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Development of a Novel Delay Discounting Task: Evaluation of Nicotinic Receptor Blockade

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Evaluation of Nicotinic Receptor Blockade.

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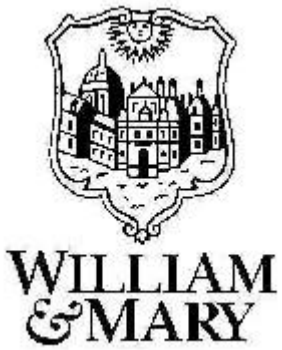

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Abstract

Delay discounting is a commonly used measure to assess impulsive decision-making. Many of the tasks used involve providing access to a small, immediate reward and a larger, delayed reward, with a choice of the immediate reward thought to reflect greater impulsivity. However, these tasks do not allow assessment of the effects of immediate versus delayed access to multiple reward levels. Moreover, a direct comparison of the effects of delay before or after reward access is confounded by reward amount. In order to address these issues, additional choices must be made available. In the present experiment, rats were trained in an 8-arm automated radial maze. Three of the arms offered immediate access to low (0.01 ml tap water), medium (0.06 ml), or high (0.10 ml) rewards with a delay imposed after reward access. Three different arms offered delayed access to low, medium or high rewards. In the standard task, the delays were matched before or after reward access for each reward amount (10 seconds for low reward, 30 seconds for medium reward, and 60 seconds for high reward). With sufficient training, animals exhibited a significant preference for immediately accessible arms. Additional task manipulations increased the probability of entering an immediately accessible arm and efforts to attenuate this preference, by increasing the delay on immediately accessible arms, had only a minor effect on performance. Finally, administration of the nicotinic receptor antagonist, mecamylamine, did not significantly affect performance. The present experiment represents an initial effort to characterize performance in a novel maze task that allows a direct comparison between immediate and delayed access for matched rewards.

Introduction

Psychologists have classified many different disorders according to a shared dysregulation of impulse control, including ADHD, substance abuse, kleptomania, and pathological gambling (American Psychiatric Association, [*DSM-IV-TR*], 2000). Impulse control, or impulsivity, is a broad construct with multiple behavioral and neurobiological components. Investigations into the neurobehavioral mechanisms that underlie impulsivity, especially as it relates to drug abuse, have led to the partitioning of this construct into impulsive action and impulsive choice. Impulsive actions generally include actions that "lack behavioral inhibition, are premature, mistimed, or difficult to suppress or control" while impulsive choice refers to actions that are initiated without "due deliberation of other possible options or outcomes" (Dalley et al., 2007). Impulsivity then is not a unitary construct; rather it encompasses a range of behaviors including aspects of hyperexcitability, behavioral disinhibition, and higher order decision making (Evenden, 1999, Winstanley et al., 2004). There are no conclusive data that examine the extent to which these impulsive behaviors are related, co-vary, or are controlled by similar neurobiological mechanisms. These factors make it difficult to assess the construct validity of a given definition of impulsivity (Mitchell, 2004).

Behavioral Measures of Impulsive Action

There are a variety of different operational measures used to assess both impulsive action (commonly referred to as behavioral inhibition) and impulsive choice. Impulsive action is generally measured using the Go/No-go task or one of its variations. In the general Go/No-go task, the delay between a Go and No-go signal is decreased until the response is accurately inhibited 50% of the time. This measure allows for individual

differences in behavioral inhibition to be quantified. This task was first applied in nonhuman studies; however, due to easy translation across species, versions of this task have also been widely tested in human studies. In general, individuals that require a longer time for inhibition to occur are classified as more impulsive (Mitchell, 2004).These findings are generally concordant among both human and nonhuman species (de Wit and Richards, 2004).

Another version of the Go/No-go task is the Stop Signal Reaction Time (SSRT) Task which has also been widely tested across both human and nonhuman subjects. One important limitation to consider in comparing performance on the Go/No-go and SSRT tasks is that they may not be measuring the same underlying construct. While SSRT tasks measure the speed at which a previously engaged response is inhibited, Go/No-go tasks assess the ability to inhibit the initiation of that start response (Eagle and Baunez, 2010). Therefore, caution must be taken when interpreting performance across tasks. Stop-signal reaction time is the critical measure on the SSRT task and is assessed indirectly from the proportion of trials on which successful inhibition is achieved in conjunction with the distribution of reaction times for Go-signals (Madden and Johnson, 2009). This measure is mapped onto a mathematical race model which assumes that as the stop-signals are presented closer to the termination of the response, the stop and go processes must compete for completion (Madden and Johnson, 2009).

A variant of the Go/No-go task in humans is the continuous performance task (CPT) and in rodents, the five-choice serial reaction time (5-CSRT) task. The CPT was first designed to assess "sustained and divided attention" in humans and shortly thereafter a version was developed for animal research (Eagle and Baunez, 2010).The 5-CSRT task

provides a behavioral measure of two types of inhibition. First, impulsive action is defined as a nose-poke before the end of the inter-trial interval (ITI), a premature response whose frequency serves as a measure of the ability to withhold a pre-potent response. Additionally, this task provides a measure of perseverative response, implicated in compulsive behavior, which is quantified according to number of nose-pokes during the ITI that occur despite absence of reinforcement. This behavior is particularly relevant to perseveration when the nose-pokes persist in the presence of negative consequences including, for example, a reduction in the total reward attainable on the task or removal of reward delivery on a correct trial (Eagle and Baunez, 2010). An important consideration in comparing results between response inhibition tasks is that successful inhibition during the No-go period of the 5-CSRT task is not reinforced, thus the variables that underlie impulsive response on this task may be qualitatively different from those controlling reinforced inhibition (Mitchell, 2004). Lastly, the reversal learning task is another operational measure of behavioral inhibition that assesses the ability to inhibit a response when the rules of a learned paradigm are reversed, or reward contingencies are adjusted. Like the 5-CSRT task, reversal learning tasks provide quantifiable measures of response perseveration and inhibition (Fillmore et. al, 2006).

One major challenge in employing these operational measures of impulsivity is that the extent to which measures are related is relatively unclear. Future studies must continue to investigate relevant neurobiology underlying task performance in order to more clearly define degree of relatedness among these tasks. Current studies rely on converging evidence in order to take different features of behavioral inhibition into account.

Behavioral Measures of Impulsive Choice

As mentioned previously, the impulsivity construct can be broadly partitioned into impulsive action and impulsive choice (IC). In contrast with impulsive action, IC is defined as the selection of a smaller, more immediate reinforcer over a larger, delayed reinforcer and is typically measured using delay discounting procedures (Madden and Johnson, 2009). Delay discounting or delay aversion refers to the "subjective devaluing of a reinforcer as the delivery is delayed in time" and occurs across species (Evenden, 1999). The delay discounting procedures and species tested vary widely; however, currently available evidence suggests that as delays increase, the value of the outcome decays according to a hyperbolic function across both hypothetical and real outcomes (Madden and Johnson, 2009). The hyperbolic function model actually provides a more accurate representation of human delay discounting than the exponential discount function proposed by economists. Thus, there is an inherent bias across species to choose the smaller, more immediate reinforcer despite the fact that it is not the most economically advantageous choice (Perry et al., 2008). Pilot testing in this field utilized non-human subjects and the procedures that were developed served as the basis for human paradigms. Presuming that these human paradigms assess similar neural processing in non-humans, these measures also serve as tools to investigate the neurobiology underlying delay aversion (Mitchell, 2004).

The first category of delay discounting measures consists of discrete-trial procedures, both adjusting-delay and fixed-delay (Madden and Johnson, 2009). These tasks allow for precise control of delay between response and reinforcer; however, these schedules also tend to produce exclusive preferences in choice behavior. One of the first

discrete-trial delay discounting tasks, pioneered by Mazur in 1987, was the adjusting-delay procedure in which the subject chooses between two levers which result in delivery of a large reward after an adjusting delay or a smaller reward delivered after a fixed delay (Madden and Johnson, 2009). This task is designed to identify the indifference point or the mean adjusted delay at which the subject is indifferent between the two rewards and thus reaches a steady level of response at 50 percent choice for each lever. A higher probability of choosing the smaller, immediate reward as the delay is increased represents a higher level of delay aversion or impulsive choice (Eagle and Baunez, 2010). Thus, subjects with larger adjusted delays at the indifference point are classified as less impulsive in comparison with smaller adjusted delays. This design improved upon previous tasks by accounting for individual differences in delay aversion more precisely. Importantly, it enabled differentiation between sensitivity to reward magnitude and sensitivity to reinforcer delay within the same trial (Ho et al., 1999). A major limitation, however, is the parametric nature of the task which resulted in concerns raised in 2002, by Cardinal, Roberts, and Everitt, over the length of sessions required to achieve stability and rate at which delays were adjusted across labs (Madden and Johnson, 2009).

In 1999, Richards pioneered the adjusting-amount task in which the experimenter adjusts the amount of immediate reinforcer until a stable indifference point is reached (Richards et al., 1999). Evenden and Ryan developed a variant of this task, an "operant, discrete-trial fixed-delays procedure" in which delay to the larger reinforcer is fixed within blocks and varied between blocks. This task is currently the most widely used behavioral assessment of delay discounting in nonhumans (Winstanley, 2009). Subjects typically show strong preference for the large reward early in the session, and stable

performance provides an accurate baseline from which to assess acute and chronic task manipulations. The design strength lies in providing measures of sensitivity to both reinforcer amount and delay to reinforcer within each session (Eagle and Baunez, 2010). In contrast to previous tasks, the trial length is kept constant to avoid a confounding increased local rate of reinforcement for choice of the smaller, immediate reward. Furthermore, this procedure is frequently employed in drug studies because it removes the bias that may result from systematic variation of delays. The most fundamental issue with this task is that choice in each trial block may be influenced by effects from previous training and exposure to reward contingencies that occur throughout the task sessions. For example, results from the above study identified carryover effects in the heightened sensitivity to delay present in trial blocks with zero delay (Winstanley, 2009). It is possible that these effects could be attenuated with an increased number of forced-choice trials within the block and/or control sessions in which no delay is paired with the larger reinforcer. Human studies that employ similar methods have investigated different types of reinforcers (i.e. monetary, drugs of abuse); however, caution must be exercised in comparing these results across humans and nonhumans because there may be fundamental differences in motivational processes that are driving task performance (Mitchell, 2004). Taken together, the aforementioned studies characterized performance on different types of delay discounting tasks with respect to both reward magnitude and delay to reinforcer.

Similar to operational measures of behavioral inhibition, a major challenge in employing these paradigms is that it is unclear to what extent the measures are related. Therefore, current studies must rely more heavily upon converging evidence while future

studies continue to investigate relevant neurobiology underlying task performance in order to define degree of relatedness between measures. Neurobiological evidence characterizes impulsive choice as a product of "decision-making processes that weigh the features of the reinforcers, attention processes that enumerate those features and working memory processes that allow the reinforcers to be compared" (Mitchell, 2004). These processes are implicated in behavioral inhibition, cognitive control, and reward-related learning.

Neural circuitry underlying performance on Delay Discounting Tasks

The nucleus accumbens (NAcb) core has been widely implicated in reward learning and maintenance of reinforcer value over a delay (Cardinal, 2006). It follows that reducing activity in this region increases both response-inhibition impulsivity and impulsive choice and suggests a role for the NAcb core in cognitive processes common to both impulsivity constructs. Lesions of the entire NAcb do not make subjects universally impulsive, as evidenced by elevated risk aversion on a probability discounting task (Cardinal and Howes, 2005). Cardinal et al. (2001) found that lesions of the nucleus accumbens core (NAcb) in rats produced impulsive choice as measured by increase in choice of smaller-sooner over larger-later reward on a delayed reinforcement task. This effect could be due to processing of delay to reward, reward magnitude, or both. The explanation including hypersensitivity to delay seems more relevant given evidence that NAcb core-lesioned rats can still discriminate large from small rewards (Cardinal et al., 2004). Cheung and Cardinal (2005) provided evidence that excitotoxic core-lesioned subjects were unimpaired when there was no delay between action and outcome, but were profoundly impaired upon incorporation of a delay in contrast to shams learning with the

same delay (Cheung and Cardinal, 2005). A decrease in reward magnitude perception was ruled out by including trials in which both levels of reward were presented with no delay to reward delivery.

Another experiment tested the effects of lesions of the two main afferents to the NAcb core, the anterior cingulate cortex (ACC) and medial pre-frontal cortex (mPFC) and found that behavior on a delay discounting task was similar to controls, implicating the core but not necessarily its afferents as necessary for maintaining the value of a reinforcer over a delay (Cardinal et al., 2001). While activity in the mPFC may not be the critical brain region in delay discounting, this area is recruited during task performance and shows greater activation in paradigms that incorporate a within-session shift in delay. Furthermore, lesions of the mPFC promote increased perseverative behavior indicating that this region plays an important role in adjusting behavior as reward contingencies change (Winstanley, 2009). It is also important to note that lesions of the NAcb shell, as well as pre-limbic and infra-limbic (PL and IL) cortical lesions did *not* result in impulsive choice on delay discounting tasks (Eagle and Baunez, 2010).

