THE EFFECT OF ETHANOL ON IMPULSIVITY IN HIGH ALCOHOL PREFERRING MICE

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DEDICATION

To my parents, Gregg and Corinne, without whom I would not have been so well equipped to deal with the challenges life has to offer. By example, they showed me that character, perseverance, and faith can overcome all difficulties. They gave me all they had to give.

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ABSTRACT

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Impulsivity is associated with addiction in many human studies. Delay discounting (DD) is often used to measure impulsive choice in humans and animals. In DD testing, a small immediate reward is pitted against a larger delayed reward, and relative preference is assesed. The relative contribution of ethanol to impulsivity in alcoholism is not well-understood, therefore I will test the hypothesis that ethanol exposure will increase impulsivity in High Alcohol Preferring (HAP) mice as measured in an adjusting amount DD task.

Selectively bred HAP mice were exposed to ethanol and tested in DD in 3 different experiments. Experiment 1: ad lib homecage ethanol drinking for 21 days and 17 days were used to expose mice to ethanol. Additionally, mice were tested in DD while "currently drinking" vs. "abstinent". In experiment 2, to achieve higher blood alcohol concentrations, mice were injected with 3.5 g/kg ethanol 8 times and tested before and after in DD. In both experiments 1 and 2, mice were tested at only 2 delays (0.5 sec and 10 sec), to maximize sensitivity to detect shifts in choice behavior. In experiment 3, mice responded for 8% ethanol or 0.01% saccharin at a full range of delays: 0, 1, 2, 4, and 8 sec.

Experiment 1 did not reveal any impact of ethanol drinking on impulsivity. Experiment 2 revealed a strong trend of reduced impulsivity in the 10 sec delay group

after ethanol injections. Experiment 3 revealed reduced impulsivity at the 8 sec delay in the group responding for ethanol, and also revealed a significant correlation between higher ethanol drinking and reduced impulsivity.

These data were unexpected, and imply that the a priori hypothesis not only should be rejected, but that the opposite hypothesis may be true: ethanol decreases impulsivity, at least with high dose exposure and in responding for it as a reinforcer. This effect was similar to the effect observed in other studies with amphetamine, which consistently decreases impulsivity. Ethanol may have been exerting an amphetamine-like effect on impulsivity at the doses tested here. There is no evidence in the data generated in these studies that ethanol increases impulsivity.

Nicholas Grahame, Ph.D., Chair

TABLE OF CONTENTS

INTRODUCTION

Excessive alcohol drinking is the third leading preventable cause of death which caused 75,766 deaths in 2001, and shortened those lives by an average of 30 years (CDC, 2001). The lifetime prevalence of alcohol dependence is from 5.4% to 14.1%, while the lifetime prevalence of alcohol abuse is from 9.4% to 13.2% of the population (Kessler et al., 2005, Kessler et al., 1997). Eighteen million Americans are physically dependent on alcohol, and alcohol abuse costs the United States an estimated $185 billion (Li, 2004). The massive costs wrought by alcoholism, both in real dollars and human happiness, combined with its high prevalence make alcoholism an important disorder to research and treat.

The heritable risk for alcoholism has been estimated to be 50% to 60% from twin studies (Hrubec and Omenn, 1981, Kendler et al., 1997, Reed et al., 1996), and as such can be considered a heritable trait with a genetic basis. Environmental effects such as *in utero* ethanol exposure, childhood stressors, and adult socialization factors also appear to play a role in the etiology of alcoholism (Alati et al., 2006, Pilowsky et al., 2009, Park et al., 2009). Alcoholism should therefore be regarded as a disorder governed by a gene by environment (G X E) interaction, which is composed of both genetic and environmental risk factors that interact in complex ways to produce the condition (Stacey et al., 2009, Clarke et al., 2008).

Attempts to better understand alcoholism as a disorder have motivated numerous attempts at stratifying alcoholism into subtypes (Morey and Blashfield, 1981, Skinner, 1982, Jellinek, 1960, Zucker, 1986, Cloninger, 1987, Babor et al., 1992). One of the more influential stratification strategies is Cloninger's Type I and Type II, which

attempts to classify alcoholics by age of onset, familial transmission, and psychiatric features (Cloninger et al., 1981). Specifically, Type I are later onset, show lower familial transmission, and display less antisocial tendencies, but more affective impairment; whereas Type II are early-onset, show high familial transmission, and display impulsive/antisocial/sensation-seeking tendencies (Gilligan et al., 1988, Gilligan et al., 1987, von Knorring et al., 1984, Cloninger, 1987). More recently, Babor developed the Type A and Type B classification system, which resulted from clustering analyses, and was heavily informed by older stratification schemes. Strikingly similar to Cloninger's distinctions, Babor's Type A are characterized by later onset, less childhood dysfunction, and reduced psychopathological dysfunction while Type B are characterized by childhood risk factors, early onset, greater severity, polydrug abuse, and greater psychopathological problems (Babor et al., 1992). Babor notes that his scheme shows considerable overlap with earlier attempts, with his Type B roughly corresponding to Jellinek's gamma subtype (Jellinek, 1960), schizoid drinkers (Morey and Skinner, 1986), Zucker's antisocial alcoholics (Zucker, 1986), and Cloninger's Type II (Cloninger, 1987) among others (Babor et al., 1992). Emergent themes in the history of classifying alcoholics are the presence, at least in the most severe subtypes, of impulsive/disinhibitory psychopathologies and high heritability.

Alcoholism, Addiction, and Comorbidity

A diagnosis of an alcohol use disorder (AUD) is often comorbid with other psychiatric disorders- in fact 85% of abstinent alcoholics reported a lifetime psychiatric disorder (this is likely a conservative estimate, as this sample was drawn from subjects with multiple years of abstinence) (Di Sclafani et al., 2007). The high rate of

cormorbidity of addiction pathologies with other disorders may give us insight to mechanisms that underlie addiction. In other words, perhaps viewing addiction (AUD and substance use disorders [SUD]) as a symptom of another, more general problem could be a useful way of identifying endophenotypes (Eisenberg et al., 2007).

One study reported that the rates of comorbidity of subjects with an AUD and an anxiety or affective disorder are 22.7% and 10.2%, respectively (Kessler et al., 1997). However, the most consistent association of other conditions with AUDs are disinhibitory pathologies such as conduct disorder, adult antisocial behavior, and antisocial personality disorder (ASPD). One epidemiological study reported that the probability of being diagnosed with one of these three disorders if a subject already has an AUD is 27.3% (Kessler et al., 1997).

The hallmarks of ASPD are lack of regard for future consequences, substance abuse, aggression, impatience, and behavioral disinhibition, among others (APA, 2000). ASPD is probably the most highly comorbid disorder with AUDs, with rates ranging from 15% to 50% (Verheul et al., 2005). Additionally, ASPD is correlated with substance use disorders (SUD) across multiple drug classes (Compton et al., 2005), which suggests a generalized risk factor. Furthermore, comorbid ASPD and SUD is associated with greater severity and poorer treatment outcomes (Goldstein et al., 2007). ASPD indicates severe behavioral problems, and as such is observed in 49% to 80% of incarcerated men (Widiger and Corbitt, 1995). Dual-diagnosis of ASPD and SUD is controversial, as substance abuse itself frequently manifests as antisocial behavior (Widiger and Corbitt, 1995). Indeed, the direction of causality is difficult to establish in these cases, i.e. does antisocial behavior cause drug abuse, or vice versa? In either case,

what is clear is that ASPD is closely related to a greater risk of development and severity of addictive pathologies. It is quite possible that ASPD is simply the 'far edge of the spectrum' of AUD/SUD neurological impairment, which is to say that minimally impaired subjects simply have AUD/SUD, while maximally impaired subjects present ASPD as well.

Before the age of 18, the symptoms of ASPD are classified as conduct disorder (CD). Supporting the viewpoint that ASPD is part of a developmental trajectory, identification of CD/ASPD symptoms before age 15 is part of the ASPD diagnosis (APA, 2000). Childhood CD is a robust predictor of later problems with substance abuse (Disney et al., 1999, Greenbaum et al., 1991). Disney et al. assessed a large sample of children 17 years old, and found that children with attention-deficit hyperactivity disorder (ADHD), CD, or both, incurred a 5-fold greater risk of later substance abuse problems. This association was almost completely mediated by CD (Disney et al., 1999). In a sample of 395 adolescents with AUD, 73% displayed 3 or more CD symptoms (Molina et al., 2002). Not only do CD-like behaviors confer a risk of substance abuse, but they also show familial transmission, much like AUD itself (Moss et al., 2001). CD, being the precursor to ASPD, is strongly predictive of AUD/SUD and is likely related to addictive pathologies in some fundamental way.

Related to CD is ADHD, co-occurring 30-50% of the time (Disney et al., 1999, Szatmari et al., 1989). ADHD is identified by inattention, disruptive behavior, impatience, distractibility, impulsiveness, and hyperactivity (APA, 2000). With some overlapping symptoms and a disinhibitory nature, it is perhaps not surprising then that ADHD has been identified as a risk factor for AUD/SUD (Sringeri et al., 2008, Wilens et

al., 1997, Goodwin et al., 1975). The effect of comorbid ADHD/CD on later AUD/SUD is controversial, with many researchers finding the risk factor entirely mediated by CD (Disney et al., 1999, Fergusson et al., 1993). However, some authors have reported that an interaction driven by ADHD worsens SUD predictions, which suggests a unique risk factor originating with ADHD symptomology (Flory et al., 2003, Molina et al., 1999).

Bipolar disorder is largely characterized by trait impulsivity (Swann et al., 2001, Najt et al., 2007) and is reported as comorbid with substance use disorders at a rate of 42.3% (Merikangas et al., 2007). Bipolar patients with a history of alcohol abuse show higher risk-taking, which means that AUD could be additive with other psychiatric disorders in maladaptive severity (Holmes et al., 2009). The high rate of AUD comorbidity with externalizing disorders suggests a common underlying mechanism that is related to impulsivity, i.e. deficits in executive control, planning for the future, inhibition of bad behavior, and impatience/delay aversion.

Alcoholism, Addiction, and Correlated Traits

Correlated traits are more subtle than psychiatric comorbidity, but they may help offer deeper insight into the etiology of addictive disorders. The well established correlation between ADHD and addiction implies a link between impaired attention/executive function and drug abuse (Lane et al., 2007, Cairney et al., 2007, Mackin et al., 2005, Goodwin et al., 1975). A particularly convincing longitudinal study in humans found that attentional impairment/executive deficit predicted later substance use, even when controlling for other factors (Tapert et al., 2002).

Novelty-seeking/sensation-seeking is another trait that has been repeatedly linked to AUD/SUD disorders (Grucza et al., 2006, Magnusson et al., 2007, Lejoyeux, 2004).

In one prospective longitudinal study that assessed novelty-seeking, harm avoidance, and reward dependence (social approval), Cloninger and colleagues found that high novelty-seeking at 11 years of age, along with low harm avoidance, were strongly predictive of alcohol problems 16 years later. These two factors alone predicted a 20-fold difference in the risk for alcohol abuse. Specifically, the subtype of alcoholism that was detected was presumed to be Type II, due to the youth of the subjects (Cloninger et al., 1988b). The description of the highest novelty-seeking category was:

> "Child is extremely curious/enthusiastic about exploration of unfamiliar or novel places, things, and situations, and seeking thrills/adventures; unable to concentrate or focus on details even when interested, and combined with extreme overactivity, cannot sit still; disorganized, disorderly, undependable, and intolerant of structure and discipline; extremely excitable, easily provoked, and quick to lose temper, with frequent violent temper tantrums even under ordinary circumstances; very talkative and quick to initiate social contacts, but unpopular because behavior is obtrusive/disruptive." (Cloninger et al., 1988b)

There is a large literature detailing a high preference for sweet solutions in alcoholics (Kampov-Polevoy et al., 1997, Kampov-Polevoy et al., 1998). For instance, in one study it was found that a majority of detoxified alcoholic men (65%) preferred the highest concentration of sweet solution (0.83M: almost 3 times as much sugar as Coca-Cola Classic®) when offered a sucrose concentration series. Only 16% of controls preferred the sweetest solution (Kampov-Polevoy et al., 1997). A very similar pattern has emerged in animal studies. Across multiple lines of rats that prefer alcohol, a high preference for sweet solutions has been consistently observed, compared to low alcohol preference lines (Sinclair et al., 1992, Overstreet et al., 1993). As certain palatable foods increase dopamine (DA) transmission similarly to drugs of abuse (Di Chiara, 1998), sweet tastes could also be thought of as having 'abuse potential'. Indeed, these studies

corroborate other studies that suggest hypersensitivity to reward is a risk factor for addiction (Brunelle et al., 2004). These traits appear to interact; for instance, sweet-liking combined with novelty-seeking were accurate predictors of an AUD diagnosis in males (Kampov-Polevoy et al., 2004).

Related to Cloninger's identification of low harm avoidance as a discriminator for Type I / Type II classification, risk taking has also been associated with addictions (Lejuez et al., 2002, Bechara et al., 2001). High risk taking can be deconstructed into two chief components: hypersensitivity to reward and insensitivity to punishment, and there is evidence to suggest that AUD/SUD patients have both of these tendencies (Bechara and Damasio, 2002, Bechara et al., 2002, Stout et al., 2005). In at least a subgroup of SUD patients, choice behavior is guided by immediacy, as there is not apparently an effect of future consequences, either positive or negative (Bechara et al., 2002).

Addictive pathologies are unusual in their high rates of comorbidity with other disorders. These disorders show considerable overlap with each other, and are rich in non-clinical correlated traits. A composite sketch begins to emerge when comorbid disorders and correlated traits are taken together and examined for common themes. A common thread that runs through the comorbid psychiatric disorders and their associated correlated traits is impulsivity. These themes include disinhibition, impatience, high reward sensitivity, inattention, tolerance to punishment, and lack of future planning. Impulsivity, broadly speaking, is a disregard for future consequences and is often defined operationally as the preference for a small immediate reward over a larger delayed reward (Ainslie, 1975). Testing the behavioral endpoint of impulsivity, therefore,

captures impatience (delay aversion), disinhibition (maladaptive behavior), and high reward sensitivity (hypersalience of immediate reward).

Alcoholism: Identification

At this juncture it will be useful to define alcoholism/AUD in terms that will be familiar to clinicians and human researchers. Most of the myriad historical attempts at diagnosing alcoholics feature many of the same criteria. A typical list of criteria used to judge AUD originates in the Diagnostic and Statistical Manual, 4th Ed. "AUD" usually includes both alcohol abuse and alcohol dependence, and sometimes polydrug abuse including alcohol, depending on the study. A diagnosis for alcohol abuse is made clinically by the following criteria:

> "A maladaptive pattern of [alcohol] use leading to clinically significant impairment or distress, as manifested by one or more of the following, occurring within a 12-month period:
> a) Recurrent alcohol use resulting in failure to fulfill major role obligations at work, school, or home (e.g., repeated absences or poor work performance related to substance use; substance-related absences, suspensions or expulsions from school; or neglect of children or household).*
> b) Recurrent alcohol use in situations in which it is physically hazardous (e.g., driving an automobile or operating a machine).*
> c) Recurrent alcohol-related legal problems (e.g., arrests for alcohol-related disorderly conduct).*
> d) Continued alcohol use despite persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of the alcohol (e.g., arguments with spouse about consequences of intoxication or physical fights).*" (APA, 2000)

A diagnosis for alcohol dependence is made clinically by:

> "A maladaptive pattern of [alcohol] use, leading to clinically significant impairment or distress, as manifested by three or more of the following seven criteria, occurring at any time in the same 12-month period:
> 1. Tolerance, as defined by either of the following:
> a) A need for markedly increased amounts of alcohol to achieve intoxication or desired effect.
> b) Markedly diminished effect with continued use of the same amount of alcohol.
> 2. Withdrawal, as defined by either of the following:
> a) The characteristic withdrawal syndrome for alcohol.
> b) Alcohol is taken to relieve or avoid withdrawal symptoms.
> 3. Alcohol is often taken in larger amounts or over a longer period than was intended.*
> 4. There is a persistent desire or there are unsuccessful efforts to cut down or control alcohol use.*
> 5. A great deal of time is spent in activities necessary to obtain alcohol, use alcohol or recover from its effects.*
> 6. Important social, occupational, or recreational activities are given up or reduced because of alcohol use.*
> 7. Alcohol use is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by the alcohol (e.g., continued drinking despite recognition that an ulcer was made worse by alcohol consumption).*" (APA, 2000)

Four out of four of the diagnostic criteria for alcohol abuse, and 5 out of 7 of the criteria for alcohol dependence are related to impulsive decision-making (asterisks added). Whether it is impulsive decision-making that causes these poor choices to be made by the alcoholic, or something else (e.g. acute intoxication) that motivates decision-making that is then interpreted as impulsivity is open to debate. What is clear, however, is that most of the traits that are used to clinically describe AUDs are descriptions of impulsive behavior.

Alcoholism: Animal Models

Considerable effort has been devoted to developing animal models of human alcoholism. Given that alcoholism in humans is highly heritable, genetic approaches have been used for quite some time, with an eye towards understanding how genes mediate individual differences in risk for this disorder. One genetic approach that is appealing both in terms of simplicity and face validity is to use selective breeding for differences in free choice alcohol drinking. To this end, beginning in the 1960's, at least 6 pairs of high and low alcohol drinking rat lines have been developed (Colombo, 1997, Le et al., 2001, Li et al., 1993, Lumeng et al., 1977, Eriksson, 1968, Mardones and Segovia-Riquelme, 1983), as well as at least 2 sets of mouse lines (Belknap et al., 1997, Grahame et al., 1999b). Lines resulting from these selections reliably show profound differences in alcohol intake resulting from fixation of divergent alleles at loci related to alcohol drinking. Mice are an appealing species for selection due to their low housing cost and well characterized genomes; however their high rate of ethanol metabolism, about 0.78 mg/dl/hr in inbred strains (Grisel et al., 2002), requires especially high intakes to reach pharmacologically relevant blood alcohol concentrations.

Inbred strains, especially the C57Bl/6J mouse, have also been widely used to understand the genetics of alcohol intake through the use of animal models. Given that all animals within an inbred strain are genetically identical, the entire strain must be viewed as the genetic equivalent of a single individual. Additionally, members of an inbred strain are homozygous at all loci, including both loci relevant and irrelevant to a trait of interest, such as alcohol consumption (Crabbe, 1989). An inbred strain is therefore a unique and rather unusual genotype, so results obtained with one inbred strain

cannot necessarily be generalized to a population as a whole (Crabbe et al., 1990; Falconer and Mackay, 1996). A more desirable population that would better model a genetically heterogeneous human alcoholic population would be a heterogeneous animal line selectively bred for high alcohol preference.

Selective Breeding for Alcohol Preference in Mice

High Alcohol Preferring mice were created in an attempt to model human alcoholism in a rodent population. To maximize genetic diversity, a heterogeneous population served as the progenitors for the high alcohol and low alcohol preferring (HAP and LAP) mouse lines. The progenitors, called HS/Ibg, were derived from an 8-way cross of inbred strains: these included A, AKR, BALB/c, C3H/2, C57BL, DBA/2, Is/Bi, and RIII (McClearn et al., 1970). HS/Ibg mice were the progenitor population for the first line HAP1, and also for the later replicate line, HAP2. The replicate line HAP2 was created as a confirmation that trait-irrelevant allele fixation was not responsible for the observed response to selection or correlated traits (Crabbe et al., 1990). The selection phenotype was based on homecage drinking: 24 hour access to both 10% (v/v) ethanol and water was used to determine volume of ethanol consumption. Initially, the selection criteria were based on drinking of <1.5 g/kg/day (LAP) and >5.0 g/kg/day (HAP). These criteria were not useful after daily drinking exceeded those parameters, so selection was performed with the low and high drinkers while maximizing heterogeneity. The breeding procedure maintained genetic heterogeneity by excluding sibling, child/parent, and first cousin matings (with few exceptions.) Response to selection was robust, as seen in figure 1, and the selected HAP1 and replicate line HAP2 both showed dramatic increases in daily ethanol consumption within the first 10 generations. Both lines continue to show a

response to selection even after 39 and 32 generations, respectively (Oberlin et al., 2010b). In the latest generations, the line 1 mice drank ~23 g/kg/day vs. ~1 g/kg/day (HAP1 vs. LAP1). The HAP2 mice show a similar response to selection, but slightly lower ethanol consumption. Indeed, it is apparent that genes relevant to ethanol preference and consumption have been concentrated in the HAP populations and largely excluded from the LAP populations.

As an animal model of human alcoholism, HAP mice show alcoholic-like traits that suggest good face validity. They do not have to be food or water deprived to drink ethanol, they will drink unsweetened ethanol, they will barpress for ethanol access (unpublished), and they achieve pharmacologically relevant blood alcohol concentrations (BACs) following free-choice access to ethanol and water (Grahame and Grose 2003; Grahame et al. 1999). The HAP1 mice prefer ethanol to water, and in fact demonstrate a preference ratio of ~80% with a 10% (v/v) ethanol solution (Grahame and Grose 2003; Grahame et al. 1999). Similarly to human alcoholics, naltrexone and memantine selectively reduce HAP ethanol drinking, which suggests good predictive validity of the HAP model (Oberlin et al., 2010a). HAP mice exhibit higher impulsivity than LAP mice, which may reflect a correlated trait observed in human alcoholics (Oberlin and Grahame, 2009). Although it is impossible to fully capture the complexity of human alcoholism in a rodent model, HAP mice fit many of the criteria deemed important to any valid animal model of alcoholism (Cicero, 1979).

