Graduate School ETD Form 9
(Revised 12/07)

PURDUE UNIVERSITY
GRADUATE SCHOOL
Thesis/Dissertation Acceptance

This is to certify that the thesis/dissertation prepared

By David Scott O'Tousa

Entitled
Adolescent and Adult Two-Bottle Choice Ethanol Drinking and Adult Impulsivity in Genetically Selected High-Alcohol Preferring Mice

For the degree of Master of Science

Is approved by the final examining committee:

Nicholas J. Grahame
Chair

Cristine L. Czachowski

Stephen L. Boehm

To the best of my knowledge and as understood by the student in the *Research Integrity and Copyright Disclaimer (Graduate School Form 20)*, this thesis/dissertation adheres to the provisions of Purdue University's "Policy on Integrity in Research" and the use of copyrighted material.

Approved by Major Professor(s): Nicholas J. Grahame

Approved by: John T. Hazer 9/30/2011
Head of the Graduate Program Date

Graduate School Form 20
(Revised 9/10)

PURDUE UNIVERSITY
GRADUATE SCHOOL

Research Integrity and Copyright Disclaimer

Title of Thesis/Dissertation:

Adolescent and Adult Two-Bottle Choice Ethanol Drinking and Adult Impulsivity in Genetically Selected High-Alcohol Preferring Mice

For the degree of Master of Science

I certify that in the preparation of this thesis, I have observed the provisions of *Purdue University Executive Memorandum No. C-22,* September 6, 1991, *Policy on Integrity in Research.**

Further, I certify that this work is free of plagiarism and all materials appearing in this thesis/dissertation have been properly quoted and attributed.

I certify that all copyrighted material incorporated into this thesis/dissertation is in compliance with the United States' copyright law and that I have received written permission from the copyright owners for my use of their work, which is beyond the scope of the law. I agree to indemnify and save harmless Purdue University from any and all claims that may be asserted or that may arise from any copyright violation.

David Scott O'Tousa

Printed Name and Signature of Candidate

9/30/2011

Date (month/day/year)

*Located at http://www.purdue.edu/policies/pages/teach_res_outreach/c_22.html

ADOLESCENT AND ADULT TWO-BOTTLE CHOICE ETHANOL DRINKING AND ADULT IMPULSIVITY IN GENETICALLY SELECTED HIGH-ALCOHOL PREFERRING MICE

A Thesis

Submitted to the Faculty

of

Purdue University

by

David Scott O'Tousa

In Partial Fulfillment of the

Requirements for the Degree

of

Master of Science

December 2011

Purdue University

Indianapolis, Indiana

ACKNOWLEDGEMENTS

I thank Nicholas Grahame, Ph.D., for primary research mentorship, Stephen Boehm, Ph.D. and Cristine Czachowski, Ph.D. for serving on my committee, Liana Matson, Nicholas Villalta, Laverne Melon, and Devon Novotney for laboratory assistance, and the AA07911 grant to David Crabb for funding this work. In addition, I would like to thank Indiana University-Purdue University Indianapolis and the University of Notre Dame, my family, friends, and the entire Psychobiology of Addictions cast and crew. I couldn't have done it without any of you.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

O'Tousa, David Scott. M.S., Purdue University, December 2011. Adolescent and Adult Two-Bottle Choice Ethanol Drinking and Adult Impulsivity in Genetically Selected High-Alcohol Preferring Mice. Major Professor: Nicholas Grahame.

Abuse of alcohol during adolescence continues to be a problem, and it has been shown that earlier onset of drinking predicts increased alcohol abuse problems later in life. High levels of impulsivity have been demonstrated to be characteristic of alcoholics, and impulsivity has also been shown to predict later alcohol use in teenage subjects, showing that impulsivity may be an inherent underlying biological process that precedes the development of alcohol use disorders. These experiments examined adolescent drinking in a high-drinking, relatively impulsive mouse population, and assessed its effects on adult drinking and adult impulsivity.

Experiment 1: Selectively bred High-Alcohol Preferring (HAP II) mice, which are shown to be highly impulsive, were given either alcohol (free choice access) or water only for two weeks during middle adolescence or adulthood. All mice were given free choice access to alcohol following 30 days without access, in adulthood. Experiment 2: Adolescent HAP II mice drank alcohol and water, or water alone, for two weeks, and were then trained to perform a delay discounting task as adults to measure impulsivity. In each experiment, effects of volitional ethanol consumption on later behavior were

assessed. We expected adolescent alcohol exposure to increase subsequent drinking and impulsivity.

Adolescent mice consumed significant quantities of ethanol, reaching average blood ethanol concentrations (BECs) of 142 mg/dl in Experiment 1 and 108 mg/dl in Experiment 2. Adult mice reached average BECs of 154 mg/dl in Experiment 2. Mice pre-exposed to alcohol in either adolescence or adulthood showed a transient increase in ethanol consumption, but we observed no differences in impulsivity in adult mice as a function of whether mice drank alcohol during adolescence.

These findings indicate that HAP II mice drink intoxicating levels of alcohol during both adolescence and adulthood, and that this volitional intake has long-term effects on subsequent drinking behavior. Nonetheless, this profound exposure to alcohol during adolescence does not increase impulsivity in adulthood, indicating that long-term changes in drinking are mediated by mechanisms other than impulsivity. Importantly, this research demonstrates that the HAP II mouse is a good candidate for a model of heavy adolescent alcohol consumption.

INTRODUCTION

Alcoholism and other alcohol-related disorders are severe problems in the U.S., affecting an estimated 17.6 million Americans and representing the third-leading preventable cause of death (Falk, 2008; CDC, 2001). Complex genetic differences and environmental influences contribute to the development of alcohol-related disorders, and research has consistently shown that multiple susceptibility genes affect variance in alcohol use (Kohnke, 2008). Discovery of correlated genes has led to experimentation with novel pharmacological treatment, improved understanding of alcoholism's development and maintenance, and the implication of potential underlying neurological processes (Oroszi & Goldman, 2004; Fehr et al., 2006). Furthermore, many key environmental risk factors such as alcohol expectancies, family influence, and educational or vocational aspiration have been implicated (Nash et al., 2005; Diaz-Anzaldua et al., 2011; van der Zwaluw & Engels, 2009). However, much of the genetic and environmental variance affecting alcoholism is still unexplained or poorly understood. Increased knowledge of the heritable psychological mechanisms and behavioral histories underlying alcoholism and alcohol abuse could aid in intervention and prevention of these destructive behaviors, improving outcomes for both alcohol-dependent adults and individuals who are predisposed to becoming dependent

Alcohol abuse among adolescents continues to be a problem in the U.S. It is suggested that earlier onset of drinking is correlated with increased alcohol abuse problems later in life; for example, one study showed that among high school students, those who drank alcohol before age 13 were 7 times more likely to consume 5 or more drinks 6 or more times per month than students who did not drink during high school (Grunbaum et al., 2004). Additionally, it is estimated that about 45% of subjects that drink alcohol before age 14 develop alcohol abuse disorders, compared with 10% who start drinking after age 21 (Grant & Dawson, 1997). Heavy adolescent drinkers tend to drink less frequently than adults with alcohol use disorders, but drinking episodes are marked by excessive binge-like consumption (Schulenberg et al., 1995). Risk factors for frequent binge drinking in adolescents include attention-deficit/hyperactivity disorder and conduct disorder, each of which is characterized by the underlying factor of high levels of impulse behavior, or impulsivity. Furthermore, strong risk factors for alcohol use disorders in adulthood are anti-social personality disorder and bipolar disorder, which also share the symptom of impulsivity (DSM-IV, 2000).

Adolescence as a Critical Period

A suggested mechanism for the correlation between an onset of drinking in adolescence and continued alcohol use problems throughout the lifespan is that adolescence represents a critical period of cortical development and a time when complex skills needed in adulthood mature (reviewed in Crews, 2007). Interestingly, common adolescent behaviors are shared across humans and other species such as rodents, such as increased social interaction which is seen in humans as high peer communication and in

animals as play behaviors or grooming (Csikszentmihalyi et al., 1977; Fassino & Campbell, 1981). Adolescents on the whole tend to be highly active and seek sensation and novelty, traits which manifest themselves differently across human individuals (Spear, 2000). For example, a low-risk individual may increase athletic or artistic interest whereas a high-risk individual may experiment with substances, among many other behaviors (Johnston et al., 2004; Csikszentmihalyi et al., 1977). It has been suggested that the activities engaged in during adolescence become habits that manifest themselves into adulthood, partially due to the shaping of the brain during this period. Profound changes in neurogenesis, cortical synaptic remodeling and pruning, receptors and transporters of neurotransmitters, and characteristic hormonal changes all occur during adolescence (Crews, 2007).

Importantly for studies of addictive behaviors and their underlying mental components, the prefrontal cortex and limbic system undergo many developmental changes during adolescence. The prefrontal cortex (PFC) is essential for higher decision making and planning, and genetic variation in catecholaminergic transmission to the PFC as well as deficits in development may increase poor, impulsive choice behaviors (Amsten & Li, 2005). Alcohol use during adolescence has been shown to cause differential brain damage to the PFC in adolescents as compared to adult rats, in turn leading to losses of executive function (reviewed in Crews & Boettiger, 2009). The limbic system includes the hippocampus, amygdala, nucleus accumbens, and the hypothalamus. Dopaminergic signaling from the nucleus accumbens is critical for reinforcement of rewarding behaviors, and levels of dopamine activity and receptor density increase during adolescence (Tarazi & Baldessarini, 2000; Tarazi et al., 1998).

These in tandem perhaps support the establishment the pleasurable activities of adolescence, potentially including alcohol use, as stable habits through modeling of attitudes and subjective reward signaling (Crews, 2007). Serotonin is another crucial neurotransmitter, principally synthesized in the raphe nucleus and implicated for its importance for mood, sleep, anxiety and other complex behaviors and exerting influences on the entire brain (Lauder and Bloom, 1974). Serotoninergic neurotransmission has been demonstrated to be altered in animals from adolescent alcohol use, potentially leading to permanent dysfunction of this important regulatory neurochemical (Monti et al., 2005).

Endophenotypes

However, a biological predisposition or environmental influence to initially use illicit substances clearly must precede this structural remodeling. Endophenotypes are heritable biological processes and trait behaviors that may underlie dysfunction including addictive disorders and other comorbid disorders. Endophenotypes are thought to be closely genetically related to the dysfunction that they precede (Gottesman & Gould, 2003). Evidence from many human and animal studies is supportive of several candidate endophenotypes of alcohol-related disorders, including impulsivity. Analysis of these endophenotypes helps to increase the understanding of the etiology and genetic susceptibility of alcoholism. It has been demonstrated that alcoholics prefer small, immediate monetary rewards and discount the value of larger, delayed rewards more than light drinkers (Petry, 2001). However, based on such results alone, it is unclear if higher impulsivity leads to the development of alcoholism or is induced by high alcohol consumption. Human studies of alcoholism etiology often present the confounding

variable of a history of drug use by subjects, though impulsivity has been shown to be a predictor of later alcohol use of teenage subjects (Ernst et al., 2006). Furthermore, animal studies using subjects that are assuredly drug-naïve have demonstrated that impulsivity is predictive of greater locomotor sensitization to alcohol (Mitchell et al., 2006). However, despite the previously discussed repercussions of alcohol's effects on the prefrontal cortex, the possibility that alcohol use in adolescence may increase impulsivity beyond the heritable high level that is correlated with alcoholism, which in turn may theoretically increase future drinking, has not been explicitly studied.

Impulsivity

Impulsivity has been separated into two or more components, including cognitive impulsivity and motor impulsivity. Cognitive impulsivity can be regarded as the impulsivity of choice and is generally represented by difficulties in either waiting for a reward or declining an immediate reward associated with later punishment, while motor impulsivity is a behavioral concept defined by the inability to withhold a response or remain motionless (Arce & Santisteban 2006). For the most part, studies of impulsivity and alcoholism have concentrated on cognitive impulsivity as measured by delay discounting (DD) or Iowa gambling task (IGT) models, which assess impulsivity as the devaluation of delayed rewards and the inability to assess future consequences, respectively (Mazur, 1987; Bechara et al., 1994). However, DD has shown higher correlations with validated impulsivity questionnaires and behavioral assessments in humans, suggesting it is a more reliable measure of impulsivity whereas the IGT may be a better measure of decision making (Luman et al., 2010; Goudria et al., 2007). Higher

levels of cognitive impulsivity as assessed by the DD task have consistently been associated with alcohol abuse in humans, and are additionally often correlated with alcohol preference and/or locomotor sensitization in animals, validating its use as a measure in studies of alcoholism and impulsive choice, where it has become fairly widespread (Petry, 2001; Oberlin & Grahame, 2009; Mitchell et al., 2006).

Delay discounting assesses impulsivity under the operational definition of impulsive behavior as the choice of smaller, immediate rewards over larger, delayed rewards (Rachlin & Green, 1972). In animals, this is commonly done by offering a choice of two levers, one linked to a delayed larger reinforce and one linked to an immediate smaller reinforce. Central to the theory behind delay discounting is the temporal value discounting function, commonly written as $V = A / (1 + kD)$, where V represents the subjective value to the animal of a reward of amount A after a delay equal to D. The hyperbolic delay parameter, k, governs the variable rate at which increasing delay causes an animal to discount the value of a reward; higher values of k represent a more impulsive animal (Green et al., 2007). A bias constant, b, is sometimes also added to the equation's numerator, which can account for the impact of factors such as lever side preference. However, this issue can also be avoided through counterbalancing or consistently setting a certain lever to be the previously-assessed preferred side. Also crucial is the concept of the indifference point, a point of trial for which the animal selects the immediate and delayed reward equally often (Helms et al., 2006). During experimentation, the values of amount and delay are manipulated in order to assess where this point lies, using procedures known respectively as adjusting-amount and adjusting-delay. These procedures have been shown to produce similar results in pigeons (Green et

al., 2007), though adjusting-amount is commonly used in rats and mice (Oberlin & Grahame, 2009; Mitchell et al., 2006; Richards et al., 1999). A reason that adjusting-amount may be preferable is that the usage of only one mean adjusted delay point is practiced, as opposed to a discounting hyperbolic function generated by multiple adjusted amounts at several delays (Perry, 2008).

Animal studies using the adjusting-amount paradigm, in further detail, feature two choices of levers to press for reinforcement: one resulting in a consistently large delayed reward and the other an immediate reward of an adjusting (and usually smaller) amount based upon reinforcer selection. Previously, high-alcohol preferring mice have been shown to be more impulsive than low-alcohol preferring mice using this paradigm when both strains are alcohol-naïve (Oberlin & Grahame, 2009). This is in agreement with human studies that have shown that a family history of alcoholism is predictive of greater impulsivity in alcohol-naïve and abstinent subjects (Ernst et al., 2006; Bjork et al., 2004). Additionally, it has been suggested that an earlier onset of alcohol abuse during adolescence correlates with increased impulsivity in adult subjects during abstinence (Bjork et al., 2004). However, this result was obtained in humans and is therefore subject to environmental confounds such as other drug use or variances in lifestyle over time. Additionally, there may be a confounding factor represented by an issue that is a variation of the previously-mentioned problem of the human literature: the possibility that increased impulsivity may predispose an individual to a younger age of the initiation of alcohol consumption (Petry, 2001; Ernst et al., 2006). Therefore, either achieving a similar result using animal models or failing to do so would prove very beneficial in

furthering the clarification of the relationship between past drinking and present impulsivity.

