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Elucidation of pharmacologically manipulated responding in the delay discounting task in high alcohol preferring mice

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of

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For my family

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Meredith Halcomb

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LIST OF ABBREVIATIONS

S-D: Sprague Dawley rats

LH: Lister Hooded rats

WIS: Wistar rats

LE: Long Evans rats

C57: C57/B6 mice

sP: Sardinian Preferring rats

sNP: Sardinain Non-Preferring rats

AA: Alco-Alcoholic rats

ANA: Alco-Non-Alcoholic rats

G: Group housed

P: Pair housed

I: Individually housed

ABSTRACT

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Impulsive behavior is the hallmark of many psychopathologies. Uncovering the neurobiological mechanisms driving impulsivity is paramount in the development of through the delay discounting (DD) task in both human and animal models. The present study is an examination of the predictive validity of the two primary types of DD procedures in animals, the Adjusting Amounts (AA) and within session Increasing Delays (ID) tasks. *Methods:* Subjects were administered either1.25 mg/kg *d*-amphetamine (AMP), 1.5 g/kg ethanol (EtOH) or saline and tested in either the AA or ID method for 15 days to evaluate drug effects on impulsive behavior. *Results:* Stimulant administration resulted in a reduction of impulsivity in the AA group, but had no effect in the ID group. There was no effect on impulsivity of EtOH administration in AA or ID groups. *Conclusion:* Given the ability of stimulant administration to reduce impulsivity in clinical studies, the AA version of DD provides the best predictive validity for the animal model

CHAPTER 1. INTRODUCTION

1.1 Introduction

Impulsive behavior is an evolutionarily developed trait which motivates individuals to act in situations where they may have otherwise remained stagnant. In moderation, impulsivity drives conduct that is essential to survival, such as the ability to adapt to changing environments and deal with complex situations that require immediate action. Impulsivity has been defined as action without forethought or regard to consequences and may be delineated into separate, yet overlapping, constituent elements, including motor and cognitive impulsivity (Evenden 1999). The inability to physically inhibit a primed action is interpreted as motor impulsivity, while the disregard for future consequences or aversion to delayed rewards is representative of cognitive impulsivity. The fractionation of impulsivity is supported by findings in self-report studies. The Barratt Impulsiveness Scale (BIS) is categorized into smaller sub-scales representing these separate aspects of impulsivity, including motor impulsivity, non-planning impulsivity and cognitive impulsiveness, which maintain only moderate correlations with each other (Miller, Joseph et al. 2004). This segregation of impulsive drives allows researchers to develop more targeted methods for evaluation, ultimately increasing knowledge of the role of impulsivity in general behavior

Unfortunately, impulsivity may also lead to detrimental behaviors that are prominent aspects of many psychological disorders. Bipolar disorder, compulsive disorders, attention deficit-hyperactivity disorder (ADHD) and substance use disorders (SUDs) are all characterized by heightened levels of impulsivity, which invariably contribute to the many maladaptive behaviors observed in these populations (Swann, Anderson et al. 2001; Alessi and Petry 2003; Dawe and Loxton 2004; Winstanley, Eagle et al. 2006). Impulsivity is considered a contributing factor in the high levels of substance abuse and incidents of suicidal behavior in patients suffering from schizophrenia (Dervaux, Bayle et al. 2001; Gut-Fayand, Dervaux et al. 2001). Impulsivity is a also a state-independent trait of bipolar disorder, influencing behavior regardless of manic or depressive episodes (Swann, Anderson et al. 2001). Drugs demonstrated to reduce impulsivity in animal models are effective in attenuating suicidal behaviors in bipolar populations, highlighting the significance of impulsivity in those behaviors (Goodwin, Fireman et al. 2003; Ohmura, Tsutsui-kimura et al. 2012; Halcomb, Gould et al. 2013).

Impulsivity is a dominant facet of ADHD, and a heightened level of impulsive behavior is one of the diagnostic criteria of the disorder (Wolraich, Hannah et al. 1996). ADHD populations typically exhibit deficits in both inhibition of pre-potent responding on a Stop Signal Reaction Task (SSRT) (Nigg 2003) and increased errors of omission on the Continuous Performance Task (CPT) (Advokat, Martino et al. 2007), both measures of motor impulsivity. When ADHD populations are analyzed with the (DD) task, the primary measure of cognitive impulsivity, they consistently display a preference for a smaller, immediate reward over a larger delayed reward, indicating an inability to delay gratification or appreciate future consequences (Sonuga-Barke, Taylor et al. 1992;

Solanto, Abikoff et al. 2001). This impulsivity trait is associated with numerous academic and social issues, including lower overall success in both verbal and mathematical arenas, and difficulties with aggression and social interaction, which can extend past adolescence into adulthood (Barkley, Anastopoulos et al. 1991; McKay and Halperin 2001; de Boo and Prins 2007). There is also evidence that children diagnosed with ADHD are more likely to develop SUDs and alcohol use disorders in adolescence and adulthood than age-matched controls (Molina and Pelham 2003). Unfortunately, ADHD populations are not only more likely to develop SUDs, they are also less likely to commit to rehabilitation (Wilens, Biederman et al. 1998).

Not surprisingly, exaggerated impulsive behavior is also prominent in the pathogenesis of alcoholism and other SUDs (Moeller, Dougherty et al. 2001; Whiteside and Lynam 2009). Studies evaluating impulsivity with selfreport questionnaires, like the BIS or Eysenck Impulsiveness Questionnaire (EIQ), have demonstrated that scores reflecting elevated levels of impulsivity are correlated with earlier onset of alcohol consumption and increased frequency of binge episodes in adolescents (Lejuez, Magidson et al. 2010). In addition, higher scores on the BIS (indicating a higher level of impulsivity) also correlate with more severe symptoms and an earlier onset of alcoholism (Dom, Hulstijn et al. 2006).

Alcoholic populations typically display elevated levels of impulsivity in behavioral measures evaluating both motor and cognitive impulsivity, as well. Individuals diagnosed as heavy drinkers displayed an inability inhibit responding in the CPT and the Stop Signal Task (SST), both of which measure motor impulsivity. There is also evidence that a family history of alcoholism is associated with impulsive responding

in the DD paradigm, indicating effects on cognitive impulsivity, as well (Fallgatter, Wiesbeck et al. 1998; Petry 2001). Scores compiled from DD measures may also be used to accurately predict responses on the Alcohol Use Disorders Identification Test (AUDIT), highlighting the substantial correlation existing between these two behaviors (Murphy and Garavan 2011). In neurobiological terms, subjects from high-risk AUD families showed decreased right and left volume of the medial orbital-frontal cortex (mOFC) than subjects from healthy control families (Hill, Wang et al. 2009). The OFC is often implicated in emotion regulation and impulsivity, particularly in updating incentive values and perceived rewards (Winstanley, Theobald et al. 2004).

This same trend is observed in animal models of impulsivity, in which more impulsive subjects self-administer higher amounts of drug than subjects displaying lower levels of impulsivity (Perry, Larson et al. 2005). Additionally, animals which display elevated levels of impulsive behavior also demonstrate addictive-like behavior, continuing to self-administer even in the face of aversive outcomes (Belin, Mar et al. 2008). In a T-maze version of DD, animals that consistently responded in an impulsive manner (high impulsive) consumed significantly more of a 12% EtOH solution than two other groups (medium and low impulsive) (Poulos, Le et al. 1995). Tasks measuring motor impulsivity in animals have also uncovered evidence that subjects exhibiting difficulty with behavioral inhibition were more likely to self- administer cocaine than low impulsivity animals (Dalley, Fryer et al. 2007).

Given the demonstrated influence of impulsivity in the development and maintenance of alcoholism and other psychological disorders, increasing knowledge of underlying neurobiological mechanisms regulating impulsive behavior is paramount for

the structuring of viable treatment options. The dissimilation of impulsivity into separate factors aids in this endeavor. There are several primary measures of motor impulsivity in both human and animal models, including the CPT and its animal analogue, the 5 Choice Serial Reaction Time Task (5CSRTT), the Go No Go Task and the SST. Cognitive impulsivity has also been heavily investigated with the DD task which allows researchers to evaluate choices and responding in a systematic, behavioral form. It has been successfully adapted for use in animal models as well, allowing evaluation and manipulation of neural processes that are not available in human studies.

1.1.1 Delay Discounting

DD is based on the premise that the ability of some reinforcer to influence behavior decreases as a function of the delay to its delivery (Ainslie and Herrnstein 1981). As the time for reward delivery becomes more imminent, the subject will reverse preference from the larger, delayed reinforcer to the small, but immediately available reinforcer. This reversal of preference and accompanying propensity to choose immediate over delayed rewards can be described empirically through the use of the hyperbolic discounting function. The equation is $V = (A/1+kD)$, wherein V represents the reward value at delay D, A is the non-discounted subjective value of the reinforcer and k is a free parameter used to typify individual variations in impulsivity (Bickel and Marsch 2001). In a DD study, participants are asked to choose between a small, immediate reinforcer and a larger, delayed reinforcer. A persistent preference for the immediate reinforcer is thought to indicate a higher level of impulsivity. This choice ultimately results in suboptimal amounts of reinforcer, suggesting the participant is disregarding the consequences. The reinforcer amounts and delay lengths are titrated throughout a session

or across several sessions and the hyperbolic function is employed to determine discounting rates for individuals or groups.

Although the influence of impulsivity in maladaptive behavior would ideally be accomplished through evaluation of human subjects, clinical studies present limitations that reduce their efficacy. They are often plagued with confounding variables and several pharmacological manipulations are not available for administration in human subjects; therefore, animal models can be a more practical method for evaluation of impulsivity. A key aspect of the viability of an animal model is its translatability to human results or predictive validity. Predictive validity is essential to establish the usefulness of the model. If data uncovered in an animal model cannot be translated to human subjects, its utility in research is severely hampered. The viability of an animal model rests in its ability to aid in understanding behavior or neural mechanisms driving behavior in humans; therefore, the subjects in those models must demonstrate similar behavioral changes or adaptations to those observed in human versions of the task.

The evaluation of predictive validity is often determined through the use of pharmacological manipulations. Drug administration should produce similar effects on impulsivity in both human and animal populations. Pharmacological predictive validity has been established for animal models of several disorders, including depression and panic disorders (Jenck, Moreau et al. 1995; Willner 1995). A great deal of research has sought to establish the predictive validity of DD for its use in animal models. Traditionally, findings from these investigations have supported the translatability of DD (Monterosso and Ainslie 1999). Animal subjects tend to discount future rewards in

the same hyperbolic manner as humans, displaying a reversal in preference for the small, immediate reward as the delay to the large reward increases.

Unfortunately, studies have thus far failed to definitively establish the predictive validity of DD through pharmacological manipulations. Numerous investigations into DD have revealed that while there are often behavioral changes associated with drug administration in the animal version of DD, these changes are not always consistent with changes observed in human populations (Winstanley 2011). These discrepancies alone are not sufficient to eliminate the suitability of the task; rather, they call into question particular aspects of the task which may be influencing results.

Although the basic premise for DD is straightforward, the procedural methodology is more malleable, which may underlie the heterogeneity observed in results. There are numerous avenues for discrepancies, including housing environment, reward magnitudes, baseline impulsivity levels and the use of cue lights to signal a reward; however, the most prominent alterable aspect of the task is the method used to present the various reinforcer amounts and delay lengths. There are two primary versions of DD utilized in animal models: the adjusting amounts (AA) method and the within session increasing delays (ID) method.

1.1.2 Adjusting Amounts

In the AA version of DD, the amount of reinforcer available immediately is titrated up and down based upon the responses of the animal, while the amount of reinforcer available after a delay does not vary. During the session, a choice of the immediate reinforcer will result in a reduction of reinforcer available immediately on the next trial. A choice of the delayed reinforcer increases the amount of reinforcer available

immediately on the subsequent trial. The length of the delay to the large reward remains stable throughout a single session. An indifference point is the amount of reward available on the immediate side when the subject is choosing both options equally. This is a measure of the subjective value of the delayed reward. These indifference points are established for several different delays and then used to create a discounting curve based upon the hyperbolic discounting equation (Rubinstein 2003). A disadvantage of this method is that testing requires several sessions since each session only represents one delay and animals are often tested for several sessions at each delay.

1.1.3 Increasing Delays

The ID method is more straightforward. During one session, the amounts of reinforcer available immediately or after a delay remain unchanged, while the length of the delay increases across blocks of trials. This allows accumulation of data for several delays in one session; however, there is a major drawback investigators must contend with when implementing this type of DD. As a session begins, there is no delay to the large reinforcer, however, after a certain number of trials, the delay is initiated and is repeatedly increased across a one- or two- hour session. It is possible that responses are influenced through a contrast effect: the tendency for an animal to perceive a reward value as diminished or enhanced relative to normal as a result of exposure to a greater or lesser value (Bower 1961). In fact, recent research suggests that contrast effects are a prominent aspect of behavior in this task (Lattal and Smith, 2011). More recent evidence indicates that responding in this task may also be directed by perseverative behavior, resulting in consistent responding that is inflexible to delay implementation and entirely independent of impulsivity (Maguire, Henson et al. 2014; Tanno, Maguire et al. 2014).

1.2 Pharmacological Manipulations

Pharmacological manipulations are important tools in investigating underlying neurobiological mechanisms driving impulsivity. DD has been used to evaluate numerous neurotransmitter systems, brain regions and drug classes. In addition, these studies help provide predictive validity for animal models. Several investigations have demonstrated that both human and animal subjects discount future rewards in a hyperbolic manner; however, pharmacologically- driven alterations in behavior in the DD task in humans and animal subjects do not always replicate.

1.2.1 Stimulants

Given the established ability of stimulant medications, such as *d*-amphetamine (AMP) and methylphenidate, to reduce impulsive behavior in ADHD populations (Greenhill, Kollins et al. 2006; Van der Oord, Prins et al. 2008) and the high prevalence of stimulant misuse in substance abusing populations, these drugs are the most commonly studied in both clinical and pre-clinical research (Maxwell and Rutkowski 2008).

In human studies, stimulants are typically successful at reducing impulsivity in the DD task (de Wit, Enggasser et al. 2002; Pietras, Cherek et al. 2003; Shiels, Hawk et al. 2009); although patients with ADHD may present difficulty introspectively assessing behavior in a hypothetical rewards paradigm (Shiels, Hawk et al. 2009). Stimulants are also the mainline treatment for ADHD and are effective at reducing the hyperactive impulsivity aspect of the disorder (Castle, Aubert et al. 2007). This suggests that stimulant administration, as noted in clinical settings, results in a decrease in impulsive behavior.

Unfortunately, when these drugs are administered in the animal model of DD, the results are largely inconsistent and contradictory.