Dalley et al. (2007) examined the NAcb core and shell. They proposed that impulsive choice on delay discounting procedures is dependent upon the functional integrity of the OFC and NAcb core, while performance on operational measures of impulsive action is dependent upon the medial PFC (including the anterior cingulate cortex (ACC) and infra-limbic cortex (IL)) as well as NAcb shell and regions of the striatum (Dalley et al., 2007). This study provides evidence of distinctive neural circuitry underlying impulsive action and impulsive choice.

The OFC is a region of the pre-frontal cortex (PFC) that projects to NAcb core and is strongly implicated in assessment of both goal-directed behavior and reward value (Cardinal et al., 2001). In human studies, lesions of the OFC have been causally linked to impulsive behavior including maladaptive decision-making, deviant social behavior, and risky choices on gambling tasks (Winstanley, 2009). Roesch and Olson (2007) recorded single neurons in the OFC and pre-motor cortex of two monkeys performing a task that was designed to distinguish motivation for reward from reward value. The study found that neuronal activation in the OFC was implicated in reward value while neuronal activity in the pre-motor cortex was mainly associated with motivation for reward. Despite clear evidence that OFC is implicated in assessment of reward value, studies in rodents produced varied results with regards to the OFC's role in impulsive behavior. Winstanley et al. (2004) found evidence of decreased impulsive choice as measured by increased preference for larger, delayed rewards in subjects with OFC lesions compared to controls; however, there are also studies to support the opposite effect, suggesting the need to further investigate sub-regions of the OFC in inhibitory control (Eagle and Baunez, 2010). The above findings support a complex role of the OFC in inhibitory control.

The basolateral amygdala (BLA) also projects to the NAcb core and has extensive reciprocal connections with the OFC. Human studies implicate the functional integrity of the connection between the BLA and OFC as critical for aspects of goal-directed behavior (Winstanley et al., 2004). In addition, a study by Schoenbaum et al. (2002) provided evidence that the OFC and BLA work together to encode the value of different stimuli in various measures of behavioral inhibition, including the Go/no-go task

(Schoenbaum et al., 2002). Similar findings have been well-documented in rodents. Excitotoxic BLA-lesioned subjects increased impulsive choice on a delay aversion task involving choice between immediate one-pellet and delayed four-pellet rewards (Winstanley et al., 2004), similar to the effects of NAcb core lesions (Cardinal et al., 2001).

Evidence for a Limbic-Motor Interface

A recent study by Roesch et al. (2009) evaluated evidence implicating different striatal and cortical regions in impulsive choice task performance. The researchers proposed that the ventral striatum (VS), through functional connectivity with brain regions implicated in both decision-making and motor behavior (including the PFC, OFC, BLA, and midbrain DA neurons) acts as a "limbic-motor interface**"** responsible for integrating information on action-outcome contingencies (Roesch et al., 2009). To test this hypothesis, rats were trained to respond on a delay discounting task to three different odor cues with two forced-choice and one free-choice alternative. Delay to reward and reward magnitude were manipulated across trial blocks such that there were four types of rewards: short-delay, long-delay, large-reward, and small-reward. Reaction time was shorter and VS neural activity higher when more valued reward (short delay or large reward) was available. The task design ruled out differences in reaction time as a result of "satiation or insufficient learning" (Roesch et al., 2009). In addition, VS neural activity following response was stronger in anticipation of delayed reward. Taken together, these results suggest that the VS neurons may play two distinct roles in delay discounting tasks. Activity in these neurons is higher prior to an immediately available reward, a result consistent with prior research that reliably demonstrates VS lesions increase the

probability that subjects will choose smaller, more immediate rewards (Winstanley et al., 2004). However, once a decision for the delayed reward has been executed, VS neural activity also increases, potentially acting to "maintain a representation of the anticipated reward" (Roesch et al., 2009). These results may be differentially relevant in the context of distinct delay discounting tasks. If, for example, the task included reversal learning, heightened VS activity after the decision for the larger, delayed reward may not be as necessary to maintain the value of that reward. These results also align with the proposed "actor-critic" model of the VS and dorsolateral striatum (DS) respectively. Through functional connectivity with midbrain dopaminergic neurons, the VS "critic" is believed to encode and store the value of a given condition and exert influence on downstream dopamine neurons which alter the DS "actor" course of action (Roesch et al., 2009). Results from this study provide strong evidence in support of the role of the ventral striatum in delay discounting tasks.

Neurotransmitter Systems Implicated in Impulsive Choice

In examining the neural circuitry that underlies delay discounting task performance, it is also relevant to investigate the role of major neurotransmitter systems implicated in impulsivity. The majority of previous research in this field was conducted based on the knowledge that psychostimulants, which are traditionally employed in the treatment of ADHD, primarily act through the dopaminergic system. Caution must be exercised, however, when interpreting the differential effects of psychostimulants. While the general trend is that psychostimulants such as amphetamines decrease impulsive behavior through increased dopamine (DA) transmission, amphetamine has the added effects of enhancing conditioned reinforcers, such as the signal during delay to

reinforcement, which may ultimately produce choice behavior in favor of the delayed reinforcer (Cardinal et al., 2004). Conversely, systemic administration of DA-D1 receptor antagonist increases impulsive choice for small, immediate rewards (Winstanley, 2009). Functional brain imaging revealed that the effect of psychostimulants on dopamine neurotransmission is mediated by the OFC in delay aversion tasks (Dalley et al., 2007). DA lesions of the OFC but neither the NAcb core nor the PFC affect delay discounting measures (Winstanley et al., 2004).

In a study assessing the role of dopaminergic and glutamatergic neural circuitry in mediating performance on a delay-based decision making task, it was determined that dopamine and NMDA receptors make "dissociable" contributions to these forms of analyses (Floresco, Tsi, and Ghods-Sharifi, 2008). Low doses of amphetamines increased the willingness to wait for larger, delayed reward. These results were likely due to an improved tolerance for delay to reward due to increased DA transmission, supported by the following evidence: "The effects of amphetamine are blocked by DA-D1, DA-D2 receptor antagonists" (Floresco et al., 2008). It is possible that higher doses of amphetamine may have resulted in excessive DA-D1 receptor activation, which may in turn have affected activity in PFC neural networks that normally integrate information about response costs and reward magnitude associated with each response option (Floresco et al., 2008). Alternatively, systemic administration of the NMDA receptor antagonist ketamine reduced preference for the larger, delayed reward indicating increased delay aversion. The experimental design ruled out the possibility that the effects were due to impaired motor functioning. In rats performing on a peak interval procedure, NMDA antagonism resulted in overestimation in time of delay to reward,

suggesting a possible mechanism whereby animal choices were biased towards the smaller, immediate rewards (Floresco, et al., 2008).

Studies that investigate the effects of administering the psychostimulant amphetamine on performance in operational measures of impulsive choice have also revealed interactions between serotonin (5-HT) and DA neurotransmitter circuits. Some overlap in activation regions for these two neurotransmitter systems exists but is not well characterized in the current literature (Adriani et al., 2010). The importance of these neurotransmitter systems in regulating impulsive behavior is supported by evidence that variability in ADHD subpopulations may be partially due to differences in relative dysfunction between DA and 5-HT systems (Winstanley et al., 2004). Global decreases in 5-HT are associated with increases in impulsivity via actions at several different receptor subtypes. 5-HT1A receptor blockade, which has been most widely investigated, increased choice of smaller, sooner reward on a delay discounting task (Winstanley, 2004). Everitt (2008) found that performance in a delayed reinforcement procedure correlates with increased 5-HT efflux in the medial prefrontal cortex, implicating distinctive brain regions in sub-forms of impulsivity (Everitt et al., 2008).

Another successful treatment for patients with the impulse control disorder ADHD is norepenephrine (NE) reuptake inhibitor atomoxetine. Systemic administration of this drug decreased impulsive action or premature responding in both 5-CSRT and SSRT tasks and decreased impulsive choice in delay aversion tasks through enhanced noradrenaline signaling (Robinson et al., 2008). This effect is likely not due to perseveration because atomoxetine generally decreases response perseveration (Winstanley, 2009). Unlike dopamine, the brain regions principally mediating this effect

have yet to be identified; however, NE neural circuitry may engage the OFC, as DOPAC is formed during the metabolism of both DA and NE (Eagle and Baunez, 2010). In summary, increased noradrenergic transmission is unique in its ability to reduce inhibitory deficits in tasks that measure different forms of impulsivity (both behavioral inhibition and impulsive choice), implying utility in the treatment of a wide range of impulse control disorders.

The preceding studies implicate dopamine and norepinephrine in the treatment of ADHD; however, recent evidence also suggests that cholinergic neurotransmission, especially involving neuronal nicotinic acetylcholine receptors (nAChRs) may also be a valuable target in treating this impulse control disorder. A role for nicotinic receptors was first investigated with respect to the association between ADHD and an increased risk and earlier onset of cigarette smoking (Wilens and Decker, 2007). It has been widely documented that administration of nicotine and nicotinic receptor agonists increases impulsivity in human and animal studies (Mitchell, 2004). Despite the caveats inherent in studies of smokers versus non-smokers, it has been reliably demonstrated that smokers display higher levels of delay aversion with regards to cigarettes versus monetary rewards, in addition to discounting monetary rewards more than controls on delay discounting tasks (Mitchell, 2004). While the neurobiology underlying this performance is still under investigation, there is evidence to support a role for nicotinic receptors in impulsivity.

The role of nicotinic acetylcholine receptors (nACHRs) in modulating other neurotransmitter systems, especially DA, provides a rationale for studying this receptor system with respect to performance on delay discounting tasks. The relationship between

nACHRs and DA release is most commonly investigated with respect to the reinforcement of smoking behaviors. The post-synaptic nicotinic receptor stimulation of DA release is robust and dose-dependent. Studies demonstrate that nAChR stimulation increases dopaminergic transmission in rat striatum; nicotine has been shown to have similar effects on striatal presynaptic DA transporters in adults with ADHD (Wilens and Decker, 2007). It is important to consider post-synaptic actions of nAChRs as well. nAChRs increase the release of other neurotransmitters implicated in cognitive function including norepinephrine, serotonin, GABA (gamma aminobutyric acid), glutamate, and acetylcholine itself (Wilens and Decker, 2007**).** A study by Gray and colleagues demonstrated that glutamate release in the rat hippocampus is modulated by presynaptic nACHRs (Gray et al., 1996). Glutamate in turn can elicit DA release via actions at NMDA and AMPA receptors on DA terminals in striatum (Cheramy et al., 1996). The above data suggest a role for cholinergic neurotransmission and rationale for targeting the nicotinic receptors in performance on delay discounting tasks. It is imperative that future studies continue to investigate neurobiology and neurotransmitter systems implicated in measures of impulsive choice in order to more precisely characterize and target treatments for impulse control disorders.

Utility of Delay Discounting Tasks in Predicting Treatment for Impulse Control Disorders

Impulse control disorders are characterized by a "dysregulation of impulse" that is most widely associated with measures of impulsive choice versus impulsive action (*DSM-IV-TR*, 2000). Recent studies support the utility of delay discounting tasks as predictors for success in the treatment of impulse control disorders including substance

abuse. The findings are as follows: "The rate at which delayed outcomes are devalued appears to be a good predictor of success in human drug treatment […] and appears to predict drug self-administration in rats" (Yoon et al., 2007). Despite the pervasive association between impulsivity and substance abuse in the literature, the causal relationship underlying this association is unclear. Importantly, while chronic drug exposure may elevate impulsivity and therefore lead to continued drug use, elevated impulsivity may also represent a liability factor for distinct phases of drug abuse. It is well-established that chronic drug users display reliable impairments in measures of cognitive control, specifically deficits in impulsive choice. This weakened control over impulsive thoughts and behavior is a phenotype that directly contributes to failure to decrease drug-taking behavior, as defined by the *DSM-IV-TR*. While past research on drug use prevention and treatment strategies has focused on mechanisms that support drug-taking, the evidence above provides a rationale for investigating mechanisms that mediate inhibition or the suppression of drug-taking behavior.

Human Studies

In a pilot study investigating the relationship between smoking cessation and impulsivity in adolescents, it was determined that adolescent smokers who were not abstinent following a four-week smoking cessation program (which included contingency management and cognitive-behavioral therapy) were significantly more impulsive prior to treatment compared to those who attained abstinence. Importantly, both self-report (Barratt impulsiveness scale (BIS-II) and behavioral measures (Kirby delay discounting measure (DDM)) of impulsivity were assessed immediately prior to program start. Only *behavioral* measures of impulsivity were significantly associated with the treatment

outcome (Krishnan-Sarin et al., 2007). This result is supported by evidence that "behavioral and self-report assessments of impulsivity may represent different level of analyses, from detailing specific behavioral processes to more general trait-like impulsive tendencies" (Eagle and Baunez, 2010). Important limitations include the small sample size and assessment of pre-treatment impulsivity only. Future studies should include both pre- and post-assessments to determine how this effect changes over time given the differential effects of acute versus chronic administration of nicotine. At a minimum, these results provide support for specific behavioral measures of impulsive choice as predictors for the ability to initiate and maintain abstinence from smoking in adolescents (Mitchell, 2004).