Much animal research using delay discounting to examine addictive behaviors and/or impulsivity has been conducted using inbred strains or heterogeneous stock (Helms et al., 2006; Mitchell et al., 2006). While such studies have been successful in demonstrating correlations between alcohol preference and impulsivity using various measures, each method has its individual drawbacks. Inbred strains are only representative of one homogeneous population, and therefore lack the genetic variance found in the population as a whole. In order to subvert this issue, research often uses many inbred strains, but this method lacks the face validity of assessing one genetically variant population. Additionally, using heterogeneous stock for the purposes of examining a strongly genetically-influenced trait provides difficulties with characterization of genetic and environmental variance, and may not model the desired population with effective power. However, with genetically selected lines such as high-alcohol preferring (HAP) and low-alcohol preferring (LAP) mice, trait relevant alleles are fixed while other alleles are free to vary. These lines were derived from heterogeneous HS/Ibg mice, and bidirectionally selected for high and low voluntary ethanol consumption while water was simultaneously available (Grahame, 1999). After many generations of selection, intakes have reached in excess of 20.0 g/kg/day (Oberlin et al., 2011). Therefore, the nature of HAP mice, of which currently several lines exist, to volitionally drink to high blood ethanol concentrations represents a unique strength of these selectively-bred animals; they represent a highly-effective animal model of a population at risk for alcohol use disorders.

Animal Models

The replicate II line of HAP mice (HAP II mice), used in the present research, represent the second mass genetic selection procedure undertaken to attempt to fix alleles relevant to high volitional ethanol consumption while simultaneously creating an otherwise-genetically diverse population. High-drinking males and high-drinking females from the progenitor strain of HS/Ibg stock were mated initially, while low-drinking males and low drinking females were mated to create the LAP II line of mice, which were not used for this research. Such bidirectional selection continued, up to over 30 generations of HAP II and LAP II mice as of the time of these experiments. Between 8-12 breeding pairs were used for each generation of each line, leading to a target of about 80 offspring for continued selection. Matings between siblings and first cousins were avoided to preserve outbreeding. Nonetheless, breeder number was usually able to maintain at >20 per line per generation (Oberlin et al., 2011). This selection procedure has created robust lines of high-drinking animals that presumably vary at non-trait relevant alleles and to this point has maintained very high fecundity. Overall, the HAP and LAP lines may provide a more valid model of the population than inbred strains, while enabling more effective study of the underlying genetic causes of alcoholism than heterogeneous stock (Grahame, 1999).

Furthermore, the ability to define a period of adolescence similar to that seen in humans is a valid strength of rodent models, including mice. Adolescent mice, along with adolescent rats, have been shown to differ from adult animals on measures of novelty-seeking, impulsivity, and stress-responsivity (Hefner & Holmes, 2007). Similarly, humans in their teenage years are generally more impulsive, more emotionally unstable,

and more likely to engage in novel or risk-taking behaviors than adults (Spear, 2000). Adolescence in mice is defined as the period of development encompassing the time period of about 24-61 postnatal days (P), and can further be divided into early (P24-P35), middle (P37-P48), and late (P50-P61) adolescence (Adriani et al., 2002). However, the adaptations in neural circuitry, neurobehavioral characteristics, and rapid body growth that are most comparable to the teenage years in humans occur during early and middle adolescence, comprising approximately P28-P42 (Spear, 2000). Therefore, this is the age range that is most frequently targeted in animal research, and the age range which this research targets as a candidate for behavioral change as a result of adolescent alcohol exposure.

A fair amount of literature has been published examining adolescent and adult drinking together using populations of rats (i.e. Bell et al., 2006; Vetter et al., 2007; Siegmund et al., 2005), or assessing behavioral changes in adulthood following adolescent alcohol exposure (ie. Salimov et al., 1996; Siegmund et al., 2005; Fullgrabe et al., 2007). Bell et al. (2006) demonstrated that adolescent alcohol-preferring (P) rats consumed more alcohol along with more water than adults when tested concurrently, though animals in this study only achieved mildly-pharmacologically relevant BECs of around 50 mg/dl. Siegmund et al. (2005), by contrast, ran an experiment with heterogeneous Wistar rats, and saw that adult animals consumed less alcohol than adolescents. Following deprivation, each age group saw a similar alcohol deprivation effect, demonstrated by transient increased intakes. Furthermore, this study showed an interesting effect of a significantly-higher increase alcohol consumption following a stressor in animals that initiated ethanol consumption in adolescence, though both age

groups increased alcohol intake following stress. Salimov et al. (1996) initiated alcohol consumption during adolescence in P rats in one group while not in another, and then following a short period of abstinence subjected all animals to measures of explorative behavior and mobility. Their results were taken to suggest that P rats exposed to alcohol during adolescence show lessened novelty-induced anxiety and lower response to stress induced by an inescapable situation, though there are alternative interpretations of the behaviors of commencing exploration earlier and spending less time attempting to escape out of an inescapable funnel. Vetter et al. (2007) used heterogeneous male Sprague-Dawley rats and demonstrated higher alcohol consumption in adolescence, and a distinctive plateau of consumption in early adolescence that was not observed when examining water or food consumption.

However, relatively few studies at this point to our knowledge have performed similar assessments of adolescent and adult drinking using mice, and none have utilized a selectively-bred free choice high-drinking strain such as HAP II mice. It has been observed that adolescent C57BL/6J mice, a commonly used high-drinking inbred strain, consume more free-choice alcohol than do their adult counterparts; however, adolescent DBA/2J mice, a low-drinking inbred strain, drink less than adults under a same limited-access paradigm (Moore et al., 2011). Another study examined mice selectively-bred to drink under a similar limited-access paradigm (Metten et al., 2011), and intermittent drinking procedures have also been assessed (Melendez, 2011). However, HAP II mice freely drink far greater levels of alcohol than do any of the aforementioned strains; adult HAP IIs show voluntary intakes in excess of 22.0 g/kg/day (Oberlin et al., 2011) while adult C57BL/6J consume about 16.0 g/kg/day and adult DBA/2J only consume about 1.0

g/kg/day (Yoneyama et al., 2008). Given these results, it would be reasonable to expect that any changes in future behavior or neurological functioning would be magnified in such a high-drinking population given the greater insult to the brain resulting from sustained exposure to elevated pharmacologically relevant levels of ethanol. Additionally, little research of adolescent drinking and its effects on adult drinking and the impulsivity of high-alcohol preferring mice has been conducted to date. Such experimentation could help to elucidate the relationship between innate and drug-induced impulsivity and help clarify adolescence's hypothesized status as a critical period, as well as lead to further validation of the HAP mouse as a reliable model of alcoholism.

Aims and Hypotheses

To characterize the adolescent free-choice drinking patterns of HAP II mice and test the effects of pre-exposure to alcohol during adolescence or adulthood on later drinking, a two-bottle choice drinking study was conducted using adolescent and adult animals. Adolescent animals were assessed at P28-P42, while adult animals were assessed at P60-P74. This experiment featured, uniquely, the characterization of adult drinking patterns alongside adolescent drinking patterns, in the same environment and timeframe. Following assessment of 24-hour drinking patterns, a specific time was selected to take blood samples in order to examine peak daily blood ethanol concentrations in adolescents and adults. A period of abstinence was included between drinking sessions to allow mice to age and withdraw from the effects of chronic alcohol consumption. Drinking behaviors were then re-examined. The presence of a group of adult animals controlled for the possibility that exposure to alcohol may increase alcohol

intake later in life regardless whether or not drinking commenced in adolescence. However, we hypothesized that adolescent pre-exposure would distinctively increase alcohol intake in adulthood, as well as that adolescent animals would drink more than the concurrently tested adult animals as suggested by the aforementioned studies using high-drinking rodent lines (Bell et al., 2006; Moore et al., 2011).

A second experiment was conducted to assess effects of free-choice adolescent alcohol drinking on adult impulsivity in HAP II mice. Mice either had free access to alcohol and water, or only to water, during early and middle adolescence (approximately P28-P42). Peak alcohol concentrations were assessed in the adolescent animals. The delay discounting task, using the adjusting-amount procedure, tested for impulsivity in adulthood following shaping. While all mice are hypothesized to discount delayed rewards to a greater extent as the delay increases, we expected to see group differences dependent upon adolescent ethanol intakes. We hypothesized that high-alcohol preferring mice who freely drink alcohol during adolescence will demonstrate greater impulsivity during adulthood (measured approximately at P90-P120), as demonstrated by steeper discounting curves. Additionally, it was expected that the degree of increased impulsivity shown by animals exposed to ethanol during adolescence will be dependent on dose and sustained blood ethanol content.

METHODS

Experiment 1: Drinking in Adolescence and Adulthood

Animals

60 replicate 2 high-alcohol preferring (HAP II) mice were used for this experiment. These animals were bred on-site from HAP II progenitors, and represent the 37th (adolescent) and 38th (adult) generation of HAP II's. As HAP II intakes have been very consistent since generation 27, the disparate generations were not considered to be a confounding variable (Oberlin et al., 2011). Eight litters of adolescent mice along with eight litters of adult mice were used, each litter yielding 1 or 2 male and 1 or 2 female mice. Dates of birth varied by four days total within each age group, and one month separated the average adolescent and adult birthday. These animals were counterbalanced across sex and litter within each age group into a water group and an alcohol group. Mice were individually housed in polycarbonate cages (27.9 x 9.5 x 12.7 cm) with Cellsorb bedding at an ambient temperature of 21 +/- 1 C. Animals were moved into the experimental room and single-housed on the same day, which was a week prior to commencement of Phase 1 drinking. Therefore, adolescent animals were 21 +/- 2 days old and adult animals were 52 +/- 2 days old. Lights were on from 20:00 to 08:00 hours. Mice had ad lib access to lab food and water during all phases. Animals in the adolescent

group were 28 +/- 2 days old at the beginning of the first drinking phase and 73 +/- 2 days old at the beginning of the second, while animals in the adult group were 60 +/- 2 days old at the beginning of the first phase and 105 +/- 2 at the beginning of the second. See Figure 1 for a timeline schematic of this experiment.

Experiment 1 Phase 1: Alcohol Pre-Exposure

Daily Procedure

Beginning at about P28 or P60, counterbalanced groups of adolescent and adult mice were allowed to drink from two 25-ml graduated cylinders, of which one contained water and one contained 10% EtOH in water solution. Another counterbalanced group of adolescent and adult mice also drank from two 25-ml graduated cylinders, but both contained water. The graduated cylinders were placed in the mice's home cages, replacing their usual water bottles, and bottle orientation was alternated every third day to eliminate effects of side preference. Weights from all animals were also taken on these days. Volume readings were taken from all graduated cylinders daily at 12:00 hours for 14 days using red illumination, with the exception of the last 2 days, when readings were taken multiple times daily, every 2 hours from 08:00 hours to 20:00 hours in order to assess bihourly drinking patterns. Food was moved from the home cage's lid to the floor for this entire phase.

Retro-orbital Bloods

Based on the results of the multiple daily readings, it was determined that retro-orbital bloods would be taken from animals at 16:00 hours to provide a measure of what was estimated to be peak blood ethanol concentrations. This procedure was executed on the 15th and final day of Phase 1 ethanol drinking. At 16:00 hours, mice were removed from the colony room and taken to the surgery room using a light shielded transporter. Bloods were taken using a retro-orbital procedure, involving applied pressure from heparinized capillary tubes to the back of one eye in order to break blood vessels and obtain a usable sample. Bloods taken from mice in the alcohol group were stored, and bloods were also taken from mice in the water group to avoid the addition of a confounding variable, but we disposed of these bloods. Mice were then returned to the colony room and water bottles were restored. Bloods taken from the alcohol group were later analyzed for ethanol concentration using gas chromatography, by centrifuging the blood and extracting a 1- µL sample of the plasma, then running samples against a 0-250 mg/dl % standard. This concluded Phase 1 of the experiment, and all animals were then left in the colony room for 30 days with no interaction other than regular bi-weekly cage changes.

Data Analysis

Readings data from the graduated cylinders of alcohol and water were recorded and organized using Microsoft Excel spreadsheets. Daily means and standard errors of intake were calculated for each group as a whole as well as separated by sex and age. Graphs of these data were created using GraphPad Prizm. Additionally, mean and

standard errors of 10% EtOH and water intake at two-hour intervals during the dark cycle were calculated and graphed. Finally, intake data was organized into SPSS and a repeated-measures analysis of variance (ANOVA) tested for between-subjects effects of group, age, and sex while also assessing the within-subjects factor of day of consumption. This phase of the experiment therefore was intended to feature a 2x2x2x14 design, consisting of 2 groups (alcohol and water), 2 ages (adolescent and adult) 2 sexes (male and female) and 14 days of analysis. However, the final 2 days of the experiment, featuring bihourly readings, were excluded from final analyses because of disparities in overall drinking behavior, so the final ANOVAs used utilized a 4-way mixed 2x2x2x12 design. Similar ANOVAs were used to assess water intakes as well as weights of animals, though since weights were taken every three days, the latter ANOVA featured a 2x2x2x4 design. Multivariate ANOVAs were also used to assess the bihourly readings data, examining all time periods as dependent variables of a 2x2x2 (Group, Age, Sex) independent variable design. An α-value of 0.05 was set as the significance threshold for all ANOVAs, t-tests, and Pearson analyses ran throughout all experiments. Data from Phase 1 were also used for later analyses following Phase 2 of the experiment.

Experiment 1 Phase 2: Adult Alcohol Drinking

This phase of the experiment was conducted similarly to the first, but all animals received 1 25-ml graduated cylinder containing 10% EtOH in water solution and 1 25-ml graduated cylinder containing water. The daily procedure in this phase was identical to that of Phase 1, except that readings every 2 hours were only taken for one full day, the

final day of a 13-day drinking measurement procedure. Data analysis again utilized Microsoft Excel, GraphPad Prizm, and SPSS. Graphs comparing data from Phase 1 and Phase 2 were created in addition to those examining Phase 2 data alone, and a repeated-measures 2x2x2x12 ANOVA was again performed. Additionally, consumption across phases was assessed using t-tests of the last four days of drinking in Phase 1 and the first four days of drinking in Phase 2. Pearson correlations of these data were also examined, and graphs were created. Four days was chosen to lessen measurement error and feature a sample of drinking that includes data spanning a side-switch, but focused upon days without the disturbance of a switch (3/4 days).

Experiment 2: Adolescent Drinking and Adult Impulsivity

Animals

48 replicate 2 high-alcohol preferring (HAP II) mice were used for this experiment. These animals were again bred on-site from HAP II progenitors, and represent the 37th generation of HAP II's. Six litters of adolescent mice were used, each litter yielding between 2 and 6 animals of each sex. These animals were counterbalanced across sex, litter, and delay discounting run order into a water group and an alcohol group. Again, mice were individually housed in polycarbonate cages (27.9 x 9.5 x 12.7 cm) with Cellsorb bedding at an ambient temperature of 21 +/- 1 C. Animals were transferred into the experimental room on P22/P23, and single-housed on P25/P26. Lights were on from 20:00 to 08:00 hours. Mice had ad lib access to lab food during all phases. During the adolescent two-bottle choice phase (Phase 1), mice had 24-hour access to water, while

during adult delay discounting phase (Phase 2), mice had restricted water access 2 hours a day after testing. Mice were 28/29 days old at the beginning of Phase 1, 46/47 days old at the beginning of Phase 2 shaping, and 105 +/- 15 days old during the collection of target delay discounting data. See Figure 2 for a timeline schematic of this experiment.

