

PURDUE UNIVERSITY
GRADUATE SCHOOL
Thesis/Dissertation Acceptance

This is to certify that the thesis/dissertation prepared

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Entitled
INCREASED DELAY DISCOUNTING TRACKS WITH LATER ETHANOL SEEKING BUT NOT
CONSUMPTION

For the degree of Master of Science

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09/23/2013

Date

INCREASED DELAY DISCOUNTING TRACKS WITH LATER ETHANOL
SEEKING BUT NOT CONSUMPTION

A Thesis

Submitted to the Faculty

of

Purdue University

by

Steven Wesley Beckwith

In Partial Fulfillment of the
Requirements for the Degree

of

Master of Science

December 2013

Purdue University

Indianapolis, Indiana

For my parents, Kristi, and my brothers.

ACKNOWLEDGEMENTS

Many thanks to my advisor, Dr. Czachowski, and my masters committee for the helpful comments and guidance. Also this work would not have been possible without the never ending support of my wife Kristi.

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ABSTRACT

Beckwith, Steven Wesley. M.S., Purdue University, December 2013. Increased Delay Discounting Tracks with Later Ethanol Seeking but not Consumption. Major Professor: Cristine Czachowski Ph.D.

Assessments of delay discounting in rodent lines bidirectionally selected for home cage intake and preference of alcohol have had mixed findings. The current study sought to examine if delay discounting related differentially to alcohol seeking versus and alcohol drinking, two processes underlying alcohol intake and preference. Three strains of rats were utilized to answer this question Long Evans (LE), high alcohol drinking rats (HAD2), and alcohol preferring P rats. All strains were compared in an adjusting amount delay discounting task. Operant self-administration of alcohol was then assessed in the sipper tube model, and finally home cage drinking was assessed in a 24 hour 2 bottle choice paradigm. In the delay discounting it was found that the P rats were steeper discounters than both the LE and HAD2. In the sipper tube model, P rats displayed higher levels of seeking than both the HAD2s and the LE, but both the P rats and the HAD2s had higher intakes than the LE. During 24 hour home cage access, the P rats and the HAD2s had higher intake and preference for alcohol than the LE, but were not different from each other. These results show that increased discounting of delayed rewards tracks with appetitive processes versus consummatory factors and home cage intake of alcohol. This builds on prior findings using selected line pairs by providing an explanation for discordant results, and supports the hypotheses that increased delay discounting is an intermediate phenotype that predisposes individuals to alcohol use disorders.

CHAPTER 1. INTRODUCTION

1.1 Cost of Alcoholism and Conceptual Overlap with Impulsivity

Alcohol abuse and dependence is a massive challenge facing modern society. According to the center for disease control's (CDC) 2011 Behavioral Risk Factor Surveillance System the median prevalence of individuals who had at least one drink in the past 30 days was 57%, 18.3% had engaged in binge drinking, and 6.6% were heavy drinkers. From 2001 to 2005 the CDC reported 98,334 alcohol-attributable deaths resulting in 2,651,431 potential years of life lost. Chronic causes, such as liver cirrhosis, hemorrhagic stroke, and esophageal cancer, were responsible for 54,603 of those deaths and 1,089,916 of the years of life lost. The annual economic impact of excessive drinking in the United States was estimated to be \$223.5 billion with 72.2% coming from lost productivity, 11% due to healthcare, 9.4% from costs associated with the criminal justice system, and 7.5% from other sources (Bouchery et al., 2011).

The DSM-IV-TR divides alcoholism based upon abuse and dependence. Both are defined in terms of specific criteria that have to be met. These include repeated use in physically hazardous situations, continued use despite social and interpersonal problems caused and/or exacerbated by use, repeated unsuccessful attempts to stop or decrease use, and continued use despite negative effects, both physical and psychological (DSM-IV-TR). These criteria show considerable congruency with various definitions of impulsivity.

For example, substance use in physically hazardous situations overlaps with the inability to stop a behavior that has negative consequences, a tendency to engage in risky behavior, and acting without forethought and/or consideration

of consequences. Repeated unsuccessful attempts to cut down can be conceptualized as an inability to stop a behavior that has negative consequences and difficulty persisting at an activity (abstaining being the activity in question). Continued use despite physical, social, psychological, and interpersonal problems caused by substance abuse aligns with a subtype of impulsivity called delay discounting. This is because it represents the selection of an immediate reward and delayed punishment over the promise of latter improved physical health, psychological well being, and interpersonal relationships.

1.2 Delay Discounting

Impulsivity, much like alcoholism, is heterogeneous. There is no one unified construct of impulsivity; rather impulsivity refers to a set of distinct concepts all relating to a general lack of behavioral regulation (Evenden 1999). Delay discounting constitutes a cognitive form of impulsivity. It is defined as a preference for immediate over later gains and rewards even if the immediate rewards are considerably smaller or less desirable. Using a child coming home from school as an example, they could study their spelling words as soon as they get home every night. This has the advantage of passing the spelling test on Friday, and not being grounded over the weekend. On the other hand, they could go play every night, be unable to pass the test as a result, and lose their weekend privileges. The former option would represent a non-impulsive decision by choosing the larger later rewards of passing the test and weekend privileges. The latter, corresponds to the impulsive decision. Self-control is not exercised and the child gets to play a little bit right away at the expense of a poor test grade and being grounded over the weekend.

This tendency to value delayed rewards less is normally adaptive. This is evidenced by its heritability (Wilhelm and Mitchell 2009; Anokhin et al., 2011; Isles et al., 2004; Helms et al., 2006), and its demonstration across species being

seen in rats (Richards et al., 1997), pigeons (Mazur 1987), and humans (Rachlin et al., 1991). Despite obvious differences in the delays that a human versus a rat can tolerate, the relationship between value and delay follows the same form across species. The value of a reward decreases in a negatively decelerating fashion with increases in delay best described by a hyperbolic equation.

Different equations have been proposed describe this relationship, but eventually the literature agreed upon a hyperbolic relationship instead of an exponential decline in value (Mazur 1987, Ainslie 1975, Rachlin et al., 1991). This is because in addition to being able to explain more variance, the function had to be concave enough to explain the preference reversal seen between two rewards (Ainslie 1975, Rachlin and Green 1972). Specifically, when both a sooner smaller reward and larger later reward are delayed, the preference is for the larger later reward. However as the time of delivery of the sooner smaller reward approaches, the preference switches to the sooner smaller reward. Going back to our spelling test example, before school the child resolves to study his spelling test that night as his weekend privileges hold more value than playing that night. However, when he or she steps off the school bus in the evening, there is a failure to follow through as the prospect of playing now outweighs going to the park on Saturday.

While there are several different methods, delay discounting is typically assessed by deriving indifference points at various delays. This is done by asking an individual if they would prefer a standard reward of fixed magnitude that is delivered after a delay or an alternative reward delivered immediately. Based on the individual's choice the magnitude of the alternative reward is adjusted and the question is asked again. If the standard reward delivered after a delay was chosen, then magnitude of the adjusting alternative reward increases, and conversely if alternative reward was chosen, its magnitude decreases. This process continues until the subject chooses indifferently between the two rewards. The magnitude of the alternative reward at this point of indifference

between the two rewards is inferred to be equal to the subjective value of the standard reward after its delay. Indifference points are then assessed at multiple delays. An individual's discount function is subsequently determined by fitting their indifference points to an equation utilizing non-linear regression. Below is the hyperbolic equation as presented by Mazur (1987) that has been generally agreed upon and is commonplace in the delay discounting literature.

$$V=(A)/(1+kD)$$

The actual magnitude of the standard reward is represented by A. On the other hand, V represents the subjective value of the reward after its delay (D). The free parameter in the equation is k. Consequently, it defines the steepness of the discount function. Larger values of k indicate steeper discounting as it would increase the size of the denominator which in turn decreases V, and smaller values of k represent shallower, less impulsive discounting.

1.3 Delay Discounting and Alcoholism

Based solely on how we define them, there is an overlap in how we view alcoholism and delay discounting. An individual chooses to drink which prevents future rewards and results in later problems be they legal, domestic, or in terms physical health. Furthermore, the preference reversal seen with delay discounting helps explain why alcoholics will often resolve to turn over a new leaf when there is no current opportunity to drink, but when faced with the choice to drink or not drink the following day, he or she fails to follow through. However, these conceptualizations are not without their drawbacks. As pointed out by Mitchell (2011) avoiding later problems and the receipt of rewards is not assured by abstaining, nor are the benefits. Also, there are more than temporal differences when resolving not to drink when no drink is present versus when a

drink is present. Examples of these factors include cues that can induce craving and one's ability to refrain from drinking regardless of what choices he or she may make.

Nevertheless, when one looks at the relationship between alcohol use disorders and increased discounting of delayed rewards, it is well replicated. In 1998 Vuchinich and Simpson were the first to examine this relationship. In two separate experiments, they found that heavy social drinkers were steeper, more impulsive discounters than light social drinkers, and problem drinkers also discounted delayed rewards faster than light social drinkers. Petry et al., (2001) expanded on this result by looking at current and abstinent alcoholics both of which showed increased delay discounting compared to controls. Mitchell et al., (2005) replicated these findings using abstinent alcoholics, and demonstrated this relationship to be independent of gender, age, socio-economic status, depressive tendencies, and years of education.

The relationship between delay discounting and alcoholism applies to more than discrete, nominal categories at opposite ends of the spectrum. In both treatment seeking and non-treatment seeking heavy drinking individuals delay discounting has a positive relationship with scores on the alcohol use disorders identification test (AUDIT; Claus et al., 2011). This is a cross culturally validated 10 item scale designed as a preliminary screening instrument for problematic levels of drinking behavior by a collaborative world health organization project (Saunders et al., 1993). Kollins (2003) demonstrated delay discounting to be related to a number of alcohol-related variables in a non-clinical sample. Specifically, increased discounting had a strong relationship with the number of times individuals passed out, a moderate relationship with lower age of first alcohol use, and other substance abuse related variables (total illicit drugs used, age of first smoke, and age of first marijuana use). Dom et al., (2006) took a different approach looking within alcoholic individuals and found early onset alcoholics, individuals with symptoms presenting by the age of 25, tended to

exhibit steeper discounting than late onset alcoholics even after controlling for other factors that could increase delay discounting such as poly-substance abuse.

1.4 Alcohol Use Disorders and Delay Discounting

In reference to both alcohol use disorders and other substance use disorders, there are three competing and non-mutually exclusive hypotheses about how they relate to impulsivity, including delay discounting (Perry and Carroll 2008, Mitchell 2011, Acheson et al., 2011).

First, heightened levels of delay discounting and other forms of impulsivity may cause or increase the risk of substance use disorders. Using delay discounting as an example, the decreased value of later rewards are outweighed by the immediately available pharmacologically rewarding effect of a drug. Moreover, it may be the case that heightened impulsivity causes problems in one's life separate from alcohol, such as problems at work or with personal relationships. The negative emotion that results coupled with expectancies of tension reduction leads to individuals drinking to cope (Cooper et al., 1995). It is also possible that heightened impulsivity leads to self-selection into peer groups, activities, or environments where substance use is prevalent without directly affecting the tendency to use drugs and alcohol (Littlefield and Sher 2010). Another possibility, specific to delay discounting, was suggested by Vuchinich and Simpson (1998). Simply by decreasing the value of other potential rewards and reinforcers in the environment, increased delay discounting decreases the number of alternative behaviors an individual is likely to engage in.

Conversely it may be that alcohol use disorders cause increases in impulsivity. This could be via direct pharmacological effect of the drug or via long term effects of a drug on brain structure and function. Again, this does not

exclude a mediated relationship. For example, the problems brought about by substance use and the stress that comes with them may increase impulsivity versus the drug itself.

The third hypotheses describes a spurious relationship, both impulsivity and alcoholism are associated via a third common factor. For example, early life stress could cause or at least contribute to alcohol dependence later in life (Enoch 2011; Young-Wolff et al., 2012; Dube et al., 2006). These same stressors early in life could increase impulsivity (Carver et al., 2011; Duckworth et al., 2013). There could also be a genetic relationship mediated by pleiotropy, such that a group of genes underlying a vulnerability to the pharmacological effects of a drug also result in increased impulsivity. Another possibility is that both are related to an increased sensitivity to reward and reinforcement in general (Yager and Robinson 2013).

1.5 Examination of Hypothesis 1

The first hypothesis, implicating delay discounting as a causal factor, is the most intriguing of the three. If true, it provides a possible avenue for intervention. Furthermore, impulsivity has been suggested as a possible endophenotype for alcohol use disorders (Eisenberg et al., 2007). Thus, identifying increased delay discounting as a causal factor would have implications in the search for the genes underlying alcoholism. The two ways to examine this possibility are to look at individuals who are at risk for developing alcoholism, and to use animal studies where the environment, genetics, and drug history can be controlled.

1.5.1 At-Risk Individuals for Alcoholism

One of the largest at risk populations is individuals who have a family history of alcoholism. This is because alcoholism is often passed on from one generation to the next. Some of this is likely due to genetics as alcoholism has a heritability of around .5 (True et al., 1999; Heath et al., 1997; Kendler et al., 1992; Prescott et al., 1999; McGlue et al., 2001), and the fact that even when adopted away from their parents in the first 6 weeks of life, children of alcoholics tend to have more alcohol related problems than their peers (Goodwin et al., 1973). However, the effect of environment should not be discounted. This is a point that Cloninger et al., (1981) stressed. When they studied the adopted away sons of Swedish alcoholics, they not only observed environmental influences but also gene by environment interactions. Thus an individual's family history positive status (FH+) could be mediated by the environment, genetics, or an interaction of the two. Either way, the thought is that by examining these individuals one is able to look at variables predisposing one to alcohol abuse.

The assessment of at risk populations prior to the development of problems to illuminate the etiology of alcoholism has a long history. In 1985 Joachim Knop published a longitudinal study assessing a cohort of Danish individuals first at birth (1959 to 1961) and again 20 years later (1980-1981). The cohort was dichotomized, identifying high risk individuals as the sons of alcoholic fathers. It was found, among other things, that the "high risk" group was rated more impulsive in both a psychopathology interview by a clinical psychologist and a school teacher questionnaire.

However, the narrowing of research to delay discounting and FH+ individuals is a more recent trend. It starts with Petry in 2002 who looked at this question with young adults (26 and 25.8 years of age in FH+ and FH- groups) classified as FH+ based upon paternal alcohol abuse. In order to examine premorbid characteristics that might lead to alcoholism, any individuals already presenting with an alcohol or substance use disorder were excluded. Petry

(2002) found that only FH+ females showed increased delay discounting. While this casts doubt on the idea that increased delay discounting may be a premorbid characteristic, the exclusion of individuals already presenting with substance use problems is a confounding factor. At the age that subjects were assessed it is highly likely that males with increased delay discounting will have already developed drinking problems. This explanation is supported by increased delay discounting being associated with the age of first use (Kollins 2003) and an early onset of alcoholism defined as before 25 years of age (Dom et al., 2006). The increased delay discounting in FH+ females is thus slightly anomalous considering the above factors until one considers that the prevalence of alcoholism is greater in males than females (Hasin et al., 2007; Kessler et al., 1994). Protective factors, be they psychological, societal, or biological, likely prevented a greater proportion of FH+ females from developing problems and thus being excluded from the study.

Given the complications in excluding adults with an alcohol use disorder, it becomes necessary to look at younger individuals before the typical age of onset in FH+ individuals. This is exactly the strategy that Herting et al., (2010) utilized. However, they found substance naïve family history positive youth only trended towards increased delay discounting. This may be a result of a small sample size (roughly 15 in each group), adolescence already being period of heightened impulsivity (Chambers et al., 2003) which could possibly cover up differences, and a sample high in socioeconomic status (SES). This last bit is problematic because SES is related to delay discounting (Anokhin et al., 2011; Herting et al., 2010). Consequently, the authors have to contend with range restriction as well.

Another factor is that if increased delay discounting is one heritable aspect of a family history positive status, it may not manifest until immediately before onset of drinking problems. This idea is substantiated by the fact that traits tend to become more heritable as an individual ages (Bergen et al., 2007; Haworth et al., 2010). Therefore, differences may not be detected in young sample.

