are several factors to consider when examining why sex effects for the number of trials to criterion and error type were not observed in both experiments. The first is that these experiments utilized different lines due to the fact that LAPs do not voluntarily consume alcohol. Potentially, female LAPs improved performance to a greater extent than male LAPs. Another possibility is that alcohol exposure caused greater impairment in female cHAPs resulting in comparable

behavior to the male mice. Considering alcohol exposure may more strongly affect female performance it is imperative that future research continues to examine potential sex differences, for behavioral flexibility and for drinking.

Forthcoming research should also aim to investigate potential genetic differences in animals that have been selectively bred to drink alcohol. To date, relatively little research has examined genetic contributes to performance in attentional-set shifting tasks. Cognitive assessment via attentional set-shifting tasks has demonstrated strain-dependent differences between male 129/SVEV and C57BL/6J mice (Colacicco et al., 2002). Other research shows transgenic mice overexpressing catechol-O-methyltransferase (COMT) were impaired compared to mice containing a null COMT mutation (Papaleo et al., 2008). These studies examined cognitive impairment in relation to genetics but lacked any addiction research component. Moreover, studies examining the effects of alcohol on attentional set-shifting tasks have not utilized alcohol-preferring rats or high drinking selectively bred mice like those assessed here that can model human voluntary alcohol consumption.

Although the exact functioning of alcohol in the brain is still unknown, several neurotransmitters that are involved in various alcohol-induced changes in the brain are of particular interest in terms of attentional set-shifting tasks. Dopamine (DA), a key neurotransmitter implicated in reward function, could also be associated with cognitive changes in patients with AUD due to modifications of DA transmission in the PFC (Fadda and Rossetti, 1998). Researchers have also determined that both a specific D2-antagonist and a D4 agonist impaired mice in shifting from an egocentric response to a visual-cue strategy indicating that multiple DA receptors in the PFC are crucial for set-shifting and by extension may mediate behavioral flexibility (Floresco, Magyar, Ghods-Sharifi, Vexelman, and Tse, 2006). Considering that attentional set-shifting

paradigms such as the one used in this experiment recruit brain regions and neurotransmitters that are associated with compulsive behaviors, as well as alcohol use, this task is ideal for further research investigating behavioral inflexibility and its possible relationship to compulsive drinking.

CHAPTER 5. FIGURES

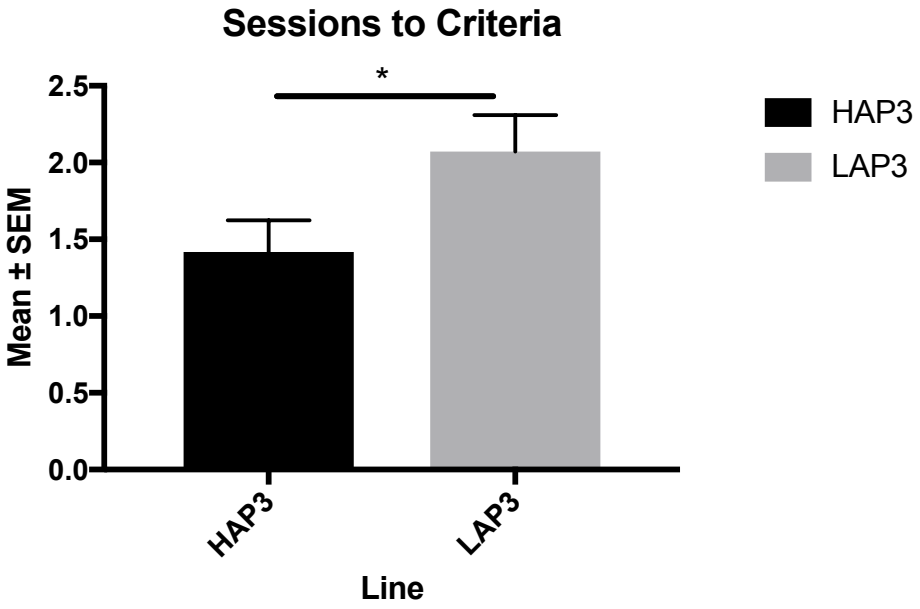

Figure 1. Sessions to Criteria by Line

Comparison of the mean ± SEM number of sessions completed reaching criteria for the shift in HAP3 and LAP3 (*p<.05). LAPs required more shift sessions than HAPs to reach shift criteria.