Table 1 Acute AMP effects on impulsivity

DRUG	DOSE (mg/kg)	STRAIN	HOUSING	VERSION	CUE	AUTHOR	IMPULSIVITY
AMP	0.03, 1.0	S-D	G	ID	No	Evenden *et al*, 1996	Increase
	0.3 - 1.6	LH	P	ID	No	Cardinal *et al* , 2000	Increase
	0.5 - 2.0	S-D	G	AD	Yes	Perry *et al* , 2008	Increase
	0.032 - 1.0	S-D	?	ID	No	Koffarnus *et al* 2011	Increase
	0.3 - 1.6	LH	P	ID	Yes	Cardinal et al, 2000	Decrease
	1.0, 1.5	LH	P	ID	No	Winstanley *et al*, 2003	Decrease
	0.3 - 1.5	LH	G	ID	No	Winstanley *et al* , 2005	Decrease
	0.2 - 1.0	WIS	P	ID	No	van Gaalen *et al*, 2006	Decrease
	0.5, 1.0	S-D	P	AA	Yes	Wade *et al* 2000	Decrease
	0.4 - 1.2	HAP II	I	AA	Yes	Oberlin *et al* , 2010	Decrease
	0.5 - 2.0	S-D	I	AD	Yes	Perry *et al* , 2008	Decrease
	0.1 - 1.7	LEWIS	I	ID	No	Huskinson *et al* , 2012	Decrease
	0.4 - 1.0	C57	G	ID	No	Isles *et al* , 2003	Dec/Inc
	0.1 -1.78	S-D	I	ID	No	Tanno *et al*, 2014	Dec/Inc
	0.32 - 1.78	S-D	I	ID	No	Maguire *et al*, 2014	Dec/Inc
	0.25 - 1.0	LE	I	ID	Yes	Barbelivien, *et al* , 2008	Decrease
	0.1 - 1.7	F344	I	ID	No	Huskinson *et al* , 2012	No Effect

The DD task may vary along many dimensions, including the type of animals used, the manner in which they are housed, the use of a cue light to signal a response and the version of the task administered. Early work was aimed at uncovering a single factor affecting results. One study found that the use of a cue light to signal a delayed response (serving as a bridge between response and reward delivery) would create a decrease in impulsivity, while eliminating the cue light would lead to an increase in impulsivity (Cardinal, Robbins et al. 2000). This observation failed to be consistently replicated, however, and further studies evaluated baseline levels of impulsivity (Barbelivien, Billy et al. 2008; Huskinson, Krebs et al. 2012). While these studies reveal an effect of pre-

drug responding on behavioral changes after administration, the data are not consistent. One study determined that only moderate levels of baseline impulsivity may be altered by drug exposure (Barbelivien, Billy et al. 2008). An alternate study observed that animals exhibiting high levels of baseline impulsivity exhibit a decrease in impulsive behavior and animals with low levels of baseline impulsivity will increase impulsive behavior after stimulant administration (Huskinson, Krebs et al. 2012). Another variable evaluated above is housing type. Whether group, pair or individually housed, no one type of housing was found to be consistently associated with either an increase or decrease in impulsivity.

Evaluation of the contribution of each of these factors to behavior revealed that only one component of the task was consistently associated with replicable results: the version of the task. When the AA task is administered, there was always a reduction in impulsivity after stimulant exposure (Richards, Sabol et al. 1999; Wade, de Wit et al. 2000; Oberlin, Bristow et al. 2010). In contrast, presenting animals with the ID version of DD leads to either a decrease or increase in impulsive responding or no effect at all after stimulant administration (Evenden and Ryan 1999; Cardinal, Robbins et al. 2000; Isles, Humby et al. 2003; Winstanley, Dalley et al. 2003; Pitts and McKinney 2005; Winstanley, Theobald et al. 2005; van Gaalen, van Koten et al. 2006; Barbelivien, Billy et al. 2008; Koffarnus, Newman et al. 2011; Slezak and Anderson 2011; Huskinson, Krebs et al. 2012; Maguire, Henson et al. 2014; Tanno, Maguire et al. 2014).

More recent research has attempted to uncover possible effects of the order of presentation of the delay in the ID method. These studies have determined that if the delays are presented in an ascending order, as they are generally given, then AMP

exposure will result in a decrease in impulsivity; however, if the delay order is reversed and the session begins with the longest delay and decreases across blocks, then AMP administration results in an apparent increase in impulsivity (Maguire, Henson et al. 2014; Tanno, Maguire et al. 2014). A possible explanation is that the animal is perseverating on the lever that is associated with the best option in the first block of trials. AMP causes increases in perseverative behavior, increasing the likelihood that the results of this study are influence by this type of responding rather than a change in impulsivity (Evenden and Robbins 1983). Although the findings from Tanno (2013) and Maguire (2014) are intriguing, it is clear that not all studies utilizing an increasing order of delays uncover decreases in impulsivity. Thus far, there is no definitive research clearly indicating a consistent factor contributing to the discrepancies found in the stimulant literature.

1.2.2 Alcohol

Although the majority of pharmacological manipulation studies in DD evaluate effects of stimulant administration, behavioral response alterations after alcohol exposure are also investigated. Given the strong association between alcohol use disorders (AUDs) and heightened impulsivity levels, most studies seek to determine if impulsivity is a consequence of or a risk factor for alcoholism. Animal research demonstrates that animals selectively bred to prefer and consume pharmacologically relevant amounts of EtOH are significantly more impulsive than animals bred to avoid alcohol intake (Oberlin and Grahame 2009). In human studies, twin data also suggest that heightened impulsive behavior is significantly associated with SUDs (Anokhin, Golosheykin et al. 2011). These studies indicate a genetic component to impulsive behavior, but inherited versus state-dependent impulsivity are not mutually exclusive. Studies evaluating alcohol effects

in human subjects have found increases in impulsive behavior in the Continuous Performance Task (CPT) and the Go No Go Task, measures of motor impulsivity (Dougherty, Moeller et al. 1999; Easdon, Izenberg et al. 2005). Unfortunately, research examining effects of acute alcohol administration on DD in human subjects has not provided any definitive answers, reflecting both increases and decreases in impulsive responding or no effect at all (Richards, Zhang et al. 1999; Ortner, MacDonald et al. 2003; Reynolds, Richards et al. 2006).

Evidence from DD tasks implementing a maze version of the task find increases in impulsivity after EtOH exposure (Poulos, Parker et al. 1998). Research in operant versions of the animal model has uncovered an increase in impulsivity whenever the ID version of DD is administered, but no effect when the AA version is administered (Tomie, Aguado et al.1998; Evenden and Ryan 1999; Hellemans, Nobrega et al. 2005; Wilhelm and Mitchell 2012).

Table 2 Acute EtOH Effects on Impulsivity

DRUG	DOSE	STRAIN	HOUSING	VERSION	CUE	AUTHOR	IMPULSIVITY
CNS DEPRESSANTS							
ETOH	0.3, 1.0 g/kg	S-D	G	ID	No	Evenden *et al*, 1999	Increase
	0.25 - 1.5 g/kg	LE	I	ID	No	Tomie *et al* , 1998	Increase
	0.3 - 1.2 g/kg	LE	G	ID	No	Hellemans *et al* , 2005	Increase
	0.3 - 1.2 g/kg	LE	I	ID	No	Hellemans *et al* , 2005	Increase
	0.25, 0.5 g/kg	sP	?	AA	?	Wilhelm, 2012	No Effect
	0.25, 0.5 g/kg	sNP	?	AA	?	Wilhelm, 2012	No Effect
	0.25, 0.5 g/kg	AA	?	AA	?	Wilhelm, 2012	No Effect
	0.25, 0.5 g/kg	ANA	?	AA	?	Wilhelm, 2012	No Effect

An evaluation of the methods employed by these studies reveals a number of possible confounding variables which likely influenced findings. In the Evenden study, there were no forced choice trials at the beginning of each block, decreasing

the likelihood that the animal was adequately informed of the new contingencies in each block prior to choice (Evenden and Ryan 1999). In the Tomie (1998) study, only 50% of the rats were deemed to be sensitive to the delay, in that they consistently chose the immediate reward even when the delay to the large reward was 0 seconds. Although this is a very rare occurrence in the literature, the authors do not attempt to explain the phenomenon and it seems clear that some confounding variable likely contributed to this anomaly. It calls into the question the results since the animals apparently had great difficulty performing the task even prior to pharmacological manipulation. The animals in the Hellemans (2005) study were exposed to the delays in a descending, rather than an ascending order, in contrast to the majority of ID studies (Hellemans, Nobrega et al. 2005). There is evidence that the presentation order of the delays can completely alter response patterns and it is difficult to rely on findings that are generated from a protocol that is not in keeping with the generally accepted parameters (Tanno, Maguire et al. 2013).

The Wilhelm (2012) study, the only investigation which employed the AA method, has two primary issues detracting from its results. The study only assessed responding at a 4 second delay, which is the median delay. This delay was evaluated to avoid floor or ceiling effects surrounding the lower or higher delays. The concern with using this delay is that there is a great deal of variability at median delay, making it more difficult to detect effects. In addition, the dose of EtOH administered, 0.5 g/kg, was lower than any effective dose from previous studies and there is no evidence that this dose elevated blood alcohol to pharmacologically relevant levels. The significance of determining the influence of alcohol on impulsivity cannot be

denied, yet from the studies described here, there is as of yet no definitive resolution. A thorough examination of the effect of acute EtOH administration in both the ID and AA methods using doses of EtOH known to produce pharmacological effects would aid in illuminating this relationship more thoroughly.

As stated above, research evaluating the effects of alcohol administration on DD in human subjects is largely inconclusive; however, these studies were conducted in healthy populations, rather than samples with a family history of alcoholism or current alcoholics. A family history of alcoholism is associated with relatively elevated levels of impulsivity and assessing alcohol effects in these populations may potentially illuminate factors contributing to overconsumption in these populations (Linnoila, De Jong et al. 1989). Binge drinking has been repeatedly correlated with increases in impulsive behavior among college students (Balodis, Potenza et al. 2009; Carlson, Johnson et al. 2010), which is particularly problematic in alcoholic populations. Understanding the relationship between alcohol overconsumption an impulsive behavior would significantly increase the ability to develop treatment options and efficacy.

One method for evaluation of this association is through the use of animal models of alcoholism. The high alcohol-preferring (HAP) mouse lines have been selectively bred to prefer EtOH over water and typically display elevated levels of impulsive behavior in the DD task, compared to animals selectively bred to be low alcohol-preferring (LAP) (Oberlin and Grahame 2009). These traits make the HAP mice ideally suited for investigation of the effects of EtOH on cognitive impulsivity. Since they consistently display high levels of impulsive behavior, there is generally

little variability in responding within cohorts, increasing the ability to detect effects of pharmacological manipulation.

Clearly the AA and ID procedures operate in drastically disparate fashions. However, at this time, there have been no investigations directly comparing the results from these two versions of DD. Although both methodologies yield discounting curves that are comparable with human findings, there are often discordant results found in studies using pharmacological manipulations. There are two primary differences between these methods that may contribute discordant findings. In the AA version, the investigator is able to derive a specific indifference point for each delay, which may lead to a more accurate description of behavior. In addition, the subject is allowed to titrate the amount of reinforcer available immediately, describing the subjective value of the delayed reinforcer, and is not required to adjust to new contingencies within a one- or two-hour session. This is particularly important when administering a drug which may impair short term memory or attentional processes.

During the ID version, the animal is rapidly exposed to increasingly longer delays and there is no mechanism for identifying an indifference point. In fact, this rather rapid method of increasing the delays within a session inherently supposes that the animal is able to correctly comprehend the new contingencies presented in each block and is responding with that knowledge. Although the ID version has been demonstrated to reflect impulsive responding in the same hyperbolic manner that human DD studies generate, recent evidence suggests that contrast effect and perseveration may be influencing behavior rather than impulsivity (LAS RESPUESTAS, DEMORADO et al. 2011). An evaluation of the possible differences in responding

prompted by the administration of a specific method of DD, particularly with pharmacological manipulations, will ultimately establish a better foundation for conclusions from those studies.

1.3 Specific Aims and Hypotheses

1.3.1 Specific Aim 1

In order to evaluate the possible impact of task administration procedures on responding, a series of experiments was designed assessing impulsive behavior after stimulant or EtOH exposure in either the AA or ID method of DD. The first aim of this study was to evaluate the ability of both versions of DD to create a hyperbolic shift in preference to the immediate reinforcer when the delay to the larger reinforcer is increased. All animals were trained to perform a series of behaviors to receive reward and then assigned to either the AA or ID version of DD and administered EtOH, (AMP) or saline. It was hypothesized that both methods of DD would result in hyperbolic discounting.

1.3.2 Specific Aim 2

Considering the controversy surrounding the results from stimulant administration in the DD task in animals, the primary goal of the second aim was to evaluate the effect of AMP administration in both versions of DD, when all other possible variables are identical. Other variables, previously investigated, such as the use of the cue light, the baseline levels of impulsivity and the housing conditions will remain constant between the two groups. It was expected that AMP administration would lead to a decrease in impulsive responding in the AA condition, particularly at the longer delays. This expectation is supported by previous work both in the literature and our own lab (Wade, de Wit et al. 2000; Oberlin, Bristow et al. 2010).

The ID version of DD is more difficult to predict. As described above, previous work with this method has sought to uncover possible confounds in administration practices to explain discrepant findings. These include varying housing conditions, evaluating baseline levels of impulsivity, the use of cue lights to signal a response or strain differences (Cardinal, Robbins et al. 2000; Barbelivien, Billy et al. 2008; Huskinson, Krebs et al. 2012). While all of these studies have added to the overall picture, none of them have definitively explicated the conflicting results and replicating results has proven difficult.

There have been no direct comparisons between AA and ID where the version of task is the only differing variable, with all others being held constant. Although the ID method of DD creates a decrease in preference for the delayed reward across time, it is possible that other factors, such as attention, perseveration and contrast, may be influencing responding as much as, if not more than, impulsivity. There is evidence that AMP withdrawal results in an increase in successive negative contrast effects, which suggests that there are likely effects of repeated exposure to AMP (Barr and Phillips 2002). In addition, AMP is known to play a role in attention and perseveration, which may hamper the ability of the animal to perform in the ID task (Evenden and Robbins 1983; Weiner, Lubow et al. 1988). If the subjects behave in a perseverative or habitual manner, the results were hypothesized to reveal an apparent decrease in impulsivity after AMP administration due to the inflexible responding on the large, delayed reward lever. If, in contrast, the animal shaped responding based upon a negative contrast effect, there was predicted to be either no effect or an increase in impulsivity.

1.3.3 Specific Aim 3

Although human studies in DD have not established the effect of acute alcohol administration on cognitive impulsivity, the research from the animal model has revealed a pattern of delineated results based upon DD version. Given the previous work conducted in our lab and the work in other labs utilizing the AA procedure , an effect of acute EtOH administration on impulsivity was not expected in the AA version of this task (Wilhelm and Mitchell 2012).