Evidence of this type of developmentally limited effect comes from Smith and Boettiger (2012) who found that age moderates the effect of variation in catechol-O-methyltransferase (COMT) genotype on delay discounting.

Crean et al., (2002) looked at adults with a family history of alcoholism based upon having a father with type II alcoholism. They did not exclude individuals with a drinking problem, but did exclude individuals with illicit drug use. They found that FH+ and FH- individuals did not differ on delay discounting. However, increased delay discounting is not simply associated with alcohol use. It is common among addictive disorders from cocaine and (Washio et al., 2011) heroin addiction (Kirby and Petry 2004), to pathological gambling (Petry and Casarella 1999; Petry 2001). Moreover, Merikansas et al., (1998) showed that it is not simply alcoholism that aggregates in families; substance abuse regardless of the drug of choice does. Thus, excluding these individuals likely altered the results.

Bjork et al., (2004) compared recently detoxified alcoholics receiving inpatient treatment to control subjects and replicated previous results by showing the alcoholics to be steeper discounters. However, when they dichotomized the recently detoxified alcoholics based on the presence of a parent with heavy drinking, they found no differences between the two subgroups. The authors suggest range restriction as one possible reason for this null result as their group of alcoholics was “Universally characterized by extremely disrupted lives and poor decision making” (Bjork et al., 2004). Also, it may be the case that how steeply one discounts delayed rewards alters the likelihood than an individual requires or seeks inpatient treatment, further clouding their results.

Most recently, in 2011 Acheson et al., looked at young adults (mean ages 24 and 23) with and without a family history positive status for delay discounting. Overall they found that family history positive individuals did tend discount delayed rewards more steeply, and discounting increased alongside the number of affected family members. However this latter relationship was small ($r=.14$),

and when additional factors were added to the model such as demographic variables, depressive and anti-social tendencies, the number of affected family members failed to predict a significant amount of unique variance in delay discounting. Interestingly, the authors found that anti-social tendencies, as measured by the Sociability scale of CPI-So, mediated the relationship between the number of affected family members and delay discounting. This is not surprising as impulsive behavior is a core feature of anti-social personality disorder, and there is a high comorbidity between anti-social personality disorder and alcoholism (Hasin et al., 2007; Sargeant et al., 2012; Sher and Trull 2002). Furthermore individuals with a multi-generational family history of alcoholism tend to have a greater degree of psychopathology (Finn et al., 1990).

1.5.2 Animal Studies: Phenotypic Correlation

In animal studies, the first and most obvious means of investigation is to look at the phenotypic correlation between delay discounting and then assess subsequent drinking of ethanol. Poulos et al., (1995) used a T maze delayed reward task in which rats choose between 2 food pellets available immediately and 12 food pellets available after a 15 second delay. They then selected the 9 most impulsive rats (defined by the selection of the immediately available 2 pellets), 9 from the middle of distribution, and the 9 most conservative rats. Then their intake in a 20 minute limited access paradigm utilizing various concentrations of ethanol was measured. Across all concentrations, there was a stepwise effect of group with increasing levels of the selection of the small immediate reward tracking with greater intake in the limited access paradigm.

Mitchell et al., (2006) examined the relationships between delay discounting and subsequent locomotor sensitization to intraperitoneal injections of alcohol in outbred WSC mice. They found that steeper discounting was

positively related to increased locomotor sensitization, which has been associated with increased ethanol consumption (Lessov et al., 2001, Grahame et al., 2000).

1.5.3 Animal Studies: Selected Lines

The breeding of selected lines is a technique used to examine additive genetic variance underlying a phenotype (for review see: Crabbe et al., 1990; Crabbe 1989; Grahame 2000). To start, a population of animals is assessed for a particular phenotype. Then the experimenter applies an artificial selection pressure by breeding individuals with one end of the phenotype together and those from the opposite end of the phenotype together as well. This bidirectional selection results in two separate populations of animals, also known as lines. In successive generations, each line is under continual selection pressure for the same end of the phenotype they for which they were originally bred. In this manner more and more of the genes underlying a particular end of phenotype are recruited and concentrated inside each line. Alleles not related to the phenotype, trait irrelevant alleles, are left unaffected (theoretically). The end result is two separate lines of animals, each with the same genetic background, and with genes underlying opposite ends of a phenotype.

In addition to being a tool to examine the genes underlying the selection phenotype, this technique allows one to examine correlated responses to selection, also known as correlated traits. Correlated responses are based on the concept of pleiotropy, one gene underlying multiple different phenotypes. Selected lines permit one to examine this possibility by comparing a pair of lines on a phenotype other than the selection phenotype. If there is a difference between the lines it indicates that the genes that underlie the selection phenotype also underlie the second phenotype that was examined. However, replication is required by demonstrating the correlated response to also exist in a

different pair of lines selected for the same phenotype. This is because during the course of selection inbreeding occurs and trait irrelevant alleles are recruited as well. Often a second pair of lines is selected from the same progenitor strain (the initial population prior to any selection). This second set of lines is called a replicate line. Replicate lines are the primary tool outside of independent replication in a separate breeding experiment to rule out genetic drift and inbreeding as confounds (for review see: Crabbe et al., 1990; Crabbe 1989; Grahame 2000).

Given the well replicated relationship of increased delay discounting in individuals with an alcohol use disorder compared to those without, it is not out of line to investigate the possibility of a genetic link between the two, especially given alcoholism large degree of heritability (True et al., 1999; Kendler et al., 1992; Prescott et al., 1999). There have been multiple selection experiments in the alcohol field using home cage intake and preference of an ethanol-containing solution as a selection phenotype. This has allowed for the examination of a genetic relationship between delay discounting and alcohol use disorders.

Wilhelm et al., (2007) assessed delay discounting in the 4th generation of the STDRH12 and the STDRLO2 lines which were derived from the F2 offspring of a C57BL/6J and DBA/2J cross (Phillips et al., 2005). They found that the lines showed no difference in delay discounting. However there are several caveats to note. When the progenitor strain is the F2 of an inbred strain cross, there is limited genetic diversity for selection to act upon as there are only a maximum of 2 different alleles at any given loci. Consequently, there is very little room for selection to move a phenotype as well as any correlated responses to selection.

In 2008 Wilhelm and Mitchell followed this study up by examining inbred high alcohol drinking (iHAD) and inbred low alcohol drinking (iLAD) rats from both replicate lines. When they looked at the indifference points they found a line by delay interaction and a main effect of delay. However, in their follow up analysis, they found the lines had large differences at the zero delay with the iHADs

overvaluing the standard reward and the iLADs undervaluing it. There were also no differences at any other delays. Then they conducted a non-linear regression analysis on the indifference points, and included a side bias term (b) in the numerator of the hyperbolic equation proposed by Mazur (1987). The purpose of this second free parameter is to account for differences at the zero delay if a subject over or undervalues the standard reward. Theoretically this prevents differences at baseline from artificially influencing k values.

$$V = (bA)/(1+kD)$$

Unsurprisingly, there were large differences in b reflecting the large differences at the zero delay. In terms of k values, iHADs of both replicate lines were larger than those for the iLADs, indicating steeper discounting. The authors drew the conclusion that the iHADs were steeper discounters than the iLADs based on the line by delay interaction and the k values. However, there was a very strong correlation approaching the criterion for test-retest reliability ($r=.687$) between the k values and the b values. This correlation indicates that the degree of under and overvaluing the standard reward at the zero second delay was highly related to the degree of discounting. Given the strength of this correlation, one has to ask if k and b are redundant and including a side bias term failed to prevent differences at the zero-second delay from skewing the results. Thus the authors' conclusion may not be entirely warranted as side bias is likely driving the results despite the use of the side bias term. The generalizability of this study is also questionable due to its use of the iHADs and iLADs, even with using both replicate lines. This is because each inbred line essentially represents a genetic n of 1 as all individuals are genetically identical, giving the study an overall n of 4. Furthermore, the inbreeding process involves at least 20 generations of brother sister mating. This results in the fixation of a large number of trait irrelevant alleles. Consequently, drawing any conclusions from this study about populations of genes which may underlie both increased delay discounting and increased alcohol intake is tentative at best.

Wilhelm and Mitchell (2012) also examined 2 other selected lines for home cage intake of alcohol. The alko alcohol (AA) and alko non-alcohol rats (ANA) did not differ in delay discounting. They also examined the Sardinian alcohol preferring (sP) and non-preferring rats (sNP) and found no difference in delay discounting as well (Wilhelm and Mitchell 2012). In 2009 Oberlin and Grahame looked at both replicates of high alcohol preferring mice (HAP) and found them to be steeper discounters than their non-selected control line (HS/lbg) and the second replicate line of the low alcohol preferring mice (LAP2). This finding is particularly robust for several reasons. First the progenitor strain for the HAPs and LAPs was derived from an 8 strain cross creating a large degree of genetic diversity for selection to act upon. Second, the mice were not inbred allowing for a greater degree of generalizability, and finally the use of both replicate lines rules out genetic drift and the unintended fixation of trait irrelevant alleles as an alternative explanation.

Another piece of positive evidence for a genetic relationship comes from Perry et al., (2007). They examined a selected line pair bred for high (HiS) and low (LoS) saccharin solution intake under 24-hour access conditions. While the selection was not for alcohol per se, increased preference for sweet solutions has been demonstrated to be a correlated trait for home cage intake of ethanol (Oberlin et al., 2011; Salimov 1999), and the HiS drink more ethanol than the LoS in a 24 hour access 2 bottle choice procedure (Dess et al., 1998). They found that the high preferring line tended to discount delayed food rewards more steeply than the low preferring line.

1.5.4 Summary of Research on the First Hypothesis

The animal and human literatures examining the first hypothesis are parallel to each other. When comparing alcoholics to controls, alcoholics and heavy drinkers have been repeatedly shown to be steeper discounters

(Vuchininich and Simpson 1998; Mitchell et al., 2005; Petry et al., 2001; Bjork et al., 2004). Furthermore delay discounting predicts variance in other alcohol related phenotypes such as levels of problem drinking (Claus et al., 2011; Kollins 2003) and age of onset (Dom et al., 2006). Similarly in animal studies looking at phenotypic correlations, increased discounting of delayed rewards is indicative of greater intake of ethanol (Poulos 1995) and addiction vulnerability (Mitchell et al., 2006). These studies also extend beyond the human studies and provide evidence that delay discounting is a predisposing factor as animals were ethanol naïve at the assessment of delay discounting.

The studies looking at delay discounting in FH+ vs. FH- individuals on the other hand were less than robust. There is only one positive result demonstrating a modest relationship with increased discounting of delayed rewards correlating with the number of affected family members. This pattern is mirrored in the selected line studies. Perry et al., (2007) and Oberlin and Grahame's 2009 paper stand in contrast with Wilhelm and Mitchell's 2007 and 2012 papers. However, Perry et al.,'s (2007) support must be taken with a grain of salt because the genes underlying both delay discounting and increased saccharin intake may not overlap with genes underlying high alcohol intake. This to some degree leaves Oberlin and Grahame (2009) standing alone. Although Wilhelm and Mitchell's 2007 paper can be set aside on grounds of a lack of genetic diversity in the progenitor strain, this confound is not present in the 2012 paper. One probable explanation for the differences between these studies is from one selection to the next different genes can be recruited. This occurring is not uncommon, and is even likely to have happened inside the HAPs and LAPs. Crossing the HAP2s and HAP1s results in a population of mice (cHAPs) that out-drink both of the two parent strains (Oberlin et al., 2011).

As different genes were likely selected for in the AA/ANA, sP/sNP, and HAP/LAP lines, and not all of the lines show differences in delay discounting, one can safely infer that the genetic relationship is limited between delay discounting

and home cage intake of alcohol solutions. Wilhelm and Mitchell's 2007 finds are also in line with this conclusion. In a situation of low genetic diversity, where genes underlying both delay discounting and intake may not have been present, there was a response to selection for alcohol intake but not delay discounting. Given that home cage intake is a gross measure that is affected by many factors, this is not entirely surprising. One way to categorize the factors affecting home cage intake is to divide them into within-bout and between-bout factors. Within-bout factors represent variables that regulate how much an animal drinks inside a single bout of consumption. By contrast, between-bout factors regulate the time between bouts and the effort put forth to obtain alcohol. Consequently, a sound question to ask is if delay discounting relates differentially to within and between bout factors that regulate alcohol intake. If this is the case, it would provide a possible explanation to the discordant set of studies cited above.

1.6 Appetitive/Consummatory Division of Behavior

The conceptualization of home cage intake as being regulated by within and between bout factors is analogous to Wallace Craig's (1918) description of behavior as being comprised of different behavioral states and patterns. The appetitive state is the first. It is characterized by a desired stimulus, the appetitive stimulus, being absent. Craig describes an individual as being agitated and ready to act exhibiting restless, varied movements, and effort directed at obtaining the desired stimulus. Obtainment occurs via trial and error. Consequently, learning helps define future behavior. However, the closer one comes to the presentation of the appetitive stimulus, the more stereotyped and innate the behavior becomes. Just prior to the arrival of the stimulus, incipient consummatory action occurs. This is when an individual performs consummatory behaviors (behaviors involved in the consumption of a substance) without the stimulus being present. An example of this would be going through the motions of drinking before any

alcohol is presented. Once the desired stimulus is presented, consummatory action ensues. This behavior is innate, and continues until the animal becomes satiated. There is no more restlessness or search behavior, and once satiated there is a state of satisfaction. These consummatory actions constitute the end of a sequence of behaviors.

Applying this model to rodent home cage drinking of alcohol, increased home cage consumption could be driven by appetitive processes and a heightened drive to obtain the appetitive stimulus (i.e., between-bout factors). On the other hand increased intake could be driven by differences in consummatory behavior (i.e., within-bout factors). An example of this would be a higher limit for satiation, which would increase intake because more alcohol would be consumed in any one bout.

1.6.1 The Appetitive/Consummatory Sipper Tube Model

The sipper tube paradigm was designed with the idea in mind that drug seeking and self-administration are two related but distinct processes (Samson et al., 1998; Samson et al., 1999). It assesses these two processes independently by utilizing a procedural separation of responding for and drinking of alcohol. Once each day subjects perform a predetermined number of lever presses (the lever press response requirement) to gain access to a sipper tube containing an alcohol solution for 20 minutes. This provides a daily intake measure. However to assess the seeking response, there are two different adaptations. The first adaptation conceived was an across session progressive ratio procedure, also known as a between sessions breakpoint (Czachowski et al., 2003; Czachowski and Samson 2002; Czachowski and Samson 1999; Samson et al., 1998). Similar to a normal breakpoint procedure for a fixed ratio (FR) schedule, the cost to

receive the alcohol solution is increased after each presentation. However, there is only one “trial” per day. The largest response requirement completed defines the strength of the seeking response.

The separate assessment of seeking is critical because when measured with consumption both measurements are confounded. As an animal becomes slowly satiated by repeated presentations of alcohol (as in a traditional progressive ratio procedure), the salience of alcohol as a reinforcer changes. Thus it is less able to drive behavior (Samson and Czachowski 2003; Samson et al., 2000). Consequently, the breakpoint value achieved is no longer a true value of alcohol’s ability to drive behavior. Simply put, the animal could “break” because it is full and does not want anymore, or because once the subject has had a little, the now higher cost is no longer worth paying, even if the higher cost would have been acceptable initially.

On the same token, the separate measurement of drinking is also necessary for measuring intake regulation (Samson and Czachowski 2003; Samson et al., 2000). This is because the animal, as opposed to the experimenter, remains in control of when and how much it drinks. This is because the schedule of reinforcement is not able to disrupt consummatory behavior, and force the animal to take breaks from drinking. Considering rodents have an increased metabolism of alcohol compared to man, this is an especially important aspect (Samson et al., 2000). Also the increasing cost can put an artificial limit on how much it drinks as the cost may become too high prior to satiation.

However, the downside to the across session breakpoint procedure is the length of time (which is also unpredictable) it takes to assess appetitive strength, and the inability to assess acute manipulations, be they pharmacological or otherwise. The second adaptation was designed to address these limitations (Samson et al., 2003a; Samson et al., 2001). This modification constitutes the use of single non-reinforced extinction sessions where the ethanol solution is

never presented, and the number of responses the animal makes defines the strength of the seeking response. Czachowski and Samson (2002) showed that responding during these extinction trials is similar to breakpoint values in the same animals, and Samson et al., (2001) showed that if they are interspersed in between normal sessions they are stable. Furthermore, similar to the breakpoint measure, they do not correlate with intake (Samson et al., 2001; Czachowski and Samson 2002; Czachowski et al., 2003; Samson et al., 2003a; Samson and Czachowski 2003).