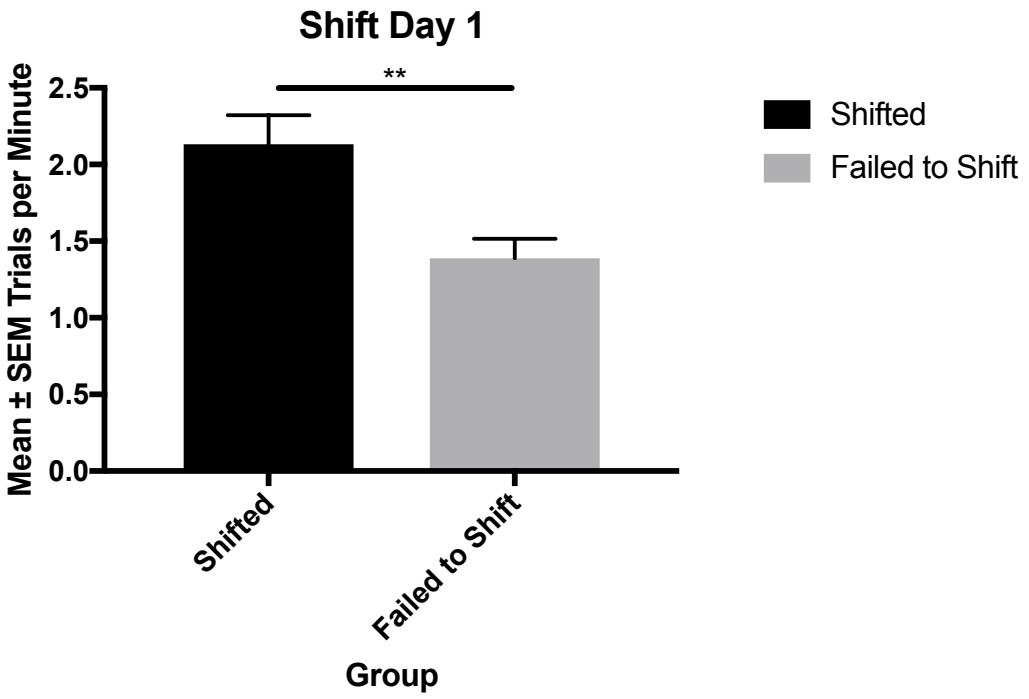

Figure 2. Trails per Minute

Experiment 1, comparison of the mean ± SEM number of trials per minute during the initial shift day, collapsed across Sex and Line (**p<.01). Animals that failed to complete the shift during the 1st shift session made fewer trials per minute than animals completing the shift.

