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By Alexis Suzanne Green

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Intravenous Self-Administration of Alcohol in Selectively Bred High- and Low- Alcohol Preferring Mice

For the degree of   Doctor of Philosophy

Is approved by the final examining committee:

Nicholas J Grahame
_____
Chair

Charles R Goodlett

James M Murphy

Julia A Chester

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INTRAVENOUS SELF-ADMINISTRATION OF ALCOHOL IN SELECTIVELY

BRED HIGH- AND LOW- ALCHOL PREFERRING MICE

A Dissertation

Submitted to the Faculty

of

Purdue University

by

Alexis Suzanne Green

In Partial Fulfillment of the

Requirements for the Degree

of

Doctor of Philosophy

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Purdue University

Indianapolis, Indiana

I would like to dedicate this to my husband, who helped me blow it away…

I would also like to dedicate this work to Thomas J. Behm, my father. Without him and his "character building" support, I would have given up long ago.

And to my mother and her never ending love.

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LIST OF ABBREVIATIONS

Acq	acquisition
ANOVA	analysis of variance
C57BL/6J	a high drinking strain of inbred mice
cm	centimeters
CPP	Conditioned Place Preference
CTA	Conditioned Taste Aversion
D.R.	Dose Response
DBA/2J	a low drinking strain of inbred mice
F	female
FR	Fixed Ratio
g	grams
G	gauge
Gen	generation
HAD	High Alcohol Drinking Rats
HAP	High Alcohol Preferring Mice
HS/Ibg	a genetically outbred progenitor stock mouse population
i.v.	intravenous
IGSA	Intragastric Self-Administration
IUPUI	Indiana University- Purdue University at Indianapolis
IVSA	Intravenous Self-Administration
Kg	kilograms
L	Liters
LAD	Low Alcohol Drinking Rats
LAP	Low Alcohol Preferring Mice
LED	Light-emitting diode
LS	Long Sleep Mice

M	male
mg	milligram
mL	milliliters
NP	Alcohol Non-Preferring Rats
º C	degree Celsius
OSA	Operant Self-Administration
P	Alcohol Preferring Rats
Posttrain	post training
Pretrain	pretraining
Rev.	Lever Reversal
SEM	Standard Error of the Mean
U	units
v/v	volume per volume measurement
WSP	Withdrawal Seizure Prone Mice

ABSTRACT

Green, Alexis Suzanne, Ph.D. Purdue University, May 2011. Intravenous Self-Administration of Alcohol in Selectively Bred High- and Low- Alcohol Preferring Mice. Major Professor: Nicholas Grahame.

Genetic vulnerability to alcoholism is theorized to be caused by multiple interacting genetic loci, each with a small to modest effect combining under certain environmental influences to contribute to vulnerability to ethanol dependence. Animal models such as selectively bred rodent lines can be used to address this hypothesis of genetic vulnerability. High-drinking lines are implicitly assumed by many to be evidence of high ethanol reinforcement without consideration for variables such as differential pre- and post ingestive effects, low response to alcohol or novelty-seeking. Therefore, it is an open question as to whether animal studies support the idea that genetic differences in free-choice drinking are correlated with genetic differences in other assessments of ethanol-reinforced behavior, including those utilizing operant and classical conditioning. Thus, the present study utilizes selectively bred High- and Low- Alcohol Preferring mice tested for operant intravenous alcohol administration to address the hypothesis that High Alcohol Preferring mice would show evidence of greater alcohol reinforcement than their selectively bred opposite, Low Alcohol Preferring mice. Evidence for greater reinforcement was supported by High Alcohol Preferring mice voluntarily pressing a lever to administer an intravenous dose of alcohol in a two lever choice paradigm, administering higher doses of intravenous alcohol, and tracking the location of the active alcohol lever during a lever reversal procedure in comparison to Low Alcohol Preferring mice. This study supports the High- and Low- Alcohol Preferring mice as a useful genetic

model of alcohol-related vulnerability even when utilizing a route of administration that bypasses the digestive system.

CHAPTER 1. INTRODUCTION

Ethanol is one of the mostly widely used drugs in the world today. While most ethanol users can be characterized as casual drinkers, the abuse rate is substantial (Grant et al., 2004), with a lifetime prevalence rate reported as high as 12.48% (Hasin & Grant, 2004). Alcoholism is a complex psychiatric disorder with an estimated heritability of 50-60% (Enoch, 2003). It has a fairly common prevalence world-wide, with the United States showing a prevalence of ethanol dependence as high as 20% in men and 8% in women (Enoch, Schuckit, Johnson, & Goldman, 2003).

Genetic vulnerability to alcoholism is theorized to be caused by multiple interacting genetic loci, each with a small to modest effect combining under certain environmental influences to contribute to vulnerability to ethanol dependence (Ginter & Simko, 2009). Animal models such as inbred strains, transgenics, knockouts/ins, and selectively bred rodent lines can be used to address this hypothesis of genetic vulnerability (see Green & Grahame, 2008 for review). Knockout and transgenic models can be used to focus on single gene alterations to address the specific pharmacogenetics of ethanol use and vulnerability to alcoholism. No matter what method is used to manipulate genes, whether through breeding strategies such as artificial selection and inbreeding, or targeted gene alterations using transgenic or knockout techniques, genetic correlations can be determined. For example, when a pair of selected lines is found to differ significantly on some trait other than the one for which they were selected, one may say that a genetic correlation between the traits exist. Taking this one step further, there may be a common set of genes or gene for the two responses (Crabbe, Phillips, Kosobud, & Belknap, 1990). This is useful for

eventually understanding underlying mechanisms of action in such complex behaviors such as ethanol abuse. These animal models may lack many aspects of human alcoholism, but experimenters are able to control their genetic and environmental history to research scientific theories difficult to address in human studies due to logistical and ethical considerations. Furthermore, these genetically-based animal models may reveal behavioral, genetic, and physiological characteristics that demonstrate genetic links to behaviors such as ethanol drinking.

Selective breeding techniques are a popular animal research method (Crabbe, 1989) and have been used to develop a number of mouse and rat lines differing in genetic sensitivity to specific effects of ethanol. Phenotypes of voluntary ethanol consumption, as well as related phenotypes such as thermoregulatory, excitatory, and dependence-producing effects of alcohol have been developed (Grahame, Li, & Lumeng, 1999b; Murphy et al., 2002; Shen, Harland, Crabbe, & Phillips, 1995). To create a selectively bred model, one ideally starts with a highly variable large population with diverse genetics on all possible alleles (Crabbe, 1989). Through selective pressure on one particular phenotype, repeated matings over several generations result in fixing alleles related to the desired phenotype while leaving non-relevant alleles variable in the population. The result are two divergent lines differing on the phenotype of choice- alcohol preference or home cage drinking, for example- that can be compared to each other with conclusions drawn based on the assumption that the selective pressure on that particular phenotype has fixed the relevant alleles and that these specific alleles are somehow related to the complex trait of interest (Crabbe, 1989). These genetic animal models of selective breeding have been utilized in numerous studies to assess the bases for those genetic differences, and to determine the specific neurochemical and neurophysiological basis for ethanol's actions and continue to be a valuable tool in addressing ethanol's mechanisms of action.

Previous studies have repeatedly demonstrated that lines genetically sensitive to one effect of ethanol are not necessarily sensitive to others, which demonstrates that no single set of genes modulates all ethanol effects. For example, LS mice, selected for sensitivity to ethanol anesthesia, are not similarly sensitive to all anesthetic drugs (Erwin, Korte, & Marty, 1987), which demonstrates that all such drugs cannot have a common mechanism of action. On the other hand, WSP mice, genetically susceptible to the development of severe ethanol withdrawal, show a similar predisposition to diazepam and phenobarbital withdrawal (Belknap, Crabbe, & Laursen, 1989; Crabbe, Merrill, & Belknap, 1993), which suggests that there may be a common set of genes underlying drug dependencies.

Replicate lines may also be produced when developing selectively-bred lines of animals. This is particularly helpful, in that we may observe phenotype stability of the genetic animal models across independently selected lines, laboratories, and generations, which increases their power as analytic tools (Crabbe, 1989).

When replicate lines, independently selected for a particular phenotype, are similar in the expression and magnitude of that phenotype, one may have high confidence regarding the fixation of multiple separate genes related to this phenotype while still expressing a genetic variability on non-phenotype traits of interest. This is one of the most valuable properties of selective breeding in comparison to other commonly used models such as inbred strains, transgenic, and knockout rodent models. Having a variable population increases the validity and generalizability of the model (Crabbe, 1989; Grahame, 2000).

Selective breeding has been implemented to produce several highly popular rodent models of high and low alcohol consumption. For example, rats selectively bred for alcohol preference and alcohol non-preference have been useful to both preclinical and clinical investigators in the alcohol research

community. Rats selectively bred for alcohol preference (alcohol preferring or "P" replicate lines) have enhanced responsiveness to the low dose reinforcing effects of alcohol, less aversion to moderate/high doses of alcohol, and are able to develop tolerance to the aversive effects of alcohol more rapidly and to maintain tolerance longer than rats selectively bred for alcohol non-preference (alcohol non-preferring or "NP" line) (Murphy, et al., 2002). As discussed in a recent review by Froehlich (2010), the low-dose alcohol may act as a particularly strong reinforcer for P rats, which might be expected to foster and maintain alcohol drinking. Weaker aversion to the pharmacological effects of moderate/high doses of alcohol in the P line would allow P rats to drink more alcohol than NP rats before the postingestional effects become aversive. Rapid induction of tolerance to the aversive effects of alcohol with repeated bouts of voluntary alcohol drinking, as well as persistence of alcohol tolerance in rats of the P line might serve to maintain alcohol drinking. See Froehlich (2010) for review.

Similarly, the high-alcohol-drinking (HAD1/HAD2) and low-alcohol-drinking (LAD1/LAD2) rat lines, derived from the N/NIH rat, were developed by using a within-family selection and rotational breeding design for alcohol preference and alcohol consumption and have been thoroughly analyzed for the above mentioned phenotypes as well (Murphy, et al., 2002). Despite their varying genetic background and differences in selective pressure during the selective breeding over generations, similarities between these rodent lines are remarkable. Indeed, the P and HAD replicate lines have met criteria for an animal model of alcoholism in that they voluntarily consume sufficient ethanol to achieve significant blood alcohol concentrations, and their alcohol-seeking behavior is reinforced by the pharmacological effects of ethanol rather than its taste, caloric content, or other properties. The P and HAD rats show an enhanced responsiveness to the stimulatory effects of ethanol and reduced

sensitivity to the aversive sedative effects of ethanol. See Murphy, et al. (2002)
for review.

Selective breeding has not been limited to rat lines, however. High Alcohol
Preferring (HAP) and Low Alcohol Preferring (LAP) replicate mice are derived
from HS/Ibg mice, a genetically outbred stock (Grahame, et al., 1999b). This
again allows a much greater variability for phenotypic behavior and related
alleles at each locus as compared with inbred strains such as C57BL/6J or
DBA/2J, two commonly used mouse models that differ in their free-choice
ethanol consumption (Grahame, 2000). Results from comparison among a
larger number of alleles are more likely to be generalizable to wider populations
(Crabbe, 1989).

The ability to produce replicate lines in selectively bred animals also increases
validity of the studies using these replicate lines and helps address the question
of genetic drift after many generations. The HAP and LAP mice are similar in the
alcohol phenotype and related phenotypes as the rat models mentioned above.
HAP mice meet criteria for an animal model of alcoholism in that they also
voluntarily consume sufficient ethanol to achieve significant blood alcohol
concentrations (Grahame, 2000; Grahame, Li, & Lumeng, 1999a; Grahame, et
al., 1999b), and their alcohol-seeking behavior is reinforced by the
pharmacological effects of ethanol rather than its taste, caloric content, or other
properties (Grahame, et al., 1999a). The HAP mice show an enhanced
responsiveness to the locomotor sensitizing effects of ethanol and reduced
sensitivity to the aversive sedative effects of ethanol (Grahame, et al., 1999b).

An important and often addressed question in the study of alcoholism and
ethanol use is that of reinforcement and alcohol reward. In these animal models,
differences in free-choice ethanol consumption have frequently been used to
study the genetic and neurobiological mechanisms underlying high ethanol

drinking behavior, whether the animal model was created using selective breeding, inbreeding, or targeted gene alteration (Crabbe, Phillips, Cunningham, & Belknap, 1992; Li, Lumeng, & Doolittle, 1993). As discussed in Green & Grahame (2008), a question often arising in the interpretation of these studies is whether high-drinking lines show greater ethanol-reinforced behavior than low-drinking lines. In other words, high drinking is implicitly assumed by many to be evidence of high ethanol reinforcement *per se.* However, other intervening variables such as anxiety tend to lead to higher alcohol drinking (Pohorecky, 1991), low response to alcohol tends to be correlated with higher rates of alcohol consumption (Schuckit & Smith, 2001), or novelty-seeking (Cloninger, Sigvardsson, & Bohman, 1988) have also been speculated to be related to excessive drinking. Therefore, it is an open question as to whether animal studies support the idea that genetic differences in free-choice drinking are correlated with genetic differences in other assessments of ethanol-reinforced behavior, including those utilizing operant and classical conditioning.

A recent review by Green & Grahame (2008) addresses this question by analyzing free-choice drinking differences and such behavioral paradigms as conditioned place preference (CPP), conditioned taste aversion (CTA), and operant self-administration (OSA). The authors concluded that there was a strong positive relationship between OSA and voluntary oral consumption of alcohol, as well as a negative relationship between CTA and voluntary oral consumption of alcohol. A weaker correlation was found between free-choice drinking and CPP, suggesting a more variable link between oral consumption of alcohol and place preference conditioning, especially when considering rat versus mouse models. In this review, intravenous self-administration (IVSA) of alcohol is also mentioned; however they were unable to assess IVSA and its relationship to differences in free-choice drinking due to the lack of relevant literature.

There are two main paradigms available for assessing the rewarding effects of alcohol that bypass the oral route of administration: IVSA and intragastric self-administration (IGSA). Both methodologies avoid most preingestive considerations, such as taste and odor of alcohol, that may be attractive or aversive, and attempt to assess the rewarding properties of the pharmacologic effects of alcohol. Neither methodology is perfect in doing so, as alcohol is excreted via exhalation. It is possible that subjects administering alcohol i.v. or IGSA may still taste or smell the alcohol and metabolites on their breath following administration (Smith, Pysanenko, & Spanel, 2010; Wilkinson, 1980). However, both of these methodologies are better equipped to address the pharmacologic effects of alcohol and the rewarding properties without interference of preingestive effects such as taste and/or somatosensory stimulation that complicates free choice drinking (Grahame & Cunningham, 2002).

Both paradigms require surgical implantation of a catheter to allow direct infusion of alcohol into the subjects. IGSA allows for administration of alcohol directly into the stomach where it is readily absorbed; IVSA allows for administration of alcohol directly into the blood stream. IVSA allows for faster, near immediate pharmacological effects in the brain while IGSA is somewhat slower and allows for first pass metabolism of the alcohol in the liver. IGSA can also be further complicated by the presence and quantity of food in the digestive tract. IVSA does not have this limitation and is therefore less variable in dose administration and time course of pharmacological effects compared to IGSA. Traditionally, IVSA is used to study drugs of abuse with stimulant properties, such as cocaine and amphetamine (Lu, Shepard, Hall, & Shaham, 2003; Osborne & Olive, 2008), with more recent literature utilizing this methodology to expand our knowledge of neurobiological substrates of a more expansive scope of drugs of abuse, including opiates and alcohol (Koob & Zorrilla, 2010; Vlachou & Markou, 2010). While IVSA is an increasingly popular way to measure reinforcing effects of

various drugs of abuse (Grahame & Cunningham, 2002; Thomsen & Caine, 2007), the alcohol literature in rodent models thus far remains limited.

IVSA is a measure of reinforcement that differs in important ways from other measures such as CPP and OSA. First, it does not rely heavily on any preingestive considerations such as taste or smell that are not components of alcohol's pharmacologically reinforcing effects, because the ethanol is administered directly into the bloodstream. Second, the ability to achieve pharmacologically-relevant blood ethanol levels is not hindered by the presence of food in the digestive tract, as it may be with free-choice drinking or intragastric administration (Cunningham, Clemans, & Fidler, 2002), and ethanol may reach the brain very quickly after it is administered. Intravenous injections allow for near-immediate passage through the blood-brain barrier. Such immediate effects are not available through any other systemic administration, including IGSA, in which the alcohol must first be absorbed through the intestinal wall into the blood stream. The rate of absorption is affected by factors such as amount and type of ingested material present in the tract, as well as quantity of adipose tissue in the peritoneal cavity. Animals are much more likely to experience the pharmacologic effects of ethanol and learn much faster to self-administer for these effects with direct administration into the blood stream (Schechter & Krimmer, 1992), bypassing the entire gastro-intestinal system.