The convergent validity of the extinction sessions and the across session progressive ratio procedure combined with their divergent validity with actual intake of alcohol goes a long way to substantiate the idea that drug seeking and drug self-administration are separate processes. Pharmacological studies performed in the sipper tube model build on this by showing the physiological substrates to differ, at least in part. For example, a serotonin (5-HT) 1A agonist administered into the nucleus accumbens core had no effect on ethanol seeking but decreased intake, and conversely a 5-HT 1B agonist decreased seeking with no effect on intake (Czachowski 2005). Inactivation of the ventral tegmental area by bilateral microinjections of tetrodotoxin and glutamate AMPA/kainite receptor antagonists decreased ethanol seeking but not intake (Czachowski et al., 2012). Furthermore, self-administered preloads of ethanol decrease seeking but not consumption (Samson et al., 2003b).

1.7 Current Investigation

The aim of the current study was to determine how and if cognitive impulsivity, in the form of delay discounting, relates to ethanol seeking and drinking separately on a phenotypic level. To accomplish this goal, the alcohol preferring P rats, HAD2 rats, and Long Evans rats were compared in a delay discounting task and then subsequently in the sipper tube model and a home

cage two bottle choice procedure. These three lines were chosen because they collectively exhibit a unique combination of ethanol related behavioral phenotypes. Specifically the alcohol preferring P rats, which were bred for high home cage intake and preference of alcohol (Murphy et al., 2002), exhibit a high degree of ethanol seeking and drinking. The HAD2s, who were bred for the same phenotype but from a NIH background (Li et al., 1993), display high levels of ethanol drinking, but only moderate levels of seeking. The Long Evans, an outbred strain derived from a captured wild rat and “several albino laboratory females,” according to Harlan Inc., round out the combination by showing both a moderate degree of ethanol seeking and drinking behavior.

The primary evidence for these classifications comes from the sipper tube model. Czachowski and Samson (2002) looked at multiple across session breakpoint determinations in both the P's and HAD2 rats. They found that the P rats showed a high level of seeking with a mean breakpoint on the second determination of 412.5 responses (+/- 72.1). This was in stark contrast to the HAD2s who's second mean breakpoint of 87.1 (+/- 20.4) was similar to previously reported Long Evans rats with a second mean breakpoint determination of 105.6 (+/-26.3; Czachowski and Samson 1999). These differences in responding were in spite of HAD2s and Ps actually consuming similar amounts of ethanol (1.25+/- .16 versus 1.41 +/- .13) which exceeded that of the Long Evans (.99g/kg +/- .1). Furthermore in a forced exposure to ethanol for 3 days prior to lever press training, the HAD2 consumed more ethanol than the P rats who subsequently out-responded them (Czachowski and Samson 2002).

Additional support is provided by Ritz et al., (1994) who showed that P's will out respond HAD2s for a range of ethanol containing solutions. Also, Files et al., (1998) essentially looked at a demand curve for ethanol by training rats to an FR1 for dipper presentations of ethanol, and then increased the response requirement. They found that P's would defend their level of ethanol intake achieved on an FR1 all the way out to an FR16 before showing any decreases in

intake. In contrast, HAD2 rats would decrease their intake with the first increase in response requirement to an FR4. Ps and HADs were also compared in a recent paper by Bertholomey et al., (2013). In this paper both strains were submitted to an extinction curve, and late into the extinction curve the P rats displayed increased responding at several points relative to the HAD2s. Another unique appetitive phenotype that only the P rats have been shown to possess (Rodd et al., 2004) is a robust spontaneous recovery of operant reinforced responding after extinction followed by a home cage wait period termed Pavlovian Spontaneous Recovery.

The comparison of these three strains in a delay discounting task will allow one to determine if increased discounting of delayed rewards tracks with appetitive or consummatory processes. To be clear, this study will not assess genetics. While genes usually follow phenotypic correlations (Crabbe et al., 1990), the design of the study precludes any conclusion about genetics because all three of the lines come from a different genetic background. Thus one cannot determine if effects are due to selection or to genetic drift.

It was hypothesized that delay discounting would track with appetitive behavior, and that only the P rats would show increased delay discounting. Then in the sipper tube model, they would display higher levels of ethanol seeking than HAD2s and Long Evans, but they should only consume greater amounts of ethanol than the Long Evans. Finally, in the two bottle choice procedure there should be limited or no difference between the HAD2s and P rats. If there is a limited difference, the HAD2s should out drink the P rats based on Czachowski and Samson's (2002) finding that the HAD2s consumed more ethanol than the P rats under forced access conditions.

This was hypothesized for several reasons. First of all, delay discounting is based on the selection of rewards, which are appetitive stimuli. Thus an animal chooses which stimulus they want to obtain in a delay discounting task. As appetitive processes are defined by the obtainment of the appetitive stimulus,

delay discounting can be said to describe appetitive behavior. Furthermore, a recent paper by Broos et al., (2012) showed that increased delay discounting was positively associated with resistance to extinction of cocaine seeking and not overall levels of cocaine intake. While this association was seen with cocaine, it may hold for alcohol seeking as well because increased discounting is seen in relation to multiple different drugs of abuse and not simply alcohol.

CHAPTER 2. METHODS

2.1 Subjects

The experiments utilized 20 HAD2's from the 60th and 62nd generations, 6 alcohol preferring P rats from the 74th and 75th generations, and 14 Long Evans (LE) rats obtained from Harlan Sprague-Dawley, Indianapolis. All animals were aged matched at approximately 50 days at the start of the experiments as Doremus-Fitzwater et al., (2012) showed that adolescent rats exhibit steeper discounting, and Simon et al., (2010) has shown that older rats exhibit shallower discounting of delayed rewards. Consequently, the P rats weighed from 185g to 242g, the Long Evans were between 180g to 235g, and the HAD2's ranged from 107g to 207g at the start of delay discounting. Subjects were run in two separate cohorts. The first cohort was run at the Institute of psychiatric research located at 791 Union Drive, Indianapolis, Indiana, 46202. The second cohort was run at the Biomedical Research and Training Center located at 1345 West 16th Street, Indianapolis, Indiana 46202. In the first cohort there were 8 Long Evans and 8 HAD2s. In the second cohort there were 6 Long Evans, 6 P rats, and 12 HAD2s.

Throughout the experiment, animals had ad libitum access to food, were individually housed plastic shoebox cages, and maintained on a 12 hour light/dark cycle with lights on at 0700. Sessions were conducted 5 days a week except where noted. During the delay discounting, animals were water restricted receiving access to water 1 hour after each session and over the weekend. Water was available for 2 hours until stage 5 of delay discounting training, after which it was only available for 1 hour. For the sipper tube appetitive/consummatory paradigm, animals were water restricted for 1 to 2

days, in the same fashion as the delay discounting, to initiate lever pressing. All procedures were approved by the institutional animal care and use committee. A timeline of the experiment can be seen below in figure 2.1.

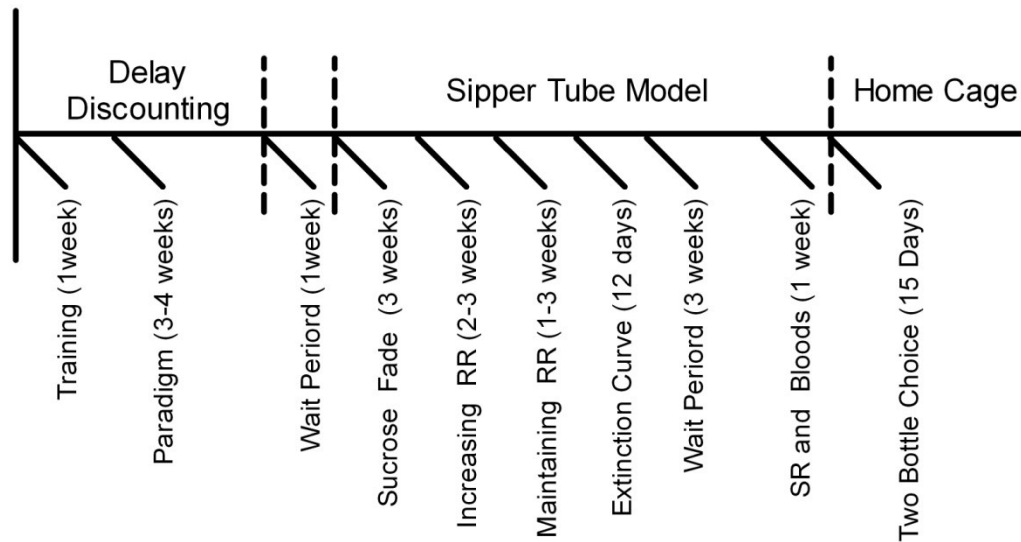


Figure 2.1: Timeline of experiment. RR=response requirement, SR=spontaneous recovery.

2.2 Apparatus

All daily sessions were conducted in modular chambers (Med-Associates; St. Albans, VT, USA, 30 x 30 x 24.5cm), and electrical inputs and outputs of each chamber were controlled using Med-Associates software (Med-Associates). All chambers had a stainless steel bar floor, a house light, and were enclosed in sound attenuating boxes with an exhaust fan for ventilation and the masking of external noise. Otherwise chambers were differentially equipped for the delay discounting and the sipper tube paradigm.

For the delay discounting, chambers were equipped with a nosepoke recess with an internal stimulus light and photocell to record beam breaks centered on the front wall (opposite the house light), and 2cm above the floor. A

retractable graduated cylinder tube with a rubber stopper, stainless steel spout with double ball bearings, and a lickometer was located 3cm above the nosepoke recess. Flanking the nosepoke recess on both sides were retractable levers each 6cm above the floor. The chambers were also equipped with stimulus lights 4cm above each lever.

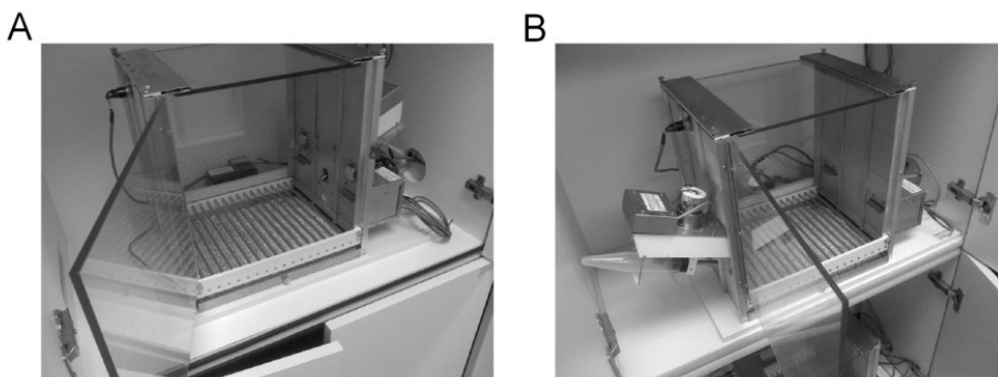


Figure 2.2: Pictures of operant chambers. A) Chamber set up for delay discounting. B) Chamber set up for sipper tube model.

In the sipper tube appetitive/consummatory paradigm, chambers were equipped with two retractable levers on the front wall (opposite the house light) each placed 6cm above the floor and 8cm from the center. A retractable graduated cylinder tube, equipped as described in the delay discounting, was placed 8cm from center, on the back wall opposite the active lever, and 6cm above the floor. Pictures of both chamber configurations can be seen in figure 2.2.

2.3 Delay Discounting

The delay discounting paradigm was an adjusting amount procedure based on Oberlin and Grahame (2009). Throughout the entire paradigm, 2% sucrose, prepared weight/volume (w/v) in tap water, was used as the reinforcer

delivered via the retractable graduated cylinder tube (sipper tube). Training was conducted in 5 stages with the fifth stage doubling as an experimental condition (0 second delay).

2.3.1 Delay Discounting: Training

Animals were handled for 2 to 4 days immediately before training began. Training consisted of 5 stages. These stages are summarized in Table 2-1. In the first stage subjects were placed into the chamber with the sipper tube already descended, levers retracted, house light on, and the nosepoke stimulus light off. After 200 “free licks” the sipper tube retracted, the nosepoke’s internal stimulus light turned on, and the subject then received 20 seconds of access to the sipper tube in response to a nosepoke (fixed ratio 1 nosepoke; FR1NP). While the sipper tube was extend the nosepoke’s internal stimulus light was turned off until the tube was retracted. The criterion for advancement to the next stage was receiving 200 free licks and the completion of 10 trials. Animals were removed from the chambers after the completion of 10 trials or if they appeared to lose interest.

The second stage was very similar to the first except that there were no free licks available, and each nosepoke only resulted in 10 seconds of access to the sipper tube. The criterion for advancement from the second stage was the successful completion of 40 trials in 60 minutes. In the third stage, the access to the sipper tube was further reduced to 5 seconds, and an inter-trial-interval (ITI) was introduced. The ITI was 10 seconds in length (beginning once the sipper tube was retracted) and during this time the nosepoke’s stimulus light was off and there were no programmed consequences for nosepoking. After the ITI was over, the nosepoke light illuminated signaling the availability of reward for nosepoking. The criterion for advancement from stage 3 was the completion of 40 trials in 60 minutes.

In stage 4, the contingency for reinforcement switched from nosepoking to a chained response. Specifically, subject had to nosepoke and then subsequently press either lever. The levers were extended at the start of the session, and remained extended for the duration of the session. At the start each trial, the nosepoke light was illuminated. Once the subject nosepoked, its internal stimulus light turned off, and the stimulus lights above both levers turned on signaling the availability of reinforcement for pressing either lever. After pressing a lever, the sipper tube descended into the chamber for 10 seconds, and the stimulus lights turned off. After the sipper tube retracted, a 10 second ITI was in place where all of the stimulus lights were off and there were no programmed consequences for making any response. The stage was completed over at least 3 sessions. During the first session, subjects were handshaped to press both levers after making the nosepoke response. On the session after the subject acquired the chained response, the access time to the sipper tube was decreased to 5 seconds, and then to 2 seconds on the next session. The criterion for advancement from stage 4 was the completion of 40 trials in 60 minutes after the access to the sipper tube had been decreased to 2 seconds. During the last 3 days of stage 4, lever preference for each subject was assessed. This was accomplished by averaging a choice ratio across the last three days of stage 4 training. In the event no clear preference was displayed ($\pm .1$ from .5), the total number of presses on each lever was used as a tie breaker.

Stage 5 of training, more aptly named magnitude discrimination, also constituted the first experimental condition. The same pattern of chained responding occurred as in stage 4. However a response on the non-preferred lever yielded a standard reward of 2 seconds of access to the sipper tube, and a response on the preferred lever resulted in the delivery of an adjusting alternative reward. The adjusting alternative reward started at one second of access to the sipper tube, but then changed pending the subject's choices. A response on the standard reward lever increased the alternative reward by 0.2 seconds on the next trial, and a response on the alternative reward lever decreased the

alternative reward by 0.2 seconds on the next trial. A variable ITI was also implemented. It took into account the amount of reward and any delays to the reward to ensure 30 seconds always elapsed between when the animal choose a reward and when the nosepoke's internal stimulus light illuminated for the next trial. While not important at this stage, the variable ITI prevents a subject from "working the system" and earning several smaller adjusting alternative rewards in the time it would take to earn 1 standard reward. Forced trials were introduced to ensure a subject would have experience with the changing reward amounts. After any two consecutive choices of the same reward, a subject was forced to choose the other reward on the subsequent trial. On these trials, after the subject nosepoked, only one stimulus light illuminated above the lever they were being forced to choose. Also, there were no programmed consequences for responding on the other lever. Finally all sessions were limited to 60 minutes or the completion of 60 free choice trials (whichever occurred first).