An inherent component of the ID version of DD is time perception. The delay to the large reward increases in approximately 10 – 20 minute intervals, forcing the subjects to adjust responding in a more rapid manner than in the AA task. EtOH affects time perception through a deceleration of time sense, possibly decreasing the preference for the large, delayed reward by increasing the perceived wait to delivery (Tinklenberg, Roth et al. 1976) Given the effects of EtOH on attention and spatial working memory, it was hypothesized that acute EtOH exposure would result in an increase in impulsivity in the ID groups (Givens 1995; Givens and McMahon 1997). The premise of the ID task includes the ability to understand new contingencies and focus attention on those current parameters, which would be greatly hampered by EtOH administration. These deficits were predicted to result in an increase in preference for the immediate reward in the ID task.

These studies were designed specifically to ascertain the most reliable animal model of DD and to confirm the impact of administration procedures on responding when all other possible variables are held constant. An animal model is considered reliable

when its results may be translated to clinical studies and used to increase knowledge about neurochemical or behavioral responding in humans.

CHAPTER 2. METHODS

2.1 General Methods

2.1.1 Training

All mice were trained identically to operate the manipulanda over the course of four stages. The operant chambers are in sound and light attenuating boxes. During Stage 1, the chamber was fitted with a nose poke illuminated with an LED light and a sipper descender containing a 0.312% saccharin in tap water solution located in the center front plate of the chamber. The animals were given non-contingent access to the reinforcer for 10 seconds every 30 seconds and any nose poke resulted in 10 seconds of access. In Stage 2, access to reinforcer is only available upon the animal completing a nosepoke. The animal was given 5 seconds of access and all nose pokes resulted in reinforcer administration. Once animals completed a minimum of 18 trials per one-hour session, they progressed to Stage 3. During this stage, once a nosepoke was completed and reinforcer access ended, the nosepoke light was extinguished and the chamber was in a timeout state for 30 seconds. Nose pokes during the timeout were recorded but did not result in reinforcer delivery. After subjects met the criterion of 18 trials completed they were moved to Stage 4. At this point, levers were inserted on either side of the nosepoke apparatus directly beneath LED lights. For each trial, the animals had to perform a

nosepoke, which extinguished the nosepoke light and resulted in illumination of the LED lights above each lever. When the animal selected a lever, the other lever light was extinguished and the animal was given reinforcer access for 5 seconds. After reinforcer delivery, a 30 second timeout began. All nose pokes and lever presses were recorded. When the subjects reached a criterion of at least 18 completed trials for three out of four consecutive days, they were separated into AA and ID groups balanced across mean completed trials for the least three days of training. Because the lever associated with the delay to reinforcement may create a conditioned place aversion, delay levers were assigned to the preferred lever for each mouse. Each of the protocols for the DD tasks were designed to represent the most common practices employed in previous studies.

2.1.2 Adjusting Amounts Procedure

In this version of DD, when a session begins, a choice of the immediate lever results in one second of access to the reinforcer immediately. A choice of the delayed lever results in two seconds of access to the reinforcer after a set delay. Each choice of the immediate reinforcer decreases access time to the immediate reinforcer on the next trial by 0.2 seconds. A choice of the delayed reinforcer increases the access time to the immediate reinforcer on the next trial by 0.2 seconds. In this way, the amount of reinforcer availability titrates up and down across the session until the subject reaches a point when it chooses both sides equally. The amount of reinforcer (adjusted amount) available at that time is called the indifference point and represents the subjective value of the delayed reinforcer. The delay to the large reinforcer remains stable throughout the session and each delay is tested for several consecutive days.

During training, the delay is set at 0 seconds and the animal must demonstrate that it has magnitude discrimination. In other words, when there is no delay to the large reinforcer, the subject should be choosing the large reward the majority of the time. This is determined by an adjusted amount of at least 1.6 for three out of four consecutive days. High adjusted amounts are indicative of a preference for the large or delayed reinforcer. Once an animal has demonstrated magnitude discrimination and completed 18 or more trials in one session, the subject moves to the testing phase wherein delays are incorporated for the large reinforcer. The primary dependent variable is the indifference point for each animal. Trials completed and volumes of reinforcer consumed are also evaluated.

2.1.3 Increasing Delay Procedure

In the ID task, there are a set number of trials per session and the reinforcer sizes remain at 0.5 seconds immediately or two seconds of access availability after the delay. The session is broken up into five blocks of 10 trials each, for a total of 50 trials. The first two trials of each block are forced choice for the immediate and delayed reinforcers. During the first block, there is 0 second delay to the large reinforcer and it is increased across the blocks.

Each trial consists of a nose poke and a lever press and is exactly 72 seconds long, which includes an inter-trial interval (ITI). The length of the ITI is dependent upon how long it takes the subject to complete the trial. The subjects have 20 seconds to initiate a nose poke. If they fail to, the light is extinguished and the chamber moves to the ITI state until the next trial. If a nose poke is performed, the nose poke light is turned off and the LED lights above the levers illuminate. The animals have 10 seconds to complete a lever

press. If they fail to complete a lever press, the lights are extinguished and the chamber returns to the ITI state until the next trial. If the animal chooses a lever, the light above the other lever is turned off and the sipper tube descends. Once the sipper access is concluded, all lights are extinguished until the next trial.

The primary dependent variable is the percent choice of the large reinforcer in free choice trials in each block. In order for the subject to proceed to the testing phase, the subject must demonstrate magnitude discrimination at the 0 second delay, choosing the large reward at least 85% of the time in the first eight free choice trials. They must also demonstrate a discounting curve, choosing the delayed amount less than 35% of the time at the longest delay. In addition, the animal was required to complete at least four free-choice trials in a block for those data to be considered accurate measures of choice for that block.

2.2 Pilot Study

2.2.1 Subjects

For the pilot study, conducted in order to verify the ability to detect a discounting curve in high alcohol-preferring (HAP) replicate line II mice in the ID version of DD, 12 male and 12 female HAP II mice, born between 1/1/14 and 1/12/14, were trained in the ID task. Training began when the animals were ~ 45 days old. They were individually housed and kept on a reverse light/dark cycle with lights out at 7:00 am and back on at 7:00 pm. They were given ad libitum access to food and access to water for two hours per day.

2.2.2 Methods

The animals were trained in the ID method outlined above. Delays to the large reinforcer were initially set at 0, 2, 4, 8 and 12 seconds. These delays were based upon previous research in our lab and available literature from other labs using mice rather than rats (Isles, Humby et al. 2003). The training progressed as outlined above, however, the mice were not consistently choosing the large reward at the 0 second delay and the trial numbers were extremely low. In order to encourage the mice to choose the large reinforcer, and to increase trial numbers, the delays were lowered to 0, 1, 2, 4 and 8 seconds. After 11 days of ID training, the animals were still not completing enough trials, so the saccharin concentration was increased from 0.032% saccharin in tap water to 0.32% saccharin in tap water, which is the preferred concentration for HAP mice (Oberlin, Best et al. 2011). Access times for the reinforcers were 0.5 seconds for an immediate reinforcer and 2.0 seconds for the delayed reinforcer. This ratio of 3:1 for reinforcer sizes is typical in many ID studies, although there is no specific standard.

2.2.3 Statistics

In order to determine gender influence, two repeated measures ANOVAs were run examining the percent choice of the large reward and trials completed per delay block. These were 2 X 5 designs used to evaluate any possible effects or interactions of sex and delay on responding. Following these analyses, two repeated measures ANOVAs were run to assess the effect of delay on percent choice of the delayed reward and trials completed per delay block, collapsed across sex. Follow up *t*-tests were used to examine differences between the 0 second and 8 second delay in percent choice of the large reinforcer and number of trials completed. Also, a paired samples *t*-test was used to

analyze any changes in trial numbers completed after delays were lengthened and saccharin concentration was increased.

A paired samples *t*-test was conducted to compare volume of saccharin solution consumed during the session prior to ID training and on the last day of ID training. An increase in volume consumed would suggest a devised strategy to increase saccharin access and demonstrate the ability of this saccharin reinforcer to motivate behavior. In addition, since this task has not been evaluated in HAP mice, it is helpful to determine the mean amount of solution consumed without any type of manipulation. To identify vigilance decrement, a paired samples *t*- test was used to analyze mean nosepoke latencies in the first block of trials compared to the last block of trials. Vigilance decrement is the tendency for subjects to increase response time across the course of a single session, decreasing the number of trials completed as the session comes to a close. This may be particularly problematic in the ID task since it results in significantly fewer exposures to the longest delay. All of these factors may be influenced by pharmacological manipulations in future studies.

2.2.4 Results

After 39 days of training, 14 mice out of 24 were able to meet criteria of greater than 85% preference for the large reward at 0 second delay and less than 35% choice of the large reward at the 8 second delay. Three mice were removed for failure to meet criteria to begin ID training (not completing the nose poke, lever press chain) and five were removed for failure to demonstrate magnitude discrimination. The remaining two mice were removed for continuing to choose the large reward at the longest delay, indicating a lack of discounting.

The statistical analyses determined no main effect of sex on percent choice of the delayed reward $F(4,48) = 1.41$, $p > 0.05$ or an effect of sex on number of trials completed, $F(4,48) = 0.18$, $p > 0.05$. There was also no interaction between sex and delay on percent choice of delayed reward, $F(4,48) = 0.89$, $p > 0.05$, nor an interaction of sex and delay on number of trials completed, $F(4,48) = 1.38$, $p > 0.05$. As a result, all further analyses were collapsed across sex. There was a main effect of delay on percent choice of the delayed reward $F(4,52) = 52.31$, $p < 0.001$ (**Figure 1**). A follow up paired samples *t*-test revealed that the subjects were choosing the large, delayed reward significantly less at the longest delay block than at the no delay block, $t(13) = 24.04$, $p < 0.001$ (**Figure 1**).

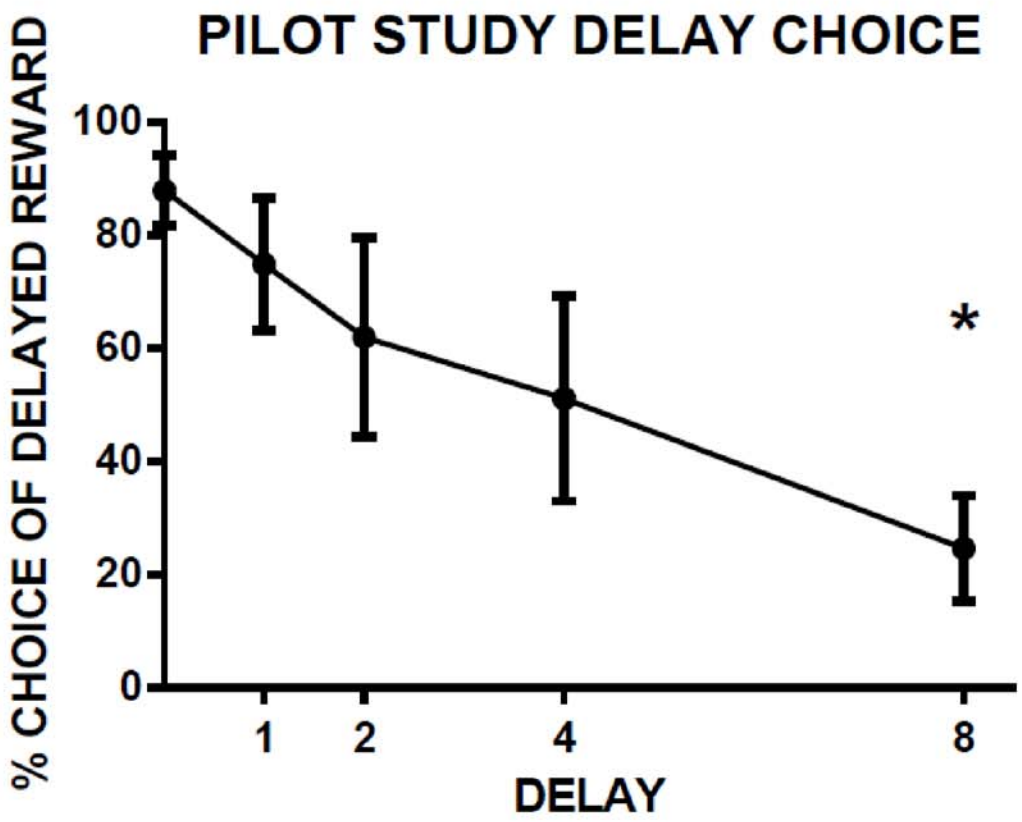

Figure 1. Percent choice of the large, delayed reward by delay (N= 14). There was a significant difference in choice of the large, delayed reinforcer between the 0 second and 8 second delay blocks. These are calculated from the mean of the final three sessions.

There was also a main effect of delay on number of trials completed, with trials numbers decreasing by block across a single session, $F(4,52) = 37.40$, $p < 0.001$ (**Figure 2**). The follow up paired samples *t*-test found a significant difference between the 0 second and the 8 second delay in number of trials completed, $t(13) = 6.39$, $p < 0.001$ (**Figure 2**).

