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For the degree of Master of Science

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DRINKING RHYTHMS IN ALCOHOL PREFERRING MICE

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This thesis is dedicated to my sisters, Helaine and Elise, two people I can always count on for love and friendship. I would also like to dedicate this thesis to Mahim for being my constant source of support; I love you with all of my heart. Finally, thank you to my parents and other family members who have always encouraged me to do what I am passionate about.

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ABSTRACT

Matson, Liana M. M.S., Purdue University, December 2011. Drinking Rhythms in Alcohol Preferring Mice. Major Professor: Nicholas Grahame.

Multiple lines of High Alcohol Preferring (HAP) mice were selectively bred for their intake of 10% ethanol (v/v) during 24-h daily access over a four-week period, with the highest drinking lines exhibiting intakes in excess of 20 g/kg/day. Drinking rhythms and corresponding blood ethanol concentrations (BEC) of the highest drinking HAP lines to those of the C57BL/6J (B6) inbred strain. Adult male and female crossed HAP (cHAP), HAP1 and B6 mice had free-choice access to 10% ethanol and water for 3 weeks prior to bi-hourly assessments of intake throughout the dark portion of a reverse 12:12 light dark cycle. In another cohort of cHAP mice, the same procedure was used to assess bi-hourly ethanol intake, and blood samples were taken across the day to look at the pattern of accumulation in these mice. Finally, considering the high level of intake by cHAP mice, we were interested in assessing whether metabolic and functional tolerance develop following chronic free-choice access, which were assessed using 2.0 and 1.75 g/kg challenge doses of 20% ethanol, respectively.

cHAP and HAP1 mice maintained an excessive level of intake throughout the dark portion of the cycle, accumulating mean BEC levels of 261.5 $\pm$ 18.09 and 217.9 $\pm$ 25.02 mg/dl at 7-8 hours following lights off, respectively. B6 mice drank comparatively

modestly, and did not accumulate high BEC levels (53.63 + 8.15 mg/dl). In the cHAP cohort, mean BECs were 112.47 $\pm$ 19.91 at 2 hours after lights off, 189.00 $\pm$ 27.40 at 6 hours after lights off, 193.80 $\pm$ 29.66 at 10 hours after lights off, and 89.68 $\pm$ 22.19 at 2 hours after lights on. Further, following 3 weeks of ethanol access, cHAP mice had a faster rate of ethanol metabolism and fewer hind slips than water-only exposed mice (*p*s $< .05$). In conclusion, the excessive free-choice drinking demonstrated by the HAP1 and cHAP lines, as well as the pattern of sustained high BECs in cHAP mice, challenge the notion that rodents will not reliably and voluntarily sustain ethanol intake at pharmacologically relevant levels. These results suggest that the highest drinking HAP lines may provide a unique opportunity for modeling the excessive intake that has been observed in alcohol-dependent individuals. Further, we observed that cHAP mice develop both metabolic and functional tolerance to the ataxic effects of ethanol following 3 weeks of free-choice access. Together, these findings support HAP mice as translational rodent model of alcoholism, and provide rationale for exploration of the predisposing factors for excessive consumption, as well as the development of physiological, behavioral, and toxicological outcomes following alcohol exposure.

CHAPTER 1 INTRODUCTION

1.1 Heavy Ethanol Consumption

Alcohol researchers have striven to model the human condition of alcoholism through developing animal models that attempt to understand some aspect or multiple aspects of the disorder. According to Cicero, an animal model should meet five criteria in order to be considered an effective model of alcoholism (Cicero, 1980). The outlined conditions include that (1) oral administration of alcohol to pharmacologically relevant blood ethanol concentration; (2) alcohol intake primarily for its pharmacological effects; (3) alcohol should be positively reinforcing; (4) chronic consumption of alcohol should produce both functional and metabolic tolerance and (5) removal of alcohol following chronic consumption should produce signs of withdrawal. These criteria are related to the present standards of the DSM-IV for defining alcohol abuse and dependence (APA, 1994). Researchers have met several of the criteria, but it has been difficult to create a model that successfully meets all of these criteria. Therefore, models exemplify particular facets of the disorder in an attempt to understand the underlying causes of and develop treatment for alcoholism in humans (Crabbe, Phillips, & Belknap, 2010). Ethanol consumption is a commonly studied aspect of the disorder, although the methods used and the resulting outcomes are incongruent between clinical, human laboratory, and

animal researchers. With regard to ethanol consumption, Leeman et al. (2010) put forth three phenotypes that need to be studied by both human and animal researchers, and pursued in a more compatible manner. These include the decision to drink or abstain, the amount consumed, and heavy drinking.

Heavy drinking can be defined in a number of ways, although heavy episodic drinking is considered four/five drinks on one occasion (SAMSHA, 2004). This definition is often used in combination with indicators such as "frequent" or "chronic" to further describe drinking patterns. Risky drinking constitutes reaching a blood alcohol level between .05-.08 gram %, while binge intake is considered reaching a BAC greater than .08 gram % or 80 mg/dl in about 2 hours (NIAAA, 2004). Human clinical research generally uses self-report to characterize heavy drinking. Although these studies do not constrain the pattern or level of drinking, they rely on survey methods to gather data. Self-report data can be problematic due to the potential for misreport, and it is often a coarse indicator of intake patterns (Sobell & Sobell, 2003). Clinical surveys also often use standard drink measurements, which are difficult to standardize across participants and do not align with either human laboratory or animal research measures (Bradley, Bush, McDonell, Malone, & Fihn, 1998; Devos-Comby & Lange, 2008). Human laboratory researchers generally assess drinking by using standardized measures of the quantity consumed, and usually obtain breath alcohol concentration (Musshoff, 2002; Sobell & Sobell, 2003). This is the preferred method of reporting because it can be measured in both humans and animals and provides a direct measure of consumption irrespective of confounding factors such as sex or weight (Sommers, 2005). Biomarkers are physiological endpoints that can be objectively measured to provide an indicator of

heavy intake, but they do not provide detailed information about the characteristics of consumption (Hannuksela, Liisanantti, Nissinen, & Savolainen, 2007).

Assessing the amount consumed during heavy drinking in human alcohol research studies is limited by constraints on the level of alcohol intake that is permissible. It is difficult to investigate chronic, unlimited alcohol intake in human laboratory studies, with the exception of being able to measure biomarkers to assess relative level and duration of intake (Sommers, 2005). A few early studies provide information for characterizing excessive, heavy drinking in alcohol-dependent individuals (Majchrowicz & Mendelson, 1970; Mello & Mendelson, 1970; Nathan, O'Brien, & Norton, 1971). These studies observed patterns of voluntary chronic intake, and found that alcoholics will self-administer up to 33 oz, or about 22-24 standard drinks, in a day. In addition, these studies demonstrate that this level of intake is observed when an individual goes on a "bender", during which a large quantity of alcohol is consumed for at least 2 days in a row (NIAAA, 2004). It is important to further characterize chronic, excessive intake as well as answer questions regarding individual susceptibility and the biological basis of heavy alcohol consumption. Due to the ethical constraints, it is presently difficult to look at this degree of intake in humans, and a deficit of knowledge exists regarding excessive, chronic ethanol consumption, or the "too often, too much" aspect of heavy drinking (Leeman, et al., 2010).

Vivian and colleagues (2001) developed a paradigm to assess chronic, excessive intake in primates. The paradigm is reflective of the human population from which only a small percentage ever heavily drinks, as about 35% of subjects acquire heavy drinking (Grant et al., 2008; Vivian, et al., 2001). In addition, patterns of intake similar to the early

human studies have been observed, notably periods of "spree" or "bender" drinking, suggesting it may be a highly translational model for learning about human excessive consumption. In certain situations, though, it may be beneficial to use a model that gathers information from an entire sample, and in a less time-consuming manner, as primate studies often last about 12 months. It may be more advantageous to use rodent models to obtain information regarding the etiology of heavy drinking. In particular, a long history of research in rodents is aimed at attempting to model human alcohol consumption.

Over the history of alcohol research with rodents, a variety of different procedures to study ethanol consumption have been developed (McBride & Li, 1998). Some of these procedures are problematic, principally because they violate the voluntary aspect of human consumption. Lynch (2010) notes that the psychoactive substances that are voluntarily consumed by humans are also usually the same as those voluntarily consumed by nonhuman animals. Models that use forced or induced intake may be difficult to interpret, because alcohol intake may occur for reasons other than for its positively reinforcing qualities.