Impulsivity and Addiction

The term ‘impulsivity’ has many meanings, and is modeled with several different behavioral tasks. For the purposes of this treatise, ‘impulsivity’ will refer to the behavior variously called ‘cognitive impulsivity’, ‘impulse control’, or ‘impulsive choice’. The hallmark feature of impulsive choice is not response inhibition per se, but rather a selection of a choice that is detrimental or maladaptive for the organism (Ainslie, 1975). Other forms of impulsivity, such as motor impulsivity (response disinhibition, commonly seen in ADHD) will not be discussed here, but may play a role in addiction (Lawrence et al., 2009, Wilhelm et al., 2007). Additionally, different forms of impulsivity can be measured independently (Winstanley et al., 2004, Wilhelm et al., 2007), implying different relative contributions to risk for addiction.

Addictive pathologies are largely defined by impulsive decision-making. Increasingly, impulsivity is thought to be a necessary component of addiction (de Wit and Richards, 2004, Goldstein and Volkow, 2002), and to be involved in all phases of human addiction (Perry and Carroll, 2008, de Wit and Richards, 2004). Increased impulsivity is seen with AUD (Petry, 2001a), abuse of cocaine (Bornovalova et al., 2005, Coffey et al., 2003), opioids (Kirby et al., 1999, Madden et al., 1997, Odum et al., 2000), methamphetamine (Hoffman et al., 2006), and nicotine (Mitchell, 1999, Bickel et al., 1999). Additionally, severity of addiction to drugs is positively correlated with the degree of impulsivity (Dom et al., 2006, Heyman and Gibb, 2006). Interestingly, addictive behaviors that are not drug-based also correlate with impulsivity, such as compulsive gambling (Petry, 2001b), overeating (Braet et al., 2007, Weller et al., 2008), and serial sex offences (Baltieri and Andrade, 2008). Neurobehavioral disinhibition,

together with familial risk group, in boys ≤12 years old correctly predicted substance use disorder at age 19 with 85% accuracy (Tarter et al., 2003). Sons of alcoholic fathers were observed to be more impulsive than controls in the premorbid stage in one longitudinal study (Knop, 1985). In one four-year longitudinal study, impulsivity was predictive of later alcohol use by teens (Ernst et al., 2006). In non-drug using female subjects, paternal drug use history predicted higher impulsivity (Petry et al., 2002).

Impulsive decision-making does not appear to be limited to one drug class, or even drugs of abuse, but rather is a general feature of the cluster of behaviors that comprise addiction pathologies. Behaviorally-defined impulsivity is a measurable endophenotype (Eisenberg et al., 2007), and one that predicts high alcohol drinking or drug taking in drug naïve animals (Oberlin and Grahame, 2009, Wilhelm and Mitchell, 2008, Perry et al., 2005, Marusich and Bardo, 2009). Impulsivity is a behavioral endpoint that will continue to allow further exploration of neurological substrates that underlie human reward selection, and ultimately addiction (McClure et al., 2004).

Delay Discounting

The matching law states that the relative rate of responding will be proportional to the relative rate of reinforcement (Herrnstein, 1961). This was further modified by the observation that the relative rate of responding is proportional to the relative rate of reinforcement, and inversely proportional to delay (Baum and Rachlin, 1969). These behavioral principles provided the basis for the empirical measurement of impulsive choice. Modern delay discounting (DD) studies evolved from earlier direct-choice and concurrent-chain procedures designed to measure self-control in pigeons (Rachlin and Green, 1972). In these studies, two choices were possible; a smaller/sooner (SS) and a

larger/later (LL). The key finding that emerged was when both reinforcers were delayed by a large amount, there was a preference for the large delayed reinforcer. However, as the delay to the SS reinforcer was shortened and approached zero, preference shifted to the SS reinforcers. Therefore, the prediction that developed from these data was that a small, immediate reward was preferable to a large delayed reward (Rachlin and Green, 1972).

However, real-life choice behavior is not so simple. Real world choices always vary in at least two dimensions: size and delay. A similar choice procedure was developed that used a delay that adjusted according to the subjects' choice (between small/immediate and large/delayed). In this new twist, the delay length of the delayed reinforcer increased after a choice of the large reinforcer, and the delay length of the delayed reinforcer decreased after a choice of the smaller immediate reinforcer. In this way, it was possible to estimate the extent to which choice behavior was affected by delay (Mazur, 1987). Mazur proposed a value discounting function to describe the observed behavior: $V = M/(1 + kD)$, where V is the subjective value of a reinforcer which is proportional to the magnitude M and inversely proportional to delay D. The fitted parameter *k* is derived from hyperbolic regression and gives an estimate of the degree of discounting, such that larger values of *k* indicate steeper discounting (greater impulsivity) and smaller values of *k* indicate shallower discounting, i.e. less impulsivity (Mazur, 1987). Considerable debate and analysis has shown that this hyperbolic discounting function is a good model for data obtained across a variety of studies (Myerson and Green, 1995, Takahashi et al., 2007) and in fact typically explains >85% of the variance in choice behavior for delayed rewards (Bickel and Marsch, 2001).

Later, a similar procedure adjusted the amount of the reinforcer according to choice behavior rather than adjusting the delay. This procedure, called the adjusting amount procedure, was successfully used in rats, and varied both reward size and delay. In these studies, the reinforcer was water which was delivered in discrete volumes to a drinking dish. The delay was fixed within a session, but was altered for other sessions. If the rat chose the immediate reinforcer, the volume of the immediate reinforcer was reduced by 10%, but if the rat chose the delayed reinforcer, the volume of the immediate reinforcer was increased by 10%. In this way the adjusted amount of the immediate reinforcer was a direct measure of the subjective value of the delayed reinforcer. Hyperbolic regression was performed on average adjusted amounts, and *k* values were determined for each rat (Richards et al., 1997). A slightly modified version of this procedure that uses sipper access time instead of discrete delivery has been successfully employed in mice by the author (Oberlin and Grahame, 2009). This version of the DD task has the added advantage of measuring actual consumption, which is only assumed in the Richards version.

The DD task is becoming an increasingly popular method of measuring impulsive choice. Using the search terms "delay discounting" in PubMed (http://www.ncbi.nlm.nih.gov/sites/entrez) yielded 1 hit for 1991, 7 hits for 1999, and 67 hits for 2009 (searches conducted Dec. 2009). The task in animals is very similar to those performed in humans, which makes the paradigm highly translational. Additionally, it is amenable to measuring behavioral effects of drugs (Pattij et al., 2009, Oberlin et al., 2010a, de Wit et al., 2002), lesions (Cardinal et al., 2001, Kheramin et al., 2003), and group differences (Dom et al., 2006, Eisenberg et al., 2007).

Delay Discounting and Alcohol History

It is well-established that AUD/heavy drinkers discount delayed rewards more steeply than controls (Dom et al., 2006, Bjork et al., 2004, Mitchell et al., 2005, Mitchell et al., 2007, Petry, 2001a, Vuchinich and Simpson, 1998, Field et al., 2007), however, two notable studies are exceptions (Bobova et al., 2009, Kirby and Petry, 2004). The difference between AUD subjects and controls can be attributed to 1) trait impulsivity, 2) alcohol history, or 3) an interaction or synergism of the two. Although it is difficult to establish the effect of trait impulsivity in human AUD subjects without the confound of alcohol history, animal studies utilizing drug-naïve subjects suggest that trait impulsivity makes a contribution (Oberlin and Grahame, 2009, Wilhelm and Mitchell, 2008). Additionally, several human longitudinal studies indicate that childhood disinhibitory traits such as high novelty-seeking and low harm avoidance predict later drug/alcohol use (Cloninger et al., 1988a, Masse and Tremblay, 1997, Tarter et al., 2003, Kirisci et al., 2004). It seems likely then that trait impulsivity is an important factor in the development of an AUD/SUD. Other studies have demonstrated that binge drinking humans show impairments in neuropsychological tasks as well as differential patterns of brain activation (Schweinsburg et al., Crego et al., 2009). However, the question of whether or not chronic alcohol use increases impulsivity as measured by DD has not been answered.

The large fetal alcohol syndrome literature indicate that *in utero* ethanol exposure in humans results in a wide range of executive impairments (Burden et al., 2005, Mattson et al., 2001, Brown et al., 1991, Streissguth et al., 1986). Similarly, in utero ethanol exposure in guinea pigs caused impaired inhibition in the Go/No-Go task, as well as

perseverative responding in the Cued Alternation task (Olmstead et al., 2009). In adult rats, chronic ethanol exposure results in learning and memory deficits, particularly spatial, reference, and working memory (Savage et al., 2000, Miller and Rieck, 1993, Matthews and Morrow, 2000, Arendt et al., 1989). These findings are mitigated somewhat by several studies that have not found such deficits (Blokland et al., 1993, Steigerwald and Miller, 1997, Fadda et al., 1999). Indeed, it is worth noting that many of the studies that assessed chronic alcohol exposure in rodents used a procedure wherein the only fluid available was 20% ethanol for many weeks. The chronic dehydration and chronic stress present in this paradigm is a confound rarely addressed.

In summary, it appears that in utero ethanol exposure often presents specific later impairments, and that chronic adult exposure is sometimes associated with impairment- but not always. Therefore, to address the question of whether or not AUD-associated impulsivity is caused by ethanol exposure similar to that seen in humans, a reasonable hypothesis can be generated: high ethanol exposure in an animal model of alcoholism (HAP mice) will increase impulsivity as measured by DD. The ethanol exposure should produce high BACs, be performed in adults, but avoid prolonged dehydration and extended periods of stress, preferably via choice drinking. This hypothesis will be tested in experiments 1 and 2.

Delay Discounting and Impulsive State: Drug Reinforcers

There is good agreement in the addiction literature that AUD/SUD subjects are more impulsive than controls, which is to say that these subjects show a greater degree of preference for smaller immediate rewards to the exclusion of larger delayed rewards. However, whether this tendency is exacerbated by choices involving drugs of abuse is of

special interest. Since it is known that addicts/alcoholics are more impulsive when choosing between non-drug rewards, an important question arises: are AUD/SUD subjects even more impulsive when selecting drug reinforcers? The answer to this question addresses the notion that impulsivity is both a cause and a consequence of drug use (de Wit, 2009), and that exaggerated impulsivity with regard to drug rewards could be contribute to much of the destructive behavior associated with addiction.

A seminal study employing the DD task in opioid-dependent human subjects found that impulsivity was more extreme in the SUD subjects than controls, but importantly that this effect was exaggerated when heroin was the expected reinforcer. This study used hypothetical money rewards (and their equivalents in heroin), delays ranging from 1 week to 25 years, and reward amounts up to $1000 (Madden et al., 1997). Their data, reproduced in figure 2, show that discounting of heroin rewards by dependent subjects is so steep that future heroin rewards are worth virtually nothing. A serious confound that was (surprisingly) not discussed was that the opioid dependent subjects received buprenorphine roughly an hour before DD testing. It is relevant to note than buprenorphine possesses high abuse potential (Mammen and Bell, 2009), and is pharmacologically similar to morphine, which can increase impulsivity (Pattij et al., 2009, Kieres et al., 2004). However, such concerns are somewhat minimized by the later finding that the opioid withdrawal state induces even greater impulsivity (Giordano et al., 2002).

Another study that used very similar parameters also found that discounting was more extreme for hypothetical heroin rewards vs. monetary rewards in opioid-dependent subjects. This study, like Madden et al. (1997) used subjects that were being treated with

buprenorphine (Giordano et al., 2002). Even more striking, in a study testing crack cocaine addicts and using the same amount and delay parameters as Madden et al. (1997), cocaine addicts showed complete discounting, i.e. indifference points of zero, of cocaine rewards that were delayed by more than 2 months (compared to $1000 monetary reward, which was discounted by only 55% for the same delay) . Indeed, the findings in this study mirror other DD studies performed in opioid addicts, in that cocaine addicts are not only more impulsive than controls, but that they discount their drug of choice to an extreme degree (Coffey et al., 2003).

Regular cigarette smokers comprise a unique model of drug addiction in humans, as addiction to nicotine does not usually result in job loss, delinquency, and homelessness often seen in addicts of opioids and ethanol (Hughes, 1993). Therefore, studies of nicotine addiction may be capturing a somewhat different population and addiction pathology. An adjusting amount DD procedure in smokers (≥20 cig/day) found that hypothetical cigarette rewards were discounted more steeply than their equivalent value in money with delays ranging from 1 week to 25 years, and a delayed amount of $1000. Smokers were instructed to smoke as usual (Bickel et al., 1999). Another adjusting amount DD study similarly found steeper discounting of hypothetical cigarette rewards vs. hypothetical money in smokers (≥10 cig/day) with delays ranging from 1 day to 6 years and a delayed amount of $10. Smokers in this study were not given any instructions about their smoking prior to testing (Odum and Baumann, 2007).

Ethyl alcohol represents another special drug, in that the majority of control subjects use it on occasion, whereas this is not generally true with opioids, cocaine, and cigarettes. In a study that tested current alcoholics, abstinent alcoholics, and controls

using an adjusting amount DD task with hypothetical money and alcohol rewards, it was found that alcohol rewards were discounted more steeply by both alcoholics and controls (Petry, 2001a). It was noted by the authors that perhaps their "bottles of alcohol" and money amounts were not equivalent, which, if true, would be consistent with steeper discounting of smaller vs. larger rewards found in several studies, i.e. a 'magnitude effect' (Green et al., 1997, Giordano et al., 2002). Other possible explanations are that there is an interaction between the consumable nature of the ethanol reward (vs. non-consumable money) and the fact that the rewards were hypothetical. Concerns about withdrawal effects were minimized by the lack of exclusion for recent ethanol use; two of the alcoholic subjects even had detectable BAC just before testing (Petry, 2001a). Field et al. used an adjusting amount procedure in adolescent light and heavy drinkers (age: 16-18 years) and found a main effect of group, such that heavy drinkers were more impulsive than light drinkers, but no significant effect of steeper discounting of ethanol rewards vs. monetary rewards in either group (Field et al., 2007).

Generally speaking, drug abusing subjects tend to discount their drug of choice more steeply than equivalent money rewards. To the author's knowledge, there have not been any published studies of discounting of actual drug rewards in AUD/SUD subjects. This experimental design would more closely mimic reality, where rewards are not hypothetical, and furthermore are available to the subject for acquisition and consumption. A related issue is that experiencing the *actual* delay, rather than a hypothetical delay, may be important (Richards et al., 1999).

An obvious confound emerges while attempting to isolate the effects of responding for a drug and the acute effects of the drug itself, i.e. as subjects respond for

ethanol reward, they become intoxicated, which likely has its own effects on decision-making. In spite of the well-accepted meme that ethanol increases impulsivity, the evidence from the laboratory has been somewhat less satisfying. One study in non-alcoholics found no effect of 0.5 or 0.8 g/kg ethanol on discounting of pseudoreal (one choice trial paid out randomly) monetary rewards of ≤$10 (Richards et al., 1999). Another study performed in non-alcoholic male humans concluded that 0.7 g/kg ethanol decreased impulsivity, but that the difference did not reach statistical significance (Ortner et al., 2003). This study used hypothetical monetary rewards of ≤$10 and delays up to one year. Employment of a somewhat different DD task, the Experiential Discounting Task (EDT) found that 0.8 g/kg ethanol increased impulsivity. The EDT is different from the more traditional DD task discussed thus far, in that it forces the subject to wait for the length of the delay for a their monetary reward, and the subjects get to keep the rewards that they earn. Additionally, this study also reported data from a DD study based on Richards et al. (1999) that showed no difference between intoxicated and sober subjects (Reynolds et al., 2006). The use of the EDT was an important step in testing the actual contingencies that DD tasks attempt to measure, i.e. actual delays and actual rewards. It is questionable how salient the $0.30 reward actually is, however. On the whole, the human literature is rather fragmented in its findings, with the only real impact on cognitive impulsivity having been detected in the EDT. A study of impulsivity using Wistar rats in a T-maze (not DD per se) found that 0.9, 1.2, and 1.8 g/kg ethanol injected i.p dose-dependently increased impulsivity (Poulos et al., 1998).

An informative study would be one that assessed impulsive responding for actual orally-administered ethanol and an alternative fluid in AUD-prone subjects. Firstly,

assessing AUD subjects, or an animal model of AUD, is of paramount importance in these studies. Alcoholics are the target population of alcoholism treatment, so therefore it is most informative to know what the relative contribution of alcohol (and alcohol-centric decision-making) is to impulsivity in an AUD sample. Secondly, the alcohol and impulsivity literature suggest that DD tasks are only sensitive to ethanol-induced impulsivity when actual delays and rewards are experienced. Lastly, given that alcohol intoxication is a common state for alcoholics, it would be important to assess impulsivity while the subjects are intoxicated- and preferably by the route of administration exclusively used by humans: oral. This study will comprise Experiment 3.

EXPERIMENTS

Experiment 1- Ethanol Drinking History and Impulsivity

Introduction

It is well-documented that alcoholics discount delayed rewards more steeply than controls. The role of trait impulsivity has been established in humans and animals, but the relative contribution of chronic ethanol exposure to cognitive impulsivity has not been well-studied. Therefore, to address the possibility that chronic exposure to ethanol increases impulsivity, we will test the hypothesis that exposure to orally self-administered ethanol in an animal model of alcoholism (HAP mice) will increase cognitive impulsivity as measured in the DD task. Experiment 1 will be divided into 3 phases to measure different aspects of ethanol drinking on impulsivity. Phase 1 will assess the effect of prolonged choice ethanol drinking on DD in currently drinking animals: this design promises good face validity. Given that there is evidence that currently drinking alcoholics are more impulsive than abstinent alcoholics (Petry, 2001a), ethanol access will be permitted until just before DD testing. Therefore, Phase 2 will model currently drinking vs. not currently drinking, i.e. abstinent in DD. Phase 3 will assess the effect of repeated prolonged choice ethanol drinking, consistent with evidence that repeated cycles of ethanol potentiates alcoholic-like behavior (Oster et al., 2006).

By testing these various permutations of alcohol drinking, I hope to therefore maximize my sensitivity to detect enhanced impulsivity in the alcoholic state, either caused by the drinking state itself (motivational effects) or by the effects of ethanol itself (neuroplasticity.) A timeline of these different phases is outlined in figure 3. Phase 1

hypothesis: prolonged exposure to ethanol will increase impulsivity in currently drinking HAP mice. Phase 2 hypothesis: stopping the drinking of ethanol will result in reduced impulsivity. Phase 3 hypothesis: repeated prolonged exposures will cause ethanol-induced increases in impulsivity that can be measured in abstinent mice.

Materials and Methods

Animals: All work was performed in accordance with, and approved by, the Indiana University School of Medicine IACUC and the IUPUI School of Science IACUC. Forty-eight HAP1 mice (24 male, 24 female) from generation 36 were used in this experiment. These mice were all born in the IUPUI School of Science animal care facility. Mice were individually housed in polycarbonate cages (27.9 x 9.5 x 12.7 cm) with Cellsorb bedding, at an ambient temperature of 21 $\pm$ 1°C and lights on from 2200 to 1000. Mice had *ad lib* access to food, and water access restricted to 2 hours per day, immediately after testing during the shaping period through Stage 5. During Stage 6 and subsequent testing, fluid was unrestricted except 2.5 hours before testing. Mice were transported in a light shielded transporter to the testing room; the mice were tested between 1100 and 1600, and were always tested in the dark.

Apparatus: The operant apparatus consisted of 12 identical boxes that measured 21.6 X 19.7 X 12.7 cm inside, with two sides constructed of clear acrylic and two sides of aluminum (MedAssociates, St. Albans, VT). The operant box was contained in a sound and light attenuated chamber that was equipped with a fan for ventilation and background noise. An LED/nose-poke infrared detector was centered on the 19.7 cm side at 6.3 cm above the floor, and illumination of that LED signaled the beginning of a trial. Below the LED/nose-poke detector was the sipper access hole, through which the sipper descended

when mice were being rewarded. The sipper tube was a 10 ml graduated plastic serological pipette fitted with a stainless steel tip (Ancare, Belmont, NY). The sipper tube could be extended into the box for varying amounts of time, which allowed precise control of sipper access. Consumption volumes (± 0.1ml) were visually read from the tube. The sipper tube was filled with a solution of 0.0136% (w/v) saccharin solution. This concentration was initially chosen to minimize differences in responding between HAP and low alcohol preferring mice in an earlier study. It is maintained here as a moderately sweet solution that promotes consistent responding. Levers were mounted 2.5 cm above the floor on either side of the sipper tube opening. Each lever had an LED 2.3 cm above it signaling that the lever was active (that is, reinforcement was available on that lever). Control of the operant boxes and collection of data was performed via the MedPC IV software and MedPC interface cards on a PC compatible computer. Data were sorted in Microsoft Excel (Redmond, WA) and statistical analyses were performed using SPSS 15.0 (Chicago, IL).