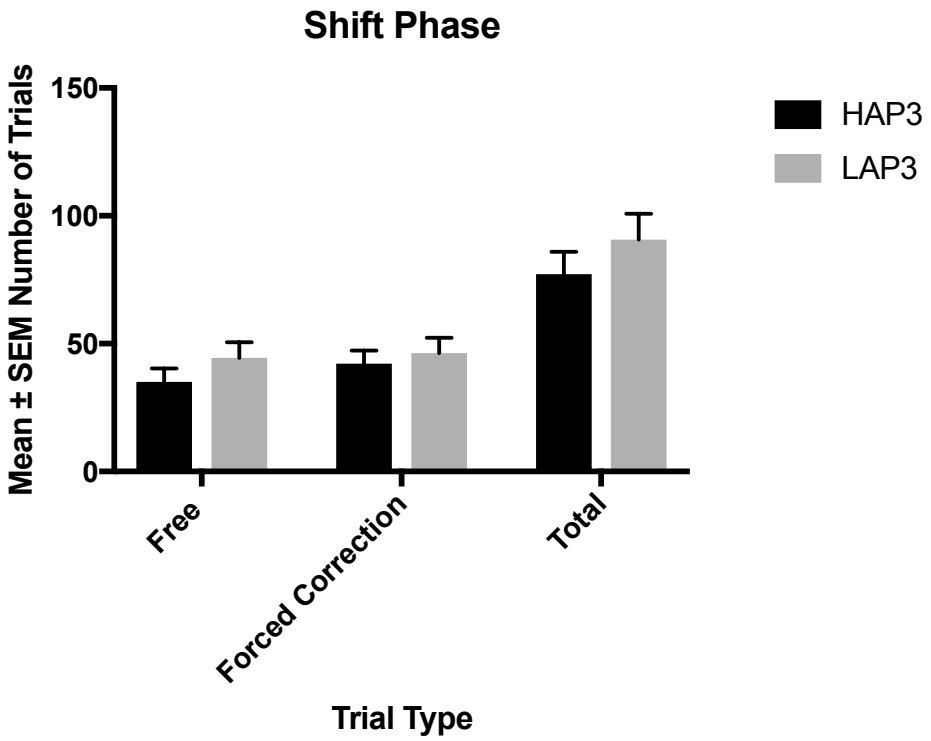

Figure 3. Number of trials be Line

Experiment 1, comparison of the mean ± SEM number of trials made during the shift line, collapsed across Sex. There were no differences between the lines for the number or types trials committed to reach shift criteria.

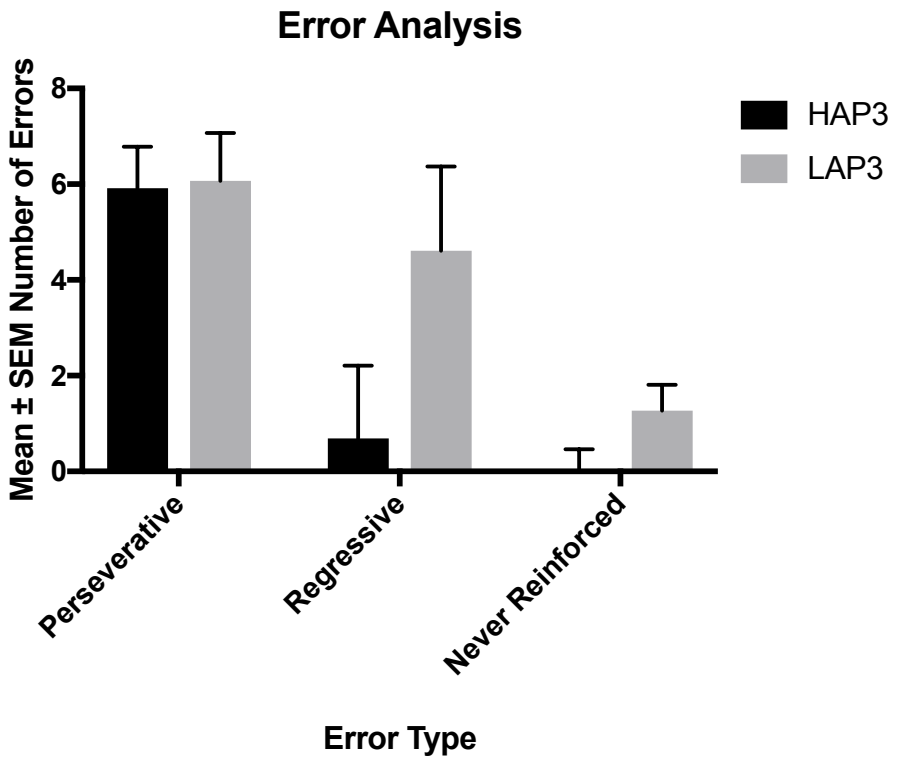

Figure 4. Error Analysis by Line

Experiment 1, comparison of the mean ± SEM number of errors made during the shift by Line, collapsed across Sex. There were no differences between the lines for the number or types of errors committed to reach shift criteria.