Third, like OSA and IGSA, intravenous administration also allows for assessment of factors related to how much the animal is willing to work for ethanol administration and in what quantities, but without the ingestive considerations and potential limitations that IGSA poses. By utilizing selectively bred mice in combination with the IVSA behavioral paradigm, we may further support the idea that genetic differences in free-choice drinking are correlated with genetic differences in other assessments of ethanol-reinforced behavior.

One such genetic model are the selectively bred High Alcohol Preferring (HAP) and Low Alcohol Preferring (LAP) mice. HAP and LAP mice were chosen here because of their selectively bred high and low preference for alcohol drinking. LAP mice do not voluntarily drink alcohol in significant quantities, but their inclusion in this study is imperative to determine if they will self-administer ethanol when ethanol's potentially aversive taste is not a factor. My hypothesis that HAP mice will find IVSA of alcohol more reinforcing than LAP mice is based on home cage and OSA drinking data. However, a previous study has shown that LAP mice show increased CPP to a 4.0 g/kg injection of ethanol (Grahame, Chester, Rodd-Henricks, Li, & Lumeng, 2001b), suggesting that while LAP mice may not drink ethanol in significant amounts, they may find some component of the drug reinforcing. This was further supported by a recent IGSA publication where LAP mice administered as much alcohol as HAP mice following a period of passive exposure intragastrically (Fidler et al., 2010). Furthermore, in a previous IVSA alcohol study, two strains of inbred mice- C57BL/6J and DBA- that differ vastly in their free choice alcohol drinking behavior, were shown to have similar IVSA alcohol administration (Grahame & Cunningham, 1997). By utilizing IVSA, we may further explore this apparent paradox.

In home-cage drinking, HAP mice are clearly able to encounter the pharmacologic effects of alcohol due to the quantities of alcohol freely consumed. The LAP mice, however, do not readily consume alcohol, limiting their capacity to experience the pharmacological effects; another route of administration is warranted to assess potential reinforcing properties of alcohol. Through IVSA, one may assess reinforcing properties of a substance, in this case alcohol, in a way that is less dependent on preingestive factors, which is clearly a consideration in all oral administration paradigms.

Therefore, this dissertation attempts to address the question of alcohol reinforcement for pharmacological effects in mice selectively bred for high and

low alcohol preference by a non-oral systemic administration. My methodology of choice was to train the mice to press a bar in order to be reinforced by a small amount of alcohol administered directly into the blood stream. Increased lever pressing for alcohol reinforcements may be interpreted as being more reinforcing. However, it is also important to note that if a subject is more sensitive to the reinforcing properties of alcohol, fewer responses might be needed to get the desired result. Therefore, through a within subjects dose manipulation, I explored what unit dose of alcohol administered directly into the blood stream resulted in the highest response rate. A demonstrated dose response curve is desirable because it addresses the question of optimal dose and/or concentration of administration and the sensitivity of such doses and concentrations and whether these differ between the high and low preferring lines. An operant training reversal task was also implemented to help address the question of whether subject may be pressing the bar less for equal reinforcement. Here, the correct lever now become inactive and the previously inactive lever becomes the target for lever pressing to gain reinforcers. The subject must learn to inhibit lever pressing on the previously correct lever, and initiate increased lever pressing on a previously non-reinforced lever. This procedure has been used successfully in many previous studies looking at learning, but can also be utilized to assess the power of reinforcement seeking (Heyser, Fienberg, Greengard, & Gold, 2000; Murray, Ridley, Snape, & Cross, 1995; Wenger, Schmidt, & Davisson, 2004). In this portion of the experiment, a faster learning curve during the reversal phase would suggest greater reinforcement, or motivation for that reinforcement. Thus, the present hypothesis was that HAP mice would show evidence of greater alcohol reinforcement than their selectively bred opposite, LAP mice.

CHAPTER 2. METHODS

2.1. <u>Subjects</u>

HAP and LAP replicate lines were originally derived from HS/Ibg mice from the Institute for Behavioral Genetic at the University of Colorado Health Science Center and served as the progenitor stock (Grahame, et al., 1999b). From this progenitor stock, 24 hour home cage preference for 10% ethanol in tap water versus plain water two bottle choice was assessed. Mice exhibiting high preference for the alcohol bottle, and concurrently mice exhibiting very low preference, were selectively bred together for repeated generations. With this selection pressure applied, after 10 generations the high alcohol preferring mice (HAP) were consuming 10 g/kg ethanol/day while the low alcohol preferring mice were consuming approximately 2 g/kg ethanol/day. HAP mice consumed approximately 70% of their daily fluid from the ethanol solution, while the LAP mice sampled from the ethanol bottle for less than 10% of their total daily fluid intake (Grahame, et al., 1999b). This selection pressure was applied three separate times, resulting in replicate HAP/LAP line 1, HAP/LAP line 2, and HAP/LAP line 3 populations. Generations and drinking scores of those respective generations used for these studies are shown below in Table 2-1.

Table 2-1

Subjects were 138 mice at the start of the study. Generation, Replicate Line, and group drinking scores are indicated along with n for each phase of the studies conducted here. All subjects were selectively bred for either High- or Low-Alcohol home cage drinking preference.

		Etoh Drinking g/kg/day		Water Pretrain	
	Gen	Mean	SEM	Start	Finish
HAP2 F	31 S	21.82	0.54	35	23
HAP2 M	31 S	16.93	0.51	32	30
LAP 2 F	31 S	0.74	0.04	28	20
LAP 2 M	31 S	0.68	0.04	15	13
LAP 3 F	12 S	1.06	0.12	16	8
LAP 3 M	12 S	0.89	0.08	12	6
		Total		138	100

		IVSA						Water Posttrain	
	Gen	Start Acq	Finish Acq	Start D.R.	Finish D.R.	Start Rev.	Finish Rev.	Start	Finish
HAP2 F	31 S	8	5	1	1	4	3	7	7
HAP2 M	31 S	15	10	5	4	5	5	5	5
LAP 2 F	31 S	10	6	--	--	4	4	5	5
LAP 2 M	31 S	3	2	--	--	2	1	5	5
LAP 3 F	12 S	6	5	5	1	--	--	--	--
LAP 3 M	12 S	4	2	2	2	--	--	--	--
		46	30	13	8	15	13	22	22

2.2. <u>Pretraining for Water Reinforcers</u>

Based on pilot data, it was determined that mice required experience learning to lever press for reinforcement prior to surgical implantation of the catheter for intravenous self-administration of alcohol. Without prior training, mice were exceeding catheter patency duration during acquisition of the task. In other words, it was taking longer for the majority of the mice to acquire set acquisition parameters than the catheters remained patent. This problem was remedied by implementing a pretraining protocol where mice were water deprived for 20 hours per day and then placed in two-lever choice operant boxes.

Over a period of seven to eleven days, mice (N=138) learned to press the correct lever in order to gain access to brief presentations of a sipper tube containing plain tap water. Response requirements were increased from a Fixed-Ratio 1 (FR1) schedule to FR3 schedule on day four and mice were required to maintain at least ten water reinforcements during the 60-minute sessions for at least three consecutive days and consume at least 0.1 mL of water each session at FR3. Mice (N=38) that had not reached criteria by day eleven were eliminated from the study (Figure 2-1). Mice that did reach criteria then underwent catheterization surgery for the next phase of the experiment.
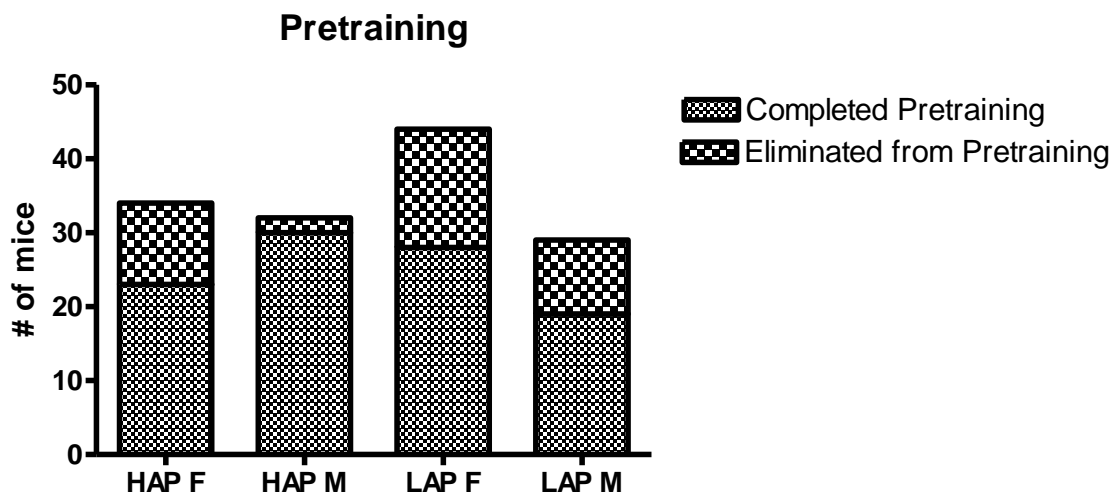
Pretraining

Figure 2-1

Mice underwent a pretraining phase to acclimate them to lever pressing for reinforcements. Those subjects not reaching criteria were eliminated from the study.

Animals. Mice were 23 HAP2 females, 30 HAP2 males, 20 LAP2 females, 13 LAP2 males, 8 LAP3 females, and 6 LAP3 males born in the IUPUI School of Science Animal Care Facility. Mice were individually housed in polycarbonate cages (27.9 x 9.5 x 12.7 cm) with Cellsorb bedding, at an ambient temperature of 21º C (± 1º) and lights on from 2000 to 0800. All mice had ad lib access to food. Water access was restricted to two hours per day, available two hours after testing. A flow chart tracking the mice from water pretraining through the rest of the study can be found in Figure A-1 in the Appendix.

Apparatus. Mice were transported in a light shielded transporter to the testing room; the mice were tested for one 60-minute session each day between 0830 and 1130 and were always tested in the dark. Twenty-four identical operant chambers that measured 21.6 x 19.7 x 12.7 cm inside, with 2 sides constructed of clear acrylic and 2 sides of aluminum (Med Associates, St. Albans, VT) were

used for water pretraining acquisition of operant responding. The operant chamber was contained in a sound- and light-attenuated box that was equipped with a fan for ventilation and background noise. An LED∕nose-poke infrared detector was centered on the 19.7 cm side at 6.3 cm above the floor, and illumination of that LED signaled the beginning of a trial. Below the LED∕nose-poke detector was the sipper access hole, through which the sipper descended when mice were being reinforced. One lever was assigned as the "correct" lever for each subject; correct lever was counterbalanced between all subjects and maintained as the "correct" lever throughout all phases of testing. Responses on the "correct" lever provided reinforcement paired with a cue light. During this phase, the reinforcement was presentation of a sipper tube containing water. The sipper tube was a 10 mL graduated plastic serological pipette fitted with a stainless steel tip (Ancare, Belmont, NY). The sipper tube could be extended into the box for five seconds of sipper access. The sipper tube was filled with tap water that served as the reinforcer.

Data Collection. At the end of each session, consumption volumes were visually read from the tube with a resolution of 0.1 mL. Levers were mounted 2.5 cm above the floor on either side of the sipper tube opening. Each lever had an LED 2.3 cm above it that remained inactive during the sessions. A centrally-located house light was illuminated during sipper tube extension, indicating presence of reinforcer. Control of the operant chambers and collection of data were performed via the MedPC IV software and MedPC interface cards on a PC-compatible computer.

Data Analysis. All data were sorted in Microsoft Excel (Redmond, WA), and statistical analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, IL). Chi Square was performed to determine any Line or Replicate differences in subjects reaching criteria. Lack of HAP3 mice prevented any Replicate analysis of variance to be assessed. Therefore, *a priori* data were separated by Line, and

LAP2 was compared to LAP3 data in a Replicate X Sex X Day ANOVAs for the final two days of pretraining for correct lever presses, incorrect lever presses, percent correct, and amount of water consumed. When no interactions were present, LAP data was collapsed across Replicate.

Next, two-way repeated measures ANOVAs of Line X Sex across Day and FR were performed for percent correct, correct lever presses, incorrect lever presses, and water consumed dependent variables. When no Sex interactions were seen, data were collapsed across sex for further analysis of variance of Line X Day X FR. Differences were considered significant when $p < 0.05$ for all analyses.

2.3. Acquisition of Intravenous Alcohol

Animals. One hundred mice underwent catheterization surgery. Of those, 46 survived surgery and the recovery phase with patent catheters. These mice consisted of 15 HAP2 males, 8 HAP2 females, 3 LAP2 males, 4 LAP3 males, 10 LAP2 females, and 6 LAP3 females. Of these, 30 completed acquisition of intravenous alcohol at a dose of 75 mg/kg/infusion with patent catheters. Three mice lost patency and were eliminated during acquisition, and thirteen mice did not meet criteria of at least 10 infusions at 75 mg/kg/infusion.

Surgery. Each subject meeting pretraining criteria was weighed and received a 5mg/kg subcuetaneous injection of carprofen non-steroidal analgesic 30 minutes prior to surgery. Mice were anesthetized via 5% (v/v) isoflurane gas vaporized in oxygen gas in a gas chamber with a flow rate of 0.5 l/min, then outfitted with a customized nose cone mask which administered the isofluane/oxygen mixture at a flow rate of 0.1 l/min throughout surgical preparation and through the remainder of the surgery. After surgical anesthesia was achieved as evidenced by loss of toe pinch flinch reflex, the area over the right external jugular vein and 2 square cm on the midline of the back halfway between the neck and tail was shaved and

disinfected using iodine. Mice were maintained at 36 º C using an isothermic heating pad, and body termperature was continuouslys monitored using a rectal probe. A 0.5 cm incision in the neck was made and the right external jugular vein was isolated using forceps. Two lengths of 5-0 surgical silk was threaded under the vein approximately 6mm apart. The rostral suture was then tied to close off the jugular vien. A 1.5 cm incision was made on the back in the shaved area. The tip of the cannula was pulled subcutaneously from the dorsal incision until it exits through the ventral incision. The subcutaneous skin button with Dacron patch (SAI part # SBD-01) surrounding the catheter was then secured beneath the dorsal skin via a single suture and the incision was closed using cyanoacrylic cement and a single 5-0 surgical silk suture. After the glue dried, the mouse was turned over and the catheter was then connected to the heparin delivery syringe and flushed to remove any air bubbles that may have formed in the line.

Sterilized silicone jugular catheters were ordered premade with Silicone beads located 11 mm and 29 mm from a beveled tip (SAI part # MJC-05), with the distance from the silicoln bead portion of the catheter premeasured to 11 mm in order to place the beveled tip of the catheter just above the right atrium on the average sized mouse once inserted. The jugular vein was then retracted using a weight attached to the tied rostral suture and a small incision was then made in the vein using a bent 27-G needle. With the needle still in the vein, the beveled end of the catheter was threaded into the vein and the needle was removed. The catheter was advanced until the silicoln bead of the catheter reached the entrance to the vein. The caudal length of the 5-0 silk was pulled tight around the jugular vein with the catheter inside. A thrid length of 5-0 silk was inserted into the musculature just dorsal to a second silicoln bead using a curved needle, and the suture was tied around the second bead to secure the catheter.

After testing to verify that blood was obtainable from the catheter by retracting on the attached syringe, approximately 20 microliters of heparinized saline (10

U/mL) was injected to prevent clotting inside the catheter. The ventral incision was then closed with medical-grade cyanoacrylic cement and the externalized portion of the catheter was immediately clamped with a vascular clip (RS 5452) to prevent backflow of blood and subsequent blockage of the catheter. Medical-grade cyanoacrylic cement (3M Vetbond item # 1469SB) was used because studies have shown its superiority to other methods of closure such as suture in mice (Sabol et al., 2010).

Each surgery lasted approximately 15-25 minutes, and mice were monitored regularly for proper recovery. Mice were fully recovered from anesthesia within 5 minutes following surgery. Following surgery, mice were given two days for recovery and monitoring of their condition. During this time, and throughout the study, mice received daily flushing of the cannula with heparinized saline to help keep the catheter clear of clots and increase length of catheter patency. Also on the second day of recovery, mice were subjected to a Brevitol test (a fast-acting, short-acting barbiturate that immobilizes the subject for a few seconds immediately after injection if cannula is patent) where 0.05 mL (16 mg/kg) was injected via the cannula into the jugular vein to ensure cannulae patency. Mice with patent catheters lost consciousness and righting reflex within two seconds of Brevitol infusion. Only mice with patent catheters were chosen to participate in the next phase of the study.