Behavioral Assessment: The time course of each trial is illustrated in figure 4. This procedure was a slightly modified version of a previously reported DD task called an adjusting amount procedure and has been previously described (Richards et al., 1997). Briefly, the center nosepoke cue light is illuminated until the mouse initiates the trial by nosepoking. After the nosepoke, the center light goes dark, and the lights illuminate above both the left and right lever, signaling availability of a choice. Both lights remain illuminated until a lever press is recorded. Once a lever press is made, the non-chosen lever light extinguishes, and the sipper containing saccharin solution descends into the drinking position. Saccharin solution was used to promote higher responding. One lever

was always assigned to “delay” and the other was assigned to “immediate” after shaping through Stage 4. A lever press on the delay side resulted in delivery of the standard, 2 second reward after the programmed delay interval, during which time the light above the delay lever remained illuminated. An immediate-side lever press resulted in delivery of the adjusting reward without any programmed delay. The immediate (adjusting reward) side was set to 1 second access time (half the standard reward) at the beginning of the session. The access time on the immediate side adjusted depending on the subject’s choice in the last trial according to the following rules: an immediate choice resulted in adjustment down of sipper access time by 0.2 seconds, and a delay choice resulted in adjustment up of sipper access time by 0.2 seconds. The adjustment increment, 0.2 seconds, was 20% of the initial adjusting side amount. As the value of the immediate reinforcer adjusts up and down throughout the session, it should have titrated to a value subjectively equivalent to the 2 sec delayed reward by the end of the session. Therefore, the adjusted amount of the immediate side gives an estimate of the subjective valuation of the delayed reward by the end of the session. The adjusted amount of the immediate side was limited to 2 seconds so that it never became larger than the amount on the delayed side, as experience with very large immediate rewards might interfere with mice being able to reliably assess reward magnitude. Therefore, the range of adjustment was always between 0 and 2 seconds sipper access time. To expose the mice regularly to both immediate and delayed reinforcers, a forced trial on the opposite lever was instituted after two consecutive selections of the same lever. In a forced trial, only one lever was active, and only that light came on after a nosepoke. Additionally, there was no adjustment of the immediate reinforcer resulting from forced trials.

Mice were shaped according to the protocol outlined in table 1. Stage 1 was performed for only 1 day; Stage 2 for 5 days; Stage 3 for 2 days; Stage 4 for 8 days; Stage 5 for 8 days; and Stage 6 for 13 days. After the mice met criterion lever pressing in stage 4 (20 trials completed), side bias was assessed by averaging the last 3 days' choices on each side. The large reinforcer was then assigned to their non preferred side, to counter any initial side bias. After shaping was completed through Stage 4, mice were assessed at the zero delay (Stage 5), which is not actually a delay but rather an assessment of magnitude discrimination. The sipper always contained 0.136% (w/v) saccharin solution, except in the case of Experiment 3, in which it contained either saccharin or ethanol.

The criterion for continued inclusion of individual mice in this study was a mean adjusted amount of 1.5 seconds or greater (75% or more of the 2 second access on the delayed side) in 3 out of 4 consecutive days. We used this inclusion criterion because any assessment of DD relies on magnitude discrimination. If individuals could not display a preference for the larger reward, then discounting of that assessment based upon time would be uninformative. Importantly, the adjusted amount derived at the zero delay was not the result of innate side bias, as the 80% adjusted amount requirement meant that the mice preferred the large reinforcer lever over the other lever, which they had initially preferred in Stage 4. Dependent variables such as total trials and response latency were calculated as means taken from the means of the 3 sessions in each condition (or 2 or 1, if they did not complete at least 20 trials during a session.) Indifference points for each mouse were determined by taking the median adjusted amount for the last 20 choice trials completed, and then averaging those values across 3 sessions. Sessions were limited to 1

hour or 60 choice trials, whichever came first. All daily data from mice that completed fewer than 20 trials on a day were excluded. For DD testing in experiments 1 and 2, Stage 6 was also implemented, which set adjusted amount criteria of ≤ 0.6 second for the 10 sec delay group, and ≥ 1.4 second for the 0.5 delay group. Mice were segregated into short delay (0.5 second) and long delay (10 second) groups to maximize our ability to detect increases or decreases, respectively, in impulsivity. Although a full range of delays, such as performed in Experiment 3, measures discounting behavior across a wider range, a short/long delay scheme allows rapid assessment with treatments that may change with many tests. Half of each of the 0.5 second group and the 10 second group were assigned to the E Group (ethanol and water in homecage) and the other half were assigned to the W Group (water and water in the homecage). Group assignment was balanced across Sex and Stage 5 adjusted amount such that zero delay adjusted amounts were similar in both groups.

Statistics: The mean of 3 days was taken to represent performance in a given condition, and delay groups were analyzed separately *a priori*, similar to another published assessment of drug effects in DD (Oberlin et al., 2010a). Mixed ANOVA will be performed on the first and second time point, i.e. 'pre' and 'post', for each treatment, with Group (E or W) and Sex as factors. Significant interactions will be followed by appropriate post-hoc tests. As each phase is separated widely in time (indifference points tend to drift with prolonged testing- unpublished) Experiment 1 will not be analyzed with an omnibus ANOVA, but rather will be analyzed separately by phase. An α value of 0.05 will be used for all analyses.

Phase 1 Methods

Mice were shaped and trained to criteria for 38 days through Stage 6. Mice were water deprived (water access limited to 2 hours per day, immediately after DD training) until the end of Stage 5. During Stage 6 and during testing they were given unrestricted access to water. The last 3 sessions comprised the 'pre-drinking' data. After shaping was completed, mice were not tested in DD for 21 days, but rather given 2-bottle access to either 10% (v/v) ethanol in tap water, or tap water alone in the homecage. After this period of ethanol drinking, mice were again tested in DD. During this portion of the experiment, ethanol bottles were removed from the homecage for 2.5 hours before DD testing, and replaced immediately after DD testing. During this time, consumption of ethanol or water in the homecage was measured by visually reading the graduations on the ethanol or water containing tubes. The DD data collected during the three sessions after DD testing commenced again were regarded as the 'ethanol-exposed and currently drinking' data.

Phase 1 Results

One mouse died for an unknown reason on day 2, and six mice were eliminated from further testing at the end of Stage 6 for not meeting behavioral criteria for advancement. This left a total of 41 mice for the remainder of the experiment (ethanol group: 0.5 second n = 10, 10 second n = 11; water group: 0.5 second n = 9, 10 second n = 11). Overall, balanced across groups, there were 20 males and 21 females remaining. The dependent variables were calculated from the mean of days 35-37 for the 'pre' condition and days 60-62 for the 'post' condition.

High ethanol consumption was observed in the homecage 24-hr access drinking portion of the experiment, with group means of 18.1 ± 1.4 and 17.1 ± 1.2 (mean ± SEM) in the 0.5 and 10 second delay groups, respectively for days 39 – 59. These intakes were slightly lower than those observed in the breeders for that generation (~ 20.2 g/kg/day), but still represented high ethanol drinking (Yoneyama et al., 2008). Choice ethanol drinking during the entire period of ethanol access in Phase 1 is illustrated in figure 5. Interestingly, ethanol consumption dropped off sharply upon DD testing, but gradually recovered to pre-DD levels. This initial drop was presumably due to competition of ethanol reward by saccharin obtained during DD.

A mixed Sex X Group (E, W) X Time (pre and post-homecage drinking) ANOVA was performed on indifference points. In the 0.5 second group a main effect of Time was detected $F(1,14) = 8.08$, $p = 0.013$, but no other main effects or interactions were significant (ps > 0.24). This effect was evidenced by indifference points of 1.63 ± 0.10 and 1.22 ± 0.15 for the pre and post times, respectively. No significant main effects or interactions were detected in the 10 second group (ps > 0.45). These data are illustrated in figure 6.

As ethanol effects on motivation may be manifested in differential trials completed, the same ANOVA was performed on total trials completed. In the 0.5 second group the only significant effect that was detected was a main effect of Sex such that males completed 40 ± 3 trials while females completed 52 ± 3 trials. Other main effects were not significant (ps > 0.17). No main effects or interactions were detected in the 10 second group (ps > 0.17).

Effects on response latency, the time to respond once the center cue becomes illuminated, may indicate ethanol-induced group differences. The same type of analysis was performed on response latency. No significant effects were detected in the 0.5 second group (ps > 0.09). A significant interaction of Sex and Group was found in the 10 second group $F(1,18) = 6.19$, $p = 0.023$, but no other effects were detected (ps > 0.54). Examination of this interaction revealed that females in the E group had much longer response latencies than those in the W group (48 ± 10 vs. 28 ± 2), while males in the E group had shorter response latencies than those in the W group (31 ± 4 vs. 46 ± 7). Closer inspection revealed that the effect appeared to be driven by a single female in the W group with very long response latencies.

Another approach to detect relationships between ethanol exposure and impulsivity is correlation. Although correlation does not give useful information about causation, it can tell us if the two variables are related in some way. If the hypothesis of interest, that ethanol exposure causes increased impulsivity, is true then greater ethanol exposure should be correlated with greater impulsivity, i.e. smaller indifference points. Pearson's correlation was performed in both delay groups between mean ethanol consumption in days 39 – 59 and indifference points measured in days 60 – 62. Pearson's r values were 0.36 and 0.20 in 0.5 and 10 second groups, respectively. These did not approach significance (ps > 0.30), but the apparent trend was in the positive direction. These data are shown in figure 7.

Phase 1 Discussion

No convincing effect of oral ethanol self-administration on impulsivity was apparent in these data. Although ethanol drinking was moderate to high, and likely was

in the range of pharmacological relevance, expected effects on impulsivity were not measured. The hypothesized interaction of Time X Group on indifference points in the 0.5 second delay was not observed. The main effect of reduced indifference points in the 0.5 second delay group appeared to be a generalized effect possibly related to the long break from operant testing, as it was seen in both the ethanol and water-only drinking groups. Transient effects of Sex emerged in total trials completed and response latency, but these were not consistent, nor seemingly related to other measures. Perhaps most convincingly, the correlation between ethanol intake and indifference point, while not significant, was in the opposite direction than predicted, i.e. higher ethanol intakes correlated with higher indifference points. Therefore, I am forced to conclude that I was unable to detect an effect of 3 weeks of high ethanol drinking on impulsivity as measured here by DD. Indeed, the lack of BAC measurement requires that brain exposure to ethanol can only be estimated.

Phase 2 Methods

The same mice were used as in Phase 1. After completion of Phase 1, mice continued drinking ethanol and performing in DD for 9 more days. The last 3 sessions comprised the 'currently drinking' data, which were experiment days 69 – 71. Two more days of drinking alone commenced, after which access to ethanol was ended. The three days following ethanol removal (days 74 – 76) were tested in DD and regarded as 'abstinent'. Mice had unrestricted access to water in the homecage during these 3 days.

Phase 2 Results

The same mice were used as in Phase 1, and there were no further exclusions. The analysis of indifference points was conducted in the same manner as in Phase 1.

Mixed Sex X Group (E, W) X Time (drinking vs. abstinent) ANOVA on indifference points in the 0.5 second group revealed a main effect of Group $F(1,15) = 4.82$, $p = 0.044$, but no other effects were detected (ps > 0.08). The ethanol group mice showed higher indifference points in both time points than the water mice. No effects were detected in the 10 second group (ps > 0.07). These data are illustrated in figure 8.

The same type of analysis was conducted on total trials. In the 0.5 second group a main effect of Time was detected $F(1,15) = 18.69$, $p = 0.001$ such that total trials increased in the abstinent condition (figure 9). A main effect of Sex was also detected, with females completing more trials than males (62.7 ± 2.8 and 44.7 ± 3.6 trials, respectively), but no other effects were observed (ps > 0.06). A main effect of Time was also detected in the 10 second group $F(1,18) = 30.93$, $p < 0.001$, but no other effects were present (ps > 0.36).

Response latency was analyzed in the same manner. In the 0.5 second group a main effect of Sex was detected $F(1,15) = 5.86$, $p = 0.029$, as well as an interaction of Time X Sex $F(1,15) = 4.96$, $p = 0.042$, and a trend of Time ($p = 0.058$), but no other effects were observed. The interaction of Time with Sex was driven by a reduction in response latency in females from the drinking condition to the abstinent condition (females: 17.8 ± 3.7 and 10.2 ± 1.5, respectively). In contrast, the males' response latencies changed very little: 23.6 ± 3.7 and 23.8 ± 3.7. No effects were detected in the 10 second delay group (ps > 0.07).

Phase 2 Discussion

In summary, the mice that had been receiving ethanol appeared to be less impulsive than the water mice, but this effect was not influenced by drinking vs.

abstinence time point. Indeed, this effect amounted to sampling error, as the group assignment was determined in Phase 1, and the E mice in the drinking condition ("pre") started off with higher indifference points than the W mice. It would have been inappropriate to reassign groups, as ethanol history was part of the independent variable. Interestingly, the female mice completed more trials than the males (in the 0.5 second delay group), and had substantially faster response latencies than males- especially when alcohol was removed. In other words, effects of Drinking vs. Abstinent were trivial, but some effects of Sex emerged. That female HAP mice completed more trials and had faster reaction times concurs with the observation that females consistently drink ~20% more ethanol than male HAP mice in free choice drinking (Grahame et al., 1999b, Oberlin et al., 2010b). The increase in performance seen in abstinent females may be explained by reward deprivation.

Phase 3 Methods

The same mice were used as in Phases 1 and 2. After completion of Phase 2, DD testing continued for 5 more days. The last 3 of these days served as the 'pre' ethanol time. Similar to Phase 1, mice were then exposed to oral ethanol access for 17 days. After this period, mice performed DD for saccharin solution, and had restricted water access. The last 3 sessions before ethanol access comprised the 'pre-drinking' data, which were experiment days 79 - 81. The three days following ethanol access (days 99 - 101) were tested in DD and regarded as 'post-drinking'.

Phase 3 Results

Ethanol consumption in 24-hr homecage access was somewhat lower than in Phase 1, but remained stable at ~15 g/kg/day (shown in figure 10). One male from the

Water Group was excluded from further analyses due to non-completion of 20 trials in all three of the 'pre' ethanol DD sessions.

The analysis of indifference points was conducted in the same manner as in Phases 1 and 2. Mixed Sex X Group (E, W) X Time (pre and post-homecage drinking) ANOVA on indifference points revealed no main effects or interactions in either group (ps > 0.06); these data are shown in figure 11.

The same analysis of total trials in the 0.5 second group revealed main effects of Time $F(1,15) = 23.00$, $p < 0.001$ and Sex $F(1,15) = 19.64$, $p < 0.001$, but no others (ps > 0.10). This main effect was driven by a greater number of trials completed in the post-drinking sessions vs. the pre-drinking sessions: 65.1 ± 2.5 and 50.7 ± 3.4 respectively. Like Phase 2, female mice completed more trials than males in the 0.5 second group (65.3 ± 2.9 vs. 49.8 ± 2.4). Indeed, the 10 second group showed a main effect of Time as well, such that 'pre' averaged 38.3 ± 2.2 as opposed to 'post', which averaged 48.1 ± 2.3 trials completed.

An analysis of response latency in the 0.5 second group revealed main effects of Time $F(1,15) = 16.06$, $p = 0.001$ and Sex $F(1,15) = 5.54$, $p = 0.033$, but no others (ps > 0.22). Mice were twice as fast in the 'post' time as the 'pre' time (10.2 ± 1.4 vs. 20.4 ± 3.6 second). Additionally, females were twice as fast as males, such that their response latencies were 10.4 ± 2.4 as opposed to 20.8 ± 3.5 second. A similar pattern emerged in the 10 second delay group. A main effect of Time was significant $F(1,17) = 8.04$, $p = 0.011$, but no others were (ps > 0.23). Although mice in the 10 second delay group were slower than the 0.5 second group, the 'post' was faster than the 'pre' (24.7 ± 2.9 vs. 30.3 ± 3.4 second.)

Phase 3 Discussion

Although the mice in the E group had repeated bouts of extended ethanol exposure, no differences in impulsivity were detected between ethanol drinking mice and water-only drinking mice. The main effects of Time and Sex that emerged in total trials and response latency were clearly related; i.e. shorter response latencies roughly correlated with more trials completed. This observation could be surmised to indicate motivation. A general trend has emerged, particularly in Phases 2 and 3: females completed more trials and had faster reaction times than males. It was unexpected to find that main effects of Time and Sex were more robust than the hypothesized effect of oral ethanol on impulsivity (Time X Group). Broadly speaking, results obtained in Experiment 1 do not support the hypothesis that oral ethanol exposure increase impulsivity.

Experiment 2- Ethanol Administration and Impulsivity

Introduction

The basic rationale from Experiment 1 is maintained here: ethanol exposure will increase cognitive impulsivity. An effect of ethanol drinking on impulsivity in Experiment 1 was not detected, and it was suspected that this could have been caused by mice never becoming sufficiently intoxicated to undergo any meaningful neural changes (either on the cellular/molecular level or the systems level). Therefore, since free choice ethanol drinking may not produce BACs in mice that are analogous to human alcoholics, at least not for comparable time periods, high doses of ethanol will be administered. A previously published study showed that administration of 2.0 g/kg ethanol i.p. resulted in a BAC of 223 mg% 45 minutes later in HAP mice (Grahame et al., 1999b). Therefore

any dose larger than that should result in intoxicating BACs, i.e. upwards of 400 mg%. To maximize sensitivity to detect effects on impulsivity, the highest reasonable dose of ethanol will be repeatedly administered. The hypothesis that will be tested for Experiment 2 is that repeated i.p. administration of high doses of ethanol will increase impulsivity in HAP mice.

Materials and Methods

Animals: Twenty-four HAP1 mice (11 male, 13 female) from generation 37 and 24 HAP2 (12 male, 12 female) from generation 30 were used in this experiment. The housing was identical to the conditions described in Experiment 1. Mice had *ad lib* access to food, and water access restricted to 2 hours per day, immediately after DD testing.

Apparatus: The same apparatus and data collection methods were used as described for Experiment 1.

Behavioral Assessment: Mice were shaped according to the protocol outlined in table 1. Stage 1 was performed for only 1 day; Stage 2 for 7 days; Stage 3 for 6 days; Stage 4 for 20 days; Stage 5 for 12 days; and Stage 6 for 11 days. DD testing was performed in the same manner described in Experiment 1. After mice met criteria in Stage 6, they were assigned to the E Group (ethanol injections) and the other half were assigned to the Sal Group (saline injections). Group assignment was balanced across Stage 5 and Stage 6 adjusted amount scores and Sex. Intraperitoneal injections of either 3.5 g/kg ethanol (20% ethanol v/v in saline, injection volume of 22.2 ml/kg) or saline (22.2 ml/kg) were given every other day for 16 days, for a total of 8 injections. A timeline showing the sequence of events is illustrated in figure 12. The injections were

given to the mice immediately before placing them in the operant boxes for 20 minutes. After 20 minutes elapsed, the mice were then placed back in their homecages. Placement in the operant chambers was done because previous unpublished data with locomotor sensitization suggests that the place where the animal experiences acute intoxication can influence the duration of ethanol-induced behavioral changes.

Statistics: The mean of the last 3 days before ethanol and saline injections and the first 3 days afterward were taken to represent performance in the pre and post-ethanol times. Delay groups were analyzed separately *a priori*, as in Experiment 1. Mixed ANOVA will be performed on the 'pre' and 'post' time for each treatment, with Group (ethanol [E] or saline [Sal]) and Sex as factors. Significant interactions will be followed by appropriate post-hoc tests.

Results

Five mice were excluded from the final analysis: 4 mice did not meet behavioral criteria for advancement in Stages 5 or 6, and one mouse died for unknown reasons. Furthermore, one other mouse did not complete 20 trials in the post-ethanol time, so this mouse was excluded from all within-subjects analyses. The mice were accidentally tested with the incorrect DD program on the second day after ethanol exposure, so that entire day, and the first 12 mice from the next day (day 68) had to be excluded from analyses. Therefore, experiment days #66, 68, and 69 were used for the post-ethanol data. Mice were profoundly impaired by the ethanol injections, such that they were unable to right themselves or walk.

The analysis of indifference points was conducted in the same manner as in Experiment 1. Mixed Sex X Group (E, W) X Time (pre and post ethanol injections)

ANOVA on indifference points revealed no main effects or interactions in the 0.5 second group (*p*s > 0.13). In the 10 second group, a strong interaction trend of Time X Group (*p* = 0.052) emerged, such that the ethanol-treated mice appeared to be less impulsive (figure 13). All other effects were non significant (*p*s > 0.07). As these data were quite unexpected, given that there appeared to be changes in opposite directions, an analysis of day by day adjusted amount was performed. These data can be seen in figure 14. As large effect sizes were apparent only on the first day after ethanol, a different type of analysis was performed, which assumed a transient effect of ethanol: either withdrawal or short-term impairment. A simple between-subjects t-test was performed on subjects on day 66 only. In the 0.5 second group, a trend was observed ($p = 0.071$), but in the 10 second group the effect was significant $t(18) = 2.47$, $p = 0.024$. Interestingly, though these changes were in opposite directions, they were similar in that they tended toward an adjusted amount of 1.0, which indicates chance responding.