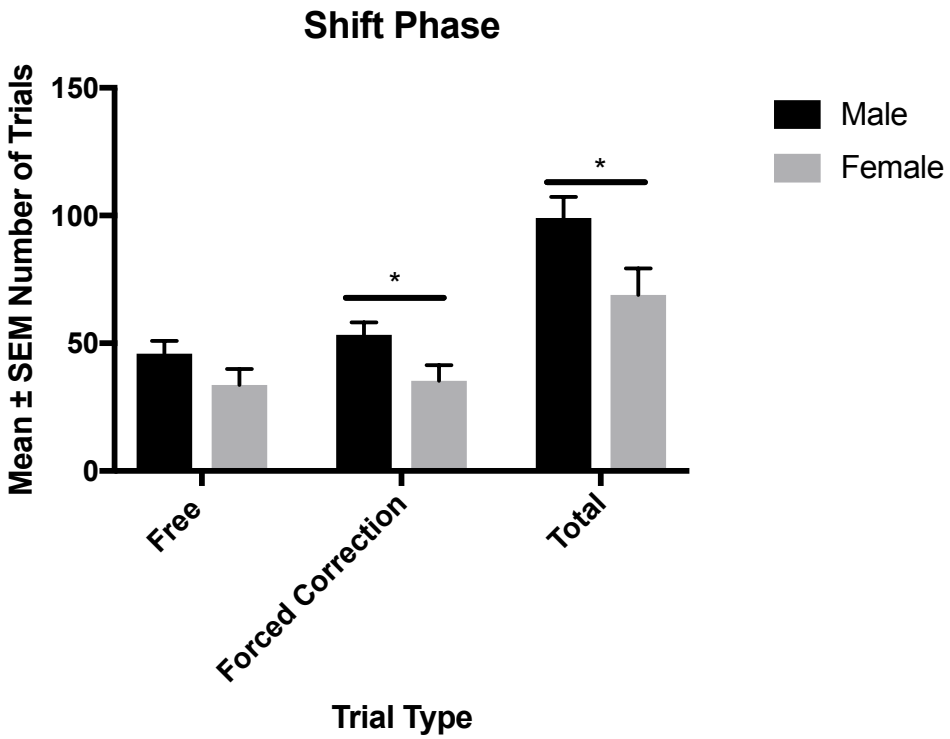

Figure 5. Trials by Sex

Experiment 1, comparison of the mean ± SEM number of trials made during the shift males and females, collapsed across Line (*p<.05). Males require more forced correction trials compared to females, resulting in a greater number of trials overall.