Apparatus. Mice were transported in a light shielded transporter to the testing room; the mice were tested for one hour each day between 0800 and 1400 and were always tested in the dark. Four identical operant chambers that measured 12.7 cm x 12.7 cm x 11.4 cm (l x w x h) with 2 sides constructed of clear acrylic and 2 sides of aluminum (Med Associates, St. Albans, VT) were used for intravenous alcohol operant administration. On the front of each modular test chamber two 1.6-cm wide ultra-sensitive mouse operant levers were mounted which protruded 0.95 cm into the chamber. Each operant chamber was

contained in a sound- and light-attenuated box that was equipped with a fan for ventilation and background noise. A signal light, which illuminated during the duration of the alcohol infusion to serve as a cue of reinforcer administration, was centered on the wall containing the levers 10 cm above the floor. The tubing connecting the mouse to the syringe pump threaded through the ceiling of the chamber via a tether and swivel system (Instec, PA). A syringe pump (Med Associates PHM-100) located outside the sound- and light-attenuating boxes was calibrated and checked once weekly for proper dose administration.

During acquisition of lever pressing for alcohol reinforcements, subjects bar pressed at a Fixed Ratio 1 (FR1) schedule for a 75 mg/kg unit dose of intravenous (i.v.) alcohol shown by Grahame & Cunningham (1997) to be effective in producing consistent responding. During the next few days, as each subject met response criteria of three consistent days of at least ten reinforcements over the sixty-minute session, the number of correct lever presses required was increased to FR 3. Training continued until each subject met one of the following: reached a set response criterion of at least 10 infusions per session for three consecutive days at FR3 with consistent responding within 25% of the average of those three days; lost cannula patency; or 9 days had passed without meeting criteria. Also, if a subject had consistently low responding, an attempt to increase responding by lowering the unit dose of alcohol to 25 mg/kg was implemented after at least four days of low (less than 10 reinforcements per session) responding. These decisions were based on previously reported response criteria for stable responding, metabolic rate of clearance for these mice reaching approximately a pharmacologically significant dose of 0.75 g/kg/hour and pilot study data from the present study (Grahame & Cunningham, 1997, 2002; Grahame, et al., 1999b; Grahame, Low, & Cunningham, 1998).

Mice were weighed daily, catheters were flushed with approximately 20 microliters of heparinized saline (10 U/ml), and Brevitol tested every other day following testing sessions to ensure canula patency.

Data Collection. Control of the operant chambers and collection of data were performed via the MedPC IV software and MedPC interface cards on a PC-compatible computer. Measures recorded included: number of days to criterion, number of lever presses on the active ("correct") and inactive ("incorrect") bars, percent correct, number of infusions, as well as distribution of bar presses throughout the session. Total dose administered for each session was extrapolated from the number of infusions and volume and concentration of alcohol of each infusion.

Data Analysis. All data were sorted in Microsoft Excel (Redmond, WA), and statistical analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, IL). Chi Square was performed to determine any Line or Replicate differences in subjects reaching criteria. Lack of HAP3 mice prevented analysis of variance with Replicate as a factor to be assessed. Therefore, *a priori* data were separated by Line, and LAP2 was compared to LAP3 data in a Replicate X Sex X Day X FR ANOVAs across IVSA acquisition for correct lever presses, incorrect lever presses, percent correct, and total dose. When no interactions were present, LAP data were collapsed across Replicate. Then, repeated measures mixed ANOVAs of Line X Sex X Day X FR were performed for percent correct, correct lever presses, incorrect lever presses, and total dose variables. When no Sex interactions were seen, data were collapsed across sex for further analysis of variance of Line X Day X FR. Additionally, FR3 data were examined *a priori* for Line X Day potential differences via analysis of variance. Finally, the possibility of Line differences to the change in FR requirement were analyzed *a priori* through a Line X Day repeated measures ANOVA for just Day 3 and Day 4 of acquisition on correct lever presses, incorrect lever presses, reinforcements,

dose, and percent correct, when the FR requirement was increased from FR1 to FR3. Differences were considered significant when $p < 0.05$ for all analyses.

2.4. Dose Response of Intravenous Alcohol

Mice having met criteria for acquisition then were subjected to a within subjects dose response manipulation. Cannula patency was confirmed upon completion of each dose. Each subject received two days each of the following doses: 25, 75, 125 mg/kg unit dose of i.v. alcohol in a counterbalanced order. These doses were chosen due to previously published literature (Grahame & Cunningham, 1997; Grahame, et al., 1998) to provide a dose-response curve for the IVSA of alcohol. The dose of 75 mg/kg was repeated as a comparison to the final three days of the acquisition phase.

Animals. 4 HAP2 males, 1 HAP2 female, 2 LAP3 males, 1 LAP3 female completed the dose response portion. These subjects were a randomly-selected subset of the subjects having successfully completed the IVSA acquisition portion of the study at 75 mg/kg/infusion and maintained catheter patency throughout this portion of the study.

Apparatus. Identical to procedure and apparatus as stated above in section 2.2. During dose response, subjects bar pressed at a Fixed Ratio 3 (FR3) schedule was used for the duration of testing. Each subject received two consecutive days at each dose as long as catheters remained patent. Mice were weighed daily, catheters were flushed with approximately 20 microliters of heparinized saline (10 U/ml), and Brevitol tested every other day following testing sessions to ensure canula patency.

Data Collection. Control of the operant chambers and collection of data were performed via the MedPC IV software and MedPC interface cards on a PC-compatible computer. Measures recorded included: number of lever presses on

the active ("correct") and inactive ("incorrect") bars, percent correct, number of i.v. infusions, as well as distribution of bar presses throughout the session. Total dose administered for each session was extrapolated from the number of infusions and volume and concentration of alcohol of each infusion.

Data Analysis. All data were sorted in Microsoft Excel (Redmond, WA), and statistical analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, IL). Mixed ANOVAs of Line X Dose at each Day were performed for percent correct, correct lever presses, incorrect lever presses, number of reinforcers, and total dose dependent variables. Differences were considered significant when $p < 0.05$ for all analyses.

2.5. Lever Reversal of Intravenous Self Administration

Cannula patency was confirmed upon completion of acquisition, and a subset of mice then underwent a lever reversal manipulation. The lever previously associated with the alcohol infusion no longer resulted in alcohol infusion (or house light illumination) and the lever that was previously inactive became the lever associated with illumination of house light and alcohol infusion at a reduced FR1 schedule for four days.

Animals. 5 HAP2 males, 3 HAP2 females, 1 LAP2 male, 4 LAP2 females, completed the reversal portion. These subjects were a randomly-selected subset of the subjects having successfully completed the IVSA acquisition portion of the study at 75 mg/kg/infusion and maintained catheter patency throughout this portion of the study.

Apparatus. Identical to procedure and apparatus as stated above in section 2.2. Subjects reached a set response criterion of at least 10 infusions per session for three consecutive days at FR3 with consistent responding within 25% of the average of those three days prior to lever reversal. Then, reducing the

requirement to an FR1 schedule for the new correct lever, subjects were assessed to determine if the mice would learn to now direct lever pressing to the previously "incorrect," or non-reinforced lever to acquire alcohol infusions. The similar criteria were set as during the acquisition phase; mice were considered to have completely reversed their preferred lever if they maintained at least 10 reinforcements over the hour-long session for at least three consecutive days and maintained a number of responses within 25% of the average of those three days.

Mice were weighed daily, catheters were flushed with approximately 20 microliters of heparinized saline (10 U/ml), and Brevitol tested every other day following testing sessions to ensure canula patency.

Data Collection. Control of the operant chambers and collection of data were performed via the MedPC IV software and MedPC interface cards on a PC-compatible computer. Measures recorded included: number of days to criterion following lever reversal, number of lever presses on the active ("correct") and inactive ("incorrect") bars, percent correct, number of infusions, as well as distribution of bar presses throughout the session.

Data Analysis. All data were sorted in Microsoft Excel (Redmond, WA), and statistical analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, IL). Data was collapsed across sex *a priori* to facilitate statistical analysis due to only having one LAP male subject complete this task with a patent catheter. Pre-lever reversal data were analyzed via Line X Day ANOVAs for correct lever presses, number of reinforcements, and percent correct. Because of differential responding between the lines pre-reversal, post-reversal analysis was also transformed into percent change from pre-reversal baseline. Using these data, Line X Day repeated measures ANOVAs were used to assess any differences in responding following correct lever reversal for reinforcers, correct lever presses,

incorrect lever presses, percent correct, and total dose. Differences were considered significant when $p < 0.05$ for all analyses.

2.6. <u>Post Test with Water Reinforcers</u>

Upon completion of the dose response or upon loss of catheter patency, healthy subjects then completed a post-test for water reinforcements as their final task. This was to ensure that lever pressing behavior was still possible. Subjects from the lever reversal portion of the study were not included because of the possibility of correct lever confusion resulting from the reversal of correct levers. Over a period of three days, mice were placed back in the original testing apparatus to lever press at an FR3 schedule for water reinforcements during 60-minute sessions. Mice were again subject to 20 hour water deprivation during these three days.

<u>Animals</u>. Mice were 7 HAP2 females, 6 HAP2 males, 6 LAP2 females, and 4 LAP2 males, born in the IUPUI School of Science Animal Care Facility. Mice were individually housed in polycarbonate cages (27.9 x 9.5 x 12.7 cm) with Cellsorb bedding, at an ambient temperature of 21º C (± 1º) and lights on from 2000 to 0800. All mice had ad lib access to food. Water access was restricted to two hours per day, available two hours after testing.

<u>Apparatus</u>. Identical to water pre-training detailed above in section 2.1.

<u>Data Collection</u>. Identical to water pre-training detailed above in section 2.1.

<u>Data Analysis</u>. Identical to water pre-training detailed above in section 2.1 except that no replicate line 3 mice were included. This negated the necessity for statistical analysis involving replicates. Also, no mice were eliminated from this portion of the study so no chi square was necessary.

CHAPTER 3. RESULTS

3.1. <u>Pretraining for Water Reinforcers</u>

As expected, aside from minor differences mentioned below, both HAP and LAP mice successfully acquired the pretraining task, associating behavior directed toward a specific lever with an outcome. Regardless of Line, approximately 72 percent of all trained subjects reached criteria for completion of pretraining, which was three consecutive days of at least 10 reinforcers delivered at FR3 plus consumption of at least 0.1 mL of water at a minimum of 80% accuracy. Mice eliminated from the study included 11 HAP2 females, 2 HAP2 males, 16 LAP females (8 of which were LAP3), and 8 LAP males (6 of which were LAP3). As shown in Figure 2-1, there was no difference between HAP and LAP mice for completing pretraining [X^2 (1,137) = 3.42, p > 0.05].

Lack of HAP3 mice prevented any Line X Replicate X Sex X FR X Day analysis of variance to be conducted. LAP2 and LAP3 mice reached completion criteria and expressed similar rates of responding and percent correct during the final two days of pretraining. This is shown in non-significant interactions in correct lever presses [$F(1,44) = 0.07$, $p = 0.80$] with no main effect of Replicate [$F(1,44) = 0.21$, $p = 0.65$], percent correct [$F(1,44) = 0.05$, $p = 0.83$] with no main effect of Replicate [$F(1,44) = 0.21$, $p = 0.65$], or number of reinforcers [$F(1,44) = 1.52$, $p = 0.22$] with no main effect of Replicate [$F(1,44) = 0.17$, $p = 0.68$]. Therefore, statistical analyses are reported both with LAP2 and LAP3 replicates combined.

Selective breeding for alcohol preference did not generally alter the acquisition of lever pressing for water reinforcements (see Figures 3-1 through 3-3). When

assessing the FR3 data only, there was a significant main effect of Line for incorrect lever presses, due to the LAP mice producing fewer incorrect lever presses than HAP mice overall [F(1,98) = 11.36, p = 0.01], and a main effect of Line for percent correct due to the LAP mice having higher percent correct overall [F(1,98) = 12.38, p = 0.01]. No significant interactions were seen except for in the amount of water consumed data, where there was a Line X Sex interaction [F(1,96) = 12.81, p < 0.01]. This was due to the male HAP mice drinking considerably more water during the sessions (see Figure 3-4).

Otherwise, both lines showed similar and significant responding on the correct lever [F(3,294) = 92.52, p < 0.01], percent correct [F(3,294) = 16.15, p < 0 .01], and consumed reinforcers [F(3,294) = 20.53, p < 0.01] main effect across days, indicating that the task was sufficiently learned.

Acquisition of Correct Lever Presses
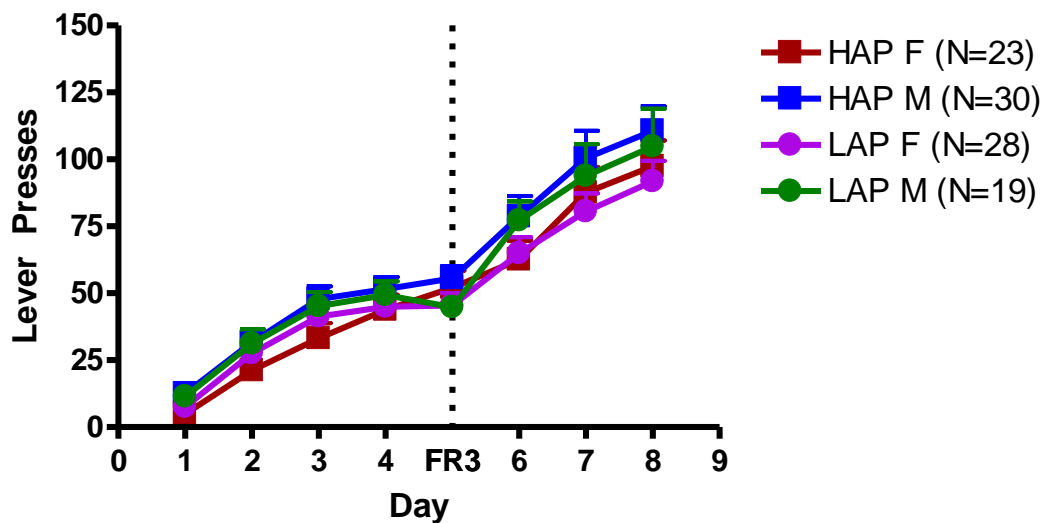
Figure 3-1
HAP and LAP of both sexes learned the pretraining task sufficiently as indicated
by number of lever presses per session.
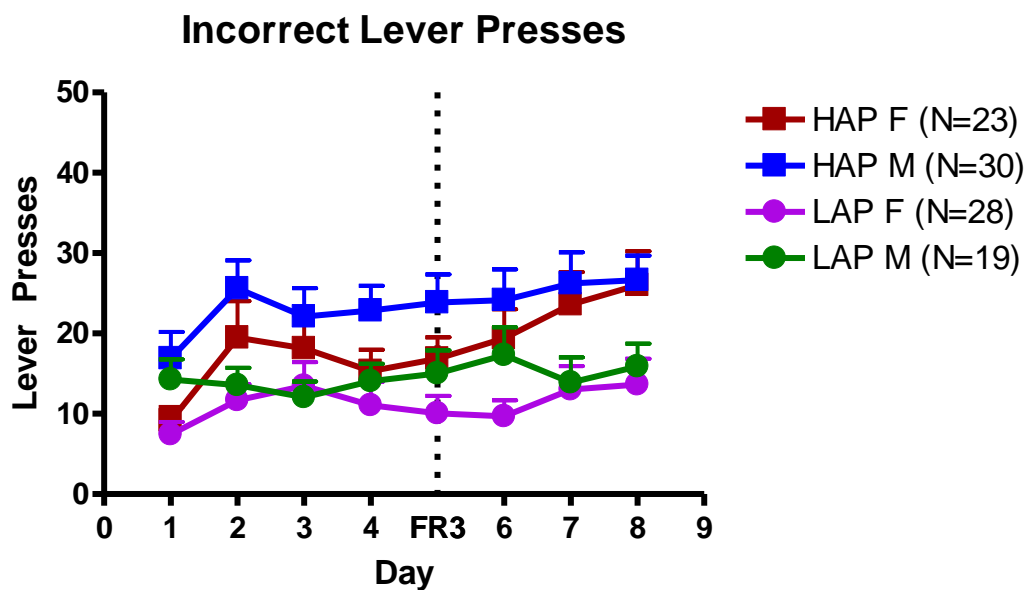
Figure 3-2
LAP subjects performed fewer lever presses on the incorrect lever as compared
to the HAP subjects.
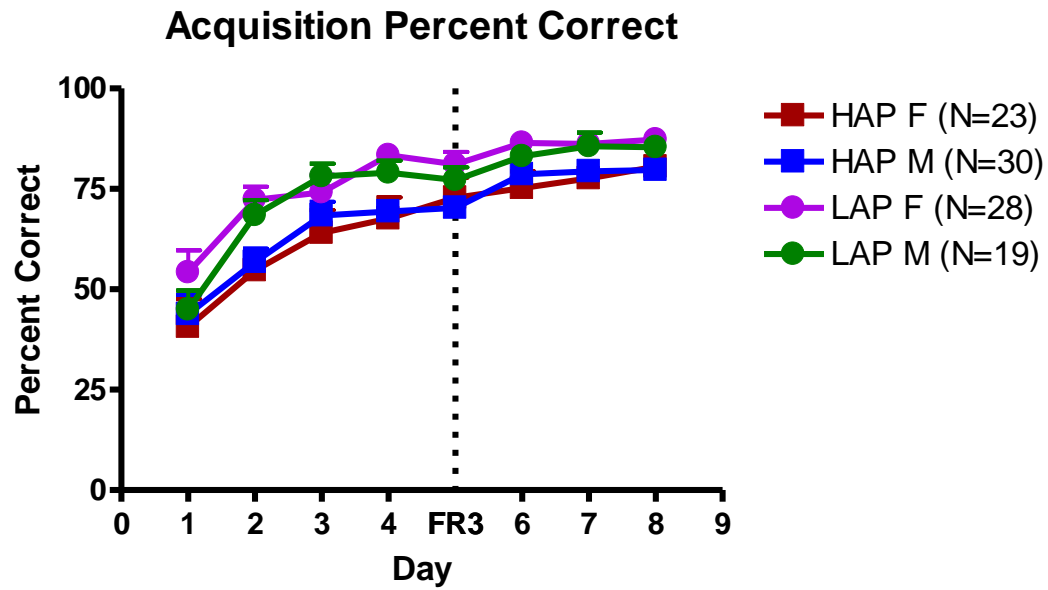
Figure 3-3

HAP and LAP of both sexes learned the pretraining task sufficiently as indicated by percent correct per session, however, LAP mice had a significantly higher percentage correct than HAP mice.

Acquisition Water Consumed
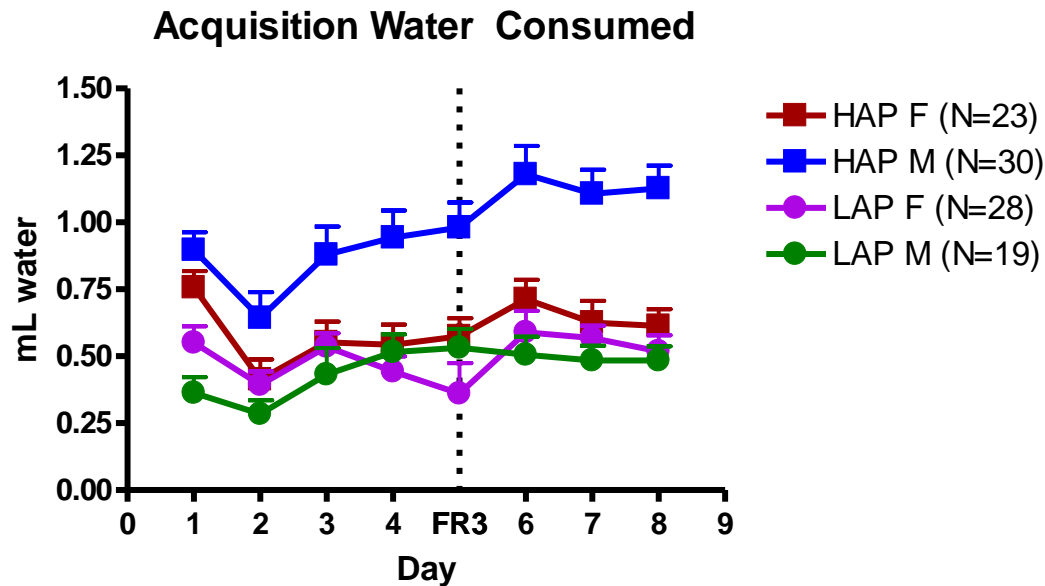
Figure 3-4

Although all mice meeting criteria consumed significant quantities of water, water consumed differed between HAP male subjects and HAP females and LAP mice.

3.2. Acquisition of Intravenous Alcohol

LAP2 and LAP3 mice did not differ in their responding on either lever, percent correct, or dose administered as indicated by lack of a 4-way (Replicate X Sex X FR X Day) interaction and data were therefore collapsed across LAP replicate lines. Male and female subjects responded similarly and no significant interactions with sex were seen, so data were then collapsed across sex and (Line X Day X FR) repeated measures ANOVAs were used to analyze all dependent variables. Furthermore, ratio schedule was separated *a priori* to analyze days where FR3 was implemented in Line X Day ANOVAs.

In support of the hypothesis that LAP mice find intravenous alcohol less reinforcing than HAP mice, there was a main effect with the LAPs in that they were less willing to do work and administer less alcohol than the HAP mice. This is evident in main effects of Line during the first three days at FR3 in the variables of correct lever presses [$F(1,22) = 13.19$, $p < 0.01$], reinforcers [$F(1,22)$

= 9.32, *p* < 0.01], and dose [F(1,22) = 9.34, *p* < 0.01] but not in a Line difference in accuracy [F(1,22) = 1.24, *p* = 0.28].

As a result of the pretraining prior to catheter placement, subjects demonstrated a less dramatic learning curve for acquisition of intravenous alcohol than normally expected. However, there was a significant increase of correct lever presses over days (Line X Day X FR) with a main effect of days [F(2,44) = 3.17, *p* = 0.05] and of correct lever press [F(2,56) = 3.76, *p* = 0.03], supporting the hypothesis that subjects find the consequence of correct lever pressing reinforcing (see Figures 3-5 through 3-9).

When assessing possible differences between Line as a result of changing the FR requirement between days 3 and 4 of acquisition, it was found there was a significant Line X Day interaction dependent on the FR change for correct lever presses [F(1,25) = 4.15, *p* = 0.05] with a significant main effect of Line [F(1,25) = 7.57, *p* = 0.01] but not Day [F(1,25) = 1.81, *p* = 0.19] in which the HAP mice increase their responding on the correct lever but the LAP mice do not immediately alter their behavior to maintain a pharmacologically significant dose of alcohol. This interaction was not present in number of reinforcers [F(1,25) = 0.08, *p* =0.77], however there was a main effect of Day [F(1,25) = 11.61, *p* < 0.01] and Line [F(1,25) = 6.20, *p* = 0.02] for number of reinforcers. There were no significant interactions for percent correct, incorrect lever presses, or dose.
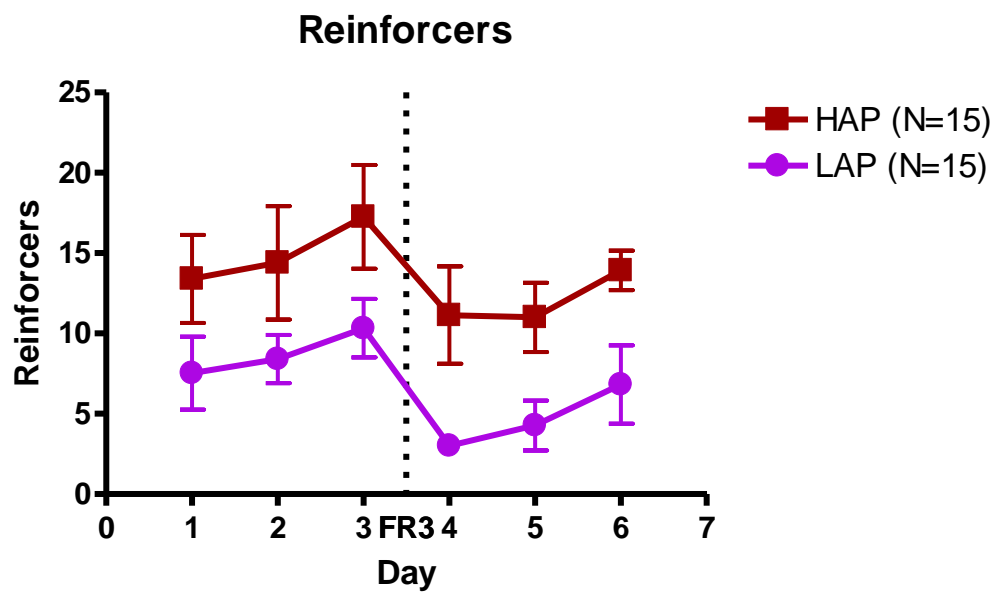
Figure 3-5
Total number of intravenous alcohol reinforcers given per session with 75 mg/kg
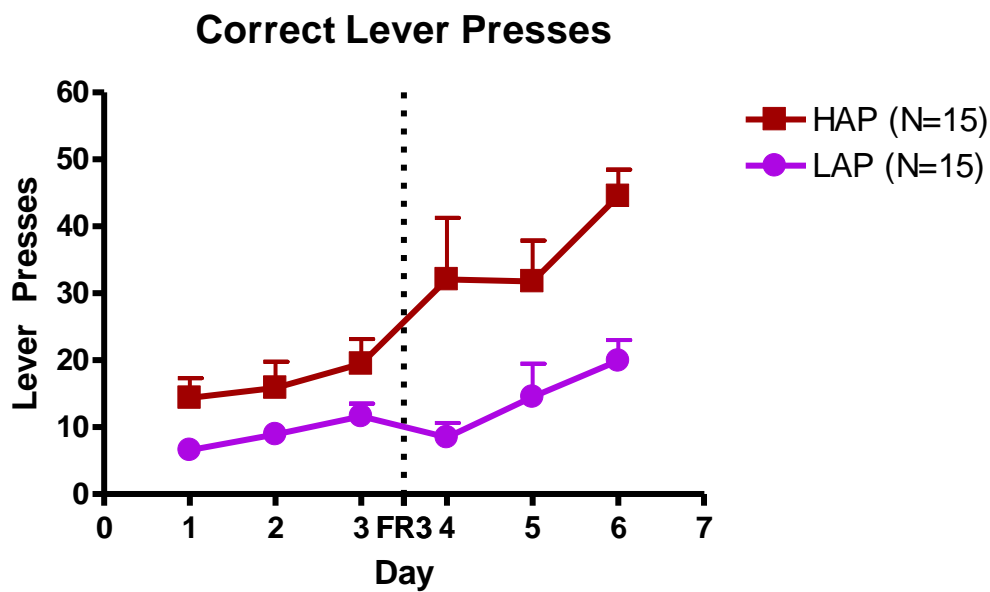per infusion dose.

Figure 3-6
Total number of correct lever presses per session during acquisition of
intravenous alcohol at 75 mg/kg per infusion dose.
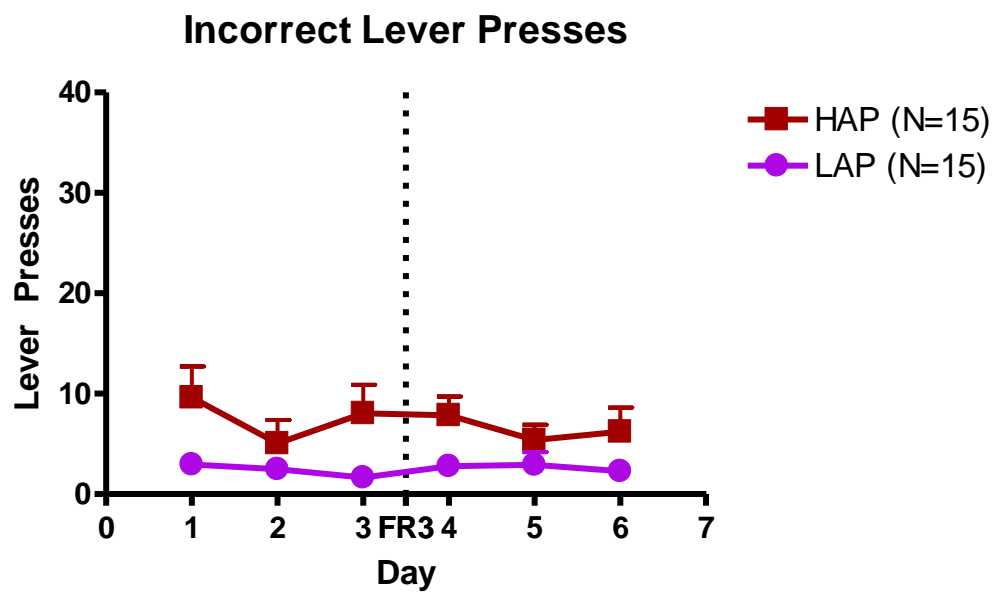
Incorrect Lever Presses

Figure 3-7
LAP subjects had fewer lever presses on the incorrect lever during IVSA acquisition.
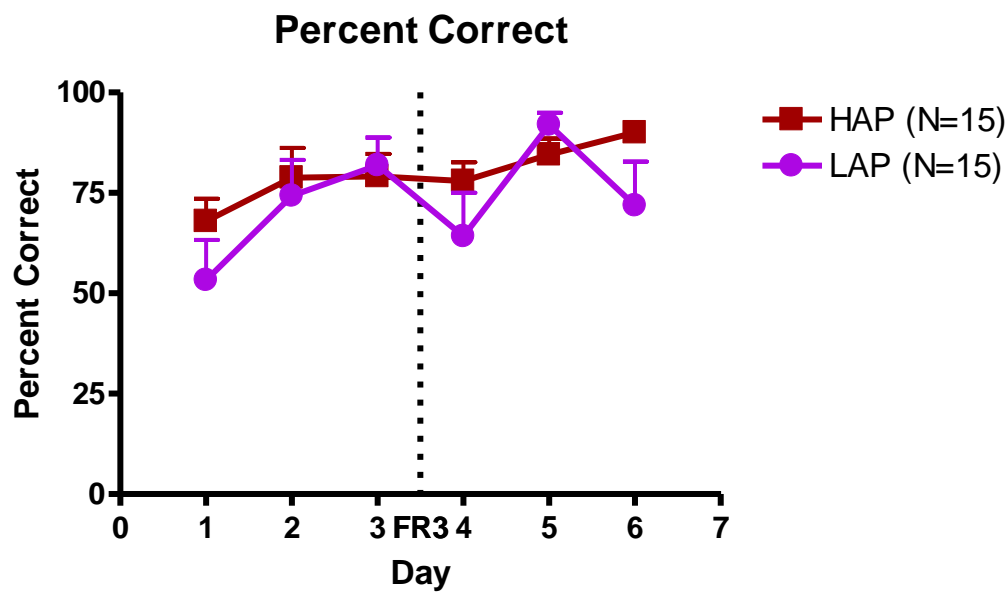
Figure 3-8
HAP mice gradually improved percent correct for lever pressing for 75 mg/kg per
infusion of intravenous alcohol during acquisition while LAP mice became
variable following the increase in FR requirement.
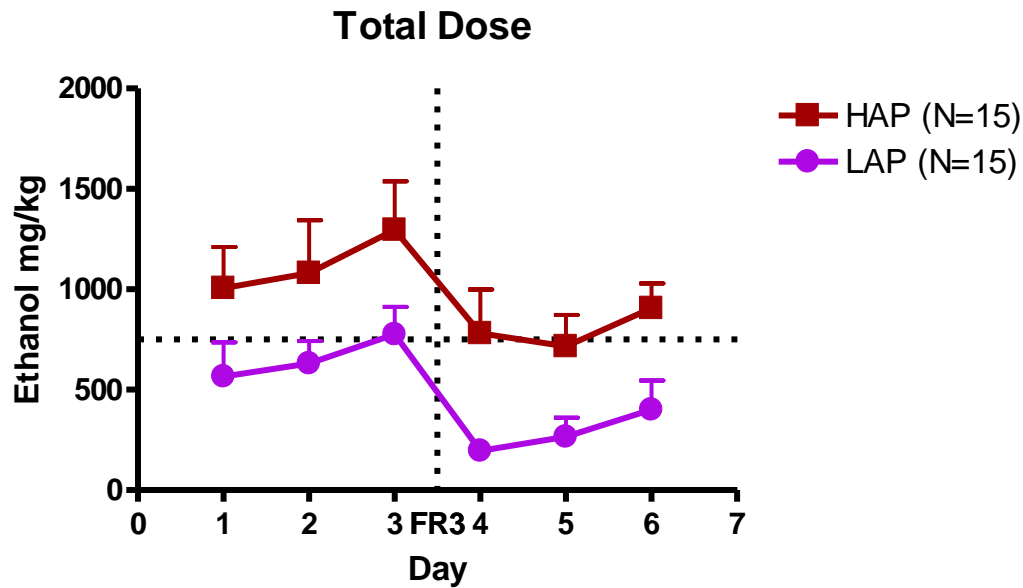
Total Dose

Figure 3-9

Total amount of alcohol administered per session during acquisition of intravenous alcohol for 75 mg/kg per infusion. Rate of alcohol metabolism/clearance is indicated at 750 mg/kg (Grahame, et al., 1999b) by the horizontal dotted line. Doses higher than this line indicate an approximate theoretical pharmacological significance for these subjects based on previously published data.

3.3. Dose Response of Intravenous Alcohol

No significant Line differences or effects of dose were seen in responding for varying doses of intravenous alcohol (see Figures 3-8 through 3-12). There were no interactions or main effects for any dependent variables measured except for a main effect of dose [$F(2,12) = 8.14$, $p < 0.01$]. Interestingly, however, the LAP mice tended to administer an overall lower dose than the HAP mice, regardless of what the infusion dose size was, and to shift their responding following the first day at a new infusion dose to regulate back to the metabolic rate of clearance, which was not evident in the HAP mice, as shown by a non-significant trend of an interaction of Day X Line [$F(1,6) = 3.73$, $p = 0.10$] and of a non-significant main effect of Line [$F(1,6) = 4.02$, $p = 0.09$].
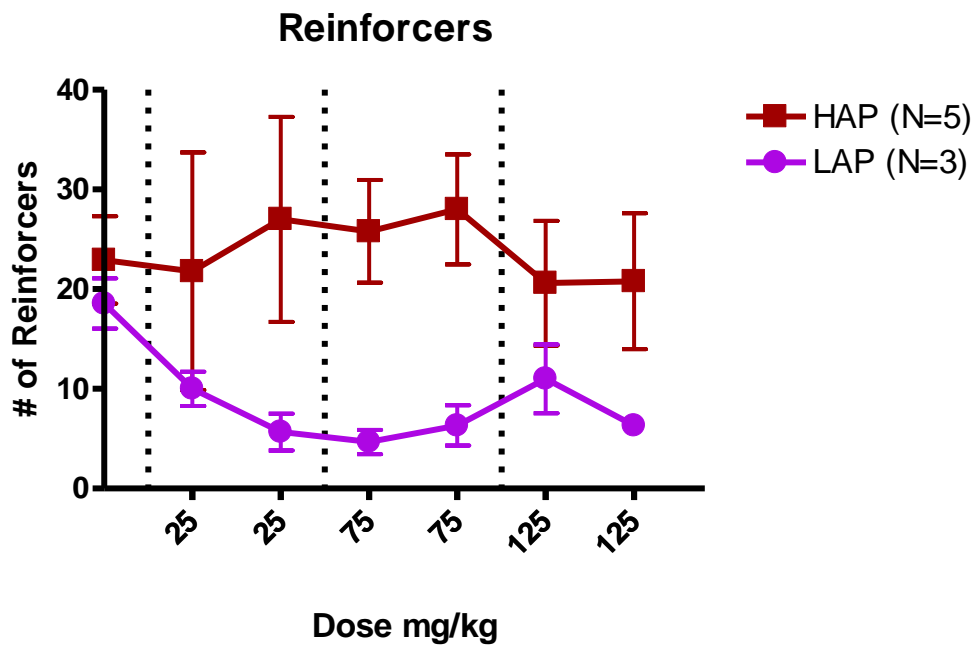
Figure 3-10
Total number of intravenous alcohol reinforcers given per session. Subjects
received two consecutive days at each dose in a counterbalanced order of
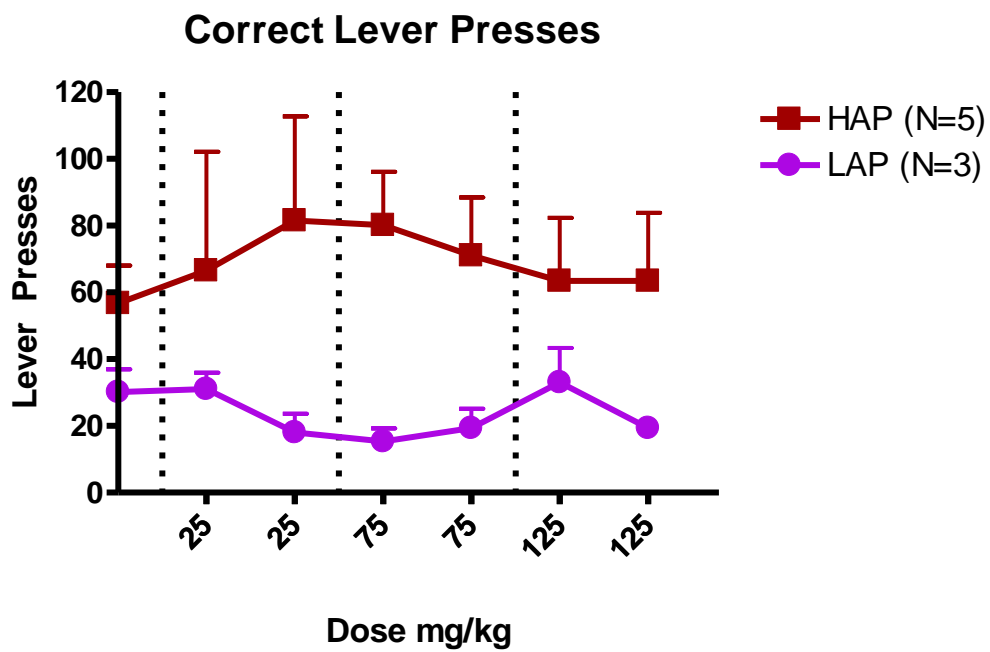presentation.

Figure 3-11
Total number of correct lever presses per session at various doses of
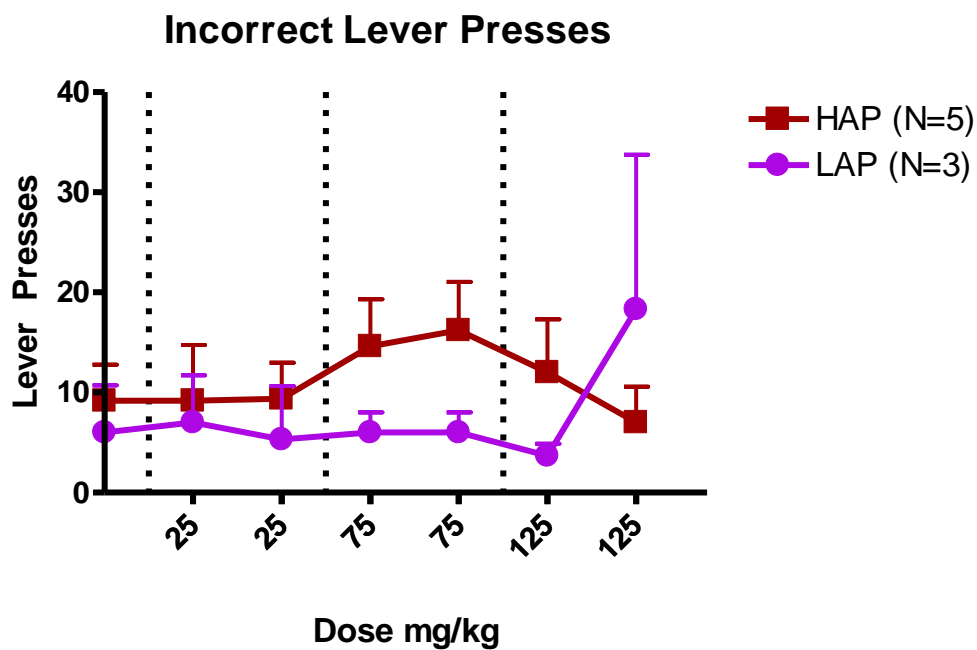intravenous alcohol infusion.

Figure 3-12
Pattern of HAP mice pressing the incorrect lever more than LAP subjects
continued through dose response, however LAP mice greatly increased their
variability and number of incorrect lever presses on the second day at 125
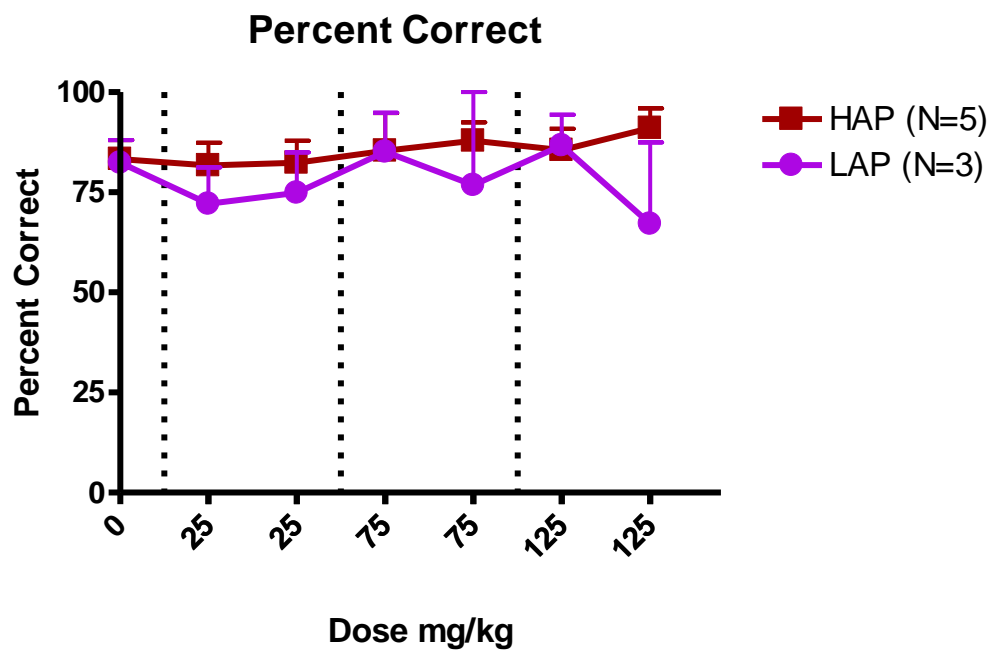mg/kg/infusion.

Figure 3-13
Percent correct per session at various doses of intravenous alcohol infusion.
Both HAP and LAP subjects maintained high percentage correct responding for
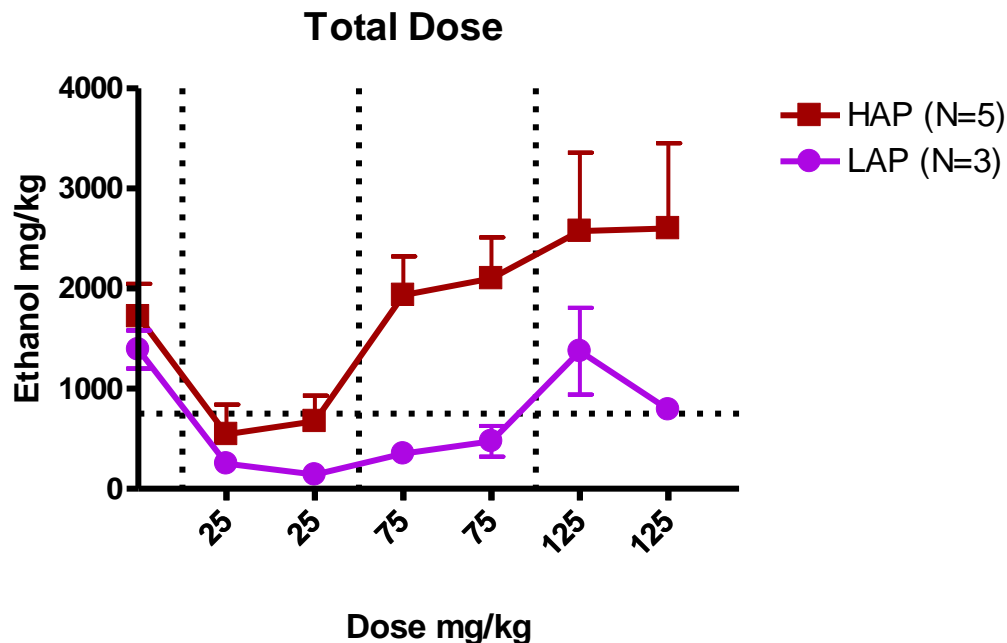i.v. alcohol at all three doses.

Figure 3-14
Total dose of alcohol administered per session, with the line at 750 mg/kg
representing the rate of clearance (Grahame, et al., 1999b). Values above this
line indicate approximate theoretical pharmacological significance for these
subjects based on previously published data.

3.4. Lever Reversal of Intravenous Self Administration

In order to assess the ability of the Lines to track the location of the lever that
would result in intravenous alcohol infusions, a lever reversal was performed on
a subset of mice. Following reversal, HAP mice show evidence of reinforcement
by intravenous alcohol via altering behavior to the new lever providing
intravenous alcohol infusions (see Figures 3-15 through 3-22). This was shown
by a main effect of Line on correct lever presses [$F(1,7) = 6.49$, $p = 0.04$)],
reinforcers [$F(1,7) = 6.49$, $p = 0.04$)], but there was not a main effect of Line for
incorrect lever presses [$F(1,7) = 1.32$, $p = 0.34$)] or percent correct [$F(1,7) = 0.00$,
$p = 0.99$]. There were no significant interactions. Because pre-reversal baseline
responding differed significantly between the HAP and LAP mice in correct lever
presses [$F(1,11) = 5.19$, $p = 0.04$)], reinforcers [$F(1,11) = 5.85$, $p = 0.03$)], and

percent correct [$F_{(1,11)}$ = 5.95, p = 0.04)], post-reversal data were transformed to percent baseline and Line X Day repeated measures ANOVAs were performed. This analysis showed significant interactions for correct lever presses [$F_{(3,21)}$ = 10.49, p = 0.02)] with a main effect of Line [$F_{(1,7)}$ = 10.22, p = 0.02)], total lever presses [$F_{(3,21)}$ = 6.17, p = 0.04)] with a main effect of Line [$F_{(1,7)}$ = 9.62, p = 0.03)], and total reinforcers [$F_{(3,21)}$ = 6.49, p = 0.03)] with a main effect of Line [$F_{(1,7)}$ = 16.23, p = 0.02)].
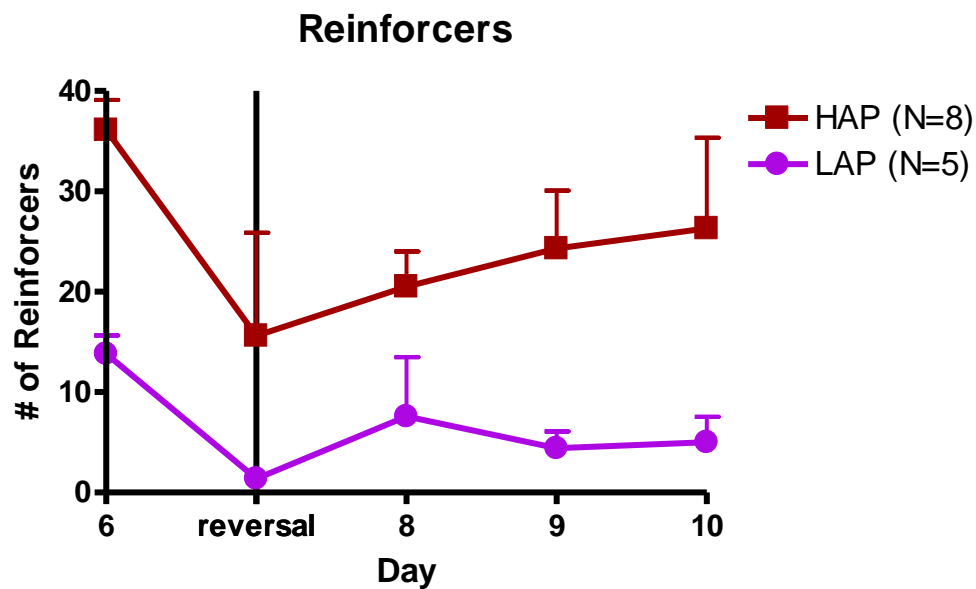

Figure 3-15
Upon lever reversal, HAP mice gradually increased their number of intravenous alcohol reinforcers over days while LAP mice did not. Lines differed significantly in their pre-reversal behavior, so analysis was divided by Line *a priori.*
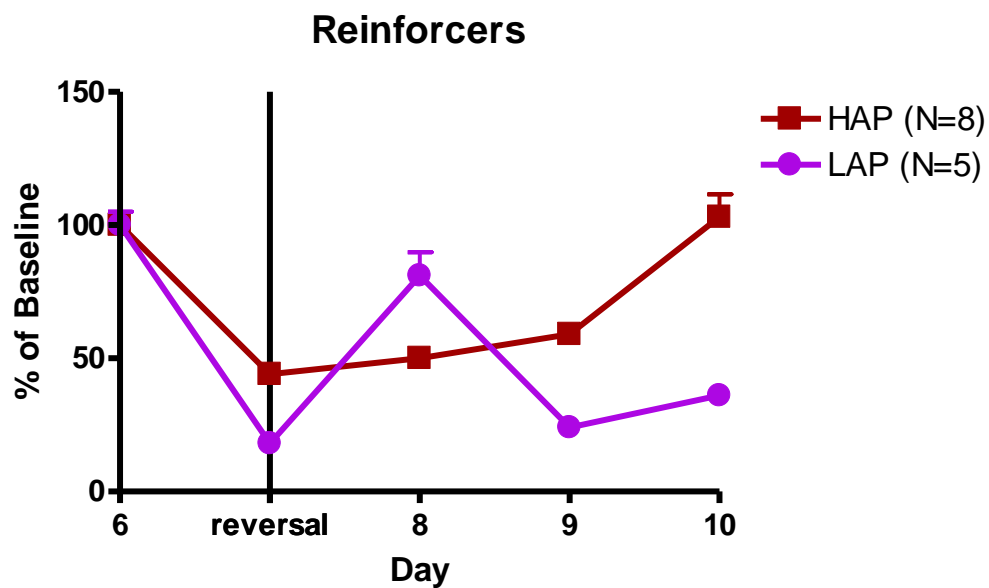
Figure 3-16
Upon lever reversal, HAP mice gradually increased their number of intravenous alcohol reinforcers over days while LAP mice did not. This graph shows data converted to percent of baseline performance to account for varying levels of performance in the lines prior to lever reversal.