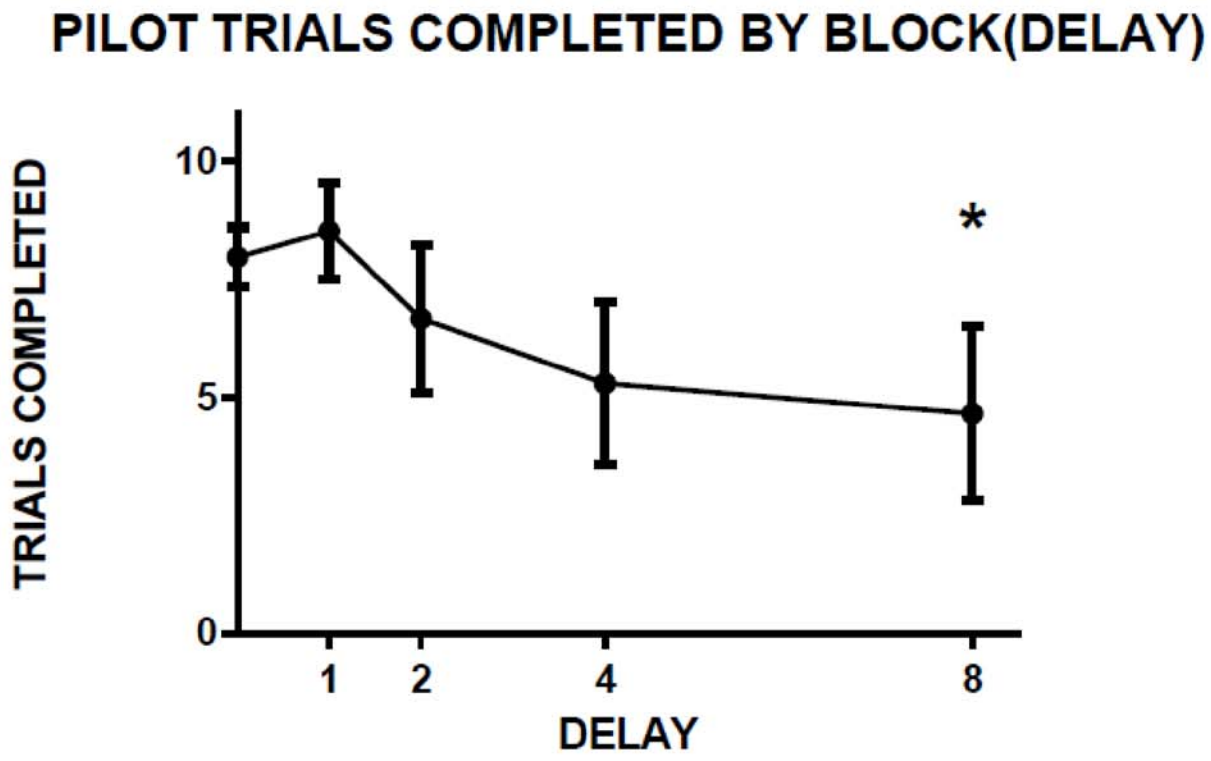

Figure 2. Mean number of trials completed per belay block across the final three sessions (n= 14). There was a significant difference between the o second and 8 second delay blocks, $p < 0.001$.

One of the most problematic aspects of the ID task during the pilot study was the significant decrease in responding across blocks (**Figure 2**). The lack of completed trials in the longer delay blocks resulted in fewer data points, inhibiting data analysis. In order for data from a subject to be available for analysis, there must be data from each data

block. This decrease in trial numbers across the session is observed in previous work as well, regardless of delay order presentation (Isles, Humby et al. 2003). This suggests that the decrease in responding is an effect of the block number rather than an effect of the longer delay.

The decrease in trials completed across blocks of trials is associated with an increase in response latency (also known as vigilance decrement). As the session progressions, there is an increase in the time it takes an animal to initiate a new trial. There is a significant difference between the response latency at the 0 second delay and the 8 second delay, $t(13) = -4.9$, $p < 0.001$ (**Figure 3**).

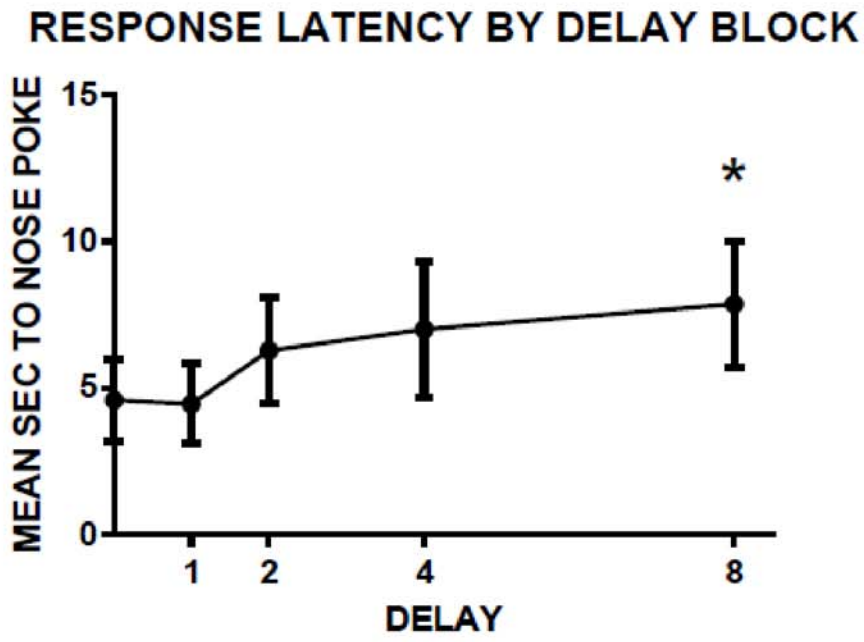

Figure 3. Response latency to initiate a new trial by delay block. There was a significance increase in response latencies, averaged across the final three sessions, $p < 0.001$.

There was also a significant difference between total number of trials completed before and after the decrease in delay time and increase in saccharin concentration, $t(13) = -4.96$, $p < 0.001$ (**Figure 4**). After these manipulations, trials completed increased from a mean of 24.5(7.9) to a mean of 34.4(6.4) per session. Although the trial numbers seen in this pilot study are not as high as previous work (Tanno, Maguire et al. 2014), they are similar to those seen in the AA version of DD in HAP mice (Halcomb, Gould et al. 2013). Since the trial numbers in AA studies are sufficient to detect effects, this suggests that ID version will also provide informative data.

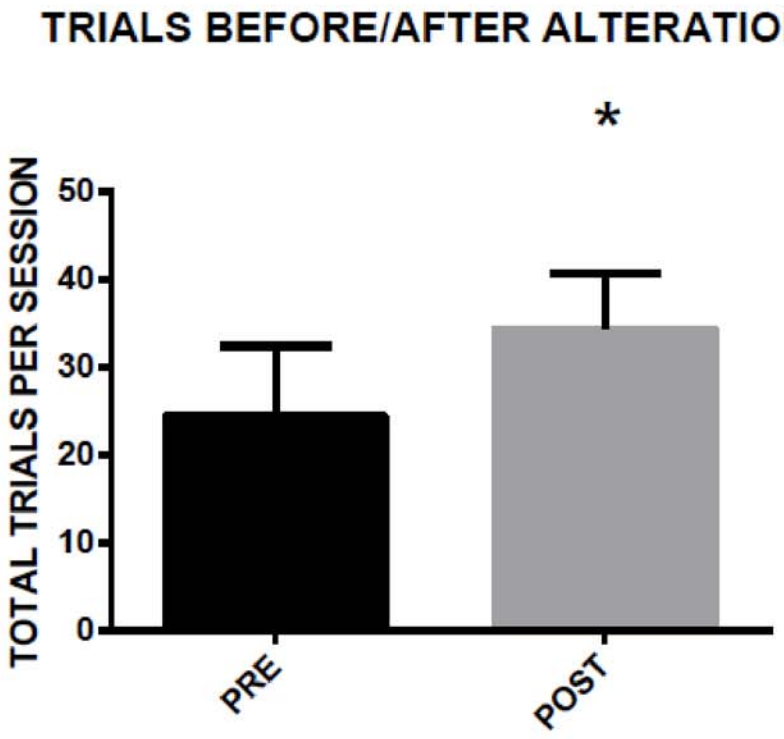

Figure 4. The mean total trials completed before and after the decrease in delay length and increase in saccharin concentration. There was a significant increase in trial numbers after these alterations, averaged across the final three sessions, $p < 0.001$.

The volume of saccharin solution consumed during a session increased significantly by the end of ID training, $t(13) = -4.87$, $p < 0.001$ (**Figure 5**). By the last day of ID training, the mean volume consumed during the session was 1.24 (0.6) ml compared to only 0.7 (0.5) ml prior to ID training.

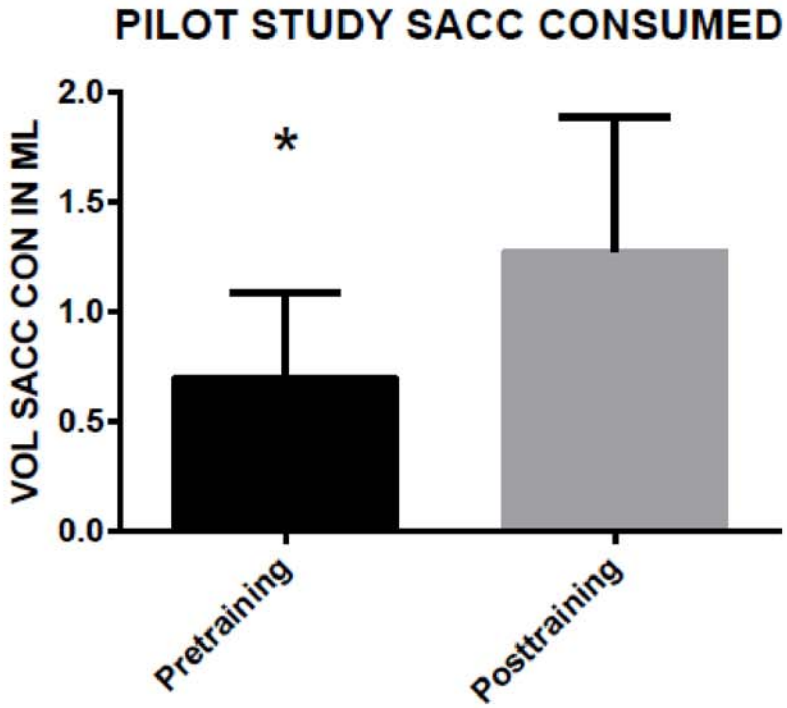

Figure 5. The mean total trials completed before and after the decrease in delay length and increase in saccharin concentration. There was a significant increase in trial numbers after these alterations, averaged across the final three sessions, $p < 0.001$.

2.3 Primary Experiment

2.3.1 Subjects

Experiments 1 and 2 were identical in general methodology, running consecutively. A total of 59 female and 59 male HAP II mice, born between 4/21/14 and

6/11/14 in cohort 1 and 8/20/14 and 8/29/14 in cohort 2, hereafter referred to as cohorts 1 and 2, were individually housed and kept on a reverse light/dark cycle with lights out at 7:00 am and back on at 7:00 pm. They were restricted to two hours of water access per day, but given ad libitum food access. Subjects were approximately 45 – 60 days old when training began.

2.3.2 Drugs

The AMP dose was set at 1.25 mg/kg dissolved in isotonic saline. The 1.25 mg/kg injection dose was determined to be a dose which most often results in pharmacological alteration of behavior in previous work; therefore it was be the only dose of AMP administered (Winstanley, Eagle et al. 2006; Oberlin and Grahame 2009). Lower doses may produce no effect while higher doses interfere with responding and may induce stereotypy (Porrino, Lucignani et al. 1984; Weiner, Lubow et a1988)

For the EtOH groups, the low dose was set at 1.5 g/kg of 20% EtOH in isotonic saline and the high dose was 2.0 g/kg. The 1.5 g/kg dose is necessary to maintain contact with earlier studies mentioned above; however, the HAP mouse lines have demonstrated an ability to metabolize EtOH at rates exceeding other strains. To ensure that the animals are maintaining pharmacologically relevant EtOH blood levels, the 2.0 g/kg dose was also administered.

In this experiment, animals were trained as outlined above and then divided into AA or ID groups, balanced across mean trials completed during training. This ensured that both high and low performing animals were present in equal amounts in both groups. Delayed levers were assigned based upon side preference during Stage 4 training. In order to compensate for a possible conditioned place aversion to the delayed lever, the

lever the subject prefers during training was assigned as the delayed lever for both the ID and AA groups.

The subjects in the AA group were trained according to the protocol described above. Briefly, during the AA training phase, the subjects could adjust the immediate reinforcer access time up or down based upon responding, which is termed the adjusted amount. At the beginning of the session, a response on the immediate lever resulted in one second of access to saccharin reinforcer (0.32% saccharin in tap water) and a response on the delayed lever resulted in two seconds of access. There was no delay to the large reward during this training. Importantly, any time during the session that the subject chose the same lever twice in a row, the subject was forced to choose the opposite lever on the following trial. Only the light for that lever was illuminated and responses on the other lever had no effect. This helps prevent perseveration by consistently reminding the animal what the contingencies are for the opposite lever and does not result in any titration of the immediate reward. Animals were trained in this method until they reach the criteria of a minimum adjusted amount of 1.6 seconds and 18 trials completed in one session.

Based upon the parameters established in the pilot study, the animals in the ID groups were exposed to initial delays of 0, 1, 2, 4 and 8 seconds, using a saccharin concentration of 0.32% saccharin in tap water with reinforcer access times of 0.5 seconds for an immediate lever press and two seconds of access for a delayed lever press. Although this framework resulted in discounting behavior in the pilot study, the subjects in cohorts 1 and 2 were not demonstrating discounting curves, even after over 25 days of ID training. The majority of subjects were responding on the delayed lever during every

delay block. In order to increase discounting behavior, the delays were doubled, becoming 0, 2, 4, 8 and 16 seconds. This change resulted in a sharp decrease in preference for the large reward across delay blocks. However, a total of 16 subjects in cohort 2 were still systematically choosing the delayed lever; therefore, two methods were implemented to subvert this behavior. First, since delay order presentation impacts responding, all animals were presented the delay blocks in a descending order (16, 8, 4, 2 and 0 seconds) for 2 days. This exposes the subjects to the aversive, long delay condition during the first block of trials, promoting choice of the immediate lever. Next, the delay blocks were returned to an ascending order and the delayed lever was removed from the operant chambers. These methods were instituted to remind the subject what the contingencies were for a response on the immediate lever. Although the first two trials of each block are forced-choice trials, there is no guarantee that the animal will complete those trials, severely limiting the exposure to immediate reinforcement. These strategies altered behavior in 14 of the 16 animals, allowing them to continue to the drug testing phase.

When training was complete for both groups, they were further subdivided into four drug administration groups, balanced across mean adjusted amounts for the AA group and percent choice of the delayed reward at the 0 second delay for the ID group:

EXPERIMENT GROUPS

AA GROUPS

- SALINE
- 1.5 G/KG ETOH
- 2.0 G/KG ETOH
- 1.25 MG/KG AMP

ID GROUPS

- SALINE
- 1.5 G/KG ETOH
- 2.0 G/KG ETOH
- 1.25 MG/KG AMP

In total, 22 female and 17 male mice met criteria for drug testing in the AA version of ID and 9 females and 14 males were tested in the ID condition. During drug testing of cohort 1, the 2.0 g/kg dose of EtOH resulted in severe response impairments. Of the six mice in the AA-2.0 g/kg EtOH group, only two continued to meet criteria after EtOH administration. In order for results from the AA version to be reliable, each animal must complete a minimum of 18 trials, allowing sufficient responding for preferred adjustment of the immediate reward; therefore the data from four of the AA mice were not useful for analysis. In the ID version, only one subject of the four assigned to the ID-2.0 g/kg EtOH group continued to complete trials. Due to this decrease of responding associated with this highest dose of EtOH in cohort 1, this dose was removed from the experiment in cohort 2, which increased group sizes for the SAL, AMP and 1.5 g/kg EtOH in that cohort. All data and analyses were completed without the 2.0 g/kg EtOH group.