No main effects or interactions were observed in either group in total trials completed (*p*s > 0.14).

Analysis of response latencies in the 0.5 second group revealed no significant effects (*p*s > 0.18). However, there was a main effect of Time in the 10 second group $F(1,16) = 5.70$, $p = 0.030$. No other effects were detected (*p*s > 0.24). The main effect of Time manifested as smaller response latencies in the post-ethanol time (24.4 ± 2.9 second) as opposed to the pre-ethanol time (29.0 ± 3.1 second).

Discussion

The *a priori* hypothesis, that ethanol exposure will increase impulsivity, was not supported, but cannot be dismissed based on definitive findings. There was no

significant interaction in the 0.5 second group (which could indicate an ethanol-induced increase in impulsivity). Contrary to predictions, the larger effect was observed in the 10 second in the direction of decreased impulsivity (although this effect did not quite meet the threshold for significance.) However, the day by day analysis indicates that this effect was driven by a tendency in both groups toward chance responding. These data suggest that there was an abolition of preference in both groups that recovered within 2 days. No differences were seen in total trials completed, which suggested that mechanisms influencing responding were unchanged. Interestingly, a main effect of ethanol treatment (Time) was detected in response latencies such that mice were faster to initiate responding after injections. A main effect of Time suggests a nonspecific effect of the treatment, i.e. the stress of being injected. This runs contrary to the idea that stressors impair performance, unless one believes that injections are a minor stressor that can augment behavioral efficiency or motivation.

Experiment 3- Responding for Ethanol and Impulsivity

Introduction

Converging evidence supports the idea that AUD/SUD subjects are more impulsive when responding for their hypothetical drug of choice. There is wide disagreement about the acute effect of ethanol on impulsive choice, but data from the 'Experiential Discounting Task' (which involves actual rewards and delays) suggests that under 'realistic' conditions ethanol can acutely increase impulsivity. The most relevant finding with regards to alcohol and impulsivity would be in alcoholics, as alcoholics are the target population for AUD treatment. Alcoholics discounting alcohol rewards can be modeled by; 1) using an animal model of alcoholism, 2) responding for their drug of

choice, and 3) becoming intoxicated during the session. Hypothesis: HAP mice will show greater impulsivity while responding for ethanol reinforcers vs. saccharin reinforcers. Saccharin was chosen as the control rather than water to minimize magnitude effects, i.e. HAP mice have high preference ratios for ethanol vs. water, but they also have high preference for saccharin over water. Therefore, although saccharin is qualitatively different than ethanol, it is rewarding in its own right. Reward in both cases is inferred from high preference ratios.

Materials and Methods

Animals: Forty-eight HAP2 mice (23 male, 25 female) from generation 29 were used in this experiment. The housing was identical to the conditions described in Experiment 1. Mice had *ad lib* access to food, and water access restricted to 2 hours per day, given immediately after DD testing.

Apparatus: The same apparatus and data collection methods were used as described for Experiment 1.

Behavioral Assessment: To acclimate mice to their relative reinforcers, they were randomly assigned to either the Sac (saccharin) or E (ethanol) groups, balanced across Sex, and given 24-hr 2 bottle access to their preferred reinforcer (either saccharin or ethanol) and water. This pre-exposure phase lasted 4 days, after which they began DD shaping. Mice were shaped for Stages 1-5 according to the protocol outlined in table 1. Stage 1 was performed for only 1 day; Stage 2 for 5 days; Stage 3 for 2 days; Stage 4 for 4 days; and Stage 5 for 25 days. The DD paradigm was the same as described for Experiment 1, except that all mice were tested at delays of 1, 2, 4, and 8 second. DD shaping through Stage 4 was performed with water reinforcement, and was changed to

saccharin (0.05% w/v) or ethanol (10% v/v) beginning on the first day of Stage 5 (day 17). After 22 days of DD Stage 5, concentrations were changed to 0.01% (w/v) saccharin and 8% (v/v) ethanol to make trial number and consumption more equitable between groups. The three days after this served as the zero delay, which were the last 3 days of Stage 5. Subsequently, mice were tested in DD with the following delays in ascending order: 1, 2, 4, and 8 seconds. Each delay was tested for 3 sessions, and means of those 3 were taken to calculate dependent variables. A timeline showing the sequence of events is illustrated in figure 15. Using discrete short and long delays is useful for rapid assessment of effects, but it comes at the cost of measuring changes across a range of delays. Thus, given that we have no *a priori* reason to expect that small daily ethanol doses will affect discounting over 15 days, a full range of delays was employed.

Statistics: Means of the 3 sessions at each delay were used to calculate dependent variables. Mixed Group (E or Sac) X Sex X Delay (0, 1, 2, 4, 8 second) ANOVA were performed on these data. Significant interactions were followed by appropriate *post hoc* tests. Additionally, hyperbolic regression was performed on indifference points within individuals to obtain *k* values for each mouse. Values for *k* were derived from the formula $V = M/(1 + kD)$ where V is the indifference point (in seconds of sipper access time) at delay D, and M is the magnitude of the delayed reinforcer (fixed at 2, as 2 seconds of sipper access was the delayed reinforcer at all delays.) Natural-log transforms were used to normalize *k* values for parametric testing. Univariate Group (E or Sac) X Sex ANOVA was performed on *lnk* values to derive overall differences in impulsive responding. Additionally, bivariate correlation was performed on mean consumption during the DD testing period and *lnk* values.

Results

Seven mice were excluded from testing because they did not meet behavioral criteria in Stage 5. Additionally, one other mouse did not complete 20 trials in any of the three 8 second delay sessions, so those data were also excluded from analyses.

Mice responding for ethanol received a pharmacologically relevant dose of ethanol, 1.73 ± 0.08 g/kg/1hr (averaged across all sessions), and most of that was obtained in the first 30 minutes (unpublished observations). Mixed Group (E, Sac) X Sex X Delay (0, 1, 2, 4, and 8) ANOVA on indifference points revealed a main effect of Delay $F(1,144) = 62.55$, $p < 0.001$, and a Delay X Group interaction $F(4,144) = 4.33$, $p = 0.002$, but no other effects (ps > 0.06). To determine which delays differed by Group, t-tests were performed between Group for each Delay. The E (ethanol) group had higher adjusted amounts at the 8 second delay $t(38) = 2.51$, $p = 0.017$, but not at the other delays (ps > 0.11). These data are illustrated in figure 16.

Mixed Group (E, Sac) X Sex X Delay (0, 1, 2, 4, and 8) ANOVA on total trials revealed a main effect of Group $F(1,36) = 23.27$, $p < 0.001$, but no other effects (ps > 0.21). This effect was evidenced by more trials completed by the mice that were responding for saccharin vs. mice responding for ethanol; 45.4 ± 2.1 and 34.1 ± 1.1 trials completed, respectively.

Mixed Group (E, Sac) X Sex X Delay (0, 1, 2, 4, and 8) ANOVA on response latencies revealed no significant effects (ps > 0.16).

To better estimate the general pattern of discounting, a Univariate Group (E or Sac) X Sex ANOVA on *lnk* values revealed a main effect of Sex $F(1,36) = 4.25$, $p = 0.046$ and a strong trend of Sex X Group ($p = 0.053$), but no main effect of Group ($p =$

0.29). The *lnk* values for males and females were -0.798 ± 0.203 and -1.497 ± 0.280, respectively. These values translate to actual *k* values of 0.450 and 0.224. Larger values of *k* indicate more impulsive responding, so these data indicate that males were more impulsive than females overall.

To determine if there was any relationship between ethanol or saccharin consumption and impulsivity, a correlation was performed between consumption and *lnk* values. The groups were analyzed separately, as an interaction with Group X Delay was detected in indifference points. Consumption was calculated as a mean across all sessions in ml/kg. No correlation was observed between saccharin drinking and *lnk* values, r = 0.071, $p = 0.76$, but a weak correlation was observed in the mice responding for ethanol, r = -0.583, $p = 0.007$. These data are illustrated in figure 17. This correlation indicates that mice that drank more ethanol were less impulsive than mice that drank less, and that this relationship did not generalize to saccharin drinking. It should be noted that much of the strength of this correlation was driven by only 2 points.

Discussion

The *a priori* hypothesis, that responding for ethanol will increase impulsivity, must be rejected. Indeed, indifference points were higher in mice responding for ethanol than in mice responding for saccharin, but it was not a large effect, and it was only detectable at the 8 second delay. The reduction in trials completed may be attributable to ethanol intoxication, but no corresponding effect of ethanol was detected in response latencies. There was not a Group effect on *lnk*, but there was an effect of Sex, such that males were more impulsive than females regardless of Group. Interestingly, a correlation emerged between ethanol consumption and *lnk*, which was not observed in the saccharin

group. One way to interpret this correlation is to conclude that ethanol makes animals less impulsive; this notion is supported by a lack of correlation in saccharin drinking. Other published data imply that we should find the same correlation in the saccharin group, given the association between ethanol drinking and sweet preference. The data generated here suggests that responding for ethanol reduces impulsivity in HAP mice, but that it is a modest effect.

GENERAL DISCUSSION

The overarching hypothesis, that ethanol exposure will increase impulsivity, was not supported. Main effects of Time on decreases in response latency and increases in total trials completed in Experiment 1, Phase 3 were likely due to the water-deprived state of the mice in the 'post' time. Experiment 2 revealed what appeared to be a transient and non-specific abolition of preference, i.e. a tendency toward chance responding in ethanol injected mice. Experiment 3 showed a statistically significant decrease in impulsivity in mice responding for ethanol, along with a correlation of reduced impulsivity and ethanol consumption. Together with the non-specific effects in Experiment 2 and the null results from Experiment 1, these findings were clearly in the opposite direction of the *a priori* hypothesis.

Ethanol Exposure and Impulsivity

The hypothesis that a history of ethanol exposure creates greater impulsivity, as tested in experiments 1 and 2, is inferred from two lines of evidence; one, that alcoholic humans are more impulsive than controls, and two, that early exposure to ethanol can cause later deficits in animals. Neither of these lines of evidence is entirely satisfying. In the first case, alcoholic humans used in studies of impulsivity are a self-selected group, i.e. it could well be that more impulsive people become alcoholics and alcohol exposure itself has little to do with the expression of high impulsivity. There is evidence from several studies in alcohol-naïve rodent models of alcoholism to suggest that impulsivity precedes alcohol use (Wilhelm and Mitchell, 2008, Oberlin and Grahame, 2009) and similarly that disinhibitory and novelty-seeking behavior precedes AUD/SUD in humans (Masse and Tremblay, 1997, Tarter et al., 2003). In the second case, that ethanol

exposure causes later cognitive deficits, has mostly been shown in cases of extremely long exposure times (some longer than half the lifespan of an average rat) (Baird et al., 1998) and/or using forced ethanol drinking (Arendt et al., 1989, Melis et al., 1996), which subjects animals to chronic dehydration and stress. Perhaps it is unsurprising then that no convincing effects of ethanol exposure were found, either via choice drinking or i.p. injections on impulsivity. In the former case it is arguable that mice did not experience high enough BACs for functional changes in neural circuitry, as their drinking of ~18g/kg/day probably equaled ~1.5g/kg/hr based on a 12 hour dark cycle. This was addressed in Experiment 2, where mice were given 3.5 g/kg in a bolus on 8 different days. Based on a previous study in HAP mice (Grahame et al., 1999a), this dose would have put the mice well over 400 mg/dl, which is beyond a pharmacologically relevant dose. Even with this exposure to high ethanol doses, mice did not show significant differences in impulsivity. A strong trend of reduced impulsivity emerged in the 10 second delay group ($p = 0.052$), which corresponded with faster response times. Neither of these effects were predicted or expected. Indeed, those results suggest that there was some facilitation of responding induced by ethanol exposure.

Responding for Ethanol and Impulsivity

The hypothesis that responding for ethanol will result in greater impulsivity, as tested in Experiment 3, was extrapolated from human studies that suggest that alcoholics/addicts are more impulsive when responding for their drug of choice (Kirby et al., 1999, Madden et al., 1997, Bickel et al., 1999, Petry, 2001a, Coffey et al., 2003). The attempt to model alcoholics responding for actual alcohol produced results (reduced

impulsivity) that may not be expected from the human literature. There are two rather different perspectives that could explain these current data.

From the standpoint of motivation, larger rewards tend to produce shallower discounting, (reduced impulsivity) in both addicts and controls: a 'magnitude effect' (Myerson and Green, 1995, Petry, 2001a, Kirby et al., 1999). Although care was taken in Experiment 3 to balance reinforcer efficacy by adjusting ethanol and saccharin concentrations to equity during training, ethanol and saccharin are qualitatively different reinforcers each with unique properties. Given the high preference that HAP mice have for ethanol, we must assume that their subjective valuation of ethanol is high, so therefore may be more highly valued than other reinforcer types. Their selection phenotype suggests as much. A high subjective valuation then would lead to the prediction, based on human studies, that discounting would be lessened. This would result in an apparent reduction in impulsivity. This confound can only be avoided if one is sure that the drug reinforcer is exactly equal to the control reinforcer, which is perhaps impossible to do.

From the standpoint of a pharmacological effect, ethanol intoxication itself may have reduced impulsivity. Human studies on the acute effect of orally consumed ethanol are mixed; one study found that 0.8 g/kg ethanol increased impulsivity (Reynolds et al., 2006), another study found that 0.7 g/kg did not increase impulsivity (Ortner et al., 2003) and that BAC was correlated with reduced *k* values, and another study found no effect of 0.8 g/kg of ethanol on discounting (Richards et al., 1999). It is worth noting that none of these studies tested alcoholics, and in fact Richards et al. specifically excluded addicts/alcoholics, while Reynolds et al. tested 'social drinkers'. It is quite plausible that the effects of alcohol on impulsivity would be very different in alcoholics vs. non-

alcoholics. Therefore, the data from human studies do not help us make clear predictions about what to expect from acute alcohol intoxication in a model of alcoholism. Indeed, in pilot studies performed by the author, injected doses of 2.0 g/kg ethanol have sometimes reduced impulsivity in HAP mice, but never increased impulsivity (unpublished observations).

This might be a good time to check the basic premise that addicts discount their drug of choice more steeply than money because of some unique qualitative difference. There is evidence from several studies that shows that money is discounted less steeply than other consumable rewards, i.e. beer, candy, soda, and food (Estle et al., 2007, Odum and Rainaud, 2003, Odum et al., 2006). Although these studies used hypothetical rewards rather than real rewards, other studies suggest that hypothetical vs. real rewards does not affect choice behavior (Lagorio and Madden, 2005, Madden et al., 2003). Although the consumable vs. non-consumable difference in reward type may be relevant, none of the studies cited here tested addicts or alcoholics. Indeed, the only appropriate test to perform for this element would be a DD task with alcoholics vs. social drinking controls for money vs. alcohol. Alcohol, after all, is the only mind-altering drug of abuse that is legal and widely used; the subjects' use of the drug to be tested in DD is important if we expect subjects to value it. One seminal study did precisely this and found that ethanol was discounted more steeply by both alcoholics and controls (Petry, 2001a), which lends credibility to the idea that ethanol is merely another consumable reward, and like other consumable rewards is discounted more steeply. The distinction comes from the idea that money is a conditioned, or secondary reinforcer, while consumable rewards are unconditioned, or primary reinforcers. So, if we assume that secondary reinforcers

like money are discounted less steeply than primary reinforcers like alcohol and food, then the human literature on discounting of consumables (drug and non-drug) is largely consistent on this basis alone. Other explanations are possible, however, when we consider the possibility of an interaction of subject type (control vs. alcoholic) and reinforcer type (hypothetical alcohol vs. real alcohol). To the author's knowledge, discounting of real alcohol by alcoholics has not been performed in humans or animal models. Experiment 3 may represent the first study of this type. In light of this very possible interaction, the unexpected results obtained here may not be viewed as so strange.

Perhaps the most parsimonious explanation for the decrease in impulsivity seen in responding for ethanol is that ethanol is acting similarly to stimulants, i.e. having an amphetamine-like effect. Operant responding for ethanol triggers mesolimbic DA release (Weiss et al., 1993, Melendez et al., 2002), as does administration of amphetamine (Pijnenburg et al., 1975). Amphetamine is the most widely-studied drug that consistently reduces impulsivity in most DD studies (Oberlin et al., 2010a, de Wit et al., 2002, Floresco et al., 2008, Wade et al., 2000, Winstanley et al., 2003). It is quite possible then that the mesolimbic dopamine system is importantly involved in choice behavior with delayed rewards, and in fact human imaging studies suggest just that (Ballard and Knutson, 2009, McClure et al., 2004). Therefore, we might speculate that at the ethanol doses seen here, ethanol is exerting a stimulant-like effect of decreased impulsivity via mesolimbic dopamine release.

CONCLUSION

Alcohol dependence and alcohol abuse affect millions of Americans and cost billions of dollars. It is one of the most costly neuropsychiatric disorders, and one of the most prevalent. A trait that is highly correlated with AUDs, and may well underlie the disorder is impulsive choice, which can be measured by DD in both humans and animals. The effect of alcohol exposure on impulsivity is unclear from the human literature, so animal models are required to answer such questions. In the HAP rodent model of alcoholism, I was able to detect an effect of reduced impulsivity when responding for ethanol, but was unable to detect an effect of ethanol exposure on later impulsivity, although a strong trend also suggested a reduction in impulsivity. The effect of reduced impulsivity with acute ethanol was unexpected, but given the fractionated nature of the human literature, does not disagree with a consensus. On the other hand, the animal literature suggests that I should be able to detect an effect of ethanol exposure, but that I was unable to measure an effect of ethanol on impulsivity. There are two obvious possibilities for this: the first is that the mice were simply not exposed to enough ethanol. This possibility raises a double-edged sword: first, if I can detect effects of very high exposure, what, exactly, am I modeling? Second, given the high rate of ethanol metabolism in mice, what is an appropriate exposure regimen to accurately model human alcoholic ethanol exposure? These are unresolved questions, in many ways, and present challenges to the researchers who use rodent models for studying alcoholism. The other possibility for why an increase (or even a difference) was not detected in impulsivity from ethanol exposure is that there is not an effect mediated toxicologically. Although I am hesitant to treat a null result as evidence for no effect, that possibility exists. Indeed,

no toxicological effect of ethanol is required to explain the higher impulsivity observed in human alcoholics, as higher trait impulsivity has already been demonstrated in several studies. Although the results found here were somewhat unsatisfying, I supposed that it is the nature of research to, sooner or later, abolish the naivety of the young scientist who expects certain results with every new experiment.

TABLES AND FIGURES

Table 1: Shaping of Operant Responses Required for Delay Discounting Testing

Stage	Description	Criterion to advancement
1 Nosepoke FR1	The only manipulandum is the nosepoke. All center nose pokes are reinforced on a FR1 schedule with 20 sec sipper access to saccharin solution. Non response-contingent reinforcement presented every 2 min. The center light is on for the entire session.	One session only, unless an individual shows a lack of consumption. (Lack of consumption ≤ 0.1 ml)
2 Nosepoke FR1	All center nose pokes are reinforced on a FR1 schedule. Sipper access time is 10 sec, and it will remain that length until Stage 5. If less than 5% are below criteria, the group will be advanced to Stage 3. The center light is on for the entire session.	Completion of 20 trials in 60 minutes
3 Nosepoke FR1 w/ ITI	All trials are cued. The center light will come on every 30 sec and stay on for the first 20 of those 30 sec, i.e. 10 sec ITI. During its illumination, a nose-poke will result in reinforcement.	Completion of 20 trials in 60 minutes
4 Nosepoke plus lever press FR1 w/ ITI	All trials are cued, and left and right levers & lights are installed. A chained nosepoke, then lever press is required for reinforcement. There is no maximum response latency or choice latency. The ITI is 10 sec. After the center nose-poke, the lights above the levers will go on, to signify that they are active. Left and right lever presses are recorded.	Completion of 20 trials in 60 minutes
5 Nosepoke plus lever press FR1 w/ ITI and Forced Choice	Immediate and 'delayed' side assignment made, and adjustment of immediate side sipper access begins. 'Delay' is zero in Stage 5. Trials are separated by 30 sec ITI. Forced choice requirement introduced: a side chosen for two consecutive trials is unavailable until the other alternative is sampled once. Available choices are signaled by illumination of lever lights.	Completion of 20 choice trials in 60 minutes AND mean AdjAmt of ≥ 1.5 sec for 3 out of 4 consecutive days
6 Nosepoke plus lever press FR1 w/ ITI and Forced Choice	Group assignments to either 0.5 sec or 10 sec delay made. Testing begins after criterion to advancement is achieved in this Stage.	Completion of 20 choice trials AND mean AdjAmt of ≤ 0.6 sec (0.5 sec group) or ≥ 1.4 sec (10 sec group)

FR1 = Fixed Ratio 1, ITI = InterTrial Interval, AdjAmt = Adjusted Amount, i.e. the median titrated value of the immediate side in the last 20 choice trials in a single session.