Operant self-administration paradigms were developed that did not involve food or water deprivation or forced access to ethanol (Samson, Pfeffer, & Tolliver, 1988). Arguably the most notable is the sucrose or saccharin-fade procedure described by Samson (1986). In addition, reinstatement paradigms can be used to model relapse-like behavior (Heilig & Koob, 2007). Although operant procedures are thought to tap into the reinforcing aspect of ethanol consumption, they involve lengthy training procedures, and

operant behavior may be driven by similar genetic determinants as it is during simple free-choice drinking paradigms (Green & Grahame, 2008).

Recently, scheduled access paradigms have been developed that take advantage of genetic predisposition to induce high BALs in both mice and rats (Bell, Rodd, Lumeng, Murphy, & McBride, 2006; Rhodes, Best, Belknap, Finn, & Crabbe, 2005). These paradigms were developed to control for the variation present in free-choice models, and involve obtaining high levels of intake in a short period of time, resulting in high BALs, which together meet the NIAAA definition of binge drinking (2004). This is accomplished by taking advantage of rodent diurnal activity and intake rhythms through presenting alcohol for distinct periods of time during the active (dark) portion of the cycle. Although the drinking in the dark (DID) paradigm has incorporated water and ethanol, higher BALs were obtained using the DID paradigm with presentation of ethanol only (Rhodes et al., 2007). These models can be very useful for several reasons, including the ability to model binge-like intake and to pharmacologically manipulate drinking (Rhodes, et al., 2005).

Scheduled access procedures have limitations, as an artificial structure of intake is created, rather than obtaining naturalistic consummatory patterns. The DID procedure also takes advantage of the nocturnal rhythms of C57/BL6 (B6) mice. Different lines and strains vary in their circadian rhythms, which may affect the degree to which the procedure can be precisely and uniformly applied to other populations of mice (Hofstetter, Grahame, & Mayeda, 2003). Further, presentation of only an ethanol bottle limits face validity with regard to human consumption, which is "free-choice" in nature (Crabbe, et al., 2010). Lastly, these methods do not result in the same degree of exposure seen in

humans with regard to both time of and amount of alcohol exposure. Although these models are advantageous for modeling binge intake, they are not as desirable for modeling chronic intake. Scheduled access paradigms are useful for learning about the binge intake, but may not be the best option for modeling the chronic, excessive aspect of heavy drinking (Leeman, et al., 2010).

Another widely used procedure takes advantage of a genetic predisposition for alcohol intake. Preference testing is a procedure during which rodents often have continuous access to both a water bottle and a particular concentration of ethanol, as well as ad libitum access to food. This procedure has been widely used in both high drinking inbred strains of mice and in selectively bred high and low alcohol-preferring lines of mice and rats (Green & Grahame, 2008). Preference testing is simple procedure, and yields high intake levels as well as information regarding the correlated traits, neurobiology, and the genetics of alcohol preference (McBride & Li, 1998). In addition, it extends a high level of face validity with regard to human alcohol consumption, considering it is voluntary, and therefore captures a more naturalistic behavior.

There are limitations to these types of studies. Rodents generally do not ingest ethanol past their capacity to metabolize it, and therefore maintain blood ethanol below relevant levels (Murphy et al., 1986). Pharmacologically relevant BECs have been found in both P rats and the B6 inbred strain, though intake occurs in discrete bouts and is not sustained in a way that models excessive human intake (Dole & Gentry, 1984; Murphy, et al., 1986). Although high-drinking rodent lines and strains have been induced to drink high amounts of ethanol, these levels are generally not demonstrated in free-choice drinking paradigms. This makes interpreting the reasons rodents drink difficult, as

ethanol consumption may not be for its pharmacologic effects, but rather for novelty, taste, or caloric value (Cunningham, Fidler, & Hill, 2000; McMillen & Williams, 1998; Rodgers, Mc, Bennett, & Hebert, 1963). Individual differences in intake throughout the light-dark cycle also make it difficult to predict periods of high intake, thereby hindering an experimenter's ability to manipulate or measure behavior concerning alcohol consumption (Crabbe, 2010). In addition, typically only total daily fluid intake values and preference ratios are reported, which does not lend insight into the patterning of ethanol consumption.

Gaining precision and detail in measuring 24-hour voluntary drinking rhythms may be helpful in providing information regarding the "too much, too often" aspect of heavy drinking. Reporting patterns of consumption is important as they may inform researchers about the distinctive characteristics of ethanol consumption in high drinking phenotypes (Grant, et al., 2008). In addition, gaining information about these patterns has the potential to aid in acquiring knowledge about the etiology of heavy alcohol intake, in that it can be utilized to design experiments involving the behavior. Bout frequency, diurnal patterns of intake, and corresponding blood ethanol levels during chronic alcohol access are useful measures that could be used to achieve a greater level of contiguity between human and rodent research (Leeman, et al., 2010).

1.2 Selectively Bred High Alcohol Preferring Mice

Selectively bred high alcohol preferring (HAP) mice were selected from the HS/Ibg line (Institute of Behavior Genetics, Boulder, CO) for their total daily intake of 10% ethanol during 24-h daily access over a four-week period (Grahame, Li, & Lumeng, 1999 1999; Oberlin, Best, Matson, Henderson, & Grahame, 2010). Recently, we demonstrated

that repeated selection of the HS/Ibg progenitor line results in lines that are consistent with respect to the selection phenotype as well as the correlated responses of impulsivity and saccharin consumption (Oberlin et al., 2010; Oberlin & Grahame, 2009). 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 the most, followed by the HAP2 and HAP3 lines, respectively. Realizing it is important to learn about the drinking behavior in these mice, we recently performed experiments to look at their circadian drinking rhythms.

Following 3 weeks of ethanol access, drinking rhythms were assessed during the dark portion of a 12:12 light-dark cycle in the HAP2 and HAP3 lines during 24-hour access to both 10% ethanol and water. We recently observed that adult HAP2 mice stabilize their level of intake following 3 weeks of access to 10% ethanol, which is why this point was chosen to assess patterning of intake (Oberlin et al., 2010). Both the HAP2 and HAP3 lines demonstrated a stable pattern of chronic ethanol consumption following 3 weeks of 2-bottle free-choice access to 10% alcohol. Following drinking rhythms assessment, blood samples were taken in both the HAP2 and HAP3 lines of mice, resulting in average BECs of 101.9 mg/dl and 69.9 mg/dl, respectively. In HAP2 mice, plasma samples were taken following several additional hours of high intake compared to the HAP3 mice, which may be responsible for the occurrence of higher BECs in this line. In addition, a higher level of alcohol consumption is generally observed in replicate 2 mice, which are farther along in the selection process than replicate 3 mice (Matson and Grahame, In press).

1.3 Tolerance

Cicero (1980) states that in order to be classified as an animal model of alcoholism, oral self-administration must occur to pharmacological levels. To our knowledge, this is the first demonstration of ethanol consumption in a two-bottle choice 24-hour access paradigm yielding BEC levels to this degree in mice. Although free-choice access studies using selectively bred high drinking rats and hybrid mice have attained pharmacologically relevant BACs, it has been difficult to attain sustained high BACs over the course of the active cycle (Aalto, 1986; Agabio et al., 1996; Blednov et al., 2005; Dole & Gentry, 1984; Murphy et al., 1986). It would be useful to gain more detailed information regarding the circadian drinking rhythms and corresponding pharmacology in the other lines of HAP mice.

Another criterion noted by Cicero (1980) that is important for a successful rodent model of alcoholism is the development of functional and metabolic tolerance following chronic self-administration of alcohol. The development of tolerance in some capacity is often characteristic of alcohol-dependent individuals, and is thought to be an important factor in the development of ethanol dependence. Tolerance is defined by the DSM-IV (APA, 1994) as a need for markedly increased amounts of a substance to achieve intoxication, or a diminished effect with continued use of the same amount of a substance. There are two general types of tolerance, which include dispositional and functional tolerance. Dispositional tolerance refers to the development of decreased sensitivity to a drug on the basis of altered absorption, distribution, inactivation, or excretion from the body (Schuster, 1978). Metabolic tolerance is a type of dispositional tolerance that involves an increase in the excretion rate of a drug, often due to induction of enzymes

responsible for its degradation (Hall et al., 2001). Metabolic tolerance to ethanol has been demonstrated in mice, rats, and humans following a variety of induction or injection procedures (Lieber, 2004). Metabolic tolerance has been demonstrated in P rats following chronic free-choice access to ethanol and water (Lumeng & Li, 1986), but to our knowledge it has not been assessed in mice following voluntary alcohol consumption. Given the high ethanol intakes observed in HAP mice, it is plausible that there is sufficient ethanol exposure to induce metabolic tolerance in mice given chronic access to ethanol.