2.3.3 Drug Testing

The AA version of the task requires a minimum of three days at each delay, requiring a total of 15 drug test days. The delays were presented in an ascending order, beginning with three days at the 0 second delay. To ensure that both AA and ID subjects had the same amount of drug exposure, the ID mice were also tested for 15 days. Injections were administered immediately prior to being placed in the operant chambers. During the testing phase, six mice from the ID group and three mice from the AA group died unexpectedly. Group numbers used in analyses were:

AA GROUPS

- SAL: 14
- AMP: 14
- 1.5 g/kg EtOH: 15

ID GROUPS

- SAL: 7
- AMP: 8
- 1.5 g/kg EtOH: 8

Unfortunately, the difficulty associated with acquiring the ID task resulted in smaller group numbers for that condition, decreasing power to detect drug effects. Only subjects completing a total of 35 trials per block across the 15 drug testing days were used in analysis. The general timeline for the entire experiment was as follows:

CHAPTER 3. STATISTICS

3.1 AA groups

Two repeated measures ANOVAs were used to identify effects of gender on responding. These were 2 X 5 designs with sex as the independent variable and adjusted amounts and trials completed as dependent variables. All other tests were conducted collapsed across sex. After eliminating gender effects, repeated measures ANOVAs were used to assess effects of drug administration on Adjusted Amount (AA), trials completed and volumes of saccharin reinforcer consumed during the session. Single *k* values representing the impulsivity level for each mouse, integrating choices over all delays, were calculated according to the formula: Mean Adjusted Amount = Delayed Reinforcer Magnitude/1 + (k*Delay). Larger values of k indicate steeper discounting. Mice with higher levels of impulsivity will generate steeper discounting curves. In addition, goodness of fit values, collapsed across groups, were calculated to determine how well the hyperbolic discounting functions describes the data. Independent samples *t* - tests were used to analyze the *k* values in SAL versus AMP group and the SAL versus EtOH group. A repeated measures ANOVA was also used to analyze delay and drug effects on response latencies. In addition, independent samples *t*-tests were used to analyze response latencies between all groups at the 0 second delay. In addition, Observed Power statistics were calculated to ensure group sizes were sufficient for detection of effects.

3.2 ID groups

Gender effects on percent choice of the delayed reward and trials completed per delay were evaluated with repeated measures ANOVAs. After analyzing the effect of gender, all further studies were collapsed across sex. Repeated measures ANOVAs were used to assess effects of drug and delay on percent choice of the delayed reinforcer and trials completed per delay. A repeated measures ANOVA was also used to analyze differences in percent choice of the large reinforcer in the 0 second delay block for all groups before drug exposure and after drug exposure. In addition, independent samples *t* – tests were used to assess differences at the 0 second delay between the SAL and EtOH groups during drug testing. Paired samples *t*-tests were also run to examine volume saccharin consumed on the final day of training and the final day of drug testing. Given the small sample sizes in the ID groups, Observed Power was also calculated to determine if the analyses had enough power to detect drug effects on percent choice of the delayed reward and number of trials completed per delay block. Correlations were also used to evaluate relationships between percent choice of the delayed reward and number of trials completed per delay block. To detect effects of repeated drug exposure on choice of the delayed reward and trials completed, repeated measures ANOVAs were run in a 3 X 15 design, with drug administered being the independent variable and percent choice or trials completed daily were the dependent variables. Response latencies were also analyzed with a repeated measures ANOVA to identify effects of drug on response times across delays. The sample sizes for all groups within the ID task were extremely modest, which decreased the ability to detect drug effects.

3.3 AA and ID GROUPS

One key aspect that differs between these tasks, and which may have greatly affected responding, is the completion of forced choice trials. While the ID version presents the subjects with forced choice trials at the beginning of every new block of trials, the completion of those trials is not mandatory. If the subject fails to complete the trials, the program simply continues to the next trial after a total of 72 seconds. On the other hand, in the AA version, if the subject chooses one option twice in a row, the next trial is forced choice on the alternate option and the subject must complete the trial in order to continue to a free choice trial. Paired sample *t*-tests were used to evaluate differences in completion of forced choice immediate and delayed trials in the both the AA and ID versions. This was followed by independent samples *t*-tests comparing completion total forced choice trials completed.

Another aspect that differed between the two groups was the number of subjects per group. The primary factor in this difference was the high attrition rate in the ID groups. During training, approximately 50% of the subjects in the ID group never met criteria for advancement to the drug test phase. In order to assess the association between DD group and attrition, a chi square test was administered using the total number mice in each group at the start of training and the number remaining during the drug test phase.

CHAPTER 4. RESULTS

4.1 Attrition

The chi square test found a significant association between the DD group and attrition rates, with subjects in the ID groups being more likely to be removed from the study, $p < 0.001$. In the ID groups, a total of 34 mice were removed from the study for failure to meet criteria. In the AA groups, only 17 mice were removed prior to drug testing.

4.2 AA Groups

Repeated measures ANOVAs found no main effect of sex on either AA, $F(1,30) = 0.08$, $p > 0.05$, or number of trials completed $F(1, 30) = 0.30$, $p > 0.05$. There were also no interactions of sex and delay on AA, $F(4, 124) = 1.20$, $p > 0.05$ or trials completed, $F(4, 124) = 0.53$, $p > 0.05$. Collapsing across sex, further repeated measures ANOVAs revealed a main effect of delay on AA, $F(4, 120) = 51.14$, $p < 0.001$, with AA decreasing across delay **(Figure 6)**. There was no main effect of drug on AA, $F(2, 30) = 0.46$, $p > 0.05$, however, there was a non-significant trend for the interaction of drug and delay, $F(8, 120) = 1.98$, $p = 0.06$. A Tukey's post hoc analysis found no significant differences between drug groups on AA, $p > 0.05$. Although there was no main effect of drug on AA, independent samples *t* – tests examining the *k* values of each group found a significant

difference between the SAL and AMP groups, $t(25) = 1.56$, $p < 0.01$. There was no significant difference between the SAL and EtOH groups, $t(25) = -0.03$, $p > 0.05$ **(Figure 7)**. The goodness of fit for the overall k value was significant, describing almost 50% of the variance (Mean = 0.48, SEM = 0.003). The k value is a free parameter used to describe the degree of discounting in the hyperbolic discounting equation. Observed Power estimates were high for effect of delay, 1.0 and delay by drug interactions, 0.78; however, they were extremely low for drug effects alone, 0.08 for adjusted amounts. For the k values, Observed power to detect drug effects was high, 0.79.

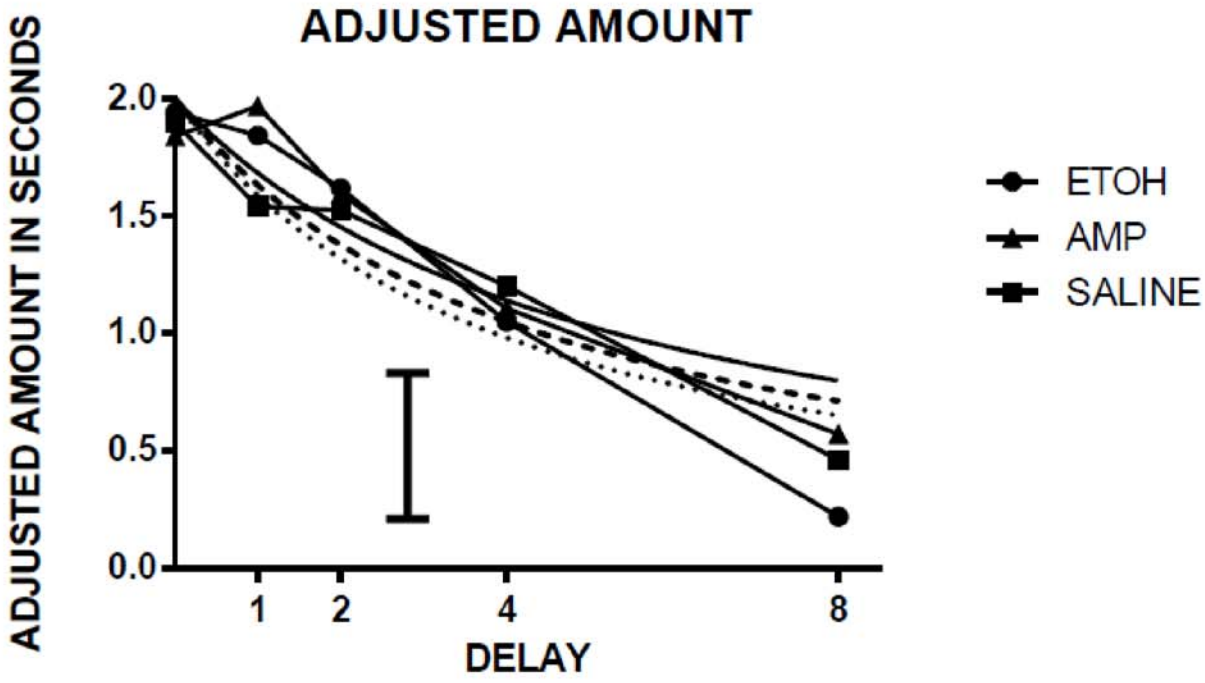

Figure 6 Adjusted amounts across delays for Saline (N = 13), AMP (N = 10) and 1.5 g/kg EtOH (N = 10) groups. There was a main effect of delay, $p < 0.01$, but no effect of drug and no interaction of drug and delay. Error bar is the mean SEM for all groups. Curves represent k values calculated for each group.

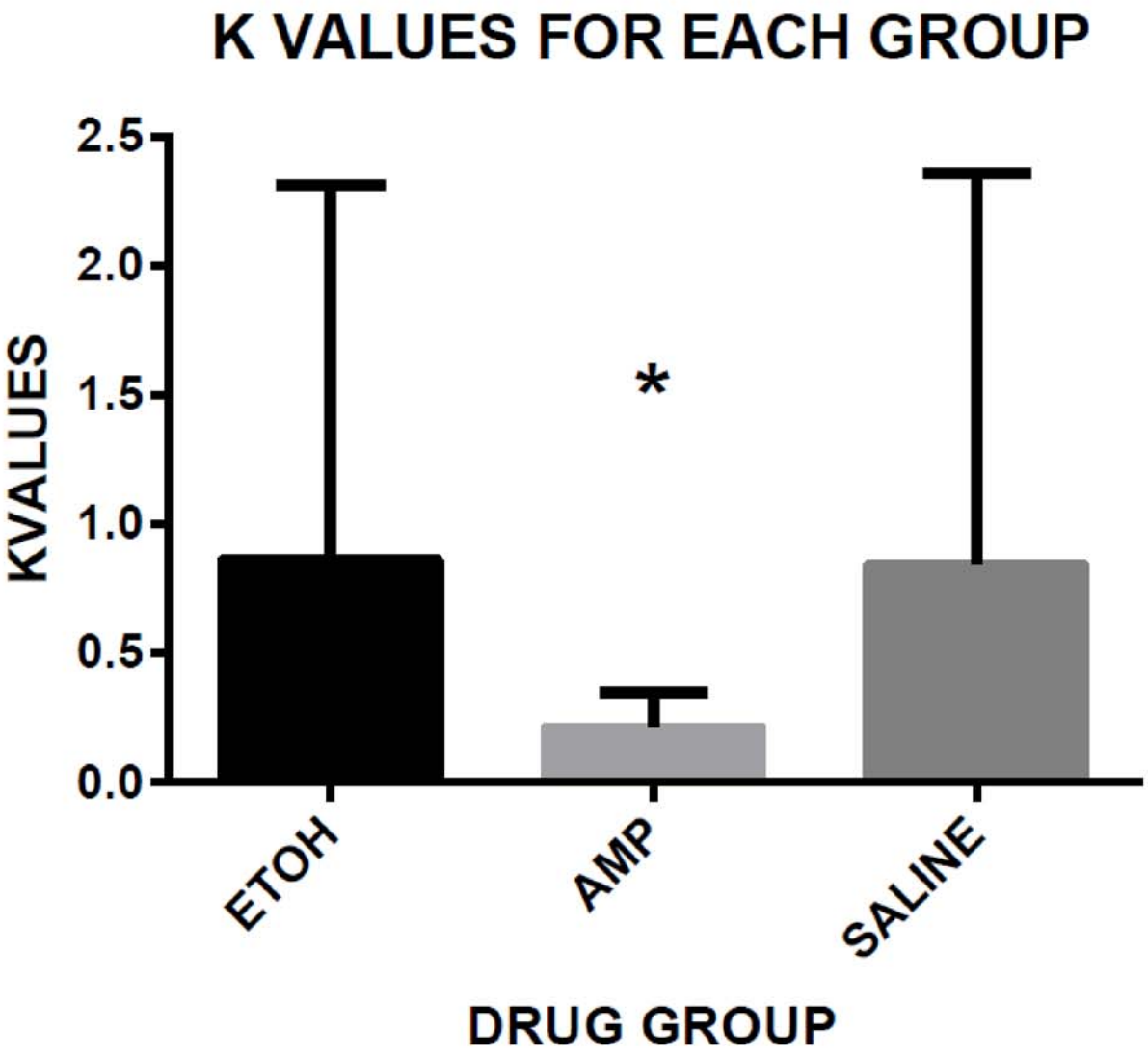

Figure 7. Calculated k values for each group. AMP groups had significantly lower k values than the SAL group, indicating a lower level of discounting, $p < 0.01$.

There was no main effect of delay on trials competed, $F(4, 120) = 0.09$, $p > 0.05$. There was also no main effect of drug on trials completed, $F(2,30) = 1.03$, $p > 0.05$, nor was there an interaction of drug with delay on trials completed, $F(8,120) = 1.26$, $p > 0.05$ **(Figure 8)**.