Figure 3-17
Upon lever reversal, HAP mice increased their number of lever presses on the
new "correct" lever gradually across days while the LAP mice did not.

Figure 3-18
Upon lever reversal, HAP mice increased their number of lever presses on the new "correct" lever gradually across days while the LAP mice did not. This graph shows data converted to percent of baseline performance to account for varying levels of performance in the lines prior to lever reversal.
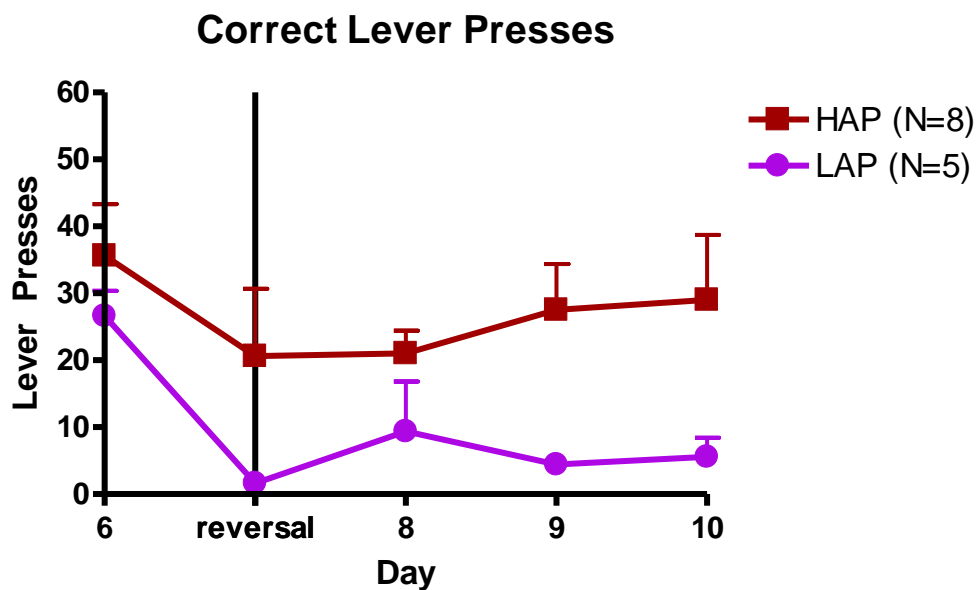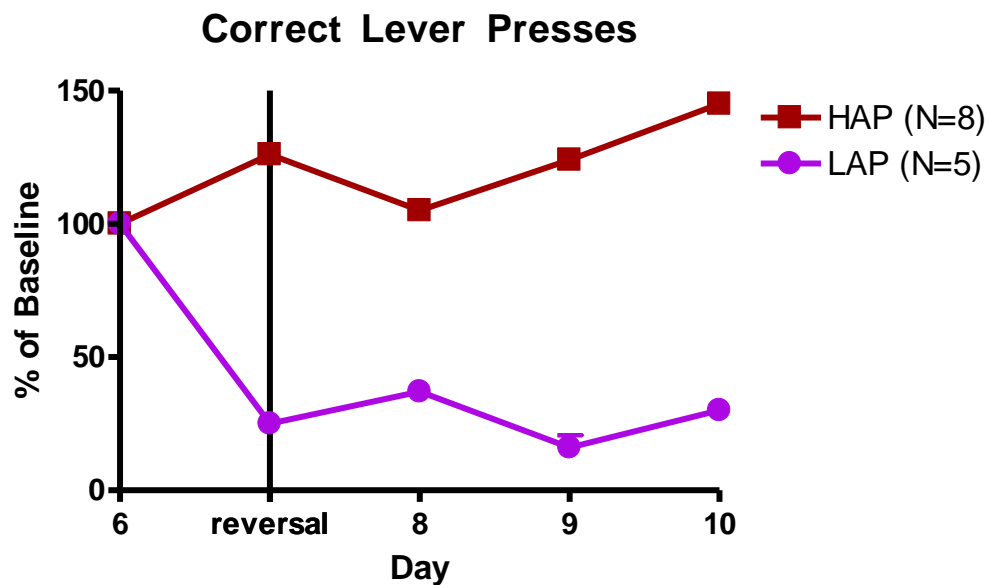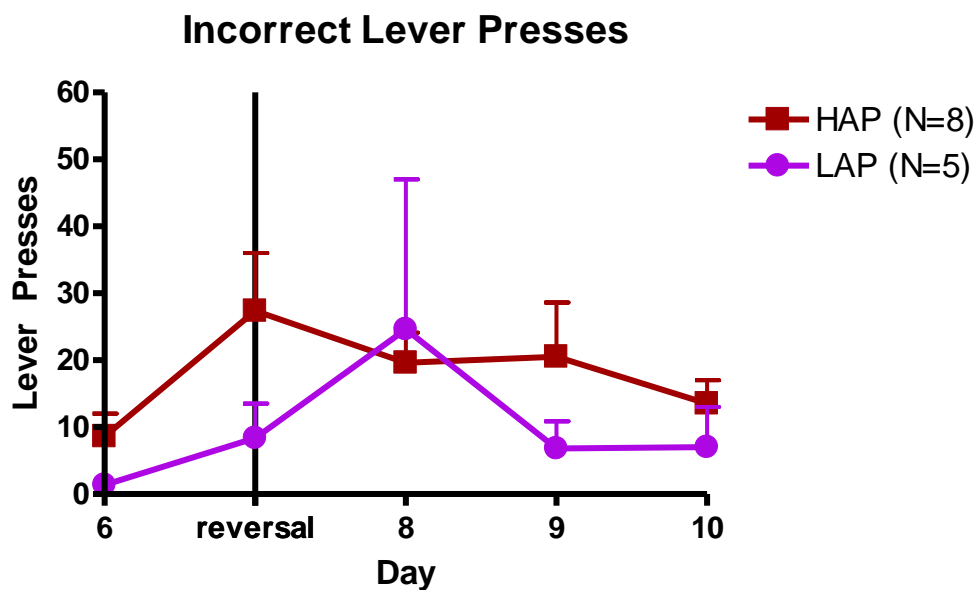
Incorrect Lever Presses

Figure 3-19
Lines did not differ in their incorrect lever responding pattern following reversal.
Incorrect lever presses increased immediately following reversal, but decreased
in both HAP and LAP subjects on subsequent days.

Total Lever Presses

Figure 3-20
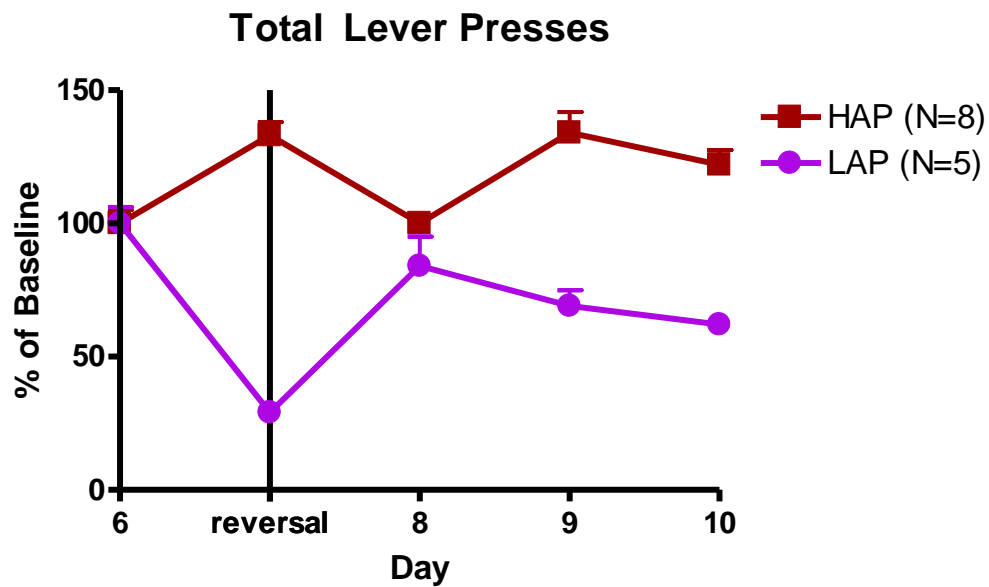As a function of percent of baseline performance, HAP mice stayed at near baseline levels following reversal. LAP mice had a drastic decrease in total lever presses, recovered, then began to decrease their total lever presses in the days following reversal.
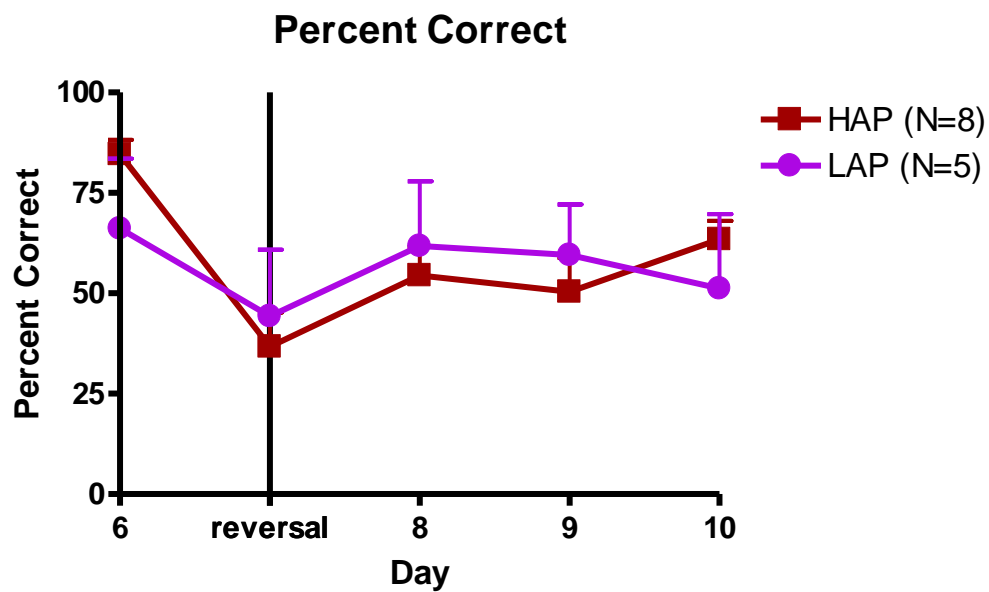
Figure 3-21
Percent correct tended to show an increase in the HAP mice, but not LAP mice, in a non-significant trend following reversal.
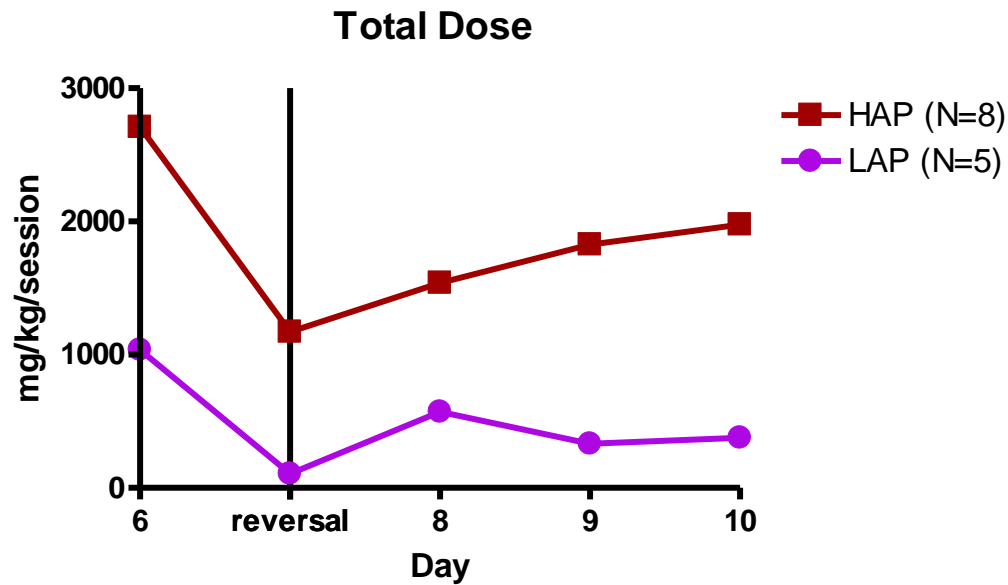
Total Dose

Figure 3-22
Total dose of ethanol received by subjects following lever reversal. HAP mice gradually worked back toward pre-reversal dose while LAP subjects failed to show this trend.

3.5. Post Test with Water Reinforcers

Both lines showed similar responding and consumption of reinforcers as compared to pretraining data. All mice maintained performance compared to the pretraining phase and met or exceeded original criteria of three consecutive days of at least 10 reinforcers with at least 0.1 mL consumed, indicating that the task remained sufficiently learned and performance was not disrupted by surgery and IVSA of alcohol (see Figures 3-23 through 3-27).

No significant interactions with Sex were seen except for in the percent correct data, where there was a Line X Sex interaction [$F(1,18) = 11.03$, $p < 0.01$] due to the female HAP mice reducing their accuracy on the last day of testing, while all other groups remained the same. There was also a main effect of Line [$F(1,18) = 14.966$, $p < 0.001$] and Sex [$F(1,18) = 4.778$, $p = .004$] for percentage correct.

The HAP mice slightly decreased a relatively high number of correct responses over the three days of testing while the LAP mice improve following the first day of testing, resulting in a Line X Day interaction for correct lever presses [$F(2,40) = 5.540$, $p < 0.01$] accompanied by a main effect of Day [$F(2,40) = 3.77$, $p = 0.03$] and Line [$F(1,20) = 7.946$, $p = 0.01$]. There were no interactions or main effects evident in amount of water consumed during the course of post training, suggesting minimal differences between the Lines for responding for a non-alcohol reinforcer.
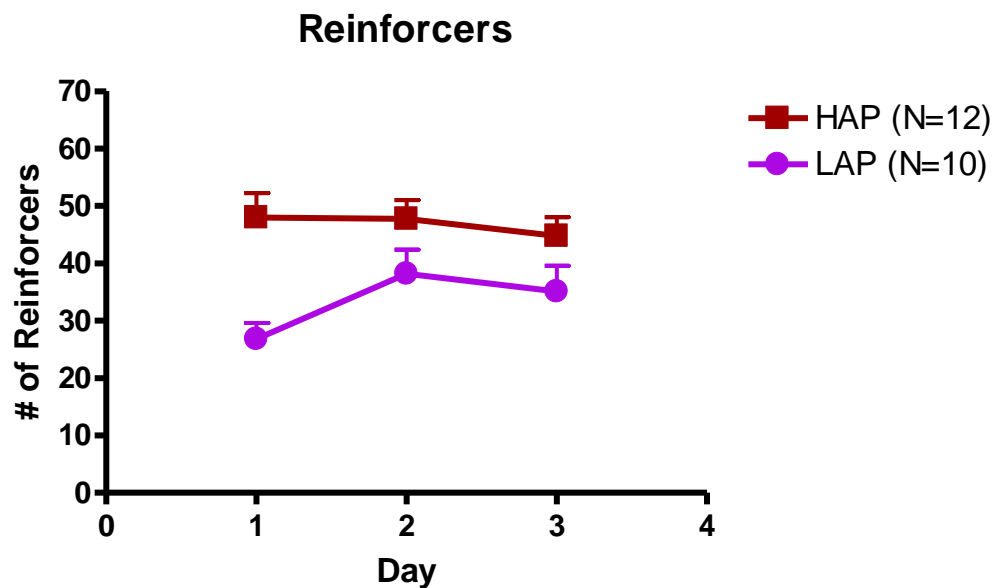
Figure 3-23
Subjects maintained similar performance for number of water reinforcers received following intravenous self administration of alcohol.

Correct Lever Presses
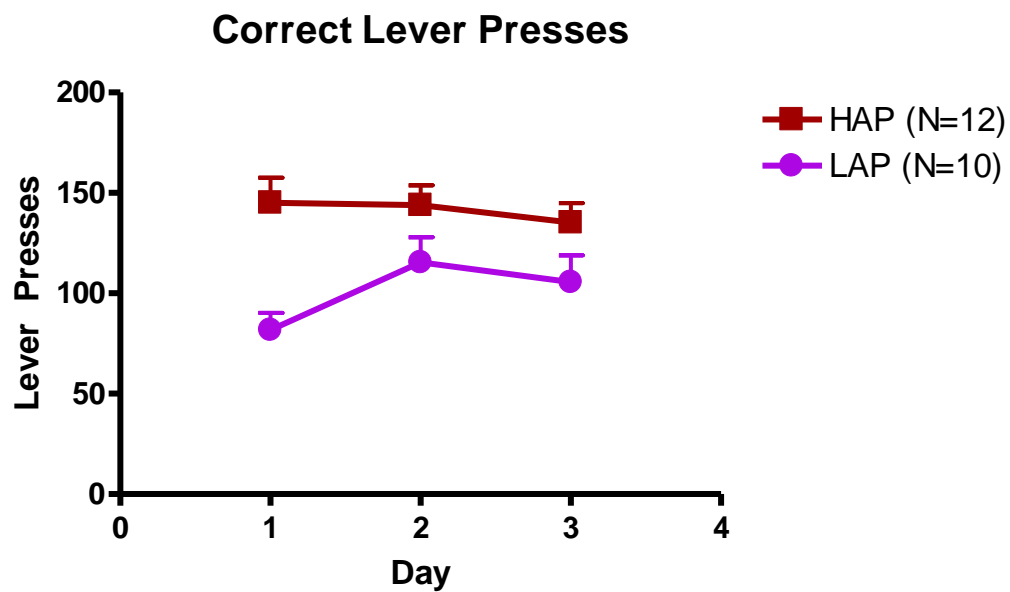
Figure 3-24
Subjects maintained similar performance for number of correct lever presses
following surgery and intravenous self administration of alcohol.

Incorrect Lever Presses

Figure 3-25
LAP subjects remained consistent with fewer lever presses on the incorrect lever
during water post testing.

Figure 3-26
All subjects maintained a high percentage correct for water reinforcers following
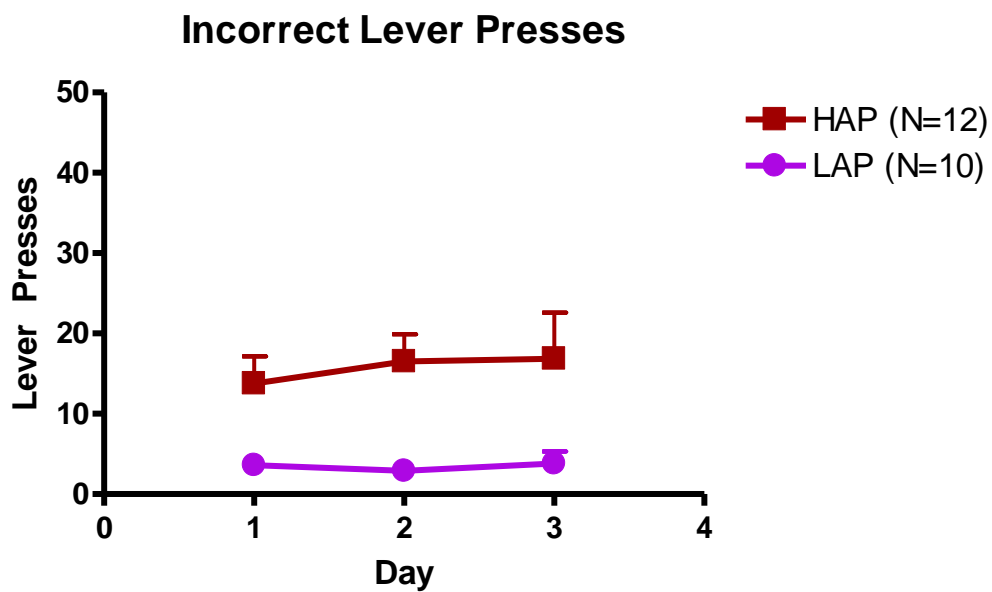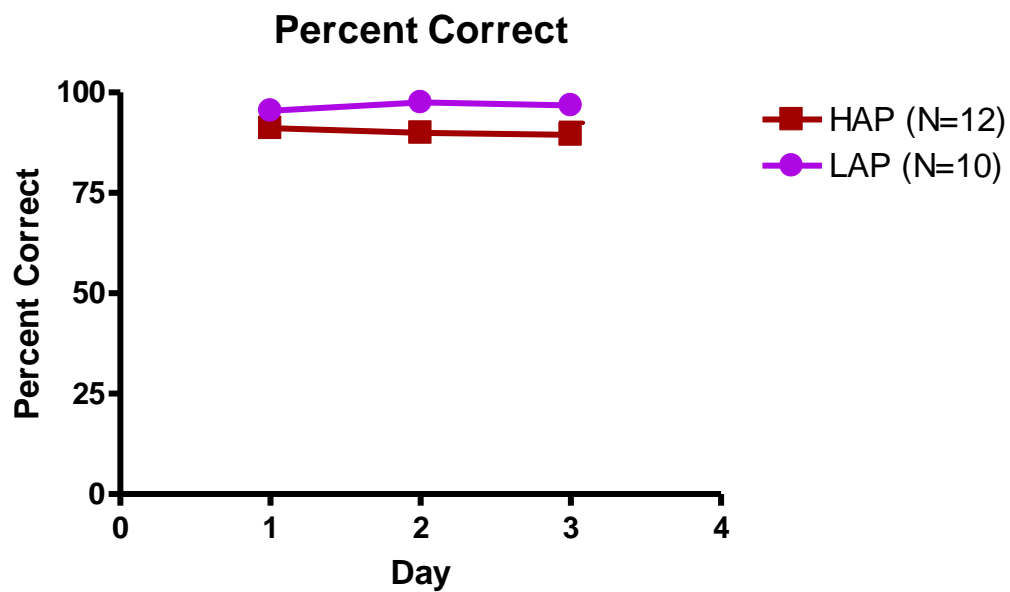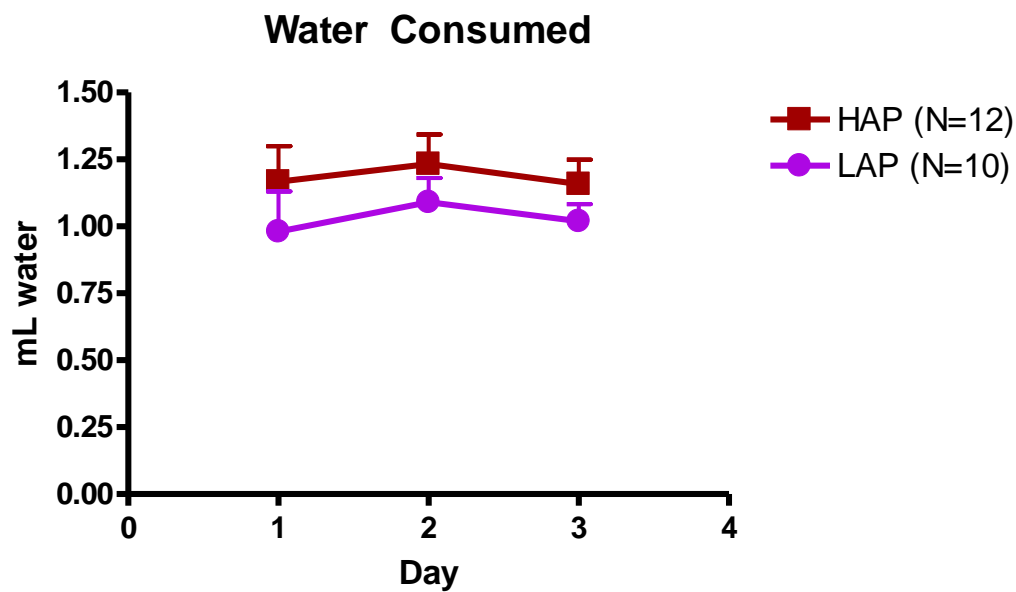intravenous alcohol administration.

Figure 3-27
There was no significant difference between HAP and LAP subjects in the
amount of water consumed during the post intravenous alcohol administration.

CHAPTER 4. DISCUSSION

Overall, these experiments support the hypothesis that mice selected for high alcohol preference administer intravenous alcohol for its reinforcing properties. This is apparent in the acquisition for HAP mice, but not LAP mice, of administration of IV alcohol at a dose of at least 75 mg/kg/infusion, exceeding that of the previously published rate of metabolism (Grahame, et al., 1999b) for HAP and LAP mice (Figure 3-9). Furthermore, HAP mice show evidence of immediately increasing behavioral output with increase of FR schedule during IVSA acquisition, which was not immediately evident in LAP mice (Figure 3-6). Additionally, HAP mice showed evidence of altering lever press behavior to track the location of the "correct" lever in order to continue IVSA alcohol following lever reversal (Figures 3-15, 3-16, 3-18). The HAP subjects increased responding on the newly correct lever, as well as inhibiting responding on the newly incorrect lever which was previously associated with i.v. alcohol. The lever reversal task has been shown effective in demonstrating reinforcing properties of stimuli, both external such as gaining access to a sweetened solution (El-Ghundi, O'Dowd, Erclik, & George, 2003) and discriminating internal drug states (Garner, Wessinger, & McMillan, 1996).

In the studies presented here, LAP mice failed to rise above what the researcher considered pharmacologically significant administration (Figure 3-9), which was based on previously reported rate of metabolic clearance in these selected lines (Grahame, et al., 1999b). However, one may argue that LAP mice did also show some evidence of IVSA ethanol reinforcement. For example, LAP mice do indeed lever press for i.v. alcohol, and in much higher quantities than voluntarily consumed in oral drinking paradigms: LAP mice here administered between

approximately 250-750 mg/kg per 60-minute session intravenously during IVSA acquisition. Considering LAP2 and LAP3 mice will orally consume generally no more than approximately 1000 mg/kg per day of oral alcohol (See Table 2-1; unpublished breeding data), which also has a much slower rate of absorption via the gastrointestinal tract, this is a significant increase of administration and at high rates of accuracy on the correct lever. Even under limited access paradigms, LAP1 mice reportedly drink approximately 1000 mg/kg per two hours of access (Grahame, et al., 1999a).

In addition, while HAP mice show immediate work load increase with increase in FR requirements, LAP mice do show evidence of a similar, albeit slower, increase back toward previous IVSA doses at a lower FR schedule (see Figures 3-5, 3-6, and 3-9). This slower change in behavior does not appear to be due to differing cognitive or learning abilities in the lines; LAP mice generally have equal, or at times higher, percent correct data throughout these studies, as well as there being no difference between the Lines in the water drinking data- both pretraining and posttraining. These data combined suggest evidence for motivation and reinforcement, not just a learning difference between the lines (Chester, Lumeng, Li, & Grahame, 2003; Grahame, Chester, Rodd-Henricks, Li, & Lumeng, 2001a).

Furthermore, both HAP and LAP mice show sensitivity to the dose of alcohol they are administering, as reflected in the change in lever pressing behavior in response to an increase in FR schedule. This is further supported by the recently published studies utilizing intragastric self-infusion of alcohol in both inbred strains and selectively bred mice that differ dramatically in their amount of home cage drinking (Fidler, et al., 2010). In this study, mice systemically administered doses of alcohol directly into the stomach, bypassing alcohol's pre-absorptive effects, such as taste and odor. High drinking inbred strain C57BL/6J mice and HAP2 mice both administered higher doses of intragastric alcohol more

than their comparison low drinking counterparts. Interestingly, both low drinking strains- DBA and LAP2 mice were shown to administer doses of alcohol equal to the high drinking mice following a period of passive ethanol exposure. The authors concluded that, especially in the selectively bred mice, expression of home cage drinking may be more related to pre-absorptive effects than postabsorptive effects. Therefore, selective breeding for home cage oral alcohol preference seems to be related, but not entirely indicative of, selecting for pharmacological reinforcing properties of ethanol. That is, preingestive properties of alcohol, such as taste, seem to play a role in the lower drinking scores exhibited by the LAP mice. These preingestive differences can be, and are, somewhat bypassed by this non-oral systemic route of administration of IVSA. Despite this, however, while LAP are showing some evidence of IVSA alcohol reinforcement, HAP mice do seem to find IVSA alcohol more reinforcing than LAP mice based on the studies presented here. The fact that LAP mice rise to HAP levels of administration in an IGSA paradigm following passive exposure (Fidler, et al., 2010) and that low drinking is negatively correlated with induction of CTA (see Green & Grahame, 2008 for review) may indicate that low drinking mice may be more sensitive to post-absorptive alcohol effects and require a buildup of tolerance before higher doses are freely administered.

Intravenous self-administration is not a simple paradigm to incorporate in research and there were several problems and limitations with this study. First, several variations in surgical technique and catheter design were explored prior to the final protocol used here, with this being ultimately the most successful. Injectable anesthesia (a ketamine cocktail of varying concentrations of components) proved to be highly problematic in maintaining a proper plane of anesthesia needed for the duration of the surgery so inhaled anesthesia was ultimately adopted. This also allowed for faster post operative recovery in the mice (Thomsen & Caine, 2007).

Next, the difficulty of maintaining catheter patency long enough for subjects to complete each portion of the study resulted in a large subject dropout rate, despite extensive training and refining of the procedure. Prior to the protocol implemented here, several variations on catheter and subcutaneous support mechanisms were explored, including a surgical steel subcutaneous saddle utilized in prior publications (Grahame & Cunningham, 1997; Grahame, et al., 1998). Finally, a commercially available subcutaneous button showed the best results and decreased occurrence of such problems as perforated and detached catheters which were seen frequently with the subcutaneous saddle.

Furthermore, commercially purchased catheters were used here after having low success with maintained patency using constructed catheters similar to those used in the previously cited articles. Purchased catheters were uniform in size, silicon bead diameter and distance, and sterile. This reduced variability seen in self-constructed catheters, as well as reduced recovery time and increased patency length.

Still, despite these changes, surgery survival and catheter patency maintenance remained low. This considerably reduced sample sizes, hindering analysis, especially when considering replicate line and sex as factors. Further development of surgical protocols, procedures, and equipment may be of use for future studies in order to improve survival and prolong catheter patency. The author noticed several non-significant trends in the data, especially with the dose-response portion of the study, that would have especially benefited by a larger n to help pull out significant results. Without this significance, interpretation of the data is severely hindered.

Additionally, the pretraining period utilized to familiarize the subjects with the operant procedure prior to surgery in order to hasten the acquisition of ethanol-reinforced responding following surgery complicates interpretation. This

pretraining for water reinforcers resulted in the introduction of additional reinforcers associated with lever pressing behavior, such as water from a sipper tube following lever pressing. This could have confounded the dependent variables during the IVSA portions of the experiment if the mice were still expecting water reinforcers following appropriate operant responding. However, it is important to note that pretraining is commonly required in time-sensitive situations and is an acceptable solution, as suggested Thomsen & Caine (2007). Additionally, operant water conditioning took place in separate operant chambers, helping to discourage place conditioning and associations.

During water pretraining, HAP male subjects consumed significantly more water than the female HAP mice or either sex of LAP mice. This difference seems difficult to interpret, as generally female HAP mice are reported to consume more fluid than males (Grahame, et al., 1999a) when difference between sexes do appear. This apparent difference does not seem to influence later operant responding, however, and this sex difference is absent during the remaining portions of the study. There is no consumption difference in the genders or Lines during the post test with water reinforcers.

Finally, it is also important to note that this study compares HAP replicate line 2 with LAP replicate line 2, as well as line 3; and only HAP replicate line 2 with LAP line 3 in the case of the dose response portion. This may cause some interpretability problems with the data, due to the inability to address genetic drift in these animals as possibly accounting for any differences we may see (Crabbe, 1989; Crabbe, et al., 1992). Future studies can and should be used to address this possibility. With the addition of appropriate replicate lines and analyses; HAP2 compared to LAP2, which were selected for simultaneously and on similar generations of selection, and additionally adding in HAP3 compared to LAP3, which are also selected for simultaneously and on similar generations of

selection, there would be more of a basis for genetic influence of phenotypes of IVSA of alcohol and generalizability to broader populations (Crabbe, 1989).