Nonhuman Studies

The hypothesis that increased levels of impulsivity may lead to increased drug intake is supported in "acquisition, escalation/ dysregulation, abstinence, treatment, and relapse" phases of human drug abuse (Perry and Carroll, 2008). Support for this hypothesis in the current literature primarily consists of animal studies on the abuse of cocaine, amphetamine, nicotine, and other stimulant drugs. This hypothesis was first studied with respect to nicotine-seeking, cued reinstatement and behavior during withdrawal. Diergaarde et al. (2008) categorized rats into high-impulsive (HI) and low-impulsive (LI) groups based on performance in a delay discounting task. HI rats displayed "diminished ability to inhibit nicotine-seeking during abstinence and enhanced vulnerability to relapse upon re-exposure to nicotine cues" (Diergaarde et al., 2008). A second experiment within this study found that rats categorized as HI based on performance on an impulsive action task displayed enhanced "acquisition and

maintenance" of nicotine self-administration. This study supports a differential relationship among sub-forms of impulsivity and vulnerability to distinct stages of nicotine-seeking in rodents (Diergaarde et al., 2008). Similarly, in human studies, individuals with impaired inhibitory control may be unable to resist environmental cues such as peer pressure that would lead them to relapse (de Wit and Richards, 2004). Taken together, these studies provide evidence that measures of impulsive choice serve as predictors for the ability to inhibit nicotine-seeking during abstinence and resist relapse upon re-exposure to nicotine cues.

In a recent study by Perry et al., (2005), impulsivity as measured by impulsive choice on an adjusting delay discounting task was used to separate groups of HI and LI rodents. The HI rodents acquired cocaine self-administration at a higher rate and required fewer days to reach that level of performance than LI rodents. The two groups were equivalent on measures of motor activity, response latency, and responses on the inactive lever, which supports the given interpretation because the results cannot be accounted for by differences in baseline levels of activity. A potential confounding factor in this study was differential learning on the delay discounting task that may have influenced rates of acquisition of cocaine self-administration; however, there was no indication that being subject to the task for a longer time period caused alterations in preference for the larger, delayed reward. Overall, these findings support the claim that elevated impulsivity may result in increased probability and more rapid cocaine self-administration (Perry et al, 2005).

Drug-Induced Neuroadaptations Increase Impulsivity

As previously stated, the causal relationship between impulsivity and drug abuse is not clear. While elevated impulsivity may represent a liability factor in distinct phases of drug abuse, chronic drug exposure may also elevate impulsivity, leading to continued drug use. Dallery and Locey (2005) reported a study in which they found chronic nicotine exposure in rodents via subcutaneous administration reliably increased impulsive choice on delay aversion tasks. It is important to note that for nicotine, these effects were not caused by acute administration because the increase in impulsive choice responses continued for approximately one month after nicotine cessation (Dallery and Locey, 2005). These results suggest a mechanism whereby elevations in impulsive choice even following cessation of drug administration could partially explain high relapse rates in smokers (Setlow et al., 2009). Chronic amphetamine exposure similarly increased impulsive choice on delay aversion tasks; however, in contrast to the effects of chronic nicotine exposure, there was no effect on impulsive choice following amphetamine cessation, indicating differential neurobiological effects over time. These findings support previous research in human amphetamine abusers who display fewer cognitive deficits than other drug users (Setlow et al., 2009).

Rationale for Current Study

To review, impulsive choice is defined as the selection of a smaller, more immediate reinforcer over a larger, delayed reinforcer and is typically measured using delay discounting procedures (Winstanley, 2009). Delay discounting or delay aversion refers to the "subjective devaluing of a reinforcer as the delivery is delayed in time" and occurs across species (Evenden, 1999). The tasks and species tested vary widely; however, recent studies support the utility of delay discounting tasks as predictors for

success in the treatment of impulse control disorders including ADHD and substance abuse (*DSM-IV-TR*, 2000). Despite this prospective role in the investigation of impulse control disorders, behavioral measures of delay discounting to date do not address several important facets of delay aversion. First, these typical two-choice tasks do not allow assessment of the effects of immediate versus delayed access to multiple reward amounts. Moreover, a direct comparison of the effects of delay preceding or following reward access is confounded by reward amount. In order to address these methodological issues and further characterize performance on delay discounting tasks, additional choices must be made available.

In the present experiment, rats were trained in an 8-arm automated radial maze. Three of the arms offered immediate access to low (0.01 ml tap water), medium (0.06 ml), or high (0.10 ml) rewards with the delay imposed following reward access, while the three remaining choice arms offered delayed access to low, medium or high rewards. In the standard task, the delays were matched before or after reward access for each reward amount. Additionally, the maze included two holding arms to control for distance traveled to each of the arms. The present experiment represents an initial effort to characterize performance in a novel maze task that allows a direct comparison between immediate and delayed access for matched rewards. A major objective was to demonstrate subject sensitivity to both reward magnitude and delay, task parameters that are known to affect performance in typical two-choice delay discounting tasks.

In addition, while prior research implicates several different neurotransmitter systems in impulsive choice, most notably dopaminergic, noradrenergic, and serotonergic systems, there has been very little investigation into the role of the cholinergic system in

performance on delay discounting tasks (Winstanley, 2009). It has been widely documented that administration of nicotine and nicotinic receptor agonists increases impulsivity in human and non-human studies (Mitchell, 2004). Thus, in the present experiment, it was hypothesized that administration of mecamylamine, a nicotinic receptor antagonist, would decrease preference for the most impulsive choice, high-reward immediate access arm four.

In order to characterize performance on this task in terms of reward and delay parameters as well as pharmacological manipulations, a within-subjects design (WSD) was employed. The nature of this task demands a WSD for several important reasons, the foremost of which is that it allows for the examination of enduring impacts of task manipulations on performance. Furthermore, due to the large individual differences (the WSD controls for subject variables) and small subject pool, within-subjects design was best suited for this data. With regards to interpreting the data, however, there are two general types of confounds that present when learning and practice effects occur in within-subjects designs. First, order effects which are defined as "changes in subject performance that occur as a function of a given treatment condition occurring in different positions in a sequence or series of treatments" present a unique confound because subjects must complete one level of an independent variable before progressing to the next (Hall, 1988). The order may have no effect, or there may be some form of "progressive error" defined as either improvement as a result of practice or familiarity with the task or a performance deficit resulting from fatigue, boredom, or decrease in attention (Hall, 1988). The second general type of confound for WSD is common to drug research. In drug administration studies using a WSD, carryover effects may occur if the

effect of a treatment condition persists after the condition ends (Hall, 1988). These effects can usually be controlled for by counterbalancing procedures. In the current experiment, we controlled for carryover effects during mecamylamine administration by randomized counterbalancing in which drug doses were administered in random orders across subjects such that carryover effects should balance out in pooling all subject data. Additional controls were applied in order to reduce confounds inherent in this experimental design and are detailed below.

Method

Subjects

In the current experiment, the subjects were ten Long-Evans male rats (Charles River Laboratories Inc., Wilmington, NC). These rats arrived at approximately two months of age, weighing 151-175 g at the vivarium of the Psychology Department at the College of William and Mary, Williamsburg, VA. Animals were housed in individual wire cages. Food (Formulab Diet 5008; W.F. Fisher & Son, Somerville, NJ) was available ad libitum throughout the experiment. After acclimating to the vivarium for approximately one week, animals were gradually exposed to water restriction, eventually receiving water access during behavioral testing and for 30 minutes immediately following each test session. The vivarium was temperature controlled and maintained on a 14:10 light/dark cycle, with light onset at 6 am. All experimental procedures were approved by the Institutional Animal Care and Use Committee at the College of William and Mary.

Behavioral apparatus

All training occurred in a computer-controlled eight arm radial maze (Med Associates, Inc., Georgia, VT, USA). Each of the eight arms (60.0 X 18.4 X 10.2 cm L X H X W) was attached to an octagonal hub (29.0 cm diameter). The floor of the maze was constructed of white polycarbonate and the walls and ceilings of clear polycarbonate. Access to each arm was controlled by motorized doors made of aluminum. Photocells and a water port were located at the end of each arm. The water port contained a dipper that could be raised to allow access to tap water (reward size varied from 0.01 to 0.1 ml). The maze was located in a windowless room that had many visual stimuli, including a computer, desk, utility cabinets, and metal shelving containing laboratory equipment. Data collection and execution of programs were controlled using a PC with Med-PC-IV software.

Procedure

Behavioral testing always took place from 11am-2pm and animals were tested in the same order each day. Rats were tested five days per week, Monday through Friday, and were allowed access to water ad libitum Friday after the test session to Sunday at 11am.

Acclimation

For the first two behavioral testing sessions animals were placed in each arm of the maze for five minutes. The animals received water access each time they broke the photocell at the end of the arm. After acclimation, all animals began training in the standard version of the six-choice delay discounting task.

Standard Task

Each session began with the rat being placed in the center hub. The gate that blocked holding arm one or five was then raised. In order to control for the distance

traveled to the arms, half of the animals had arm one as the holding arm and the other half of the animals had arm five as the holding arm. When the animal broke the photocell at the end of the holding arm, the dipper was raised for 3.0 s with 0.01 ml water. The gates blocking arms two, three, and four were simultaneously raised, thus they were referred to as the immediate access arms. The gates blocking arms 6-8 were raised after variable delays (arm six, 60 second delay; arm seven, 30 second delay; arm eight, 10 second delay) (*Fig. 1*). The cups attached to the dippers at the end of each arm varied in size. Arms two and eight were low reward (0.01 ml), arms three and seven were medium reward (0.06 ml), and arms four and six were high reward (0.10 ml). Thus, the animals could enter arms 2-4 immediately after leaving the holding arm or could enter arms 6-8 when the gates were raised after the respective delay for that arm. If the rats entered an arm that was immediately accessible (arms 2-4), when the rat broke the photocell at the end of the arm, the dipper was raised and the gate for that arm was lowered for a delay. The delays for these immediately accessible arms were as follows: for arm two, the delay was 10 seconds, for arm three, the delay was 30 seconds, and for arm four, the delay was 60 seconds. After the delay, both the gate to that arm and to the holding arm were raised and the animal could enter the holding arm and receive water access. If the rat broke the photocell at the end of an arm which was not immediately accessible (i.e. after the delay for arms 6-8), then the dipper was raised for 3.0 seconds and the gate blocking the holding arm was raised. All other gates closed except for the holding arm. The animal could then enter the holding arm without additional delay. After breaking the photocell at the end of the holding arm, another trial began with the gates to the immediate access arms being raised. The session continued until either 10 responses were recorded or 15

minutes elapsed. At the end of the session, all gates were lowered, the rat was removed from the maze and returned to the hanging wire cages and received 30 minutes water access.

Equivalent Delay Task

After 10 sessions testing in the standard task, rats were trained and tested in the equivalent delay task. The delays were equivalent at 10 seconds for each of the six choice arms. The rats were tested on the equivalent delay task for five sessions under conditions described for the standard task above. The rats were then returned to the standard task for 10 sessions.

Long Hold Immediate Access Arms Tasks

Subjects were then trained and tested in the long hold immediate access arms task. The delays for the immediately accessible arms were as follows: arm two for 30 seconds, arm three for 60 seconds, and arm four for 90 seconds. The gates blocking arms 6-8 were raised after variable delays (arm six, 30 second delay; arm seven, 20 second delay; arm eight, 10 second delay). The rats were tested on the immediate access arm delay task for 12 sessions under the same conditions as described above for the standard task. For the subsequent five sessions, the animals were again tested on the long hold immediate access arms task.

Subjects were then trained and tested on the second long hold immediate access arms task in which the delays associated with immediate access (arms 2-4) were further exacerbated. The delays for the immediately accessible arms were as follows: arm two for 60 seconds, arm three for 120 seconds, and arm four for 240 seconds. The gates blocking arms 6-8 were raised after variable delays (arm six, 30 second delay; arm seven,

20 second delay; arm eight, 10 second delay). The rats were tested on the immediate access arm long delay task for a total of 10 sessions.

Mecamylamine Administration

After completing the long hold immediate access arms task the effects of mecamylamine, a nicotinic receptor antagonist, on task behavior was assessed by administering rats injections of vehicle (saline, 0.0 mg/kg drug, i.p.) or mecamylamine (1.0 and 5.0 mg/kg, i.p.) prior to performance in the long hold immediate access arms task (delays of 60, 120, and 240 seconds in immediate access arms). Sham intraperitoneal (i.p.) injections were administered to each rat three days prior to testing. The needle was inserted into the body cavity without any fluid in order to prepare the animals for the stress of the injection. The order of drug administration was randomized for each rat according to a counterbalancing procedure. After injections, rats were placed in their home cage for 10 minutes, and then transported to the maze, placed in the hub, and the long hold immediate access arms task began. Rats received training in the long hold immediate access arms task without any injection on days between drug administration days.