Functional tolerance can be defined as decreased physiological sensitivity to a drug following one or more exposures (Schuster, 1978). Behavioral tolerance is an associative process that results in decreased effects of the drug on performance with continued exposure (Bitran & Kalant, 1991). Differentiating between behavioral and functional tolerance can be difficult, and behavioral endpoints are often an inter-play of the two phenomena. It has been particularly difficult to model behavioral or functional tolerance following free choice access to ethanol and water, because it is dependent upon finding initial evidence of behavioral intoxication. Procedures involving induction, injections, or limited access have been used to model both behavioral intoxication and tolerance in mice (Suwaki et al., 2001). There are a number of different assays that have been developed to assess different types of tolerance, such as to the hypothermic, positively reinforcing, or ataxic effects of ethanol (Koob & Le Moal, 1997; Rustay et al., 2001). The balance beam is one procedure that has been consistently employed to detect evidence of behavioral intoxication through assessing the motor in-coordinating effects of acute alcohol; as well as whether tolerance develops to the motor in-coordinating effects

of chronic alcohol administration (Crabbe et al., 2003; Rustay, Wahlsten, & Crabbe, 2003).

Only one study has examined the relationship between the development of chronic functional or behavioral tolerance and voluntary consumption of ethanol. Linsenbardt and colleagues (2011) demonstrated evidence of functional tolerance in B6 mice following repeated exposure to ethanol using the DID procedure. In this study, the length of ethanol access was limited in order to achieve intoxicating levels of intake, which proved to be a sufficient exposure for functional tolerance to develop. To our knowledge, no studies have examined whether functional tolerance develops following 24-hour, free-choice exposure to ethanol in either mice or rats, which is most likely due to the fact that it has been difficult to achieve pharmacologically relevant levels of ethanol intake using this type of procedure. Preliminary studies in HAP mice lead us to believe that all of the HAP lines achieve intoxicating levels of intake throughout the dark portion of the cycle (Matson and Grahame, In press). The level of exposure we observe in HAP mice leads us to believe that functional tolerance will develop following chronic free-choice access to ethanol. Further, it seems most likely that the highest drinking HAP lines will develop functional tolerance, therefore we would like to explore this possibility in the highest drinking line.

1.4 Hypotheses

In the following experiments, we are interested in characterizing the drinking rhythms of our high drinking lines, cHAP and HAP1 mice. This is of particular interest, because we hypothesize that the cHAP and HAP lines farther along in the selection process will maintain a higher rate or level of intake throughout the dark portion of cycle.

In addition, if these mice maintain a level of intake above the metabolic rate of ethanol, accumulation of blood ethanol should occur throughout the active period. Further, we compare the drinking rhythms and corresponding BECs of these lines to those of the B6 inbred strain, an inbred strain commonly used for modeling high alcohol intake (Belknap, Crabbe, & Young, 1993; McClearn & Rodgers, 1959). B6 mice have been observed to consume in excess of about 10 g/kg/day, and it is among the highest drinking inbred strains (Yoneyama, Crabbe, Ford, Murillo, & Finn, 2008). Although B6 mice will consume ethanol to pharmacologic levels, intake occurs in bouts across the dark cycle (McClearn & Rodgers, 1959). Therefore, although we expect the HAP1 and cHAP lines to accumulate high levels of blood ethanol, we expect the B6 mice to maintain a level of intake that will not surpass metabolic capacity, resulting in relatively low BECs. We also seek to further characterize the behavior of the highest drinking line by assessing if pharmacologically relevant BEC levels can be sustained throughout the active portion of the light-dark cycle following chronic access to 10% alcohol. Finally, we assess whether this line develops both metabolic and functional tolerance to the ataxic effects of alcohol following free-choice alcohol access. Demonstrating that the highest drinking HAP line maintains pharmacologically relevant levels of intake across the day resulting in both the development of metabolic and functional tolerance would provide support for this line as a unique and highly translational animal model of excessive ethanol consumption.

CHAPTER 2 COMPARING CHRONIC DRINKING RHYTHMS IN B6, HAP1, AND CHAP MICE

2.1 Materials and Methods

2.1.1 Subjects

Mice were male and female C57Bl6/J (B6) and HAP lines of mice born in the IUPUI Animal Care Facilities; parents for the B6 mice were obtained directly from Jackson Laboratories. The experiment consisted of 20 B6 mice, 24 cHAP mice from generation 17, and 23 HAP1 mice from generation 43, aged 69-89 days at analysis of drinking rhythms. Two weeks before testing, mice were single-housed in a 12:12 reverse light-dark cycle. Water and food were available ad libitum, and ambient temperature was maintained at 21 $\pm$ 1° C. All experiments were performed in drug-naïve mice, and were conducted in the same colony room.

2.1.2 Procedure

Prior to ethanol access, total fluid intake of water was measured in mice for two days to assess baseline levels of water intake using 25 mL tubes. The mice were then given 24-hour free-choice access to water and a 10% ethanol solution for 3 weeks; intakes were measured using 25- or 50-ml graduated cylinders mounted on the wire cage

tops. Intakes were recorded and bottles sides were switched 3 times per week. 10% ethanol was used in all experiments involving consumption because it is the concentration used during the selection process for the HAP lines (Grahame et al., 1999).

After three weeks of access to ethanol, we continued 24-hour free-choice access to 10% ethanol (v/v) and water, but used 10 mL stereological pipettes readable to $\pm$ 0.1 ml. Drinking rhythms were observed for 3 days, with readings every 2 hours beginning at 8 am (lights off) and ending at 8 pm (lights on). Pipettes were not removed during readings except to refill, and readings were performed as rapidly as possible in order to complete them within 20 minutes. The ethanol and water tube positions were switched on the second day.

Both lines of HAP mice maintained a stable, high level of intake throughout a majority of the dark period, therefore we chose to sample at the highest average point of intake from the previous 3 days in B6 mice, which also corresponded to the time of day we acquired samples in HAP2 mice. Intake was assessed every other hour beginning at 8:00 am, and then hourly intakes were assessed in the 2 hours prior to testing for behavioral intoxication and taking blood samples (Figure 1). Blood ethanol concentrations were assessed using the gas chromatography procedure described by Lumeng and colleagues (1982).

2.1.3 Analyses

The alpha level was set at .05. In addition, Mauchly's Test of Sphericity was significant for repeated measures ANOVAs (ps < .05), therefore we proceeded with the Greenhouse-Geisser test for repeated measures. Baseline water intakes were compared using a Univariate Line x Sex Analysis of Variance (ANOVA). Ethanol intake during

acquisition was analyzed using a mixed Line x Sex x Day ANOVA. Trend analyses were used to assess the trajectory of ethanol drinking in each line, and planned comparisons were used to assess for differences in intake between the lines at the initial and last points of intake.

Because alcohol intakes varied minimally, we collapsed across the multiple days of bihourly drinking assessments a priori to get the most reliable measure of circadian drinking patterns. To compare the circadian pattern of ethanol intake between lines during drinking rhythm assessment, we used a mixed Line x Sex x Time ANOVA. Planned comparisons were performed between the highest mean point of intake and all other points of intake, to determine if there was a peak in intake. A Line x Sex ANOVA assessed for line differences in BEC, and a Pearson correlation was performed to examine the relationship between ethanol intake and BEC. We also report average 24-hour water, ethanol, and total fluid intakes during the drinking rhythms assessment, as well as the animal weights and preference ratios for ethanol in Table 1.

2.2 Results

Baseline water intake did not differ between the lines, although females had higher water intake than males. A Line x Sex ANOVA for baseline water intake indicated there was a main effect of Sex, $F(1, 61) = 6.48, p < .001$. There was no main effect of Line, $F(2, 61) = 2.91, p > .05$ or an interaction of Line and Sex, $F(2, 61) = 1.11, p > .05$ (Figure 2a.) Ethanol intake in B6 mice did not increase across the 3-week period, but HAP1 and cHAP intakes increased. A mixed Line x Sex x Day ANOVA was performed on ethanol intakes on the last day of acquisition.