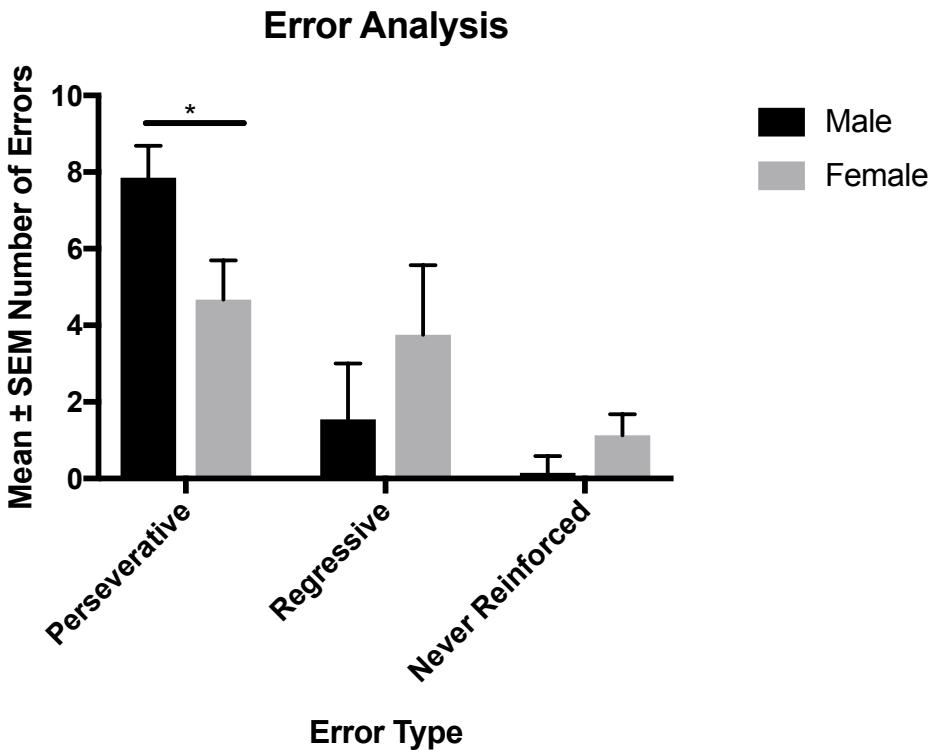

Figure 6. Error Analysis by Sex

Experiment 1, comparison of the mean ± SEM number of errors made during the shift by males and females, collapsed across Line (*p<.05). Males committed more Perseverative errors relative to females during the shift.

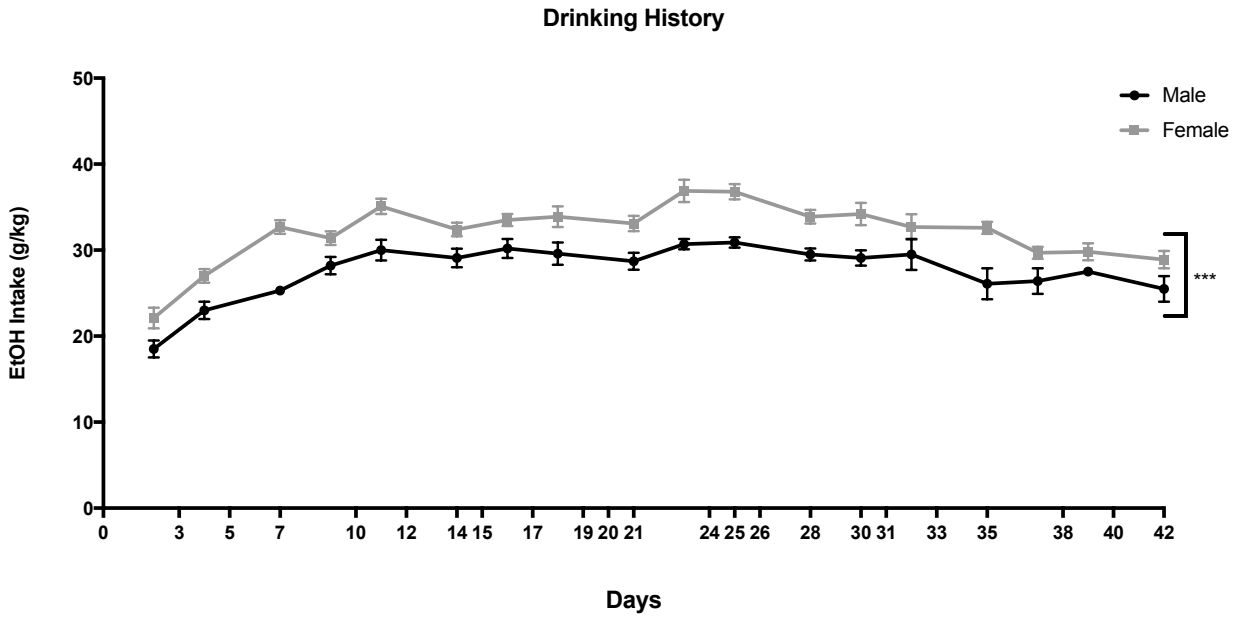

Figure 7. Drinking History

Experiment 2, Mean ± SEM g/kg/day ethanol intake for males (27.5g/kg/day) and females (31.75g/kg/day) over the seven-week drinking history (***p=0.000).