Effects of pre-session water access

As a final manipulation, rats were tested for one session in the immediate access arm delay task with regular water restriction. After testing, rats were given access to water bottles until being tested in the immediate access arm delay task the next day. A summary of delay/holding times for each task manipulation and sessions per task can be found in *Tables 1 and 2*.

Statistical Analyses

For each of the task manipulations, choice behavior during the final three sessions on each task was averaged in order to compare performance between tasks. ANOVAs were used to investigate the effects of task manipulations on arm choice. All ANOVAs were conducted with arm as one factor including six levels (2, 3, 4, 6, 7, and 8). Additional factors (e.g. drug dose or task type when testing the effects of some task manipulations) were also included in some analyses. See *Table 3* for a summary of ANOVA test values for each task manipulation. A level of $\alpha = 0.05$ was used to determine statistical significance for all ANOVAs. The *P*-values were corrected with the Huynh-Feldt procedure. Because the average number of choices made per session differed significantly between each of the tasks, probability of entering each arm was used as the behavioral measure in the following analyses; therefore, the main effect of week was not significant for any of the task manipulation ANOVAs because the probability of choosing at least one of the six arms was always one. Paired sample *t*-tests were then conducted for each significant task x arm interaction. These tests compared choice for each arm in order to more explicitly define any shifts in choice behavior between consecutive tasks. A Bonferroni correction was applied to these *t*-tests. A summary of significant *t*-tests can be found in *Table 4*.

Results

Task Manipulations

Standard Task

All analyses reflect data from nine animals as one animal did not reliably enter arms during the testing sessions. During the first week of training in the standard task,

rats did not exhibit a significant preference for entering any of the arms. This observation was assessed with a one-way ANOVA including arm (2, 3, 4, 6, 7, and 8) as a factor. This analysis yielded no significant main effect of arm (*Fig. 2*).

Preference for arms three and four, immediate access medium and high-reward arms was established in week two of the standard task. To test the observed preference for arms three and four in comparison with all other arms, a week (standard task week one, standard task week two) x arm ANOVA was conducted. This analysis yielded a main effect of arm (F (5, 40) = 4.491, P<0.05) and a significant week x arm interaction (F (5, 40) = 3.383, P<0.05). In contrast to the lack of observed main effects for the one-way ANOVA for standard task week one, a one-way ANOVA conducted for standard task week two revealed a significant main effect of arm (F (5, 40) = 6.747, P<0.01). Subsequent paired sample t-tests comparing the probability of choosing each of the arms revealed that arm 4 was chosen significantly more frequently than arms 2 and 6 (t (8) = 4.450, P<0.01; t (8) = 4.549, P<0.01). Arm 3 was chosen significantly more frequently than arm 2 (t (8) = 4.256, P<0.01) (*Fig. 3*).

Equivalent Delay Task

Preference for arm four, immediate access high-reward arm, increased in comparison to all other arms when all delays and hold times were equivalent at 10 seconds. To test this observation, a task (standard task, equivalent delay task) x arm ANOVA was conducted. This analysis yielded main effects of arm (F (2, 15) = 11.73, P<0.01) and a significant week x arm interaction (F (5, 40) = 5.573, P<0.01). Subsequent paired sample *t*-tests revealed that choice behavior shifted significantly away from arm eight (t (8) = 5.704, P<0.01). Behavior shifted in a manner that approached significance

towards arm four (t (8) = -3.390, P=0.009 [Bonferroni correction significance level = 0.0083]). Thus, when all delays and hold times were equivalent, subjects increased choice for the immediate access high reward arm four and decreased choice for the low reward delayed access arm eight (*Fig. 4*).

Standard Task Two

Preference for immediate access high-reward arm four increased in comparison to all other arms upon return to the standard task. To test this observation, a task (equivalent delay task, standard task two) x arm ANOVA was conducted. This analysis yielded main effects of arm (F (5, 40) = 34.429, P<0.01) and a significant week x arm interaction (F (5, 40) = 5.319, P<0.01). Subsequent paired sample t-tests revealed that choice behavior shifted significantly towards arm four (t (8) = -3.934, P<0.01) (*Fig. 5*).

Long Hold Immediate Access Arms Task (30, 60, and 90s)

Efforts to attenuate this preference for immediate access arms by increasing the hold times on those arms had only a minor effect on performance. Preference for immediate access medium-reward arm three decreased, while preference for delayed access low-reward arm eight increased. To test this observation, a task (standard task two, increasing delay on immediate arms task) x arm ANOVA was conducted. This analysis yielded main effects of arm (F (5, 40) = 47.643, P<0.01) and a significant week x arm interaction (F (5, 40) = 9.076, P<0.01). Subsequent paired sample t-tests revealed that choice behavior shifted significantly away from arm three (t (8) = 4.064, P<0.01) and towards arm eight (t (8) = -3.486, P<0.01) (*Fig. 6*).

Long Hold Immediate Access Arms Task (60, 120, and 240s)

When the immediate access arm hold times were *further* increased, preference for immediate access high-reward arm four decreased significantly while preference for delayed access medium-reward arm seven increased. To test this observation, a task (long hold immediate access arms task one, task with increased hold times) x arm ANOVA was conducted. This analysis yielded main effects of arm (F (5, 40) = 20.671, P<0.01) and a significant week x arm interaction (F (5, 40) = 9.316, P<0.01). Subsequent paired sample *t*-tests revealed that choice behavior shifted significantly away from arm four (t (8) = 4.223, P<0.01) and towards arm seven (t (8) = -3.766, P<0.01) (*Fig.7*). Thus, when the subjective value of the reward was manipulated by further increasing the hold times on immediately accessible arms, subjects displayed decreased preference for immediate access high reward arm four and increased preference for arm seven, the medium-reward delayed access arm.

Across both long hold immediate access arms tasks, subjects decreased preference selectively for immediate access arms, moving away from medium-reward arm three then high-reward arm four; likewise, subjects selectively increased preference for delayed access arms, significantly elevating choice for low-reward arm eight then medium-reward arm seven. These data support sensitivity to delay as an important factor in task performance.

Response Latencies to Arm 4

Due to both elevated choice and considerable shifts in preference for high-reward immediate access arm 4 across task manipulations, additional analyses were conducted to further characterize subject performance. Response latencies for choice of arm 4 were compared across tasks using paired sample *t*-tests. These analyses revealed a significant

decrease in response latency between the second week on the standard task and the equivalent delay task (t (8) = 4.314, P<0.01). There was a significant increase in response latency between the first long-hold immediate access arms task and the second task long-hold immediate access arms task with further increased holding times (t (8) = 2.524, P<0.01) (*Fig. 8*).

Effect of Injection

Preferences did not vary due to injection. In order to test this observation, an injection condition (saline injection, no injection) x arm ANOVA was conducted. This analysis yielded a main effect of arm (F (5, 40) = 6.755, P<0.01), but no significant interaction. It is worth noting that injection data error bars were, on average, approximately twice the size of error bars on saline trials (*Fig. 9*).

Mecamylamine Administration

Preferences did not shift significantly as a result of mecamylamine administration at any dose. To test this observation, a dose (0.0, 1.0, 5.0 mg/kg) x arm ANOVA was conducted. This analysis yielded no significant main effects or interaction (*Fig. 10*).

Pre-session Water Access

Preference for arm seven decreased as a result of pre-session water access. To test this observation, a water access condition (pre-session water access, water deprivation) x arm ANOVA was conducted. This analysis yielded a significant main effect of arm (F (5, 40) = 6.895, P<0.01) and a significant water access condition x arm interaction (F (5, 40) = 4.901, P<0.01). Subsequent paired sample t-tests revealed that choice behavior shifted significantly away from arm seven (t (8) = -4.168, P <0.01).

Additional one-way ANOVAs were conducted for both water access and water deprivation conditions with arm as the factor including six levels (2, 3, 4, 6, 7, and 8). These analyses yielded a significant main effect of arm for the water deprivation condition (F (5, 40) = 9.432, P<0.01). There was a significant main effect of arm for the water access condition as well (F (5, 40) = 2.785, P<0.05); however, the effect was weaker at P = 0.03. Thus, pre-session water access tended to produce more equivalent probability of entering each of the arms (*Fig. 11*).

Discussion

With sufficient training, animals exhibited a significant preference for immediate access medium and high-reward arms 3 and 4, established during week two on the standard task. Training on the equivalent delay task increased preference for immediate access high-reward arm 4 in comparison to all other arms and decreased preference for delayed access low-reward arm 8. If subjects were simply sensitive to reward, they should exhibit increased preference for high-reward arm 4 as well as arm 6, the high-reward *delay access* arm. Likewise, subjects should exhibit decreased choice for low-reward arm 8 as well as *immediate access* arm 2. Because subjects selectively increased choice for the immediate access high-reward arm 4 and decreased choice for the delayed access low-reward arm 8, these data support an interaction between delay aversion and reward sensitivity in task performance.

When subjects were returned to the standard task in which delays were matched with reward magnitude, choice behavior did not return to levels consistent with original standard task performance. Preference shifted significantly further towards immediate

access high-reward arm 4 (*Fig. 5*). It was hypothesized that this preference for arm 4, now a more "automatic" response that dominated choice behavior on average 59% of each session, could be attenuated by introducing longer hold times on the immediate access arms, thereby reducing the subjective value of immediately accessible rewards. Upon introducing longer hold times on immediate access arms, subjects decreased preference for immediate-access medium reward arm 3. When the hold times on immediate access arms were *further* increased, subjects decreased preference for immediate access high reward arm 4. In both the long-hold immediate access arms tasks, arm 4 represented the most impulsive choice as it was the immediate access arm with the longest holding time (120 and 240 seconds on tasks one and two respectively). Conversely, arm 6 was the most logical choice for the large-reward with only a 30-second delay prior to reward access. The resistance to decreasing choice for arm 4 in the first long-hold immediate access arms task may be best explained by the interaction of delay aversion and maintenance of an "automatic" response through perseveration, habit learning, or some combination of the two. The pattern of increased choice for the delayed access arms in long-hold immediate access arms task is also important to note. As the value of the reward on immediate access arms was subjectively diminished with increasing holding times (30, 60, and 90 seconds) preferences first shifted towards the arm with the shortest delay, arm 8. Preference increased for the medium-sized delay arm 7 only after hold times were *further* increased on immediate access arms (60, 120, and 240 seconds). In general, this behavioral pattern suggests that animals exhibit aversion to delays *prior to* reward access, such that when increased hold times were introduced on

immediate access arms, subjects selectively increased preference for delayed access arms starting with the shortest delay arm 8.

While discussing the probability of entering into different arms above, it is worth noting for which arms preferences did not change over the course of task manipulations. Arms 2 (immediate access low-reward) and 6 (delayed access high-reward) were the lowest choice arms overall. Arm 6 remained the lowest-choice arm from standard task week one through the return to the standard task, and then preference increased slightly as immediate rewards were devalued in long-hold immediate access arms tasks. Arm 2 was the next lowest choice arm overall. Preference for this arm decreased further in the long-hold immediate access arms tasks compared with previous tasks, a logical progression because this low reward was further devalued with increased hold times. In the long-hold immediate access arms task two, access to high-reward arm 6 was available after a 30-second delay in comparison with 240-second hold time for the same size reward on immediate access side (arm 4). Persistent choice for arm 4 provides evidence of a specific type of delay aversion in task performance. When given a choice among matched rewards, subjects endured longer holding times or delays *following* reward compared with delays *preceding* reward. Thus, the findings across task manipulations suggest that subjects are more sensitive to delays *prior to* reward access. When entering an immediately accessible arm, however, preference was largely based upon reward magnitude.

There are likely at least two different types of learning processes that have explanatory value in the context of task performance. The established preference for immediate access, high-reward arm 4 was possibly maintained due to habit learning;

however, when increased hold times were introduced for the immediate access arms, behavior shifted significantly away from this "automatic" response, indicating that the learning process could be manipulated and that choice behavior was still somewhat sensitive to reward contingencies. Thus, we postulate that the relative amount of time spent in different learning processes changed over the course of task manipulations. In contrast to goal-directed behavior that is sensitive to action-outcome or reward contingencies and typically defined as involving a "decision making" process, habitual behavior is associated with automatic processing that is implicit and progressively controlled by conditioned reinforcers (Eagle and Baunez, 2010). Habitual behavior is often defined as compulsive when it leads to response perseveration in the face of negative consequences, resistance to punishment, and/or lack of sensitivity to manipulation of reward contingencies (Yin et al., 2006).