The criterion for advancement from this stage was twofold. First the animal had to perform at least 20 free choice trials. Furthermore it had to display the ability to discriminate between the two rewards based upon magnitude. In order to do so, subjects need to have an indifference point, median alternative reward in the last 20 trials, of 1.5 or greater. Both of these criteria had to be met in 3 of 4 consecutive sessions. The data from the 3 sessions in which this occurred was used as the measurement for the zero delay condition.

Table 2.1: Delay discounting training procedure. In short training starts with nosepoking. After nosepoking is established the levers are introduced. Once animals are responding reliably on the chained schedule, forced trials and the adjusting amount are introduced. Abbreviations: FR=Fixed Ratio, NP=Nosepoke, LP=Lever Press.

Stage	Procedure	Criterion for Advancement
1	200 "Free Licks" then FR1NP for 20 seconds of access	200 licks + 10 trials
2	FR1NP for 10 seconds of access	40 trials in 60 minutes
3	FR1NP for 5 seconds of access and 10 seconds ITI	40 trials in 60 minutes
4	Nosepoke cues levers and lights & 10 second ITI Day 1: Handshaped to lever press for 10 seconds access Day2: FR1LP for 5 seconds access Day 3: FR1LP for 2 seconds access	40 trials in 60 minutes while responding for 2 seconds of access
5	Full program: Adjusting amount, forced trials, variable length ITI	20 Free choice trials in 60 minutes and an indifference point of 1.5 or greater

2.3.2 Delay Discounting: Experimental phase

The experimental phase continued the same as stage five except a programmed delay was implemented before the delivery of the standard reward. During this delay, the stimulus light above the standard reward lever remained illuminated until the reward was delivered, and the stimulus light above the alternative reward lever was turned off. Delays of 0 (stage 5), 2, 4, 8, 12, and 16 seconds were assessed. Each delay was assessed for 3 sessions each, and delays were assessed in ascending order similar to a breakpoint procedure.

2.3.3 Delay Discounting: Statistical Analysis

Inclusion of results from a given session was dependent upon the successful completion of 20 free choice trials. A minimum of two sessions completed at each delay was required for a subject to be included. If subjects violated the contingencies of reinforcement by "stealing licks" during the ITI or the delay to the standard reward they were excluded. Data were averaged across

each delay and subjected to a 3 way mixed analysis of variance with delay as a within subjects factor and cohort and strain as between subjects factors. Significant between subjects main effects were examined using Fisher's LSD tests and significant interactions were followed up by student T tests with a Bonferroni corrected alpha. Subjects mean indifference points at each delay were used for least squares non-linear regression to determine the discount function as described by the following equation (Mazur 1987):

$$V=(A)/(1+kD)$$

In the above equation, V is equal to the subjective value of the standard reward after a given delay (represented by the mean indifference point). A is the actual magnitude of the standard reward. D is the delay to the standard reward. Finally, k is the fitted parameter that describes the steepness of the discount function. A univariate analysis of variance with both cohort and strain as factors was used to examine k values. Significant main effects were examined with Fisher's LSD tests. Data were sorted using Microsoft Excel 2007. Non-linear regression was carried out in Prism 4, and all other statistical procedures were carried out in IBM SPSS statistics 19.

2.4 Sipper Tube Model

2.4.1 Sipper Tube Model: Training

After completion of the delay discounting task, animals were left in their home cages for at least one week. Then animals were initially trained on a fixed ratio (FR) 1 schedule of reinforcement for 10 seconds of access a sipper tube containing 10% sucrose (w/v) in tap water while water restricted. Over the next three weeks subjects underwent a modified sucrose fading procedure (Samson 1986) in which the concentration of sucrose was incrementally decreased as

ethanol was introduced. The ethanol concentration was increased until it reached 10% (v/v), and the sucrose was completely removed. Table 2.2 shows the specific concentrations of sucrose and ethanol throughout this procedure. Over the course of the sucrose fade animals were increased from an FR1 to an FR4, the second, inactive lever was extended into the chamber, and on 2nd to last day the procedural separation between seeking and drinking was implemented. Animals now completed a single response requirement (RR) of 4 lever presses to receive 20 uninterrupted minutes of access to the sipper tube. Over the course of the next 2-3 weeks the RR was increased to 15 responses, and responding was maintained for 1 to 3 weeks at this level.

Table 2.2: Sucrose fade and schedule of reinforcement. Fixed ratio (requirements) and response requirements (RR) represent the target schedule of reinforcement. If a subject needed an additional D it was given. However instances of such exception as oppose to the rule. Inactive lever introduced on W3D2. Procedural separation between seeking and drinking introduced on W3D4. W=Week, D=Day.

Session	W1 D1	W1 D2	W1 D3	W1 D4	W1 D5	W2 D1	W2 D2	W2 D3	W2 D4	W2 D5	W3 D1	W3 D2	W3 D3	W3 D4	W3 D5
Sucrose Concentration	10%	10%	10%	10%	10%	10%	5%	5%	5%	5%	5%	2%	2%	0%	2%
Ethanol Concentration	0%	2%	2%	5%	5%	5%	5%	5%	10%	10%	10%	10%	10%	10%	10%
Schedule of Reinforcement	FR1	FR2	FR2	FR2	FR2	FR2	FR3	FR3	FR3	FR3	FR3	FR4	FR4	RR4	RR6

2.4.2 Sipper Tube Model: Drinking Measurement

Ethanol drinking variables were measured during the last 4 days of responding at an RR15 and then averaged across each subject. Total intake of alcohol was determined from the change in volume in the graduated cylinder tube (ml), and intake in grams per kilogram was calculated using daily measurements of body weight. Licking behavior was characterized by the total number of licks as well as divided into 2 minute time bins. If a subject failed the

response requirement on any one of the days, an average of the remaining three days was taken. If the response requirement was not met on 2 of the 4 days, the subject was excluded.

2.4.3 Sipper Tube Model: Seeking Measurement

After the four days of drinking measurement, subjects underwent a 12 session extinction curve. During extinction sessions rats received 20 minutes of access to the active and inactive levers both of which had no programmed consequences. Responses were recorded in the forms of total responses, responses in 2 minute bins, and cumulative records. The graduated cylinder tube was still filled with 10% ethanol, but was in a retracted state to control for olfactory and visual cues. After completion of the extinction curve, subjects were left in their home cages for three weeks and then tested in extinction one more time to assess spontaneous recovery.

2.4.4 Sipper Tube Model: Blood Ethanol Concentration

After testing for spontaneous recovery, animals responded for ethanol on a RR1 for 4 to 5 sessions. On the last session, tail bloods were taken while animals were briefly restrained using heparinized capillary tubes. Samples were immediately stored on ice, centrifuged, and then frozen. The concentration of ethanol was determined from a 5 μ L volume of plasma using an AM1 Analyzer (Analox Instruments, Lundenburg, MA).

2.4.5 Sipper Tube Model: Statistical Analysis

Intake in g/kg, ml, licks, and number of responses on the first day of extinction were analyzed with a univariate ANOVA with both strain and cohort as

factors. Significant main effects were followed up by Fisher's LSD tests. Responding and licks in 2 minute bins were analyzed with a mixed factorial ANOVA with strain and cohort as between subjects factors and bin as the within subject factor. Significant main effects were followed up by Fisher's LSD tests and interactions were examined with student t tests using a Bonferroni corrected alphas. For BEC's the Pearson correlation between intake in g/kg and BEC was examined overall and within each line with a bonferroni corrected alpha.

2.6 Home Cage Two Bottle Choice

2.6.1 Two Bottle Choice: Procedure

The day after BEC determination, all rats were given free-choice access to both water and 10% Ethanol (v/v) in bottles holding approximately 450ml of fluid. Animal, water, and ethanol bottle weights were obtained daily using an Ohaus CS 2000 electronic scale (Ohaus Corporation, Pine Brook, NJ). This was done daily at the same time during the light cycle for 15 days (14 24 hour periods). Bottle sides were alternated daily to eliminate side preference. Two additional "leak" cages also were set up with bottles.

2.6.2 Two Bottle Choice: Statistical Analysis

Intake of a given fluid during any 24 hour period was determined by the change in bottle weight minus the average change in bottle weight of the two "leak" cages for the same fluid. The volume consumed was then determined by dividing the adjusted weight change by the density of the fluid. Daily body weights taken at the beginning and end of every 24hr period were averaged and then used to calculate g/kg intake for a given period. Ethanol preference was determined by dividing the volume of ethanol consumed by the total fluid volume

consumed. All measures were subjected to a mixed factorial ANOVA with strain and cohort as between subjects factors and- 24hr period as a within subjects factor. Days in which there was a clear perturbation of a subject's intake measure for any reason were replaced with the average of the period before and the period after.

CHAPTER 3. DELAY DISCOUNTING RESULTS AND DISCUSSION

3.1 Delay Discounting: Results

One P rat and 3 Long Evans rats were excluded for stealing licks from the sipper tube during the inter-trial interval and during the delay to the standard reward. Five HAD2 rats were excluded due to problematic levels of performance preventing them from completing the task, and one HAD2 rat was excluded due failing to learn the task. The final group sizes for the delay discounting were 11 Long Evans, 5 P rats, and 14 HAD2s.

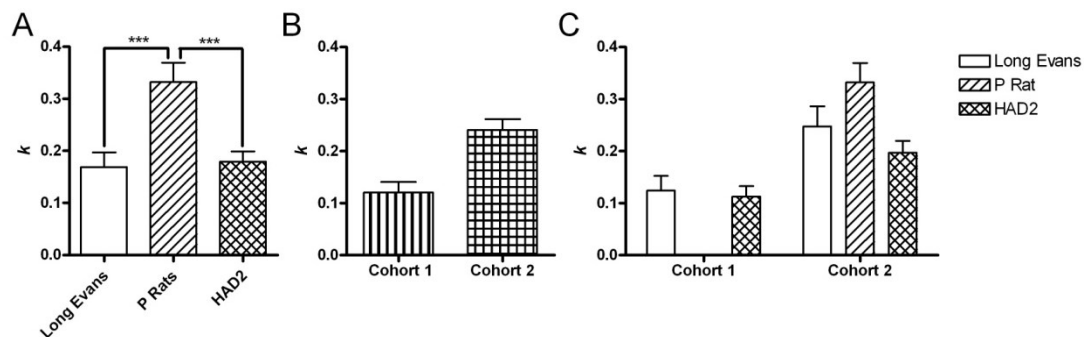


Figure 3.1: Graph of k values. A) k values broken down by group. B) k values broken down by cohort. C) k values broken down by both group and cohort. ***p<.001 on Fishers LSD.

A univariate ANOVA for k values had a main effect of strain ($f(2,25)=4.791$, $p=.017$, partial $\eta^2=.277$), and a main effect of cohort ($f(1,25)=9.595$, $p=.005$, partial $\eta^2=.277$). The first cohort had lower k values than the second. Fisher's LSD follow up comparisons for strain revealed that the Long Evans ($M=.17$, $SEM=.03$) had significantly lower k values than the P rats ($p<.001$; $M=.33$, $SEM=.04$), but not the HAD2s ($p=.735$; $M=.18$, $SEM=.02$). The P rats also

had higher k values than the HAD2s ($p=.001$). There was no interaction ($f(1,25)=.336$, $p=.567$, partial $\eta^2=.013$). These data are displayed in figure 3.1.

To insure the main effect of cohort was not driving differences in strain as there were no P rats in the 1st cohort, a sensitivity analysis was run with within the second cohort. This univariate ANOVA replicated the main effect of strain ($f(2,20)=5.273$, $p<.001$, partial $\eta^2=.383$). Fisher's LSD tests identified the P rats ($n=5$) to have higher k values than the HAD2s ($n=11$; $p=.005$), but not compared to the Long Evans ($n=4$; $p=.120$). The Long Evans were not significantly different from the HAD2s ($p=.280$).

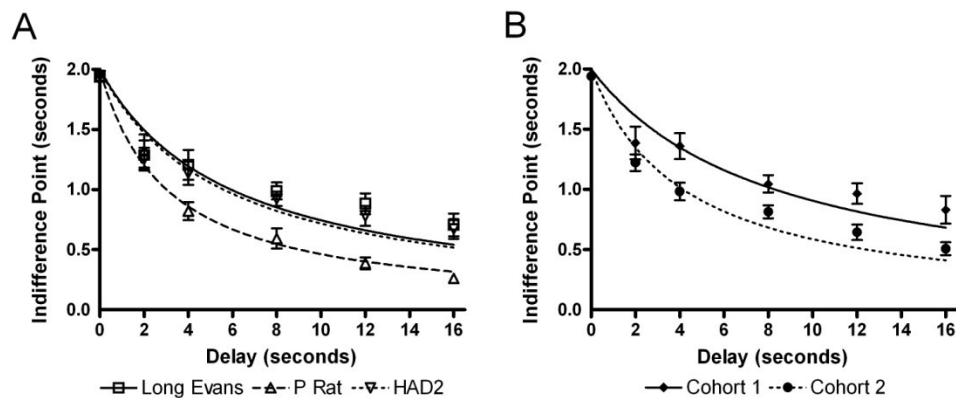


Figure 3.2: Indifference points plotted with hyperbolic curve. The curve is defined by the mean k value. A) Divided by group. B) Divided by cohort.

The median adjusting amount of the last 20 trials (indifference points) had a main effect of delay showing that values decreased with increasing delay ($f(3.12,77.91)=76.2$, $p<.001$, partial $\eta^2=.753$), no delay by strain ($f(6.23,77.91)=1.238$, $p=.295$, partial $\eta^2=.09$), delay by cohort ($f(3.12,77.91)=.93$, $p=.433$, partial $\eta^2=.036$), or three way interaction ($f(3.12,77.91)=.538$, $p=.664$, partial $\eta^2=.021$). There was no effect of strain ($f(2,25)=2.793$, $p=.08$, partial $\eta^2=.183$), but there was an effect of cohort ($f(1,25)=11.918$, $p=.002$, partial

$\eta^2=.323$) with the first cohort showing higher indifference points. However, there was no interaction ($f(1,25)=.549$, $p=.466$, partial $\eta^2=.021$). Indifference points are plotted in figure 3.2.

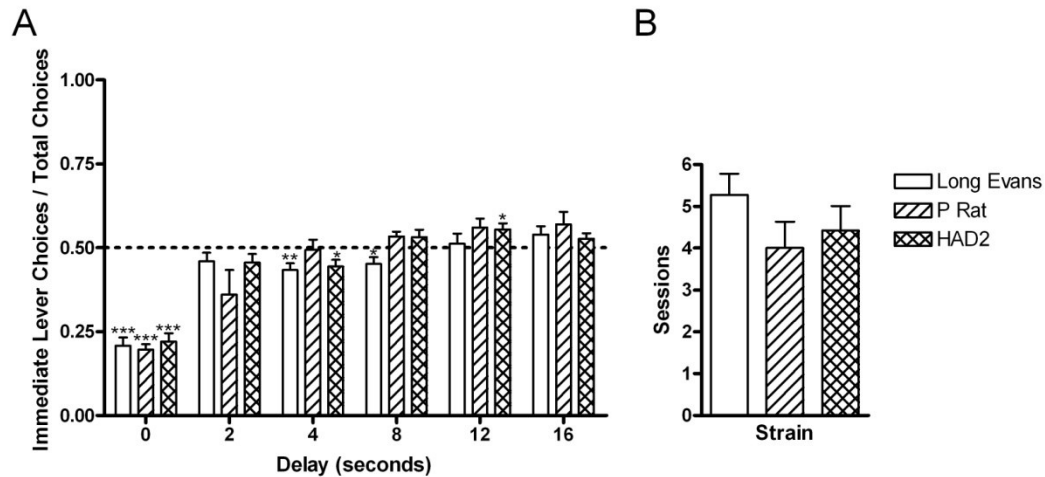


Figure 3.3: Delay discounting manipulation checks. A) Choice ratio in the last 20 trials. B) Sessions until the completion of magnitude discrimination from the implementation of the adjusting amount. * $<.05$, ** $<.01$, *** $<.001$ compared to .5.