There were main effects of Line, $F(2, 61) = 76.78, p < .001$, Sex, $F(1, 61) = 34.04, p < .001$, and Day, $F(5.7, 349.1) = 10.12, p < .001$. There was an interaction of Line and Day, $F(11.4, 349.1) = 3.10, p < .001$, but there were no interactions of Sex and Day, $F(5.7, 349.1) = 1.08, p > .05$, or Line, Sex, and Day, $F(11.4, 349.1) = .20, p > .05$. To analyze the trajectories of drinking in each line and the interaction between Day and Line, orthogonal ANOVAs were performed on each line for intake during acquisition and followed up with trend analyses when significant. A repeated measures ANOVA was performed for Day in B6 mice, and indicated no change in intake across days, $F(7.7, 147.0) = 1.27, p > .05$. The repeated measures ANOVA of Days in cHAP mice showed an increase in intake across days, $F(4.8, 112.0) = 11.79, p < .001$. The trend analysis showed significant linear and quadratic effects for intake in cHAP mice across days $F(1, 23) = 36.84, p < .001$ and $F(1, 23) = 18.65, p < .001$ respectively. A repeated measures ANOVA for Day in HAP1 mice indicated a significant change in intake, $F(4.8, 105.8) = 5.79, p < .001$, and demonstrated a significant linear increase, $F(1, 22) = 0.21, p < .001$. A one-way ANOVA indicated there were no significant differences in intake at the initial point in any of the lines, $F(2, 66) = 2.796, p > .05$. A one-way ANOVA indicated there were significant differences at the last point of intake, $F(2, 66) = 28.361, p < .05$. Planned comparisons indicated that B6 mice intake was significantly lower at the last point of intake than cHAP and HAP1 mice (ts < -4.54, $p < .001$); also HAP1 intake was lower than cHAP mice ($t = 2.97, p < .05$) (Figure 2b). The preference ratios for ethanol during acquisition are also reported for each line (Figure 2c).

During the active period, B6 mice drank at a relatively consistent level that did not result in high BECs, while HAP1 and cHAP mice drank at an excessive level,

resulting in high BECs. A repeated measures Line x Sex x Time ANOVA was performed on ethanol intake; there were main effects of Line, $F(2, 61) = 8.00$, $p < .001$, Time, $F(4.8, 293.7) = 103.91$, $p < .001$, and Sex, $F(1, 61) = 48.69$, $p < .001$. There were interactions of Line and Sex, $F(2, 61) = 8.00$, $p < .005$, and Time and Line, $F(9.6, 293.7) = 5.66$, $p < .001$, but there were no interactions between Sex and Time, $F(4.8, 293.7) = 1.98$, $p > .05$ or between Line, Sex, and Time, $F(9.6, 293.7) = 0.98$, $p > .05$. Post-hoc comparisons were conducted by Line for each time point using a Bonferroni correction (.05/21 = .002). Ethanol intake in B6 mice at the highest point of intake (2-4 pm) was not significantly different from all points of intake ($p > .002$), except during overnight ($p < .002$). The HAP1 highest point of intake (8-10 am) was only significantly different from intake at 6-8 pm and overnight ($p < .002$), but was not significantly different from all other points ($p > .002$). In cHAP mice, the highest point of intake (8-10 am) was not significantly different from intake during 10-12 and 12-2 pm ($p > .002$), but was different from all other points ($p < .002$) (Figure 3). As evidenced in Table 1, B6 had higher water intake than the HAP lines during the ethanol drinking rhythms assessment. Preference ratios for the HAP1, and cHAP lines were extremely high, with ethanol preferences over 90%, while B6 mice had a 74% preference for ethanol. Total fluid intake was higher with greater ethanol intake.

A Sex x Line ANOVA indicated there were significant main effects of line and sex on BEC, $F(2,66) = 31.26$, $p < .05$ and $F(1, 61) = 4.37$, $p < .05$, respectively, although there was no interaction between Sex and Line, $F(2, 61) = .340$, $p > .05$. Females had higher mean BECs, reaching 200.23 ± 15.29, versus 154.69 ± 15.51 mg/dl for males. Post-hoc comparisons were conducted using a Bonferroni correction (.05/3 = .02), and

indicated that B6 BEC was lower than cHAP and HAP1 (ps < .001), but HAP1 and cHAP BECs were not significantly different (p > .05) (Figure 5b). The correlation between intake and BEC specified a positive relationship between intake and BEC (r = .48, p < .001) (Figure 4a, b).

CHAPTER 3 PATTERN OF BLOOD ETHANOL ACCUMULATION AND METABOLIC TOLERANCE IN CHAP MICE

3.1 Materials and Methods

3.1.1 Subjects

Experiment 2 included 47 female and male cHAP mice from the 17th generation, aged 75-86 days at the time of drinking rhythms assessment. Two weeks before testing, mice were single-housed in a 12:12 reverse light-dark cycle, with lights off at 8 am and lights on at 8 pm. Water and food were available ad libitum, and ambient temperature was maintained at 21 $\pm$ 1° C.

3.1.2 Procedure

We were able to show that cHAP mice had the highest level of intake by the end of acquisition; therefore this line was used in experiments 2 and 3. Mice were assigned to two groups, Ethanol (n = 36) and Water (n = 11), balanced for sex and family of origin. Total fluid intake of both 10% ethanol and water was assessed in the ethanol-assigned mice for 3 weeks using the same procedure described for Experiment 1. Total fluid intake of water will be assessed in the control group during this time using 25 mL tubes (n = 12). Following 3 weeks of access, bihourly readings of ethanol were taken from 6 am to

10 pm for one day. We chose to take an additional reading prior to lights off and following lights on in order to assess when intake increases and decreases in this line. Based on our observations from the previous day, blood samples were taken at 10 am, 2 pm, 6 pm, and 10 pm (n = 8-9 at each time-point). These points were chosen in order to capture BECs following the time that intake first increases (10 am), during time-points of high intake across the day (2 pm and 6 pm), and then when BECs should begin to decrease (10 pm), to get a sense of the pattern of daily BECs. Retro-orbital blood samples were taken in a between-subjects manner in order to avoid disrupting normal drinking behavior. Following sampling, mice were placed back in the home-cage with access to water and ethanol. The following day, ethanol bottles were removed within an hour of lights on, and mice were given water-only access in order to clear all remaining blood ethanol in preparation for metabolic tolerance testing (Figure 5).

Beginning at 8 am (lights off) on the following day, the 6 water-only access mice and 6 ethanol-access mice were each given a 2 g/kg intra-peritoneal injection of 20% v/v ethanol. Each mouse was sampled twice via the retro-orbital sinus at 25 and 75 minutes. The dose and time parameters were adapted from Grahame and colleagues (1999), and will be used to obtain linear regressions of each group's ethanol elimination rates.

3.1.3 Analyses

To look at the time-course of BECs, a Sex x Time-point (10 am, 2 pm, 6 pm, 10 pm) ANOVA were performed for BEC values. Pearson correlations were performed to assess whether there was a correlation between the rate of intake and BEC at each time-point, as well as across all time-points.

Individual metabolic rates were calculated by deriving the change in BEC across minutes, using the following formula for slope (BEC at 75 min - BEC at 25 min)/ (75 min- 25 min). To assess whether metabolic tolerance develops following chronic access to ethanol, an Group x Sex ANOVA was used to determine if there were significant differences in the slope between the sexes, as well as between ethanol-exposed and water-exposed mice.

3.2 Results

As is evidenced by Figure 6, cHAP mice increase their intake during the 3 weeks of ethanol acquisition, and maintain a stable drinking rhythm during the dark portion of the light-dark cycle following chronic access. Both observations are very similar to the patterns of intake we observed by cHAPs in experiment 1. In addition, it is clear that the rate of intake is low before lights off, and then rapidly increases to a high level during the two hours following.

cHAP mice accumulate blood ethanol to differing degrees across their light-dark cycle. A Sex x Time between-subjects ANOVA indicated there were significant differences in BEC values across the four sampling points, $F(3, 27) = 5.049, p < .05$, but there was no difference in BEC between the sexes, $F(1, 27) = 2.434, p > .05$. There was also no interaction of Time and Sex, $F(3, 27) = .584, p > .05$. Post hoc analyses were performed for Time using a Bonferroni correction (.05/4 = .013), and indicated that the BEC values at 2 pm and 6 pm were not different from each other, but were significantly higher than BEC at 10 pm. The BEC values from the 10 am sampling point were not

significantly different from any other time-point. Mean BEC values were 112.47 $\pm$ 19.91 at 10 am (6/9 above 80 mg/dl), 189.00 $\pm$ 27.40 at 2 pm (8/9 above 80 mg/dl), 193.80 $\pm$ 29.66 at 6 pm (7/8 above 80 mg/dl), and 89.68 $\pm$ 22.19 at 10 pm (6/9 above 80 mg/dl). A Bonferroni correction was used to assess whether there were significant Pearson correlations for rate of intake and BEC (.05/5 = .01). There were no significant correlations between rate of intake and individual sampling points (ps > .01), but there was a significant correlation between rate of intake across all sampling points and BEC (p < .01) (Figure 7a, b).