With respect to choice behavior surrounding immediate access-high reward arm 4, it may be pertinent to investigate the possibility of response perseveration. One way in which experimenters have defined response perseveration is the inability to adapt behavior to changes in reward contingencies (Fillmore et. al., 2006). In the present experiment, when reward contingencies were altered by increasing the hold times on immediate access arms in long-hold immediate access arms task, behavior first shifted away from arm 3 only, providing evidence of response perseveration with respect to arm 4. Choice behavior shifted significantly away from arm 4 when reward contingencies were altered again to *further* decrease the subjective value of immediate access rewards. Persistent choice for arm 4, the most impulsive choice in the context of increased hold times on immediate access arms, supports a baseline level of response perseveration on

these tasks; however, behavior remained at least somewhat sensitive to reward contingency manipulation.

In order to further investigate the role of different learning processes across task manipulations, response latencies for choice of arm 4 were calculated. The resulting trend supported the findings for probability of entering arm 4. With respect to immediate access high-reward arm 4, preference was established during week two on the standard task, increased on the equivalent delay task, and increased further upon return to the standard task, accounting for approximately 59% overall choice. Response latencies for arm 4 on week two of the standard task decreased in comparison with week one in a manner that approached significance. Response latencies for choice of arm 4 significantly decreased from week two on the standard task to the equivalent delay task. This trend of decreased response latencies for choice of arm 4 in combination with increased choice supports an interpretation of increased habitual, automatic processing in subject responses on both the standard and equivalent delay tasks. At this point in the experiment, reward contingencies were manipulated. In the first long-hold immediate access arms task, subjects did not significantly decrease choice for arm 4. Response latencies for choice of arm 4 did not shift significantly. Choice for arm 4 persevered in the face of increased hold times for immediate access arms. In the second long-hold immediate access arms task, however, preference for arm 4 decreased significantly while response latencies for choice of arm 4 increased significantly. With respect to immediate access high-reward arm 4, this decrease in choice in combination with increased response latencies supports a shift away from habitual response and toward a response that more heavily engages the decision making process.

To summarize, the above results offer evidence for the interaction of sensitivity to both reward magnitude and delay *prior to* reward access as determinants in task performance. The trends for choice behavior and response latency surrounding immediate-access high reward arm 4 provide evidence of differential learning and behavioral response patterns across task manipulations. While we made postulations concerning the relative influence of different learning processes throughout task manipulations, it is not possible to make any definitive conclusions without assessments of functional brain regions underlying task performance. In future studies, it may be particularly relevant to characterize performance on this novel maze task in terms of neural circuitry and neurotransmitter systems.

As mentioned briefly in the Introduction, several studies have attempted to define the locus of control for different types of learning. Understanding the progression of behavior from impulsive to compulsive, particularly in the context of substance abuse disorders, has served as the impetus for characterizing the neural substrates of habit formation. Previous studies hypothesized several different models to elucidate this process in terms of neural networks. Yin et al., (2006) postulated a hierarchically-related system of three networks: The limbic system, consisting of OFC and ventral PFC, is characterized by "goal- directed behavior that is sensitive to action-outcome contingencies and affective valence, and it has strong dopaminergic control" (Yin et al., 2006). The dorsal striatal network is characterized by stimulus-response (SR) associations and receives inputs from PFC and parietal cortical areas in humans. The sensorimotor network, alternatively, is located in a different region of dorsal striatum (putamen) and is associated with "action schemata and well-established habits" (Yin et

al., 2006). Of particular interest is the characterization of the connections between these systems, particularly in the context of habit formation. Yin et al. (2006) hypothesized that the dopaminergic connections between ventral (VS) and dorsal striatal regions of the nucleus accumbens (NAcb) comprise the following pathway. The NAcb shell projects to NAcb core whose projections to immediately dorsal regions include caudate-putamen. From that region, dopaminergic connections continue into more lateral areas of the dorsal striatum and terminate in regions wherein dopamine receptor antagonists impair drug-seeking behavior. In studies by Yin et al. (2002, 2006), lesions of the dorsolateral striatum (DS) in a task wherein subjects previously exhibited habitual response resulted in the reinstatement of behavioral sensitivity to reward contingency. These results are consistent with the DS-VS "actor-critic" model in which dopaminergic connections are hypothesized to underlie long-term action selection and behavioral consolidation (Roesch et al., 2009). It may be especially relevant to consider the role of these brain regions and functional connectivity in the context of delay discounting task performance. With regards to the present experiment, future studies may gain insight into the relative prevalence of different learning processes during the task by characterizing the neural circuitry and neurotransmitter systems implicated in performance.

Factors Affecting Performance on Delay Discounting Tasks

In characterizing performance on delay discounting tasks, particularly in terms of the psychological processes that may be driving responses, it is useful to consider a few of the complexities in interpreting results from these measures. It is well-documented that animals exhibit both stimulus-response (procedural) and truly goal-directed or action-outcome (declarative) responding. To differentiate, in the declarative method, the subject

must encode both the action-outcome relationship and value of the outcome such that these two factors interact to determine the probability of selecting a given action (Cardinal, 2006). It is not clear to what extent immediate versus delayed reinforcement in the context of delay discounting tasks is controlled by habit versus goal-directed action. Several competing theories exist. Subjects may never fully learn the action-outcome contingency for the longer delay condition, they may value the delayed reinforcer less, or the delay itself may slow the "acquisition of a procedural stimulus-response (SR) habit" (Cardinal, 2006). A recent study provided evidence to support the following trend for environmental context: the longer the delay, the greater the probability that contextual competition will impair the acquisition of the action-outcome contingency (Cardinal, 2006). Alternatively, cues or signals that occur during the delay may either be associated with or act as conditioned reinforcers (CRs) themselves, speeding the acquisition of habitual responses in the case of delayed rewards. In this study, it is more likely that the first relationship applies. Subjects may not have acquired the action-outcome contingency as rapidly or completely for delayed access rewards. This disparity may in turn reflect a bias towards immediate access arms from the earliest stages of learning on the task. These factors are important to consider when investigating the extent to which decision making versus habitual responses act as determinants in task performance.

Additionally, operational measures of impulsive choice in the rat share several other variables of interest that may be useful in characterizing the psychological processes that underlie task performance. These variables include: "omissions in starting trials by nose poke, nose poke latency to start trial, number and duration of nose pokes during delays and inter-trial interval (ITI), reward collection latency, omissions in lever

pressing, and lever response latency for immediate versus delayed reinforcers" (Mar and Robbins, 2007). These measures may provide evidence of differences in attention, arousal, motivation, and/or other psychomotor processes taxed during a particular procedure and may suggest "complimentary or global behavioral effects of experimental manipulations" (Cardinal et al., 2001). Evaluating subjects on these measures may assist both in identifying the psychological processes that underlie task performance and identifying potential confounds. Future studies may more precisely characterize performance on the present task by measuring additional variables of interest.

Furthermore, future studies may incorporate additional measures of impulsivity to more precisely characterize task performance. Most two-choice operant delay discounting tasks assess subjects on only one principal measure of impulsivity: percent larger-later choice versus percent smaller-sooner choice. In a study by Adriani et al., (2010) researchers provided evidence to support an incorporation of additional measures including: "(1) average spontaneous waiting before a choice is made (response time, RT, occurring between the end of a timeout (TO) and the next nose-poke) and consequently (2) time elapsing between two consecutive reinforcing events (mean ITI: TO + RT)" (Adriani et al., 2010). Experimenters proposed that delay aversion may be promoted by the tendency to rely on a regular rate of reinforcement; thus, assuming this rate is imposed by the individual mean ITI, individual differences may arise from the extent to which altering the delay modifies internal pacing (Adriana et al., 2010). In the current study, probability of entering each arm as well as response latencies towards high-reward immediate access arm four (equivalent to principal percent choice measure and RT

measure in the aforementioned study) were compared across tasks to characterize impulsivity according to multiple measures.

In characterizing performance on this novel maze task, several caveats need to be emphasized with regard to interpretation of the present data. As was previously mentioned, a within-subjects design (WSD) was chosen to achieve the objectives of this study. WSD was primarily employed due to small sample size and individual differences in delay discounting tasks utilized in the literature. Moreover, WSD enabled some examination of the effects of exposure to varied task parameters on subsequent performance. However, confounds present in this design made interpretation complex.

One confound inherent in task design was the inability to determine to what extent the order in which task manipulations were performed may have contributed to the final results, especially with regards to choice behavior surrounding high-reward immediate access arm 4. This limitation is consistent with previously characterized delay discounting tasks in which prior learning affects task performance (Winstanley, 2009). The present results for each task are likely due to a combination of training on the current task and learning on previous tasks, especially in cases wherein choice behavior progressed towards more "automatic" or habitual responses. In the present experiment, the value of each reward was not altered directly; however, delays/ holding times were manipulated between tasks which altered the subjective value of the reward. To challenge the critique that delay aversion is dependent upon the particular order in which delays are presented, future studies using this maze task may include procedures in which the order of delay presentation is reversed (ascending to descending or vice versa) in order to compare observed trends (Robles et al., 2009). Another important variable to consider is

the magnitude of immediate and delayed rewards to which subjects are exposed. The magnitude effect, demonstrated reliably in humans, predicts that delay discounting should be greater with ascending presentation of delays. In the current experiment, the magnitude effect may be relevant to long-hold immediate access arms task performance in which delays following reward (or hold times) were progressively increased relative to delays preceding reward in an attempt to shift choice behavior away from immediate access arms.

In considering order and magnitude effects, we cannot rule out the possibility that contrast effects could factor into task performance. Contrast effects occur when "the value or effectiveness of a stimulus varies inversely with the surrounding context" (Dai et al., 2009). If this same phenomenon is applied to delay contrast, it follows that delays would seem subjectively longer when the previous task schedule included shorter delays. In the present experiment, subjects were trained on the equivalent delay task in which all delays were set at 10-seconds, the shortest delay time, and when subjects returned to the standard task, behavior never recovered to the original standard task performance. Instead, preference increased for immediate access arms. Combined with baseline levels of delay aversion evident in previous task performance, contrast effects predict this shift towards higher choice for immediate access arms as delays prior to reward seem subjectively longer due to previous experience on the equivalent delay task. This pattern could similarly augment evidence for shift in preferences away from immediate access arms in the long-hold immediate access arms tasks. In summary, the presence of order, magnitude, and contrast effects can effectively alter the subjective value of reward and thus, should be considered in characterizing task performance.

A final caveat to emphasize with regards to the current task is the lack of post-consequence inter-trial interval (ITI). Included in typical delay discounting procedures, a post-consequence ITI allows the experimenter to rule out an increased local rate of reinforcement. In the present task, animals did not exhibit choice behavior consistent with a preference for increased local rate of reinforcement. If this was the case, arms 2 and 8, the low-reward arms which had the shortest delay/hold times, should have been most highly preferred throughout the task manipulations. As mentioned previously, arm 2 was consistently one of the two lowest choice arms, and while preference for arm 8 did shift over task manipulations, neither of these arms was highly preferred throughout the experiment.

Mecamylamine Administration

It has been widely documented that administration of nicotine and nicotinic receptor agonists increases impulsivity in human and non-human studies (Mitchell, 2004). Thus, in the present experiment, it was hypothesized that administration of a nicotinic receptor antagonist would result in decreased preference for the most impulsive choice, high-reward immediate access arm 4; however, preferences did not shift significantly as a result of mecamylamine administration at any dose (0-5.0 mg/kg). This result suggests that drug administration did not impair subjects' ability to respond based on the rules of the task and training to date. It is also possible that prior learning may have decreased the role of the nicotinic receptors in mediating task performance (*Fig. 10*). While the cholinergic system has not been widely investigated with respect to performance on delay discounting tasks, nicotinic receptors are reliably implicated in attention (Hanh and Stolerman, 2002). As previously stated, it is possible that the more

"automatic" response of immediate access high-reward arm 4 was maintained by habit learning, thus reducing the probability that disruptions of attention at the nicotinic receptors would significantly affect task performance. In a study by Sarter et al., (2003) mecamylamine administered prior to task performance at similar doses to the present experiment resulted in increased omissions but did not affect accuracy (see also McQuail and Burk, 2006). Mizra and Stolerman (2000) also reported increased omissions over a similar dose range (1.6-5.0mg/kg). Unfortunately, the causes of drug-induced elevated omissions are difficult to determine as these effects may be due to attentional variables or other nonspecific variables (e.g., alterations in motivation or motor functioning).

Because mecamylamine was administered to subjects after training on the long-hold immediate access arms task, we cannot rule out the possibility that drug effects were masked by the continuing shift of choice behavior on the task. Administration of mecamylamine in the context of previously lowered level of preference for immediate access arms, specifically arm 4, may have biased results towards floor effects or lack of drug effect due in part to a previously lowered baseline. It is possible that a drug effect would be more prominent if testing were completed even a week later. The present experiment investigated the role of the cholinergic system in an initial attempt to make informed conclusions about neurobiology underlying task performance. In the future, it may be relevant to investigate different neurotransmitters and receptor systems through lesions studies, drug manipulations, and the like, controlling for potential confounds detailed above.