The choice ratio of the last 20 trials of every session was analyzed within each strain utilizing a one sample t test at each delay that assessed whether the data was different from .5. For the Long Evans they were different from .5 at the 0 ($t(10)=11.668$, $p<.001$), 4 ($t(10)=3.256$, $p=.009$), and 8 second delay ($t(10)=2.240$, $p=.036$). The P rats were only different at the 0 delay ($t(4)=18.772$, $p<.001$), and the HAD2s were different at the 0 ($t(13)=11.071$, $p<.001$), 4 ($t(13)=2.802$, $p=.015$), and 12 second delay ($t(13)=3.024$, $p=.01$). While all lines show difference from “indifferent” choice at some point, all significant mean differences were less than .1 away from .5 except at the 0 second delay. At the 0 delay a univariate ANOVA was carried out to ensure that these large differences from the zero delay were no different between strains. There was no main effect of strain ($f(2,25)=.155$, $p=.857$), cohort ($f(1,25)=.373$, $p=.547$) or strain by cohort interaction ($f(1,25)=.133$, $p=.718$). A univariate ANOVA for the number of

sessions until the criteria for magnitude discrimination were met revealed that there was no main effect of strain ($f(2,25)=.505$, $p=.609$) or strain by cohort interaction ($f(1,25)=.231$, $p=.635$). This result and the indifference point choice ratio data is plotted below in figure 3.3.

The number of free trials per session was non-normally distributed and was subjected to log transformation. There was a significant main effect of delay on the log transformed trial completion values ($f(2.03,50.65)=7.691$, $p=.001$, partial $\eta^2=.235$). Trials completed tended to decrease with increasing delay. There was no delay by strain interaction ($f(4.05,50.65)=1.608$, $p=.186$, partial $\eta^2=.114$), no delay by cohort interaction ($f(2.03,50.65)=.336$, $p=.719$, partial $\eta^2=.013$), and no three way interaction ($f(2.03,50.65)=.555$, $p=.580$, partial $\eta^2=.022$). There was a main effect of strain ($f(2,25)=5.039$, $p=.014$, partial $\eta^2=.287$), no main effect of cohort ($f(1,25)=1.529$, $p=.228$, partial $\eta^2=.058$), and no interaction of strain and cohort ($f(1,25)=.674$, $p=.419$, partial $\eta^2=.026$). Follow up analysis on strain differences showed the P rats to have completed more trials than the HAD2 rats ($p=.009$), but the Long Evans were not different from the P rats ($p=.181$) or the HAD2 rats ($p=.076$). The raw score trial completion data are graphed in figure 3.4.

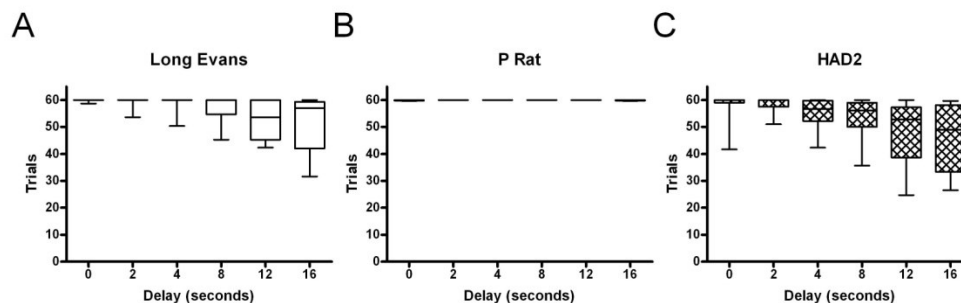


Figure 3.4: Untransformed trial completion. Plotted by delay within each group. A) Long Evans B) P rat C) HAD2.

For the number forced choice trials there was a main effect of delay with values decreasing with increasing delay ($f(2.75,68.84)=22.821$, $p<.001$, partial

$\eta^2=.477$). There were no interactions of delay and strain ($f(5.51,68.84)=.934, p=.471$, partial $\eta^2=.07$), delay by cohort ($f(2.75,68.84)=.222$, $p=.865$, partial $\eta^2=.009$), or a three way interaction ($f(2.75,68.84)=.193, p=.887$, partial $\eta^2=.008$). There was however a main effect of strain ($f(2,25)=4.855$, $p=.017$, partial $\eta^2=.280$). There were no cohort ($f(1,25)=1.282$, $p=.268$, partial $\eta^2=.049$) or strain by cohort effects ($f(1,25)=.034, p=.855$, partial $\eta^2=.001$). Fisher's LSD tests comparing the strains revealed the HADs to complete fewer forced trials than both the Long Evans ($p=.034$) and the P Rats ($p=.012$).

Intake of 2% sucrose in ml showed a main effect of delay ($f(3.2,79.99)=21.452$, $p<.001$, partial $\eta^2=.462$). Intake tended to decrease with delay. There were no delay by strain ($f(6.4,79.99)=1.356$, $p=.240$, partial $\eta^2=.098$), delay by cohort ($f(3.2,79.99)=.576$, $p=.643$, partial $\eta^2=.023$), or three way interactions ($f(3.2,79.99)=.309$, $p=.831$, partial $\eta^2=.012$). There was a main effect of strain ($f(2,25)=14.258$, $p<.001$, partial $\eta^2=.533$), no main effect of cohort ($f(1,25)=.260$, $p=.615$, partial $\eta^2=.01$), and no interaction ($f(1,25)=1.635$, $p=.213$, partial $\eta^2=.061$). Fisher's LSD test following up the main effect of strain revealed the P's to drink more than both the Long Evans ($p=.005$) and the HAD2s ($p<.001$), and the Long Evans drank more than the HAD2s as well ($p=.003$). These data are displayed in figure 3.5.

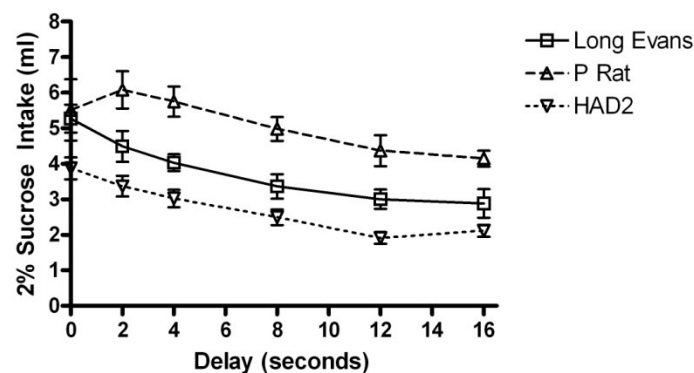


Figure 3.5: Delay discounting reward consumption. Intake of 2% sucrose (weight/volume) solution in the delay discounting paradigm plotted as a function of delay.

The amount of sipper access resulting from free choice trials showed a main effect of delay ($f(5,125)=72.941$, $p<.001$, partial $\eta^2=.745$) with the amount of access decreasing with increasing delay. There were no effects of strain ($f(2,25)=2.598$, $p=.094$, partial $\eta^2=.172$), cohort ($f(1,25)=.411$, $p=.527$, partial $\eta^2=.016$), delay by strain ($f(10,125)=.741$, $p=.684$, partial $\eta^2=.056$), cohort by delay ($f(5,125)=.698$, $p=.626$, partial $\eta^2=.027$), strain by cohort ($f(1,25)=.863$, $p=.362$, partial $\eta^2=.033$), or three way interactions ($f(5,125)=1.153$, $p=.336$, partial $\eta^2=.044$).

The same exact pattern was seen for the total sipper access (resulting from both free and forced trials) with the amount of access decreasing with increasing delay ($f(3.31,82.72)=83.237$, $p<.001$, partial $\eta^2=.769$), no strain by delay interaction ($f(6.62,82.72)=.923$, $p=.490$, partial $\eta^2=.069$), no cohort by delay interaction ($f(3.31,82.72)=.357$, $p=.803$, partial $\eta^2=.014$), no three way interaction ($f(3.31,82.72)=.842$, $p=.484$, partial $\eta^2=.033$), no main effect of strain ($f(1,25)=2.644$, $p=.091$, partial $\eta^2=.175$), cohort ($f(1,25)=.178$, $p=.677$, partial $\eta^2=.007$) or interaction of strain by cohort ($f(1,25)=.762$, $p=.391$, partial $\eta^2=.03$). Both total and free choice trial sipper access are plotted in figure 3.6.

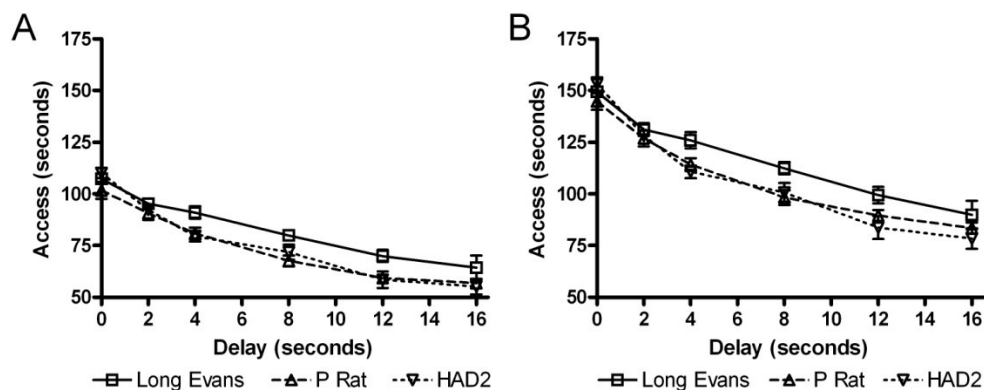


Figure 3.6: Delay discounting sipper access. Amount earned inside a single session plotted as a function of delay. A) Sipper access resulting from free choice trials only. B) Sipper access resulting from both free and forced choice trials combined.

Trial initiation latencies were positively skewed, had unequal variances across strains, and were subjected to a log transform. The raw score trial initiation latencies are graphed in figure 3.7. A mixed factorial ANOVA of the log transformed latencies revealed a main effect of delay ($f(3.1,77.46)=51.336$, $p<.001$, partial $\eta^2=.673$). Latencies tended to increase with delay. There was also a delay by strain interaction ($f(6.2,77.46)=3.071$, $p=.009$, partial $\eta^2=.197$), but there was no delay by cohort interaction ($f(3.1,77.46)=2.305$, $p=.081$, partial $\eta^2=.084$) or three way interaction ($f(3.1,77.46)=1.482$, $p=.225$, partial $\eta^2=.056$). There was also a main effect of strain ($f(2,25)=23.697$, $p<.001$, partial $\eta^2=.655$), but there were no effects of cohort ($f(1,25)=.148$, $p=.704$, partial $\eta^2=.006$) or interaction of strain and cohort ($f(1,25)=.056$, $p=.814$, partial $\eta^2=.002$). T tests with bonferroni corrected alphas were used to follow up the delay by strain interaction. They revealed that the P rats had shorter latencies than the Long Evans and the HAD2s at all delays except the zero second delay. The Long Evans and the HAD2s were not different at any delays.

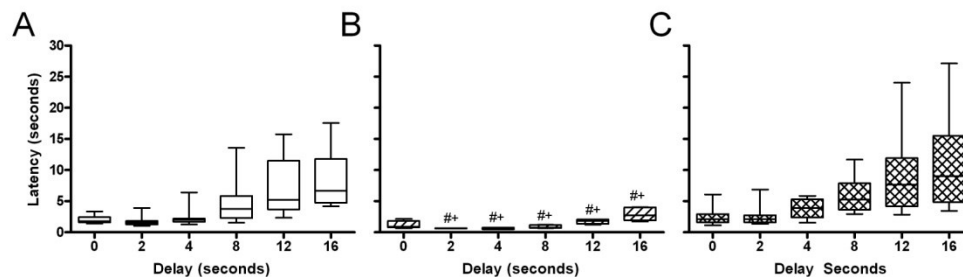


Figure 3.7: Untransformed trial initiation latencies. Plotted within each group as a function of delay. A) Long Evans B) P Rat C) HAD2. #, + different from Long Evans and HAD2 respectively after Bonferroni correction.

Choice latencies also showed a main effect of delay ($f(2.64,65.98)=15.854$, $p<.001$, partial $\eta^2=.388$) with latencies tending to increase with delay, no delay by strain ($f(5.28,65.98)=1.259$, $p=.291$, partial $\eta^2=.092$), no delay by cohort ($f(2.64,65.98)=.224$, $p=.856$, partial $\eta^2=.009$), and no three way interaction was found ($f(2.64,65.98)=.181$, $p=.888$, partial $\eta^2=.007$). There was however a main

effect of strain ($f(2,25)=4.049$, $p=.03$, partial $\eta^2=.245$). There was no main effect of cohort ($f(1,25)=.485$, $p=.949$, partial $\eta^2=.019$) or an interaction of the two ($f(1,25)=.004$, $p=.949$, partial $\eta^2<.001$). Follow up analysis with Fisher's LSD tests showed the P rats to have shorter latencies than the HAD2s ($p=.009$), but not the Long Evans ($p=.164$). The HAD2s and Long Evans also were not different ($p=.094$). The data are graphed in figure 3.8.

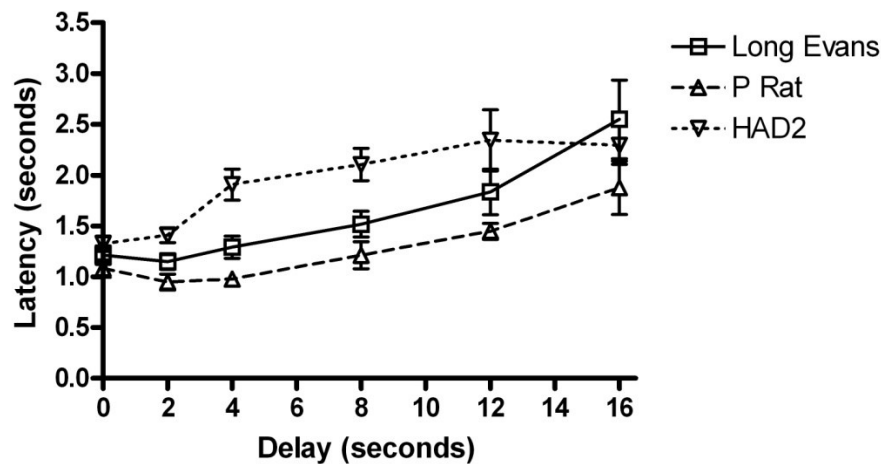


Figure 3.8: Choice latency. Latency to lever press after initiating a trial plotted as a function of delay.

3.2 Delay Discounting: Discussion

The main variable of interest in the delay discounting paradigm is k , the free parameter from the hyperbolic analysis that describes the steepness of the discount function. Consequently, it is used as an index of the impulsiveness of an individual subject. The alcohol preferring P rats had the highest k values, and there was no difference between the Long Evans and HAD2s. This indicates that P rats exhibit steeper discounting of delayed rewards, and thus are more impulsive. The indifference points that are used to derive the k values did not

show statistically significant differences between strains, although they did approach it. However this is not a major concern given that there are differences in k .

One of the most interesting and unpredicted effects found from examining the k values was the main effect cohort. This demonstrates how incredibly sensitive this phenotype is to slight environmental differences. Unbeknownst to the author until trying to interpret this effect, there is support in the literature for environmental sensitivity in delay discounting. Perry et al., (2008) found that environmental enrichment could decrease delay discounting or conversely a lack of could increase impulsivity. Studies looking into the heritability of delay discounting also support the notion of environmental impact. Wilhelm and Mitchell (2009) used a six line inbred rat strain panel to estimate delay discounting's heritability to be approximately .4, and Isles et al., (2004) estimated it to be .16 using a 4 strain panel of inbred mice. In both cases the majority of the variance in choice behavior was due to the environment or any gene by environment effects. This holds translationally as well. Anokhin et al., (2011) estimated the heritability of delay discounting in adolescent twins to be between .3 and .51.

The choice ratio in the last 20 trial is used to look at if subjects were actually choosing indifferently when the indifference point was measured. If this manipulation check is different from .5, it calls the validity of the indifferent point into question. There were differences from .5 at several delays in all lines except the P rats. However, with the exception of the 0 delay these differences were modest and within 0.1 of 0.5. These differences likely arise because delay discounting describes behavior in a molar versus molecular fashion. In other words, delay discounting will describe the overall trend in choice behavior, not an individual choice. Consequently, it does not predict perfect alternation between rewards once the indifference point is reached, and minor differences from .5 are not a major concern. Furthermore the number of trials is not in danger of falling

below the amount needed for rats to reach their indifference points which Richards (1997) found to be approximately 30 trials using much smaller adjustments.