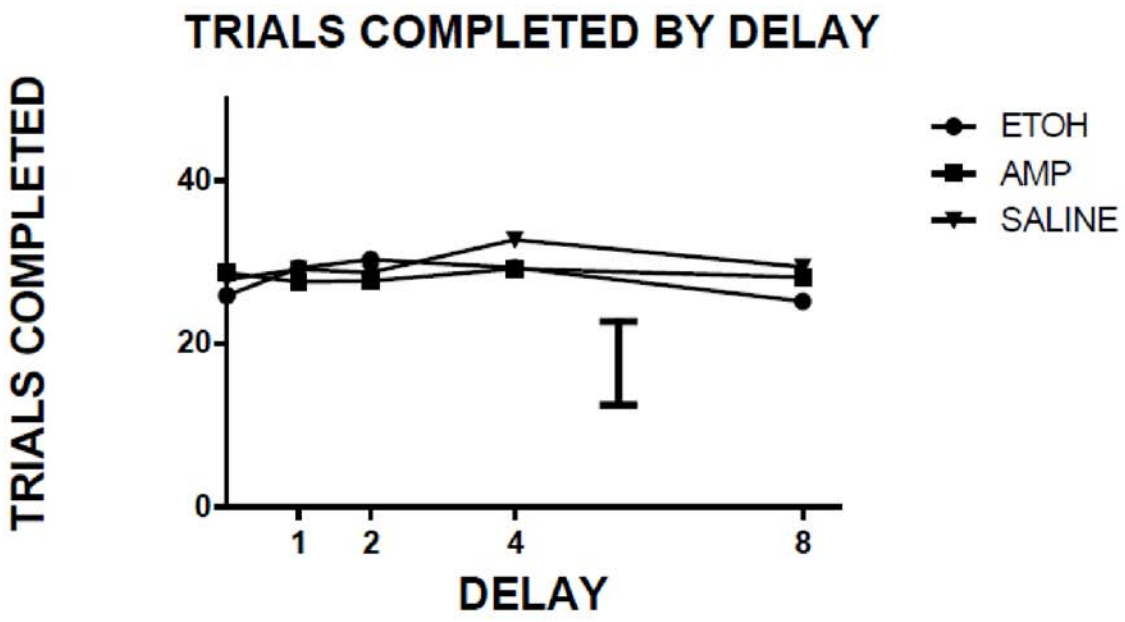

Figure 8. Trials completed across delays for each drug group. There was no main effect of delay and no significant difference between groups, $p > 0.05$. Error bar is the mean SEM for all groups.

There was a main effect of delay on volume of reinforcer consumed during the session, $F(4,160)$ 7.80, $p < 0.001$ and a main effect of drug on volume consumed $F(2,40) = 5.56$, $p < 0.01$; however, there was no interaction between drug and delay on volume consumed, $F(8,160) = 1.07$, $p > 0.05$ (**Figure 9**). Follow-up *Tukey's* post hoc analyses revealed that the AMP and 1.5 g/kg EtOH groups both consumed significantly less reinforcer during the session overall than the SAL group, $ps < 0.01$.

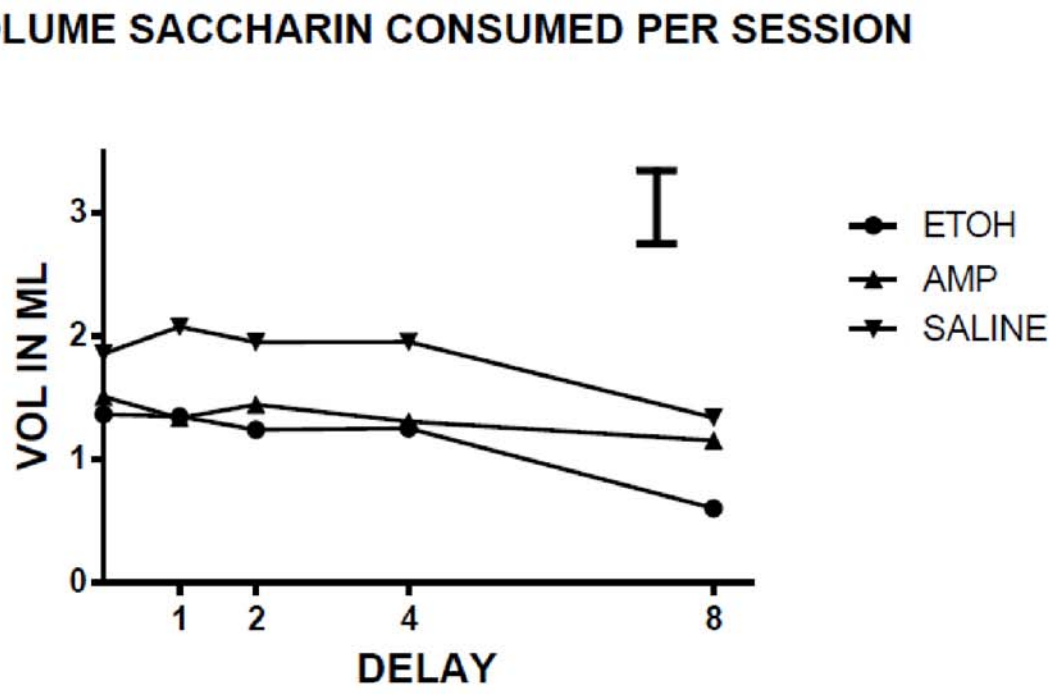

Figure 9. There was a significant effect of delay on mean volume reward consumed during a session, $p < 0.001$. There were also significant differences in mean reward consumption between the AMP and SAL groups and the 1.5 g/kg EtOH and SAL groups, $ps < 0.01$.

There was a main effect of delay on response latencies, $F(4,160) = 11.67$, $p < 0.001$, with latency to initiate a new trial increasing across delays. There was also a main effect of drug on response latency, $F(2,40) = 6.24$, $p < 0.01$ and a significant drug by delay interaction, $F(8,160) = 2.53$, $p < 0.05$. A Tukey's post hoc analysis revealed that response latencies for the EtOH group were significantly longer than the SAL group, $p < 0.01$.

4.3 ID Groups

Repeated measures ANOVAs found no effect of sex on percent choice of the delayed reward, $F(1,21) = 0.03$, $p > 0.05$, nor was there an interaction of sex and delay on percent choice, $F(4,84) = 1.62$, $p > 0.05$.There was also no main effect of sex on number of trials completed, $F(1,21) = 1.44$, $p > 0.05$ or an interaction of sex and delay on trials completed, $F(4,84) = 0.39$, $p > 0.05$. Given these results, all other analyses were collapsed across sex.

There was a main effect of delay on percent choice of the delayed reinforcer, with preference for the delayed reinforcer decreasing as delay increased, $F(4,80) = 28.10$, $p < 0.001$; however, there was no significant main effect of drug, $F(2,20) = 2.82$, $p > 0.05$ **(Figure 10).** There was a significant interaction of drug group and delay, $F(4,80) = 2.17$, $p < 0.05$ and a Tukey's post hoc analysis found a non-significant trend toward a difference between the SAL group and the 1.5 g/kg EtOH group, with the EtOH group showing lower percent choice, $p = 0.07$.

A repeated measures ANOVA found a significant difference in percent choice for the delayed reward at the 0 second delay from pre-drug training in all groups, $F(2,20) = 6.98$, $p < 0.01$. During the 0 second delay block under drug testing, all groups significantly decreased percent choice for the delayed reward compared to training. While the observed power to detect an interaction between delay and drug group was high, 0.82, the observed power to detect drug effects alone was only moderate, 0.48.

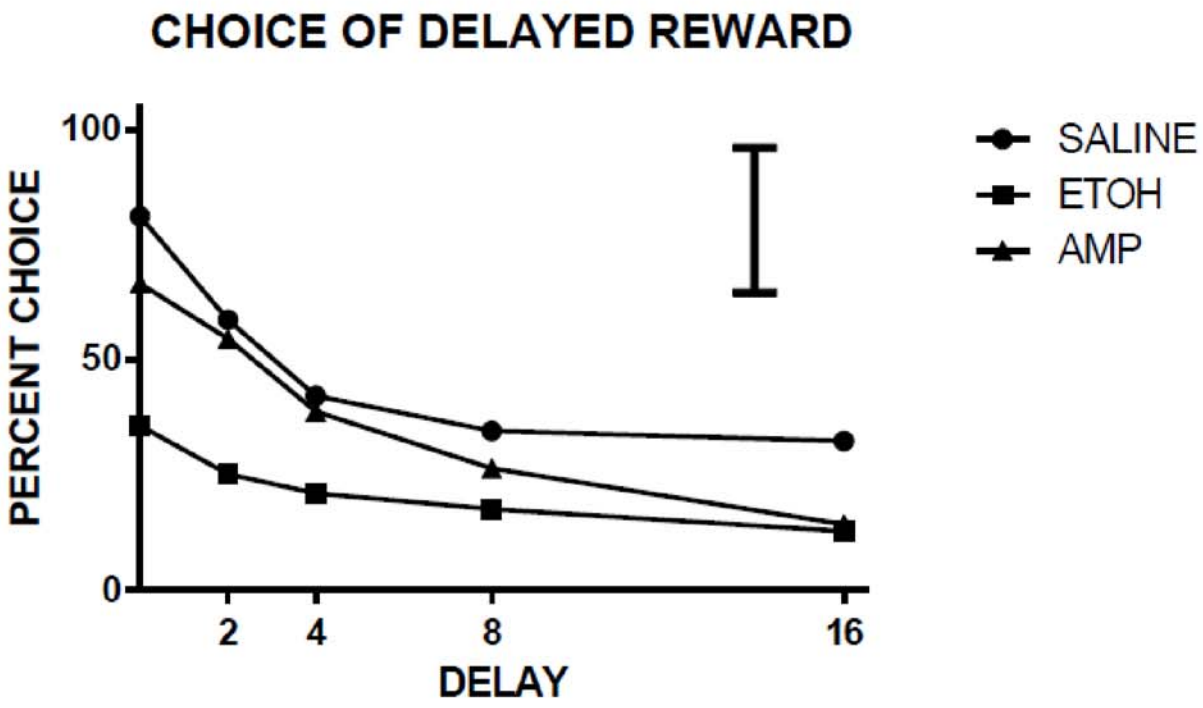

Figure 10. Percent choice of the delayed reward across delay blocks by drug group. There was a main effect of delay, $p < 0.001$, and a drug by delay interaction, $p < 0.05$, but no main effect of drug group. All groups significantly decreased their choice of the delayed reward at the 0 second delay block, as compared to pre-drug choice. Error bar represents the mean SEM for all groups.

There was a main effect of delay on number of trials completed per delay block, with trial numbers decreasing as delay lengthened in the AMP and SAL groups and increasing in the EtOH group, $F(4,80) = 5.73$, $p < 0.001$, but no main effect of drug group, $F(2,20) = 0.16$, $p > 0.05$ **(Figure 11).** There was a significant interaction of drug and delay on trials completed per delay block, $F(8,80) = 2.99$, $p < 0.01$, however a Tukey's post hoc analysis found no significant differences between drug groups. There was no significant correlation between the percent choice of the delayed reward in the EtOH or

SAL groups, R square = 0.14 and 0.23, respectively, *p*s > 0.05; however, there a significant positive correlation in the AMP group, R Square = 0.78, $p < 0.05$.

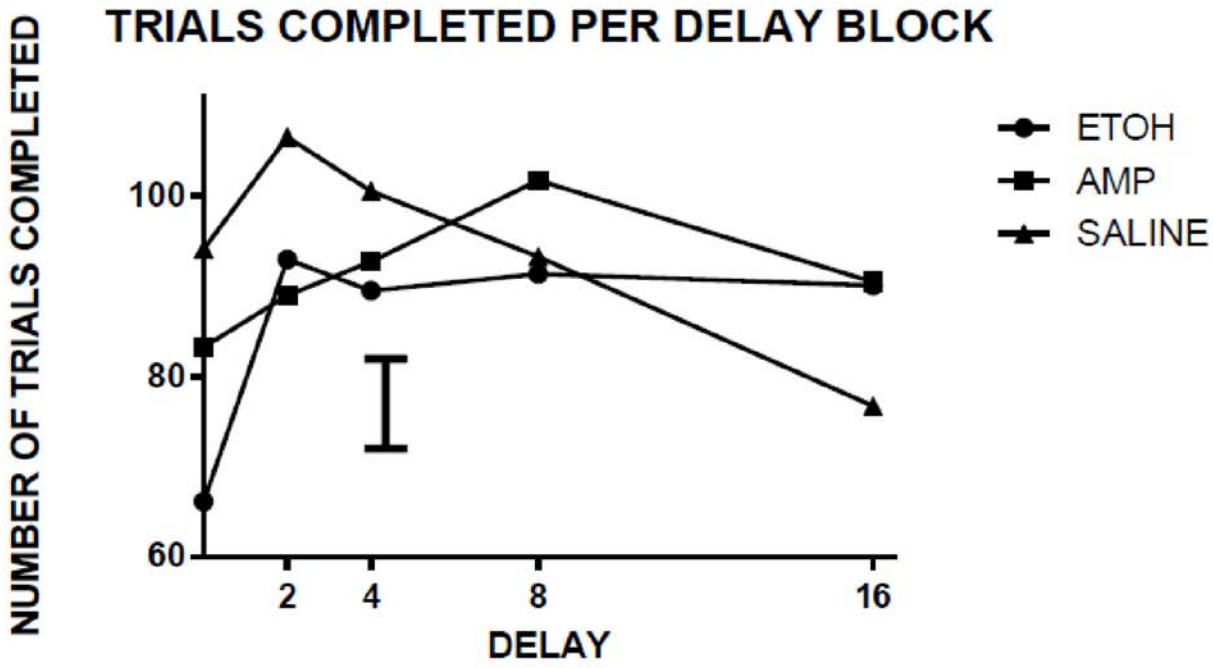

Figure 11. Total trials completed, per delay block, over the course of drug testing. There was a main effect of delay and a delay by drug group interaction, but no main effect of drug group. Error bar represents the mean SEM for all groups.

There was no main effect of delay on response latency across delay blocks, $F(4,80) = 1.45$, $p > 0.05$. There was no main effect of drug group, $F(2,20) = 0.08$, $p > 0.05$ or an interaction of delay by drug group, $F(4,80) = 0.274$, $p > 0.05$ (**Figure 12**).