Experiment 2 Phase 1: Alcohol Pre-Exposure

All data collection procedures were modeled after those described for Experiment 1 Phase 1, with the important differences being that fewer animals were tested and all animals were in the same age group. Intake data was organized into SPSS and a repeated-measures analysis of variance (ANOVA) tested for between-subjects effects of group and sex while also assessing the within-subjects factor of day of consumption. Thus, this phase of the experiment featured a 2x2x13 design, consisting of 2 groups (alcohol and water), 2 sexes (male and female) and 13 days of analysis. A similar ANOVA was run on water data. In this experiment, unlike Experiment 1, bihourly readings were consistent with prior data and thus deemed acceptable for inclusion. Again, these data were also used for later analyses following Phase 2 of the experiment.

Experiment 2 Phase 2: Delay Discounting

Daily Procedure

Delay discounting shaping and testing began at P46/47. During this phase, mice were moved from the colony room to the testing room using a light-shielded transporter

unit at 10:00 hours, 5 days a week; animals were not tested on weekends. Each operant box was wiped with a wet sponge prior to each squad. Four squads of 12 mice each were run in 12 total operant boxes. At the end of daily testing, all mice received 2 hour water access in their home cage. Additionally, mice were weighed and had their ear tags checked each Monday prior to running. The bedding in each cage was changed following testing on Fridays. All sipper tubes were rinsed in bleach at this time. During weekends, they had ad lib access to water. Water bottles were then removed at 5 PM on Sundays to prepare for five days of testing beginning on Monday.

Apparatus

12 identical boxes measuring 21.6 x 19.7 x 12.7 cm were used for Phase 2 of this experiment, each with 2 sides of clear acrylic and 2 sides of aluminum (Med Associates ENV 307W, St. Albans, VT). Operant boxes were contained in sound and light attenuated chambers equipped with fans. An LED nose-poke infrared detector is centered on the 19.7 cm of each box at 6.3 cm above the floor. Below this light is a sipper access hole. The sipper tubes that were used for this experiment were 10-ml graduated plastic serological pipettes fitted with stainless steel tips. All tubes were filled with 0.0316% (w/v) saccharin solution. Two levers were mounted in each operant chamber for the latter stages of shaping and testing, each 2.5 cm above the floor on either side of the sipper tube opening. These levers had an LED 2.3 cm above them signaling activation following a nose-poke. Operant boxes were controlled using MedPC IV software on a Windows computer.

Behavioral Assessment

Overall, mice underwent 5 stages of shaping, with the final stage serving as 0-second delay testing. During the first stage, nose pokes were reinforced on a FR1 schedule with 20 seconds of sipper access, and non-contingent reinforcement was presented every 120 seconds. This stage was only run for 1 session. One reward of sipper access constituted one "trial" throughout all stages and testing, and following Stage 1, all trials represented contingent reinforcement. Stage 2 consisted of reinforcement of nose pokes on a FR1 schedule with 10 seconds of sipper access, and was run until 95% of mice had achieved 20 trials at least once. However, for all stages, mice that continuously failed to meet criteria were eliminated from the study. Typically, this results in the loss of less than 10 percent of subjects, a result which was again observed in this experiment. Stage 3 introduced cued trials; in this stage, the nose-poke light comes on every 30 seconds and remains illuminated for 20 seconds, representing a 20-second period when levers were active and an inter-trial interval (ITI) of 10 seconds. During this time, a nose poke resulted in 10 seconds of sipper access. This stage also was run until 95% of mice had completed 20 trials at least once. Stage 4 introduced the two levers, and a nose poke as well as a lever press was required for 10 seconds of sipper access. There again was a 10-second ITI. Side preference was assessed individually for each mouse in this stage. 95% of mice were required to complete 20 trials at least once to progress to Stage 5.

Stage 5 of shaping introduced a 30-second ITI, adjustment of sipper access time, and forced trials. Forced trials occurred when one lever had been chosen for two consecutive trials; this lever is unavailable until the alternative has been sampled once. During this stage, the preferred lever in Stage 4 became the "adjusting" (immediate) lever,

which adjusted sipper access time in increments of 0.2 seconds, downward for immediate choices and upwards for delayed choices. The other "standard" (delayed) lever always granted 2 seconds of sipper access, while the adjusting lever began at 1 second and adjusted accordingly. However, no adjustment of the standard lever resulted from forced trials. Adjusted amount values based upon the final 20 trials performed by each mouse were calculated and represented the main data point taken from that session. These values range from 0 seconds to 2 seconds. Stage 5 of training also served as the beginning of delay testing. Once animals reached adjusted amounts of 1.6 seconds or higher on three out of four days, delays of 1, 2, 4, 8, and 12 seconds were introduced, and each delay received 4 days of testing. The standard lever became the delayed, large reward lever for each mouse. When it was selected, the delay was subtracted from the 30-second ITI, so that selection of either lever resulted in the same amount of time that must pass before a mouse is able to receive another reward. During Stage 5 and testing, sessions were limited to 1 hour or 60 choice trials, whichever occurred first. Data from mice that did not complete 20 trials on a given day was excluded from analysis on that particular day, and mice that completed fewer than 20 trials on consecutive days were excluded from analysis of a delay and eliminated if substandard performance continued.

Data Analysis

Adjusted amounts, saccharin intake, and number of trials were recorded and organized into Microsoft Excel spreadsheets, and all data were subsequently analyzed. Graphs and charts were made using GraphPad Prizm, including the standard delay discounting hyperbolic curve created using average adjusted amount values for each

group from each delay. Following completion of all testing, *k* values, overall constants representing impulsivity in which higher values are equal to elevated impulsivity, were generated for each animal by using non-linear regression to assess adjusted amounts. The *k* value is derived from the formula $V = A/ (1 + kD)$, where *V* is the subjective value of the reward, *A* is the size of the delayed reward, *D* is the length of the delay, and *k* is an adjusted parameter (Mazur, 1987). Therefore, by using non-linear regression once all other parameters are known, including *V* representing the adjusted amount for each delay and $A = 2$, *k* values are able to be calculated. All data was organized into SPSS and a multivariate analysis of variance (ANOVA) examined for between-subjects impulsivity effects of group and sex, as well as any interaction effects, on all adjusted amount data, while a univariate ANOVA assessed effects on *k* values. Individual t-tests were also performed between groups on each delay. Additionally, *k* values were correlated with both the first day of drinking in adolescence to assess the potential effect of novelty on impulsivity as well as the final four averaged days of drinking to assess overall high consumption's potential effect on impulsivity, and graphs were generated of these data.

RESULTS

Experiment 1 Phase 1

An acquisition effect occurred in the alcohol group, as g/kg alcohol intakes increased steadily throughout the first 12 days of consumption (See Figure 3 and Fig 5 A and B). The final 2 days, when bihourly readings were taken, were excluded from these overall analyses and graphs because of an unexpected decrease in g/kg/day assessments, likely caused by the environmental disturbances of an experimenter entering the room to take repeated readings. A repeated-measures ANOVA demonstrated the observed acquisition curve, as it revealed significant main effects of Day ($F_{(1,26)} = 100.2$, $p < .01$) and Sex ($F_{(1\ 26)} = 7.8$, $p = .01$), but a lack of a Day by Sex interaction. However, importantly, this sex effect was entirely in the adult animals, as a significant Day by Age effect was noted ($F_{(1,26)} = 1.8$, $p = .04$) in the main ANOVA, and a second repeated-measures ANOVA ran on only adolescent animals showed no effect of sex, whereas a similar ANOVA considering adult data only showed a significant effect ($F_{(1,13)} = 12.7$, $p < .01$). Contrary to our hypothesis, adult animals drank more than adolescent animals over all 12 days ($F_{(1,26)} = 8.6$, $p = .01$). Additionally, there was neither a significant Age by Sex interaction, nor a Day by Sex by Age interaction. After the first 12 days, once the procedural change to bihourly readings was made, intakes appeared to be fairly level. Final adult consumptions over the first 12 days were consistent with prior data gathered using HAP II's, as females drank about 25.0 g/kg/day and males about 20.0 g/kg/day (see

Oberlin et al., 2011; Figure 1-B, 1-C). An ANOVA ran on water drinking data in all animals revealed significant effects of Age ($F_{(1,52)} = 44.2$, $p < .01$), Group ($F_{(1,52)} = 76.7$, $p < .01$), and Day ($F_{(1,52)} = 88.6$, $p < .01$) on water consumption. Overall, averaged data over the 12 days reveals an increase in adolescent water-only consumption, who consume more water than adults, steady water intake behavior in adults only given the choice of water, and decreased water intake in mice given a choice of ethanol (See Figure 4; as no Sex effects were seen, data are collapsed across sex in this table). Additionally, a Day by Group ($F_{(1,52)} = 14.0$, $p < .01$) interaction was seen, likely suggestive of the marked decrease shown in ethanol animals outweighing the effects of an increase in adolescent consumption.

On days 13-14 of acquisition, a multivariate ANOVA conducted using bihourly readings data revealed a significant difference between males and females throughout the dark or light cycle, representative of greater consumption by females in general ($F_{(3,26)} = 11.1$, $p < .01$). Interestingly, however, no significant difference between ages was found, nor was there a Sex by Age interaction. The similarity between adolescent and adult animals in drinking patterns is illustrated in Figure 6-A. Peak consumption was observed from 08:00-16:00 hours, though elevated (greater than metabolic rate) intake was seen throughout the dark cycle. Retro-orbital B.E.C. data taken at 16:00 hours on Day 14 showed remarkably high levels of intoxication in adolescents (M = 141.8 mg/dl, SEM = 25.3) and adults (M = 154.3 mg/dl, SEM = 10.0); the difference between age groups was not statistically significant (See Figure 6-B). B.E.C.'s are correlated with the averaged intake over the 8 hours of the dark cycle preceding retro-orbital bloods in Figure 7;

Pearson correlation analysis showed a significant correlation between g/kg/hr and B.E.C. in adolescents (R = .576, p = .050) and overall (R = .529, p < .01).

A repeated-measures ANOVA inspecting averaged weights revealed main effects of Sex ($F_{(1,52)}$ = 21.5, p < .01), Age ($F_{(1,52)}$ = 76.7, p < .01), Day ($F_{(1,52)}$ = 88.6, p < .01), Day by Sex ($F_{(1,52)}$ = 3.1, p = .02), and Day by Age ($F_{(1,52)}$ = 8.6, p < .01). Importantly, no Group effects were seen, demonstrating the strength of the HAP II 2-bottle free choice drinking model as compared to other high intake models that affect the weights of animals. Overall, weight data suggests that adolescent animals gained more weight than adults over the course of the 12-day period, and males are heavier than females (See Figure 8).

Experiment 1 Phase 2

During this phase, several data outliers representative of fluid spills were adjusted to the median of the day they occurred. This happened three times, twice on Day 6 and once on Day 8, and represents only 0.4% of total data points, or 3/750. As shown in Figure 9-A and-B, each group previously exposed to alcohol consumed significantly more alcohol on two of the first four days of post-exposure testing than the ethanol-naïve groups, while trending toward increased consumption on the other two, and intakes otherwise remained fairly level and similar across groups. Mixed ANOVA results revealed significant main effects of Day ($F_{(1,52)}$ = 13.0, p < .01), Group ($F_{(1,52)}$ = 5.2, p = .03), and Sex ($F_{(1,52)}$ = 15.4, p < .01), and an unexpected interaction effect of Day by Sex ($F_{(1,52)}$ = 5.8, p = .02; see Figure 8). However, no Day by Group interaction was seen. Interestingly, all groups failed to reach the typical level of intake for HAP II mice, as

consumptions for adolescents and adults reached only about 15.0 g/kg/day. One day of bihourly readings at the conclusion (Day 13) of testing failed to reveal any group differences, and bihourly readings were not continued nor were bloods taken, as at this point between-group variance other than sex effects was considered to be insignificant.

Experiment 1 Phase 1 to Phase 2 Comparisons

A t-test using data from the final four days of Phase 1 drinking and the first four days of Phase 2 drinking revealed a significant mean difference ($M_1 = 21.2$, $M_2 = 14.5$; $t = 7.99$, $p < .01$), unexpectedly suggestive of a negative alcohol-deprivation effect in each alcohol pre-exposed group. An age difference in this effect was also detected by comparing difference scores of the averaged last four days of Phase 1 and the first four days of Phase 2 ($M_{adolescent} = 4.0$, $M_{adult} = 9.5$, $t = -3.99$, $p < .01$). Bivariate correlation analysis using the Pearson statistic showed a significant correlation of the final four days of Phase 1 drinking to the first four days of Phase 2 drinking ($R = .49$, $p < .01$), as shown in Figure 11.

Experiment 2 Phase 1

A less pronounced acquisition effect was observed in this experiment compared to Experiment 1 (Figure 12); intakes initially appeared to be fairly stable throughout the two weeks of drinking around the levels generally seen in HAP II mice. Nonetheless, a repeated-measures ANOVA revealed a main effect of Day on g/kg/day consumption ($F_{(1,22)} = 54.2$, $p < .01$). However, no main effect of Sex was seen, nor was there a Day by Sex interaction. A repeated-measures ANOVA ran on water data revealed main effects of

Group ($F_{(1,44)} = 483.6$, $p < .01$) and Day ($F_{(1,44)} = 12.1$, $p < .01$). Contradictory to Experiment 1, animals in both of the water and ethanol groups decreased water consumption, though this effect was more marked in the ethanol group (See Figure 13). Bihourly readings showed peak ethanol consumptions from 08:00 hours to 13:00 hours, representing the first five hours of the dark cycle, though elevated intakes were seen throughout the dark cycle (Figure 14-A). These data also suggested the emergence of a sex effect as noted by trends toward increased female consumption at all hours of the dark cycle. Retro-orbital bloods were taken earlier in the day than in Experiment 1, at 12:00 hours, during this experiment because of the observed peak in drinking levels from 11:00-13:00 hours. Retro-orbital bloods again showed high levels of intoxication in adolescents (M = 108.8 mg/dl, SEM = 13.3), and the lower overall mean compared to Experiment 1 was likely a result of blood sampling earlier in the dark cycle. Interestingly, males showed a higher mean ($M_{male} = 120.8$, SEM = 17.6) than females ($M_{female} = 95.7$, SEM = 20.1) despite females' consumption being significantly higher throughout bihourly readings (See Figure 13-B). One mouse died during blood sampling, but a usable sample was obtained. B.E.C.'s are correlated with four hours of intake, assessing intake since the commencement of the dark cycle, in Figure 15; this correlation was significant ($R = .613$, $p < .01$).

Experiment 2 Phase 2

Animals shaped very efficiently, consistent with prior studies using HAP II mice of the same age. There were not any Group or Sex differences through the stages of shaping, or in the initial "delay" testing of 0 second delay. Stages 1-3 of shaping took 10

days, while Stage 4 took 5 days. Nine days of Stage 5 occurred before delay testing started, in which all remaining mice were able to meet criteria to advance. Three mice, in total, had their data excluded from consideration. As mentioned, one died during retro-orbital bloods; otherwise, one failed to meet criteria during shaping, and another's weight dropped below 15 grams during delay testing and became unresponsive, possibly because of a tooth problem. All three of these mice were in the alcohol pre-exposure group. As seen in Figure 16, significant differences were not observed at any delay between the pre-exposed alcohol group and the water group, though a trend was observed at 2 sec ($p = .12$). A univariate ANOVA also revealed no Group or Sex differences in impulsivity as measured by *k* values, while a multivariate ANOVA replicated this result for all delays tested. Pearson correlation analysis also showed a relationship between neither alcohol intake during the first day of Phase 1($R = -.132$, $p = .57$) nor alcohol intake over the last four days of Phase 1 ($R = -.132$, $p = .55$) and the *k* value determined in Phase 2 (see Figure 17).