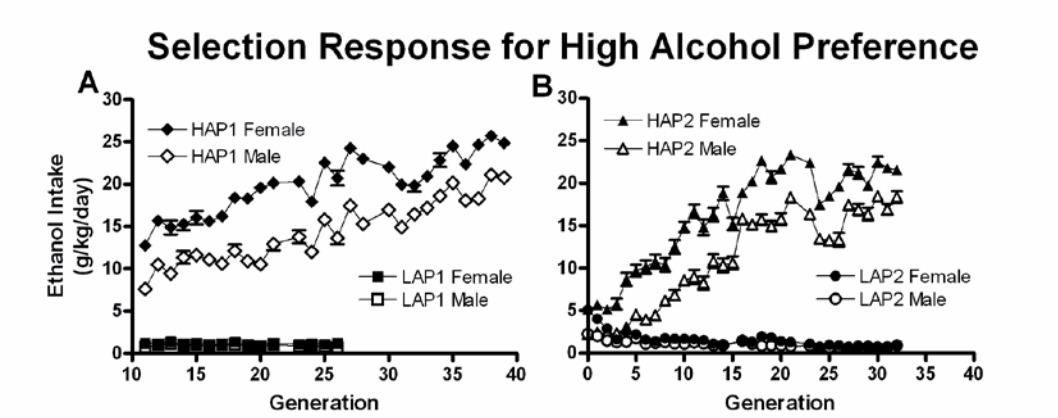
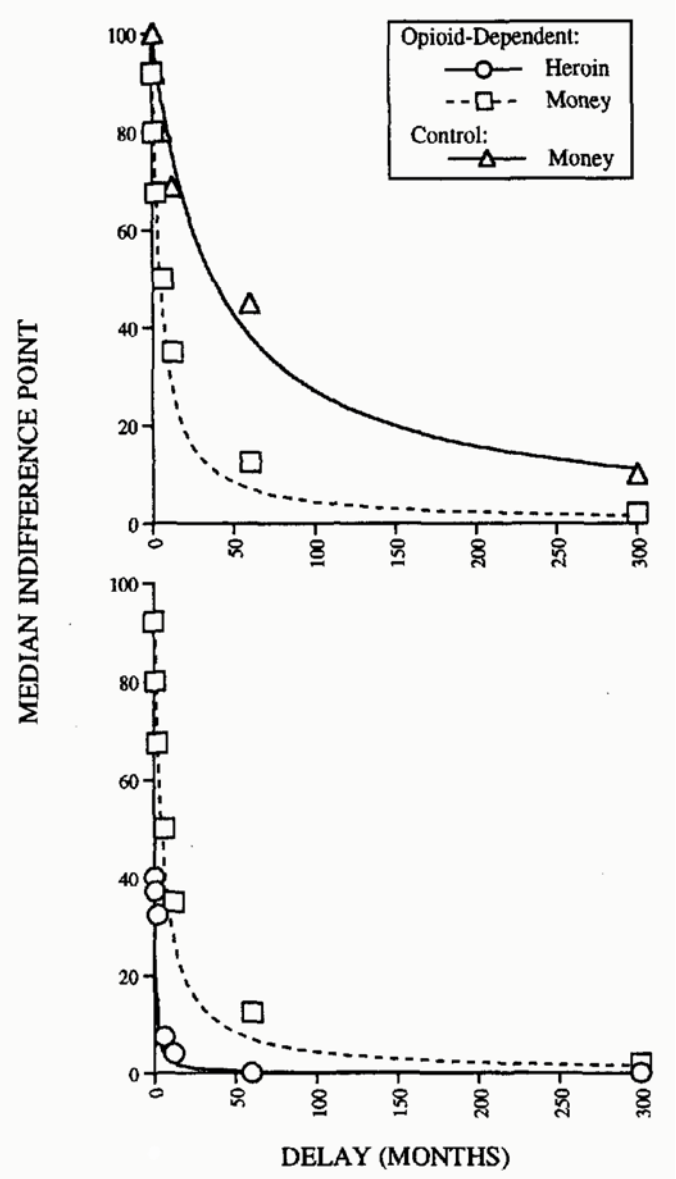


Figure 1. The left panel (A) illustrates the response to bidirectional selection for alcohol preference in replicate line 1 by sex and line as a function of selection generation (HAP1 ns = 62 to 109, LAP1 ns = 70 to 102 per generation). Mice were assessed for 24 hour homecage drinking of 10% (v/v) ethanol for 28 days. On the right panel (B) the selection response of the replicate line 2 is shown (HAP2 ns = 66 to 197, LAP2 ns = 46 to 159 per generation). The same selection procedure was used for both lines.



Median indifference points between large delayed and small immediate heroin and monetary rewards. Opioid-dependent and control participants' monetary choice data are shown at the top, whereas the bottom shows opioid-dependent participants' monetary and heroin choice data. Indifference points reflect the present value of the larger, more delayed rewards (i.e., the value of the delayed rewards in immediate-reward units). So that heroin and monetary rewards could be compared on a common axis, the vertical axis shows the percentage of the large delayed reward (this transformation did not affect the delay-discounting parameter estimates).

Figure 2. Reproduced from Madden et al. 1997

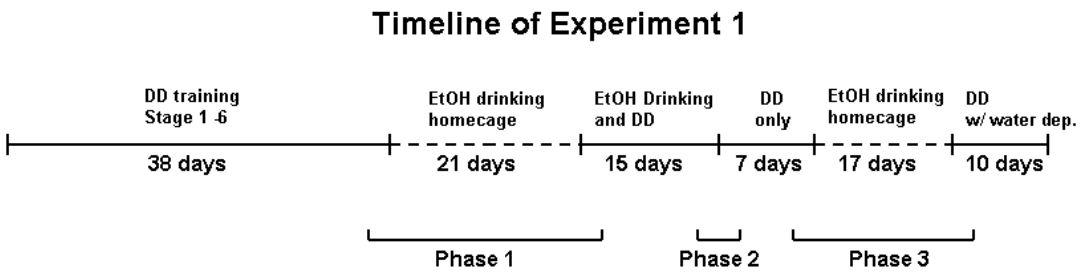


Figure 3. In Experiment 1, Phase 1, HAP1 mice were first trained in Delay Discounting (DD) through 6 Stages, given 10% (v/v) ethanol in the homecage, and tested again in DD while still having access to ethanol. In Phase 2, mice were compared when they had access to ethanol vs. when they had only water. In Phase 3, mice were again given ethanol in the homecage, then tested in DD while water deprived. The number of days that the mice spent in each portion of the experiment is indicated. The dotted line indicates periods of no DD testing and homecage drinking only.

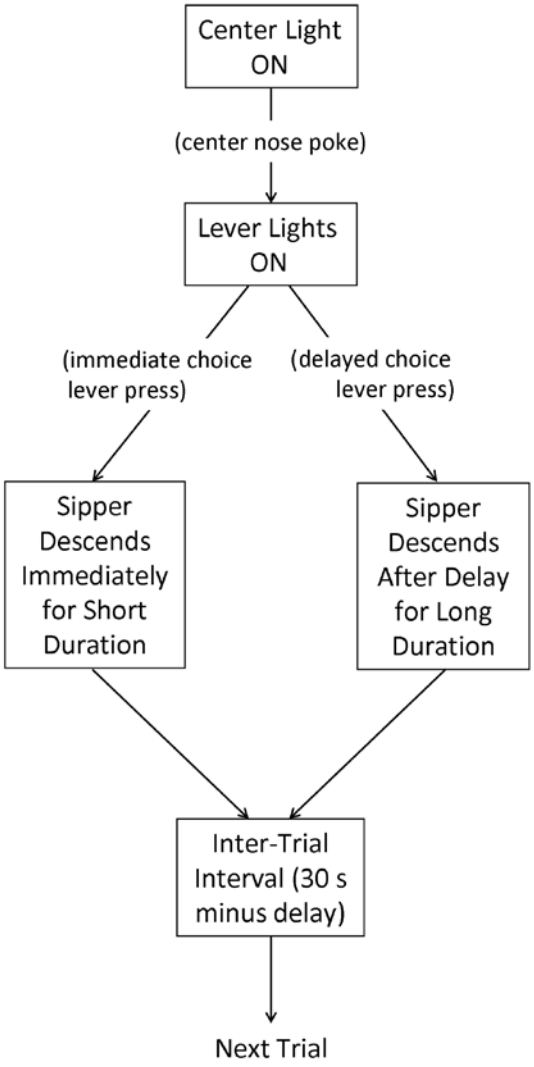


Figure 4. Response requirements in a trial. The text in parentheses show the operant responses required to proceed to the next step. The sipper tube contains 0.0136% (w/v) saccharin solution.

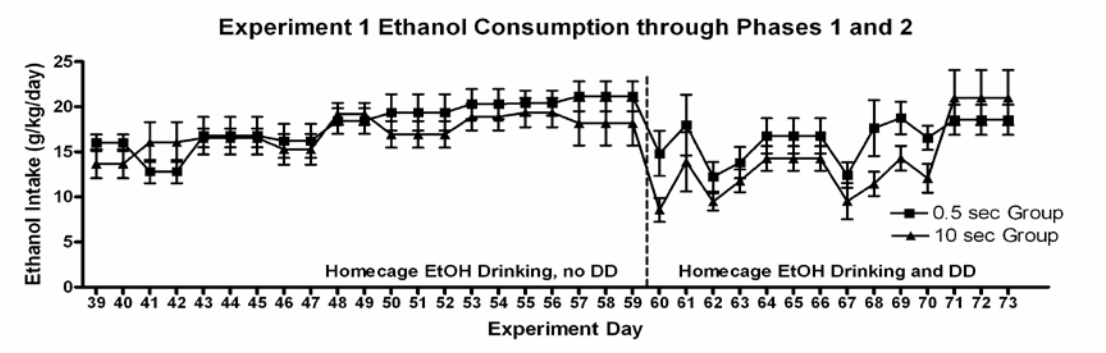


Figure 5. Two-bottle choice consumption of unsweetened 10% (v/v) ethanol intake during homecage drinking is shown by Delay Group. The alternative fluid is tap water (not shown). Days 39 to 59 represent 24-hour ethanol access, while days 60 – 73 represent 20.5 hours per day ethanol access, as ethanol was removed 2.5 hours prior to DD testing. Day numbering corresponds to the first day of shaping.

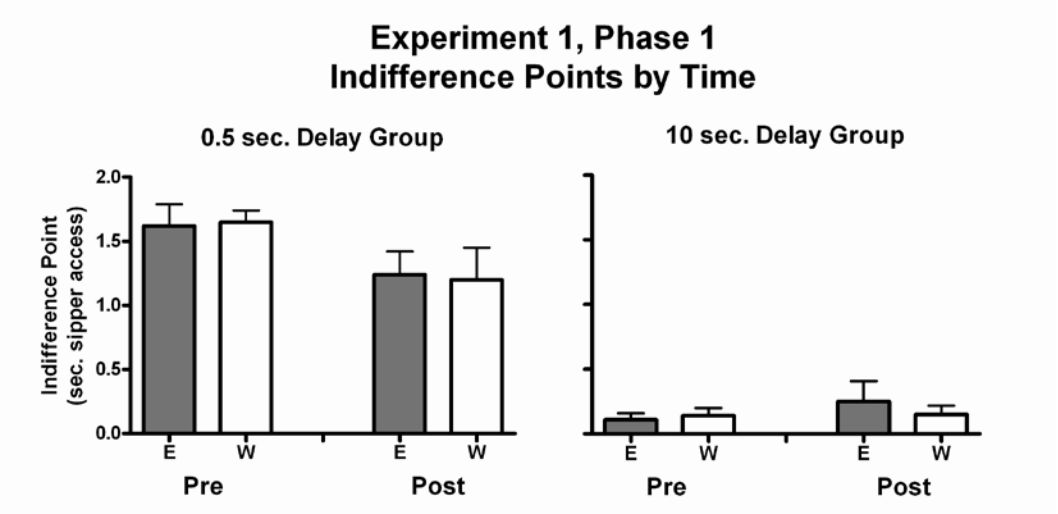


Figure 6. Indifference points are shown by Delay (.5 second on left and 10 second on right) before (pre) and after (post) homecage ethanol/water (E) or water/water (W) for 21 days. E (ethanol) group n = 21, and W (water) group n = 20. All animals were assigned to their respective delay before ethanol or water exposure. Note that decreased indifference points from Pre to Post indicate increased impulsivity, and vice versa.

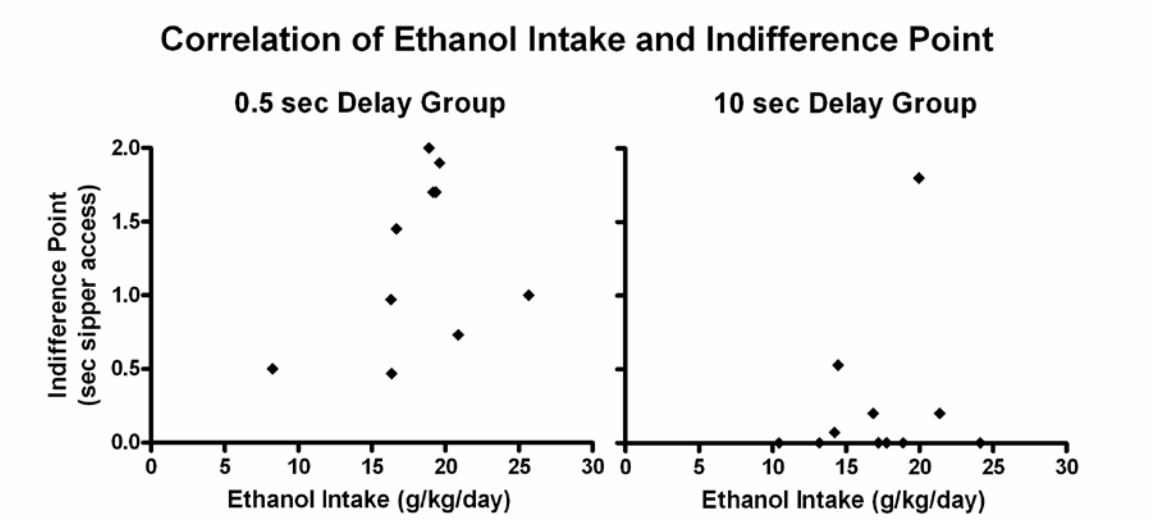


Figure 7. Mean ethanol intake for homecage drinking days 39 – 59 are uncorrelated with DD indifference points derived from days 60 – 62 is shown for the 0.5 second delay group (left) and 10 second delay group (right) in the E groups only.

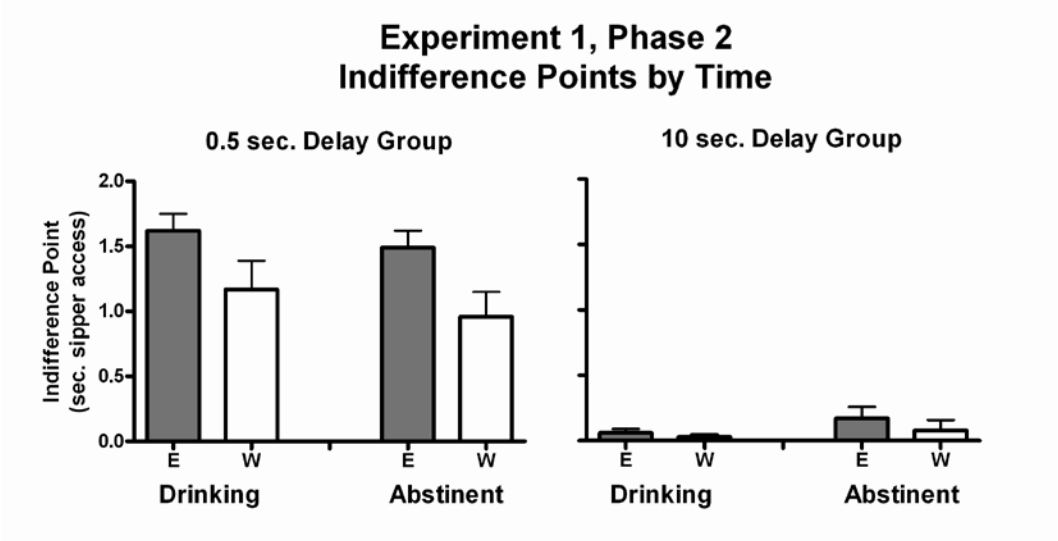


Figure 8. Indifference points are shown by Delay during an ethanol drinking period (Drinking) and with only water (Abstinent) for the 0.5 second delay group (left) and the 10 second delay group (right). E (ethanol) group n = 21, and W (water) group n = 20. All animals were assigned to their respective delay before ethanol or water exposure. Note that decreased indifference points from Pre to Post indicate increased impulsivity, and vice versa.

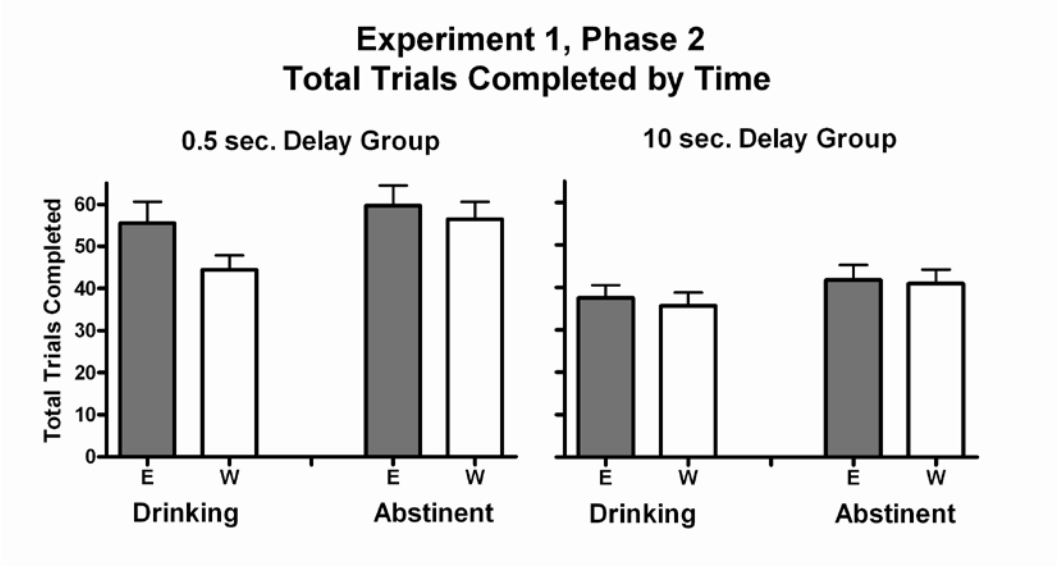


Figure 9. Total trials completed by Delay are shown during an ethanol drinking period (Drinking) and with only water (Abstinent) for the 0.5 second delay group (left) and the 10 second delay group (right). E (ethanol) group n = 21, and W (water) group n = 20. All animals were assigned to their respective delay before ethanol or water exposure. Note that decreased indifference points from Pre to Post indicate increased impulsivity, and vice versa.

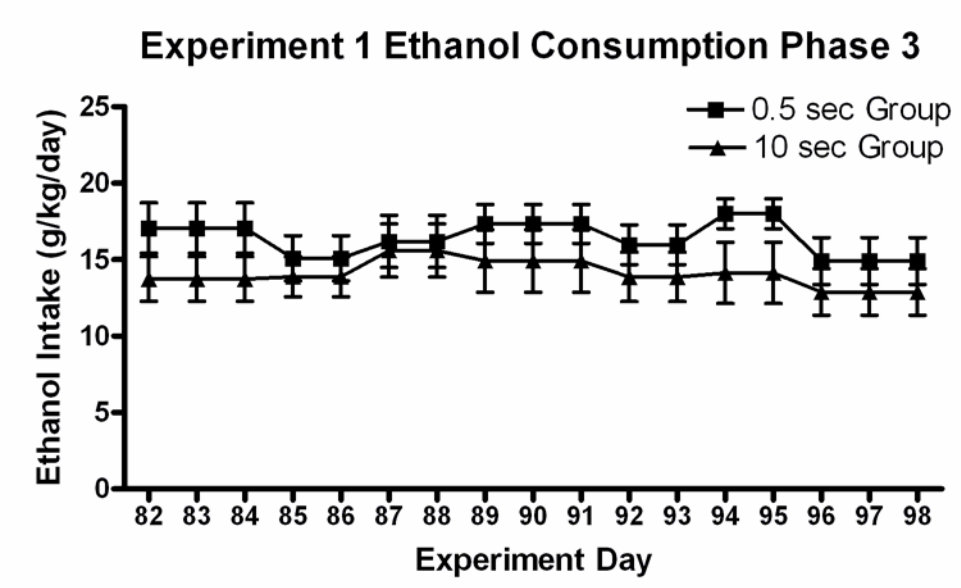


Figure 10. Two-bottle 24-hr choice consumption of unsweetened 10% (v/v) ethanol intake during homecage drinking is shown by Delay Group. The alternative fluid is tap water (not shown). Day numbering corresponds to the first day of shaping.