A Sex x Group ANOVA indicated there was a significant difference in metabolic rate between the sexes, $F(1, 7) = 7.188$, $p < .05$, with metabolic rates of 2.29 $\pm$ .31 mg/dl/min for females and 1.50 $\pm$ .30 mg/dl/min for males. There was also a significant difference in ethanol metabolism between the chronically ethanol-exposed and water-exposed mice $F(1, 7) = 10.343$, $p < .05$, with metabolic rates of 2.33 $\pm$ .38 mg/dl/min and 1.46 $\pm$ .18 mg/dl/min, respectively, although there was no interaction of Sex and Group, $F(1, 7) = 3.141$, $p > .05$ (Figure 8).

CHAPTER 4 FUNCTIONAL TOLERANCE IN CHAP MICE

4.1 Materials and Methods

4.1.1 Subjects

Experiment 3 included 24 female and male cHAP mice from the 18th generation, aged 83-89 days at the time of testing for functional tolerance. Two weeks before testing, mice were single-housed in a 12:12 reverse light-dark cycle, with lights off at 8 am and lights on at 8 pm. Water and food were available ad libitum, and ambient temperature was maintained at 21 $\pm$ 1° C.

4.1.2 Procedure

Mice were assigned to two groups, Ethanol (n = 12) and Water (n = 12), and balanced for family of origin and sex. The 12 male and female water-assigned mice were given ad libitum access to water for three weeks using 25 ml graduated cylinders. The 24 ethanol-assigned male and female had 24-hour free-choice access to 10% ethanol and water for three weeks using the procedure previously described for chronic ethanol access. On the night before functional tolerance testing, ethanol was removed within an hour of lights on to ensure ethanol clearance prior to behavioral testing (Figure 9).

Beginning at 8 am, training on the balance beam was performed, which involved gently prodding mice to traverse the beam in both directions. This procedure has been shown to be sufficient in training mice to traverse the beam without difficulty during testing (Crabbe et al., 2003). The balance beam consisted of a 122 cm long x 2 cm wide x 4 cm tall wood block attached at both ends to two 48 cm ring stands. Immediately following training, all mice received a 1.75 g/kg intra-peritoneal injection of 20% v/v ethanol. 10 minutes following the injection, each mouse was placed on the end of the balance beam and allowed to traverse it in both directions while an independent observer assessed for number of hind-slips on the balance beam. Blood samples were taken immediately after the procedure via the retro-orbital sinus to assess BEC.

4.1.3 Analyses

In order to assess for behavioral tolerance, Group x Sex ANOVAs were run to assess whether there are differences in the number of footslips or BEC between the ethanol and water groups and/or between the sexes.

4.2 Results

As is evident in Figure 7, cHAP mice increase their intake of 10% ethanol during 3 weeks of access with a similar acquisition pattern to that observed in experiments 1 and 2. Chronically exposed ethanol mice had fewer hindslips than water controls, as was indicated by a Sex x Group ANOVA, $F(1, 20) = 4.350, p \leq .05$, but there was no main effect of Sex $F(1, 20) = .272, p > .05$ (Figure 8). There was also no interaction of Sex and Group, $F(1, 20) = .272, p > .05$. There was no significant difference in BEC between

ethanol-exposed and water groups, $F(1, 15) = 1.764, p > .05$, or between the sexes, $F(1, 15) = .002, p > .05$. Additionally, there was no significant interaction between Drug or Sex, $F(1, 15) = .001, p > .05$.

CHAPTER 5 DISCUSSION

These studies document excessive levels of alcohol drinking in both the cHAP and HAP1 lines of mice, and demonstrate that this level of free-choice drinking leads to the development of tolerance. Experiment 1 demonstrates that the HAP1 and cHAP lines exhibit a predictable and stable pattern of chronic ethanol intake, and importantly, that these lines achieve pharmacologically relevant levels during the active portion of the light-dark cycle. HAP1 and cHAP lines drink considerably more than the widely used B6 strain, in the absence of water intake differences at baseline, resulting in accumulation of extremely high BECs. Further, cHAP mice maintain these high blood ethanol levels through the entire dark portion of the light dark cycle. Conversely, the BECs we observed in B6 mice are similar to the levels previously published in a 24-hour free-choice paradigm (Dole & Gentry, 1984). In addition, cHAP mice demonstrate evidence of both metabolic and functional tolerance following chronic 24-hour, free-choice ethanol access. These data suggest that HAP mice, particularly the cHAP line, may provide an avenue for modeling alcoholism in a highly translational manner. Further, they provide an opportunity to study the "too much, too often" aspect of excessive alcohol consumption in mice that has been unattainable to model in rodents (Leeman et al., 2010).

Crabbe (2010) notes that a major problem of two-bottle choice preference testing is that rodents will rarely self-administer ethanol to pharmacological levels. Previous

studies in mice have not been able to demonstrate high intake for extended periods of time. Rodents tend to drink in bouts, rarely maintaining levels of alcohol intake above the rate of metabolism (Murphy et al., 1986). This type of drinking is not necessarily translational in nature, in that chronic alcoholics report being unable to control drinking (McKinley & Browne-Mayers, 1968), with higher levels of intake often observed in at-risk and dependent individuals (Dick, Aliev, Viken, Kaprio, & Rose, 2011; Harford, Grant, Yi, & Chen, 2005). The first and second experiments challenge the notion that with free-choice access, rodents will not self-administer past their capacity to metabolize consumed ethanol. Further, the BECs observed in cHAP and HAP1 mice are reminiscent of the excessive levels of intake previously observed in dependent humans using an almost unlimited procedure (Mello & Mendelson, 1970). In this respect, the cHAP and HAP1 lines may provide an unprecedented opportunity for studying causes, consequences of, and treatments for volitional alcohol consumption.

In the second study, we attempted to address the "area under the curve" with regard to blood ethanol levels in the cHAP line. Across both consummatory studies, we demonstrate that a majority of cHAP mice maintain pharmacologically relevant BECs throughout the dark portion of the light-dark cycle and even up through 2 hours following lights on. Although BECs decrease following lights on, 67% of the mice still had pharmacologically relevant levels (> 80 mg/dl) at 10 pm. In addition, BEC is strongly and positively correlated with rate of intake across the day, and when taken with the replication of the drinking pattern in cHAP mice, suggests that predicting intake and BEC ranges across the day in this line of mice is possible. Quantification of the daily pattern of

alcohol exposure in these mice may allow for behavioral, neurobiological, or genetic changes to be examined in a dose- and time-dependent manner.

For example, consistent daily intake patterns are also useful for investigation of pharmacotherapies for alcoholism. A principle advantage of the DID model is that the timing of intake is predictable because alcohol is presented for 2-4 hours, making it clear when to administer putative medications and allowing for pharmacological modulation of binge drinking (Moore & Boehm, 2009). The predictable and pharmacologically relevant intake patterns during 24-hour access in HAP mice suggest that medication administration can be timed. Theoretically, administration of a pharmacological agent could occur prior to the rise in intake to attempt to stop a bout before it starts, while administration during the dark period could be useful to address stopping an ongoing bout of drinking. Further, observing the time-course of intake following administration may provide an indication of relative efficacy and duration of action of a pharmacological agent.

Demonstrating high BECs in HAP mice not only allows for exploration of questions surrounding drinking and the presumed intoxication that results, but also for studying behaviors that may result from chronic alcohol intake. The presence of tolerance is a DSM-IV criterion for diagnosing alcohol dependence in humans, and is thought to be a causal factor in the escalation of use that occurs with drug dependence (APA, 1994). Few studies have assessed whether tolerance results following 24-hour, free-choice administration in rodents, as this type of drinking paradigm is often limited by the amount of ethanol rodents will voluntarily consume. Induction or injection procedures have typically been necessary to induce metabolic tolerance (Lieber, 2004). Although

these models result in high BECs, voluntary consumption may be less stressful and more translational. Consuming ethanol results in more exposure to the gastrointestinal tract and liver, and ethanol metabolism occurs in both of these tissues (Caballeria, Baraona, & Lieber, 1987). Therefore, it is possible that liver and stomach enzyme induction may be higher in a continuous consummatory procedure than it would be in paradigm involving intra-venous or intra-peritoneal ethanol administration, simply due to the increased tissue exposure to ethanol. It has been shown that P rats demonstrate metabolic tolerance following free-choice access to ethanol, and further, the degree of metabolic tolerance did not differ from P rats fed an alcohol liquid diet (Lumeng & Li, 1986). Our results demonstrate that cHAP mice also develop a significant amount of metabolic tolerance following voluntary access to ethanol, which is a novel finding in mice.