DISCUSSION

Experiment 1

Drinking Assessment

Results from Experiment 1 are demonstrative of a significant effect of free choice pre-exposure to alcohol on alcohol consumption later in life in HAP II mice. Adolescent and adult mice achieved pharmacologically relevant blood alcohol concentrations throughout the dark cycle, representative of a sustained level of heavy intoxication. Following a month of abstinence, a main effect of increased intake was seen in pre-exposed animals compared to ethanol-naïve animals. However, individual t-tests demonstrated that this effect disappeared entirely during the fifth day of free choice drinking, and was only seen in two out of the first four days of drinking. Additionally, it was observed that pre-exposed animals failed to reach their previous intakes from the earlier phase of the experiment. This latter finding is in contradiction to previous studies demonstrating sustained increased within-subjects intakes in adulthood following binge-like alcohol exposure in C57BL/6J mice (Strong et al., 2010; Moore et al., 2010), but is largely in agreement with adolescent drinking studies using Wistar rats (Siegmund et al., 2005), DBA/2J mice (Moore et al., 2010), WSC-1 mice (Tambour et al., 2008), and a vapor exposure study using Sprague-Dawley rats (Slawecki & Betancourt, 2002) in which exposure did not generate a continued effect. Altogether, these findings appear to

be suggestive of genetics as the prime contributor to heavy alcohol consumption in animal models, as opposed to any consequence of early-onset drinking such as neurological changes or behavioral adaptation, but also that certain genotypes may be prone to the latter-mentioned effects. Alcohol consumed during adolescence may also be a factor, but the contradiction between the two highest-drinking strains assessed, HAP II mice and C57BL/6J mice, seems suggestive of some genetic difference.

General Discussion and Implications

Because P rats are selectively bred to drink high levels of alcohol, like HAP II mice, results of adolescent drinking studies using these animals should likely be considered in the same vein as our results. Bell et al. (2005), however, showed significantly higher intakes in adolescents as compared to adults P rats. The blood ethanol concentrations achieved by this study were significantly lower than in our research, however, showing means of 47.5 mg/dl in adolescent animals and 55.5 mg/dl in adults. Drinking patterns throughout a 22-hour period were also assessed in this study, but results again seemed contradictory to the present research, as P rats showed elevated drinking behaviors throughout the entire dark cycle, even demonstrating increase towards its conclusion, rather than the high g/kg intakes early in the dark cycle of HAP II mice. However, importantly, Bell et al. (2005) used a lick-o-meter to measure animals licking the sipper tube, rather than measuring actual mls of alcohol consumption. Therefore, it cannot be determined exactly where peak drinking periods were. This study also did not assess intakes of adolescent animals exposed to alcohol later during adulthood. However, another study with P rats showed that adolescents pre-exposed to alcohol acquired EtOH

operant responding quicker than non-exposed animals, a result seemingly in agreement with our assessed transient increase in adult drinking (Rodd-Henricks et al., 2002). Overall, our results are newly suggestive of an initial, transient increase in alcohol drinking caused by pre-exposure to alcohol in adolescence or adulthood in a selectively bred high-drinking population such as HAP II mice, and that these increased levels remain stable in the pre-exposed group while the non-exposed group's intakes rise to similar levels. This effect is likely either due to established tolerance in pre-exposed animals, or a behavioral or neurobiological difference that manifests itself in the other group of mice quickly once they are exposed to alcohol.

This experiment was also distinctively important because it was the first to assess adolescent and adult 24-hour free choice drinking concomitantly in a high-drinking population of mice. Other similar experiments have looked at adolescent and adult alcohol consumption using the drinking in the dark (DID) paradigm (Metten et al., 2011; Moore et al., 2010), and daily and intermittent drinking (Melendez, 2011). Metten et al. (2011) demonstrated that adult mice selectively bred for high alcohol consumption using the DID paradigm drank more than adolescents, while Melendez (2011) and Moore et al. (2010) showed that adolescent C57BL/6J mice had higher intakes of alcohol using each paradigm than their adult counterparts. Given these data along with the aforementioned increased intakes in adulthood seen following adolescent pre-exposure in this strain, adolescent C57BL/6J mice appear to be uniquely affected by alcohol consumption in adolescence. However, the blood ethanol concentrations measured by Moore et al. (2010) after two weeks of consumption were much higher in adults (M = 129.0 mg/dl) than adolescents (M = 95.7 mg/dl). Additionally, Metten et al. (2011) observed that once

adolescent mice entered adulthood their intakes matched the ones that initiated as adults; thus, all the results of this experiment were in agreement with our current research. It appears that whether the adolescent or adult animal voluntarily consumes more alcohol varies between populations, though further research involving differentiated alcohol access initiation ages across multiple genotypes is required to elucidate the relationship between alcohol intake and age.

Our research also showed that HAP II adolescent and adult mice reach roughly the same blood ethanol concentrations voluntarily (M = 141.8 mg/dl and M = 154.3 mg/dl, respectively). These adolescent B.E.C.'s were higher than those previously mentioned in the study by Moore et al. (2010), though this study showed lower intakes on the blood sampling day than in other days of the experiment. Additionally, g/kg/day consumptions were higher than those seen by Siegmund et al. (2005), Melendez et al. (2011), and Metten et al. (2011); these experiments did not include B.E.C. assessment. Furthermore, g/kg/day consumption was higher than those observed in forced administration studies that have demonstrated neurological changes resulting from adolescent alcohol exposure (Coleman et al., 2011; Crews et al., 2000), though it is important to note that these studies used intragastric injections either once or multiple times a day which causes a more rapid onset of intoxication and only the Coleman et al. (2011) study used mice as opposed to rats. The question of whether extremely high peak blood alcohol levels or sustained, but potentially much lower, blood alcohol levels leads to a greater neurological insult is relevant to such studies. Importantly in our current research, it is very evident that adolescent HAP II mice voluntarily drink to heavily pharmacologically relevant levels of intoxication, validating this study's opportunity to

detect an effect in adulthood based on adolescent exposure while simultaneously supporting the adolescent HAP II mouse as a candidate for future research involving free-choice adolescent drinking.

Furthermore, the observation that the weights of neither adolescent nor adult animals were affected by alcohol consumption is a crucial strength of the HAP II model of alcoholism. Other rodent models that cause similar sustained blood ethanol concentrations to the ones that all replicates of HAP mice achieve voluntarily, such as liquid diet or vapor chamber exposure, are sources of stress to animals and/or cause marked weight loss (Kang et al., 2004; Anji & Kumari, 2008). This issue may represent another crucial strength of the HAP mouse model of adolescent drinking, as adolescence represents a particularly sensitive time period for marked effects of stressors such as the emergence of depressive-like symptoms (Andersen & Teicher, 2009; Leussis & Andersen, 2008). Rodent models of adolescent drinking generally require at least one stressor, that of single-housing the animals at a young age, so it is beneficial to keep other stressors at a minimum to avoid confounding variables. For example, as will be discussed with the results of Experiment 2, the timing of single-housing animals in adolescence may have effects on alcohol intake, and it would have improved the current research to single-house adolescent animals on the same postnatal day in each experiment.

An interesting post-hoc result of Experiment 1 was the apparent observation of a negative alcohol deprivation effect. In rats (Sinclair & Richman, 1969), mice (Salimov et al., 1993), and humans (Burish et al., 1981), a period of abstinence sometimes produces an alcohol deprivation effect, or ADE, represented by an initial increase in ethanol intake following reinstatement. Periods of deprivation vary and are often between several days

and several weeks. The ADE, in mice, is often observed in strains that drink far less than high-alcohol preferring mice and is slight in overall magnitude, though relatively large (Ozburn et al., 2008; Tambour et al., 2008). With 30 days of deprivation, it was observed that HAP II mice intakes significantly decreased, though intakes at reinstatement were still far higher than in mice where a positive ADE is seen, such as WSC-1 mice that cease drinking at about 3.25 g/kg/day and resume at about 4.75 g/kg/day (Tambour et al., 2008). In contrast, the HAP II mice in this research ceased drinking at about 21.0 g/kg/day and resumed at about 14.0 g/kg/day. The final four days of Phase 1 drinking correlated with the first four days of Phase 2 drinking, averaged across days to lessen the effects of environmental noise and measurement error, suggest a consistent negative ADE across our sample and suggest that the alcohol consumption of HAP II mice is a state variable rather than a trait variable. The size of this effect was greater in pre-exposed adolescent animals than adults, perhaps demonstrating that drinking behavior in HAP II mice is more stable across long periods of abstinence if the age of onset is adulthood.

However, serious caveats exist regarding the consideration of this effect. Another surprising result of Experiment 1 was that animals never reached the expected intake levels for HAP II mice during Phase 2. Furthermore, given that the adolescent control group commenced alcohol consumption at about the same age as the adult alcohol group, it would be logically expected that each group would show similar drinking patterns, but this was not supported by the data. The lack of an acquisition effect, except for a minor one that can only be seen in females collapsed across groups and ages, is particularly interesting. HAP II mice usually show a gradual increase in drinking that is likely due to the progression of tolerance or sensitization, and further research into these prospective

mechanisms is a current goal of our lab. If the result of a lack of acquisition could be replicated in a similar experiment to Experiment 1 with the addition of continuous-drinking control groups, it may suggest a potential effect leading to lessened and altered alcohol intake brought about by being single-housed for an extended period of time prior to the initiation of drinking, as HAP II mice (and most other strains of mice) are generally given alcohol shortly after their removal from group housing. Previous research using rats has suggested that social isolation increases alcohol consumption (Apter & Erikkson, 2006) while another study showed no effect (Thorsell et al., 2005), though to our knowledge no similar experiments have been conducted using mice. Again, a continuous-drinking control group, in addition to a group that begins drinking at the beginning of the experiment and one that begins drinking following the former group's abstinence period, is required to properly assess this effect. The observation of similar results in the future would warrant further consideration of social and/or epigenetic factors in the drinking behaviors of HAP II mice or other strains.

Experiment 2

Adolescent Alcohol Drinking

Drinking results in the adolescents in Phase 1 of Experiment 2 were similar to the adolescents in Phase 1 of Experiment 1, with the notable difference of a clear lack of as pronounced of an acquisition effect, although the ANOVA did show a main effect of Day. The animals in Experiment 2 were single-housed more closely to the commencement of drinking (P25 as compared to P21), and the immediately elevated drinking may represent

an effect of single-housing stress. Importantly, both experiments showed a lack of a sex difference in adolescents. This result had not been documented in HAP II mice previously (Oberlin et al., 2011; Grahame et al., 1999) and may be suggestive of sex differences in intake being dependent upon pubertal changes occurring in late adolescence, as adolescent drinking had previously not been examined in HAP mice before this research. Further examination into this phenomenon is warranted; research into adolescent consumption using more subjects and examining hormonal changes within each sex is suggested. The high blood ethanol levels measured earlier in the day than Experiment 1 demonstrate that HAP II mice reach high levels of intoxication four hours or less after the beginning of the dark cycle, and the g/kg/hour consumptions measured suggest that they maintain these levels throughout the rest of the dark cycle. Alternatively, taken with the blood ethanol concentrations from Experiment 1, our results show that HAP II mice drink to intoxication quickly, and then further increase blood ethanol concentrations throughout the dark cycle. As earlier discussed, the blood ethanol levels obtained were highly pharmacologically relevant, and were consistent with or greater than prior research demonstrating neurological and behavioral changes caused by adolescent alcohol use in mice (Coleman et al., 2011) and rats (Crews et al., 2000).

Delay Discounting

Delay discounting results demonstrated that there is no effect on an adult HAP II mouse's level of cognitive impulsivity caused by alcohol consumption during adolescence. Previous unpublished results from our lab have demonstrated that adult alcohol exposure using forced injections does not have an effect on delay discounting in

high-alcohol preferring (HAP) and low-alcohol preferring (LAP) mice. It appears from our data that a history of past or present alcohol consumption does not increase impulsivity in high-alcohol preferring mice. However, several experiments have suggested that strains of animals who display high intakes of alcohol are naturally more impulsive than low-drinking strains, including a study using HAP mice (Oberlin et al., 2009) and another experiment using high-alcohol drinking rats (Wilhelm & Mitchell, 2008). Additionally, research using outbred WSC mice has shown that animals who display greater levels of impulsivity later show greater locomotor sensitization to alcohol than non-impulsive animals (Mitchell et al., 2006). Taken together, these results are suggestive of the trait of innately high cognitive impulsivity as measured by the delay discounting task being a causal factor of predisposition to alcoholism, rather than high levels of impulsivity resulting from prior alcohol use in adolescence or adulthood.

Implications and General Discussion

These findings also appear to be in accord with longitudinal human research that has failed to observe significant impulsivity differences among alcohol drinkers and non-drinkers in adolescence (Squeglia et al., 2009). A study involving high-and-low binge drinking college students noted poorer decision making as measured by the Iowa Gambling Task in the binge drinking group, but no differences in the impulsivity questionnaires issued (Goudria et al., 2007). Additionally, McQueeny et al. (2008) found only modestly decreased behavioral inhibition as measured by the Go-No Go task in drinkers. However, other cognitive changes as a result of adolescent binge drinking have been noted. Decrements in verbal encoding abilities in teenage drinkers, such as a lack of

differential cortical activation to novel stimuli as opposed to non-novel, and the ability to encode fewer words than non-drinkers have been observed (Schweinsburg et al., 2010). Deficits in spatial working memory function as well as abnormalities in brain response to the task have also been observed in adolescents with alcohol use disorders (Tapert et al., 2004). Overall evidence, therefore, is indicative of a lack of an effect on impulsivity as a result of adolescent binge alcohol use, but supportive of other various and detrimental cognitive changes.

This research was very relevant to the ever-growing knowledge base regarding concurrent alcoholism and impulse control disorders. Given the animal studies (Oberlin et al., 2009; Mitchell et al., 2006) as well as the human studies (Ernst et al., 2006; Bjork et al., 2004) previously discussed as well as the results of the current research, the phenotype of high impulsivity appears among high-drinking lines of animals and the relatives of alcoholics. Our results show that alcohol exposure during the critical period of adolescence fails to increase impulsivity in HAP II mice, though it does increase adult drinking. A potential caveat to this finding is that adult drinking also increases drinking following abstinence, which suggests that adolescence is not as critical of an exposure period as is commonly thought. Nonetheless, the observed transient increase in drinking in either case is seemingly mediated by an effect other than higher levels of impulsivity. Other potential mechanisms include the development of metabolic tolerance, functional tolerance, or appetitive tolerance, or perhaps sensitization to the pharmacological effects of alcohol. Additionally, the change in another behavioral construct, perhaps in the same vein as impulsivity or fundamentally different, may have occurred. These questions should be addressed by future research.