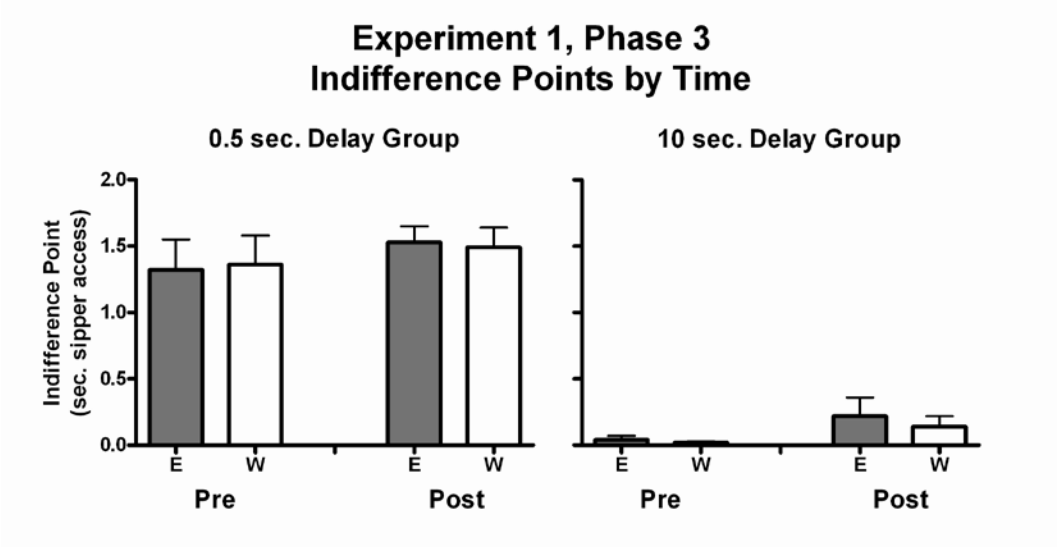


Figure 11. Indifference points are shown by Delay (.5 second on left and 10 second on right) before (pre) and after (post) homecage ethanol/water (E) or water/water (W) for 17 days. E (ethanol) group n = 21, and W (water) group n = 19. All animals were assigned to their respective delay before ethanol or water exposure. Note that decreased indifference points from Pre to Post indicate increased impulsivity, and vice versa.

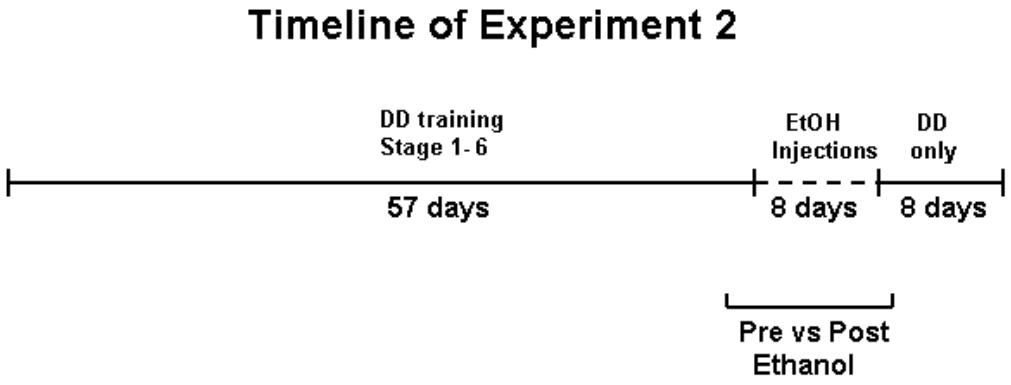


Figure 12. In Experiment 2, HAP1 and HAP2 mice were first trained in Delay Discounting (DD) through 6 Stages, injected with 3.5 g/kg ethanol i.p. 8 times, and tested again in DD. All DD responding was for saccharin solution. The number of days that the mice spent in each portion of the experiment is indicated. The last 3 days of Stage 6 comprised the 'pre' condition, while the first 3 days after ethanol injections comprised the 'post' condition. The dotted line indicates periods of no DD testing and ethanol injections only.

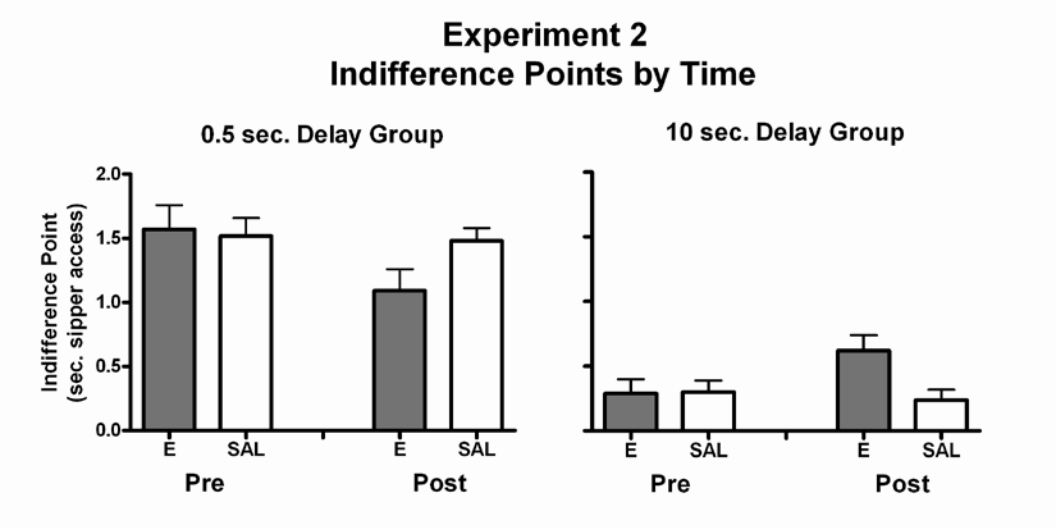


Figure 13. Indifference points are shown by Delay (.5 second on left and 10 second on right) before (pre) and after (post) 3.5 g/kg ethanol (E) or saline (SAL) injection for 8 days. E group n = 21, and SAL group n = 21. All animals were assigned to their respective delay before ethanol or water exposure. Note that decreased indifference points from Pre to Post indicate increased impulsivity, and vice versa.

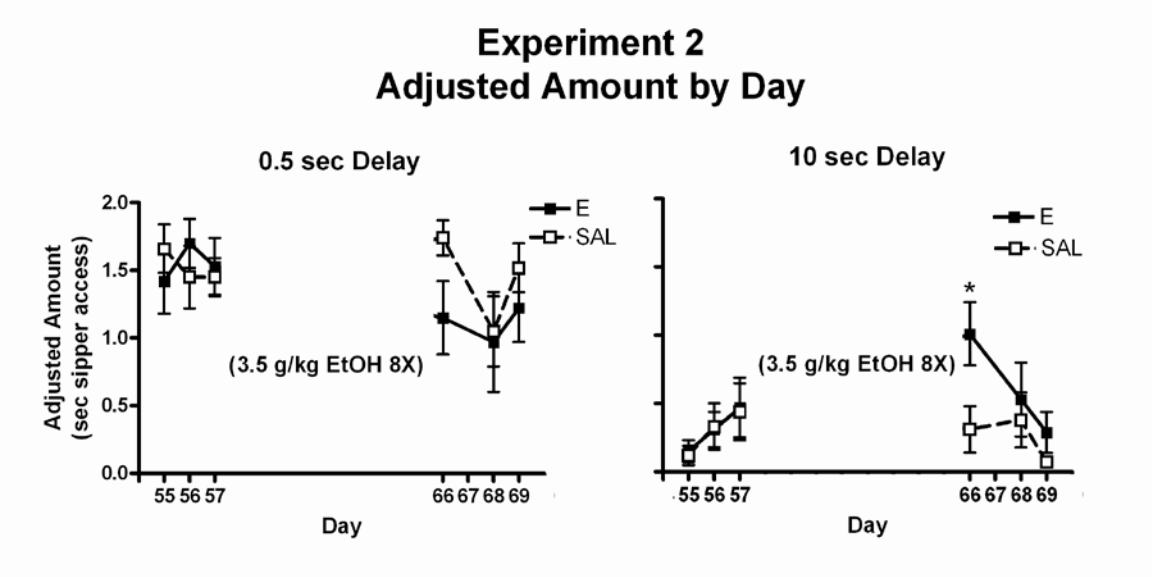


Figure 14. Indifference points are shown by Day and Delay (.5 second on left and 10 second on right) before (pre) and after (post) 3.5 g/kg ethanol (E) or saline (SAL) injection for 8 days. E group n = 21, and SAL group n = 21. Note that decreased adjusted amounts indicate increased impulsivity, and vice versa. A significant increase in adjusted amount was observed in the 10 second group on the first day after ethanol treatment ($p < 0.05$).

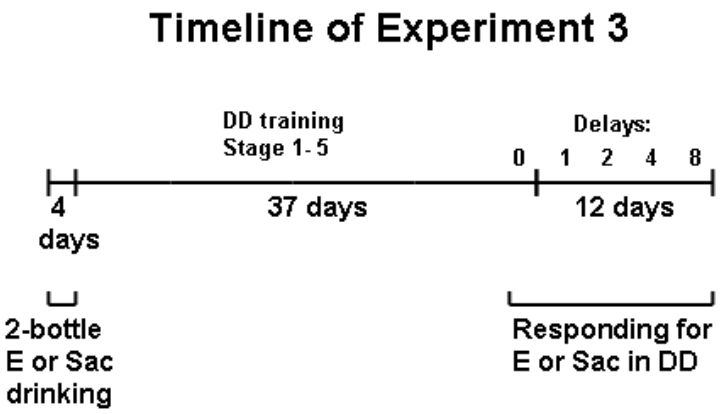


Figure 15. In Experiment 3, HAP2 mice were first given 24-hr 2-bottle access to water and either E (ethanol) or Sac (saccharin). They were then shaped in Delay Discounting (DD) through 5 Stages. Delays were then introduced, and mice were tested at each delay for 3 sessions using their respective reinforcers: 8% ethanol (v/v) or 0.01% saccharin (w/v). The number of days that the mice spent in each portion of the experiment is indicated.

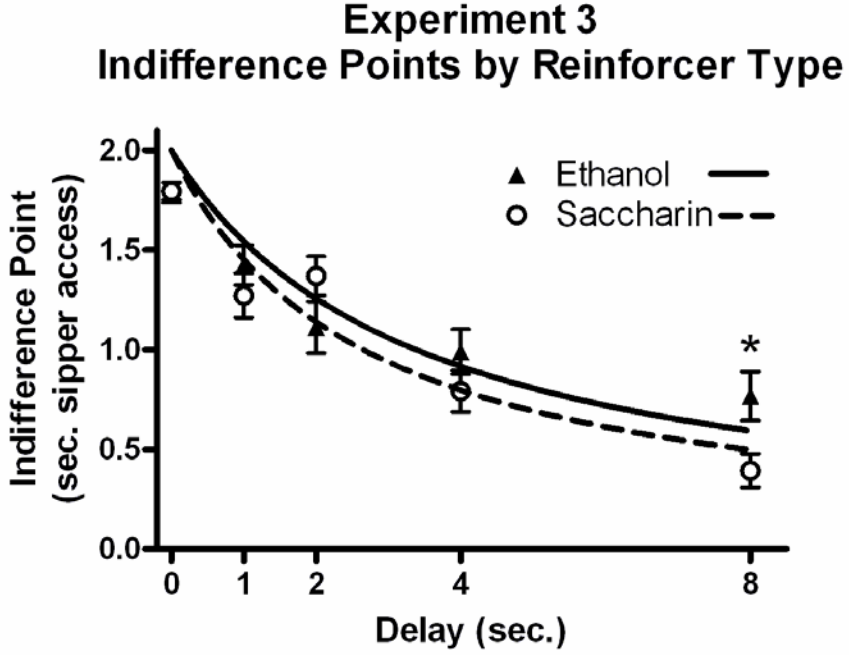


Figure 16. Indifference points were derived from delays of 0, 1, 2, 4, and 8 seconds. Hyperbolic regression was used to derive best-fit lines. Mice discounted delayed rewards of either 8% ethanol (v/v) or 0.01% saccharin (w/v). E group n = 20, and Sac group n = 20. Both rewards were discounted as a function of delay length, but ethanol was discounted less at the longest delay (* $p < 0.05$).

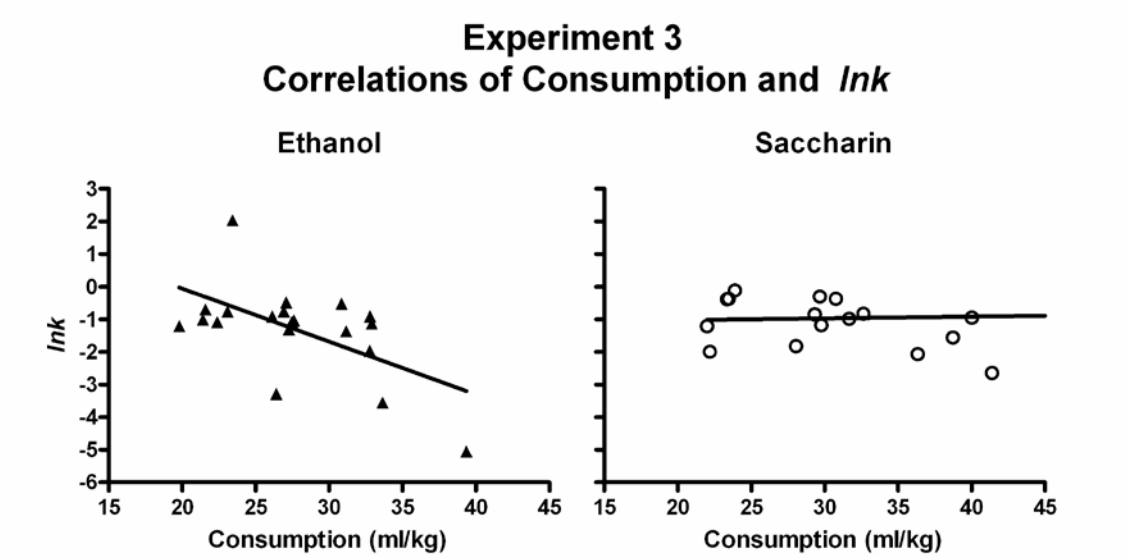


Figure 17. Correlations between ethanol consumed (left) during DD, saccharin consumed (right), and *lnk* values. Note that smaller values of *lnk* represent reduced impulsivity. A strong correlation was observed in mice responding for ethanol ($r = -0.583$), but not in mice responding for saccharin. Ns = 20 for both groups.

APPENDIX

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High-Alcohol Preferring Mice Are More Impulsive Than Low-Alcohol Preferring Mice as Measured in the Delay Discounting Task

B. G. Oberlin and N. J. Grahame

Background: Repeated studies have shown that high impulsivity, when defined as the tendency to choose small immediate rewards over larger delayed rewards, is more prevalent in drug addicts and alcoholics when compared with nonaddicts. Assessing whether impulsivity precedes and potentially causes addiction disorders is difficult in humans because they all share a history of drug use. In this study, we address this question by testing alcohol-naïve mice from lines showing heritable differences in alcohol intake.

Methods: Replicated selected lines of outbred high-alcohol preferring (HAP) mice were compared to a low-alcohol preferring (LAP) line as well as the low-drinking progenitor line (HS/Ibg) on an adjusting amount delay discounting (DD) task. The DD task employs 2 levers to present subjects with a choice between a small, immediate and a large, delayed saccharin reward. By adjusting the quantity of the immediate reward up and down based on choice behavior, the task allows an estimate of how the subjective value of the delayed reinforcer decreases as delays increase. Latency to respond was also measured for each trial.

Results: Both HAP2 and HAP1 lines of mice were more impulsive than the LAP2 and HS/Ibg lines, respectively. Hyperbolic curve-fitting confirmed steeper discounting in the high-alcohol drinking lines. In addition, the high-alcohol drinking lines demonstrated greater within-session increases in reaction times relative to the low-alcohol drinking lines. No other differences (consumption of saccharin, total trials completed) consistently mapped onto genetic differences in alcohol drinking.

Conclusions: Alcohol-naïve outbred mice selected for high-alcohol drinking were more impulsive with saccharin reinforcers than low-alcohol drinkers. These data are consistent with results seen using inbred strain descendents of high-alcohol drinking and low-alcohol drinking rat lines, and suggest that impulsivity is a heritable difference that precedes alcoholism.

Key Words: Alcohol Drinking, Alcoholism, Behavioral Economics, Endophenotype, Behavioral Genetics.

EXCESSIVE ALCOHOL DRINKING is the third leading preventable cause of death, and caused 75,766 deaths in 2001, and shortened those lives by an average of 30 years (CDC, 2001). Impulsivity is correlated with alcoholism (Petry, 2001), abuse of cocaine (Bornovalova et al., 2005; Coffey et al., 2003), heroin (Kirby et al., 1999; Madden et al., 1997), methamphetamine (Hoffman et al., 2006), and nicotine (Mitchell, 1999). Additionally, severity of addiction to drugs is positively correlated to the degree of impulsivity (Dom et al., 2006; Heyman and Gibb, 2006). While impulsivity appears to be linked to addiction, the relative contributions of innate and induced impulsivity to addiction is controversial and of great interest to researchers (Petry, 2006).

Impulsivity may be viewed as the behavioral output of neurological processes that likely contributes to addictive disorders, and as such might be thought of as a candidate endophenotype. Endophenotypes are measurable, heritable biological states that are hypothesized to underlie and precede the development of a disorder, and are presumably closely related to the particular alleles that cause a disorder (Gottesman and Gould, 2003). Endophenotypes are valuable analytical tools, as they potentially allow greater power to detect genes and/or systems of interest than the clinical phenotype itself (Dick et al., 2006), as well as increasing understanding of the etiology of complex disorders such as alcoholism and other addictions.

To avoid the confounding variable of drug history found in most human studies, we may turn to studies of drug-naïve subjects to assess whether impulsivity could be considered a valid endophenotype rather than a result of a history of drug use. Sons of alcoholic fathers were

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observed to be more impulsive than controls in the premorbid stage in one longitudinal study (Knop, 1985). In another 4-year longitudinal study, impulsivity was predictive of later alcohol use by teens (Ernst et al., 2006). In nondrug using female subjects, paternal drug use history predicted higher impulsivity (Petry et al., 2002). In rats, impulsivity in drug-naïve animals was predictive of later cocaine self-administration (Perry et al., 2005) and, in another rat study, later alcohol drinking (Poulos et al., 1995).

Impulsivity, along with heightened reward, forms a crucial component of addiction. Although much attention has been devoted to drug reward and reward-related brain circuits, drug reward alone may not be sufficient to establish drug addiction. An emerging hypothesis is that addiction results from the interaction of reward mechanisms and impaired inhibition (Finn, 2002; Goldstein and Volkow, 2002; Olmstead, 2006; Perry and Carroll, 2008; de Wit and Richards, 2004). The inhibition addressed in this study is of maladaptive choice behavior that results in a net loss to the organism.

One way to define impulsivity is the tendency to value smaller immediate rewards over larger delayed rewards (Ainslie, 1975; Rachlin and Green, 1972). According to this theoretical framework, impulsivity is an inability to defer reward, even when waiting consistently results in greater success, or reward density. This tendency can be measured empirically using the delay discounting (DD) task. The DD task is widely used in both human and animal studies, and the task is remarkably similar between species, lending good face validity to assessments of impulsivity in experimental animal models. Impulsivity in the DD task is defined as the extent to which time degrades the subjective rewarding value of the delayed reinforcer. The steepness of this relationship between time and subjective value is usually referred to in the literature as k. Larger values of k equal greater impulsivity, that is, greater discounting of delayed rewards. The parameter k is derived from the formula:

$$V = \frac{A}{(1 + kD)}$$

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 the fitted parameter (Mazur, 1987). The DD task is uniquely well suited to measuring impulsive choice, or cognitive impulsivity, which can be differentiated from prepotent response inhibition, or motor impulsivity (Mitchell et al., 2005; Winstanley et al., 2004). The DD task is a robust measurement tool, as discounting curves remain stable for months, and have high test-retest reliability (Ohmura et al., 2006).

An ideal animal model of alcoholism would be one that demonstrated high volitional ethanol consumption in the presence of an alternative fluid. Bidirectional selection of 2 replicated lines of HAP and LAP mice both began with heterogeneous HS/Ibg mice; these are low ethanol drinkers which consume about 3.5 g/kg/day (Behm et al., 2003; Grahame et al., 1999a). The HAP mice do not have to be food or water deprived to drink ethanol, they will drink unsweetened ethanol, and they achieve pharmacologically relevant blood alcohol concentrations following free-choice access to ethanol and water (Grahame and Grose, 2003; Grahame et al., 1999a). Both HAP1 and HAP2 mice prefer ethanol to water. Parents of the HAP mice used in the current study (generations 34 and 23 for lines 1 and 2, respectively) have an 84% preference ratio when drinking a 10%(v/v) ethanol solution, as compared to a 6% preference ratio observed in generation 23 LAP2 and a 15% preference ratio in the HS/Ibg mice.

The selection criterion for the HAPs was high consumption of 10% ethanol solution during 4 weeks of free-choice access, while the criterion for LAPs was low consumption of the same solution during the same period (Grahame et al., 1999b). Over generations, alleles that increase ethanol preference become concentrated in the population of HAPs and become excluded from the population of LAPs. Generally speaking, bidirectional selection results in divergence on the trait of interest as well as divergence on correlated traits (Crabbe et al., 1990; Falconer and Mackay, 1996). Traits correlated with ethanol preference include motor impulsivity (Wilhelm et al., 2007), anxiety-like behaviors (Izidio and Ramos, 2007), sweet preference (Dess et al., 1998; Piasecki et al., 1998), and conditioned taste aversion (Chester et al., 2003), among others (Green and Grahame, 2008). These correlated traits may reveal endophenotypes. If impulsivity is an endophenotype for addiction/alcoholism, then selection for high and low ethanol preference should result in parallel differences in impulsivity.