Inherent to assessing functional tolerance to ethanol is the demonstration of intoxication, which often requires mice to reach BECs of at least 100 mg/dl (Cronise, Finn, Metten, & Crabbe, 2005). Linsenbardt and colleagues (2011) were able to demonstrate evidence of functional tolerance following consumed alcohol, using a procedure that has been shown to produce drinking to intoxication in B6 mice. Finding that HAP mice will voluntarily drink to intoxicating BECs allowed us to also explore the relationship between voluntarily consumed ethanol and the development of tolerance. Results from experiment 3 suggest that cHAP mice develop functional tolerance to the ataxic effects of ethanol, which is a novel finding, in that no study has been able to demonstrate this effect using a 24-hour, free-choice procedure. This is important because we can now use a free-choice paradigm to look at what may be driving the development of functional tolerance in these mice. For instance, this may be used to examine whether

a differing length or dose of ethanol exposure determines the level of tolerance or the speed of its development. This finding may also be important because the level of ethanol exposure HAP mice receive during this procedure is analogous to the degree of exposure seen in chronic, excessive human consumption.

Important to consider in interpreting the functional tolerance experiment, is the possibility that these results could reflect the development of behavioral tolerance (Bitran & Kalant, 1991). Although mice were not exposed to the balance beam prior to testing, it is possible that being intoxicated in the home cage results in learning, which decreases the ataxic effects of ethanol in both the home cage and during the balance beam procedure. It is highly plausible that learning could occur in a 24-hour access procedure, as continuous ethanol exposure provides a multitude of opportunities for intoxicated practice to occur. It is also likely that functional tolerance is occurring, as physiological alterations in receptors or signaling have been shown to occur in response to repeated ethanol exposures (Kumar et al., 2009). Although it would be difficult to separate the two processes, it may be interesting to explore the degree to which the observed effect is driven by both associative processes and physiological adaptations.

In conclusion, the HAP1 and cHAP mice demonstrate stable, excessive patterns of ethanol intake across the dark portion of the cycle, during which both lines exhibited intakes considerably higher than B6 mice. Achieving stable, high intake in the HAP mice to the observed level of intoxication provides support for these lines as a rodent model of alcoholism, and may provide a unique opportunity for modeling chronic, excessive human intake. This excessive intake results in the development of both metabolic and functional tolerance in the cHAP line. Demonstrating that the cHAP mice display

evidence of both metabolic and functional tolerance following free-choice access to ethanol provides more support for the cHAP mice as a highly translational rodent model of alcoholism. These effects may at least partially explain the increase in drinking over weeks seen in both cHAP and HAP1 mice, but not B6 mice. These findings provide rationale for further exploration into other predisposing factors surrounding excessive consumption, as well as the development of other physiological and behavioral adaptations, and toxicological effects outcomes following voluntary alcohol exposure.

TABLES

TABLES

Table 1. Average 24-hour intake during chronic drinking rhythms. Average 24-hour intakes of water and ethanol during drinking rhythms across 2-3 days of intake readings, reported in ml/kg/day and g/kg/day, respectively. We also report the mean preference ratios for ethanol during drinking rhythms, weights (in grams), and total fluid intakes (in mls).

Line	Ethanol Intake	Water Intake	Preference Ratio	Weight	Total Fluid
H1	21.85 ± .49	25.14 ± 2.81	.92 ± .01	23.94 ± .36	7.11 + .15
Females	23.07 ± .59	23.77 ± 2.50	.93 ± .01	22.75 ± .33	7.17 + .22
Males	20.51 ± .60	26.62 ± 5.33	.91 ± .02	25.25 ± .39	7.05 + .21
cHAP	23.85 ± .47	29.44 ± 4.51	.92 ± .01	24.15 ± .43	7.96 + .16
Females	25.22 ± .58	35.32 ± 6.67	.90 ± .02	22.53 ± .34	8.02 + .27
Males	22.47 ± .50	23.56 ± 5.86	.93 ± .02	25.78 ± .44	7.90 + .20
B6	14.16 ± .82	62.52 ± 6.79	.74 ± .03	24.03 ± .47	5.73 + .18
Females	17.13 ± .69	65.16 ± 10.18	.77 ± .03	22.37 ± .42	6.30 + .20
Males	11.18 ± .64	59.88 ± 9.45	.71 ± .04	25.69 ± .38	5.16 + .15

FIGURES

FIGURES

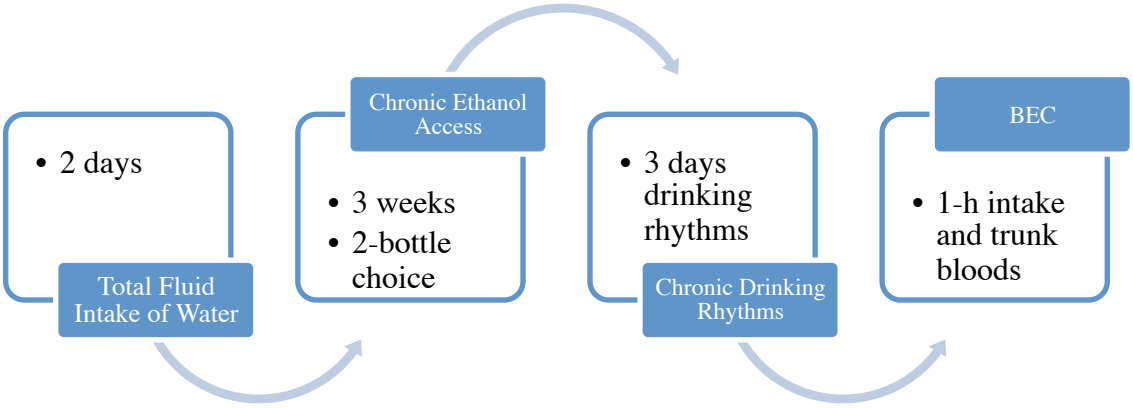

Figure 1. Schematic of experiment 1 procedure.

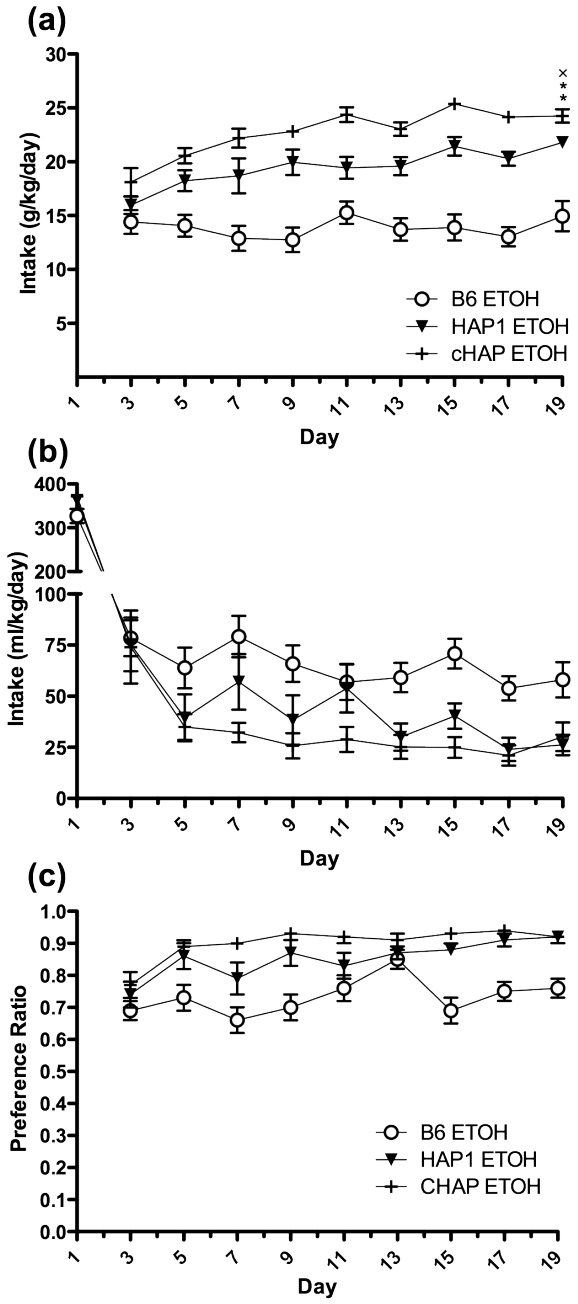

Figure 2. Ethanol and water intake during acquisition. (b) Water intake during baseline and acquisition of ethanol in B6, HAP1, and cHAP mice. (a) Ethanol acquisition for B6, HAP1 and cHAP mice, with intake reported in g/kg/day. Daily intake was derived from dividing intake readings by the appropriate number of days, in order to obtain a daily intake score. Astericks (*) indicate a difference from B6, (×) indicate a difference from HAP1. (c) Preference ratio for 10% ethanol during acquisition in B6, HAP1, and cHAP mice.