Conclusions

In sum, this study and past research taken as a whole is very suggestive of high levels of impulsive choice as an endophenotype of a predisposition to drink heavily and possibly develop alcohol-related problems. Furthermore, alcohol use earlier in life appears to promote later alcohol use, though whether or not adolescence is a critical period for this increase remains unclear. Evidence suggests that different animal populations may be differentially affected by the age of onset of alcohol consumption, a result which may theoretically generalize to humans. Additionally, the possibility that alcohol use may increase impulsivity in non-predisposed individuals warrants consideration. Thus, future delay discounting research should test IP injections or otherwise forced administration of pharmacologically relevant alcohol levels to low-drinking animal strains. While such a procedure lacks the face validity of free choice drinking, it would further elucidate the relationship between alcohol and impulsivity, perhaps suggesting an important social or epigenetic component to human comorbidity of alcoholism and impulsivity. Future studies examining adolescent and adult alcohol consumption should utilize a variety of genotypes and a range of ages of drinking onset in each genotype. Additionally, cognitive tasks such as those requiring working memory should be tested in animals exposed to alcohol during adolescence. Lastly, abnormalities in brain structure and function should be researched in each population, especially if differences in behavior are observed.

TABLES

Table 1. Water drinking in ml/kg/day throughout the first 12 days of Experiment 1 Phase 1. These data are suggestive of an increase in water drinking in adolescents, who consume more water than adults, and steady water drinking in adults that were not offered ethanol, and a decline in water drinking in all animals that were given a choice of ethanol or water.

Water Consumption

Day	1	2	3	4	5	6	7	8	9	10	11	12
Adol W	385.7	378.4	404.8	399.8	372.8	418.5	421.6	443.8	424.2	375.9	441.0	440.0
Adult W	361.5	335.5	337.4	348.9	318.6	342.1	350.5	341.6	351.3	330.3	361.3	362.0
Adol E	183.1	229.3	173.6	166.6	171.9	145.4	117.8	112.9	79.5	132.2	121.6	90.6
Adult E	132.9	87.3	79.4	67.7	37.4	23.3	28.4	17.9	9.4	19.9	16.1	13.5

Table 2. Weights in grams throughout Experiment 1 Phase 1, assessed on experimental days 3, 6, 9, and 12, which correspond to, on average, P30, P33, P36, and P39 for adolescents and P62, P65, P68, and P71 for adults. Significant differences were assessed between ages and sexes, and as a result of the progression of time, but no differences were seen between the alcohol and water groups.

Averaged Weights

		Day 3	Day 6	Day 9	Day 12
Males	*Adult E*	26.1	26.4	26.2	27.0
	Adol E	20.3	21.2	21.6	22.3
	Adult W	25.4	26.0	26.0	26.2
	Adol W	21.0	21.5	22.2	22.7
Females	*Adult E*	22.8	23.2	23.5	24.2
	Adol E	18.9	19.5	20.4	20.7
	Adult W	22.5	23.7	23.7	24.5
	Adol W	18.6	19.4	20.2	21.1
Overall	*Adult E*	24.6	24.9	24.9	25.7
	Adol E	19.5	20.3	21.0	21.5
	Adult W	23.9	24.8	24.7	25.3
	Adol W	19.8	20.5	21.3	21.9

Table 3. Experiment 2 Phase 1 adolescent ml/kg/day water consumption. These data are interestingly suggestive of a decrease in water consumption in both 2-bottle choice groups, though it should be noted that the last three days considered consisted of bihourly readings.

Water Consumption

Day	1	2	3	4	5	6	7
Water	422.1	371.7	367.4	367.4	391.6	359.7	412.7
Ethanol	166.0	91.2	84.9	84.9	67.4	61.2	60.4
Day	8	9	10	11	12	13	
Water	357.6	374.8	334.4	359.4	329.3	316.5	
Ethanol	47.9	42.9	30.6	49.3	39.4	45.5	

FIGURES

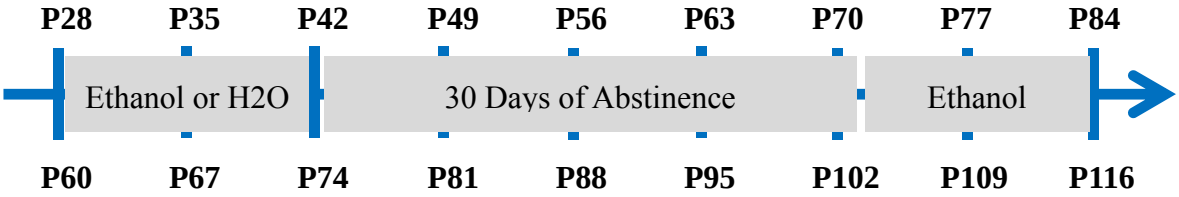

Figure 1. Overall timeline schematic of Experiment 1. The post-natal days listed on the top of the timeline correspond to adolescent animal ages, while those on the bottom correspond to adult animal ages. (Phase 1: Ethanol or H2O, Phase 2: Ethanol.)

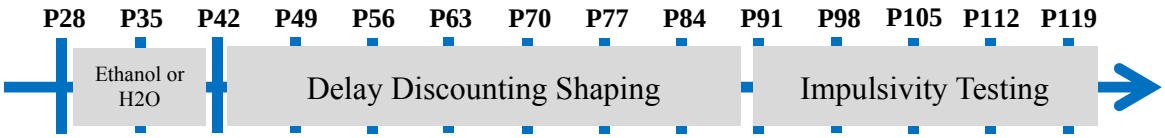

Figure 2. Overall timeline schematic of Experiment 2. Phase 1 data collection occurred from P28-P42, while Phase 2 data collection occurred from P90-P122.

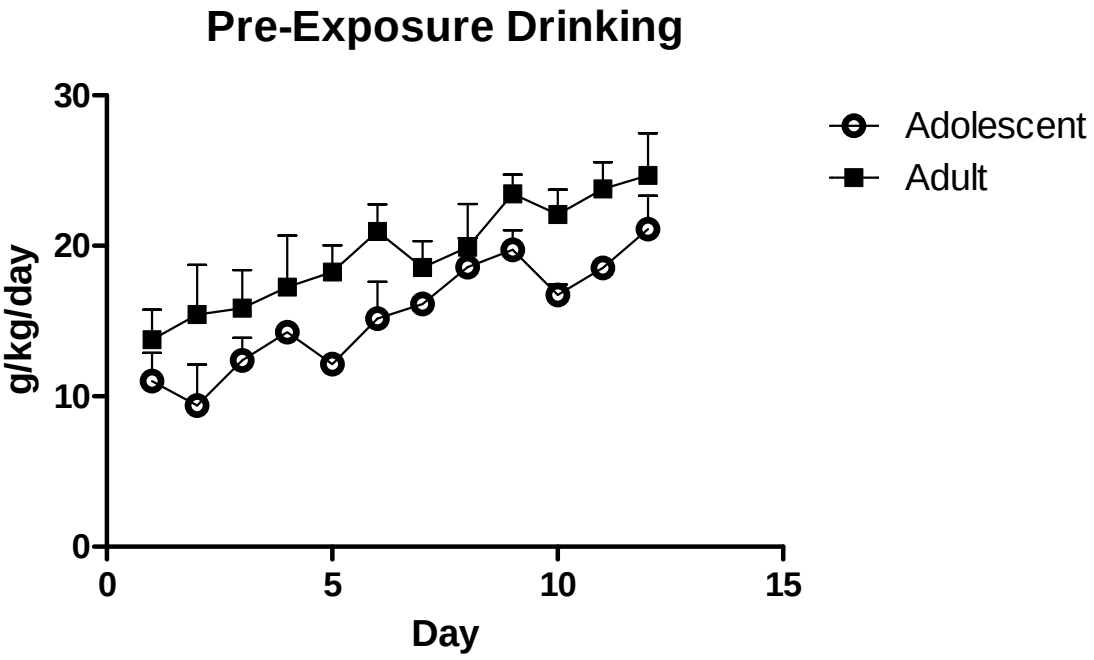

Figure 3. Experiment 1 Phase 1 overall drinking in adolescent and adult animals, collapsed across sex, over the first 12 days. Adult animals consumed significantly more alcohol over this time period. Data in these figures and others are expressed as mean + SEM.

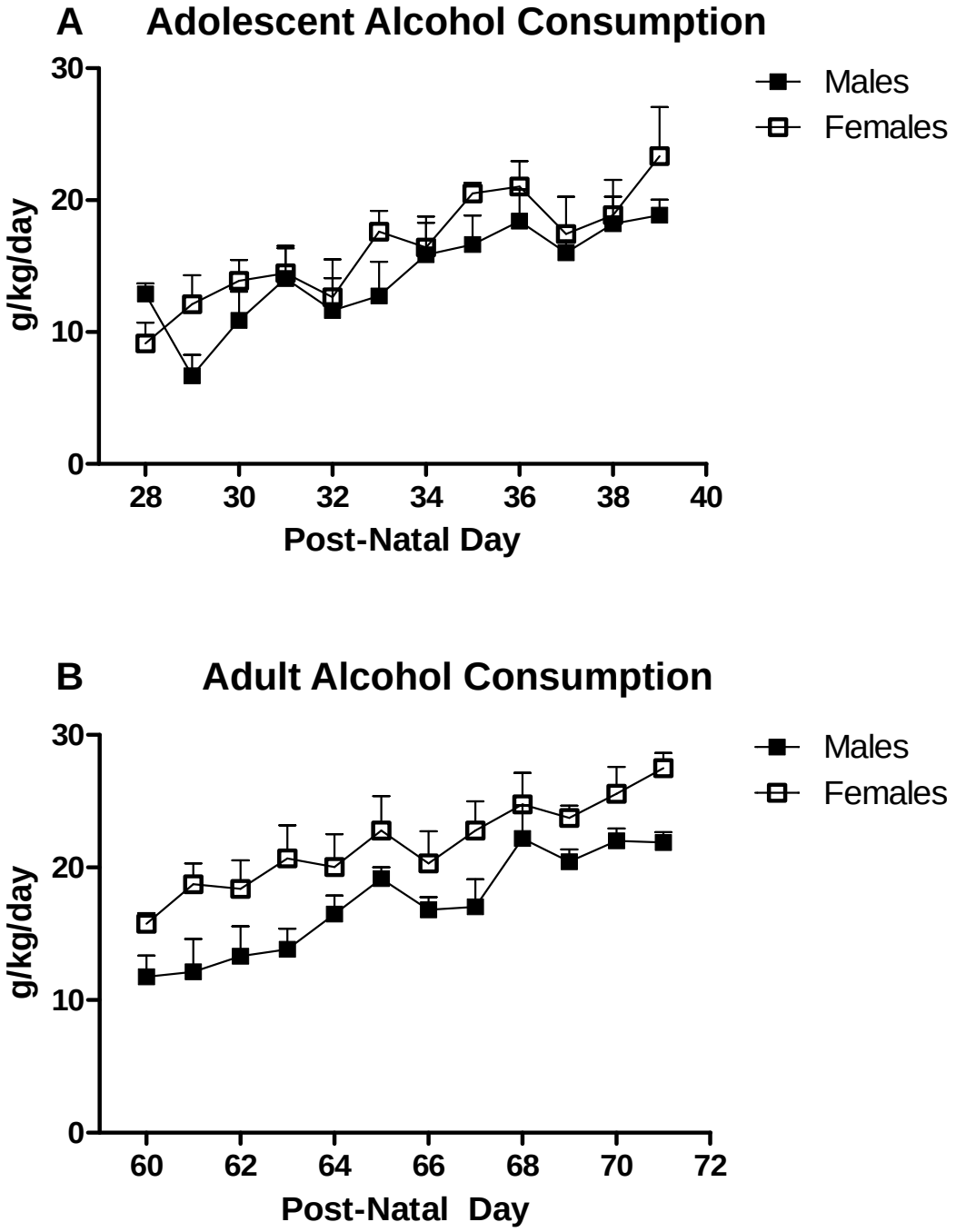

Figure 4. Ethanol intake in male adolescents (n = 7) and female adolescents (n = 8) groups over 12 days. B. g/kg/day drinking in male adults (n = 8) and female adults (n = 7).

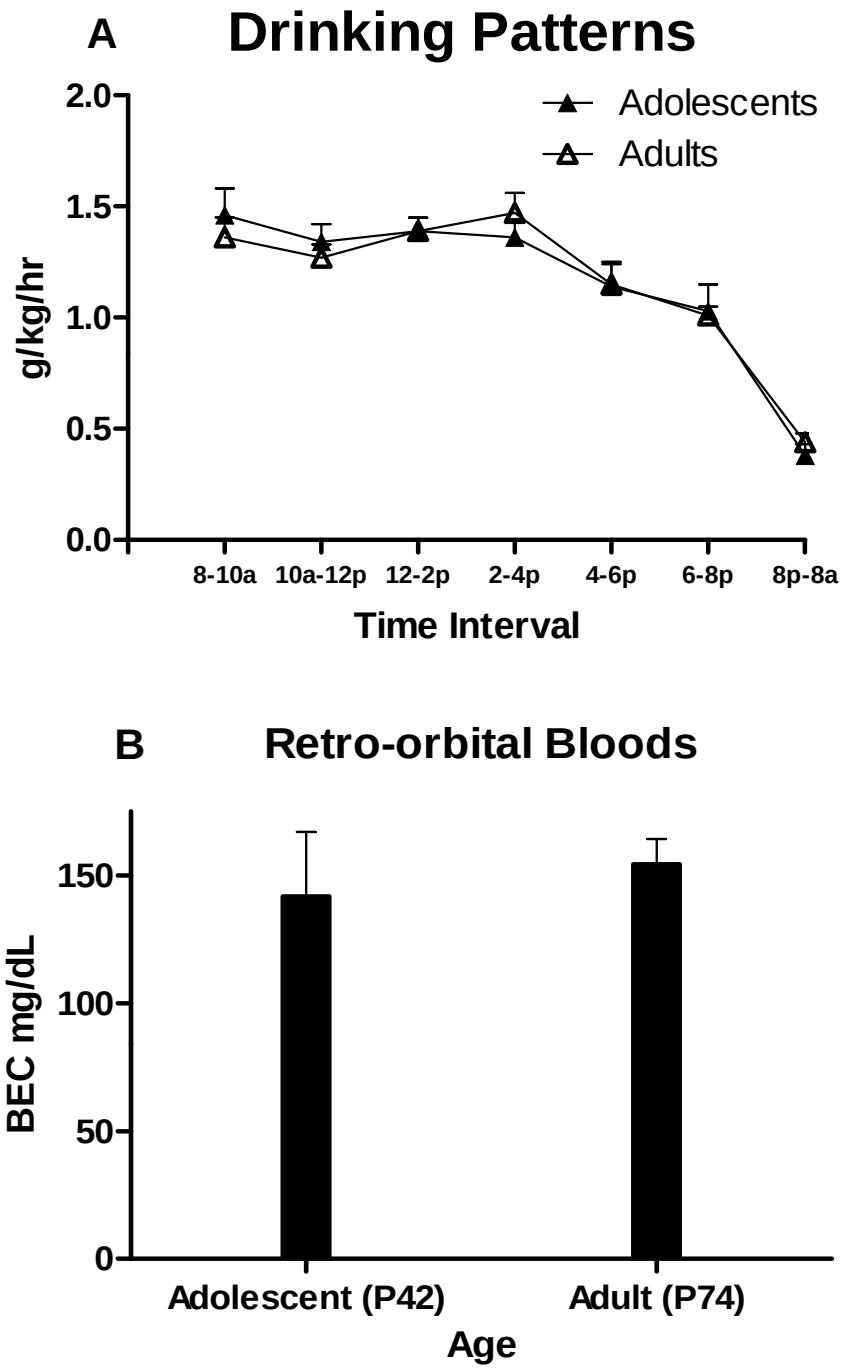

Figure 5. A. Experiment 1 Phase 1 g/kg/hr drinking patterns over 2 days. No significant differences were observed between adolescent (n = 15) and adult (n = 15) groups. B. Average blood ethanol concentrations measured via retro-orbital bloods at 4 PM of the final day of pre-exposure (P42 or P74). ($M_{adolescent}$ = 141.8, SEM = 25.3; M_{adult} = 154.3, SEM = 10.0)

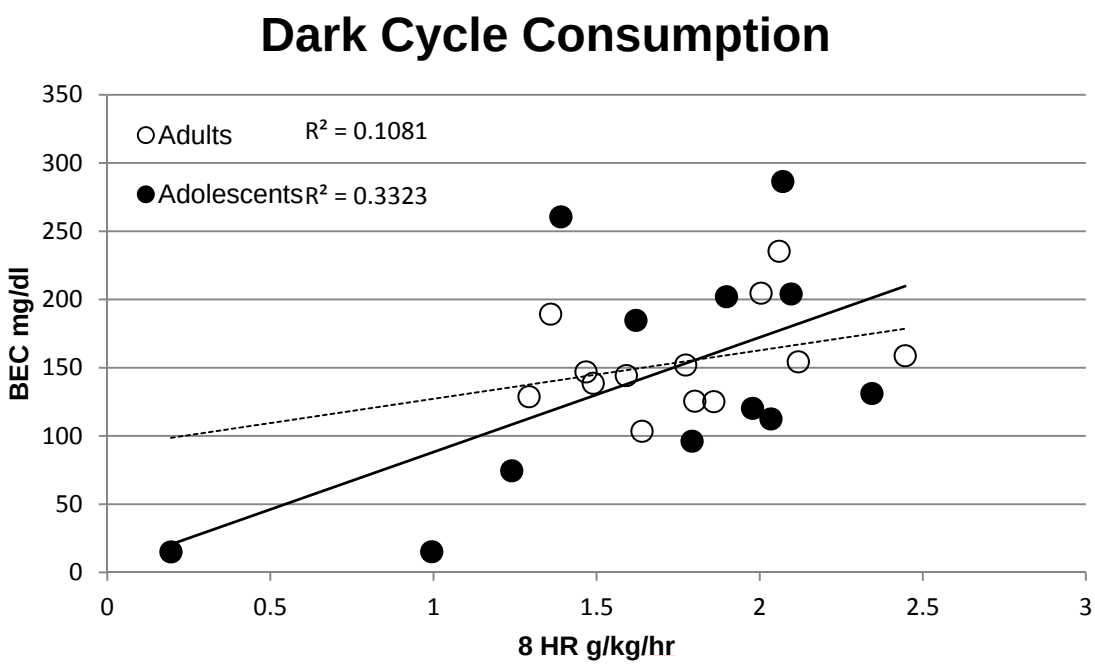

Figure 6. Retro-orbital bloods in adolescents and adult animals assessed against g/kg/hr from the beginning of dark cycle consumption in Experiment 1 over 8 hours, from 08:00 hours to 16:00 hours. R^2 values are indicated for adults and adolescents in the figure; Pearson analysis for adults indicated a non-significant correlation ($R = .329$, $p = .27$) while correlation for adolescents was significant ($R = .576$, $p = .050$). Additionally, combined data showed a significant correlation ($R = .529$, $p < .01$).

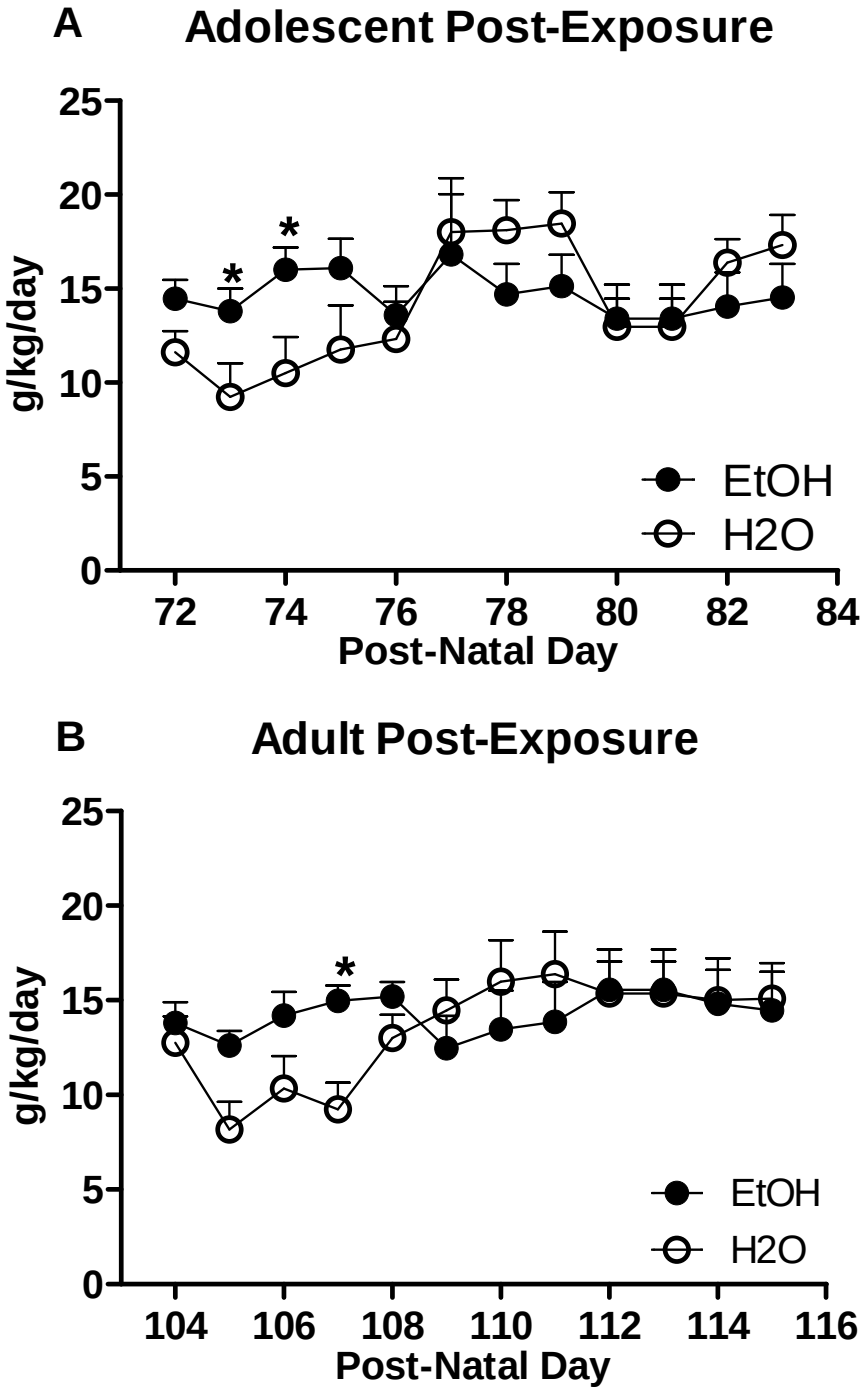

Figure 7. Experiment 1 Phase 2. Alcohol pre-exposed mice drank more alcohol than water pre-exposed mice in both age groups. Significant group differences are marked by *'s. A. Animals pre-exposed to alcohol (n = 15) or not (n = 15) as adolescents. B. Animals pre-exposed to alcohol (n = 15) or not (n = 15) as adults.

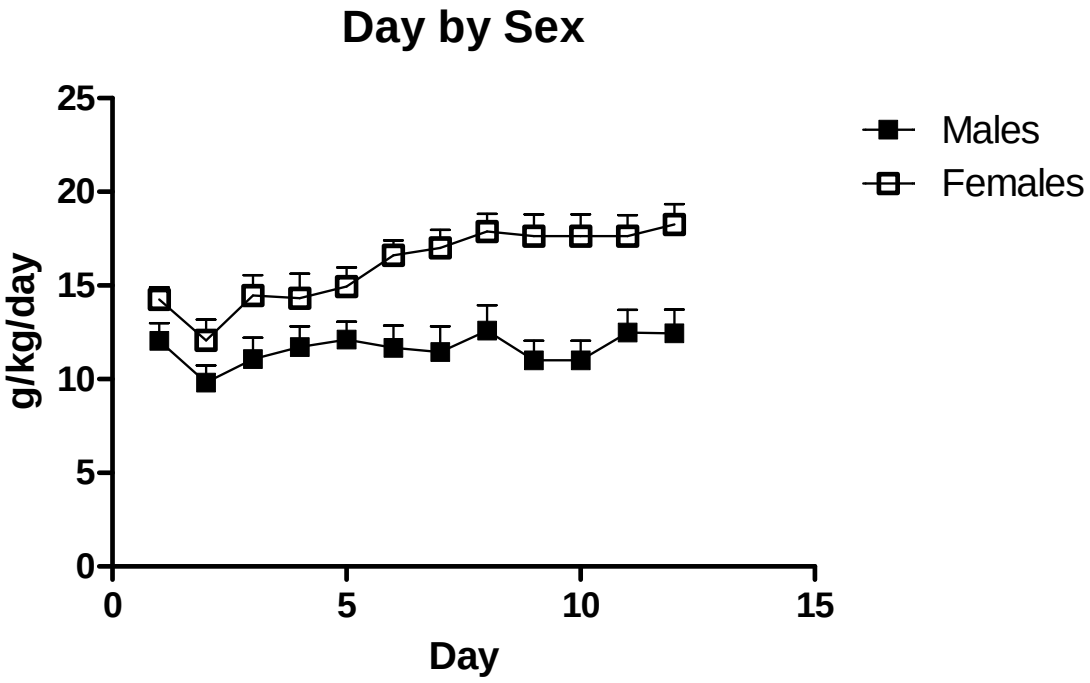

Figure 8. Collapsed across ages and groups, females steadily increased g/kg/day consumption in Phase 2 while males remained at baseline level, representative of a significant day by sex interaction.

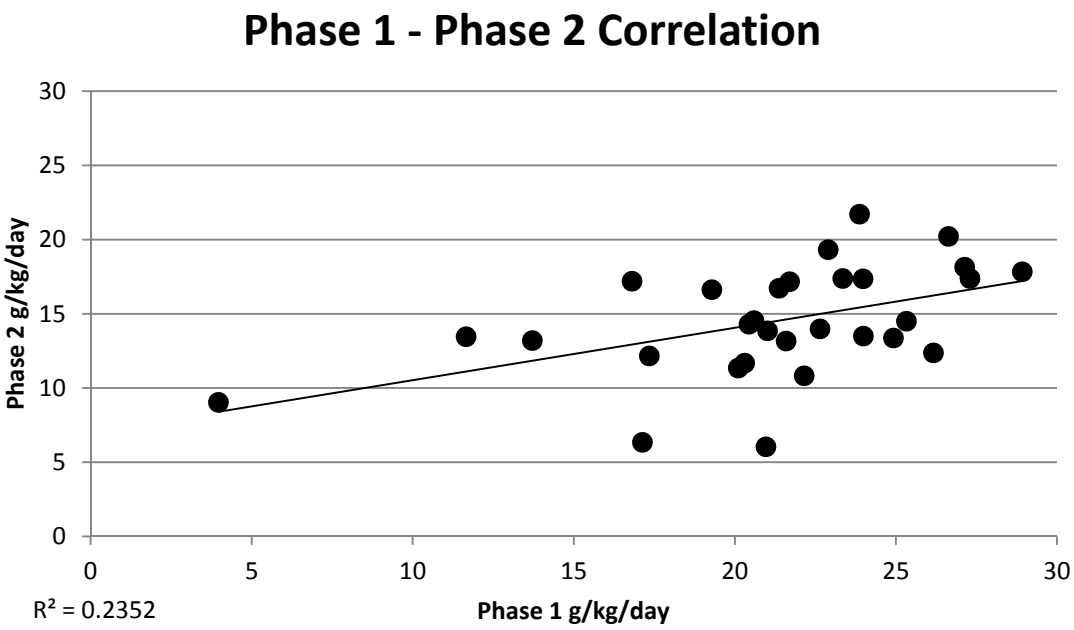

Figure 9. Experiment 1 Phase 1 to Phase 2 comparisons. The averaged last four days of pre-exposure drinking were correlated with the averaged first four days of post-exposure drinking in ethanol pre-exposed animals. Pearson analysis showed this correlation to be significant ($R = .49$, $p < .01$).

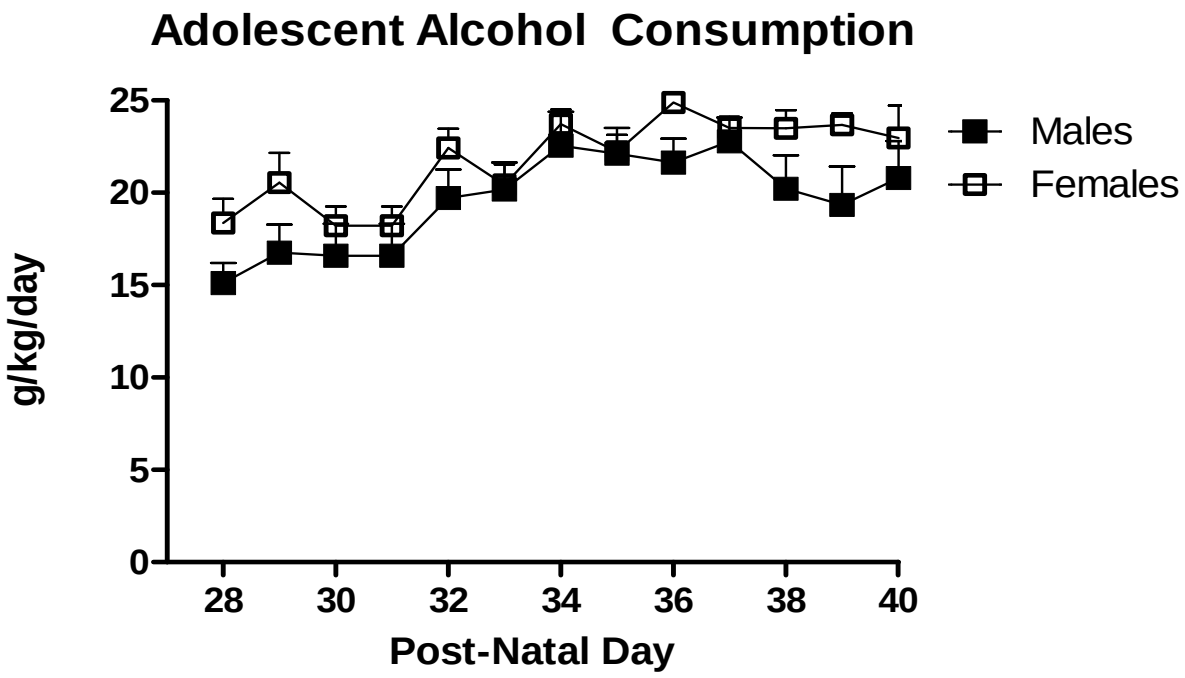

Figure 10. Experiment 2 Phase 1 adolescent g/kg/day ethanol intake in male (n = 12) and female (n = 12) groups over 13 days, the last 3 of which consisted of drinking pattern observation. No sex effect was observed in these data.

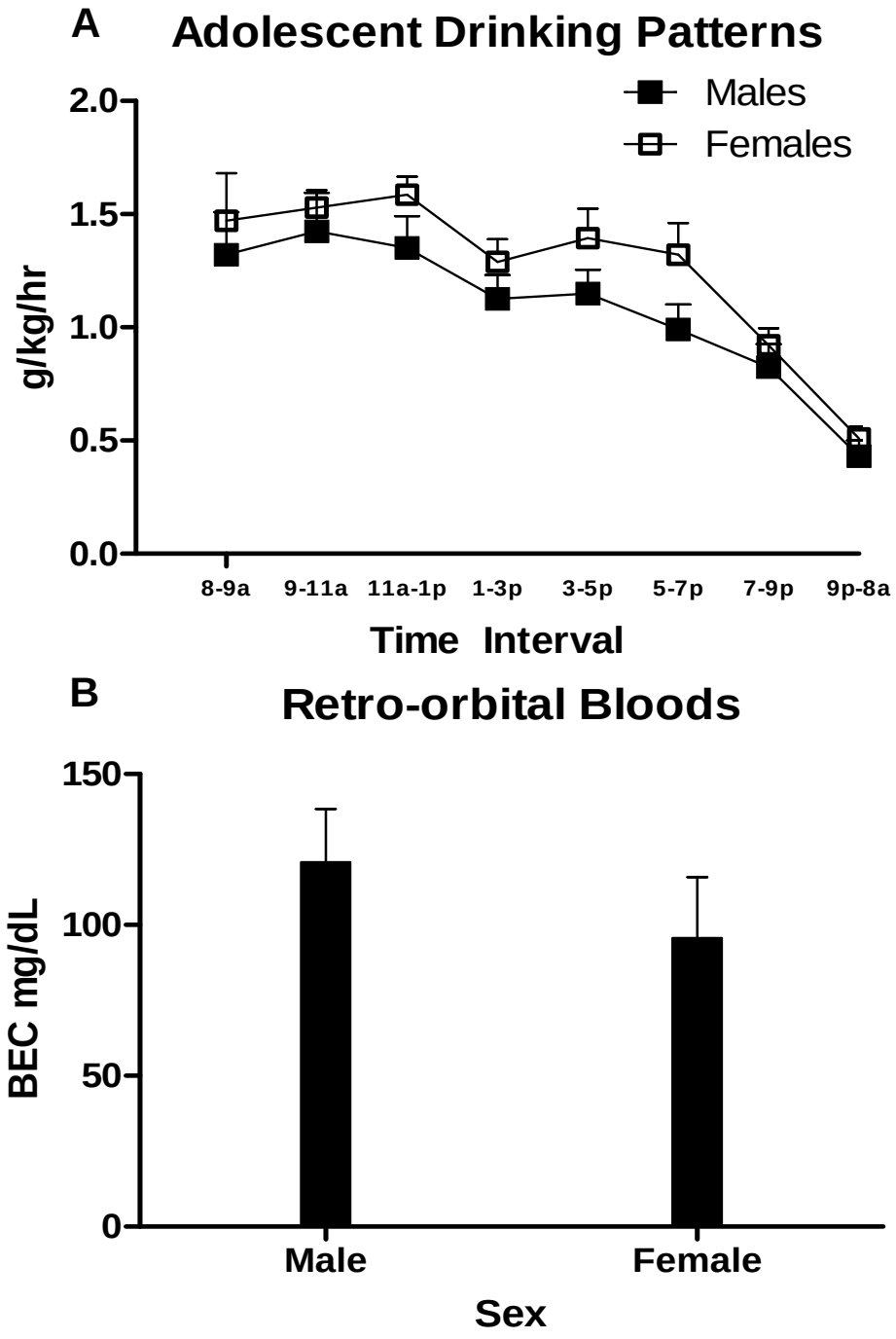

Figure 11. A. Experiment 2 Phase 1 g/kg/hr drinking patterns in male and female groups over 3 days, from P38-P40. Data suggests a trend toward a sex effect is emerging. B. Blood ethanol concentrations assessed at 12 PM on P41. Interestingly, males demonstrated a higher mean. (M_{male} = 120.8, M_{female} = 95.7)

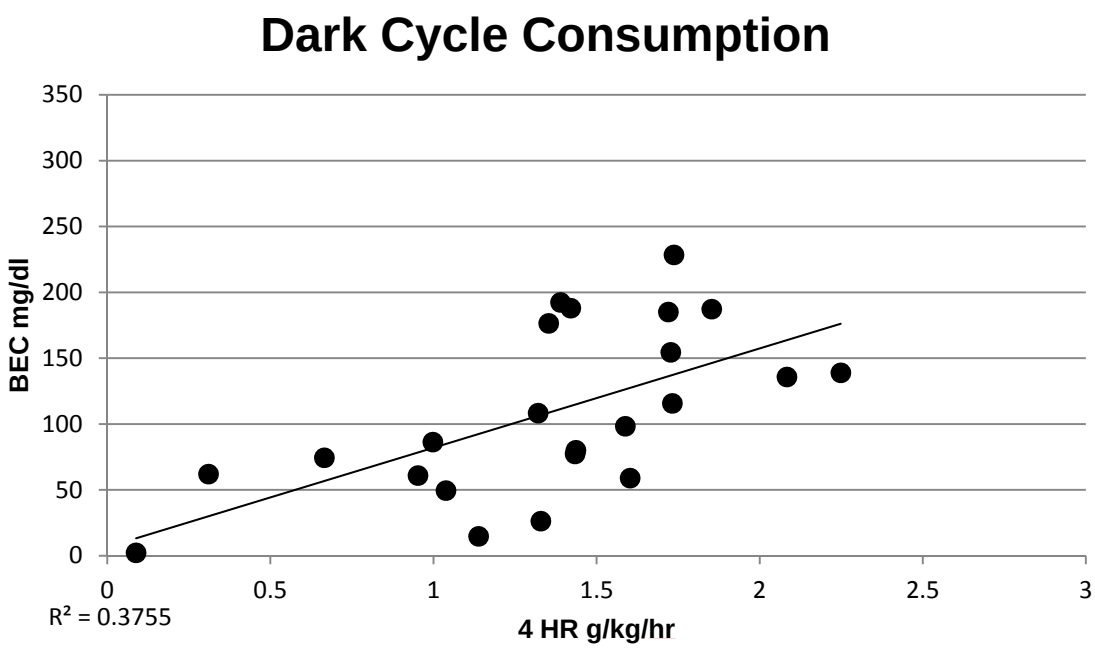

Figure 12. Retro-orbital bloods in adolescent animals assessed against g/kg/hr from the beginning of dark cycle consumption in Experiment 2 over 4 hours, from 08:00 hours to 12:00 hours. R^2 value is indicated; Pearson analysis showed a significant correlation ($R = .613$, $p < .01$).

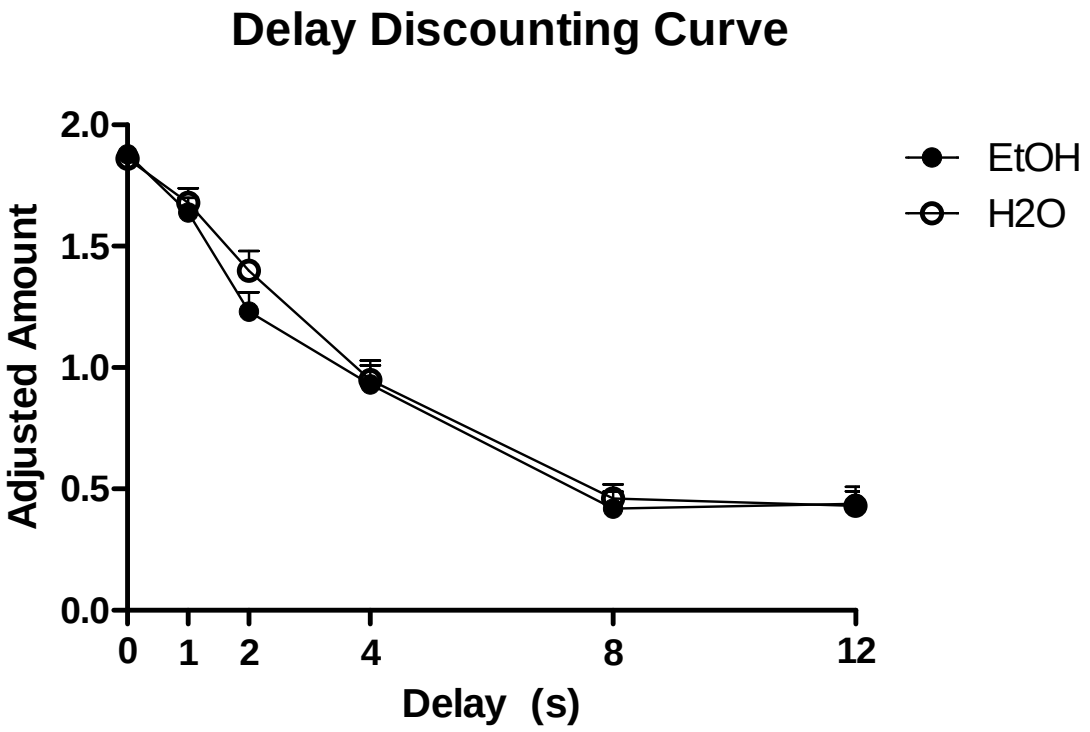

Figure 13. Experiment 2 Phase 2 group mean adjusted amounts + SEM in seconds of access shown at each delay tested. While a trend toward increased impulsivity in the EtOH group is seen at the 2-second delay ($p = .12$), no significant group differences were observed at any delays measured.

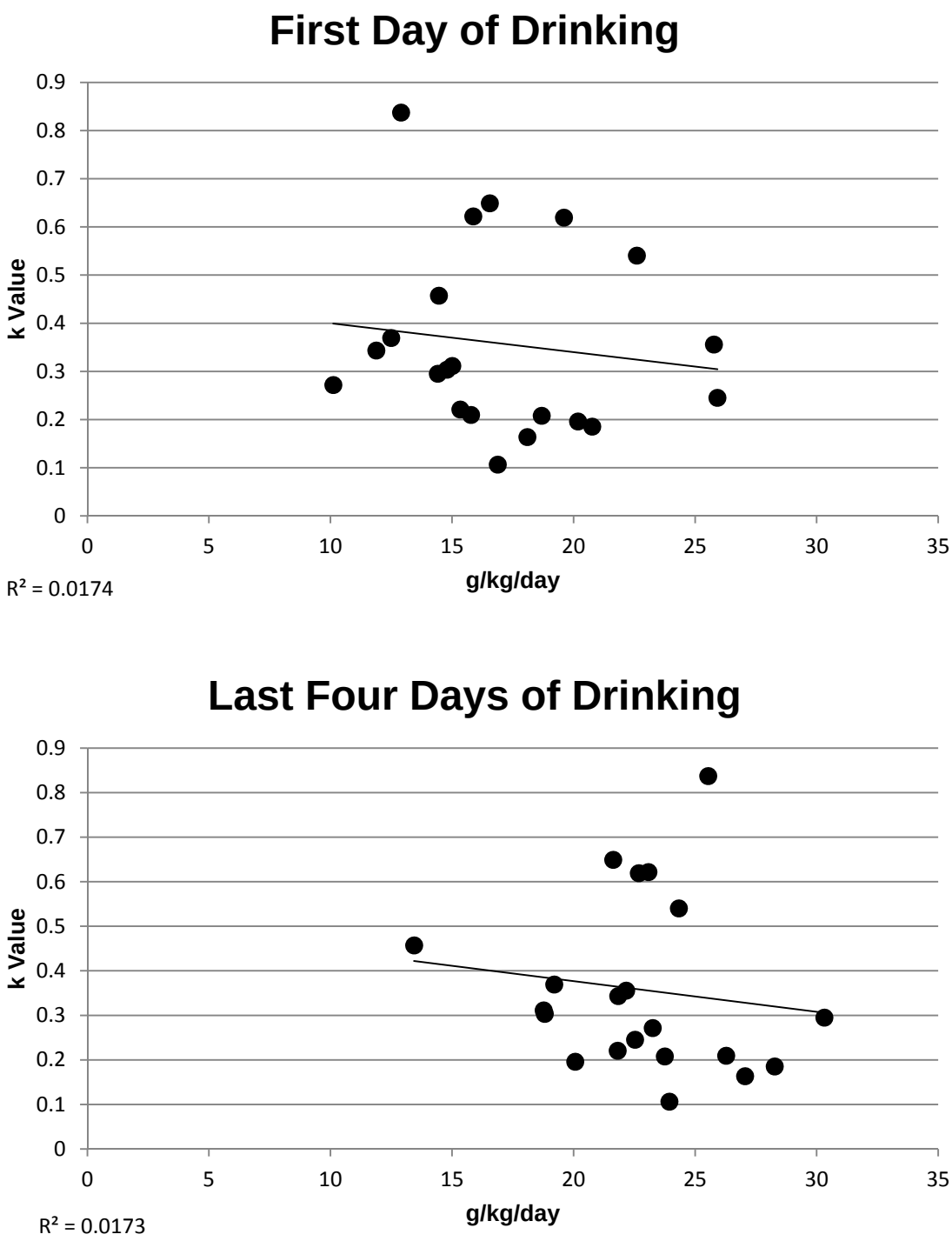

Figure 14. Significant correlations were demonstrated between neither the first day of alcohol intake in adolescence (A; R = -.132, p = .57) nor the last four days of alcohol intake in adolescence (B; R = -.132, p = .55) and the *k* values assessed in adulthood**.**

REFERENCES

REFERENCES

Adriani W, Macri S, Pacifici R, Laviola G (2002) Peculiar vulnerability to nicotine oral self-administration in mice during early adolescence. Neuropsychopharmacology 27(2):212-224

Apter SJ, Eriksson CJP (2006) The role of social isolation in the effects of alcohol on corticosterone and testosterone levels of alcohol-preferring and non-preferring rats. Alcohol and Alcoholism 41(1):33-38

Arnsten AFT, Li BM (2005) Neurobiology of executive functions: Catecholamine influences on prefrontal cortical functions. Biological Psychiatry 57(11):1377-1384

Andersen SL, Teicher MH (2009) Desperately driven and no brakes: Developmental stress exposure and subsequent risk for substance abuse. Neuroscience and Biobehavioral Reviews 33(4):516-524

Anji A, Kumari M (2008) Supplementing the liquid alcohol diet with chow enhances alcohol intake in C57BL/6 mice. Drug and Alcohol Dependence 97(1-2):86-93

Arce E & Santisteban C (2006) Impulsivity: a review. Psicothema 18(2):213-220

Bechara A, Damasio AR, Damasio H, Anderson, SW (1994) Insensitivity to future consequences following damage to human prefrontal cortex. Cognition 50:7-15

Bell RL, Rodd ZA, Sable HJK, Schultz JA, Hsu CC, Lumeng L, Murphy JM, McBride WJ (2006) Daily patterns of ethanol drinking in peri-adolescent and adult alcohol-preferring (P) rats. Pharmacology Biochemistry and Behavior 83(1):35-46

Bjork JM, Hommer DW, Grant SJ, Danube C (2004) Impulsivity in abstinent alcohol-dependent patients: relation to control subjects and type 1-/type 2-like traits. Alcohol 34:133-150