Greater impulsive choice was not detected using the DD task in mice selected for alcohol drinking in one study, although those authors did find higher motor impulsivity in the high drinkers (Wilhelm et al., 2007). As the selection was performed over only 4 generations, this study may have lacked a large enough response to selection to detect a correlated trait such as impulsivity. Another study showed that 2 strains of inbred high-alcohol drinking (iHAD) rats had steeper discounting curves than 2 strains of inbred low-alcohol drinking (iLAD) rats (Wilhelm and Mitchell, 2008), suggesting a genetic relationship between drinking and impulsivity. However, because an inbred strain represents a single genetic individual that is homozygous at all loci, extrapolating findings from even 2 pairs of strains to a wider population may not be as straightforward as more outbred lines.

Given that HAP mice freely drink relatively large amounts of alcohol, and alcoholism in humans correlates with impulsivity, our first hypothesis is that ethanol-naïve HAP2 mice will be more impulsive than LAP2 mice on the DD task using saccharin rewards. Our second hypothesis is that HAP1 mice will be more impulsive than the relatively abstinent HS/Ibg mice, which would allow replication of the line 2 result, showing that line differences in impulsivity are not likely to be a

chance result of differences arising from inbreeding and genetic drift. If these hypotheses were supported, it would provide additional evidence for impulsivity as an endophenotype in alcoholism.

MATERIALS AND METHODS

All work was performed in accordance with, and approved by, the Indiana University School of Medicine IACUC. Mice were offspring of HAP1, HAP2, LAP2, and HS/Ibg breeders, and were all born in the Indianapolis Veterans Administration Animal Care Facility. HS/Ibg breeders were supplied from the founding colony in Boulder, CO, and HS/Ibg mice used in this study were no more than 3 generations removed from those original breeders. Mice were all individually housed in polycarbonate cages (27.9 × 9.5 × 12.7 cm) with Cellsorb bedding, at an ambient temperature of 21 ± 1°C and lights on from 22:00 to 10:00 hours. Mice had ad lib access to food, and water access restricted to 2 hours per day, immediately after testing. Mice were transported in a light shielded transporter to the testing room; the mice were tested between 11:00 and 16:00 hours, and were always tested in the dark.

Experiment 1 and experiment 2 were conducted in the same way using the same shaping protocol, equipment, software, and handling procedures. The only difference between them was the type of mice tested. Experiment 1 was conducted with HAP2 and LAP2 mice, while experiment 2 was conducted with HAP1 and HS/Ibg mice.

Apparatus

The operant apparatus consisted of 12 identical boxes that measured 21.6 × 19.7 × 12.7 cm inside, with 2 sides constructed of clear acrylic and 2 sides of aluminum (MedAssociates, St Albans, VT). The operant box was contained in a sound and light attenuated chamber that was equipped with a fan for ventilation and background noise. An LED/nose-poke infrared detector was centered on the 19.7 cm side at 6.3 cm above the floor, and illumination of that LED signaled the beginning of a trial. Below the LED/nose-poke detector was the sipper access hole, through which the sipper descended when mice were being rewarded. The sipper tube was a 10-ml graduated plastic serological pipette fitted with a stainless steel tip (Ancare, Belmont, NY.) The sipper tube could be extended into the box for varying amounts of time, which allowed precise control of sipper access. Consumption volumes (±0.1 ml) were visually read from the tube. The sipper tube was filled with a solution of 0.0316% (w/v) saccharin solution. Levers were mounted 2.5 cm above the floor on either side of the sipper tube opening. Each lever had an LED 2.3 cm above it signaling that the lever was active (that is, reinforcement was available on that lever). Control of the operant boxes and collection of data was performed via the MedPC IV software (MedAssociates, St Albans, VT) and MedPC interface cards on a PC compatible computer. Data were sorted in Microsoft Excel (Redmond, WA) and statistical analyses were performed using SPSS 15.0 (Chicago, IL).

Animals

Two experiments were conducted. Experiment 1 used 2 cohorts each of HAP2 and LAP2 mice, and Experiment 2 used 1 cohort each of HAP1 and HS/Ibg mice. In both experiments, both lines of mice were tested concurrently. In Experiment 1, the first cohort of mice were 12 HAP2 and 12 LAP2, (generation 23) 6 male and 6 female of each line, and were an average of 110 ± 25 days old at the beginning of shaping. The second cohort were 12 HAP2 and 12 LAP2 (generation 24) 6 male and 6 female of each line, and were an average of 137 ± 3 days old at the beginning of shaping. Experiment 2 used 1 cohort of 24 HAP1 (generation 34) and 24 HS/Ibg (generation 76), 12 males and 12 females of each line, which were 70 ± 4 days old at the beginning of the experiment.

Behavioral Assessment

The time course of each trial is illustrated in Fig. 1. This procedure was a slightly modified version of a previously reported DD task called an adjusting amount procedure and has been previously described (Richards et al., 1997). Briefly, the center nosepoke cue light is illuminated until the mouse

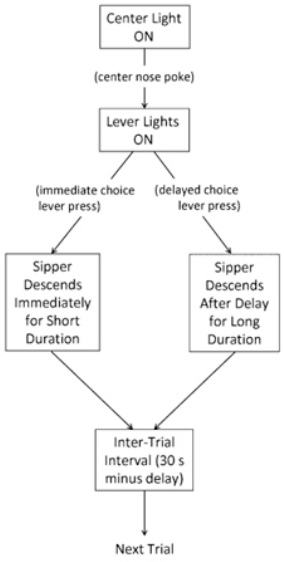

Fig. 1. Response requirements in a trial. The text in parentheses show the operant responses required to proceed to the next step. The sipper tube contains 0.0316% (w/v) saccharin solution.

initiates the trial by nosepoking. After the nosepoke, the center light goes dark, and the lights illuminate above both the left and right lever, signaling availability of a choice. Both lights remain illuminated until a lever press is recorded. Once a lever press is made, the nonchosen lever light extinguishes, and the sipper containing saccharin solution descends into the drinking position. Saccharin solution was used to promote higher responding. One lever was always assigned to "delay" and the other was assigned to "immediate." A lever press on the delay side resulted in delivery of the standard, 2-second reward after the programmed delay interval, during which time the light above the delay lever remained illuminated. An immediate-side lever press resulted in delivery of the adjusting reward without any programmed delay. The immediate (adjusting reward) side was set to 1 second access time (half the standard reward) at the beginning of the session. The access time on the immediate side adjusted depending on the subject's choice in the last trial according to the following rules: an immediate choice resulted in adjustment down of sipper access time by 0.2 seconds, and a delay choice resulted in adjustment up of sipper access time by 0.2 seconds. The adjustment increment, 0.2 seconds, was 20% of the initial adjusting side amount. As the value of the immediate reinforcer adjusts up and down throughout the session, it should have titrated to a value subjectively equivalent to the 2-second standard reward by the end of the session. Therefore, the adjusted amount of the immediate side gives an estimate of the subjective valuation of the delayed reward by the end of the session. The adjusted amount of the immediate side was limited to 2 seconds so that it never became larger than the amount on the delayed side, as experience with very large immediate rewards might interfere with mice being able to reliably assess reward magnitude. This was a departure from the Richards and colleagues (1997) paper, which also included a side bias term in the numerator. As we capped the adjusted amount at 2 seconds (the size of the delayed reward), we were not sensitive to biases toward the delayed lever, so we excluded the bias term. Therefore, the range of adjustment was always between 0 and 2 seconds sipper access time. To expose the mice regularly to both immediate and delayed reinforcers, a forced trial on the opposite lever was instituted after 2 consecutive selections of the same lever. In a forced trial, only 1 lever was active, and only that light came on after a nosepoke. In addition, there was no adjustment of the immediate reinforcer resulting from forced trials.

Mice were shaped according to the protocol outlined in Table 1. After the mice met criterion lever pressing in stage 4 (20 trials completed), side bias was assessed by averaging the last 3 days' choices on each side. The large reinforcer was then assigned to their non preferred side, to counter any initial side bias. After shaping was completed, mice were assessed at the zero delay, which is not actually a delay but rather an assessment of magnitude discrimination. The criterion for continued inclusion of individual mice in this study was a mean adjusted amount of 1.5 seconds or greater (75% or more of the 2 seconds access on the delayed side) in 3 of 4 consecutive days. We used this inclusion criterion because any assessment of DD relies on magnitude discrimination. If individuals could not display a preference for the larger reward, then discounting of that assessment based upon time would be unreliable. Importantly, the adjusted amount derived at the zero delay was not the result of innate side bias, as the 75% adjusted amount requirement meant that the mice preferred the large reinforcer lever over the other lever, which they had initially preferred in the beginning of shaping. Within 5 days of reaching the zero delay criterion, delays of 1, 2, 4, and 8 seconds were introduced in ascending order. Mice were tested for 3 consecutive sessions at each delay, and each session had a single fixed delay for the duration of that session. Dependent variables such as total trials, percent forced trials, consumption, and choice sipper access were means taken from the means of those 3 sessions (or 2, if they did not complete at least 20 trials during a session.) Indifference points for each mouse were determined by taking the median adjusted amount for the last 20 choice trials completed, and then averaging those values across 3 sessions within a programmed delay. Sessions were limited to 1 hour or 60 choice trials, whichever came first. All daily data from mice that completed fewer than 20 trials on a day were excluded. If a mouse did not complete ≥20 trials on 2 of 3

Table 1. Shaping Protocol

Stage	Description	Criterion to advancement
1	All center nose pokes reinforced on a FR1 schedule with 20 seconds; sipper access. No levers	Stage 1 is run for only 1 session
2	All center nose pokes reinforced on a FR1 schedule with 10-second sipper access. No levers	Completion of 20 trials
3	All trials are cued. The center light is illuminated for 20 seconds, with 10-second ITI. No levers	Completion of 20 trials
4	Nosepoke and then lever press required for 10-second sipper access. Levers are reinforced equally, and the ITI is 10 seconds. Levers added	Completion of 20 trials in 60 minutes
5	Levers present. Delay is zero, and ITI is 30 seconds. Adjustment and forced trials are introduced. The first 3 days that the mouse meets criterion becomes the "zero delay" score	Completion of 20 choice trials in 60 minutes, AND mean adjusted amount of 75% of the standard side in 3 of 4 sessions.

FR1, fixed ratio 1; ITI, intertrial interval.

sessions at a delay, that mouse was excluded from the experiment for nonperformance.

Statistics

Parametric statistics were performed, and an α-value of 0.05 was set as the significance threshold, unless corrected by the Bonferroni method. Our main hypothesis was that selection for differences in alcohol preference changed temporal discounting. Because each experiment was performed with a high-alcohol drinking line and a low-alcohol drinking line, we assessed the effect of Alcohol Preference in each study in an overall Experiment × Sex × Alcohol Preference ANOVA, but collapsed across Experiment and Sex in the absence of significant interactions between alcohol preference and these other 2 independent variables. The *n*s for experiment 1 were HAP2 10(m), 6(f); LAP2 10(m), 7(f), and for experiment 2 they were HAP1 8(m), 10(f); HS/Ibg 10(m), 7(f). In the presence of significant interactions, data were stratified by factor and analyzed separately for simple main effects and/or group differences. Huyn-Feldt corrections were used where appropriate.

Analyses of response latencies used medians, because medians are less sensitive than means to the positive skew inherent in reaction time (RT) data sets. Analyses of median response latencies used trials pooled from all sessions, and latencies greater than 30 seconds were excluded as omissions. In the presence of simple main effects of trial block within Line, *t*-tests back to the first bin were performed to assess differences in response times.

RESULTS

Two mice were excluded from experiment 1 for nonperformance and 11 mice were eliminated due to equipment failure. Additionally, 2 mice died from unknown causes. In experiment 2, 13 mice were excluded for nonperformance. Not including the equipment problem, 16% of the total mice were excluded from the experiments for nonperformance. Six sessions were discarded from experiment 1, and 2 sessions were discarded from experiment 2 for noncompletion of 20 trials, amounting to 1.2 and 0.4% of the total sessions, respectively. There were no line differences in side preference during shaping in any experiment (ps > 0.39). For the critical last 20 trials data, the 95% confidence interval for mean delay preference (number of delayed choices divided by delayed plus immediate choices) was 45.7 to 51.6%, indicating a lack of preference for either lever. Therefore, adjusted amounts recorded on the immediate lever should accurately reflect indifference points.

The dependent variables that were most relevant to choice impulsivity were indifference points and k values. A mixed Experiment (1 or 2) × Sex × Alcohol Preference (High or Low) × Delay (0, 1, 2, 4, or 8) ANOVA was performed on indifference points. Main effects of Delay $F(4.0,240.0) = 178.68$, $p < 0.001$ and Alcohol Preference were detected $F(1,60) = 14.04$, $p < 0.001$, but no main effects of Experiment ($p = 0.20$), Sex ($p = 0.58$), or interactions with Alcohol Preference, Experiment, or Sex were observed (ps > 0.17). Mean indifference points and discounting curves are presented in Fig. 2; for clarity, we have included data from each of the 4 lines plotted separately, as well as the data collapsed across Alcohol Preference (bottom panel). These data indicated a consistent effect of a genetic difference in alcohol preference on indifference points in the DD task, consistent with impulsivity's role in high alcohol consumption.

The free parameter k is the value that generates the best fit with the hyperbolic discounting function for each individual. In the present study, k described much of the variance in discounting (mean ± SEM R^2 values = 0.73 ± 0.024). The k

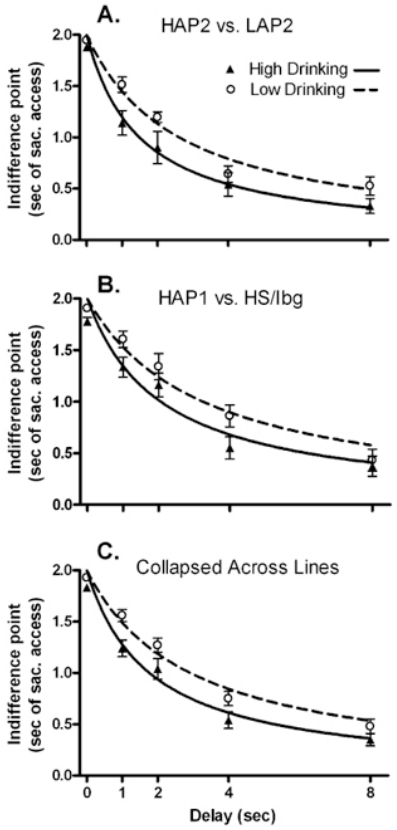

Fig. 2. Group mean indifference points ± SEM as a function of delay (0, 1, 2, 4, and 8 seconds). The y-axis is in units of "seconds of access to saccharin solution." Hyperbolic discounting curves were fitted using calculated k values derived from nonlinear regression. (**A**) Data derived from experiment 1: HAP2 ($n = 16$), LAP2 ($n = 17$). (**B**) Data derived from experiment 2: HAP1 ($n = 18$), HS/Ibg ($n = 17$). (**C**) Data collapsed across experiment and shown by Alcohol Preference.

values were skewed right (skew = 5.28), so they were normalized with a natural-log transform to allow parametric testing (skew = 0.28). An Experiment × Sex × Alcohol Preference univariate ANOVA on natural-log transformed k values revealed a main effect of Alcohol Preference $F(1,60) = 12.14$, $p = 0.001$, but no main effects of Sex ($p = 0.55$), Experiment ($p = 0.09$), or interactions with these factors ($ps > 0.39$). A follow-up t-test between the high and low drinking phenotypes revealed a difference by Alcohol Preference $t(66) = 3.41$, $p = 0.001$. Mean $ln\ k$ values were −0.520 and −1.091, which correspond to k values of 0.594 and 0.336 for high and low-alcohol preferring, respectively. Larger values of k represent steeper discounting, i.e., greater impulsivity.

Trials completed is a measure of operant behavior that may reflect any motivational differences among groups, but is unlikely to be related to our central measures of impulsivity. To assess whether there were group differences in this dependent variable, we conducted a mixed Experiment (1 or 2) × Sex × Alcohol Preference (High or Low) × Delay (0, 1, 2, 4, or 8) ANOVA on total trials. Main effects of Alcohol Preference $F(1,60) = 9.00$, $p = 0.004$ and Experiment $F(1,60) = 5.47$, $p = 0.023$ were detected, but Sex was not ($p = 0.09$). There were no interactions with Sex ($ps > 0.21$) so data were collapsed across Sex. Experiment interacted with Delay $F(4.0,240.0) = 5.71$, $p < 0.001$, so the 2 experiments were analyzed separately. In experiment 1, there was no main effect of Delay ($p = 0.06$) or Line ($p = 0.12$), nor was there an interaction of Delay and Line ($p = 0.62$). In experiment 2, main effects of Delay $F(3.4,113.7) = 5.40$, $p = 0.001$, Line $F(1,33) = 6.96$, $p = 0.013$ and an interaction of Delay × Line $F(3.4,113.7) = 3.09$, $p = 0.024$ were detected. Data were stratified by Line, and a main effect of Delay was detected in HAP1 $F(4,68) = 4.49$, $p = 0.003$ and HS/Ibg $F(4,64) = 4.02$, $p = 0.006$ such that HAP1s generally completed fewer trials at intermediate delays, and HS/Ibgs completed more trials at longer delays. Additionally, follow-up t-tests confirmed that HAP1s and HS/Ibgs differed at 0 and 1-second delays $ts(33) > 3.05$, $ps < 0.005$ using a Bonferroni corrected α of 0.01. These data are summarized in Table 2. The general pattern showed more trials completed by high-alcohol drinkers, but this effect was marginal, and did not reveal a consistent relationship between genetic differences in alcohol preference. An additional analysis was performed to assess whether there was any correlation between trials completed and k values, our central measure of impulsivity. We ascertained this correlation by determining the mean number of trials completed by each mouse from both experiments, and correlating it with that mouse's lnk value. Across all 68 mice included in the study, this correlation (Pearson's) was $r = 0.219$, $p = 0.073$.

Forced trials can influence the relationship between actual consumption and indifference points, especially in the case of low indifference points and high trial number. Percentage of forced trials may be a measure of behavioral rigidity or perseveration. Although we did observe some line differences in the proportion of trials that were forced, this was seen only at the 0-second delay, in which low preference mice had more forced trials (see Table 2). To assess whether there were group differences in this dependent variable, we conducted a mixed Experiment (1 or 2) × Sex × Alcohol Preference (High or Low) × Delay (0, 1, 2, 4, or 8) ANOVA on average percentage of forced trials. A main effect of Delay was detected $F(4,240) = 20.52$, $p < 0.001$, but Sex ($p = 0.07$), Experiment ($p = 0.27$), and Alcohol Preference ($p = 0.56$) were not. An interaction of Delay × Alcohol Preference was also

Table 2. Summary of Trials, Consumption, and Sipper Access

		Delay				
	Line	0 seconds	1 second	2 seconds	4 seconds	8 seconds
Total trials	HAP2	56.1 ± 2.51	56.6 ± 2.30	56.4 ± 2.59	52.6 ± 2.46	52.8 ± 1.94
	LAP2	49.4 ± 2.57	50.4 ± 2.52	50.4 ± 2.89	49.3 ± 3.22	48.5 ± 2.69
	HAP1*	52.9 ± 2.50[a]	52.3 ± 2.65[a]	46.3 ± 2.51	48.8 ± 2.51	50.1 ± 2.99
	HS/Ibg*	41.5 ± 1.96[a]	41.9 ± 2.10[a]	39.7 ± 2.15	42.2 ± 1.93	46.4 ± 1.79
Percent forced trials	HAP2[b]	27.4 ± 0.65[b]	26.3 ± 0.40	25.2 ± 0.34	25.3 ± 0.55	25.3 ± 0.50
	LAP2[b]	28.8 ± 0.41[b]	26.2 ± 0.39	24.4 ± 0.66	25.6 ± 0.52	25.0 ± 0.60
	HAP1[b]	26.8 ± 0.52[b]	25.5 ± 0.38	25.7 ± 0.34	26.2 ± 0.43	25.8 ± 0.30
	HS/Ibg[b]	28.6 ± 0.48[b]	26.4 ± 0.54	25.8 ± 0.54	24.4 ± 0.68	25.3 ± 0.36
Consumption (ml)	HAP2*	1.57 ± 0.10[a]	1.39 ± 0.10	1.29 ± 0.12	0.91 ± 0.08	0.90 ± 0.06
	LAP2*	1.13 ± 0.08[a]	1.13 ± 0.08	1.07 ± 0.07	1.00 ± 0.08	0.77 ± 0.06
	HAP1	0.97 ± 0.07	0.91 ± 0.07	0.79 ± 0.07	0.78 ± 0.06	0.77 ± 0.07
	HS/Ibg	0.88 ± 0.08	0.92 ± 0.09	0.81 ± 0.09	0.81 ± 0.08	0.71 ± 0.07
Choice sipper time /trial (s/trial)	HAP2[c]	1.36 ± 0.02	1.16 ± 0.03	1.05 ± 0.05	0.87 ± 0.05	0.74 ± 0.04
	LAP2[c]	1.36 ± 0.02	1.27 ± 0.02	1.18 ± 0.03	0.97 ± 0.03	0.81 ± 0.04
	HAP1[c]	1.34 ± 0.02	1.24 ± 0.03	1.16 ± 0.04	0.87 ± 0.06	0.73 ± 0.05
	HS/Ibg[c]	1.36 ± 0.01	1.30 ± 0.03	1.22 ± 0.05	1.02 ± 0.05	0.82 ± 0.04

Ns: HAP2 10(m), 6(f); LAP2 10(m), 7(f); HAP1 8(m), 10(f); HS/Ibg 10(m), 7(f). Data are shown as mean ± SEM by Line and Delay. In the presence of a Delay × Line interaction within Experiment, a simple main effect of Delay was detected within Line (*$p < 0.05$). Line differed within Delay ([a,b]$p < 0.01$, Bonferroni corrected).