An alternative explanation of the interpretation of the IVSA acquisition, dose response, and lever reversal portions of this study could be that the house light becomes a secondary reinforcer during the pretraining phase. It could be said that HAP and LAP mice do still exhibit some lever pressing, at a reduced amount compared to water pretraining phase levels, because of the reinforcing properties now associated with the house light, which is illuminated during infusions. It may be that the subjects do not find the addition of i.v. alcohol reinforcing, thus decreasing the amount of lever pressing as compared with pretraining rates, as well as failing to discriminate between doses in the dose response portion of the study. In other words, they still lever press the same amount no matter what dose of alcohol is being infused because they still maintain the same production of light presentation.

This alternative interpretation has support in that other mouse models have shown operant sensation seeking for visual stimuli (Olsen & Winder, 2009). However, it is important to note that Olsen & Winder (2009) used C57BL/6J mice and complex visual stimuli, rather than a single illuminated house light. Additionally, mice are not water deprived during IVSA portions of the present study because additional motivation for lever pressing is not necessary as it was during the acquisition of a novel behavior during instrumental acquisition during the water pretraining phase. Also, the subjects are now responding for a non-oral reinforcer as they were in the pretraining phase, and extensive water deprivation may have complicated post operative recovery. Furthermore, the consequence of lever pressing is qualitatively much different than in the pretraining and post test phases, which may account for the change in amount of lever pressing. Mice do resume greater amounts of lever pressing for the post test, but they are again operating under the extra incentive of lever pressing for

access to water under water deprivation conditions. Also, IVSA of alcohol has been shown to be reinforcing, not aversive, in previous mouse IVSA studies (Grahame, et al., 1998), as well as in humans (Morzorati, Ramchandani, Flury, Li, & O'Connor, 2002).

The lack of a demonstrated dose response curve following exposure to a range of intravenous alcohol doses here, while unfortunate, is not unusual and has been previously observed in mouse literature (Grahame & Cunningham, 1997). Other studies exhibiting a dose-response curve did so after several consecutive days (Ikegami et al., 2002), rather than just two, as presented here. A demonstrated dose response curve is desirable because it addresses the question of optimal dose and/or concentration of administration and the sensitivity of such doses and concentrations and whether these differ between the high and low preferring lines. It may be argued that LAP mice are administering lower doses of alcohol per session because they are more sensitive to the reinforcing effects and need less alcohol to be reinforced. This would be a logical concern based on previous studies showing LAP mice being more sensitive to CTA resulting from 2 g/kg or 4 g/kg injections of alcohol (Chester, et al., 2003). However, this contradicts the CPP data suggesting that LAP mice find *higher* doses of alcohol more reinforcing (Grahame, et al., 2001). Therefore, it would likely be beneficial to carry out more consecutive days at each dose to see if mice are able to eventually adjust behavior based on the infusion dose, a procedure that was difficult here due to the limited catheter patency duration. In a successful dose response curve, one would expect to see adjustment of lever pressing behavior based on infusion dose, such that the subjects would adjust lever pressing and number of reinforcers to correct for change in infusion dose size (Thomsen & Caine, 2007). Differential behavior at each infusion dose size between Lines would suggest differing reinforcement properties.

This series of experiments provides support for HAP mice finding IV alcohol more reinforcing than LAP mice. However, based on previously published CPP data where LAP mice developed CPP to a high dose of alcohol (Grahame, et al., 2001b), LAP mice may find higher doses more reinforcing. Furthermore, data from inbred strains of varying alcohol preferences in the IVSA paradigm show equal self-administration regardless of home-cage alcohol preference (Grahame & Cunningham, 1997). In this study, C57BL/6J mice, which readily consume alcohol orally, and DBA mice, which do not readily consume alcohol orally, were subjected to a similar IVSA alcohol paradigm to the methodology presented here, using nose poke behavior rather than lever pressing, and it was found that DBA mice self-administered IV alcohol in quantities similar to those of the higher drinking C57BL/6J strain. Therefore, it may be concluded that preingestive effects may limit, or artificially enhance, self-administration, and must be taken into account when assessing reinforcing properties of alcohol using oral administration paradigms.

The present study was limited by the small volume of ethanol able to be administered and the concentration of such. This limitation required high concentrations of solution (up to 75% v/v) that may have produced undesirable systemic sensations for the subjects. As such, subjects may have been less inclined to lever press for the alcohol infusions. It must also be noted that the motor suppression effects of extremely high doses, such as those used in the above mentioned CPP studies, were not operantly attained here. So while LAP mice may find extremely high doses of ethanol reinforcing, such doses are not realistically obtained using this paradigm due to the small infusion doses necessary so as to not overwhelm the vasculature and volume limits of the mouse physiology, and for the fact that infusion concentrations were already notably high, especially for the 125 mg/kg/infusion dose at 75% v/v. Furthermore, those high doses do not necessarily reflect human administration studies (Morzorati, et al., 2002).

Blood alcohol concentrations (BAC's) were not taken from any subjects in this series of studies. Due to the pharmacokinetic considerations of IVSA alcohol administration; immediate crossing of the blood brain barrier and equally fast clearance of alcohol from the system, measureable BAC's would depend heavily on time course of administration. Future studies should address this concern by taking into account time course of administration and assess BAC's in subjects undergoing similar IVSA of alcohol as presented here to make comparisons. Limited access to oral alcohol solutions in the home cage elicited differing patterns of drinking between HAP and LAP subjects, with HAP subjects "loading up" early in the session with higher rates of drinking early on in the session that tapered off toward the end. In contrast, LAP subjects maintained a low rate of oral administration throughout the session. It would be interesting to see if such time course differences exist with this paradigm of administration as well. With time courses of administration and BAC's further conclusions can be drawn as to the pharmacological significance of the levels of administration attained here.

Selective breeding has been repeatedly shown to be a valuable tool for assessing the genetic contribution of complex genetic traits such as alcoholism (Crabbe, 2008; Crabbe, Phillips, & Belknap, 2010; Froehlich, 2010) as well as other behavioral traits (Brush et al., 1985; Carroll, Anderson, & Morgan, 2007; Harmon et al., 2008; Hitzemann, Malmanger, Belknap, Darakjian, & McWeeney, 2008; Jonas et al., 2010; Touma et al., 2008; Zombeck, Deyoung, Brzezinska, & Rhodes, 2010). HAP and LAP replicate lines of mice have helped broaden our understanding of the underlying genetics of alcoholism (Bice et al., 2006) and correlated phenotypes such as impulsivity (Oberlin & Grahame, 2009), locomotor sensitization (Grahame, Rodd-Henricks, Li, & Lumeng, 2000), and associative learning (Chester, et al., 2003). The present study helps further broaden our understanding of this well-characterized mouse model of high- and low-alcohol preference as a legitimate animal model of human alcoholism. By showing that genetic basis for alcohol preference in home cage drinking can transfer to similar

administration patterns and doses, especially in the HAPs, when using a route of administration that bypasses traditional means of consumption further supports the idea of alcohol administration can be, in part, due to post-ingestive pharmacological reinforcing effects. By using selectively bred subjects that have a diverse genetic background except for the loci that have been selectively fixed with regards to high- and low- alcohol drinking preference, we have a much more generalizable and valid model compared to inbred or transgenic/knockout strains that only differ in a single gene (Crabbe, 1989, 2008; Grahame, 2000).

Human studies on family history positive and family history negative individuals have revealed genetic differences in brain activity in response to i.v. alcohol (Kareken et al., 2010) with heavy drinkers with a positive family history of alcoholism responding differently than heavy drinkers with a negative family history of alcoholism. Other human studies have suggested individuals with a positive family history are more sensitive to the intoxicating effects of alcohol, but develop a tolerance for those effects more quickly than individuals with a negative family history (Morzorati, et al., 2002). The alcohol clamp i.v. infusion technique is a valuable tool in humans for assessing differences in genetic background and physiologic response. It would be interesting to see a parallel study done in humans to see what total dose they voluntarily administer and what infusion dose is most reinforcing and if this differs between family history positive or negative for alcoholism.

The methodological paradigm of IVSA alcohol, both in humans as well as non-human subjects, has another application for research in that IVSA may be implemented as a second-order schedule of drug administration, as suggested by Howell & Fantegrossi (2009). Human drug use, including intake of alcohol, often involves a ritualized sequence of behaviors that occurs in a specific environment. The environmental stimuli associated with drug use are believed to play a major role in the maintenance of drug-seeking behavior (Schindler, Katz, &

Goldberg, 1988). Second-order schedules of drug self-administration have been used in nonhuman primates to maintain extended sequences of responding between drug injections (Howell & Byrd, 1995; Kelleher & Goldberg, 1977) analogous to patterns of drug use in humans. These schedules can be implemented using the IVSA protocol to help further our understanding of the post-ingestive reinforcing properties of alcohol.

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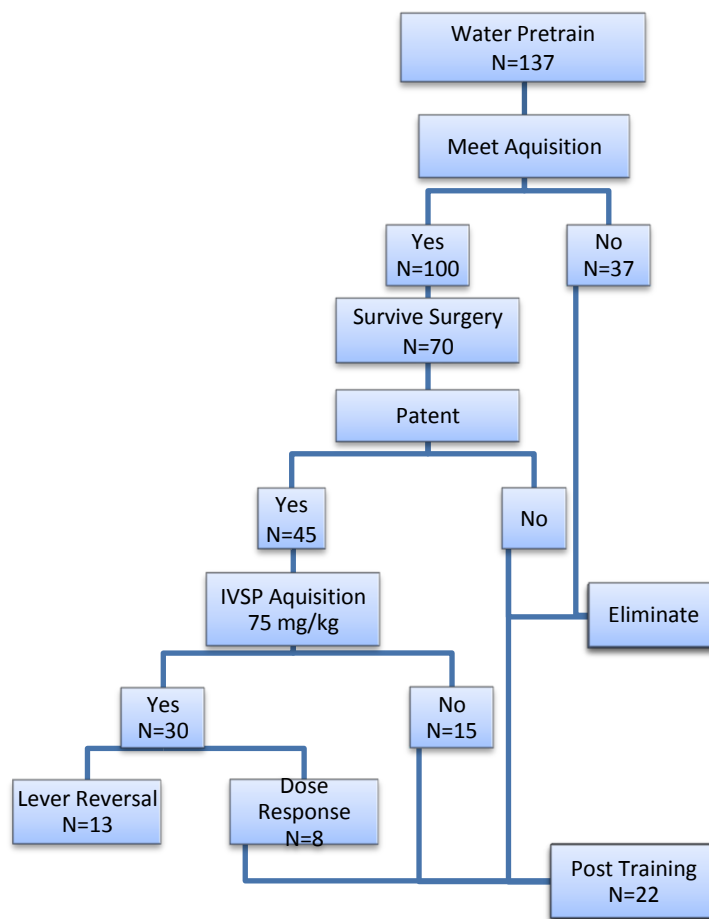
APPENDIX

APPENDIX

Figure A-1
Illustration of flow for methodologies used in this dissertation project, tracking
subjects through various phases of research

VITA

VITA

Alexis Suzanne Green

<u>Education</u>
Doctor of Philosophy, Psychobiology of Addictions 2004-2011
     Indiana Univ.-Purdue Univ. at Indianapolis
     Dissertation: <u>Intravenous Self-Administration of Alcohol in Selectively Bred High- and Low- Alcohol Preferring Mice</u>
Master of Science, Behavioral Psychology 2001-2004
     Indiana Univ.-Purdue Univ. at Indianapolis
     Thesis: <u>Learning and Memory Assessment in Mice Transgenic for the Human *tau* Mutation</u>
Bachelor of Science 1997-2001
     Alma College, Alma Michigan
     Major: Biology; Minor: Psychology
     Senior Thesis: <u>The Effects of Estrogen Deprivation on Learning, Memory, and the Morphology of the Hippocampus and Pituitary in Rats</u>

<u>Experience</u>
Visiting Psychology Instructor at Hanover College Fall 2009-Present. Courses taught include Neuropsychology, Learning, Introductory Psychology, Childhood and Adolescence, Senior Seminar. Duties include course development; choosing proper textbooks; constructing and following course syllabi; designing engaging and effective lectures, labs, and class projects; constructing and grading exams, homework assignments, lab and class projects; mentoring seniors and advising senior research projects
Coordinator and participating Instructor for Rivers Institute Summer Academy 2010: Psychology of the Media. Duties included developing coursework, lectures, and activities for a week-long summer workshop of high school juniors and seniors
GK-12 Fellow, placement at Crispus Attucks Middle/High School in Indianapolis Fall 2008-Spring 2009. Duties included designing and implementing lesson plans to 6th, 7th and 8th grade middle school science students with the goal of increasing their interest in neuroscience
Instructor for Introduction to Psychology as a Social Science Fall 2006-Spring 2007, including an accelerated section

Teacher Assistant for Introduction to Psychology as a Biological Science 2003-2004. Duties included setting up and implementing online class components including homework, quizzes, exams, and grade books, tutoring students, leading review study sections both online and in person, grading, composing exam questions

Teacher Assistant for Physiological Psychology 1999-2001. Duties included mentoring students, setting up lab, giving lab lecture and guiding experiments, grading

Visiting lecturer for Psychology as a Biological Science 2004-2008

Selection Committee Head, Session Chair, Organizer for NIAAA 2005 Trainee Workshop

Presenter at Local Society for Neuroscience 2004, 2005, 2007 Conferences, Indianapolis IN

Presenter at Regional Society on Alcoholism 2003 and 2004 Conferences, Fort Lauderdale FL, Vancouver BC

Presenter at Society for Neuroscience 2004 and 2006 Conferences, San Diego CA, Atlanta GA

Selection Committee Member, Session Co-Chair, Presenter for NIAAA 2003 Trainee Workshop

Presenter at International Society for Developmental Psychobiology 2002 Conference, Orlando FL

Presenter at 2001 National Conference of Undergraduate Research

Presenter at 2001 Honors Day, Alma College

Proficient in SPSS, MS Office software including Outlook, Word and Excel, GraphPad Prism, Explorer, Mozilla Firefox

Labs and Techniques

Lab of Dr. Grahame (2002-2009): research involving selectively bred HAP and LAP line I and II mice

Techniques: Behavioral observations using locomotor boxes, Morris Water Maze, Plus Maze, Object Recognition task, operant training and reversal tasks, brain and spinal cord extractions, perfusions, tissue processing using immunocytochemical techniques such as *c-fos*, sectioning, light microscope stereology, and alcohol drinking study techniques, surgical catheter implantation and intravenous administration studies

Lab of Dr. Murrell (2002-2004): research involving mutant tau-induced mechanisms of neurodegeneration in a transgenic mouse model with the human P301S tau mutation

Techniques: Morris Water Maze, Object Recognition, and Operant Reversal learning tasks to quantify cognitive decline

Lab of Dr. Neal-Beliveau (2001-2002): research involving Methamphetamine Sensitization in rats; AMPT prenatal exposure (G13-G21) and adult D1 and D2 expression in adulthood using SKF 38393, SKF 82958, and Eticlopride in rats

Lab of Dr. Hill-Beagley (1998-2001): research involving learning and memory, hippocampal morphology, ageing and memory, and estrogen related influences on learning and memory

Techniques: Cannula implantation for drug infusion, electrode implantation for stimulation, behavioral observations using Morris Water Maze and Object Recognition test, operant training, perfusions, tissue processing, sectioning, transmission electron microscope and light microscope analysis

Honors/Awards

NSF GK-12 Fellowship 2008-2009
Predoctoral Traineeship NIAAA Training Grant 2003-2008
Dr. Mori Aprison Presentation Award, Second Place, 2003
Graduate Student 2002 Travel Fellowship
International Society for Developmental Psychobiology 2002 Travel Scholarship
Radiation Safety Certified
University (RIF) Fellowship 2001-2002
Alma Professional and Business Women's Club Scholarship 1997-2001
Cum Laude, Class of 2001 Alma College
Trustee Honor Scholarship 97-01

Memberships

Student member of International Society of Developmental Psychobiology 2001-present
Student member of Regional Society on Alcoholism 2003-present
Student member of the Indianapolis Chapter of the Local Society for Neuroscience 2002-2009
Student member of the Society for Neuroscience 2003-present
Lynhurst Gardens Home Owners Association, Treasurer 2005-2010
Madison First Assemblies of God Church member

Publications

Intravenous Self-Administration of Ethanol in Mice Selectively Bred for High- and Low-Alcohol Preference (in progress)

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