Burish TG, Maisto SA, Cooper AM, Sobell MB (1981) Effects of voluntary short-term abstinence from alcohol on subsequent drinking patterns of college students. Journal of Studies on Alcohol 42:1013-1020CDC (2001) Alcohol-attributable deaths and years of potential life lost-United States, 2001. Morbidity and Mortality Weekly Report (Centers for Disease Control and Prevention) 53:866-870

Coleman Jr LG, He J, Lee J, Styner M, Crews FT (2011) Adolescent binge drinking alters adult brain neurotransmitter gene expression, behavior, brain regional volumes, and neurochemistry in mice. Alcoholism: Clincal and Experimental Research 35(4):671-688

Crews FT, Boettiger CA (2009) Impulsivity, frontal lobes and risk for addiction. Pharmacology Biochemistry and Behavior 93(3):237-247

Crews FT, Braun CJ, Hoplight B, Switzer RC 3rd, Knapp DJ (2000) Binge ethanol consumption causes differential brain damage in young adolescent rats compared with adult rats. Alcoholism-Clinical and Experimental Research 24(11):1712-1723

Crews F, He J, Hodge C (2007) Adolescent cortical development: A critical period of vulnerability for addiction. Pharmacology Biochemistry and Behavior 86(2):189-199

Csikszentmihalyi M, Larson R, Prescott S (1977) The ecology of adolescent activity and experience. Journal of Youth and Adolescence 6:284-94

Diaz-Anzaldua A, Diaz-Martinez A, Diaz-Martinez LR (2011) The complex interplay of genetics, epigenetics, and environment in the predisposition to alcohol dependence. Salud Mental 34(2):157-166

Ernst M, Luckenbaugh DA, Moolchan ET, Leff MK, Allen R, Eshel N, London ED, Kimes A (2006) Behavioral predictors of substance-use initiation in adolescents with and without attention-deficit/hyperactivity disorder. Pediatrics 117:2030-2039

Falk D, Y HW (2008) An epidemiologic analysis of co-occurring alcohol and drug use and disorders. Alcohol Research and Health 31:100-110

Fassino M, Campbell B (1981) The ontogeny of play in rats. Presented at the meeting of the Eastern Psychological Association, New York, NY

Fullgrabe MW, Vengeliene V, Spanagel R (2007) Influence of age at drinking onset on the alcohol deprivation effect and stress-induced drinking in female rats. Pharmacology Biochemistry and Behavior 86(2):320-326

Gottesman II & Gould TD (2003) The endophenotypes concept in psychiatry: etymology and strategic intentions. American Journal of Psychiatry 160:636-645

Goudria AE, Grekin ER, Sher KJ (2007) Decision making and binge drinking: A longitudinal study. Alcoholism-Clinical and Experimental Research 31(6):928-938

Grahame NJ, Li TK, Lumeng L (1999) Selective breeding for high and low alcohol preference in mice. Behavior Genetics 29(1):47-57

Grant BF, Dawson DA (1997) Age at onset of alcohol use and its association with DSM-IV alcohol abuse and dependence: results from the National Longitudinal Alcohol Epidemiologic Survey. Journal of Substance Abuse 9:103-110

Green L, Myerson J, Shah AK, Estle SJ, Holt DD (2007) Do adjusting-amount and adjusting-delay procedures produce equivalent estimates of subjective value in pigeons? Journal of the Experimental Analysis of Behavior 87:337-347

Grunbaum J, Kaun L, Kinchen S, et al. (2004) Youth Risk Behavior Surveillance-United States, 2003. MMWR Surveillance Summary 53:1-96

Hefner K, Holmes A (2007) Ontogeny of fear-, anxiety- and depression-related behavior across adolescence in C57BL/6J mice. Behavioral Brain Research 176:210-215.

Helms CM, Reeves JM, Mitchell SH (2006) Impact of strain and D-amphetamine on impulsivity (delay discounting) in inbred mice. Psychopharmacology 188:144-151

Johnston LD, O'Malley PM, Bachman JG, Schulenberg JE (2004) Monitoring the future, national survey results on drug use, 1975-2004. NIH Publication 05-5727 1

Kang SS, Cole M, Lee S, Rivier C (2004) Development of individual alcohol inhalation chambers for mice: Validation in a model of prenatal alcohol. Alcoholism-Clinical and Experimental Research 28(10):1549-1556

Kohnk MD (2008) Approach to the genetics of alcoholism: a review based on pathophysiology. Biochemical Pharmacology 75(1):160-177

Lauder JM, Bloom FE (1974) Ontogeny of monoamine neurons in the locus coeruleus, raphe nuclei and substantia nigra of the rat. I. Cell differentiation. The Journal of Comparative Neurology 155:469-81

Leussis MP, Andersen SL (2008) Is adolescence a sensitive period for depression? Behavioral and neuroanatomical findings from a social stress model. Synapse 62(1):22-30

Luman M, Tripp G, Scheres A (2010) Identifying the neurobiology of altered reinforcement sensitivity in ADHD: A review and research agenda. Neuroscience and Biobehavioral Reviews 34(5):744-754

Mazur JE (1987) An adjusting procedure for studying delayed reinforcement. In: *The Effect of Delay and of Intervening Events on Reinforcement Value* (Commons ML, Mazur JE, Nevin JA, Rachlin H eds.) Vol. 5, Quantitative analysis of behavior series pp 55-73, Lawrence Erlbaum, Hillsdale NJ

McKinzie DL, Nowak KL, Yorger L, McBride WJ, Murphy JM, Lumeng L, Li TK (1998) The alcohol deprivation effect in the alcohol-preferring P rat under free-drinking and operant access conditions Alcoholism-Clinical and Experimental Research 22(5):1170-1176

Melendez R (2011) Intermittent (every-other-day) drinking induces rapid escalation of ethanol intake and preference in adolescent and adult C57BL/6J mice. Alcoholism: Clinical and Experimental Research 35(4):652-658

Metten P, Brown LL, Crabbe JC (2011) Limited access ethanol drinking in the dark in adolescent and adult mice. Pharmacology, Biochemistry and Behavior 98:279-285

Mitchell SH, Reeves JM, Li N, Phillips TJ (2006) Delay discounting predicts behavioral sensitization to ethanol in outbred WSC mice. Alcoholism: Clinical and Experimental Research 30(3):429-437

Monti PM, Miranda Jr R, Nixon K, Sher KJ, Swartzwelder HS, Tapert SF, et al. (2005) Adolescence: booze, brains, and behavior. Alcoholism-Clinical and Experimental Research 29:207-20

Moore EM, Mariani JN, Linsenbardt DN, Melon LC, Boehm SL (2010) Adolescent C57BL/6J (but not DBA/2J) mice consume greater amounts of limited-access ethanol compared to adults and display continued elevated ethanol intake into adulthood. Alcoholism-Clinical and Experimental Research 34(4):734-742

Nash SG, McQueen A, Bray JH (2005) Pathways to adolescent alcohol use: family environment, peer influence, and parental expectations. Journal of Adolescent Health 37(1):19-28

Oberlin BG, Best C, Matson LM, Henderson A, Grahame NJ (2011) Derivation and characterization of replicate high- and low-alcohol preferring lines of mice and a high-drinking crossed HAP line. Behavioral Genetics 41(2):288-302

Oberlin BG & Grahame NJ (2009) High-alcohol preferring mice are more impulsive than low-alcohol preferring mice as measured in the delay discounting task. Alcoholism: Clinical and Experimental Research 33(7):1-10

Oroszi G & Goldman D (2004) Alcoholism: genes and mechanisms. Pharmacogenomics 5(8):1037-1048

Ozburn AR, Harris RA, Blednov YA (2008) Wheel running, voluntary ethanol consumption, and hedonic substitution. Alcohol 42(5):417-424

Perry JL, Nelson SE, Carroll ME (2008) Impulsive choice as a predictor of acquisition of IV cocaine self-administration and reinstatement of cocaine-seeking behavior in male and female rats. Experimental and Clinical Psychopharmacology 16(2):165-177

Petry NM (2001) Delay discounting of money and alcohol in actively using alcoholics, currently abstinent alcoholics, and controls. Psychopharmacology 154:243-250

Rachlin H & Green L (1972) Commitment, choice and self-control. Journal of the Experimental Analysis of Behavior 17:15-22

Richards JB, Sabol KE, de Wit H (1999) Effects of methamphetamine on the adjusting amount procedure, a model of impulsive behavior in rats. Psychopharmacology 146(4):432-439

Rodd-Henricks ZA, Bell RL, Kuc KA, Murphy JM, McBride WJ, Lumeng L, Li TK (2002) Effects of ethanol exposure on subsequent acquisition and extinction of ethanol self-administration and expression of alcohol-seeking behavior in adult alcohol-preferring (P) rats: I. Periadolescent exposure. Alcoholism-Clinical and Experimental Research 26(11):1632-1641

Salimov RM, McBride WJ, McKinzie DL, Lumeng L, Li TK (1996) Effects of ethanol consumption by adolescent alcohol-preferring P rats on subsequent behavioral performance in the cross-maze and slip funnel tests. Alcohol 13(3):297-300

Salimov R, Salimova N, Klodt P, Maisky A (1993) Interaction between alcohol deprivation and morphine withdrawal in mice. Drugs and Alcohol Dependence 34:59-66

Schulenberg J, O'Malley PM, Bachman JG, Wadsworth KN, Johnston LD (1996) Getting drunk and growing Up: trajectories of frequent binge drinking during the transition to young adulthood. Journal of Studies on Alcohol 57(3):189-304

Schweinsburg AD, McQueeny T, Nagel BJ, Eyler LT, Tapert SF (2010) A preliminary study of functional magnetic resonance imaging response during verbal encoding among adolescent binge drinkers. Alcohol 44(1):111-117

Siegmund S, Vengeliene V, Singer MV, Spanagel R (2005) Influence of age at drinking onset on long-term ethanol self-administration with deprivation and stress phases. Alcoholism-Clinical and Experimental Research 29(7):1139-1145

Sinclair JD, Richman CL (1969) Induced consumption of high concentration ethanol in rats. Quarterly Journal of Studies on Alcohol 30:330-335

Spear LP (2000) The adolescent brain and age-related behavioral manifestations. Neuroscience and Biobehavioral Reviews 24:417-463

Squeglia LM, Jacobus J, Tapert SF (2009) The Influence of Substance Use on Adolescent Brain Development. Clinical EEG and Neuroscience 40(1):31-38

Strong MN, Yoneyama N, Fretwell AM, Snelling C, Tanchuck MA, Finn DA (2010) "Binge" drinking experience in adolescent mice shows sex differences and elevated ethanol intake in adulthood. Hormonal Behavior 58(1):82-90

Tambour S, Brown LL, Crabbe JC (2008) Gender and age at drinking onset affect voluntary alcohol consumption but neither the alcohol deprivation effect nor the response to stress in mice. Alcoholism Clinical and Experimental Research 32(12):2100-206

Tapert SF, Schweinsburg AD, Barlett VC, Brown SA, Frank LR, Brown GG, Meloy MJ (2004) Blood oxygen level dependent response and spatial working memory in adolescents with alcohol use disorders. Alcoholism-Clinical and Experimental Research 28(10):1577-1586

Tarazi FI, Baldessarini RJ (2000) Comparative postnatal development of dopamine D(1), D(2) and D(4) receptors in rat forebrain. International Journal of Developmental Neuroscience 18:29-37

Tarazi FI, Tomasini EC, Baldessarini RJ (1998) Postnatal development of dopamine and serotonin transporters in rat caudate-putamen and nucleus accumbens septi. Neuroscience Letters 1998:254:21-4

Thorsell A, Slawecki CJ, Khoury A, Mathe AA, Ehlers CL (2005) Effect of social isolation on ethanol consumption and substance P/neurokinin expression in Wistar rats. Alcohol 36(2):91-97

van der Zwaluw CS (van der Zwaluw, Carmen S.)1, Engels RCME (2009) Gene-environment interactions and alcohol use and dependence: current status and future challenges. Addiction 104(6):907-914

Vetter CS, Doremus-Fitzwater TL, Spear LP (2007) Time course of elevated ethanol intake in adolescent relative to adult rats under continuous, voluntary-access conditions. Alcoholism-Clinical and Experimental Research 31(7):1159-1168

Wilhem CJ, Mitchelle SH (2008) Rats bred for high alcohol drinking are more sensitive to delayed and probabilistic outcomes. Genes Brain and Behavior 7(7):705-713

Yoneyama N, Crabbe JC, Ford MM, Murillo A, Finn DA (2008) Voluntary ethanol consumption in 22 inbred mouse strains. Alcohol 42(3):149-160

VITA

VITA

David Scott O'Tousa

Education

2009-2011	***IUPUI***, Indianapolis, IN M.S. in Psychobiology of Addictions (2011) Thesis: Adolescent and Adult Two-Bottle Choice Ethanol Drinking and Adult Impulsivity in Genetically Selected High-Alcohol Preferring Mice
2004-2008	***University of Notre Dame***, Notre Dame, IN B.A. in Psychology and Pre-Professional Studies

Honors & Distinctions

2011	Research Society on Alcoholism Student Merit Travel Award
2010-2011	Research Investment Fund (RIF) Fellowship, IUPUI
2009-2010	University Fellowship, IUPUI

Manuscripts in Progress

O'Tousa, D.S., Matson, L.M., Villalta, N.A., Grahame, N.J. Adolescent and Adult Two-Bottle Choice Ethanol Drinking and Adult Impulsivity in Genetically Selected High-Alcohol Preferring Mice. *Awaiting submission, Alcohol Clin Exp Res.*

Accepted Abstracts/Poster Presentations

O'Tousa, D.S., Villalta, N.A., Matson, L.M., Grahame, N.J. Adolescent and Adult Two-Bottle Choice Ethanol Drinking and Adult Impulsivity in Genetically Selected High-Alcohol Preferring Mice. (Research Society on Alcoholism Meeting, Atlanta, GA, June 2010)

Matson, L.M., **O'Tousa, D.S.**, Heighton, M.E., Villalta, N.A., Grahame N.J. Drinking Rhythms in Alcohol Preferring Mice. (Research Society on Alcoholism Meeting, Atlanta, GA, June 2010)

O'Tousa, D.S., Grahame, N.J. Adolescent Drinking in High Alcohol Preferring Mice Does Not Affect Adult Impulsivity as Measured by the Delay Discounting Task.

(Indianapolis Society For Neuroscience Local Neuroscience Meeting, Indianapolis, IN, October 2010)

O'Tousa, D.S., Fenlon, B.T., Mansion, A.D., Davies, P.T., Cummings, E.M. The Link Between Adolescents' Perceptions of Marital Conflict and Conflict Strategies in Their Dating Relationships. (Society for Research in Child Development Meeting, Denver, CO, April 2009)

Rife, T.R., Geier, M.H., **O'Tousa, D.S.**, Davies, P.T., Cummings, E.M. Marital Satisfaction and Child Adjustment as a Bidirectional Effect: Child Behavior Effects on Marital Satisfaction. (Society for Research in Child Development Meeting, Denver, CO, April 2009)

Experience

Fall 2011	Teaching Assistant, Graduate Statistics and Undergraduate Statistics, IUPUI
Spring 2011	Teaching Assistant, Undergraduate Introductory Psychology and Undergraduate Behavioral Neuroscience, IUPUI
Fall 2010	Teaching Assistant, Graduate Statistics and Undergraduate Learning, IUPUI