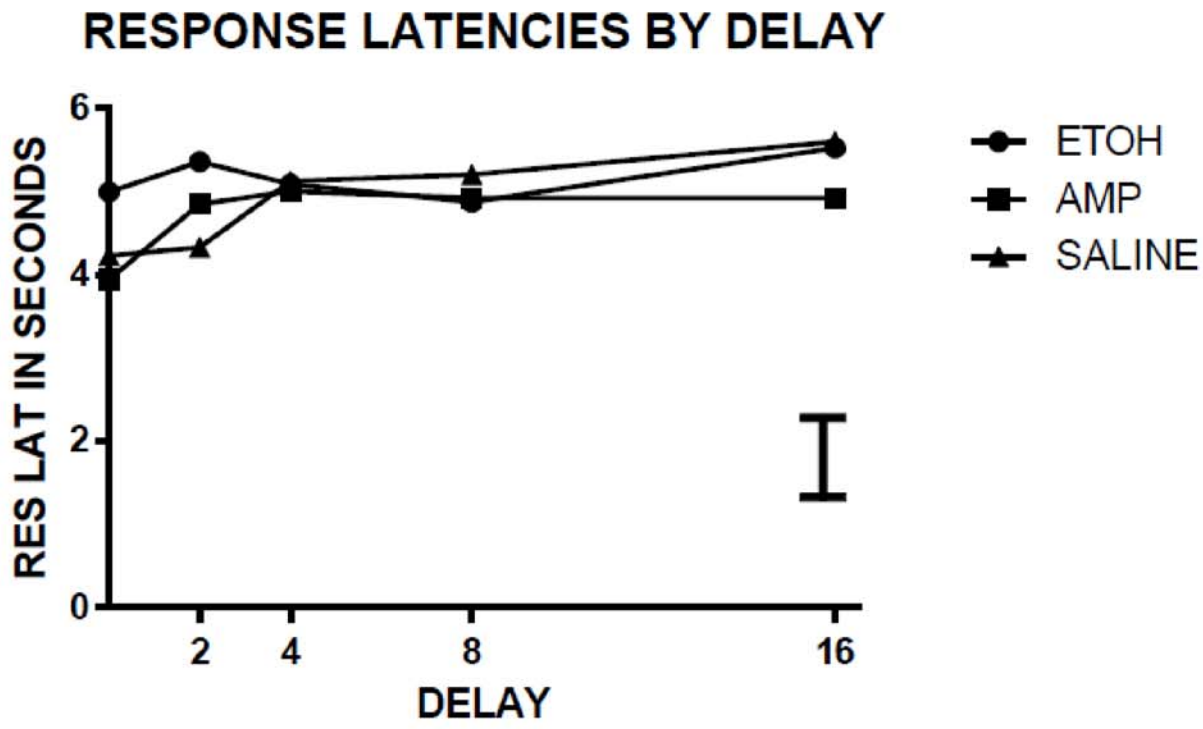

Figure 12. Response latencies to initiate a new trial. There were no main effects of delay or drug group, nor were there any interactions, ps > 0.05. Error bar represents mean SEM for all groups.

The paired samples *t*-test found no significant difference in volume reinforcer consumed in pre-drug versus drug test stages, $t(22) = 1.06$, $p > 0.05$. There was also no effect of day of injection on percent choice of the delayed reinforcer, $F(13,247) = 0.63$, $p > 0.05$, indicating there was not an effect of repeated drug exposures on percent choice. Percent choice numbers for this analysis were averaged across blocks of three days each to compensate for missing data points. There was no effect of day on number of trials completed, $F(14,266) = 0.63$, $p > 0.05$, nor was there an interaction of day and drug group, $F(26,247) = 0.95$, $p > 0.05$. These findings indicate that repeated drug exposure did not affect responding.

4.4 Both Groups

A one sample *t*-test revealed a significant difference between the AA and ID groups in total number of forced choice trials completed over the 15 days of drug testing, $t(36) = 17.26$, $p < 0.001$ **(Figure 13).** Subjects in the AA version completed significantly more forced choice trials during the testing phase.

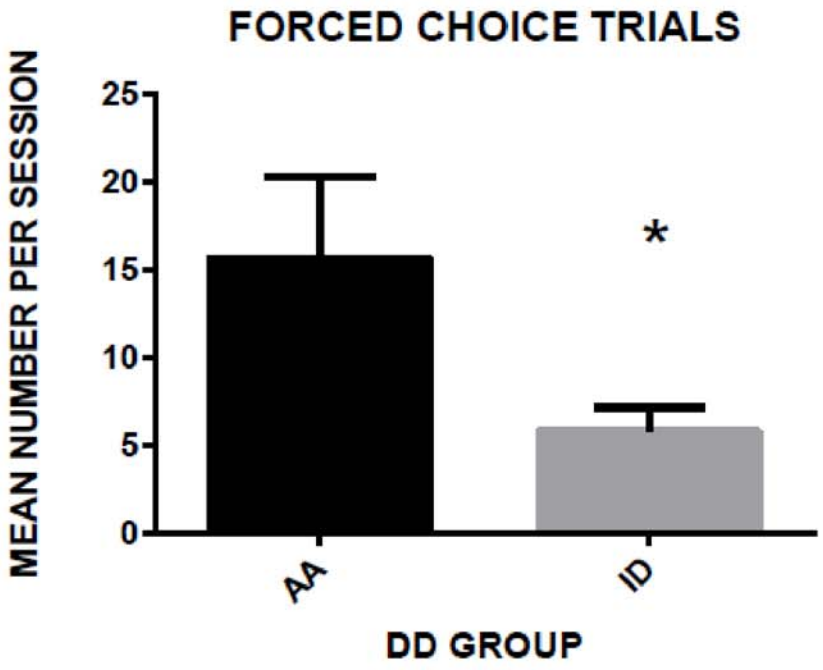

Figure 13. Response latencies to initiate a new trial. There were no main effects of delay or drug group, nor were there any interactions, ps > 0.05. Error bar represents mean SEM for all groups.

CHAPTER 5. CONCLUSIONS

5.1 General conclusions

Specific aim 1 of this study predicted that both the ID and AA versions of DD would result in a decrease in preference for the large reward as delay to the reward increased, which was supported by the findings here. Both types of DD created a main effect of delay, with subjects decreasing preference for the large, delayed reward as delay increased.

Specific aim 2 proposed that AMP administration would result in a decrease in impulsive responding in the AA group, but would not decrease impulsivity the ID group. As predicted, there was no decrease in impulsive responding in the ID group after AMP administration, since there was no significant difference between the AMP and SAL groups in percent choice of the delayed reward at any delay. AMP administration in the AA group resulted in a significant decrease in discounting, as indicated by the lower *k* values in the AMP group versus the SAL group. This implies that AMP administration resulted in lower levels of impulsivity as measured by the AA task, further supporting the hypothesis of specific aim 2.

Specific aim 3 hypothesized that EtOH administration would have no effect on impulsivity in the AA group, but would create an increase in impulsive responding in the ID group. Although this hypothesis was partially supported by the finding of no effect on

impulsivity in the AA groups, neither in adjusted amounts nor *k* values, there was also no effect on impulsivity found in the ID groups. The absence of effect of EtOH on impulsive responding in the ID task is likely perpetrated by a lack of power to detect effects, as described above.

5.2 Drug effects

5.2.1 Specific Aim 2

5.2.1.1 AA Group

Although there was no significant effect on adjusted amounts in the AA task, the AMP group had significantly lower *k* values. Subjects with higher *k* values discount rewards at a steeper rate and the *k* value is used to describe the discounting curve for individuals or groups of subjects. This difference implies that despite the lack of effect demonstrated for adjusted amounts, subjects in the AMP curve were not discounting as steeply as subjects in the SAL group. Since this indicates that there is indeed a difference between the AMP and SAL groups, it is possible that a larger cohort may have attenuated the impact of the high rates of variability and allowed detection of effects on adjusted amount. It is unlikely that this effect was driven by motor or motivational effects, since there were no differences in the number of trials completed or the latency to respond. There was a decrease in volume of saccharin consumed during the session; however, this was likely due to the appetite suppressant effects of AMP administration (Epstein 1959). These findings are consistent with previous work evaluating the effect of AMP on impulsivity utilizing the AA task and strengthen the predictive validity of the method (Wade, de Wit et al. 2000; Oberlin, Bristow et al. 2010).

5.2.1.2 ID Groups

There was no significant difference in percent choice of the delayed reinforcer between the AMP and SAL groups in the ID task. Since there were also no significant differences in trials completed or response latencies, it is possible that some extraneous variable was impairing the ability to detect effects. Two primary concerns are the effects of injections themselves and insufficient group sizes.

Previous studies, which did find reductions in impulsive responding with the ID version typically used cohort sizes of at least 12 -14 subjects per group (Evenden and Ryan 1999; Cardinal, Robbins et al. 2000; Koffarnus, Newman et al. 2011). Unfortunately, the difficulty associated with training the animals to criteria greatly reduced the number of subjects available for testing. The current study was only able to provide 8 mice for AMP administration. Not only does the small sample size reduce the power to detect effects, power was also further hampered by high levels in variability. This is reinforced by the low to moderate level of observed power calculated. Increasing the cohort size would increase the power to detect effects, if any, and reduce the impact of variability in responding.

During drug testing, there was a significant difference in the percent choice of the delayed reward from pre-drug training in both the AMP and SAL groups at the 0 second delay. This indicates that the change in response behavior was an effect of the injection itself, rather than an effect of AMP exposure. The change in behavior at the 0 second delay in the ID groups reduces the ability to detect effects as the delay increases. A possible way to circumvent this would be to administer the drug 10 - 15 minutes prior to

testing, as seen in other studies (Evenden and Ryan 1996; Winstanley, Theobald et al. 2005).

While previous work has shown that repeated AMP administration leads to perseverative behavior, there was no evidence that repeated exposure to AMP affected perseveration in the drug testing phase of the study(Evenden and Robbins 1983). There was no significant change in responding across the 15 day testing period and no indication that the subjects were perseverating on a specific lever, since there was a decrease in preference for the large reinforcer as the delay increased during the session.

5.2.2 Specific aim 3

5.2.2.1 AA Group

Specific aim 3 predicted there would be no effect on impulsive responding following EtOH administration, in adjusted amounts or *k* values, which was validated here and is consistent with previous work evaluating the effects of acute EtOH exposure in the AA paradigm in animals (Wilhelm and Mitchell 2012). This finding is also consistent with results from clinical studies utilizing the AA version of DD (Richards, Zhang et al. 1999; Reynolds, Richards et al. 2006). Although anecdotal evidence would suggest a high correlation between an increase in impulsive behavior and alcohol intake (Duffy 1995), there is evidence that this association may only be evident is tasks involving high levels of conflict with excessive levels of alcohol intake in human subjects (Steele and Southwick 1985). Consistent with that observation, the animals in the 1.5 g/kg dose in this study displayed a non-significant trend toward increased impulsivity at the longest delay.

Although there was no effect on the mean number of trials completed, the latencies to initiate a new trial were significantly longer in the EtOH group than the SAL group across delays. In addition, the subjects in the EtOH group consumed significantly lower amounts of saccharin reinforcer than the SAL group. These findings are likely a result of the motor impairments often associated with EtOH administration. There is evidence that even the moderate dose used here can have depressant effects on animals selectively bred to prefer alcohol (Waller, Murphy et al. 1986). These data confirm that while there was no effect on impulsive behavior, subjects were experiencing pharmacological effects of EtOH.

5.2.2.2 ID Group

Contrary to the hypothesis in Specific aim 3, there was no increase in impulsivity after EtOH administration in the ID task. There were also no significant differences in number of trials completed per block or response latencies. Similar to the findings in Specific aim 2, there are likely several contributing factors affecting results here. The most probable element influencing results was the extremely modest sample size. ID studies finding an increase in impulsive responding after EtOH exposure had sample sizes ranging from 12 – 15 animals per group (Evenden and Ryan 1996; Tomie, Aguado et al. 1998). The group size for EtOH administration in the present study was only 8 subjects, and greatly reduces the ability to detect effects, as noted by the low power established above. In addition, responding in the EtOH group was extremely variable between subjects.

There was also a significant decrease in percent choice of the delayed reward at the 0 second delay block compared to pre-drug training. As this decrease was also present in the SAL group, it was likely an effect of the injection itself. Since the 0 second delay is only tested for the first 12 minutes of the session, this change in behavior greatly inhibits the ability to accurately analyze responding for this delay. Previous work analyzing acute EtOH effects on impulsivity in ID task administered the drug 15 minutes prior to testing and did not observe this difference in responding at the 0 second delay (Evenden and Ryan 1996). This earlier work did not find this decrease in preference at the zero delay. Since there was no correlation between the percent choice of the delayed reinforcer and number of trials completed, the results suggest that EtOH was inhibiting the ability of subjects to demonstrate magnitude discrimination.

Lastly, EtOH not only creates motor impairments, it also impacts both spatial and working memory through disruption of hippocampal-dependent behaviors (Givens and McMahon 1997). The differences observed at the 0 second delay may reflect an influence of EtOH on the subject's ability to complete the forced choice trials and remember those contingencies during the free choice trials.

5.3 Comparison between AA and ID groups

Direct comparison of responding between the AA and ID versions of DD is limited, given the inherent differences in administration. For instance, while both types of sessions were one hour in length, giving subjects the same amount of exposure to all delays, the method of presentation of those delays may present problems, particularly when analyzing effects of acute pharmacological manipulations. In the AA version, there is only one delay to the large reward for the entire hour, allowing the subject ample time

to recover from any negative injection effects. In the ID version, the behavior during the 12 minutes of access to the 0 second delay was hampered by injection effects, rendering data from that delay block unreliable. Additionally, it is not possible to analyze the volume of saccharin reward consumed during the session across delays in the ID version.

In the AA version of the task, there was a significant decrease in volumes consumed as the delay increased, which was not accompanied by any decrease in trial numbers completed. This may represent a decline in motivation at the longest delay, which cannot be measured in the ID task. Finally, the lengths of the actual delays to the large reward were twice as long in the ID task as in the AA task. Although this difference was instituted simply to aid in successful training of the ID mice, these delays are more similar to previous work done in mice, which allowed for greater contact with established literature (Isles, Humby et al. 2003).

One feature compared between ID and AA groups was the number of forced choice trials completed. Subjects in the ID groups completed significantly fewer forced choice trials during the session. This is problematic since the forced choice trials serve to decrease perseveration, which was particularly strong in the ID task. Unfortunately, the available literature from previous work does not describe or mention completion of forced choice trials, so it is unclear if this aspect impacts results consistently.

Finally, although the study began with equal numbers of subjects in the ID and AA groups, there were significantly smaller group numbers in the ID groups during the drug test portion due to the high rate of attrition. Subjects in the ID groups were consistently failing to demonstrate discounting behavior. Instead, they were choosing the delayed reward throughout the session. This type of behavior was also observed in a

previous study assessing EtOH effects on impulsivity in the ID task (Tomie, Aguado et al. 1998). Rather than removing the subjects from the study, they were labeled “insensitive to delay” and continued into the drug test phase. The original purpose of that study was to associate impulsive responding in the DD task with autoshaping, thus the subjects not discounting future rewards were an essential aspect of the investigation. The development of the Tomie (1998) study suggests that non-discounting behavior is often an issue in the ID task, a problem not observed in studies implementing the AA version of DD.

CHAPTER 6. DISCUSSION

6.1 Discussion

This study is the first to directly analyze the impact of DD administration type on responding and the results clearly suggest that the AA version of DD has greater predictive validity than the ID version. In clinical studies, stimulant administration is typically associated with a decrease in impulsive behavior, which is consistent with these findings in the AA group here (de Wit, Enggasser et al. 2002; Pietras, Cherek et al. 2003; Shiels, Hawk et al. 2009). Additionally, the AA version of DD is the preferred method of assessing DD in the computerized version in human studies. Utilizing a similar paradigm in the animal model increases translatability of findings to those observed in clinical studies. Importantly, the AA version allows investigators to calculate *k* values with the hyperbolic discounting equation, which is not possible with the ID method. The *k* value is generated using the specific adjusted amount of each subject. Since the ID task does not allow subjects to adjust the amount of the immediate reward, there is no value available to complete the equation for the discounting function.