The difference seen in all strains at the 0 delay in the indifference point choice ratio is larger for several additional reasons. One is a procedural artifact. The delayed lever is assigned to their non-preferred lever in training. This tends to result in the subjects learning how to titrate the adjusting amount the hard way. Specifically, they tend to take the adjusting amount to zero and persevere on it for quite some time before switching to the standard reward lever. At which point, they tend to titrate the alternative reward up until it reaches its upper limit of 2 seconds. In addition to carry over effects from essentially responding in extinction on the alternative lever, now they are choosing between two rewards of equal magnitude, and rats tend to have an aversion to the adjusting alternative (Wilhelm and Mitchell 2010). Consequently, the difference seen at the zero delay is essentially a measurement artifact corresponding to a slight preference for fixed magnitude rewards, and a temporary shift due recent experience on the other lever. This could be problematic if it was different between lines. Fortunately, there are no differences between the strains at the zero delay in the indifference point choice ratio.

Another alternative explanation for differences seen in k is the possibility differential learning ability between the strains. The number of sessions it took to meet criteria for magnitude discrimination once the adjusting amount was implemented was used as a general assessment of learning ability. The lack of a difference in this criterion is pertinent because it rules out differential learning as an alternative explanation for differences seen in delay discounting. This is because it shows that each of the lines was able to learn the task, specifically the adjusting amount, at roughly the same rate.

The time it takes to initiate a trial is a gross measure that can be interpreted similar to a runway procedure in that it measures how motivated a

subject is to obtain a reinforcer. However, it also assesses the attention they are paying to the task and their vigilance. When interpreted in this fashion, the interaction seen in this variable is paradoxical. One would expect the line with the steepest discounting to exhibit the greatest increases in trial initiation latency. This is because the schedule of reinforcement is essentially leaning out the fastest for the strain that discounts the steepest. However the opposite is observed in the trial initiation latencies. The P rats, whose choices are affected the most by delay and have the fastest decrement in value of the delayed reward, are the most resistant to the effects of delay in trial initiation latencies. It may be the case that the P rats are very delay aversive even if it is self-imposed, and responding as quickly as possible to decrease any delay. Alternatively they could simply be responding compulsively without consideration of the schedule of reinforcement.

Another possibility is that their appetitive drive is so great that even the smaller reinforcers are able to overcome the effect of delay. In other words, regardless of the delay they “got to have it, and got to have it now.” This brings up the possibility that the different strains may actually value the access to the sipper tube differently from the start. If such is the case, the P rats would be inferred to want the reward the most based on their trial initiation latencies. In delay discounting, the size of the reward affects the rate of discounting. This has been termed the magnitude effect, and larger rewards typically have shallower discounting (Baker et al., 2003). Based on the P rats potentially valuing 2% sucrose more than the other lines and greater magnitude rewards being discounted slower, one would predict the P rats to have shallower discounting than the HAD2s and Long Evans. However, steeper discounting was observed.

This is probably because magnitude effects are a point of divergence of the human and animal literature on delay discounting. Several groups have tried and failed to show a magnitude effect in animals. Green et al., (2004) attempted to demonstrate a magnitude effect in both rats and pigeons by changing the

number of pellets delivered for the standard reward from 5 to upwards of 20. This same group also attempted to show a magnitude effect by changing the quality of the reinforcer using sucrose, cellulose, and precision pellets (Calvert et al., 2010) to no avail. Also when Richards et al., (1997) first used the adjusting amount procedure in rats, they varied the amount of the standard reward as well as the level of deprivation and were only able to show a trend.

A similar pattern was seen for choice latencies as trial initiation latencies. Specifically the P rats had shorter choice latencies than the HAD2s, and the Long Evans were not different from either the P rats or the HAD2s. The decreased choice latency may be a result of the P rats choosing without fully assessing their choice, which is impulsive in and of itself. Another possibility is that the choice is easier for the P rats. Therefore there is less mental load, and the choice is made faster. Considering the P rats were steeper discounters, they would have more trials where there is a greater value difference between the current alternative reward and their indifference point. This is because they had to titrate the adjusting amount down further as their indifference point is lower. Another way the choice could be easier is if the P rats find waiting for the standard reward so aversive that it weights the choice heavily in favor of the immediate, alternative reward.

Either explanation relies on the P rats being more impulsive. However, the P rats were also more impulsive than the Long Evans, but were not any different in choice latency. A possibility that would conserve the two different explanations above and explain the Long Evans data would be that the Long Evans were actually deciding before the choice link.

CHAPTER 4. SIPPER TUBE MODEL RESULTS AND DISCUSSION

4.1 Sipper Tube Model: Results

For the sipper tube model 3 Long Evans and 3 HAD2s were excluded for failing to train up to and maintain responding at a response requirement of 15. The 5 HAD2 rats who failed to perform the delay discounting task also failed to acquire responding in the sipper tube model as well. This left the final group sizes for the sipper tube model as 11, 6, and 12 for the Long Evans, P rats, and HAD2s respectively.

Responding on the 1st day of extinction was examined with a univariate ANOVA due to the a priori hypotheses concerning differences on this day. For the active lever, there was a main effect of strain ($f(2,24)=5.179$, $p=.013$, partial $\eta^2=.301$). Fisher's LSD post hoc tests revealed that the P rats ($M=118.8$, $SEM=14.2$) responded more on the active lever than either the Long Evans ($p=.001$; $M=53.3$, $SEM=9.4$) and the HAD2s ($p=.036$; $M=81.7$, $SEM=9.4$) who were not different from each other ($p=.053$). There was no effect of cohort ($f(1,24)=.333$, $p=.569$, partial $\eta^2=.014$) or interaction of the two ($f(1,24)=.211$, $p=.650$, partial $\eta^2=.009$). On the inactive lever there was no effect of strain ($f(2,24)=.673$, $p=.519$, partial $\eta^2=.053$), cohort ($f(1,24)=1.537$, $p=.227$, partial $\eta^2=.06$), or interaction of strain and cohort ($f(1,24)=.120$, $p=.732$, partial $\eta^2=.005$).

When broken down into 2 minute bins there was a main effect of bins on responding ($f(3.19,46.44)=19.476$, $p<.001$, partial $\eta^2=.448$), but there was no bin by strain ($f(6.37,76.44)=1.664$, $p=.137$, partial $\eta^2=.122$), bin by cohort ($f(3.19,76.44)=.168$, $p=.926$) or three way interaction ($f(3.19,76.44)=.458$, $p=.724$, partial $\eta^2=.019$). When responding on the inactive lever was broken down, there

was a main effect of bin ($f(3,72.1)=2.838$, $p=.044$, partial $\eta^2=.106$), and no bin by strain ($f(6,72.1)=.839$, $p=.544$, partial $\eta^2=.065$), bin by cohort ($f(3,72.1)=1.464$, $p=.231$, partial $\eta^2=.058$), or three way interactions ($f(3,72.1)=1.245$, $p=.3$, partial $\eta^2=.049$). Lever pressing data from the first day of extinction are graphed in figure 4.1.

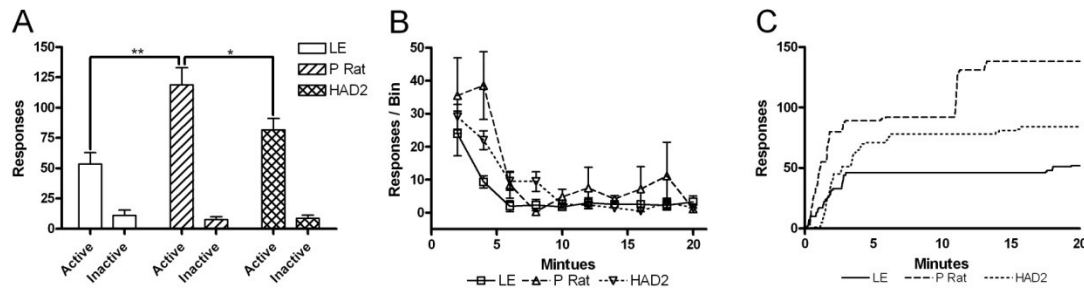


Figure 4.1: Lever pressing on the first day of extinction. A) Mean values for both active and inactive levers plotted as a function of group. B) Responding on both the active (filled symbols) and inactive (unfilled symbols) levers. C) Representative cumulative records of individual subjects from each group. * $p<.05$, ** $p<.01$ on Fisher's LSD test.

Responding throughout the entire extinction curve had main effect of day ($f(4.27,102.51)=53.388$, $p<.001$, partial $\eta^2=.690$) and a day by strain interaction ($f(8.54,102.51)=3.215$, $p=.002$, partial $\eta^2=.211$). There was no day by cohort interaction ($f(4.27,102.51)=.850$, $p=.503$, partial $\eta^2=.034$) or three way interaction ($f(4.27,102.51)=.618$, $p=.661$, partial $\eta^2=.025$). There was also a main effect of strain ($f(2,24)=5.999$, $p=.008$, partial $\eta^2=.333$), but there was no effect of cohort ($f(1,24)=.163$, $p=.69$, partial $\eta^2=.007$) or strain by cohort interaction ($f(1,24)=.014$, $p=.907$, partial $\eta^2=.001$). Student t tests with a bonferroni corrected alpha were used to examine the interaction. They revealed that the P rats continued to exhibit greater responding later into the curve. The inactive lever had a main effect of day ($f(4.22,101.22)=3.773$, $p=.006$, partial $\eta^2=.136$), but no effects of strain ($f(2,24)=.1$, $p=.905$, partial $\eta^2=.008$), cohort ($f(1,24)=.008$, $p=.929$, partial $\eta^2<.001$). There were also no strain by cohort ($f(1,24)=.246$, $p=.624$, partial $\eta^2=.01$), strain by day ($f(8.44,101.22)=.854$, $p=.562$, partial $\eta^2=.066$), cohort by

day ($f(4,22,101.22)=1.549$, $p=.191$, partial $\eta^2=.061$), or three way interactions ($f(4,22,101.22)=.749$, $p=.568$, partial $\eta^2=.03$). The curve is graphed in figure 4.2.

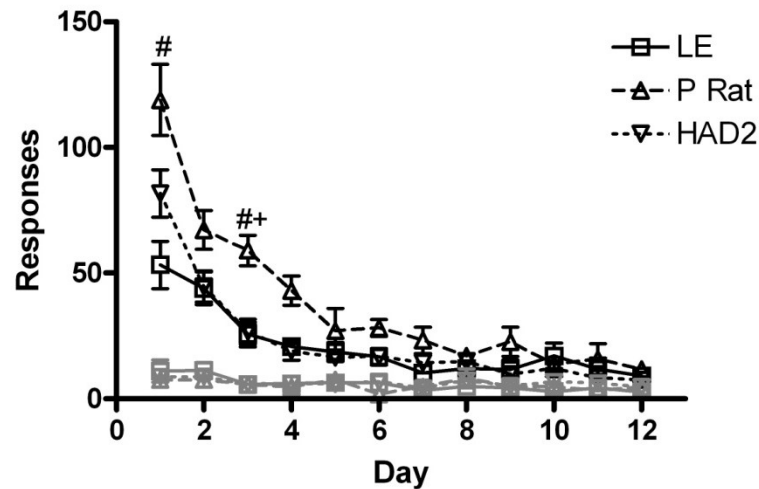


Figure 4.2: Responding during extinction. Plotted as a function of day. #, + different from Long Evans and HAD2 respectively on Bonferroni corrected student t tests. Grey lines represent Inactive lever.

When spontaneous recovery was tested by comparing the last day of extinction to the spontaneous recovery test day, the active lever showed a main effect of day ($f(1,24)=9.176$, $p=.006$, partial $\eta^2=.277$) such that there tended to be more responses on the spontaneous recovery test day, but there was no effect of strain ($f(2,24)=.501$, $p=.612$, partial $\eta^2=.04$), cohort ($f(1,24)=.068$, $p=.797$, partial $\eta^2=.003$), strain by cohort ($f(1,24)=.004$, $p=.951$, partial $\eta^2<.001$), Day by strain ($f(2,24)=.435$, $p=.652$, partial $\eta^2=.035$), day by cohort ($f(1,24)=1.566$, $p=.223$, partial $\eta^2=.061$), or cohort by strain by day interactions ($f(1,24)=.004$, $p=.951$, partial $\eta^2<.001$). The same pattern held for responses on the inactive lever. There were more responses in general on the spontaneous recovery test day as evidenced by a main effect of day ($f(1,24)=7.299$, $p=.012$, partial $\eta^2=.233$), but it was not specific with no effects of strain ($f(2,24)=.985$, $p=.388$, partial $\eta^2=.076$), cohort ($f(1,24)=.718$, $p=.405$, partial $\eta^2=.029$), strain by cohort ($f(1,24)=.5$, $p=.486$, partial $\eta^2=.02$), day by strain ($f(2,24)=.031$, $p=.969$, partial $\eta^2=.003$), day by cohort

($f(1,24)=.145$, $p=.707$, partial $\eta^2=.006$), or three way interaction ($f(1,24)=.5$, $p=.486$, partial $\eta^2=.02$). Responding under spontaneous recovery is displayed in figure 4.3.

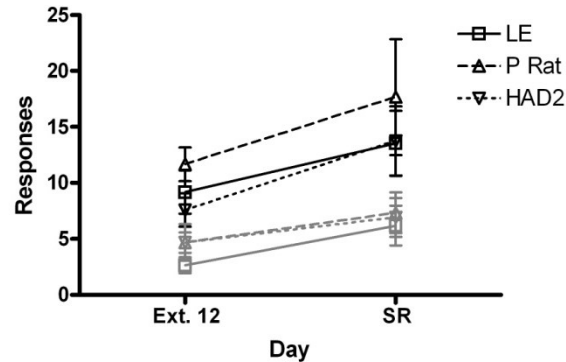


Figure 4.3: Spontaneous recovery responding. Active lever (black) and inactive lever responses (grey) on extinction day 12 (Ext. 12) and on the spontaneous recovery test day (SR) three weeks later.

Intake in ml showed a main effect of strain ($f(2,24)=43.408$, $p<.001$, partial $\eta^2=.783$). Fisher's LSD tests revealed that the P rats drank more than both the Long Evans ($p<.001$) and the HAD2s ($p<.001$). There was no difference between the Long Evans and the HAD2s ($p=.906$). There was no main effect of cohort ($f(1,24)=.009$, $p=.927$, partial $\eta^2= <.001$) or an interaction of strain and cohort ($f(1,24)=3.248$, $p=.084$, partial $\eta^2= .119$). Intake of alcohol in grams/kilogram had a main effect of strain $f(2,24)=14.992$, $p<.001$, partial $\eta^2=.555$). Fisher's LSD follow up tests revealed both the P rats ($p<.001$) and the HAD2s ($p=.003$) had higher intakes than the Long Evans. The P rats also had a higher intake than the HAD2s ($p=.001$). Intake data are displayed in figure 4.4.

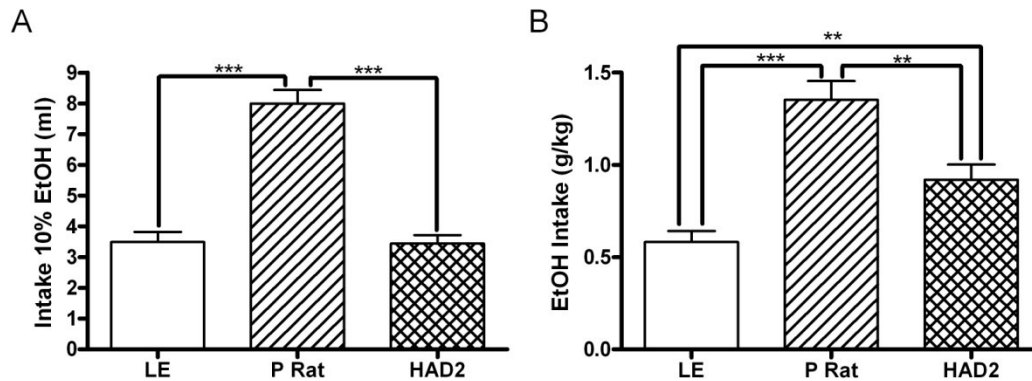


Figure 4.4: Sipper tube model ethanol intake. Intake of 10% ethanol solution prepared (volume / volume) in water. A) Intake in ml. B) Intake in g/kg. ** $p < .01$, *** $p < .001$ on Fisher's LSD test.