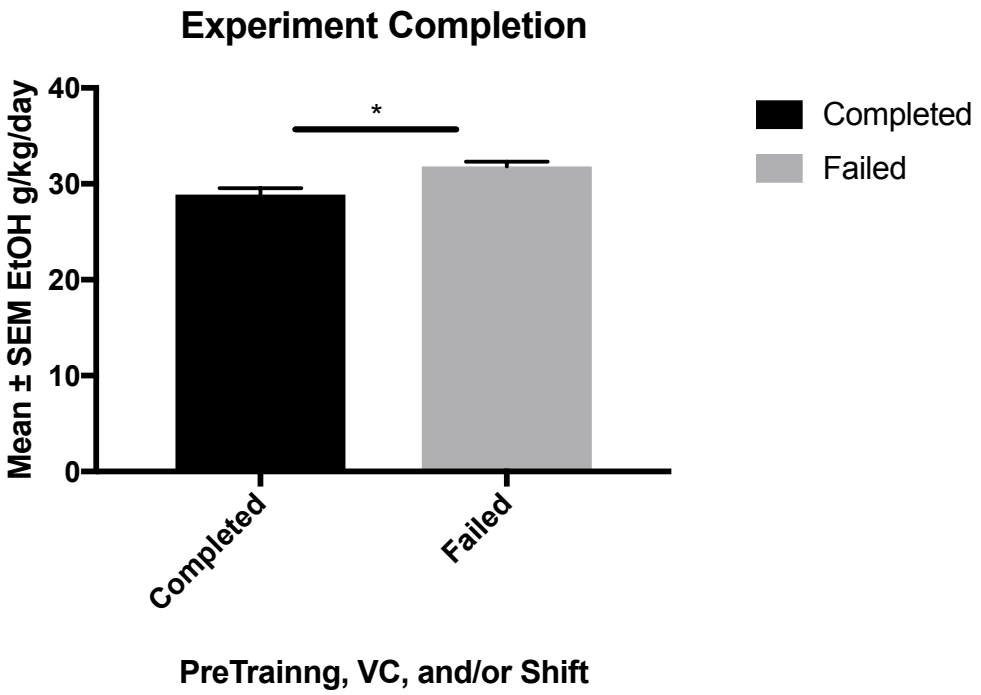

Figure 8. Alcohol Intake by Experiment Completion

Mean ± SEM g/kg/day ethanol intake by animals that completed all phases of the experiment versus those that failed any stage including pre-training, the attentional set (visual-cue discrimination) and shift collapsed across Line and Sex drinking history (*p<.05). Animals that failed to complete any of the stages of the experiment drank on average more than animals that successful completed all aspects of the experiment.

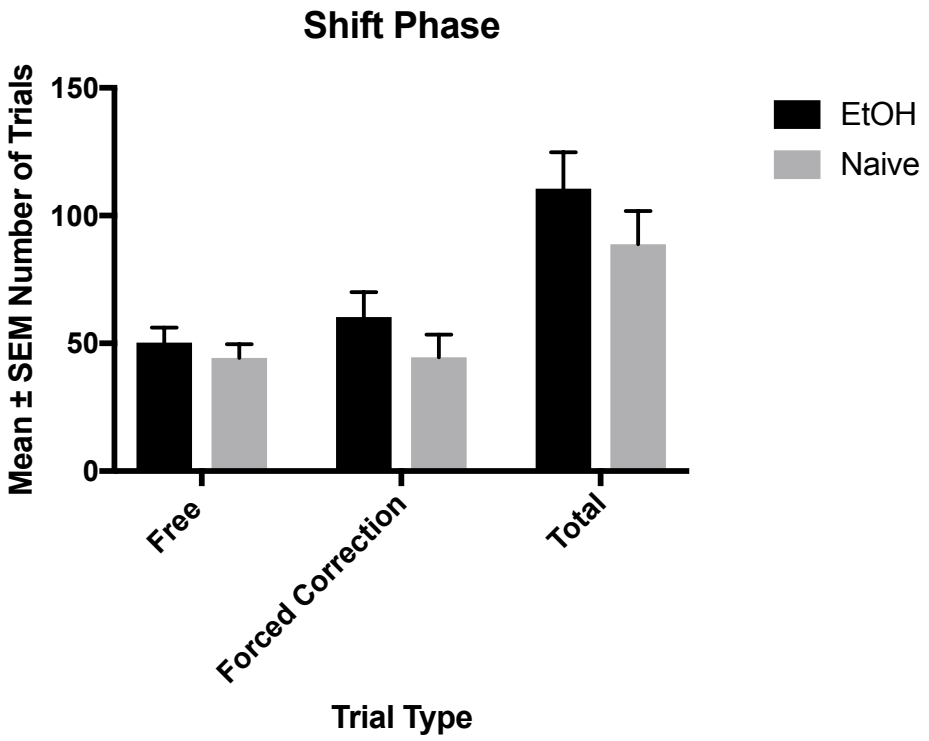

Figure 9. Number of Trials by Group

Experiment 2, comparison of the mean ± SEM number of trials during the shift by Group collapsed across Sex. There were no differences in the type or number of trials completed during the shift between alcohol-exposed and naïve animals.