[b]Percent forced trials showed no interactions with Experiment, so data were analyzed by Alcohol Preference rather than Line.

[c]Main effects of Delay and Alcohol Preference were detected.

detected $F(4,240) = 3.61$, $p = 0.007$, but interactions with Sex ($ps > 0.17$), and Experiment ($ps > 0.27$) were not. Therefore, data were collapsed across Sex and Experiment and analyzed by Delay and Alcohol Preference. A main effect of Delay was present $F(4,264) = 21.61$, $p < 0.001$, as well as a Delay × Alcohol Preference interaction $F(4,264) = 4.30$, $p = 0.002$, but the main effect of Alcohol Preference ($p = 0.72$) was not. Therefore, we assessed the effect of Alcohol Preference at each by Delay using *t*-tests, again Bonferroni-correcting for multiple comparisons to $p = 0.01$. The 0 delay differed by Alcohol Preference $t(66) = 3.16$, $p = 0.002$, but the other delays did not ($ps > 0.14$). These data indicate that the percentage of forced trials was higher in the low-alcohol drinkers at the 0 delay, but that they were generally similar at other delays.

If the DD task is set up correctly, there should be a decrease in reward received with lower adjusted amounts, because a more impulsive strategy is self-defeating (Ainslie, 1975). Actual consumption of reward declined as a function of Delay $F(3.4,202.9) = 31.17$, $p < 0.001$, and showed interactions of Delay × Alcohol Preference $F(3.4,202.9) = 5.09$, $p = 0.001$, Delay × Experiment $F(3.4,202.9) = 7.38$, $p < 0.001$, but not interactions with Sex ($ps > 0.12$). There was a main effect of Experiment $F(1,60) = 15.49$, $p < 0.001$, but not of Sex ($p = 0.25$), Alcohol Preference ($p = 0.12$), or interactions with Alcohol Preference ($ps > 0.13$). Therefore, data were collapsed across Sex, and to follow up on the interactions with Alcohol Preference and Experiment, experiments were analyzed separately by Line. Experiment 1 showed main effects of Delay $F(2.6,82.2) = 24.55$, $p < 0.001$ and Line $F(1,31) = 4.23$, $p = 0.048$ as well as a Delay × Line interaction $F(2.6,82.2) = 5.19$, $p = 0.004$. To follow up on this interaction, we assessed whether there were line differences at each delay, again using Bonferroni-corrected *t*-tests. The 0 delay was found to differ $t(33) = 3.38$, $p = 0.002$, but the lines did not differ at the other delays ($ps > 0.046$). In experiment 2 there was a main effect of Delay, $F(3.5,114.1) = 9.95$, $p < 0.001$, but no main effect or interaction with Line ($ps > 0.343$). In experiment 2, Delay was less potent in decreasing consumption than in experiment 1. These data are shown in Table 2. The pattern of results suggests that at longer delays, impatience impairs the ability of the mice to correctly choose the delayed reward and that at least between HAP2 and LAP2 mice, the steeper discounting curve observed did result in a loss of ability to obtain the reinforcer.

Given that the high-alcohol preferring lines generally completed more trials and also received more forced trials on the delayed side, actual consumption did not track perfectly with the main effect of Alcohol Preference on indifference points. Perhaps a better index of optimal behavior is the amount of sipper access time that mice earned on choice (nonforced) trials. To cancel the effect of differential total trials between Alcohol Preference groups, the choice sipper access time was divided by total trials completed to yield a measure of behavioral efficiency: that is, ability to gain access to the sipper tube.

A mixed Experiment (1 or 2) × Sex × Alcohol Preference (High or Low) × Delay (0, 1, 2, 4, or 8) ANOVA was performed on choice sipper access time per trial. Main effects of Delay $F(3.9,231.9) = 185.81$, $p < 0.001$ and Alcohol Preference $F(1,60) = 14.63$, $p < 0.001$ were detected, but there were no interactions ($ps > 0.16$). This measure of behavioral efficiency corresponded well with indifference points, and is illustrated in Table 2. Again, this dependent variable suggests the costs, in terms of a loss of reinforcer access time, of the high preferring lines' more impulsive strategy. As differences in drinking efficiency could cloud interpretations by allowing faster mice to get more reward in less sipper access time, an ANOVA was conducted to assess this. Mean drinking efficiencies were 0.0149 ± 0.0006, 0.0134 ± 0.0006, 0.0112 ± 0.0006, and 0.0121 ± 0.0009 ml/s sipper access ± SEM in HAP2, LAP2, HAP1, and HS/Ibg lines, respectively. No effect of Line was detected ($p = 0.25$).

To examine possible differences in RT during the 1-hour operant sessions, average median response latencies were calculated in 5-trial blocks up to 40 trials. We selected 40 trials as the upper limit, as there were some subjects that did not complete more than 40 trials and would therefore have been excluded from the analysis. A mixed Experiment (1 or 2) × Sex × Alcohol Preference (High or Low) × Block (1 to 8) ANOVA was performed on these data. Main effects of Experiment $F(1,60) = 33.73$, $p < 0.001$, Alcohol Preference $F(1,60) = 8.59$, $p = 0.005$, and Block $F(4.8,288) = 40.30$, $p < 0.001$ were detected, but Sex was not ($p = 0.62$). No interactions with Sex were found ($ps > 0.16$), but interactions of Block × Experiment $F(4.8,288) = 2.36$, $p = 0.043$ and Block × Alcohol Preference $F(4.8,288) = 7.33$, $p < 0.001$ were detected. In experiment 1, a main effect of Block and an interaction of Block × Line $F(4.0,124.8) > 3.97$, $ps < 0.005$ were detected, therefore HAP2 and LAP2 were stratified. Simple main effects were detected in both HAP2 $F(3.1,46.4) = 19.71$, $p < 0.001$ and LAP2 $F(4.5,72.3) = 8.63$, $p < 0.001$ lines, so paired *t*-tests were performed by Block back to the first block within Line. To control for multiple comparisons, we used a Bonferroni-corrected α of 0.00714. In HAP2s, the RTs were slower in the seventh and eighth blocks than the first block $ts(15) > 4.93$, $ps < 0.001$. In LAP2s, RTs improved in the second block, but were then unchanged through the last block. Large increases in RTs were only seen in the HAP2s. In experiment 2, a main effect of Block and an interaction of Block × Line $F(4.5,148.7) > 4.39$, $ps < 0.002$ were detected, therefore HAP1 and HS/Ibg lines were stratified. Simple main effects were detected in both HAP1 $F(3.0,51.3) = 20.70$, $p < 0.001$ and HS/Ibg $F(5.4,87.0) = 3.43$, $p = 0.006$ lines, so paired *t*-tests were performed by Block back to the first block, again Bonferroni correcting for multiple comparisons. In HAP1s, the fourth through the seventh blocks differed from the first $ts(17) > 3.43$, $ps < 0.004$. In HS/Ibgs, the fifth, sixth, and seventh blocks differed from the first $ts(16) > 3.07$, $ps < 0.007$. Generally, there was an increase in RT later in the session in both lines, but this

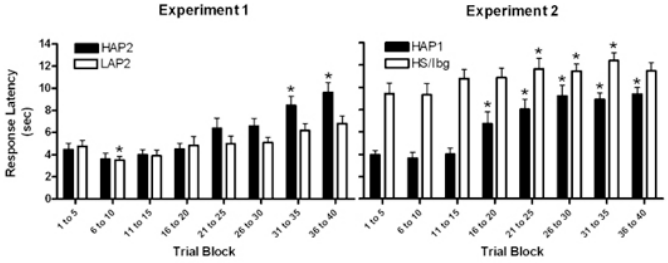

Fig. 3. Average median reaction times (RTs) in 5-trial blocks were analyzed with paired *t*-tests to the first block. HAP2 RTs increased more throughout the session than the LAP2s' (Experiment 1, left panel). HAP1 RTs increased sooner in the session, and by a larger percentage than HS/Ibgs' (Experiment 2, right panel). *$p < 0.0071$, which is the Bonferroni-corrected α value.

happened earlier and to a larger degree in the HAP1 versus the HS/Ibg. These data are illustrated in Fig. 3.

DISCUSSION

These studies demonstrated that genetic differences in alcohol drinking among 4 independent, outbred mouse lines was positively correlated with differences in impulsivity. In contrast, while we occasionally observed differences among the lines in measures of general motivation, such as amount of reinforcer consumed, this difference was not consistently related to genetic differences in alcohol preference and was uncorrelated with our central measure of impulsivity.

Although the use of sipper tube access for reinforcement rather than discrete delivery introduces drinking efficiency as a possible confound, we did not observe differing efficiencies between the lines, meaning that this was an unlikely source of line differences in discounting behavior. However, the HAP mice did generally consume more reinforcer than the LAP mice in spite of lower adjusted amounts. The most reasonable explanation for this result is the larger number of total trials, as well as forced trials completed by the high-alcohol drinkers as compared to the low drinkers. When we assessed reinforcer access time only on nonforced trials, we again observed no line differences.

The exclusion of 16% of the mice raises the possibility that the current findings do not generalize to the whole population. This issue was carefully weighed against the problem of magnitude discrimination, i.e., mice that cannot discriminate between large and small cannot produce useful data in a discounting task. We decided to use only the 84% of the population that could reliably learn to discriminate the large reinforcer from the small one.

Results found here were somewhat complicated by the fact that we did not have access to 2 complete sets of selected lines maintained in Indianapolis for equivalent numbers of generations; on the other hand, we were able to observe the behavior of a nonselected control line, the HS/Ibg. With respect to DD, the line difference was slightly larger when the bidirectionally selected lines were compared to each other as opposed to the high drinking line versus the progenitor line. This may suggest that the largest response to selection was in the direction of high drinking leading to high impulsivity, rather than low drinking leading to low impulsivity. Overall, however, HAP2 differed from LAP2 with a similar magnitude as HAP1 differed from HS/Ibg. Typically, using a nonselected progenitor stock instead of another LAP line would tend to diminish our ability to detect correlated responses; the fact that we were nonetheless able to observe the DD and RT differences suggests that they are fairly robust.

Another study indicated that selection for ethanol drinking had no effect on impulsive choice in mice. Although procedurally that study was very similar to the present experiment, that study used a murine population quite different from the present sets of selected lines (Wilhelm et al., 2007). Specifically, they used the fourth generation of their selected mice (STDRHi2 and STDRLo2) for DD testing. They observed an effect of selection for alcohol drinking (high drinkers: 10.5 ± 0.67 g/kg/d), but compared with the current generations of HAP mice (Line 1: 20.8 ± 0.55 g/kg/d and Line 2: 17.2 ± 0.62 g/kg/d) intake of the high drinking parental line was relatively low. Another possibility is that both their response to selection and their ability to detect genetic correlations may be limited by low genetic diversity compared to the HS/Ibg, as the progenitor population in the Wilhelm et al. study was an F2 derived from 2 inbred strains (C57Bl/6J and DBA2/J), compared to the 8 inbred strain cross that was used to derive the HS/Ibg. Overall, any genetic correlation (or lack thereof) is specific to the population in which it is observed, although greater genetic diversity may allow a greater ability to generalize from a particular population to the species in general (Falconer and Mackay, 1996).

A different study performed by the same group found that ethanol-naïve iHAD rats were more impulsive in a DD task than iLAD rats (Wilhelm and Mitchell, 2008). Together with the current study, these data suggest that impulsive choice is heritable and could be an endophenotype for high alcohol consumption. The genetic risk factor for alcoholism in humans may be accounted for, at least in part, by an enhanced tendency to choose impulsively. Whether or not

higher impulsivity causes higher alcohol drinking is unknown, but these data indicate that higher impulsivity precedes alcohol drinking and may be associated with it.

Other studies have suggested a link between the genetic influence on various forms of impulsivity and drug abuse. Trait impulsivity, i.e., high premature responding, was associated with low D2/3 receptor availability and later cocaine self-administration (Dalley et al., 2007). In another study, rats selectively bred for high saccharin intake, a correlated trait to alcoholism, were more impulsive in a DD task than low saccharin drinking rats (Perry et al., 2007). Alcohol-preferring Lewis rats, which show conditioned place preference to cocaine (Kosten et al., 1994) and reduced conditioned taste aversion to alcohol (Roma et al., 2006) also showed differences in autoshaping that suggest higher impulsivity (Kearns et al., 2006).

Our measure of behavioral efficiency, choice sipper access time per trial, showed effects of both delay and alcohol preference. Greater impulsive choice, both with longer delays and in high-drinking lines, resulted in losses in sipper access time. This relationship demonstrates that the inability to wait for delayed rewards has detrimental consequences in this task. In the case of human impulsive choice, this behavior may manifest itself as the choice to drink when this will result in later loss of other sources of reinforcement, such as drinking on the job, choosing immediate intoxication over recreational activities, or choosing drug use over human relationships.

We found that the high drinking lines showed a greater increase in RT during the session than the low drinking lines. The results of experiment 2 are more difficult to interpret due to long baseline RTs in the progenitor HS/Ibg line. While it is typical to see increases in RT later in a session, larger relative increases in RT are interpreted by some researchers as impaired sustained attention (Pattyn et al., 2008; Shimizu et al., 2008; Whyte et al., 1995). If these data were regarded as deficits in sustained attention, it would be consistent with studies performed in humans that have shown a correlation between drug abuse and impaired attention (Aharonovich et al., 2003; Cairney et al., 2007; Lane et al., 2007; Mackin et al., 2005). Error rates rather than RT are the most common method of evaluating sustained attention, which is impossible to assess in this version of the DD paradigm, as there is no "incorrect" response. Other reasonable explanations for line differences in RT increases would be fatigue, boredom, working memory, or differences in motivation and/or satiation. Without additional measures of attentional capacity, it is impossible to conclude that these observed differences are due specifically to differences in attention.

The present data show that replicate lines of outbred selected high-alcohol drinking mouse lines are also more impulsive than low drinking lines. Evaluating the neural substrates that modulate choice behavior between small/immediate versus large/delayed may be important to understanding many different neuropsychiatric disorders (Boyle et al., 1992; Dervaux et al., 2001; Noordsy and Green, 2003), and especially the addiction disorders. Impulsive choice may be a more useful construct with which to study the inhibitory aspect of addiction than self-administration, as inhibitory processes are assessed independently of drug reward and in a drug-free state. The current data are consistent with viewing impulsivity as a trait predictive of alcoholism.

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CURRICULUM VITAE
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Education:

2004-2010	Ph.D. Medical Neuroscience, Indiana University
1999-2003	B.S. Biology, with Honors, University of Oregon, Eugene, OR

Professional Experience:

2005-2010	Graduate Research Assistant	Nicholas Grahame, Ph.D. IUPUI, Indianapolis, IN
2005 (Spring)	Graduate Research Assistant	William Truitt, Ph.D. IUPUI, Indianapolis, IN
2004 (Fall)	Graduate Research Assistant	Nicholas Grahame, Ph.D. IUPUI, Indianapolis, IN
2003-2004	Research Assistant	Chris Cunningham, Ph.D. OHSU, Portland, OR
2002-2003	Research Assistant	Richard Marrocco, Ph.D. U of Oregon, Eugene, OR
2002	Research Assistant	William Roberts, Ph.D. U of Oregon, Eugene, OR
2001	Research Assistant	Richard Marrocco, Ph.D. U of Oregon, Eugene, OR
1999	Technician	CH2M Hill, Bioassay Division; Corvallis, OR

Ad Hoc Reviewer – Translational Research

Teaching:

2009 (Fall)	Instructor	PSY-B 344	Learning
2001 (Spring)	Lab T.A.	BI 252	Molecular Genetics

Awards:

2006-2010	Predoctoral Fellow, Kirchstein National Research Service Award for individual predoctoral fellows (F31AA016430); *Assessing Impulsivity in High Alcohol Preferring Mice*. NIAAA
2006-2007	Larry Kays Award for research using delay discounting ($5000; IUPUI)
2006	Guzé Symposium Meeting Awardee (Washington University, St. Louis, MO)
2004-2005	Graduate Fellowship (Indiana University School of Medicine)
2002-2003	McNair Scholar Fellowship (University of Oregon)

Memberships:

2004 to current	Research Society for Alcoholism
2005 to current	American Psychological Association, Division 28

Presentations:

Selectively bred high-alcohol-preferring mice are more impulsive than low-alcohol-preferring mice in a delay discounting task. 20th Annual Tri-State Conference on Animal Learning and Behavior. Purdue University, West Lafayette, IN; April 27, 2007.

Discounting of delayed saccharin rewards by high alcohol preferring mice. 19th Annual Tri-State Conference on Animal Learning and Behavior. Indianapolis, IN; April 8, 2006.

Gene Structure and Expression of CtBP2/RIBEYE in Danio Rerio. 11th Annual National McNair Research Conference and Graduate Fair. Delavan, WI; Sept 2002.

Invited Addresses:

High Alcohol Drinking mice are more impulsive than Low Alcohol Drinking mice: Pharmacological Manipulations. Indianapolis Chapter, Society for Neuroscience, Indianapolis, IN; Nov 7, 2008.

High Alcohol Preferring mice are more impulsive than Low Alcohol Preferring mice: Genetic Differences. Indianapolis Chapter, Society for Neuroscience, Indianapolis, IN; Oct 25, 2007.

Publications:

Research Papers:

• **Oberlin BG**, Bristow RE, Heighton ME, Grahame NJ (2010) Pharmacologic dissociation between impulsivity and alcohol drinking in High Alcohol Preferring mice. Alcoholism: Clinical and Experimental Research (in press).

• Fidler TL, **Oberlin BG**, Struthers AM, Cunningham CL (2009) Schedule of passive ethanol exposure affects subsequent intragastric ethanol self-infusion. Alcoholism: Clinical and Experimental Research: Aug 10 (Epub ahead of print).

• **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.

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• **Oberlin BG**, Alford JL, Marrocco RT (2005) Normal attention orienting but abnormal stimulus alerting and conflict effect in combined subtype of ADHD. Behavioural Brain Research 165(1):1-11.

Submitted / In Progress:

• **Oberlin BG**, Henderson AN, Grahame NJ. Selective breeding for high and low alcohol preference in mice. (in progress). Manuscript will be submitted to Behavior Genetics.

Abstracts / Posters:

• **Oberlin BG**, Frye CJ, Grahame NJ (2009) Responding for ethanol reduces impulsivity in female High Alcohol Preferring mice in a delay discounting task. Alcoholism: Clinical and Experimental Research. 33, Suppl(6):157A.

• **Oberlin BG**, Bristow RE, Grahame NJ (2008) D-amphetamine reduces impulsivity in High Alcohol Preferring mice in a modified delay discounting task. Alcoholism: Clinical and Experimental Research. 32, Suppl(6):160A.

• **Oberlin BG**, Grahame NJ (2008) Selection for high alcohol preference in mice reliably causes greater impulsivity during a delay discounting task. Alcoholism: Clinical and Experimental Research. 32, Suppl(6):16A.

• **Oberlin BG**, Henderson AN, Grahame NJ (2007) High Alcohol Preferring mice are more impulsive than low alcohol preferring mice as measured by a delay discounting task. Alcoholism: Clinical and Experimental Research. 31, Suppl 1(6): 215A.

• **Oberlin BG**, Mitchell SH, Grahame NJ (2006) Discounting of delayed saccharin rewards by high alcohol preferring mice. Alcoholism: Clinical and Experimental Research. 30, Suppl(6): 84A.

• **Oberlin BG**, Grahame NJ (2005) Validation of selectively bred High Alcohol Preferring mice for discovery of potential pharmacotherapies. Alcoholism: Clinical and Experimental Research. Res 29, Suppl 1(5): 99A.

• **Oberlin BG**, Truitt WA, Dietrich A, Fitz SD, Minick P, Shekhar A. Involvement of Basolateral Amygdalar Interneurons in Top Down Cortical Modulation of Amygdalar Function. Indianapolis Chapter, Society for Neuroscience, Indianapolis, IN; May 2005.

Service:

NIAAA Trainee Workshop, Nov. 4-5, 2005, Selection Committee; Organizer

International Brain Bee (Indianapolis), Jan 23, 2010; Judge