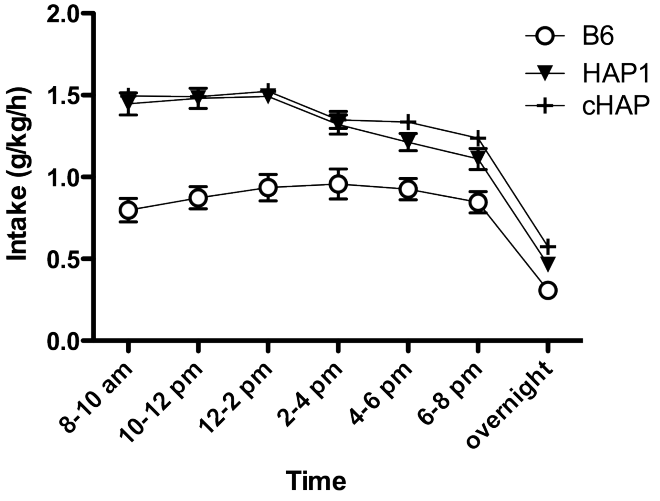

Figure 3. Drinking rhythms in B6, HAP1, and cHAP mice. Intakes are reported in mean g/kg/h, and are obtained from data averaged across 2-3 days. Drinking rhythms in B6 (open circles), HAP1 (filled triangles), and cHAP mice (hatches).

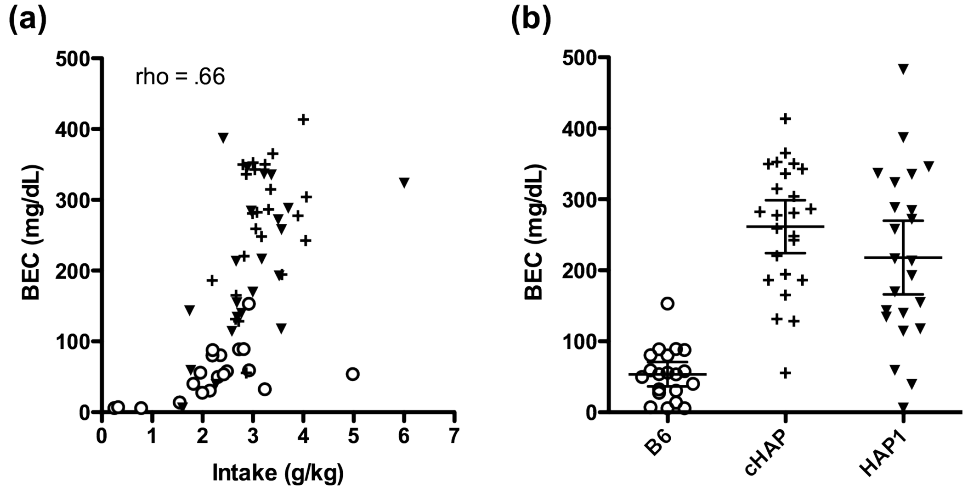

Figure 4. BEC in B6, HAP1, and cHAP mice. (a) Correlation between 2-hour intake and BEC in B6, HAP1, and cHAP mice (b) 95% CIs for B6, HAP1, and cHAP mice with mean BECs of 56.63 $\pm$ 8.149, 217.9 $\pm$ 25.02, and 261.5 $\pm$ 19.09, respectively.

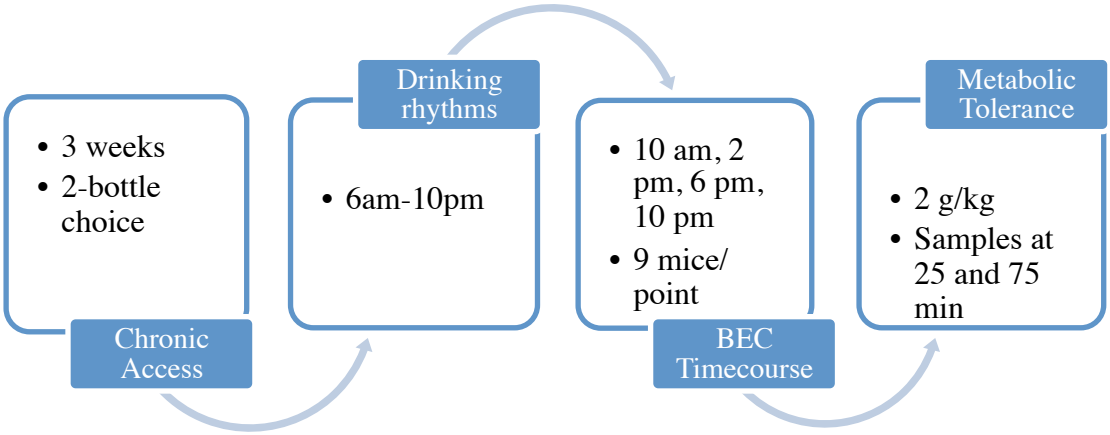

Figure 5. Schematic of experiment 2 procedure.

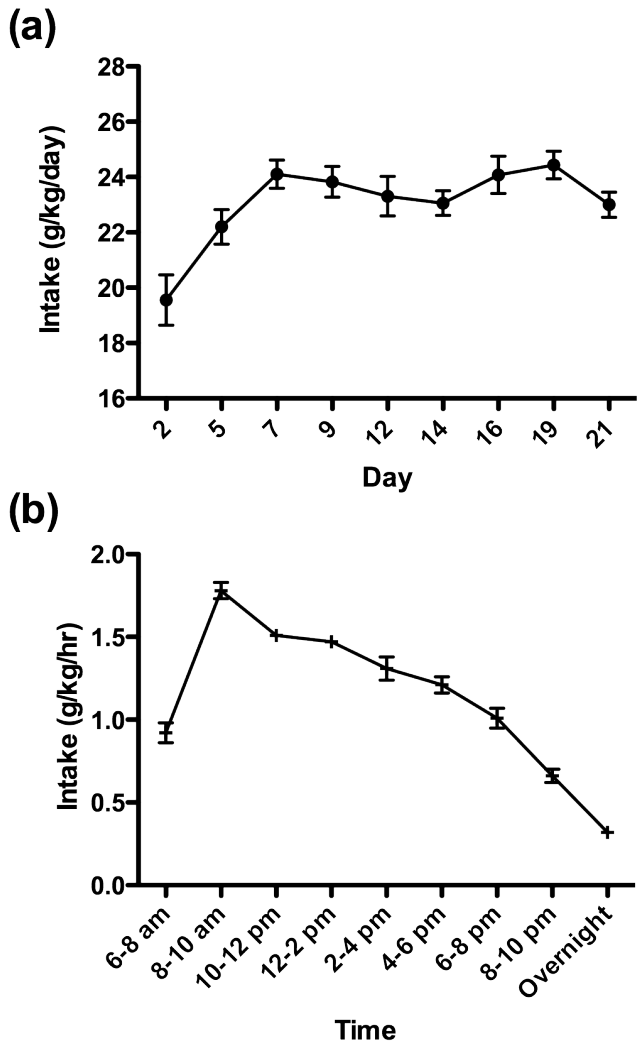

Figure 6. Ethanol acquisition and chronic drinking rhythms in cHAP mice. (a) Ethanol acquisition cHAP mice over 3 weeks with intake reported in g/kg/day. Daily intake was derived from dividing intake readings by the appropriate number of days, in order to obtain a daily intake score. (b) Chronic drinking rhythms (g/kg/h) in cHAP mice including 2 hours before and after the dark portion of the cycle.