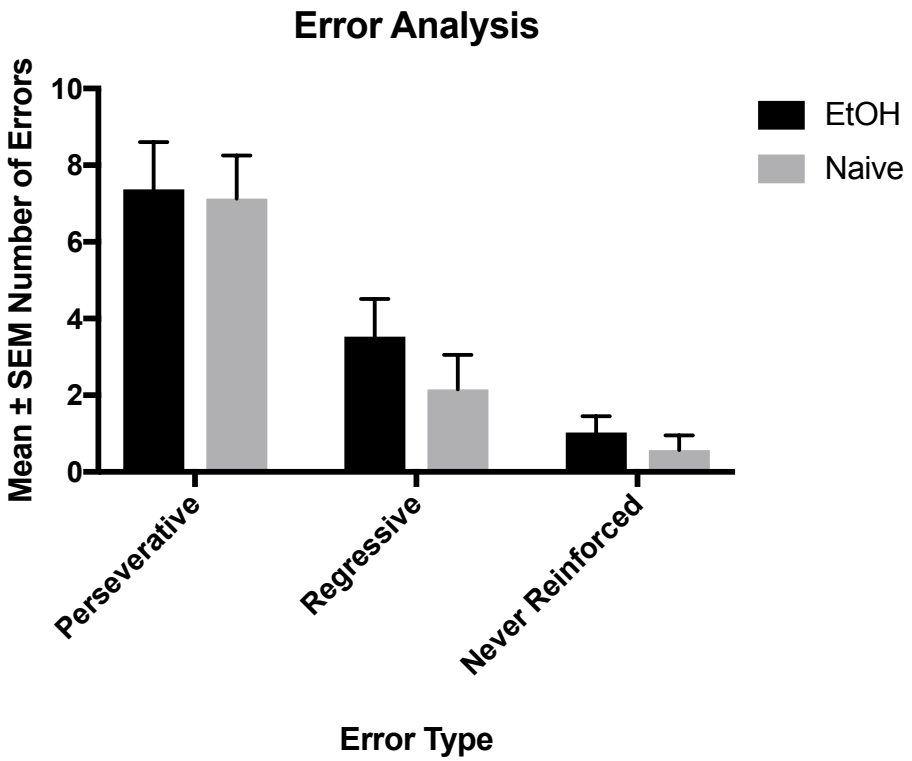

Figure 10. Error Analysis by Group

Experiment 2, comparison of the mean ± SEM number of errors made during the shift by Group, collapsed across Sex. There were no differences in the type or number of errors made during the shift between alcohol-exposed and naïve animals.

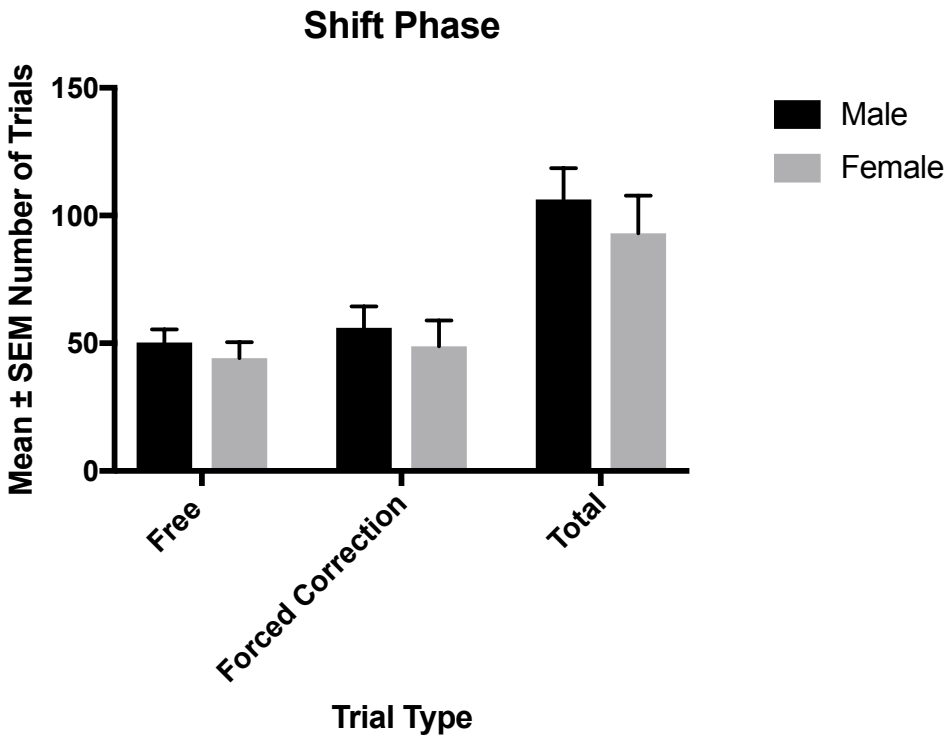

Figure 11. Number of Trials by Sex

Experiment 2, comparison of the mean ± SEM number of trails made during the shift by Sex, collapsed across Group. There were no differences in the type or number of trials completed during the shift between males and females.

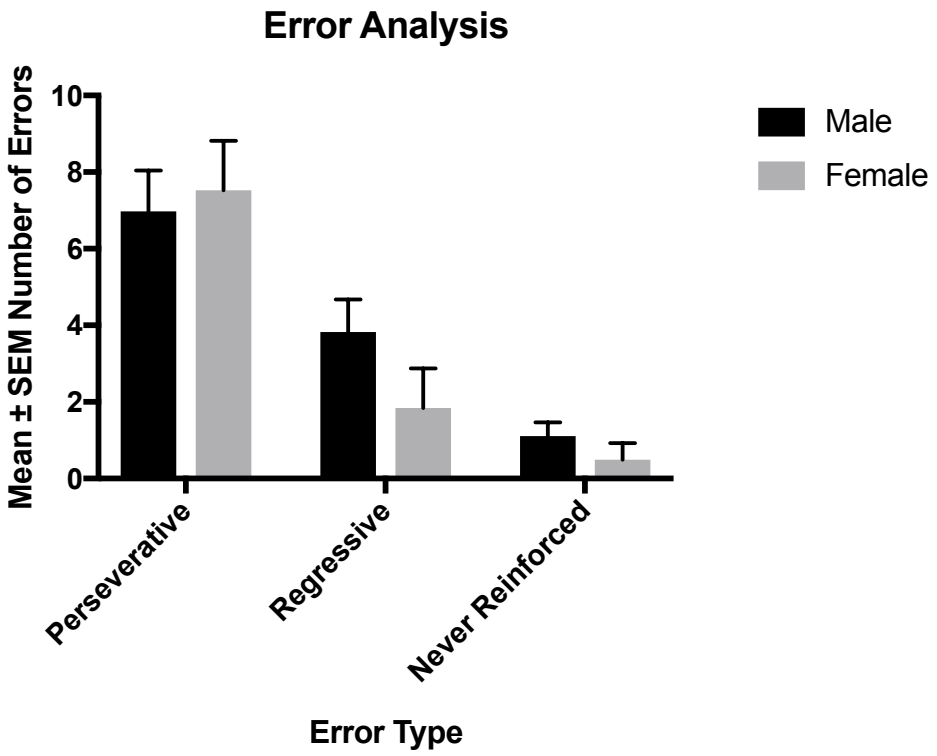

Figure 12. Error Analysis by Sex

Experiment 2, comparison of the mean ± SEM number of errors made during the shift by Sex, collapsed across Group. There were no differences in the type or number of errors made during the shift between males and females.

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