The average number of licks during baseline showed a main effect of strain ($f(2,24)=21.211$, $p < .001$, partial $\eta^2 = .639$), no effect of cohort ($f(1,24) < .001$, $p = .993$, partial $\eta^2 < .001$), and no interaction of the two ($f(1,24)=2.052$, $p = .165$, partial $\eta^2 = .079$). Fisher's LSD post hoc tests revealed the P rats to have more licks than both the Long Evans ($p < .001$), and the HAD2s ($p < .001$). The Long Evans and the HAD2s were not different ($p = .055$). When the average number of licks was broken down into 2 minute bins, there was a main effect of bin ($f(2.16,51.78)=113.031$, $p < .001$, partial $\eta^2 = .825$) and a bin by strain interaction ($f(4.32, 51.78)=5.78$, $p < .001$, partial $\eta^2 = .325$). However there was no bin by cohort ($f(2.16,51.78)=.114$, $p = .905$, partial $\eta^2 = .005$), or bin by cohort by strain interaction ($f(2.16,51.78)=1.130$, $p = .334$, partial $\eta^2 = .045$). For intake in ml per lick, there was a main effect of strain ($f(2,24)=6.776$, $p = .005$, partial $\eta^2 = .361$). There was no effects of cohort ($f(1,24)=1.092$, $p = .306$, partial $\eta^2 = .044$) or an interaction of strain and cohort ($f(1,24)=.981$, $p = .332$, partial $\eta^2 = .039$). Post hoc analysis revealed that the HAD2s drank more per lick than the Long Evans ($p = .002$), but not the P rats ($p = .081$). The Long Evans and P rats were not different ($p = .337$). These data are graphed in figure 4.5.

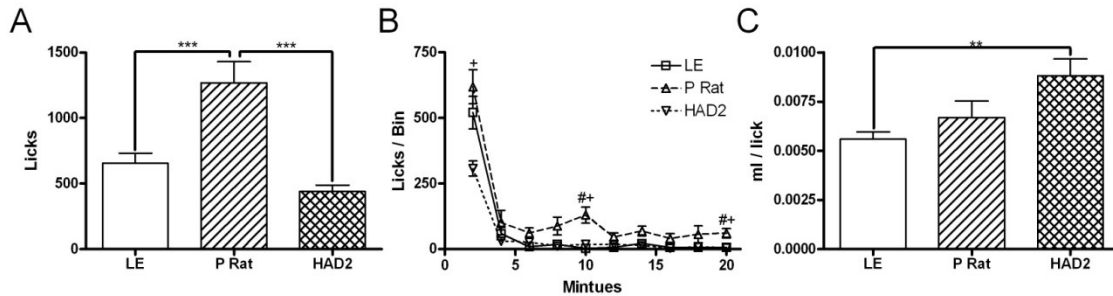


Figure 4.5: Baseline characterization of licking behavior. A) Mean total daily licks. B) Licks in 2 minute bins. C) Intake of 10% ethanol in ml per lick. ** $p < .01$, *** $p < .001$, #, + different from Long Evans and HAD2 on student t tests with a Bonferroni corrected alpha.

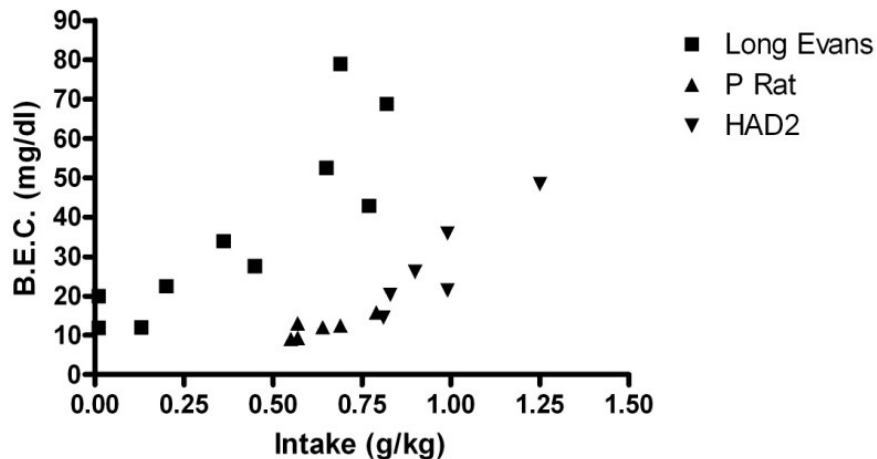


Figure 4.6: Sipper tube model blood concentrations (BEC). BECs plotted as a function of intake in g/kg. Long Evans are graphed as squares, HAD2s as inverted triangles, and P rats as triangles.

For BECs, due to experimenter error not all samples were able to be tested. This left an n of 22 across all strains, 10 in the Long Evans, 6 in the P rats and 6 in the HAD2s. Across all strains the correlation between BEC and intake in g/kg was not significant ($r(20) = .345$, $p = .115$). However it was significant inside both the Long Evans ($r(8) = .872$, $p = .001$), and HAD2 rats ($r(4) = .919$, $p = .01$) using a bonferroni corrected alpha, but not inside the P rats ($r(4) = .833$, $p = .039$). Visual inspection of the scatter plot identified an anomaly. Long Evans rats that drank virtually no alcohol on the day of BEC determination had the same BECs as P

rats drinking between .5 and .75 g/kg, and HAD2s drinking roughly 1 g/kg. Given that subjects tend to drink the most at the beginning of the session, and intakes on the day BECs were determined was on the low side to begin with, one has to question whether this measurement is representative of the normal exposure an animal would experience. This data can be seen in figure 4.6.

4.2 Sipper Tube Model: Discussion

The results from the sipper tube model confirmed the combination of behavioral phenotypes between the P rats, HAD2s, and Long Evans. The first day of extinction is the most direct evidence of the increased appetitive drive of the P rats. Their higher level of responding on this day showed that they were willing to pay a larger price whereas the HAD2s and Long Evans were not. This finding was corroborated by the resistance to extinction the P rats displayed. In the face of non-reinforcement the P rats continued to out respond the other two lines. Testing for spontaneous recovery failed to replicate the PSR phenotype of the P rats. While all lines showed an increase in responding on the test day it was far from robust. However, there is a key methodological difference. The previous studies demonstrating the PSR used a traditional fixed ratio procedure as oppose to implementing any form of procedural separation of seeking and drinking.

The P rats had an increased intake of ethanol compared to the Long Evans and the HAD2s. The difference for the latter strain was not predicted. Never-the-less, the HAD2s did still out drink the Long Evans. Furthermore, a closer examination of the licking behavior sheds light on this discrepancy. All of the lines had a large initial bout of drinking inside of the first 2 to 4 minutes. However, only the P rats came back to the sipper tube for a second and third bout of drinking. The presence of multiple bouts within a session of drinking demonstrates that the P rats reengaged in focal search behavior, and indicates

that they have smaller inter-bout intervals than the two other lines. Both of these correspond to between bout, appetitive factors, and not consummatory ones. Consequently, the “extra high” intake in the P rats is likely to be due to an additive interaction of heightened appetitive drive and consummatory processes. Regardless of being different, both the HAD2s and the P rats had high levels of intake compared to the Long Evans moderate intake.

CHAPTER 5. TWO BOTTLE CHOICE RESULTS AND DISCUSSION

5.1 Two Bottle Choice: Results

For the two bottle choice procedure no HAD2s from the first cohort were measured due to a logistical necessity. This left only Long Evans rats in the first cohort, and as a result SPSS was unable to calculate three way interactions or strain by cohort interactions. Final groupwise n's were 6 for the P rats, 14 for the Long Evans, and 12 for the HAD2s. For water intake, there was a main effect of day ($f(5.89, 158.89)=2.811, p=.013, \text{partial } \eta^2=.094$), a day by strain interaction ($f(11.77, 158.89)=2.166, p=.016, \text{partial } \eta^2=.138$), but no day by cohort interaction ($f(5.89, 158.89)=.710, p=.639, \text{partial } \eta^2=.026$). There was a main effect of strain ($f(2,27)=9.286, p=.001, \text{partial } \eta^2=.408$), but no effect of cohort ($f(1,27)=1.902, p=.179, \text{partial } \eta^2=.066$). Follow up analysis was conducted using student t tests with a bonferroni corrected alpha due to the interaction. They revealed an increase in water intake in the Long Evans driving the result while the HAD2s and P rats remained relatively constant.

For ethanol intake, there was a main effect of day ($f(4.73, 127.79)=2.438, p=.004, \text{partial } \eta^2=.083$), no day by strain interaction ($f(9.47, 127.79)=1.206, p=.295, \text{partial } \eta^2=.082$), and no day by cohort interaction ($f(4.73, 127.79)=.299, p=.905, \text{partial } \eta^2=.011$). There was a main effect of strain ($f(2,27)=8.728, p=.001, \text{partial } \eta^2=.393$), but no effect of location ($f(1,27)=.714, p=.406, \text{partial } \eta^2=.026$). Fisher's LSD tests used to follow up the main effect of strain revealed that both the P rats ($p=.005$) and the HAD2s ($p<.001$) consumed more ethanol than the Long Evans. The P rats and HAD2s however did not consume different amounts of ethanol ($p=.560$).

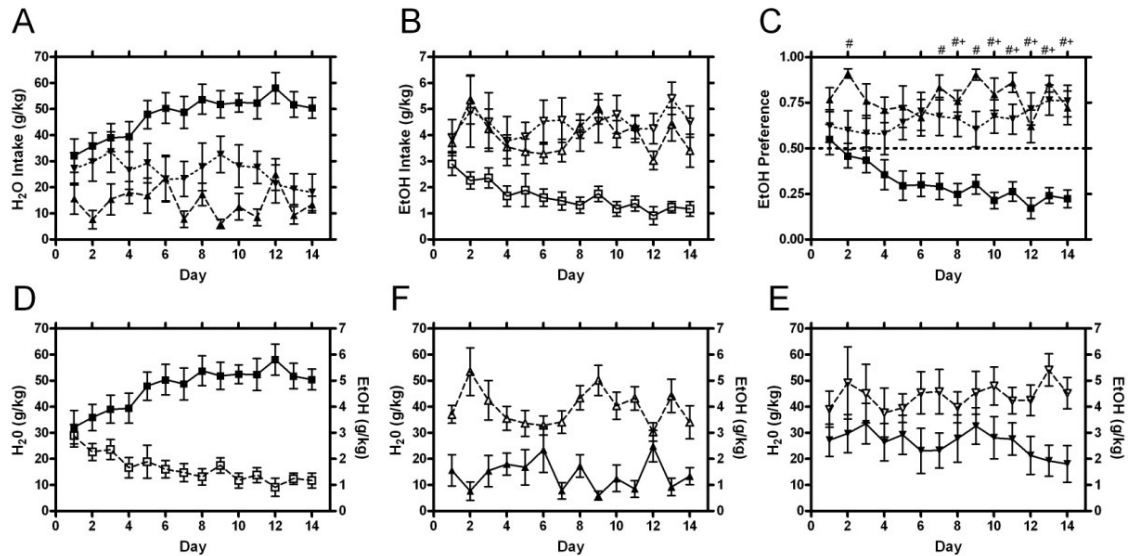


Figure 5.1: Two bottle choice drinking patterns. A) Water intake across days in g/kg. B) Ethanol intake in g/kg. C) 10% Ethanol over water preference score. D) Water and Ethanol intake in Long Evens rats. E) Ethanol and water intake in P rats. F) Ethanol and water intake in HAD2 rats. In all graphs where water and ethanol intake are graphed together: filled symbols correspond to water intake (graphed on left axis) and unfilled symbols correspond to ethanol intake (graphed on right axis). Long Evens are graphed with squares. P rats are graphed with triangles. HAD2s are graphed with inverted triangles. #, +, and @ signify P vs. Long Evens, HAD2 vs. Long Evens, and P vs. HAD2 after bonferroni correction for multiple comparisons.

Ethanol preference showed a main effect of day ($f(6.48, 174.82)=3.173$, $p=.004$, partial $\eta^2=.105$), an interaction of day and strain ($f(12.95, 174.82)=2.83$, $p=.001$, partial $\eta^2=.173$), but no day by cohort interaction ($f(6.48, 174.82)=.521$, $p=.805$, partial $\eta^2=.019$). There was a main effect of strain ($f(2,27)=11.145$, $p<.001$, partial $\eta^2=.452$), and no effect of cohort ($f(1,27)=1.559$, $p=.222$, partial $\eta^2=.055$). Follow up analysis with student t tests with bonferroni corrected alphas revealed that the Long Evens begin to separate out from the P rats and the HAD2s at roughly the 7th to 8th 24hr period.

5.2 Two Bottle Choice: Discussion

The results from the two bottle choice procedure were in keeping with the initial hypotheses that the Long Evans would exhibit decreased intake and preference for 10% ethanol compared to water, and the P rats and HAD2s would show increased intake and preference. These results conflict somewhat with Czachowski and Samson's (2002) report of their home cage drinking behavior in that the HAD2s did not out drink the P rats, but they used forced access where the ethanol solution was the only available fluid. However in this assessment rats had access to water as an alternative to the ethanol solution.

An interesting anomaly to the results from the two bottle choice is a lack of an acquisition curve in any of the lines for alcohol. This is probably due to having previous experience with 10% ethanol in the operant chamber. Also going through a sucrose fade will alter home cage intake. This was evident in the first paper describing a sucrose fade in 1986 by Samson. He found that having animal undergo a sucrose fade in the operant chamber and then return to 24hr access in the home cage increased their intake and preference for alcohol. These two factors are a likely explanation for the Long Evans initially had a level of preference equal to that of the P rats and the HAD2s.

However these explanations are unable to explain why the Long Evans decrease their preference. The Long Evans decrease in preference is likely mediate by their increase in water intake. While their mean ethanol intake appears to decrease, their water intake increases robustly. One possible explanation for this decrease in preference is that the effects of the sucrose fade on home cage drinking are time dependent.

CHAPTER 6. SUMMARY AND CONCLUSIONS

6.1 General Discussion

Taken together, the main finding from the study is that increased discounting of delayed rewards tracks with ethanol seeking and appetitive processes versus consummatory factors and home cage intake of alcohol. This can be inferred based on the following points of evidence. First, only the P rats showed increased discounting of delayed rewards compared to the Long Evans and HAD2s who were not different in delay discounting. Second, in the sipper tube paradigm, P rats showed elevated appetitive drive compared to both the HAD2s and the Long Evans who again were not different from each other. By contrast, consummatory measurement had a stepwise effect where the P's had a higher intake than HAD2s (although this is most likely due to appetitive influence) who in turn consumed more ethanol than the Long Evans. During 24 hour access with no appetitive requirement, both the P rats and the HAD2s had an equal elevated preference and intake of ethanol compared to the Long Evans. The patterns match between ethanol seeking and delay discounting, not consumption in the operant chamber or in the home cage.

These findings and experimental design are similar to that of Broos et al., (2012). They looked at delay discounting in relation to subsequent cocaine self-administration by comparing the most impulsive quartile to the least impulsive quartile. The two opposing quartiles were also not different in their overall levels of cocaine intake or have different dose response functions. This is in line with the current study where increased discounting of delayed rewards did not track with overall intake. Broos et al., (2012) also found that the two quartiles

responded the same on a progressive ratio test. While this is seemingly in conflict with the current study, an important point to consider is that a traditional progressive ratio breakpoint procedure is a combined measurement. The upper limit of responding reached could have been driven by attaining the desired dose.

In fact, closer examination indicates that the upper limit of responding was affected by reaching a specific dose. While Broos et al., (2012) do not report the actual dose obtained, or the unit dose per reinforcer for the test, calculation of the average number of rewards earned based on the breakpoint values and the equation used to calculate the fixed ratio requirement yield a value around 30 presentations. This is also the number of rewards earned during the dose response curve for the unit dose administered during their baseline measurement, the most logical dose to test a breakpoint on. Given that the dose response was assessed on an FR1 and the animals were only willing to work for 30 rewards in three hours, it seems unlikely that in the progressive ratio test, which could last up to 4 hours, rats would press an additional 2012 times to earn a 31st reward. Thus the lack of a difference on this breakpoint is actually consistent with the current findings as high levels of intake did not track with increased delay discounting. Furthermore, Broos et al., (2012) examined extinction responding for cocaine in both a neutral, and in the administration context. In both cases the high impulsive quartile showed greater responding. This occurred on the first day of extinction and later on into the extinction curve. They also measured context induced reinstatement. Again the high impulsive strain out responded the low impulsive strain.

Mackillop et al., (2010) examined heavy drinkers' demand curves for alcoholic beverages in a hypothetical bar situation in addition to delay discounting. They found that delay discounting was associated with the intensity of demand. The "intensity of demand" is a specific measure defined by the amount an individual would consume when alcohol is free. Other variables describing the curve such as breakpoint (the price at which individuals would no

longer purchase a single drink), the maximum total spending, the drink price at which maximum total spending occurs, and elasticity were not related to delay discounting. These results seemingly conflict with the idea that increased delay discounting tracks with appetitive behaviors as alcohol intake when it is free is directly relatable to consummatory behaviors. The price one is willing to pay (i.e., the strength of the seeking response) would fall under the appetitive domain. However, delay discounting was not the only variable that failed correlate with the demand curve variables. With the exception of the intensity of demand (consumption when free), all of the behavioral economic variables describing the demand curve failed to correlate with any of their alcohol related measures including drinks per week, alcohol use disorder severity, self-reported craving, and the number of heavy drinking days. The only correlations seen were with other variables describing the demand curve and income such that individuals who had more money were willing to pay more money. In this respect their results are an excellent demonstration of marginal utility theory, which in short states that the more one has of something the less utility or worth one unit of it possess (Pine et al., 2009). Thus individuals with a higher income were willing to spend more because a single dollar has less utility and is worth less to them because they have more money. Rather, alcohol use disorder severity and self-reported craving were related to delay discounting.

The results of Oberlin et al., (2010) also support delay discounting being related to ethanol seeking as oppose to simply consumption. Using the HAP mice they looked at the effects of amphetamine, naltrexone, and memantine on delay discounting and a limited access (30 minutes) two bottle choice procedure. They found that both naltrexone and memantine decreased ethanol intake but did not affect delay discounting. Conversely amphetamine did not affect drinking until the highest dose of 3 mg/kg, but affected delay discounting at only .4 mg/kg. However some caution must be taken as Czachowski and DeLory (2009) found that naltrexone would selectively attenuate ethanol seeking, and using similar doses Kieres et al., (2004) found it would not affect delay discounting except to

block a morphine induced increase. A possible reason for this incongruence comes from Mitchell et al., (2007) who found the effect of naltrexone to be moderated by an individual's locus of control. Another possibility is that delay discounting may be related to appetitive drive in general and not necessarily specific appetitive drive for alcohol. This idea is supported by the fact that Czachowski and DeLory (2009) saw that naltrexone did not decrease seeking for the control sucrose solution. Also Perry et al.,'s (2007) demonstration of delay discounting being different between rats selected for saccharin intake is in line with this idea.

Furthermore, increased delay discounting being related to seeking of multiple different drugs and not one specific one supports the idea that delay discounting is related to reinforcer seeking in general. The current findings demonstrate delaying discounting and seeking to be related for alcohol, Broos et al., (2012) for cocaine, and Dieraade et al., (2008) provides further evidence. Dieraade et al., (2008) looked at delay discounting and nicotine seeking and self-administration. Most interestingly, using a between session progressive ratio they found rats that were more impulsive in delay discounting emitted the same number of responses as low impulsive rats at low fixed ratios, but when the fixed ratio increased to upwards of 20 they responded more. This is same pattern Files et al., (1998) saw with the P rats compared to the HAD2s. Files et al., (1998) also examined the AA rats and found their pattern of responding be similar to the HAD2s and not elevated like the P rats.

That the P rats also "out responded" the AA in similar fashion as they did the HAD2s is an interesting point. Especially so when one considers Wilhelm and Mitchell's 2012 null result for delay discounting as a correlated trait with home cage intake when examined in the AA/ANA and sP/sNP. As determined by Files et al., (1998) the AAs do not possess the same heightened level of seeking as the P rats. If selection in the AA rats did not recruit alleles underlying increased appetitive drive, but only consummatory factors, this may explain why the lines

did not separate on delay discounting. Furthermore, comparison of the secondary variables on these two delay discounting tasks provides additional support for delay discounting being linked to appetitive drive. Unlike the P rats in the current investigation, neither the AAs nor the sPs exhibited decreased choice latencies, decreased trial initiation latencies nor a resistance to the effect of delay in increasing them (Wilhelm and Mitchell 2012). Rather the opposite was shown with both of the preferring lines being slower to initiate trials and had larger increases in latency with delay compared to their non-preferring counter parts as shown by a delay by line interaction.

However care should be taken, for this pattern was not seen in Perry et al., (2007) when the HiS and LoS were examined and found to be different. Furthermore, while Oberlin and Grahame's (2009) examination of the HAPs and LAPs looked at latencies very differently by breaking them up into bins within in a session, it appears there was no systematic effect across both replicate lines on latency measures. Overall the HAP1s appeared to have shorter trial initiation latencies than the HS/lbgs, their non-selected control line, but the opposite was seen with the HAP2s as the LAP2s showed shorter latencies. Another factor to consider with regard to with trial initiation latencies, is that trial initiation latencies are not solely a measure of appetitive drive. The attention a subject is paying is highly influential. It is quite possible that the HAPs, AAs, and sP exhibit attentional deficits that could be assessed in the five choice serial reaction time task. Using the HAP and LAPs as an example, attentional deficits would be pushing the reaction times in the opposite direction that increased appetitive drive would, hiding any effect.

One unexpected but highly interesting finding from the delay discounting analysis was the main effect of cohort. As mentioned before this shows how incredibly sensitive this phenotype is to environmental variability. The environmental sensitivity seen by delay discounting is what one would expect in a phenotype that mediates a heritable form of alcoholism. As mentioned before,

a family history of alcoholism does not guarantee that one will become an alcoholic. As pointed out by Cloninger et al., (1981) there are large environmental and gene by environment effects. The environmental sensitivity seen by delay discounting provides a means by which adopted away children of alcoholics, who likely have a genetic loading for alcoholism, may have fewer or no problems with alcohol.

This assumes that delay discounting is a causal factor, which brings us back to the three competing and non-mutually exclusive hypotheses of how impulsivity is related to alcohol abuse. Impulsivity causes drug abuse. Drug abuse causes impulsivity. The relationship between drug abuse and impulsivity is spurious. The results from the current study cannot speak to the second hypotheses that drug abuse causes increases in impulsivity as there was no reassessment of delay discounting after the sipper tube model and home cage drinking, nor was there proper control group necessary to make such an inference. A group drinking and responding for a nondrug reinforcer such as 2% sucrose during both the sipper tube model and 2 bottle choice procedure would have been necessary to rule out carry over, and developmental effects as confounds. However, the current findings are in support of both the 1st and 3rd hypothesis, but are unable to differentiate between the two.

While it was shown that increased delay discounting is related to appetitive process versus consummatory, one cannot infer that one causes the other. Furthermore, there could still be another variable that links the two. The decreased choice latencies seen by the P rats in the delay discounting task offers one possibility. The increased appetitive drive of the P rats causes them to choose faster. This, in turn, could result in a suboptimal decision that was not fully evaluated. On the other hand, pharmacological tests by Broos et al., (2012) are in support of a spurious relationship. At the end of their study they used their context reinstatement tests for cocaine to determine the effect of SCH-23390, a dopamine D1 receptor antagonist, and methylphenidate. They also tested these

same drugs for their effect on delay discounting. The D1 antagonist increased impulsivity and decreased responding in the context reinstatement test. Methylphenidate on the other hand decreased delay discounting, but increased cocaine seeking by several orders of magnitude. In both cases, the manipulations of delay discounting and seeking suggest that one is not causing the other. This is because one increased and the other decreased versus both either decreasing or increasing.

Another important factor to consider is whether the nature of the relationship depends on if one is looking at humans as opposed to animals. Using delay discounting as an example, there is a good theoretical reasoning as to why increased delay discounting could be causal in humans. They are making choices which have both delayed and immediate consequences. However, when looking at a rat this is not the case. One could argue that increased delay discounting is decreasing the value of alternative reinforcers. This idea is substantiated by Ping and Kruzich's (2008) finding that concurrent access to sucrose pellets decreased methamphetamine reinforcement in rats, and Comer et al., (1996) showing that access to a glucose plus saccharin solution decreased cocaine self-administration.

However unless one is specifically looking for an effect of alternative reinforcers, animals are usually in a "reinforcer desert" in self administration studies. This includes positive results examining the relationship between delay discounting and addition liability such as the current one, Broos et al., 2012, Dieraade et al., (2008), and Poulos et al., (1995). A better rationale comes from a decrease in the salience of delayed punishing stimuli. Woolverton et al., (2013) looked at histamine injections given at various delays after a cocaine injection. They found that delayed histamine punishment could decrease self-administration, and with increasing delays after the cocaine injection the histamine's ability to do so decreased. For alcohol, the mechanism of increased delay discounting causality could be via decreasing the influence of the

descending limb of blood ethanol concentration. However, while we know the descending limb is aversive (Holdstock et al., 2000), it would need to be shown to be punishing as well. Considering that in order to have a descending limb one has to have a reinforcing ascending limb first, methodologically this would be very difficult. Furthermore, the idea that an animal may discount the negative after effects of a drug, making the relationship a causal one, is difficult to reconcile with the previously stated evidence supporting delay discounting as being related to appetitive process in general versus drug specific. Not all appetitive stimuli have negative after effects.

Ultimately as the hypotheses are not mutually exclusive, it may be that all the hypothesized relationships between delay discounting and addictive disorders are true. This lends the possibility of a snowballing effect on addictive disorders. If there is a genetic relationship such that genes underlying pharmacological sensitivity to alcohol also underlie delay discounting in animals this could appear spurious. However, if delay discounting is causal in humans, then these alleles would be underlying two separate phenotypes that are both causal.

Furthermore increased appetitive drive and delay discounting could have an interactive effect. In an individual without steep discounting and a high appetitive drive, consideration of future consequences would keep increased seeking for a drug in check due to the substance's negative after effects. Also if value equates to reinforcing strength, other delayed reinforcing stimuli would take effort away from seeking the drug in favor of working for them. Conversely, if this individual was a steep delay discounter, the negative after effects and consequences of using the drug would no longer keep seeking the drug under control. Moreover, delayed alternative reinforcing stimuli would lose their strength due to their value being discounted. This, in turn, would result in responding and effort previously directed at obtaining these alternative reinforcers to be directed elsewhere. The immediate drug reinforcer now has a greater proportion of the

relative rates of reinforcement due to devaluation of the alternative reinforcers, and discounting of negative consequences associated with the drug. Consequently, the effort directed at obtaining the alternative reinforcers would now be directed at obtaining the drug. Thus increased delay discounting would disinhibit seeking from punishers, and take responding away from delayed alternative reinforcers. This hypothetical moderating effect of delay discounting on the relationship between appetitive process and addictive disorders would fit into the already existing framework comprised of the three hypotheses relating impulsivity to drug abuse. What this would look like is drawn out in figure 6.1.

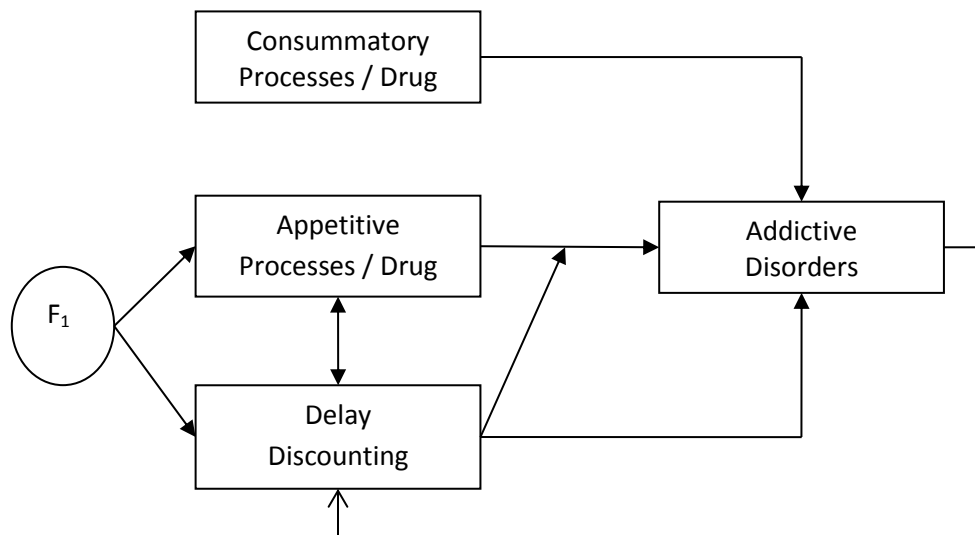


Figure 6.1: Model of possible interrelationships between delay discounting, appetitive processes, consummatory processes, and addictive disorders.

6.2 Future Directions

The model suggested for the interrelationships between delay discounting, appetitive process, and addictive disorders is still highly speculative and will need considerable amounts of future research to verify. One interesting study that would assess the interaction starts with an assessment of delay discounting. Then subjects would self-administer a drug under a fixed ratio schedule of

reinforcement with concurrent access to an alternative reinforcer also on a fixed ratio schedule of reinforcement. The key variables of interest would be the amount responding for both the drug and the alternative reinforcer. Also the proportion of the total responding for each option would be examined.

Initially the alternative reinforcer is delivered immediately to determine the effect of the alternative reinforcer in general. Then a delay is added to the presentation of the alternative reinforcer (ideally multiple different delays would be assessed). Once the delay is implemented the interaction can be assessed. More impulsive subjects should show greater increases in responding for the drug (in terms of total responses and the proportion of total responding) than less impulsive subjects.

The experiment also could be expanded to include a punishment aspect tied to drug responding. The question this would address is if the punisher can not only decrease responding for the drug reward but increase responding for the alternative reward. Similar to the alternative reinforcer, the punisher would be delayed. Then one would look to see if delay discounting moderated the effectiveness of the punisher. Furthermore under all conditions, single trial extinction sessions could be used to obtain an unadulterated seeking measure free from consummatory influence.

Another possible line of investigation comes from the main effect of cohort observed, and the environmental sensitivity of delay discounting. Perry et al., (2008) showed environmental enrichment decreased delay discounting behavior, and Deehan et al., (2007; 2011) recently showed that environmental enrichment can decrease responding on a within session breakpoint procedure for ethanol. As Deehan et al., (2007, 2011) did not use any means of procedural separation between seeking and drinking, an interesting question to answer is whether or not environmental enrichment differentially affects seeking or specifically drinking of ethanol.

Another interesting question along this line of thought is if the protective influence of environmental enrichment can be reversed by self-administration of alcohol or another drug of abuse. This also raises another question; given that heroin self-administration can increase delay discounting, (Schippers et al., 2012) could prior environmental enrichment decrease the heroin mediated increase in delay discounting?

The context reinstatement test, done by Broos et al., (2012) , brings up an important point. Current treatments that decrease impulsivity (i.e., methylphenidate), would not be appropriate to give to individuals with certain drugs of choice. Methylphenidate substitutes for cocaine in drug discrimination procedures (Rush et al., 2001). The multiplicative increase in seeking seen is to be expected since one is reinstating with both drug and context. Consequently, if the first hypothesis is shown to be true, that increased impulsivity is a causal factor, new treatments that decrease impulsivity need to be found. Currently stimulant medications, such as methylphenidate, are the frontline. However their pharmacological similarity to other drugs of abuse and their abuse liability make them unacceptable treatments. Atomoxetine as it is more selective for norepinephrine as opposed to dopamine and serotonin (Bymaster et al., 2002) may have more promise. Furthermore atomoxetine has been shown to decrease delay discounting and other forms of impulsivity (Robinson et al., 2008).

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