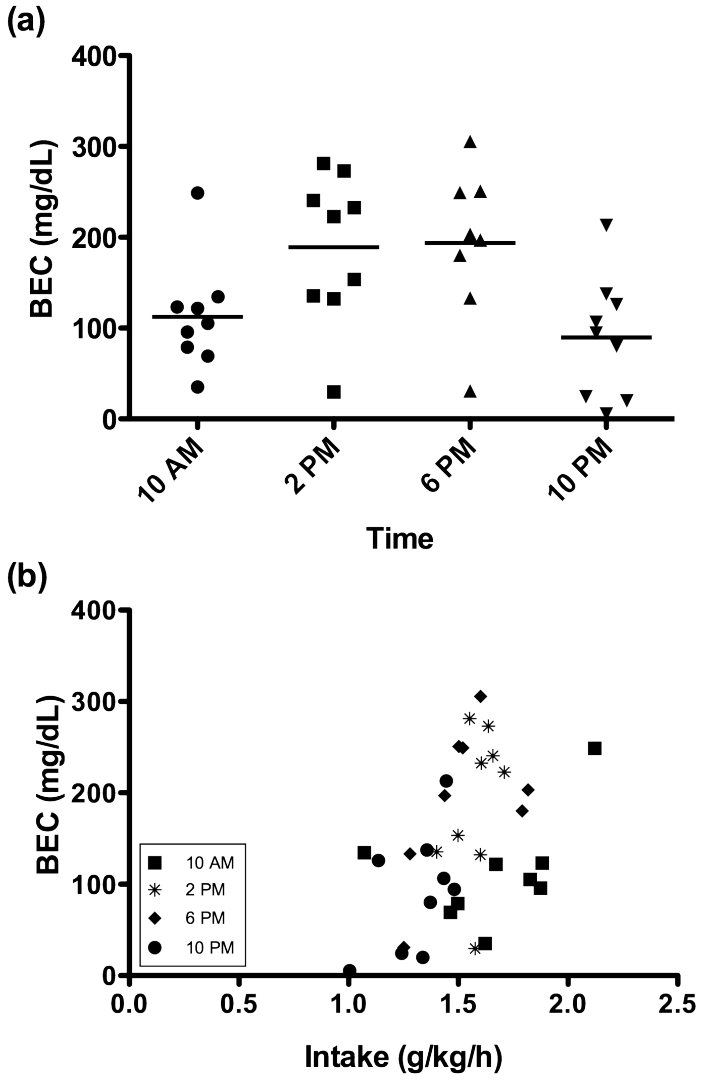

Figure 7. Pattern of BEC accumulation in cHAP mice. (a) BECs across the day in cHAP mice (n = 8-9 per time point). Mean BECs were 112.47 $\pm$ 19.91 at 10 am, 189.00 $\pm$ 27.40 at 2 pm, 193.80 $\pm$ 29.66 at 6 pm, and 89.68 $\pm$ 22.19 at 10 pm. (b) Correlation of BEC with rate of intake across the day (g/k/h) through the point of blood sampling.

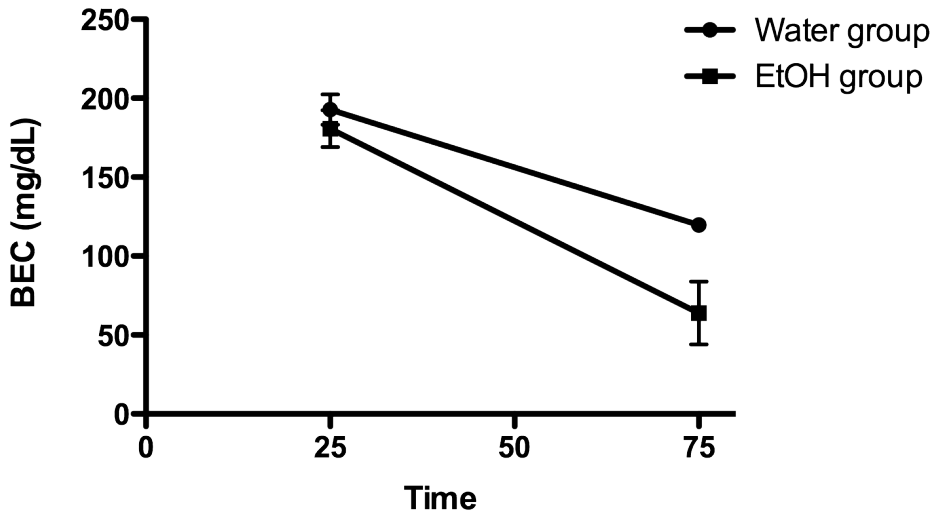

Figure 8. Metabolic tolerance in cHAP mice. Ethanol and water mice had mean metabolism rates of 2.33 ± .38 mg/dl/min (about 139.8 mg/dl/h) and 1.46 ± .18 mg/dl/min (about 87.6 mg/dl/h), respectively.

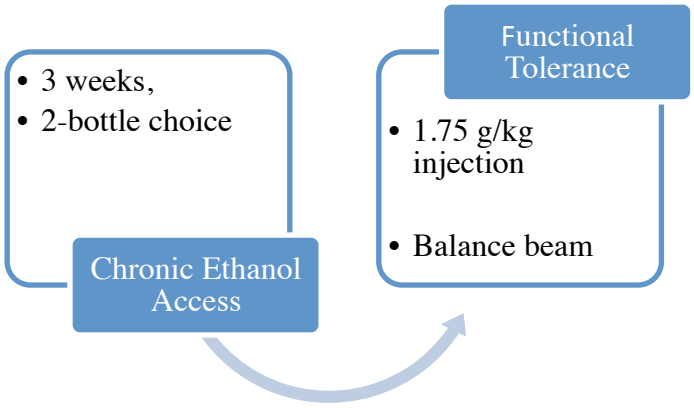

Figure 9. Schematic of experiment 3 procedure.

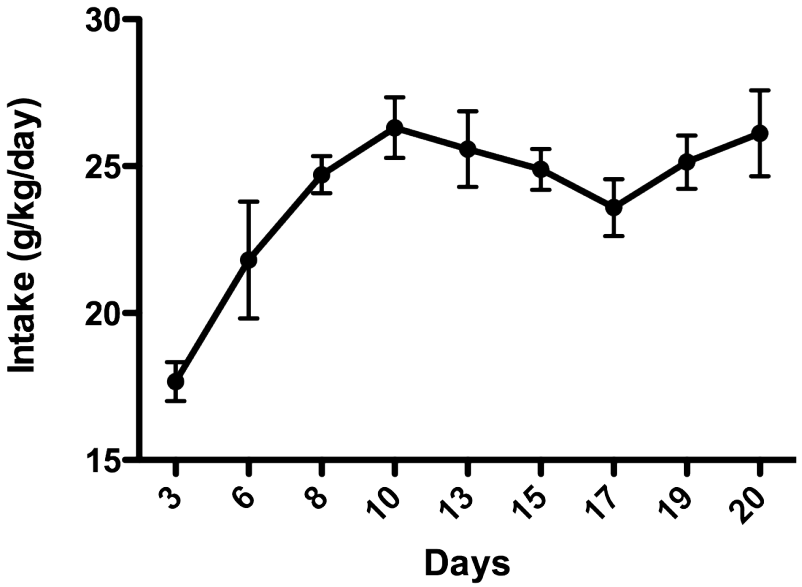

Figure 10. Ethanol acquisition cHAP mice over 3 weeks. Daily intake is reported in g/kg/day, and was derived from dividing intake readings by the appropriate number of days, in order to obtain a daily intake score.

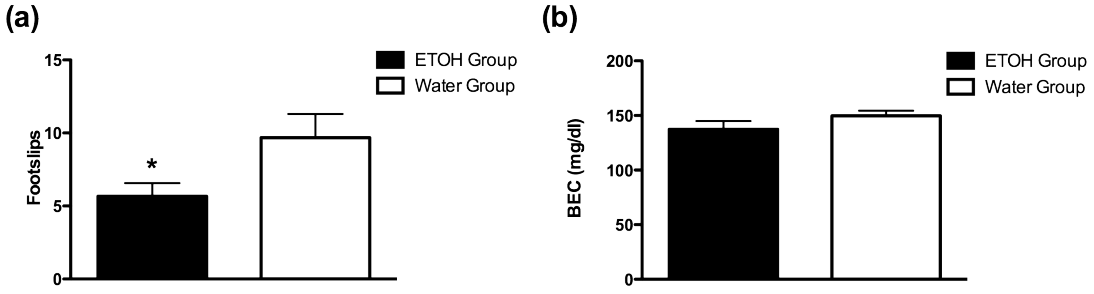

Figure 11. Functional tolerance in chronically ethanol-exposed cHAP mice. (a) Number of hindslips in the water and ethanol groups following a 1.75 g/kg ethanol injection. (b) BEC in water and ethanol groups immediately following balance beam procedure. Asterisk (*) indicates significant difference from the water group ($p < .05$)

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Publications

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

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.

Matson, L., Liangpunsakul, S., Buckingham, A., Crabb, D., Grahame, N. (In preparation) Chronic ethanol consumption in selectively bred crossed high alcohol preferring (cHAP) mice results in sustained blood ethanol concentrations and metabolic tolerance.

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Matson, L., Halcomb, M., O'Tousa, D., Buckingham, A., Villalta, N., N. Grahame (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.

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