There are numerous issues in training and acquisition of the ID task which limits its ability to assess pharmacological manipulation of behavior. It is clear, from findings reported here, that detecting effects in the ID task is complicated by the difficulty in achieving and maintaining stable criteria responding. In fact, subjects in the ID version of

DD were significantly less likely to reach criteria for responding than subjects in the AA version. Additionally, there are several other factors likely contributing to behavior in this task, including perseveration, decrease of trials completed across delay blocks and contrast effects associated with the delay order presentation.

6.1.1 Problems with the ID task

6.1.1.1 Forced choice trials and perseveration

The use of forced choice trials is essential in the DD task. They serve the purpose of reminding the subject what the alternate contingencies are and aid in the prevention of perseverative or habitual responding. They are particularly imperative when using pharmacological manipulations, given the propensity for many drugs to increase these types of behaviors. An unfortunate aspect of the ID version of DD is that, while each new block of trials is initiated with at least two forced-choice trials, the nature of the task permits the subjects to omit those trials. If the subject does not respond during the set amount of time, the program moves into an ITI state and the trial is omitted. The exact number of forced choice trials per block varies from two per block to six per block across studies, as does the length of time the response is available, and completion rates of the forced choice trials are rarely reported (Evenden and Ryan 1996; Winstanley, Dalley et al. 2003; Koffarnus, Newman et al. 2011). The fact that the training and administration of this task varies so widely hinders accurate analysis of the methodology.

In contrast, in the AA version, forced choice trails are mandatory for subjects whenever they choose one option twice consecutively. In order to continue with the

session, the animal must complete the forced choice trial on the opposite lever. In the present study, subjects in the AA groups completed significantly more forced choice trials than subjects in the ID groups, leading to significantly higher numbers of exposure to both contingencies throughout a session. Subjects in the ID groups were completing only approximately 50% of the available forced choice trials, decreasing the ability of the following free choice trials to accurately measure preference for that block. The omission of forced choice trials is especially problematic for those studies utilizing only five free choice trials, since it could be argued that almost half of those choices were made without prior exposure to the new contingencies in that block if forced choice trials were not completed (Winstanley, Dalley et al. 2003; Maguire, Henson et al. 2014).

While the forced choice trials serve to decrease perseverative responding in the animal model of DD, they are not included in DD models used in human studies. This difference leads to a slight reduction of the face validity of the task. Face validity is used to describe how well a test measures what it is claiming to measure. In this case, the task should appear to measure impulsivity in both humans and animals and use similar standards and practices. Maintaining only slight procedural variations allows for greater translatability of findings, thus also supporting predictive validity. Unfortunately, this is seldom possible with animal models; however, the AA of version of DD in animals is analogous to the version used in clinical studies in function and time course. Although the forced choice trials may influence behavior while the subjects are training, there is not any evidence at this point to suggest that they influence impulsive behavior.

6.1.1.2 Delay order presentation

Perseveration was a particular problem during training of the ID task. Subjects in the pilot experiment and both cohorts in the primary experiment required numerous manipulations in training protocol to establish discounting curves. During the first block of trials, there is no delay to the large reward, promoting preference for this option across the first 10 free choice trials. According to DD theory, preference for the large reward should decrease as a function of the delay to its delivery (Ainslie and Herrnstein 1981); however, the majority of subjects in the current study were not demonstrating this reversal of preference. Subjects continued to choose the large delayed reward exclusively, even at the longest delays. In order to facilitate discounting behavior, the order of the delay presentation was reversed to descending delays rather than ascending delays.

The presentation of the delays was reversed for two sessions so that subjects were exposed to the longest delay during the first block of trials, promoting choice of the immediate reward. When the delay order presentation was returned to an ascending order, several subjects displayed preference reversal at the longest delays, highlighting the importance of exposure to both contingencies. Prior to reversing the delay order presentation, subjects were not choosing the immediate reward during forced or free choice trails, focusing instead on the larger reward. Other studies evaluating the presentation of delay order have revealed effects after AMP administration, with AMP exposure decreasing impulsive responding in an increasing order paradigm and increasing impulsive responding in the descending order paradigm. (Tanno, Maguire et al. 2013; Maguire, Henson et al. 2014). This suggests that the behavioral alterations observed in the ID task are not indicative of changes in impulsivity, but rather an effect

of perseveration. For an increasing delay paradigm, subjects initially prefer the large reinforcer then continue to perseverate on this lever across the session, while in descending delay paradigm, subjects prefer the immediate reward to avoid the long delay and then perseverate on that lever across the session. Although no effect of AMP was uncovered in the current study, the change in behavior observed after the delay order reversal implies that the perseveration impacted training behavior in this task.

The order of delay presentation may also create a negative contrast effect. Recent evidence reveals that negative contrast effects decrease responding to delayed reinforcement (LAS RESPUESTAS, DEMORADO et al. 2011). Rapidly exposing subjects to increasing longer delays may also decrease the preference for the delayed reward as a result of this negative contrast effect rather than impulsivity.

6.1.1.3 Decrease in trials across blocks

A notable issue with the ID task is the decrease in trial completions as the session progresses. In the present study, subjects in the SAL and the AMP groups, and all subjects during training, significantly decreased responding by the final block of trials. The lack of responding in the longest delay block presents two problems. First, if enough trials are not generated, there arenot enough data for analysis. Indeed, in order to accumulate sufficient data points for this study, percent choice of the large reward was averaged across the entire drug test period for each delay block. Second, if the subjects are not completing trials at the longest delay, there is significantly more exposure to the short delays, increasing the likelihood of perseveration. This, in turn decreases the

probability that the subject will reverse preference to the immediate reward at the long delays.

6.2 Future Directions

Although the use of HAP mice in DD tasks likely decreases the variability in response patterns often observed in heterogeneous lines (Barbelivien, Billy et al. 2008), it may also inhibit the ability to learn the ID version of DD. The high level of perseveration observed in training for the ID task indicates an inherent difficulty in interpretation of the new contingencies presented in each block of trials. Several subjects in the ID groups continued to choose the delayed reward exclusively, even at the longest delays. One way to circumvent this possibility would be to assess differences between the AA and ID methods using the LAP II line of mice rather the HAP II line. The LAP mice typically display lower levels of impulsive behavior, maintaining a low rate of variability within the cohort, while eliminating the correlated trait of high alcohol preference.

An alternate method for assessing differences in behavior between the AA and ID versions is the use of cortical lesions to influence response patterns. Work in human studies evaluating reward discrimination following damage to the orbitofrrontal (OFC) cortex reveals deficits in reward processing, an ability that is essential in accurate completion of a DD task (Manes, Sahakian et al. 2002). These lesions are also associated with increased impulsive behaviors in humans while lesions of the nucleus accumbens core promote increases in impulsivity in animal models (Cardinal, Pennicott et al. 2001; Berlin, Rolls et al. 2004). Future work evaluating the validity of both the AA and ID versions of DD in animals could focus on lesioning specific brain regions known to influence responding the DD task, such as the OFC and the nucleus accumbens core.

Confirming findings in previous work would greatly increase the predictive validity of the paradigm.

Overall, the ID task was extremely difficult to train and administer and there were no significant effects on impulsive-like responding after pharmacological manipulation, suggesting that the predictive validity of this task is questionable. Although ID testing for multiple delays may be much shorter than the AA task, the longer training periods and the unreliability of results should discourage the use of this task for examining impulsivity. The AA animal model of DD consistently replicates findings from clinical studies and is more similarly modeled after those studies. In order to ensure accurate findings, investigators should administer the AA version of DD for analysis of impulsivity.

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Wolraich, M. L., J. N. Hannah, et al. (1996). "Comparison of Diagnostic Criteria for Attention-Deficit Hyperactivity Disorder in a County-Wide Sample." Journal of the American Academy of Child & Adolescent Psychiatry **35**(3): 319-324.

VITA

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Education

Bachelor of Science, Indiana State University (May, 1998)
Major: Criminology

Bachelor of Arts, Indiana University-Purdue University Indianapolis (December, 2009)
Major: Psychology

Master of Science, Indiana University-Purdue University Indianapolis (May, 2013)
Psychobiology of Addictions

PhD Candidate, Indianapolis University-Purdue University Indianapolis
Addiction Neuroscience
Projected graduation date: May, 2015

Research Experience

Research technician for Dr. Nick Grahame (Fall, 2007 to 2010)

Projects: During my undergraduate career, I conducted or was involved with several projects examining ethanol consumption and its effects on tasks of cognitive impulsivity. I engineered several studies investigating how various pharmacotherapies used to treat alcoholism affect cognitive impulsivity and voluntary ethanol consumption. I have also studied the effects of ethanol on motor impairments in transgenic mice. During my undergraduate career, I gained valuable experience designing and implementing entire experiments and analysis of data. These skills have contributed greatly to my success as a graduate student.

As a graduate student, I completed a year-long study evaluating the effects of two common pharmacologic treatments for bipolar disorder, lithium and valproate, on cognitive impulsivity in high alcohol-preferring mice. I then implemented a follow-up study observing the effects of lithium on ethanol intake in the same strain of mice. In addition, I conducted several additional delay discounting studies evaluating effects of various pharmacological agents, including clozapine, olanazapine and a triple monamine uptake inhibitor, followed up by analysis of effects on ethanol consumption in a two-bottle choice paradigm. I have also contributed to several other discounting and ethanol consumption projects which have since resulted in publications.

I have recently initiated my dissertation project analyzing the effects of various pharmacological manipulations on impulsivity as measured by two separate versions of the delay discounting task. The ultimate underlying goal of the study is to determine differences in behavioral responding in these tasks to further clarify the version which more reliably predicts behavioral responses in human subjects.

Teaching Experience

Graduate teaching assistant for several Introductory Psychology classes, IUPUI. 2011 – 2013
Duties: Teach labs, grade papers

Teaching assistant for Dr. Drew Appleby (Spring, 2009)
Duties: Help individual students understand assignments
Be a mentor
Help advise students and encourage them to excel

Teaching assistant for Professor Brandon Oberlin (Fall, 2009)
Duties: Help proctor exams
Grade exams

Substitute teacher, Decatur Township of Indianapolis (2005-2007)

Abstracts

L. Matson, M. Halcomb, H., June, N. Grahame. Manipulation of pharmacologically relevant free-choice drinking in HAP I mice using the triple monoamine re-uptake inhibitor amitifadine (DOV 21,947). Research Society for Alcoholism. San Francisco, CA. June 2012

L, Matson, S. Liangpunsakul, D. Crabb, A. Buckingham, R. Ross, M. Halcomb, and N. Grahame. Chronic free-choice drinking in crossed-HAP mice leads to metabolic tolerance and CYP2E1 enzyme induction without evidence of liver damage. Research Society for Alcoholism. San Francisco, CA. June 2012.

M. Halcomb, T Gould, N Grahame. Lithium, but not valproate, reduces impulsive choice in a delay discounting task in mice. Society of Biological Psychiatry. Philadelphia, PA. May 2012.

M. Halcomb, T Gould, N Grahame. Lithium, but not valproate reduces impulsive choice in a delay discounting task in mice. Indianapolis Society For Neuroscience Conference, Indianapolis, IN, May 2013.

M. Halcomb, N Grahame. Effects of lithium administration on ethanol intake in high alcohol-preferring mice. Research Society for Alcoholism. Orlando, FL June, 2013.

M. Halcomb, T Gould, N Grahame. Lithium, but not valproate reduces impulsive choice in a delay discounting task in mice. STEM, Women in Science Poster Session. Indianapolis, IN. April, 2014.

M. Halcomb, N. Grahame. Effects of ethanol exposure on impulsivity in a high alcohol-preferring (HAP) mouse line. Research Society for Alcoholism. Bellevue, WA. June, 2014.

M. Halcomb, B. Oberlin, N. Grahame. Chronic ethanol exposure does not alter impulsive responding as measured by the delay discounting task. Indianapolis Society for Neuroscience Conference. Indianapolis, IN, Oct. 2014.

Invited Presentations

M. Halcomb. Lithium Effects in Impulsive Mice. Indiana University School of Medicine, Department of Psychiatry Seminar Jan 8, 2013.

M. Halcomb. Lithium Effects in Impulsive Mice. Purdue University, Psychology Department Seminar March 4, 2013.

M. Halcomb. Behavioral Genetics. Annual Ann Daughtry Addiction Symposium, TARA Rehabilitation Center. May 24, 2013.

Publications

Oberlin, B.G., Bristow, R.E., ** Heighton, M.E., and Grahame, N.J**.** (2010). Pharmacologic dissociation between impulsivity and alcohol drinking in High Alcohol Preferring mice. *Alcoholism: Clinical and Experimental Research*, 34, 1363-1375.

Liana Matson, Suthat Liangpunsakul, David Crabb, Amy Buckingham, Ruth Ross, Meredith Halcomb, Nicholas Grahame. (2012). Chronic free-choice drinking in crossed HAP (cHAP) mice leads to sustained blood ethanol levels and metabolic tolerance without evidence of liver damage. *Alcoholism: Clinical and Experimental Research.* 37(2) 194-201.

David S. O'Tousa, Kaitlin T. Warnock, Liana M. Matson, Ojas A. Namjoshi, Michael Van Linn, Veera Venkata Tiruveedhula, Meredith E. Halcomb, James Cook, Nicholas J. Grahame and Harry L. June (2013). Triple monoamine uptake inhibitors demonstrate a pharmacologic association between excessive drinking and impulsivity in high-alcohol-preferring (HAP) mice. *Addiction Biology*. Oct 13, 2013.

Halcomb ME, Gould TD, Grahame NJ. (2013) Lithium, but not valproate, reduces impulsive choice in the delay discounting task in mice. *Neuropsychopharamcology,* 38, 1937 – 1944.

Halcomb ME, Grahame NL. (2014) Pharmacological predictive validity in delay discounting: A meta-analysis of the role of task procedures in studies of psychostimulants and alcohol in humans and animals. *Neuroscience and Biobehavioral Reviews.* In revision.

Honors and Awards

Psi Chi member since 2007
Alpha Sigma Lambda member since 2008
Scholar’s List every semester enrolled during undergraduate career
University Fellowship 2010
First place, Women in Science Poster Session, 2014
Paul J Mckinley Award for Outstanding Graduate Student, 2015

Service

Ad hoc Reviewer: Journal of the Experimental Analysis of Behavior, 2014

Audio Visual Aide, Research Society for Alcoholism Conference, San Francisco, CA, June, 2012;

Audio Visual Aide, Research Society for Alcoholism Conference, Orlando, FL, June, 2013.

Audio Visual Aide, Research Society for Alcoholism Conference, Bellevue, WA, June, 2014.

**My previous name was Meredith E Heighton.