Pre-Session Water Access

Access to water prior to test sessions did not significantly decrease the total number of choices made in the maze task in comparison with the water deprivation condition; however, decreased motivation for reward was evident in the altered distribution of reward magnitude choices. While there was a significant main effect of arm for one-way ANOVAs conducted for both water access and water deprivation conditions, the effect was weaker for pre-session water access condition. The preference for high-reward immediate access arm 4 in the water deprivation condition was less pronounced in the pre-session water access condition in which choice behavior was approximately evenly distributed between reward levels. These findings support motivation for reward as an important factor in task performance.

In summary, there are a variety of different factors to take into account when characterizing performance on delay discounting tasks. Although the tasks and species tested vary widely, recent studies support the utility of delay discounting tasks as predictors for success in treatment of impulse control disorders including ADHD, substance abuse, and pathological gambling (DSM-IV-TR, 2000). Despite this prospective role in the diagnosis and treatment of impulse control disorders, behavioral measures of delay discounting to date do not address several important facets of delay aversion. The present experiment represents an initial effort to characterize performance in a novel maze task that allows a direct comparison between immediate and delayed access for matched rewards. Collectively, the data suggest that performance in this task is sensitive to many manipulations of task parameters that are known to affect performance in typical two-choice delay discounting tasks, including both delay to reward and reward magnitude. Thus, the present task appears to be a useful complement to existing delay

discounting procedures. However, our results also suggest that there are multiple factors that likely impact task performance in complex ways.

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Tables

Table 1: Summary of Training Time in Sessions for Each Task

Task	**Number of Sessions**
Standard Task	10
Equivalent Delay	5
Standard Task	10
Long Hold Immediate Access Arms	20
Long Hold Immediate Access Arms (2)	12

*Session number corresponds to approximately five sessions per week.

Table 2: Summary of Delay/Holding Times for Task Manipulations

	Immediate Access Arms			**Delayed Access Arms**	
Task	**Arm 2**	**Arm 3**	**Arm 4**	**Arm 6**	**Arm 7**	**Arm 8**
Standard Task	10	30	60	60	30	10
Equivalent Delay Task	10	10	10	10	10	10
Standard Task (return)	10	30	60	60	30	10
Long-Hold Immediate Access Arms Task 1	30	60	90	30	20	10
Long-Hold Immediate Access Arms Task 2	60	120	240	30	20	10
Mecamylamine Administration (0-5mg/kg)	60	120	240	30	20	10
Pre-Session Water Access	60	120	240	30	20	10

Table 3: Summary of ANOVA Test Values for Task Manipulations

	Main Effect of Arm		**Interaction**	
Task	**F**	**P**	**F**	**P**
Standard Task 1	1.787	0.178	n/a	n/a
Standard Task 2	6.747	**0.007**	n/a	n/a
Standard Task (1-2)	4.491	**0.030**	3.383	**0.012**
ED Task	11.73	**0.002**	5.573	**0.002**
Standard Task 2	34.429	**0.000**	5.319	**0.004**
Long Delay	47.643	**0.000**	9.076	**0.000**
Increasing Delay	20.671	**0.000**	9.316	**0.000**

*Values significant at the level $\alpha = 0.01$ are in **bold**.

Table 4: Summary of T-tests for Task Manipulations

	Standard Week Two	**ED Task**	**Standard Two**	**Long-Delay**	**Increasing Long-Delay**
2					
3	**			*	
4		**	**		*
6					
7					**
8		*		**	

*__Statistically significant at p<0.01__: Choice behavior moves away from arm.
**__Statistically significant at p<0.01__: Choice behavior moves towards arm.

Figure Captions

Fig. 1

Each session began with the rat being placed in the center hub. The gate that blocked holding arm one or five was then raised. When the animal broke the photocell at the end of the holding arm, the gates blocking arms two, three, and four were simultaneously raised, thus they were referred to as the immediate access arms. The gates blocking arms 6-8 were raised after variable delays (arm six, 60 second delay; arm seven, 30 second delay; arm eight, 10 second delay). Thus, the animals could enter arms 2-4 immediately after leaving the holding arm or could enter arms 6-8 when the gates were raised after the respective delay for that arm. The cups attached to the dippers at the end of each arm varied in size. Arms two and eight were low reward (0.01 ml), arms three and seven were medium reward (0.06 ml), and arms four and six were high reward (0.10 ml).

Fig. 2

The figure depicts the probability of entering each of the six arms on the standard task during week one. A week x arm ANOVA yielded no significant interaction. During the first week of training in the standard tasks, rats did not exhibit a significant preference for entering any of the arms. Error bars represent standard error (SE) of the mean.

Fig. 3

The figure depicts the probability of entering each of the six arms on the standard task during weeks one and two. A week x arm ANOVA yielded a main effect of arm ($P<0.05$) and a significant week x arm interaction ($P<0.05$). The asterisk signifies that there was a

significant shift in choice behavior towards arm three. Subjects established preference for medium-reward immediate access arm three during week two of the standard task. Subsequent paired sample t-tests comparing the probability of choosing each of the arms revealed that arm 4 was chosen significantly more frequently than arms 2 and 6 ($P<0.01$), and arm 3 was chosen significantly more frequently than arm 2 ($P<0.01$). Error bars represent SE of the mean.

Fig. 4

The figure depicts the probability of entering each of the six arms on both standard task week two and equivalent delay task. A week x arm ANOVA yielded a main effect of arm ($P<0.01$) and a significant week x arm interaction ($P<0.01$). The asterisk denotes a significant shift in choice behavior away from arm eight and shift that approached significance towards arm 4 ($P<0.01$). Behavior shifted in a manner that approached significance towards arm four ($P=0.009$ [Bonferroni correction significance level = 0.0083]). Preference for arm four, immediate access high-reward arm, increased in comparison to all other arms when all delays and hold times were equivalent at 10s.

Fig. 5

The figure depicts the probability of entering each of the six arms on the equivalent delay task and return to the standard task. A week x arm ANOVA yielded a main effect of arm ($P<0.01$) and a significant week x arm interaction ($P<0.01$). The asterisk denotes a significant shift in choice behavior towards arm four ($P<0.01$). Error bars represent SE of the mean.

Fig. 6

The figure depicts the probability of entering each of the six arms on the standard task and long-hold immediate access arms task one. A week x arm ANOVA yielded a main effect of arm ($P<0.01$) and a significant week x arm interaction ($P<0.01$). Asterisks denote a significant shift in choice behavior away from arm three and towards arm eight ($P<0.01$). Error bars represent SE of the mean.

Fig. 7

The figure depicts the probability of entering each of the six arms on the long-hold immediate access arms tasks one and two. A week x arm ANOVA yielded a main effect of arm ($P<0.01$) and a significant week x arm interaction ($P<0.01$). Asterisks denote a significant shift in choice behavior away from arm four and toward arm seven ($P<0.01$). Error bars represent SE of the mean.

Fig. 8

The figure depicts the median response latency when arm four was entered. Averaged across all subjects (n=9), symbols represent the means of median latency for each animal during each of the tasks. Asterisks denote a significant *decrease* in response latency between the second week on the standard task and the equivalent delay task ($P<0.01$) in addition to a significant *increase* in response latency between the first long-hold immediate access arms task and the second task long-hold immediate access arms task with further increased delays ($P<0.01$). Error bars represent SE of the mean.

Fig. 9

The figure depicts the probability of entering each of the six arms for both saline injection condition and no injection condition. An injection condition x arm ANOVA yielded a main effect of arm ($P<0.01$), but no significant interaction. It is worth noting, however, that injection data error bars were, on average, approximately twice the size of error bars on saline trials, indicating greater variability in the injection condition irrespective of mecamylamine administration. Error bars represent SE of the mean.

Fig. 10

The figure depicts the probability of entering each of the six arms for three different dose conditions (saline, low, and high; 0.0, 1.0, 5.0 mg/kg respectively). A dose x arm ANOVA yielded no significant effects. Preferences did not shift significantly as a result of mecamylamine administration at any dose. Error bars represent SE of the mean.

Fig. 11

The figure depicts the probability of entering each of the six arms on the long-hold immediate access arms task during two conditions: pre-session water access and water deprivation. A water access condition x arm ANOVA yielded a significant main effect of arm ($P<0.01$) and a significant water access condition x arm interaction ($P<0.01$). The asterisk signifies that there was a significant shift in choice behavior away from arm seven. One-way ANOVAs were conducted for both water access and water deprivation conditions and yielded a significant main effect of arm for the water deprivation condition ($P<0.01$) There was a significant main effect of arm for the water access condition as well ($P<0.05$). Error bars represent SE of the mean.

Figures

Fig. 1: Standard Task Schematic

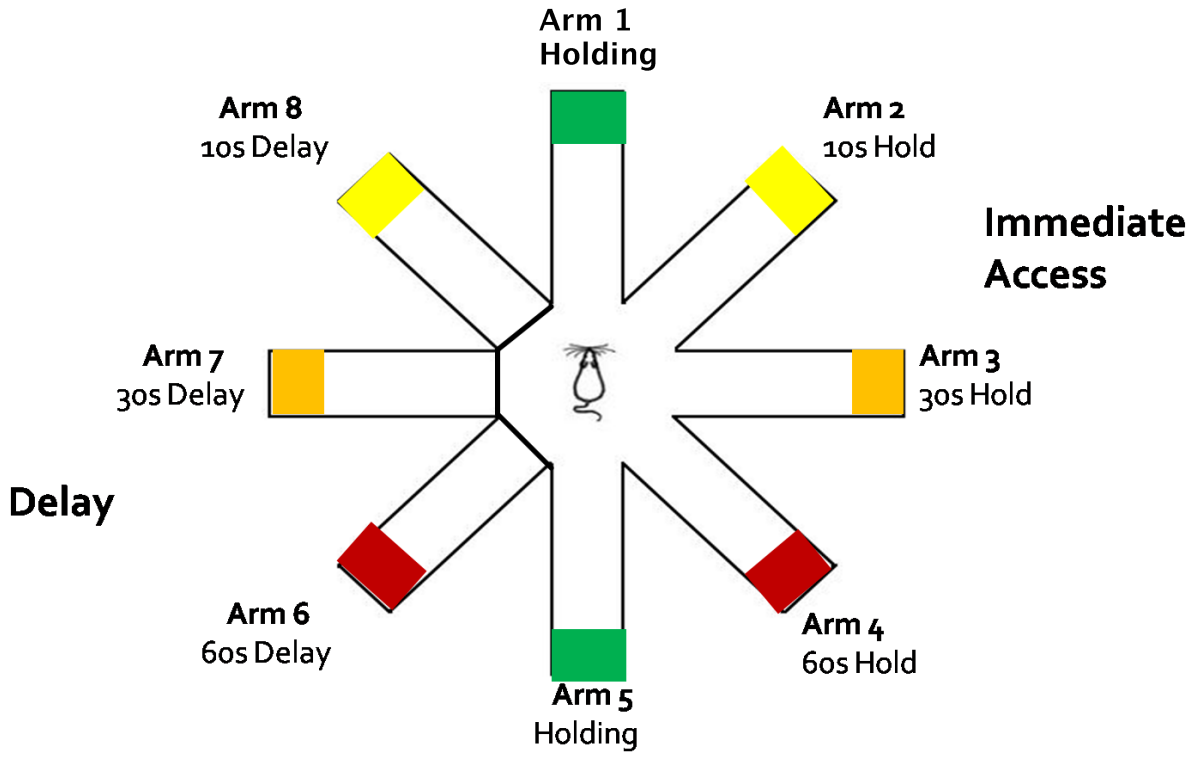


Fig. 2: Standard Task Week One:

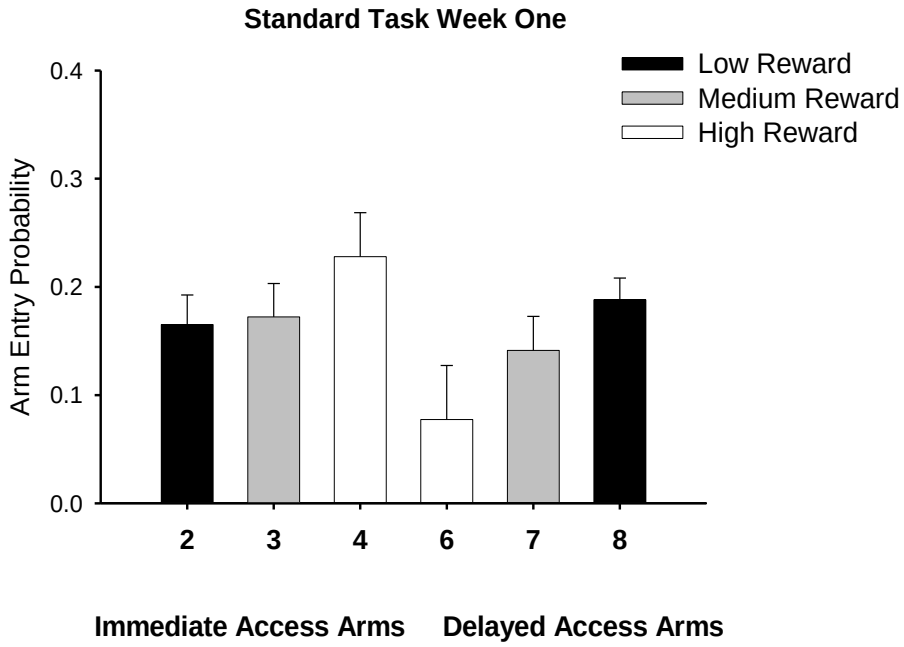
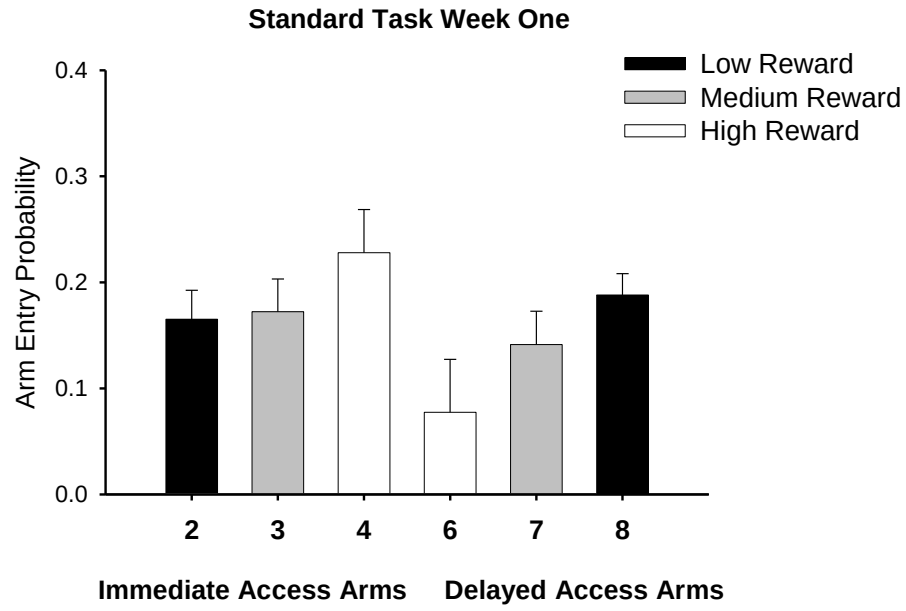


Fig.3: Standard Task Weeks One to Two:



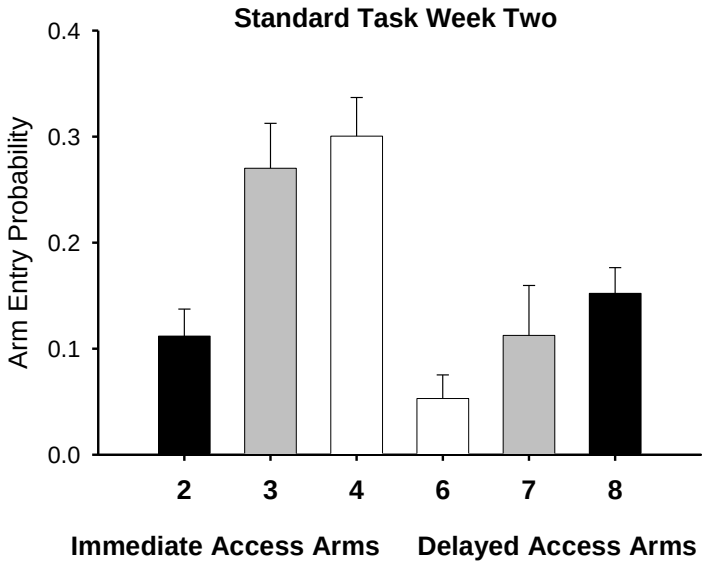
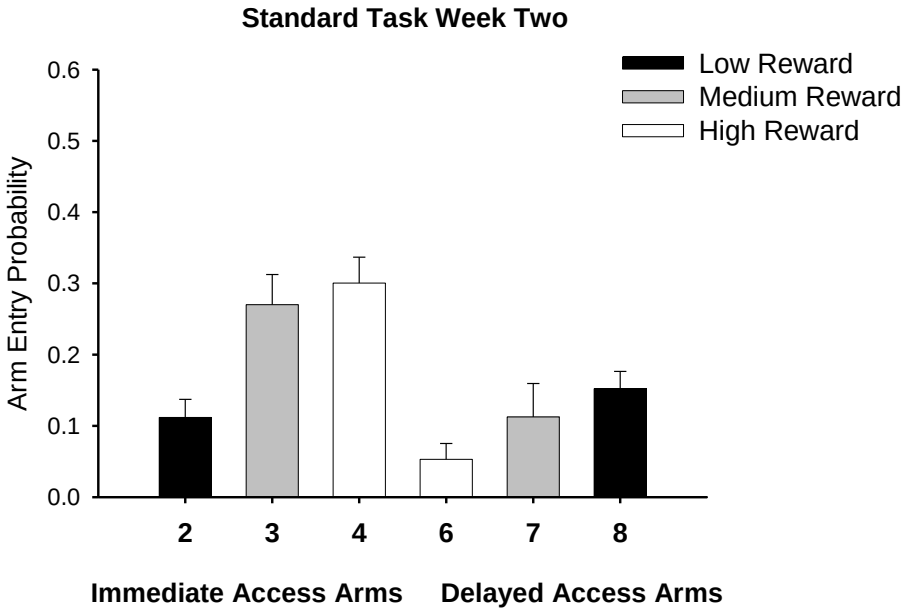


Fig. 4: Standard Task Week Two to Equivalent Delay Task:



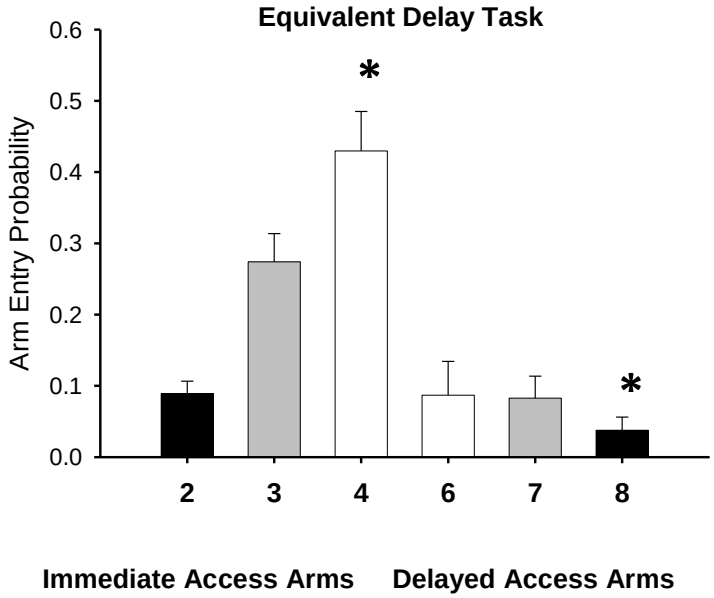
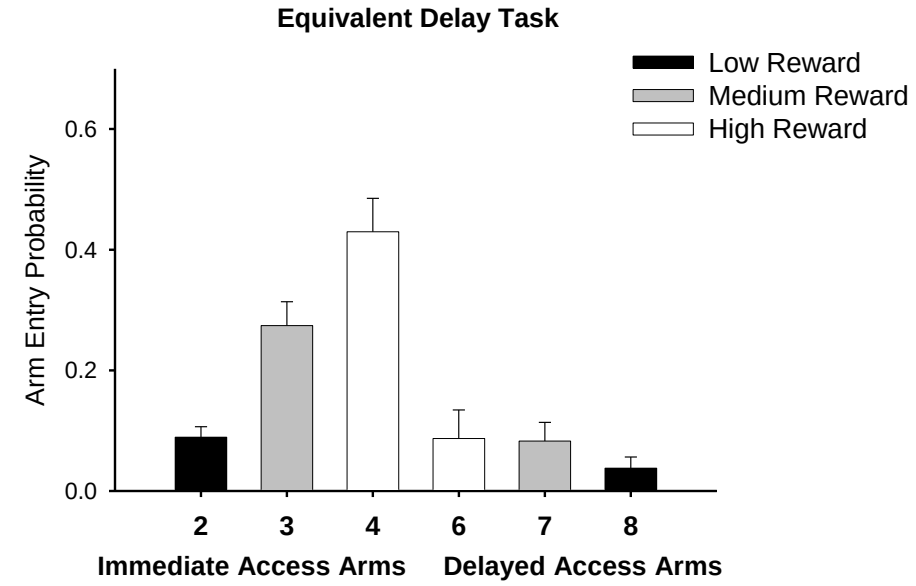


Fig. 5: Equivalent Delay Task to Standard Task (Two):



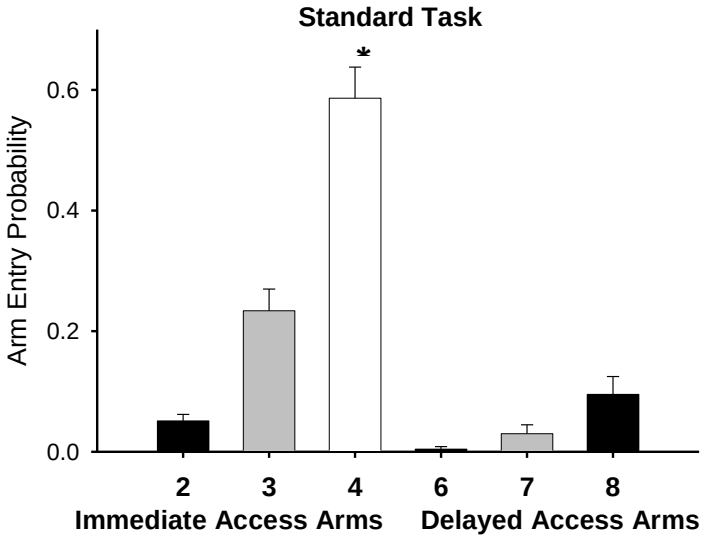
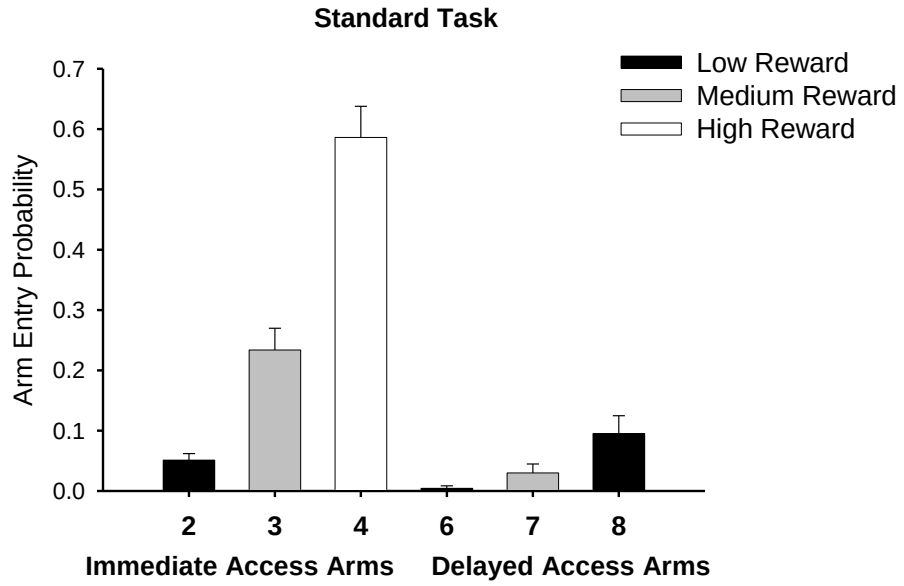


Fig. 6: Standard Task (Two) to Long Hold Immediate Access Arms Task One:



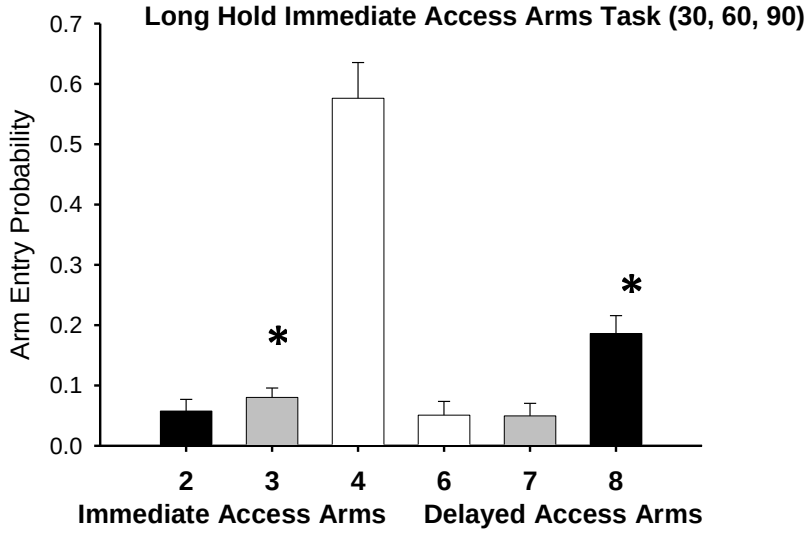
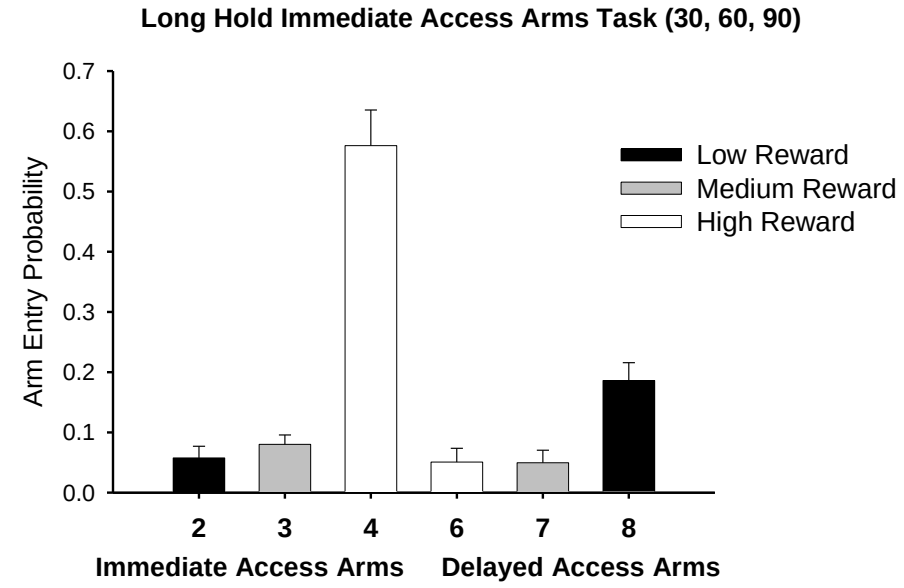


Fig. 7: Long Hold Immediate Access Arms Tasks (One to Two):



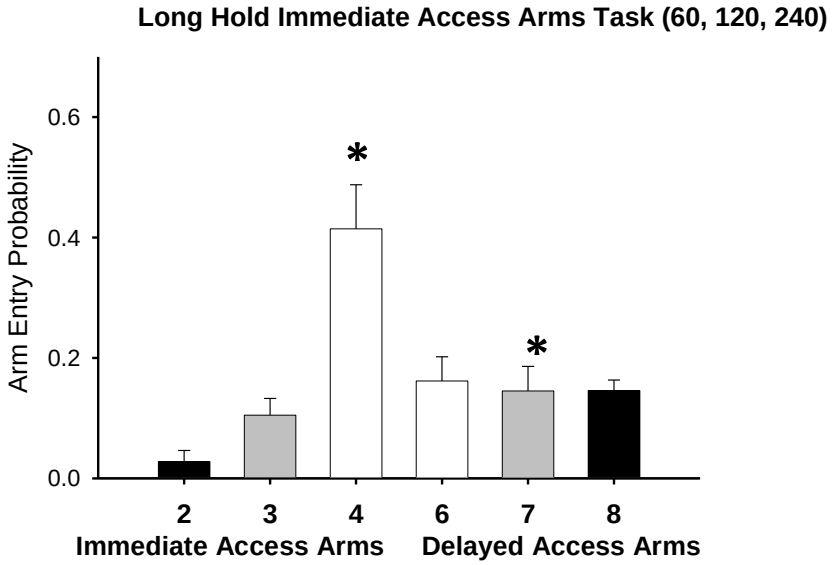


Fig. 8: Response Latencies to Arm 4

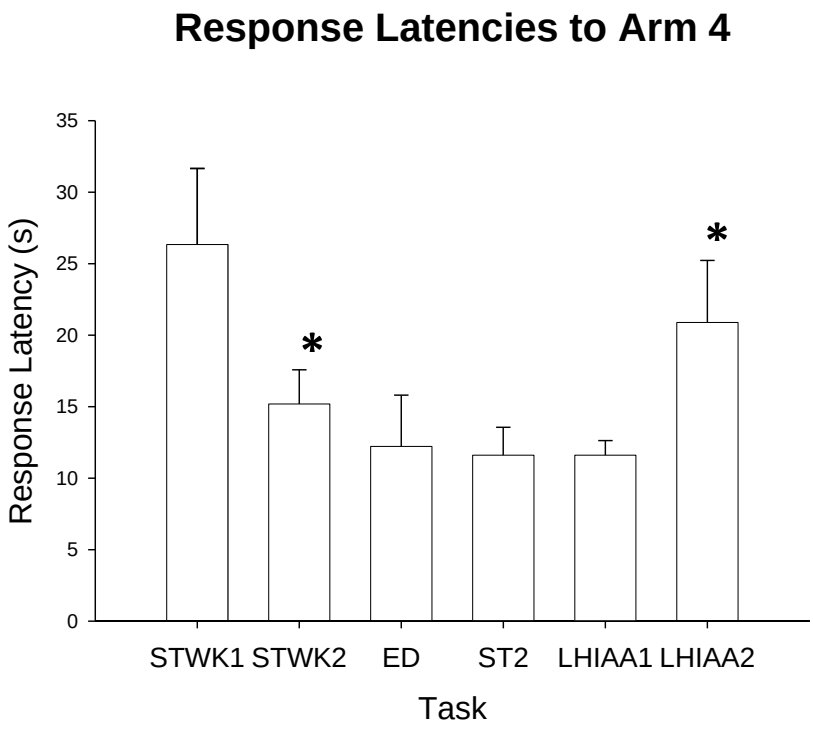
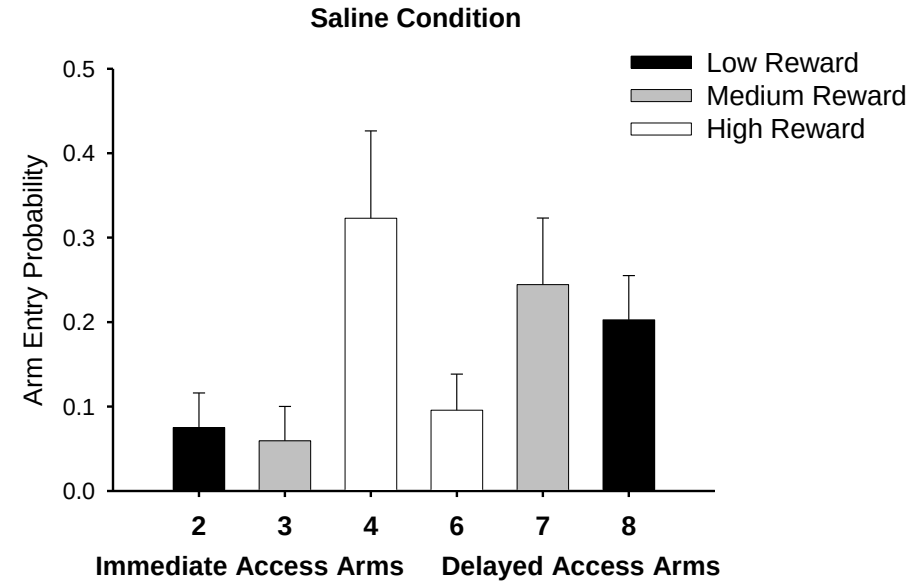


Fig. 9: Saline Administration versus No Injection Condition:



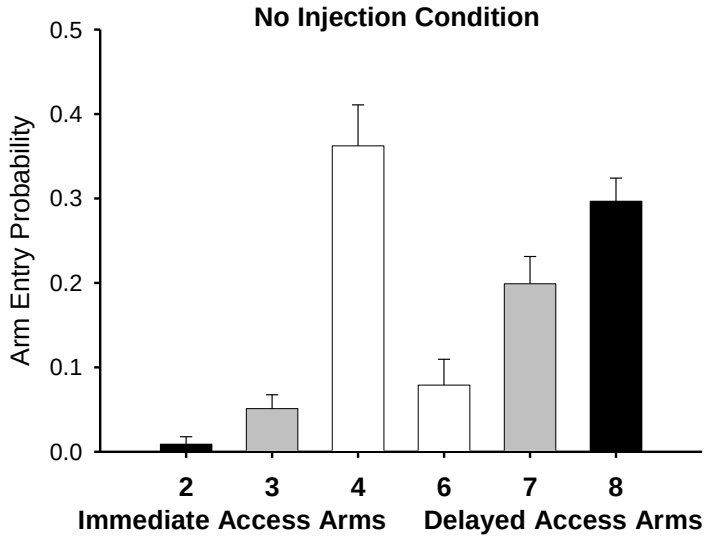


Fig. 10: Mecamylamine Administration (Saline, Low-Dose, High-Dose conditions):

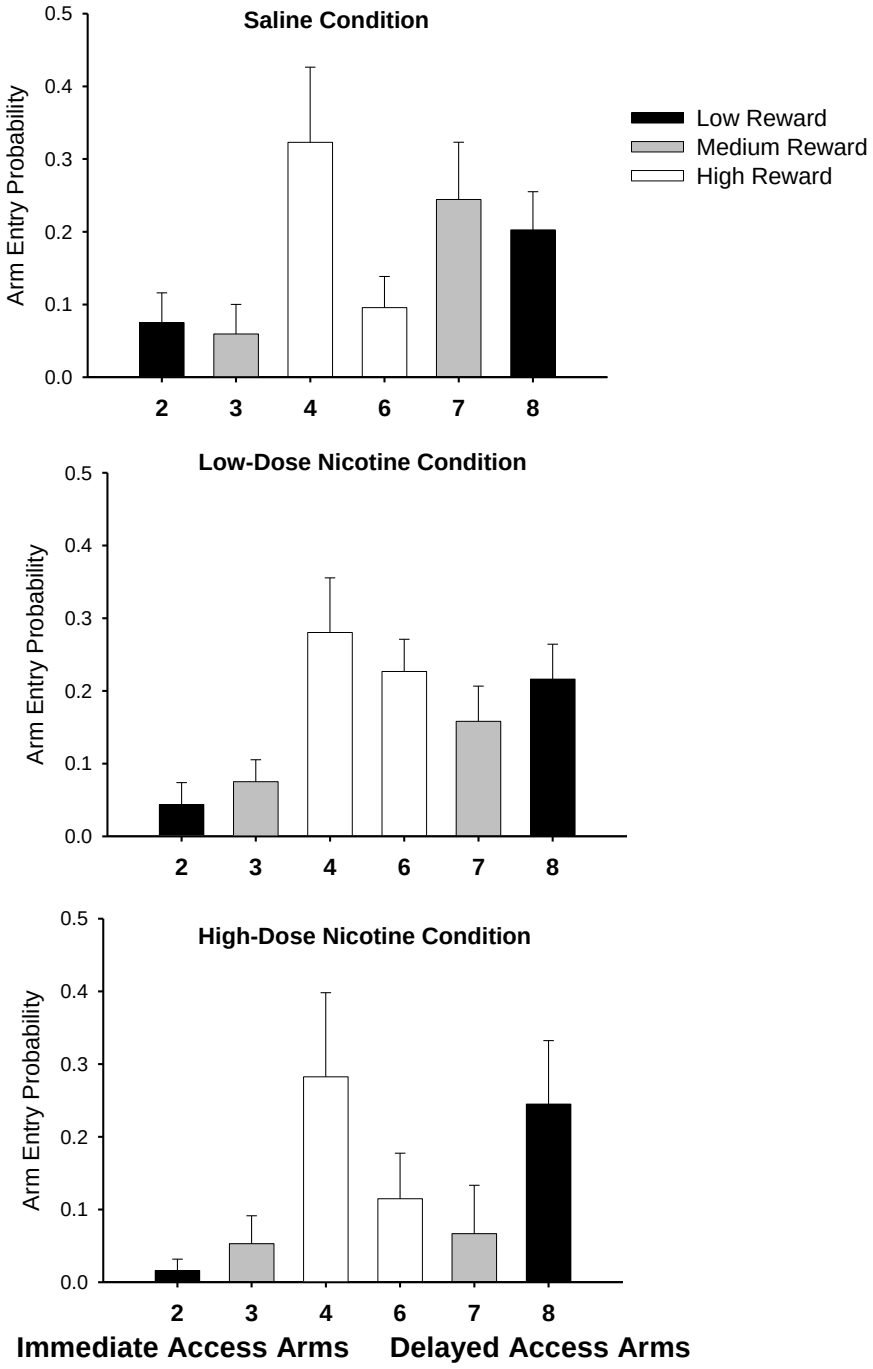


Fig. 11: Pre-Session Water Access Compared to Water Deprivation: