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The role of muscarinic cholinergic signaling in cost-benefit decision making

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Abstract

The role of muscarinic cholinergic signaling in cost-benefit decision making

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Animals regularly face decisions that affect both their immediate success and long term survival. Such decisions typically involve some form of cost-benefit analysis and engage a number of high level cognitive processes, including learning, memory and motivational influences. While decision making has been a focus of study for over a century, it's only in the last 20 years that researchers have begun to identify functional neural circuits that subserve different forms of cost-benefit decision making. Even though the cholinergic system is both functionally and anatomically positioned to modulate cost-benefit decision circuits, the contribution of the cholinergic system to decision making has been little studied. In this thesis, I investigated the cognitive and neural contribution of muscarinic cholinergic signaling to cost-benefit decision making. I, first, re-examined the effects of systemic administration of 0.3 mg/kg atropine on delay and probability discounting tasks and found that blockade of muscarinic acetylcholine

receptors by atropine induced suboptimal choices (impulsive and risky) in both tasks. Since the effect on delay discounting was restricted to the No Cue version of the delay discounting task, I concluded that muscarinic cholinergic signaling mediates both forms of cost-benefit decision making and is selectively engaged when decisions require valuation of reward options whose costs are not externally signified. Second, I assessed the impact of inactivating the nucleus basalis (NBM) on both forms decision making and the effect of injecting atropine locally into the orbitofrontal cortex (OFC), basolateral amygdala (BLA), or nucleus accumbens (NAc) core during the No Cue version of the delay discounting task. I discovered that although NBM inactivation failed to affect delay discounting, it induced risk aversion in the probability discounting task; and blockade of intra- NAc core, but not intra-OFC or intra-BLA, muscarinic cholinergic signaling lead to increased choice of the delayed reward. While those findings implicate the NBM in supporting risky choices and intra-NAc core muscarinic signaling in discouraging delayed choice, more work is needed to fully elucidate the underlying mechanisms.

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DEDICATION

In loving memory of Tracy Jackson Boone and Johnny Fobbs.

Chapter 1. INTRODUCTION

Every day, animals are faced with numerous decisions. Most involve some form of cost-benefit analysis and engage a number of high level cognitive processes, including learning, memory and motivational influences. While decision making has long been a focus of scholars and scientists, it's only in the last 20 years that researchers have begun to identify functional neural circuits that subserve different forms of cost-benefit decision making. In this introduction, I will review the decision literature relevant to my thesis research, focusing on intertemporal choice and data from rodent studies, and I will present a justification for investigating the role of the muscarinic cholinergic system in cost-benefit decision making.

1.1 HISTORY OF DECISION SCIENCE

Among the countless behaviors that are studied by neuroscientists, decision making engenders a lot of fascination because of the ease with which people can think of examples of the behavior from their own lives. A decision can be as routine as selecting what to eat for lunch or as profound as choosing a long term partner or determining the right time to invest in a home. Even those few examples illustrate how ubiquitous and central to survival decision making is, as well as the variety and complexity that the term encompasses. It is that very breadth that's made it so difficult to fully understand how people make decisions.

In order to simplify the problem and make it more tractable, the pioneers of decision science embraced a normative approach. Those early 20th century neoclassical economists worked to develop models that could predict the best or optimal choice for a given economic decision problem. Although their models were elegant and logical, able to predict the option that would maximize utility (von Neumann and Morgenstern, 1944); they were also bounded by unrealistic

assumptions, like expecting the decision maker to be rational and self-interested, which made them unable to actually predict or explain the real choices of humans and animals. As the decades unfolded and more and more exceptions were catalogued, it became clear that a new approach was necessary; one that could figure out what caused individuals' choices to deviate from those of the idealized decision maker the neoclassicists had in mind.

Embracing the challenge in the second half of the 20th century, psychologists and behavioral economists adopted a new approach that flipped their predecessors' process on its head. Instead of developing axiomatic models that predicted decision behavior, they generated descriptive models, like prospect theory (Kahneman & Tversky, 1979), that were based on empirical evidence- the real choices of decision makers faced with different problems. Not only was this approach better at accounting for the choices of humans and animals, but it also lead to a deeper exploration of the internal and external factors that can shape a decision.

Although some factors received immediate attention; it wasn't until the early 1990s and the work of Antoine Bechara, Antonio Demasio, and colleagues that the neural basis of economic decision making became a focus of study (Antoine Bechara, Damasio, Damasio, & Anderson, 1994; Antoine Bechara, Damasio, Damasio, & Lee, 1999). By showing that patients with prefrontal cortical damage made poor decisions on a gambling task, they sparked an interest in applying the cutting edge technologies of neuroscience to understanding the fundamental neurobiological processes that drive normal and abnormal decision making.

That pursuit is still ongoing today and has even been recognized as its own field of study called Neuroeconomics. The field draws information from economics, psychology, neuroscience and mathematics in order to understand 1) the computations the brain must carry out to make economic, or value-based, decisions and 2) how the brain implements those computations (Rangel

2008). This work is conducted using a variety of species. Human research provides insight into the general involvement of brain structures and cognitive processes; while animal research allows the use of invasive techniques and manipulations that can tease apart the exact contributions of neural structures/systems to different aspects of the decision process.

1.2 Cost-Benefit Decision Making

Within the broad category of economic or value-based decisions, decisions between options that vary in reward size and cost to obtain reward are called cost-benefit decisions. In order to solve these complex decision problems, decision makers must weigh the value of possible rewards against their associated costs in order determine which option is worth pursuing. The costs that are studied the most in the decision literature are delayed reward receipt (intertemporal choices or delay-based decisions) and probabilistic/uncertain reward delivery (risk-based or probabilistic decisions).

1.2.1 *Intertemporal choice*

Humans and other animals (primates, pigeons, pigs, and rats) behave irrationally when faced with intertemporal choices. Even though they prefer the objectively more valuable reward when presented with a simple choice between small and large rewards, they don't maintain that preference during more complex, intertemporal choices; such as between a small, immediately available reward and a larger, delayed reward. Instead, as the delay to the large reward increases, the likelihood of selecting the large reward decreases (Ainslie, 1975; Evenden & Ryan, 1999; Hwang, Kim, & Lee, 2009; Mazur, 1997; Melotti, Thomsen, Toscano, Mendl, & Held, 2013).

The decreased preference for the large reward is called delay discounting and is reflective of reduced or discounted subjective valuation of the delayed reward. The amount of delay

discounting can be determined using either the shape of or area under the delay discounting curve: the graphical relationship between the delay to the reward and the likelihood of selecting the reward (subjective value) (Figure 1.1). The quantitative model that best captures that relationship is Mazur's (1987) hyperbolic delay discounting equation:

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

The subjective value (V) of a given magnitude (A) of reward is a decreasing hyperbolic function of the delay (D), with k functioning as the discount rate.

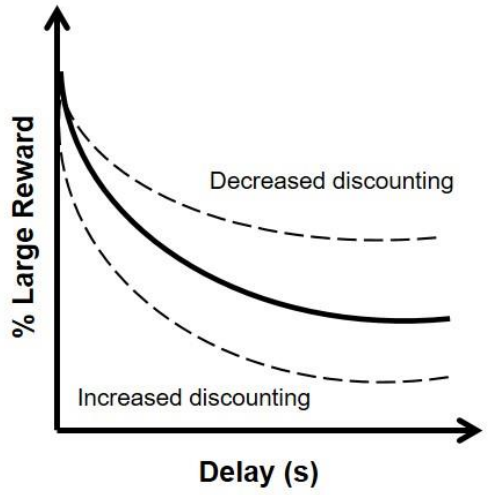


Figure 1.1. Delay discounting curves. The solid black line represents a typical discounting curve, while the dashed lines represent a decreased discounting curve (top) and an increased discounting curve (bottom).

Understanding delay discounting is important because intertemporal decisions are critical to many arenas of life, including health, wealth, and politics. Although delay discounting is normative, excessive delay discounting (increased k) is indicative of poor cost-benefit decision making and described colloquially as "impulsive choice". The amount of delay discounting exhibited by a given individual appears to be both fixed *and* malleable: stable within the subject for weeks, months, years (Anokhin, Golosheykin, Grant, & Heath, 2011; Kirby, 2009; Ohmura,

Takahashi, Kitamura, & Wehr, 2006); while also sensitive to environmental factors and manipulations (Peters & Büchel, 2011). Those observations coupled with the fact that elevated delay discounting is predictive of and correlated with a wide variety of psychiatric conditions (Koffarnus, Jarmolowicz, Mueller, & Bickel, 2013), suggests that intertemporal choice is mediated by many cognitive and neural processes.

1.2.2 *Measuring delay discounting*

The methods used to investigate intertemporal choice depend on the species under investigation. Whereas humans are asked directly about their preferences between novel choices about food or money rewards; animals must be trained to learn and express their preferences for food rewards. There are four behavioral tasks that are typically used to measure delay discounting in rodents. They are similar in that they present choices between a small food reward that is associated with little/no delay and a large food reward that is associated with delays; but they are different because each presents animals with a different number/order of choices and range of delay lengths.

The first and most popular task is called, appropriately, a delay discounting task, and there are two versions. In the first, a within-session version, animals encounter a prescribed pattern of choices and the delay to the large reward is systematically increased within the session from 0s (Cardinal, Robbins, & Everitt, 2000). Each session is broken into blocks of trials, comprised of forced and free choice trials with a particular, fixed delay preceding the large reward (e.g. block 1, 0s; block 2, 4s; block 3, 8s; block 4, 16s; block 5, 32s). At the end of every session, the percentage of large rewards chosen per delay block is recorded in order to generate delay discounting curves and calculate discount rates. Alternatively, in the second version –a between-session version- the delays are systematically increased between sessions, which allows for a greater number of forced and free choices (that are not well-trained) to be assessed and used to

generate discounting curves (Day, Jones, & Carelli, 2011). The delay discounting task can be further customized by including cues during the delay periods.

Unlike the delay discounting task, the adjusting delay task presents animals with choice options that depend on their selections (Cardinal, Daw, Robbins, & Everitt, 2002; Mazur, 1997; Papale, Stott, Powell, Regier, & Redish, 2012; Piras et al., 2014). Within a session, trials are usually grouped into blocks of four- 2 forced choice trials and 2 free choice trials. If the small reward is chosen during both free choice trials, the delay to the large reward is decreased; whereas if large reward is chosen, the delay is increased. Subjects are free to 'adjust' their delays daily until they titrate around an indifference point, which is defined as the delay at which the discounted values match. Instead of discount rate being derived from the curve, the indifference point is used as a proxy for the discount rate; with lower indifference points indicating higher discounting and vice versa.

Finally, the last task used to assess delay discounting is a quantitative analysis task (Bezzina et al., 2007). It is a combination of a within and between session design: animals encounter groups of sessions with delays to the large reward systematically increasing from block-to-block within a session (e.g. small reward at 1s delay vs large reward at 1.00, 1.75, 3.06, 9.38s delay by block) and the delay to the small reward increasing as rats complete groups of session (e.g. First 100 sessions, 1s delay; next 50 sessions, 2s delay, etc.). The abundance of choice information from this task (150-300sessions) allows experimenters to gain a comprehensive quantitative account of the factors the drive an individual's choice: not only are indifference points for both rewards calculated, but measures of delay discounting and reward sensitivity (ability to distinguish reward sizes) are also derived.

With the exception of the quantitative analysis task, the tasks can be performed either in an operant chamber or on a T-maze. In the chamber, left and right levers are pressed to get large and small rewards, respectively; while on the maze, the rat must run to the end of the left and right arms to collect large and small rewards, respectively. In the within session delay discounting task, neuroanatomical and neurochemical manipulations can be employed to test whether they alter choice behavior by increasing or decreasing delay discounting; whereas the other three tasks are more conducive to neural recordings and lesions. Finally, it's been shown that the tasks measure slightly different levels of discounting, likely because rats rely on different strategies to perform each task: a well-learned behavioral choice pattern versus flexible, instantaneous choice (Peterson, Hill, & Kirkpatrick, 2015).

1.3 Cognitive Basis of Intertemporal Choice

While computational models capture the relationship between the option(s) features and an animal's choice, conceptual models provide a framework for thinking about the cognitive and computational processes that contribute to decisions.

One such conceptual model, proposed in Rangel, Camerer, & Montague (2008), divides the cost-benefit decision process into five subprocesses that decision makers perform (Figure 1.2). First, an individual represents the decision problem, which means they identify the features of the available options (actions required to obtain rewards, costs that must be endured, reward sizes, etc.). Next, once the options are identified, they assign each option a value through a valuation process (also called cost-benefit analysis) that involves integrating reward and cost information and calculating subjective values. Third, they compare the computed values and select the action associated with the greatest value. After they perform the action and receive the reward, they then compare the experienced value against that which they expected; and finally, they update their

decision representations and valuations based on learning, memory, and motivational influences and what has/hasn't changed externally (actions, outcomes) or internally (hunger, thirst, cost sensitivity, reward sensitivity, etc.). Not only does such a model of decision making broadly implicate certain cognitive and computational processes (reinforcement learning, delay perception, etc.), but it allows for the derivation of specific computational values (choice, action, reward values) that can be used as explanatory variables in neural recording studies (electrophysiology, fMRI, etc.). Although, it's easy to conceive of valuation and action selection (choice) as distinct processes, it's difficult to disentangle the two behaviorally because a basic assumption of decision theory is that choice, especially in animals, reflects valuation.

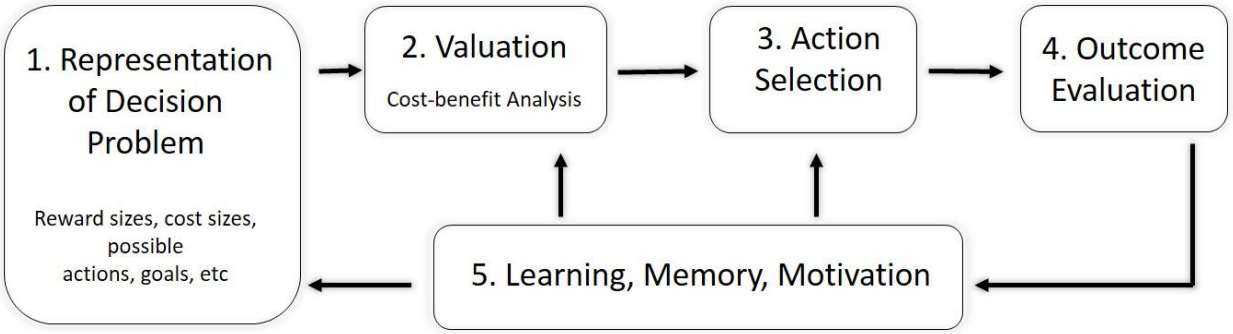


Figure 1.2. A schematic of the five subprocesses that underpin cost-benefit decision making. Adapted from (Rangel et al., 2008).

Another way to implicate cognitive processes in intertemporal choice is to look for connections between behavioral measures and delay discounting and/or test whether manipulating those behavioral measures alters delay discounting. We know from prospect theory that valuation is greatly influenced by decision context, such as the framing of options (Kahneman & Tversky, 1979). This is true of intertemporal choices as well; manipulating the context of the time that a future reward will be delivered can reduce discount rates in human subjects. Specifically, when

choices were framed with an emphasis on the time of the outcome by using exact dates of delivery (Read, Frederick, Orsel, & Rahman, 2005), subjects encouraged to view a delay in terms of accelerating its arrival (Weber et al., 2007), or subjects presented with delays paired with episodic cues (Peters & Büchel, 2010), discount rates were diminished. Each of those framing devices targeted the temporal focus of the individual, causing them to engage prospective processes and project themselves forward, which in turn made the future outcome more salient and desirable (Peters & Büchel, 2011). Similarly, when humans were trained to directly estimate durations of a series of activities prior to choice procedures, their discounting rates were also reduced (Zauberman, Kim, Malkoc, & Bettman, 2009). The later finding is not surprising considering many of the same disorders characterized by impulsivity and associated with increased delay discounting have also been linked to dysfunctional timing (Allman & Meck, 2012; Berlin, Rolls, & Kischka, 2004; Meck, 2005; Piras et al., 2014; Rubia, Halari, Christakou, & Taylor, 2009).

In addition to temporal context cues, human and rodent researchers have shown that general contextual cues can also affect decision making through activation of learned behavioral patterns. With human subjects, researchers showed that gamblers exhibited decreased discounting when large rewards were offered in the context of a positively conditioned cue (Dixon & Holton, 2009). In rats, a similar contextual effect is observed when animals are trained with a cue present during the delay-to-reinforcement in the delay discounting task. The cue acts as a conditioned reinforcer by changing the reward association, speeding task learning, and influencing discounting (Cardinal et al., 2000; Zeeb, Floresco, & Winstanley, 2010). In both cases, the cues seem to alter the valuation likely by influencing affective reward processing and strengthening action-outcome associations.

Finally, beyond temporal and affective processing, researchers have also been successful at linking working memory to intertemporal choice. Not only is working memory capacity negatively correlated with delay discounting in humans and rats (Renda, Stein, & Madden, 2015; Shamosh et al., 2008); but manipulating human's working memory by taxation or training leads to increased and decreased discounting rates, respectively (Hinson, Jameson, & Whitney, 2003; Wesley & Bickel, 2014).

1.3.1 *Caveat*

When considering the cognitive processes that are involved in a given decision paradigm, it's important to recognize the specifics of the decision process being engaged. For example, in the rodent delay discounting task, animals are well-trained, having encountered the exact same choices over and over again by the time they are tested. They likely already have mnemonic representations of the action-outcome associations for the options they will encounter in a given session; and thus, might rely on a subtly different set of cognitive processes to make their choices than the humans or rodents in tasks with unpredictable or adjusted delay options for whom valuation is more instantaneous and flexible (Hayden, 2015; Killeen, 2011).

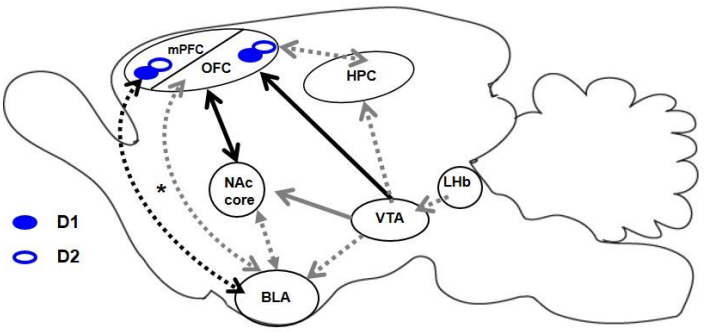
1.4. Neural Basis of Intertemporal Choice

To gain a complete view of the neural basis of cost-benefit decision making, consideration of both a high level neural circuit view (interactions between brain structures) and a low level neuronal view (coding and interaction of specific subpopulations within brain structures) will be essential (Orsini, Moorman, Young, Setlow, & Floresco, 2015). Human decision studies permit recording of high level circuit activity, while animal studies permit confirmation/determination of the

necessity of brain structures for normal levels of choice and recording of intra-structural single-unit signals.

Based on the activity patterns observed in human fMRI experiments, two interesting models for value encoding during intertemporal decisions (Figure 1.1, subprocess 2) were proposed. Overall, the studies found that when normal, healthy humans are faced with intertemporal choices, they show BOLD activation of large scale neural networks, including prefrontal cortex, amygdala and ventral stratum (Kable & Glimcher, 2007; McClure, Ericson, Laibson, Loewenstein, & Cohen, 2007; McClure, Laibson, Loewenstein, & Cohen, 2004). The first model, the β-δ model, suggested that the network influenced choice based on the relative activation of separate, distinct substructures that either responded to immediate or delayed options, with immediate-choice promoting activity in limbic structures (β) and delayed-choice promoting activity in prefrontal structures (δ) (McClure et al., 2007; McClure et al., 2004). Alternatively, the second model found correlates of subjective values (single, scaled values calculated from subjects' choices) within both limbic and prefrontal structures (Kable & Glimcher, 2007). Those two models highlight a fundamental question about how value is computed in the brain during inter-temporal choices: are dual values integrated at the network level, are subjective values represented within structures throughout the network, or is some combinatorial approach at play? (Peters & Büchel, 2011).

Human studies alone cannot fully answer that question, but rodent studies have helped expand our understanding of which structures and what information contributes to the interconnected neural circuit that underlies intertemporal choice (Figure 1.3).



Structure/ Connection	Delay Discounting	DA manipulation		Delay Discounting
OFC	↓ ↑ NE	Flupenthixol / D1 antagonist	--	↓
mPFC	Flattens curve	Amphetamine	+	↑
BLA	↓	DA lesion in OFC	--	↓
Nac core	↓	D1/D2 antagonist in OFC	--	↓
HPC	↓	D1/D2 antagonist in mPFC	--	↓
OFC → NAc	↓	DA lesion in NAc	--	NE
LHb	Indifference	D1/D2 agonist/ antagonist in NAc	+ --	NE

Figure 1.3. Anatomical circuit representation showing structures and pathways involved in rodent intertemporal decisions (Left). Structures and DA receptors are only included if silencing with lesions, inactivation, or blockade altered rodent delay discounting. *Solid black lines* indicate the pathways that are required for normal discounting performance; whereas *solid grey lines* represent pathways that are not necessary for normal performance. Pathways marked by *dotted grey lines* have yet to be tested during delay discounting tasks; and the *dotted black line (and asterisk)* shows a pathway that's been implicated in choice of a single delayed choice but has yet to be tested during a discounting task. The Table lists the changes to the delay discounting curves that resulted from silencing structures, disconnecting pathways, or pharmacologically manipulating DA signaling during the delay discounting task (Right). ↑ decreased discounting; ↓ increased discounting/ impulsive choice; + stimulating DA signaling; -- blocking DA signaling; BLA-basolateral amygdala; HPC-hippocampus; LHb- lateral habenula (inactivation leads to indifference (Colin M Stopper & Floresco, 2013)); mPFC- medial prefrontal cortex; NAc- nucleus accumbens; OFC-orbitofrontal cortex; VTA-ventral tegmental area.

1.4.1 *Prefrontal cortex monitors and adjusts intertemporal choices*

1.4.1.1 Orbitofrontal cortex (OFC)

The OFC is the region of the prefrontal cortex (PFC) that has been most extensively studied for its role in intertemporal choice. Although humans with OFC lesions consistently exhibit impulsive choices (Bechara, Tranel, & Damasio, 2000; Berlin et al., 2004; Sellitto, Ciaramelli, & di Pellegrino, 2010), there are discrepancies between reported effects of manipulating the rodent OFC. Following lesion or inactivation of the whole OFC, rodents' discounting increased (Mobini et al., 2002; Rudebeck, Walton, Smyth, Bannerman, & Rushworth, 2006), decreased (Kheramin

et al., 2002; Mar, Walker, Theobald, Eagle, & Robbins, 2011; C. A. Winstanley, 2004), or didn't change (Abela & Chudasama, 2013; Churchwell, Morris, Heurtelou, & Kesner, 2009; Jo, Kim, Lee, & Jung, 2013; Mariano et al., 2009; Moschak & Mitchell, 2014). The reasons for the inconsistencies are not fully known, but one possibility is that the OFC was differentially engaged based on the experimental design. Specifically, differences might've arisen because rats encountered a limited number of delays (Churchwell et al., 2009); mixed delay order(Jo et al., 2013); no pre-operative training (Abela & Chudasama, 2013); lengthy post-op training (Mar et al., 2011); a cue during the delay-to-reinforcement (Mariano et al., 2009; Zeeb et al., 2010); or interactions with their basal levels of impulsivity (Zeeb et al., 2010). A second possibility is that the medial and lateral portions of the OFC exert heterogeneous influence on intertemporal choice. Mar et al (2011) showed that mOFC lesions decreased rodent discounting while lOFC lesions increased rodent discounting; but it took 6 days of post-lesion retraining for those effect to be observable and inactivation of mOFC by another group was not successful at changing rodent discount rates (Colin M. Stopper, Green, & Floresco, 2014).

Single unit electrophysiological recordings have furthered our understanding of why the OFC is necessary for normal intertemporal decisions by pinpointing OFC signals that likely underpin its contribution. Specifically, two types of OFC reward signals were recorded in rats during a chamber-based intertemporal choice task in which an odor cue and the spatial location of a reward indicated whether the delay preceding the reward would be short or long. While the majority of OFC neurons exhibited reward evoked firing that reflected the delay-discounted reward value, meaning they fired stronger to the more preferred, immediate rewards than to the delayed reward (immediate-preferring neurons); a second, smaller subset showed sustained increases in firing during the delay to the large reward, i.e. a larger response to delayed rewards

(delay-preferring neurons) (Roesch *et al*., 2006). However, neither response type could be categorized as encoding a common value because they failed to also respond differentially to reward size (i.e. immediate-preferring neurons didn't fire more for the large reward when offered without delay). The authors concluded that the two reward representations support two separate processes: the delay-discounted signal broadcasts the reduced value of delayed rewards and promotes choice of the immediate reward; and the second ramping signal represents an outcome expectancy signal that tracks delay, facilitates and updates associative representations and outcome predictions in other brain areas, and promotes choice of the delayed reward (Roesch, Calu, Burke, & Schoenbaum, 2007). In support of the suggestion that OFC neurons signal outcome expectancy during intertemporal decisions, Stott & Redish (2014) observed that OFC neuron ensembles signaled covert reward representations just after the choice point of a spatial adjusted delay discounting task.

Consistent with the previously mentioned human study that showed that the degree of OFC population activation correlated with choice of delayed rewards (McClure *et al*., 2004), there's evidence to suggest that the two reward representations in the rodent OFC also work together to value options and guide choice. When the two reward signals were identified, the overall OFC representation favored the immediate rewards (the majority of the recorded cells were immediate-preferring) and was accompanied by a behavioral preference for the immediate rewards. Indeed, if the overall OFC reward representation is responsible for guiding choice of delayed rewards, then neural manipulations that change choices during inter-temporal decisions probably do so by changing the balance of the two reward representations in the OFC (increasing or decreasing the proportion of delay-preferring reward representation). The hypothesis has already been tested once by recording OFC neurons in aged rats that are believed to have OFC dysfunction because they

exhibit age-related changes in OFC-mediated behaviors. Consistent with the hypothesis, the aged rats displayed an increased preference for delayed rewards that was accompanied by an increased percentage of delayed- preferring neurons and no change to the overall percentage of delay-sensitive reward neurons (Roesch *et al*., 2012).

1.4.1.2 Medial prefrontal cortex (mPFC)

The mPFC is a region of the PFC that has been less-studied during intertemporal choice because it isn't necessary for valuation/choice during delay discounting tasks. Although several human imaging studies have observed activation of the mPFC during intertemporal choice tasks (Ballard & Knutson, 2009; Peters & Büchel, 2011), silencing the mPFC in rodents doesn't affect discounting during a within-session delay discounting task. When the mPFC was lesioned, animals' discounting curves were flattened- their choice of the large reward was reduced at short delays and increased at long delays (Cardinal, Pennicott, Lakmali, Robbins, & Everitt, 2001). At first glance, that might seem attributable to a change in discounting; but when combined with the fact that the lesion also failed to impact reward discrimination and that a recent study showed temporary mPFC inactivation had no effect on discounting, it is clear that the mPFC contributes to timing or working memory rather than valuation (Feja & Koch, 2014). Since the mPFC is known to track interval durations (Kim, Ghim, Lee, & Jung, 2013; Meck, Church, Wenk, & Olton, 1987) and support tolerance during reaction time tasks (Narayanan, Horst, & Laubach, 2006), it's probable that the mPFC monitors delay information that in turn helps animals determine when to pursue rewards. Additionally, there is a rich literature implicating the mPFC in working memory processes that support the linking actions with outcomes over delays (Yang, Shi, Wang, Peng, & Li, 2014). While animals with their mPFC temporarily inactivated during one test session can rely on well-established delay-reward associations, which remain supported by stored temporal

memory information from other neural systems (Matell & Meck, 2000); animals with lesioned mPFC, whose delay-reward associations are likely inappropriately updated during retraining (probably lengthened according to results of mPFC lesions during interval timing tasks (Meck et al., 1987)), no longer base their behavior on accurate temporal information. Consistent with a timing contribution, temporary disconnection of the mPFC and BLA reduced waiting tolerance for a single 15s delayed reward on a T-maze choice task during which the small, immediate reward was constantly available (Churchwell et al., 2009).

1.4.2 *Amygdala-ventral striatal circuits promote choice of large, delayed rewards*

1.4.2.1 Basolateral Amygdala (BLA)

Even though the amygdala is primarily studied for processing negative emotions and linking environmental stimuli to aversive sensory experiences, the amygdala, and the BLA in particular, is also known to play an essential role in positive emotions and reward-related behavior (Baxter & Murray, 2002). Thus, it's not surprising that the BLA is required for intertemporal choice. Specifically, its known that the BLA is responsible for biasing choice toward the large, delayed reward because BLA-lesioned rodents shifted their choice away from the large reward during a within-session delay discounting task (Winstanley, 2004). Their increased discounting was not attributable to an inability to discriminate reward sizes nor an inability to remember the stimulus (lever)- reward association for two reasons: first, BLA-lesioned rodents in the same study retained their preference for the large reward when it was immediately available; and second, previous studies have shown the BLA is not necessary for appetitive Pavlovian conditioning (Parkinson, Robbins, & Everitt, 2000).

However, since BLA recordings have yet to been performed during a rodent delay discounting task, the precise role of the BLA in intertemporal choice remains unknown; but the most compelling hypothesis is that it contributes through its role in assigning incentive or motivational significance to instrumental cues, just as it does in other tasks. BLA neurons recorded in rodents during instrumental tasks exhibited cue and delay-related activity that differentially responded based on the motivational significance of the stimuli (Schoenbaum, Chiba, & Gallagher, 1998). Additionally, lesion studies have found that the BLA is required for animals to adjust their instrumental responses to conditioned cues when faced with changes in affective information, such as conditioned reinforcers or reinforcer devaluation (Hatfield, Han, Conley, Gallagher, & Holland, 1996; Hitchcott & Phillips, 1998; Geoffrey Schoenbaum, Roesch, & Stalnaker, 2009). Therefore, BLA-lesioned animals probably decreased their choice of the large reward because they no longer had access to incentive signals that would normally invigorate them to pursue those rewards despite their associated delays.

1.4.2.2 Nucleus Accumbens (NAc)

The role of the NAc in intertemporal choice has received more attention than the BLA. Time and time again, irrespective of the task used to measure delay discounting (all four described above), rats with NAc core lesions displayed increased discounting that wasn't linked to altered reward sensitivity nor satiety (Bezzina et al., 2007; Cardinal et al., 2001; da Costa Araujo et al., 2009; Valencia-Torres et al., 2012). Curiously, when the NAc core was instead temporarily inactivated during the within-session version of the delay discounting task, the effect was the opposite – rats exhibited *decreased* discounting (Moschak & Mitchell, 2014). While it's possible that means temporary silencing of the NAc core affects choice differently than permanent lesions, it's more likely that it is an outlier and the NAc is required work with the BLA and OFC to promote choice

of the large reward. The evidence for the OFC-NAc projection's involvement comes from a disconnection study that showed that serial information transfer between the OFC and the NAc core is also required to maintain normal levels of delayed reward choices and avoid excessive discounting. (Bezzina et al., 2008).

Recording studies have observed several neural responses within the NAc that further detail its contribution to intertemporal choice. Population level analyses of the NAc across species have identified reward representations that likely bias choice during intertemporal choices. Human fMRI studies found subjective value correlates as well as activity that was predictive of the choice of immediate rewards (Ballard & Knutson, 2009; Kable & Glimcher, 2007; McClure et al., 2004); while more recent recordings of rodent NAc ensembles at the choice point of a spatial adjusted delay task uncovered signals that combined reward value and chosen action– reward representations that not only distinguished reward size but also preferentially responded to the chosen reward over unchosen reward (Stott & Redish, 2014). Integration of value and action information has also been seen at the single cell level in the NAc. In a second rodent study, performed during a chamber-based intertemporal choice task with odor cues associated with short or long delayed rewards, NAc cells exhibited cue-evoked firing that was both reflective of delay-discounted value and sensitive to whether the cue-associated action was performed, meaning, for example, they fired more strongly to cues associated with more preferred immediate rewards but only when the nosepoke action was taken to pursue the immediate reward (Roesch, Singh, Brown, Mullins, & Schoenbaum, 2009). In addition to responding preferentially to cues whose associated rewards were obtained, the cue responses were also correlated with the speed of the nosepoke action. Thus, the study directly demonstrated how an action –dependent cue signal in the NAc core can trigger downstream motor targets and encourage the animal to perform actions and pursue a

given reward. Said another way, it's a great example of how the NAc functions as a 'limbic-motor interface' able to integrate reward, value and cue information during intertemporal choice tasks (Mogenson, Jones, & Yim, 1980).

1.4.3 *Hippocampus provides temporal and prospective information*

The final cortico-limbic structure currently implicated in intertemporal choice is the hippocampus (HPC). In three separate within- session delay discounting experiments, HPC lesions caused rats to decrease their choice of the large, delayed reward (Abela & Chudasama, 2013; Cheung & Cardinal, 2005; Mariano et al., 2009; McHugh, Campbell, Taylor, Rawlins, & Bannerman, 2008). Their increased discounting was not specific to either subregion, as lesions of both the dorsal and ventral HPC produced the same impairment (McHugh et al., 2008); nor could the effect be interpreted as stemming from deficits in magnitude or spatial discrimination or, by extension, memory (Mariano et al., 2009). A slightly different, yet complimentary effect was observed in the spatial adjusted delay task: even though HPC-lesioned animals were eventually able to titrate around a similar indifference delay as the controls; their training took much longer and their preference for the large reward was much more variable than controls (Bett, Murdoch, Wood, & Dudchenko, 2015). Finally, HPC-lesioned rodents faced with a choice between an immediate uncertain reward and a delayed certain reward were less tolerant of delayed rewards, shifting their preference from the certain reinforcement to less certain, but immediate reinforcement (Rawlins, Feldon, & Butt, 1985). Taken together, the lesion data suggests that the HPC is required for animals to tolerate delays in order to obtain larger rewards.

A precise functional characterization of the HPC neurons during a rodent delay discounting task has yet to be performed, but based on related observations, it seems likely that the HPC contributes both temporal and prospective information to intertemporal choice. Evidence for the

former role comes from the fact that HPC neurons in both primates and rodents encode the duration of elapsed time (MacDonald, Lepage, Eden, & Eichenbaum, 2011; Naya & Suzuki, 2011). Given that HPC-lesions failed to affect timing behavior in some studies (Dietrich, Allen, & Bunnell, 1997; Dietrich & Allen, 1998; Port, Romano, Steinmetz, Mikhail, & Patterson, 1986; J. N. Rawlins, Winocur, & Gray, 1983), while others have reported that lesions to the HPC or fimbria/fornix have shortened duration estimates (Meck, Church, & Olton, 2013; Meck, 1988; Olton, Meck, & Church, 1987); it's not clear what the exact role of the HPC is to timing behavior and/or whether it's even required (Yin & Troger, 2011).

The role of the HPC in prospection is more straightforward, stemming from complimentary human and rodent studies. Behavioral psychologist have long considered the HPC an important component of the imagery and prospection network because it's engaged when individuals 'self-project' or 'mentally time travel' and imagine or predict future events like reinforcement (Peters & Büchel, 2011; Peters & Büchel, 2010). In fact, when such 'future thinking' was stimulated by a behavioral intervention that encouraged episodic prospection during an human intertemporal choice task, subjects discounted less and their fMRI BOLD signals revealed enhanced PFC-HPC coupling (Peters & Büchel, 2010). Additionally, at decision points in a maze-based decision task, rodent HPC neuron ensembles are known to show forward sweeping spatial representations that 1) are sensitive to task demands and experience and 2) likely signal the potential forward paths and associated actions and choices (Johnson & Redish, 2007; van der Meer, Johnson, Schmitzer-Torbert, & Redish, 2010).

1.4.4 *The dopamine system transmits expected value information throughout the decision circuit*

The dopamine (DA) system is best known for its ability to broadcast reward information throughout the brain, including to structures described above (Figure 1.3). DA neurons fire in response to predictive cues and encode the magnitude of future rewards (Schultz, 2006). In fact, direct stimulation of DA neurons can invigorate reward-seeking behavior (Phillips, Stuber, Heien, Wightman, & Carelli, 2003), just as inactivation of DA neurons cam impair animals ability to respond to reward-predicting cues (Yun, 2004). As DA neurons respond to cues, they continue to track reward information by firing at higher rates for greater than expected rewards and at lower rates for smaller than expected rewards, a phenomena called reward prediction error signaling (Schultz, 2006). Finally, recordings made during two rodent delay discounting tasks showed that DA neurons' cue responses are able to integrate delay information and signal expected value information- in this case, a single value signal that encodes both reward size and delay-discounted value (Day, Jones, Wightman, & Carelli, 2010; Roesch, Calu, & Schoenbaum, 2007). Thus, in addition to contributing to outcome evaluation, the DA neurons phasic responses provide expected value information that is transmitted throughout the decision circuit where it can update outcome predictions, modulate other decision signals, and support normal levels of delay discounting.

Pharmacological manipulations confirm that DA neurotransmission influences intertemporal choice through tonic signaling as well. Blocking DA receptors with the antagonist flupenthixol caused animals to increase discounting; whereas, stimulating DA receptors with amphetamine or a DA reuptake inhibitor decreased delay discounting (Floresco, Tse, & Ghods-Sharifi, 2008; Winstanley, Dalley, Theobald, & Robbins, 2003). Since systemic injection of the D1, but not D2, antagonist also increases discounting, it's likely that DA biases choice toward the delayed reward through global D1 receptor activation (van Gaalen, van Koten, Schoffelmeer, &

Vanderschuren, 2006). Additionally, through its capacity as a neuromodulator, the DA system is also capable of influencing important signals and computations locally by modifying activity within single structures. So far, it's been reported that DA input into the OFC but not the NAc is necessary for normal levels of choice, as eliminating DA input using 6-hydroxydopamine in the OFC and not NAc caused rats to decrease choice of the delayed reward (Kheramin et al., 2004; Winstanley, Theobald, Dalley, & Robbins, 2005). Finally, two experiments found that blocking both D1 and D2 receptors in the OFC and mPFC can increase discounting (Loos et al., 2010; Zeeb et al., 2010).

1.5 Risk-Based Choice

Although it was initially expected that all forms of cost-benefit decision making rely upon the same neural circuit irrespective of the cost involved (delay, risk/uncertainty, effort, etc.), rodent studies have revealed that is not the case. So, even though I intended to only focus on intertemporal choice, I have also considered risk-based choice as a control and/or counterpoint that may help illuminate the generalizability or clarify the specificity of any observations related to intertemporal choice.

When faced with risk-based choices, decisions between a certain and a risky option, decision makers go through the same basic subprocesses outlined in Figure 1.2. However, because valuation and outcome evaluation during risk-based choice require consideration of reward risk/uncertainty rather than delay, they depend on a different set of cognitive and neural processes.

1.5.1 *Measuring probability discounting*

To asses risk-based choice, rodent researcher employ probability discounting tasks, which are similar to delay discounting tasks but differ in important ways. First, the probability of reward

delivery is decreased (descending version) or increased (ascending version) within/across the session(s). Second, unlike the delay discounting tasks for which rats and humans never perform optimally because they discount, there *is* an achievable optimal discounting solution to a probability discounting task that is based on calculations of expected value (expected value =reward size x probability of reward receipt). Rats should select the large, risky lever when it's more advantageous at 100 or 50%; select arbitrarily at 25% because neither option is mathematically favorable; and switch to the small, certain lever when the large reward lever becomes disadvantageous at 12.5%. Of course, not all rats conform to that rational choice pattern. Instead, they exhibit a probability discounting pattern that reflects their unique, subjective valuation of the risky rewards, with excessive and diminished probability discounting indicating risk averse and risk seeking choice tendencies, respectively (Figure 1.4). A final thing that distinguishes the probability discounting task from the delay discounting task is the fact that insight into underlying cognitive processes can be gained from trial-to-trial choice analysis. Specifically, the impact on current choice of being rewarded (win-stay ratio; measure of reward sensitivity) or not being rewarded (lose-shift ratio; measure of loss sensitivity) during the preceding trial can reveal whether a neural structure or circuit mediates sensitivity to rewards or losses.

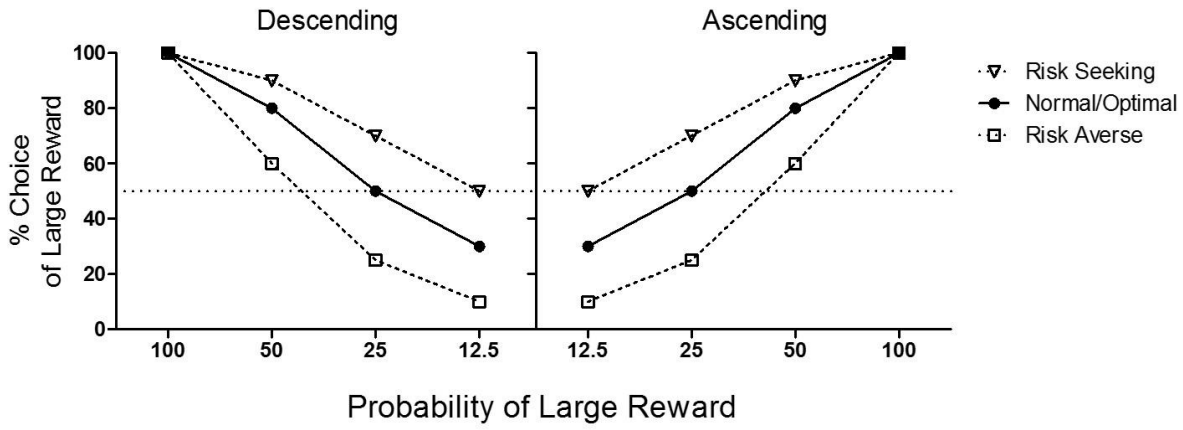
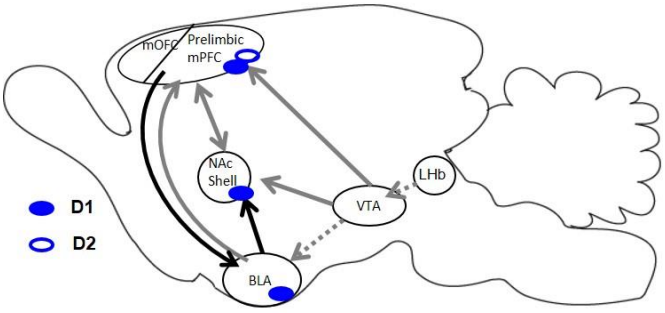


Figure 1.4. Probability discounting curves for the descending and ascending versions of the probability discounting task. The *solid black lines with filled black circles* are the optimal discounting curves, while the *dashed lines with empty triangles* represents decreased probability discounting or a risk seeking choice profiles and the *dashed lines with empty squares* represents an increased probability discounting or a risk averse choice profile.

1.5.2 *Neural basis of risk-based decision making*

Finally, the neural circuit underlying risk-based choice differs from that which subserves intertemporal choice (Figure 1.5). To briefly summarize - prefrontal regions attenuate and adjust risky decision; the BLA and NAc shell promote choice of the large, risky rewards, with the BLA mitigating loss sensitivity and the NAc shell mediating reward sensitivity; and DA transmission shapes and sculpts risky choices through tonic and phasic signals (reviewed in more detail elsewhere (Floresco, St Onge, Ghods-Sharifi, & Winstanley, 2008; Fobbs & Mizumori, 2014; Larkin, Jenni, & Floresco, 2016; Orsini et al., 2015)).



Structure/ Connection	Probability Discounting		DA manipulation		Probability Discounting	
	D	A			D	A
mPFC	↑	↓	Flupenthixol	--	↓	↓
mOFC	↓	↓	Amphetamine	+	↑	↓
NAc shell	↓		D1 antagonist in mPFC	--	↓	
BLA	↓		D2 antagonist in mPFC	--	↑	
BLA→NAc	↓		D1 antagonist in NAc	--	↓	
mPFC → BLA	↑		D1 antagonist in BLA	--	↓	↓
LHb	Indifference		DA lesion in mPFC or NAc	--	NE	

Figure 1.5**.** Anatomical circuit representation showing structures and pathways involved in rodent risk-based decisions (Left). Structures and DA receptors are only included if silencing with lesions, inactivation, or blockade altered rodent probability discounting. *Solid black lines* indicate the pathways that are required for normal discounting performance; whereas *solid grey lines* represent pathways that are not necessary for normal performance. Pathways marked by *dotted grey lines* have yet to be tested during probability discounting tasks. The Table lists the changes to the descending (D) and ascending (A) probability discounting curves that resulted from silencing structures, disconnecting pathways, or pharmacologically manipulating DA signaling during the delay discounting task (Right). ↑ risk seeking; ↓ risk averse; + stimulating DA signaling; -- blocking DA signaling; BLA-basolateral amygdala; LHb- lateral habenula (inactivation leads to indifference (Colin M Stopper & Floresco, 2013)); mPFC- medial prefrontal cortex; NAc- nucleus accumbens; mOFC-medial orbitofrontal cortex; VTA-ventral tegmental area.

1.6 The Central Cholinergic System

The central cholinergic system, which innervates the entire decision circuit, is essential for numerous complex behaviors like arousal, attention, and cue detection and is implicated in the etiology and treatment of several neuropsychiatric conditions that are characterized by suboptimal decisions. All of which together make it a compelling candidate to consider for its role in cost-benefit decision making.

1.6.1 *The central cholinergic system is positioned to influence the decision circuit*

The central cholinergic system projects widely and is capable of modulating target structures, including structures in the intertemporal and risk-based decision circuits (Figure 1.6). Specifically, it does so by activating two acetylcholine (ACh) receptors- muscarinic and nicotinic acetylcholine

(ACh) receptors (m/nAChRs) (Picciotto, Higley, & Mineur, 2012). The exact nature of cholinergic influence on the decision circuit will depend on the site of innervation, the composition of the targeted neural populations, and the receptor subtype(s) activated. Sources of ACh include projection neurons of the medial septum (ms) and nucleus basalis magnocellularis (nbm) that innervate the HPC and PFC/amygdala, respectively; cholinergic interneurons in the striatum and cortex (Hasselmo & Sarter, 2011; Parikh & Sarter, 2008; Picciotto et al., 2012); and LDTg and PPTg projections to VTA DA neurons. Broadly, cholinergic neurotransmission can alter neurotransmitter/neuromodulator release, modify neuronal excitability, change the strength of synaptic plasticity, and synchronize neuronal networks (Picciotto et al., 2012).

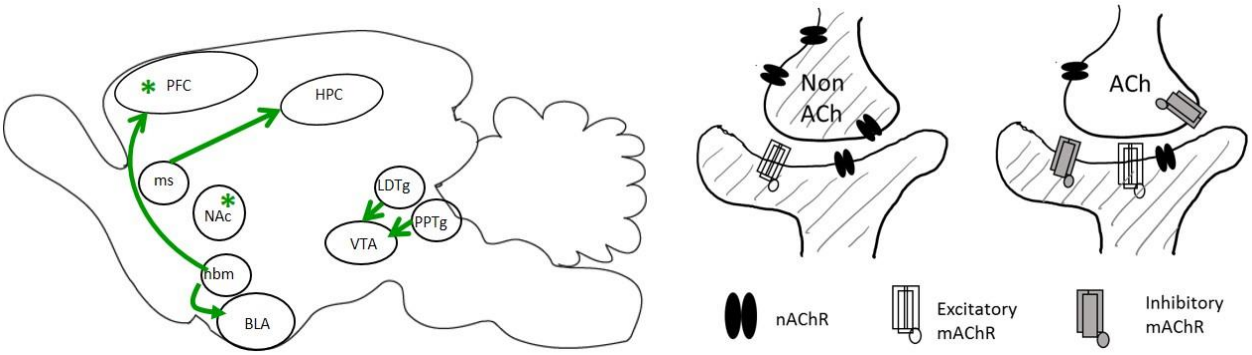


Figure 1.6**.** Anatomical representation of how the cholinergic system anatomically and functionally interacts with structures implicated in intertemporal and risk-based choices. Solid green lines represent cholinergic projections, and the green asterisks represents cholinergic interneurons (although found in the rodent and human neocortex, they aren't found in the primate cortex (Benagiano et al., 2003)). mAChRs and nAChRs are located pre-and postsynaptically and have overlapping but distinct distribution patterns throughout the brain (Barak & Weiner, 2010; Levin, 2002; Mark, Shabani, Dobbs, & Hansen, 2011; Zhou, Wilson, & Dani, 2003). While nAChRs are fast-acting and excitatory ionotropic receptors, mAChRs are slower, metabotropic receptors that can excite (G_q coupled; M1, M3, and M5 subtypes; typically found postsynaptically, except for M5 which is located presynaptically on DA neurons in striatum) or inhibit ($G_{i/o}$ coupled; M2 and M4 subtypes; found pre-and postsynaptically) (Hoon, Adrover, Wess, & Alvarez, 2015; Picciotto et al., 2012). Presynaptic mAChRs are typically autoreceptors on cholinergic terminals (with M2 in the HPC and M4 in the striatum), whereas presynaptic nAChRs typically stimulate the release of many neurotransmitters, including DA, ACh, serotonin, glutamate, GABA, and norepinephrine (Picciotto et al., 2012). PFC- prefrontal cortex; NAc- nucleus accumbens; BLA-basolateral amygdala; VTA-ventral tegmental area; HPC-hippocampus; ms-medial septum; nbm-nucleus basalis magnocellularis; LDTg- laterodorsal tegmentum; PPTg- pedunculopontine tegmentum.

1.6.2 *Evidence of cholinergic involvement in cost-benefit decision making*

Only a couple studies have considered whether endogenous cholinergic signaling is required for normal decision making. The first, a lesion study sought to determine the long-term effects of prolonged cholinergic hypofunction on impulsivity. They found that when ACh neurons of the basal forebrain were selectively lesioned in neonates, the neonates grew to be adults that were more likely to discount delays than their non-lesioned counterparts, suggesting that early cholinergic hypofunction reduced tolerance for delays and increased impulsivity. Of course, there are a couple aspects of the experiment which make it difficult to compare to other lesion studies

we've covered in previous sections: first, since the lesions were made when the animals were neonates, the effect may be attributable to altered neuronal development; and second, the lesions were made prior to task exposure, meaning that the effects could be driven by impaired action-outcome learning rather than valuation or choice (Scattoni, Adriani, Calamandrei, Laviola, & Ricceri, 2006). Nonetheless, it was the first study to suggest that cholinergic signals were important for normal decision making. In support, a subsequent pharmacological study revealed that signaling through the mAChRs, and not nAChRs, is required for optimal delay and risk-based decision making: blockade of mAChRs, but not nAChRs, altered performance on a delay discounting and probability discounting task (Mendez, Gilbert, Bizon, & Setlow, 2012).

Thus, the goal of my thesis was to expand upon those findings and focus on both the cognitive and neural contribution of muscarinic cholinergic signaling to cost-benefit decision making.

Chapter 2. THE NEURAL AND COGNITIVE CONTRIBUTION OF WHOLE BRAIN MUSCARINIC CHOLINERGIC SIGNALING TO COST-BENEFIT DECISION MAKING

2.1 INTRODUCTION

Cost–benefit decision making is a process by which an individual chooses between two or more options that differ in reward magnitude and costs to obtain reward, such as delays to reward delivery or probabilistic (i.e. uncertain/ risky) reward delivery. When faced with such choices, animals evaluate their options, integrate reward and cost information, and select the option associated with maximal value. However, some individuals make poor choices and instead choose options associated with fewer rewards and larger costs (suboptimal delay-based decisions are called 'impulsive' decisions whereas suboptimal probability-based decisions are called "risky" decisions). The fact that impulsive and risky decisions are associated with drug addiction, neuropsychiatric disorders, and behavioral disorders suggests that both a vast neural circuitry and numerous cognitive processes mediate normal and abnormal cost-benefit decision making (Koffarnus et al., 2013; Lee, 2013). In fact, preclinical studies with rodents have long-defined the decision circuit as the prefrontal cortex, limbic regions, striatum, and dopamine system, with distinct substructures and/or interactions recruited to solve different types of decision problems (Floresco, St Onge, et al., 2008; Fobbs & Mizumori, 2014). Such studies rely on a suite of sensitive psychological assessments capable of modeling "real world" decisions in a laboratory setting. The most popular are discounting tasks that present choices between a small food reward associated with little or no response cost and a large food reward linked to a bigger cost that varies within or across sessions (Mazur, 1987; Evenden & Ryan, 1999). Animals prefer the large reward option

when costs are absent or equal, but their preference decreases as the cost increases, reflecting how costs 'discount' the value of the large reward option (Rachlin, 2006).

Mendez, Gilbert, Bizon, & Setlow, (2012) used discounting tasks to assert that the muscarinic cholinergic system should also be considered part of the decision circuitry. The report showed that systemic administration of the muscarinic antagonists scopolamine and atropine biased choices in delay and probability discounting tasks. Scopolamine shifted choices toward the small reward in both tasks, while atropine induced distinct effects in the two tasks. In the delay discounting task, atropine caused animals to exhibit impulsive choices- increased preference for the small, immediate reward; whereas in the probability discounting task, it flattened animals' choice profiles- reducing choice of the large reward at high probabilities and increasing choice at low probabilities (Mendez et al., 2012). Given those observations, it is tempting to conclude that scopolamine and atropine changed animals' choices by specifically disrupting the valuation of the two options. But, that is likely too simplistic an explanation because it fails to acknowledge the drugs' effects on 1) non-choice aspects of task performance and 2) more basic cognitive processes that could also explain the change in choice behavior and deepen our understanding of how and when muscarinic cholinergic signaling is recruited during cost-benefit decision making (Bezzina et al., 2007; Cardinal et al., 2000; Ho, Mobini, Chiang, Bradshaw, & Szabadi, 1999; Kheramin et al., 2002; Killeen, 2011). In fact, Mendez et al. (2012) found that the muscarinic antagonists also affected three other aspects of task performance: the antagonists increased the number of omitted trials and decreased locomotion; and, in the case of scopolamine, significantly reduced choice of the large reward when it was guaranteed (immediately available or certain). Together, those effects support an alternative conclusion that scopolamine and atropine changed animals' choices by

impairing motor performance, attention, motivation, and/or reward discrimination instead of just valuation.

In order to reconcile these possible interpretations and to more thoroughly characterize the role of muscarinic cholinergic signaling in cost-benefit decision making, we re-examined the effects of atropine on delay and probability discounting, paying special attention to the implications of non-choice task variables. Specifically, we asked whether the addition of a cue during the delay-to-reinforcement in the delay discounting task differentially engaged the muscarinic cholinergic system, as it did with other structures/systems (Cardinal et al., 2000; Zeeb et al., 2010) and whether changes to choice behavior in the probability discounting task were due to changes in feedback sensitivity (St. Onge, Stopper, Zahm, & Floresco, 2012; St. Onge et al., 2011; Stopper & Floresco, 2011). Further, we considered whether deficits to two cognitive processes- interval timing and working memory, for which the muscarinic cholinergic system is implicated (Heise, Hrabrich, Lilie, & Martin, 1975; Meck et al., 1987; Meck & Church, 1987; Meck, 1983) and which have recently been correlated with impulsivity (Heilbronner & Meck, 2014; Marshall, Smith, & Kirkpatrick, 2014; J. McClure, Podos, & Richardson, 2014; Renda, Stein, & Madden, 2014; Shimp, Mitchell, Beas, Bizon, & Setlow, 2014)- might also explain the role of the muscarinic cholinergic system in cost-benefit decision making.

2.2 Materials and Methods

2.2.1 *Subjects*

Fifty six male Long-Evans rats, weighing between 250-300g upon arrival (Simonsen Labs, Gilroy, California, USA), were housed individually in Plexiglas cages. The rats took part in four separate experiments: 27 rats in experiment 1; 15 rats in experiment 2; 8 rats in experiment 3; and 10 rats in experiment 4. 15 of the rats in experiments 1 and 4 participated in 1-3 behavioral tasks in no

consistent order, but the exact order of experience did not produce significant changes to task performance or treatment effect. Additionally, 25 rats in experiments 1 and 4 were surgically implanted with cannula in their nucleus basalis prior to training on the two discounting tasks (details of surgeries and data not included). Following surgery, they were trained to baseline and given infusions of saline and a muscimol/baclofen mixture prior to being retrained to a new baseline before the saline and atropine injections relevant to this paper. Although, the cannula infusions neither interfered with the atropine treatment nor changed the rat's baseline choice levels, it did cause rats to experience a larger number of training sessions prior to atropine injections. Every rat had unrestricted access to water and was food-restricted to 80-83% of their free-feeding body weight to ensure motivation to work for food rewards. Prior to the start of behavior training, they were exposed to 10 sugar pellets (Test Diet, 45mg) in their cages for 3 days to accustom them to the new food rewards. Behavioral training and testing took place during the light cycle of a 12h light/dark schedule (lights on 0700-1900) during 5 to 6 days per week. Procedures for this study were reviewed and accepted by the Institutional Animal Care and Use Committee at the University of Washington.

2.2.2 *Experiment 1- delay discounting*

2.2.2.1 Behavioral Apparatus

All of the behavioral testing was performed in 7 standard rat operant chambers (30.5 x 31.8 x 29.2cm; Med Associates, St. Albans, VT, USA) enclosed in sound attenuating boxes with fans that masked external noise and supplied ventilation. One wall of each chamber contained two retractable levers, one on either side of a central food cup into which sucrose pellet rewards were delivered by pellet dispenser and over which an infrared photo beam was located to record nosepokes. Cue lights and a traylight were also located above the two levers and food cup,

respectively. In contrast, the opposite wall of each chamber was equipped with a single houselight and a speaker system. All experimental data were recorded by computers connected to the chambers through the MED-PC interface and software.

2.2.2.2 Behavioral Procedures: Training and testing were modeled off the delay discounting task previously described in Cardinal, Robbins, and Everitt (2000).

Pre-training: Prior to training on the delay discounting task, rats underwent two pre-training procedures designed to teach them to lever press for rewards and familiarize them with the task structure. In the first, they were trained to press both levers, one after the other, for pellets using a fixed-ratio (FR) 1 schedule. Once they met the criterion of 60 presses in 30 min on each lever, they continued to the retractable lever training, which was a 96 trial-long simplified version of the full delay discounting task. During the retractable lever training, the trial start was signaled by the illumination of the houselight and traylight every 50s. Rats learned to nosepoke above the food cup within 10s of the trial start in order to prevent the lights from being extinguished and the trial being counted as an omission. When they nosepoked in a timely manner, the traylight was turned off, a single lever was extended, and they again had 10s to respond before the lever was retracted and the trial recorded as omitted. However, if they responded within the window, they were immediately rewarded with a single pellet and the traylight was again turned on until the pellet was collected (or the 10s collection period ended). Finally, the houselight was switched off for the remainder of the time left in the 50s long trial, the inter-trial interval (ITI). Trials were presented in pairs, with each pair consisting of random orders of left and right lever trials. Rats were trained to a stable baseline performance allowing no more than 1 omitted trials for 3 consecutive sessions.

Side preference test: After pre-training, rats were tested for their side bias using a 1 session task described by Haluk and Floresco (2009). In total, rats were presented with 7 trials that were structured similarly to the retractable lever training, except that both levers were inserted simultaneously and rats had to press each lever once in order to move onto the next trial. The trial began by rewarding rats for a press to either lever and then proceeded until the opposite lever was pressed and rewarded (with presses to the same lever turning the houselight off and not being rewarded). The first lever pressed (left or right) in every trial was recorded and counted, so that we could determine each rat's preferred side (the lever they chose four or more times) and assign that lever as their small reward lever to ensure that their ultimate choice preference for the large reward lever during the delay discounting task was due to the task rather than side bias.

Delay discounting task: The full delay discounting task used the same lights schedule and response windows as the retractable lever training (described above), but it differed from that task by presenting rats with a series of choices between two levers associated with different rewards and delays. Sessions were 50 min long, consisting of 5 blocks of 12 trials that included 2 forced-choice trials during which only one lever was extended (one trial per lever) and 10 free-choice trials during which both levers were extended simultaneously. Pressing one lever always resulted in the immediate delivery of a small reward (1 pellet), while pressing the other lever resulted in the delivery of a large reward (4 pellets) after a delay that increased with each block (0, 4, 8, 16, 32s). Lever assignments were based on the side preference test and counterbalanced within groups. Two different versions of the delay discounting task were used. In the Cue version, the stimulus light above the large reward lever was illuminated during the delay, signaling the duration of the delay; whereas the cue light remained off in the No Cue version. For every session, choice behavior for

each delay block (percentage of large reward choices made during completed free-choice trials) was recorded to generate delay discounting curves. The time it took rats to perform different aspects of the task was also recorded for every session. This included the time it took to initiate the trial with a nosepoke (nosepoke latency); time it took to choose a lever (choice latency), and the time it took to start consuming pellets once delivered (collection latency). Drug testing started as soon as individual rats reached criterion, which was stable baseline performance for 3 consecutive sessions as analyzed by ANOVA (see 'Data Analysis' section for further details of ANOVA structure). (See Figure 2.1a for task schematic)

No delay task: Following the delay discounting task, a subset of rats was tested in a variant of the delay discounting task designed to assess reward magnitude discrimination and satiety. In this control version of the delay discounting task, the only task feature that was changed was the delay to the large reward: instead of the delay increasing with each block, it was held constant at 0s throughout the entire session. Rats were trained until they exhibited stable performance for 3 consecutive session and then drug testing began.

Matched delay task: A subset of rats was also tested on a second variant of the delay discounting task designed to assess reward-delay integration and willingness to wait for rewards. Like the no delay control, this control version differed from the delay discounting task in only one way: the delay to both the large and small rewards was matched at 8s in blocks 2-5. Again, rats were trained until they exhibited 3 consecutive sessions of stable performance before drug testing commenced.

2.2.3 *Experiment 2- timing*

2.2.3.1 Behavioral Apparatus: Operant chamber (See 'Experiment 1 Behavioral Apparatus' for detailed description)

2.2.3.2 Behavioral Procedure: Our temporal bisection procedures were modeled off of those described in Meck (1983) and Santi, Miki, Hornyak , and Eidse (2006).

Pre-training: Rats underwent two pre-training procedures designed to teach them to lever press and collect rewards. In the first procedure, which only took one session, a single pellet was delivered every minute for 60 minutes and rats were only expected to passively experience the pellets' temporally specified delivery and collect them from the food cup. Next, rats were trained to press alternating levers for pellets using a FR-10 schedule until they pressed the levers 60 times within 60 minutes. Rats performed two versions of the second training procedure- one with the left lever extended first and another with the right lever extended first.

Duration discrimination training: Following pre-training, rats were divided into two groups and trained to discriminate between short (4s) and long (16s) durations of either Filled or Empty intervals (See Figure 2.3a). Rats assigned to the Filled version of the task were presented with 4 or 16s long intervals that were signaled by a continuous 1 Hz tone; whereas rats assigned to the Empty version were presented 500ms 1Hz tones played on either side of 4 or 16s long empty intervals. Each trial of the 2hour daily sessions began with the onset of either the 4 or 16s interval followed immediately by the extension of both levers. Rats were trained to press one lever ("short" response) following the 4s interval and the other lever ("long" response) following the 16s interval, with the exact left/right lever assignment counterbalanced across rats. The 4 or 16s interval

durations were randomly presented with a probability of 0.5. When the correct lever was selected within 10s, the levers were retracted and 1 pellet was delivered. However, when the incorrect lever was pressed, both levers were retracted and no reward was given. Each time the levers were retracted, the trial ended and a variable ITI (30-70s) began. Any incorrect responses were followed by correction trials, which were so-called because the same interval was presented again. For every trial of a training session, we recorded the interval duration, lever pressed, and response latency. Rats were trained for 15 sessions until they were able to discriminate between the two durations by pressing the correct lever on greater than 85% of the non-correction trials.

Temporal bisection procedure: Once trained to discriminate the anchor durations, rats continued to the full temporal bisection procedure, which tested their perception of intermediate durations. The trial structure of the temporal bisection procedure was identical to the discrimination training, but in addition to presenting the anchor durations, half of the trials were probe trials with intermediate durations (5.04, 6.35, 8, 10.08, and 12.7s). Over the course of the session, trials with the anchor durations were presented with a probability of 0.25 and trials with each of the five intermediate durations were presented with a probability of 0.1. Lever responses on probe trials caused the levers to retract without reinforcement, and no correction trials were included. Every day of the temporal bisection procedure, psychophysical curves were generated that reflected the relationship between the likelihood of judging a given duration "long" and the actual length of that duration. In keeping with tradition, responses (lever presses) made with latencies greater than 3s were excluded from analysis (Meck, 1983). Rats were tested for 15 days prior to drug testing, at which point their psychophysical curves were smooth and their judgments of anchor durations accurate (>85%) for 5 consecutive sessions. Temporal bisection procedures measure two aspects

of interval timing: fist, the accuracy of timing is reflected by the perceptual midpoint (the point of subjection equality, PSE) when the likelihood of judging an interval "long" is 50%; while the imprecision of timing is reflected by the variance of the function (called the Weber Fraction, WF), which is calculated by dividing the standard deviation by the PSE. Choices during baseline, drug, and saline sessions were fit to a cumulative normal curve before PSE's and WFs were calculated. (See Figure 2.3 a for task schematic)

2.2.4 *Experiment 3- working memory*

2.2.4.1 Behavioral Apparatus

The delayed alternation task was performed on an elevated T-maze with three black Plexiglas arms (a trunk and two arms; 58 x 5.5 cm each) radiating from a raised circular platform (19.5 cm in diameter and 79 cm from the floor). The maze was surrounded by 4 black drapes adorned with distinct visual cues and lit by 4 15-W bulbs located at the corners of the enclosure.

2.2.4.2 Behavioral Procedure

Habituation: During habituation, rats were placed on the trunk of the T-maze and permitted to traverse the maze and collect pellets sprinkled throughout. They were trained until they successfully ate all the pellets and freely visited both arms.

Alternation task: Once habituated, rats were trained to alternate between maze arms to collect rewards. The alternation task consisted of pairs of trials, one forced-choice trial and one free-choice trial. Prior to the start of each pair of trials, the ends of both arms were baited with 4 pellets and one arm was obstructed with a wooden block. The forced-choice trial started when rats were placed on the start location (end of the T-maze trunk) and permitted to run into the unobstructed

arm to collect pellets. After collecting the pellets, rats were trained to run back to the start location to begin the free-choice trial, during which both arms were accessible and they could choose either arm to enter for reward. The order in which arms were blocked during the forced-choice trial was random. Since we baited the ends of both arms at the start of each pair of trials, rats received the maximum amount of reinforcement when they learned to enter (choose) the previously blocked arm on every free-choice trial, alternating arms relative to the forced-choice trial. Initially, we presented rats with 10 pairs of trials with 30s inter-trial intervals and recorded how frequently they chose the correct arm. Once they chose correctly 80% of the time over 2 consecutive days, we increased the number of trial pairs to 24. Again, we trained them until their performance stabilized at 80% for another 2 days.

Delayed alternation task: After learning to successfully alternate, we increased the complexity of the task to assess working memory capacity across delays. The delayed alternation task was structured like the alternation task, except that a delay was added between the forced- and free-choice trials of the trial pairs. Instead of simply returning to the start and immediately beginning the free-choice trial, rats were held at the start location with a wooden block until a delay period (4, 6, 16 s) elapsed. Since there were 24 trial pairs in total, rats encountered 8 pairs with each delay. Rats were trained until they chose correctly on 80% of the trials for 3 consecutive days before drug testing started. (See Figure 2.4a for task schematic).

2.2.5 *Experiment 4- probability discounting*

2.2.5.1 Behavioral Apparatus: Operant chamber (See 'Experiment 1 Behavioral Apparatus' for detailed description)

2.2.5.2 Behavioral Procedure: Our probability discounting protocols were adapted from those of St. Onge and Floresco (2009).

Pre-training: Two pre-training procedures were used to train rats to lever press for rewards in a timely manner. First, they were trained under a FR-1 schedule to a criterion of 60 presses in 30 min to press both levers, one after the other, for pellets. Then, they were trained on a 90 trial, 50s task that started with the illumination of the houselight and the insertion of one of two levers into the chamber. Rats learned to press the lever within 10s because failure to do so resulted in the lever being retracted, the houselight extinguished, and the trial counted as an omission. When they pressed the lever within the window, the lever retracted and a single pellet was delivered immediately and the houselight was switched off 4s later and the ITI started for the remainder of the trial. Each trial lasted 40s with no more than two consecutive presentations of the same lever. Rats were trained to a strict criterion of 89 or 90 successful trials over 3 consecutive sessions.

Side preference task: After pre-training, rats were tested for their side bias using a 1 session task described above for Experiment 1.

Probability discounting task: The full probability discounting task used the same light schedule and response window as the pre-training (described above) but presented rats with a series of choices between two levers associated with different reward sizes and probability of reward. Sessions were 48 minutes long, consisting of 5 blocks of 18 trials that included 8 forced-choice

trials during which only one lever was extended (one trial per lever) and 10 free-choice trials during which both levers were extended simultaneously. Pressing one lever (small/certain lever) always resulted in the delivery of 1 pellet, while pressing the other lever (large/risky lever) resulted in delivery 4 pellets in a probabilistic manner that varied systematically descended across the session (100, 50, 25, 12.5%) Lever assignments were based on the side preference test and counterbalanced. After every session, choice behavior for each probability block (percentage of large reward choices made during completed free-choice trials) and choice latencies (free and forced choice) were recorded and probability discounting curves generated. Drug testing started as soon as individual rats reached criterion, which was stable baseline performance for 3 consecutive sessions as analyzed by ANOVA (see 'Data Analysis' section for further details of ANOVA structure). To clarify whether changes in risky choice were driven by changes in feedback sensitivity, we determined how choice of the large/risky option was influenced by receiving or not receiving reinforcement on preceding trials. Specifically, we calculated the proportion of trials during which rats chose the large/risky lever after choosing it and being rewarded on the preceding trial (win-stay ratio) and the proportion of trials during which the rat chose the small/certain lever after not receiving reinforcement for their risky choice on the preceding trial (lose-shift ratio). (See Figure 2.5 a for a task schematic)

2.2.6 *Drug Testing*

Since our intention was to re-examine the effects of blocking the muscarinic cholinergic system with the antagonist atropine on choice reported in Mendez et al. (2012) and to determine whether they were specific to valuation, we chose to use the dose of atropine that was effective in both discounting tasks, 0.3 mg/kg. We purchased the atropine from Sigma-Aldrich and administered it (intraperitoneally) mixed in 0.9% saline at a volume of 1ml/kg. The schedule of within-group drug

testing differed for each experiment. For experiments 1 and 4, drug testing took place over two days: saline was injected on the first day and atropine was injected on the second. Alternatively, drug testing for experiment 2 occurred over 3 days and included injections with either saline or atropine separated by a single retrain day with no injection. Lastly, we employed a six day injection schedule for experiment 3 that consisted of an ABBA injection design interrupted in the middle by 2 retrain days with no injection. All injections were conducted 30 minutes prior to the start of the behavior training session; and while the order of saline and atropine were fixed for experiments 1 and 4, the orders were counterbalanced for experiments 2 and 3.

2.2.7 *Data Analysis*

All statistical analyses and curve fitting were conducted in Graph Pad. Stable baseline performance in discounting tasks was determined using two-way repeated-measures ANOVA (day x delay/ probability) and was defined by 1) a main effect of delay and 2) the absence of a main effect or interaction for day. For all experiments, reported baselines were calculated by averaging across the number of stable days assessed for criterion (3 days for discounting tasks and delayed alternation task and 5 days for the temporal bisection procedure). Analyses of choice behavior, response latencies, and feedback sensitivity for discounting tasks and delayed alternation task was also conducted using two-way repeated-measures ANOVA (atropine x delay/probability/feedback sensitivity ratios) with Bonferroni post hoc tests used when warranted. In contrast, we used a two-tailed paired t-test to analyze the effects of atropine on the PSE and WF in the temporal bisection procedures. In all cases, p values <0.05 were considered significant. The main effect of delay on choice was always for discounting tasks and saline injections did not significantly changed behavior when compared to baseline in any of the experiments, so neither will be reported further.

2.3 RESULTS

2.3.1 *Experiment 1- delay discounting*

The goal of the first experiment was to determine whether atropine induce impulsive choice in both versions of the delay discounting task.

2.3.1.1 Basal levels of choice during the delay discounting task

The 10 rats in the No Cue group (40 $\pm$ 5.2) and 9 rats in the Cue group (45.3 $\pm$ 5.0) took a similar number of days to reach stable baseline. Comparison of their baselines revealed that the Cue group displayed a lower basal level of large reward choices than the No Cue group. A non-repeated measure ANOVA showed that there was no group x delay interaction ($F_{4, 85}$=1.00, not significant [ns]) but that there was a main effect of group ($F_{1, 85}$=13.09, $p<0.001$) that post hoc analysis revealed was driven by a significant difference in the 8s block ($p<0.05$).

2.3.1.2 Effects of atropine on choice during the delay discounting task

Atropine injections had differential effects on choice that depended on whether the delay-to-reinforcement was cued. Specifically, atropine significantly decreased choice of the large reward when the delay to its delivery was uncued (No Cue group; atropine: $F_{1, 45}$=37.90, $p<0.0001$; atropine x delay: $F_{4, 45}$=2.78, $p<0.05$; Figure 2.1b) and failed to affect choice when the delay to the large reward was signaled by a cue light (Cue group; atropine: $F_{1, 40}$=0.04, ns; atropine x delay: $F_{4, 40}$=0.55, ns; Figure 2.1c). The choice reduction in the No Cue group was not observed when both rewards were available immediately (block1); and, as post hoc analysis revealed, the increase in impulsive choice was particularly evident at the intermediate delays of 4 and 8s ($p<0.05$ and $p<0.001$, respectively). Additionally, the change in the No Cue group was not accompanied by an increase in the number of omitted trials, suggesting it was not attributable to a reduction in the

number of choices available for analysis ($F_{4, 45}$= 0.6298, ns; Figure 2.1d). Similarly, the number of omissions made by the Cue group was unaffected by atropine ($F_{4, 40}$=0.8, ns; Figure 2.1e).

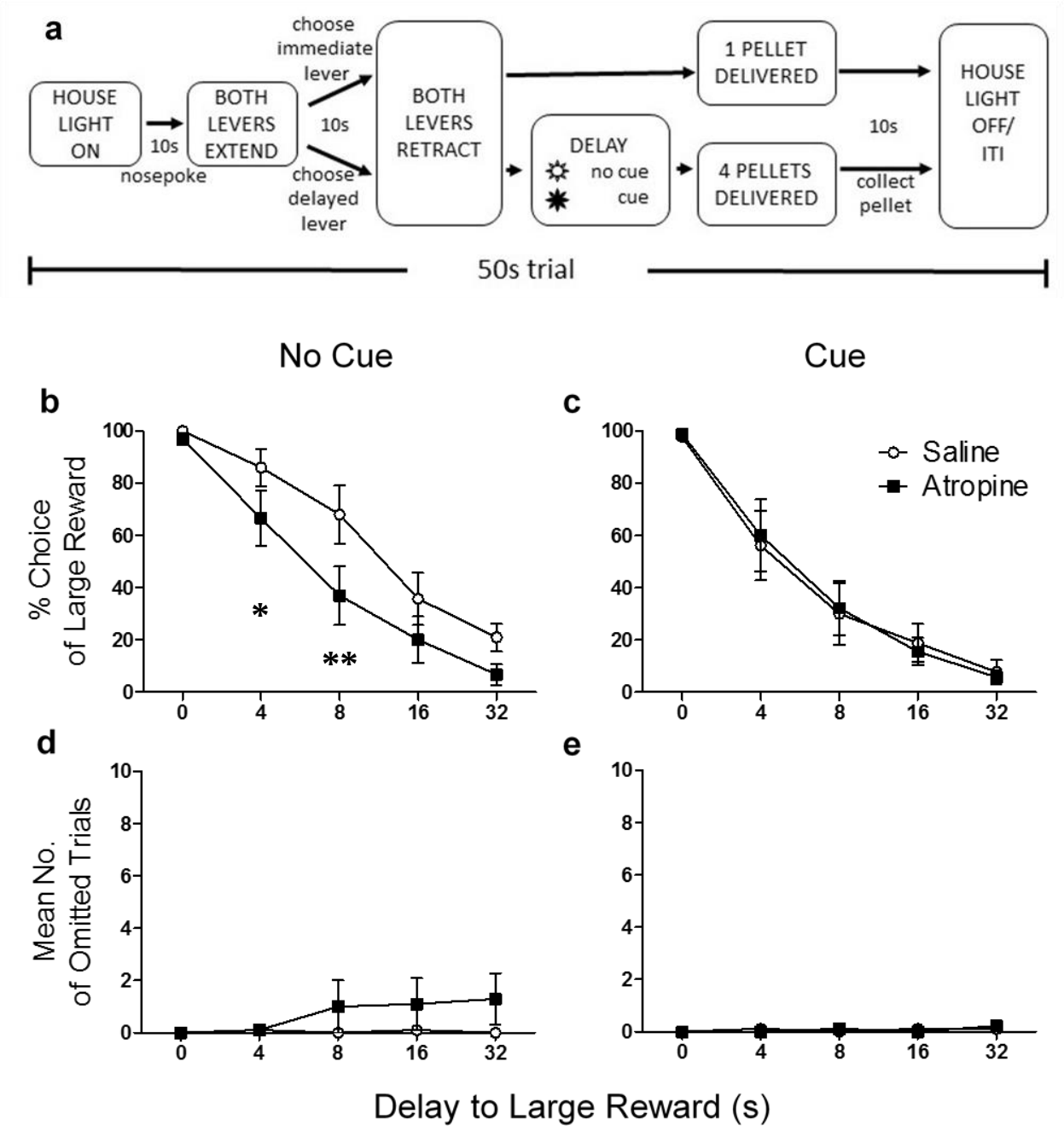


Figure 2.1**.** Effect atropine on choice behavior during the two versions of the delay discounting task. Detailed schematic of the delay discounting task (a). Atropine caused rats in the No Cue group to decrease preference for the large reward (b) without increasing the number of omitted trials (d); whereas in the Cue group, atropine failed to alter choice (c) and the number of omissions (e). Data shown are mean ± SEM. * $p < 0.05$; ** $p<0.001$.

2.3.1.3 Effect of atropine on performance controls

A priori, we decided that if atropine induced impulsivity in either version of the delay discounting task, we would conduct supplementary analyses and control tasks to clarify the underlying cognitive mechanism. Since atropine's effects were only seen in the No Cue group, each of the following analyses involved rats that experienced an uncued delay-to-reinforcement. First, we tested whether the reduced choice of the large reward observed in the No Cue group was accompanied by changes to non-choice aspects of task performance. We evaluated the impact of atropine on several measures of response latency, including nosepoke latency, forced- and free-trial large and small reward choice latencies, and collection latency, but none were changed by atropine ($F's<1.82$, ns; Table 2.1).Thus, we weren't able to find evidence to directly link the observed impulsivity to motor, attention, and/or motivation deficits just by analyzing the task performance.

Table 2.1. Summary of mean response latencies, including the time taken to nosepoke and initiate trials (i), to press levers for large and small rewards during forced-(ii) and free- choice (iii) trials, and to start consuming pellets (iv). Atropine injections didn't impact task performance as measured by response latencies.

Performance Measure	Reward	Treatment	Latencies by delay block (s)					Latencies for session (s)
			0	4	8	16	32	
i. Trial Initiation		Saline	0.68	0.77	0.87	1.01	1.09	0.89
		Atropine	0.83	0.86	0.83	0.99	1.57	1.02
ii. Forced-choice	Large	Saline	0.79	0.75	1.80	1.69	3.11	1.63
		Atropine	0.73	0.77	0.97	1.81	3.25	1.51
	Small	Saline	1.31	1.96	1.56	1.28	1.16	1.45
		Atropine	1.31	1.80	1.25	1.50	1.03	1.38
iii. Free-choice	Large	Saline	0.82	1.04	1.15	1.28	1.06	1.07
		Atropine	0.85	0.90	1.23	1.14	1.19	1.06
	Small	Saline		0.84	1.27	1.17	1.28	1.14
		Atropine		1.19	1.04	1.03	1.09	1.09
iv. Pellet Consumption	Large	Saline	2.31	15.54	10.77	2.21	0.61	6.29
		Atropine	2.25	11.17	7.51	2.79	1.56	5.06
	Small	Saline	0.38	6.34	6.92	3.84	0.62	3.62
		Atropine	0.79	5.84	9.00	4.23	0.45	4.06

Next, to further establish whether the diminished preference for the large reward in blocks 2-5 was attributable to other cognitive deficits, we evaluated the impact of atropine on the performance of rats in a control task in which the delays to the large reward were fixed at 0s throughout all 5 blocks of the session. 11 rats were trained for 5.4 $\pm$ 0.6 days, and at baseline they exhibited 100% preference for the large reward over the small reward. Atropine injections didn't reduce their preference for the large reward in any block of the session ($F_{1, 50}$=1.312, ns; Figure 2.2a) nor did it increase the number of omitted trials ($F_{4, 50}$= 1.14, ns; Figure 2.2c). As a result, atropine did not appear to bias choices toward the small reward by increasing satiety, and thus decreasing motivation to pursue the large reward, or by causing the rewards to become indistinguishable through a lost ability to perceive the reward size difference (Cardinal et al., 2001; Ho et al., 1999)

Having addressed reward discrimination and satiety with the no delay control, we next trained rats to perform a control task in which the delays to the large and small rewards were matched at 8s in blocks 2-5. This second control task was designed to address 2 additional interpretations of the atropine induced impulsivity: first, that atropine made rats generally deficient at integrating delay and reward information, either by means directly related to poor cost-benefit valuation or by triggering an inability to use memory to bridge the spatiotemporal gap between lever press and reward (McHugh et al., 2008); and second, that atropine induced an aversion to waiting for reward. 9 rats were trained in the matched delay task for 6.3 $\pm$ 0.5 days until they chose the large reward over the small reward nearly 100% of the time. Consistent with the no delay task, atropine induced no change in choice preference ($F_{4, 40}$=0.24, ns; Figure 2.2b) nor number of omissions ($F_{4.40}$=0.33, ns; Figure 2.2d), and thus, lent no support to the explanations for which the task was designed to address.

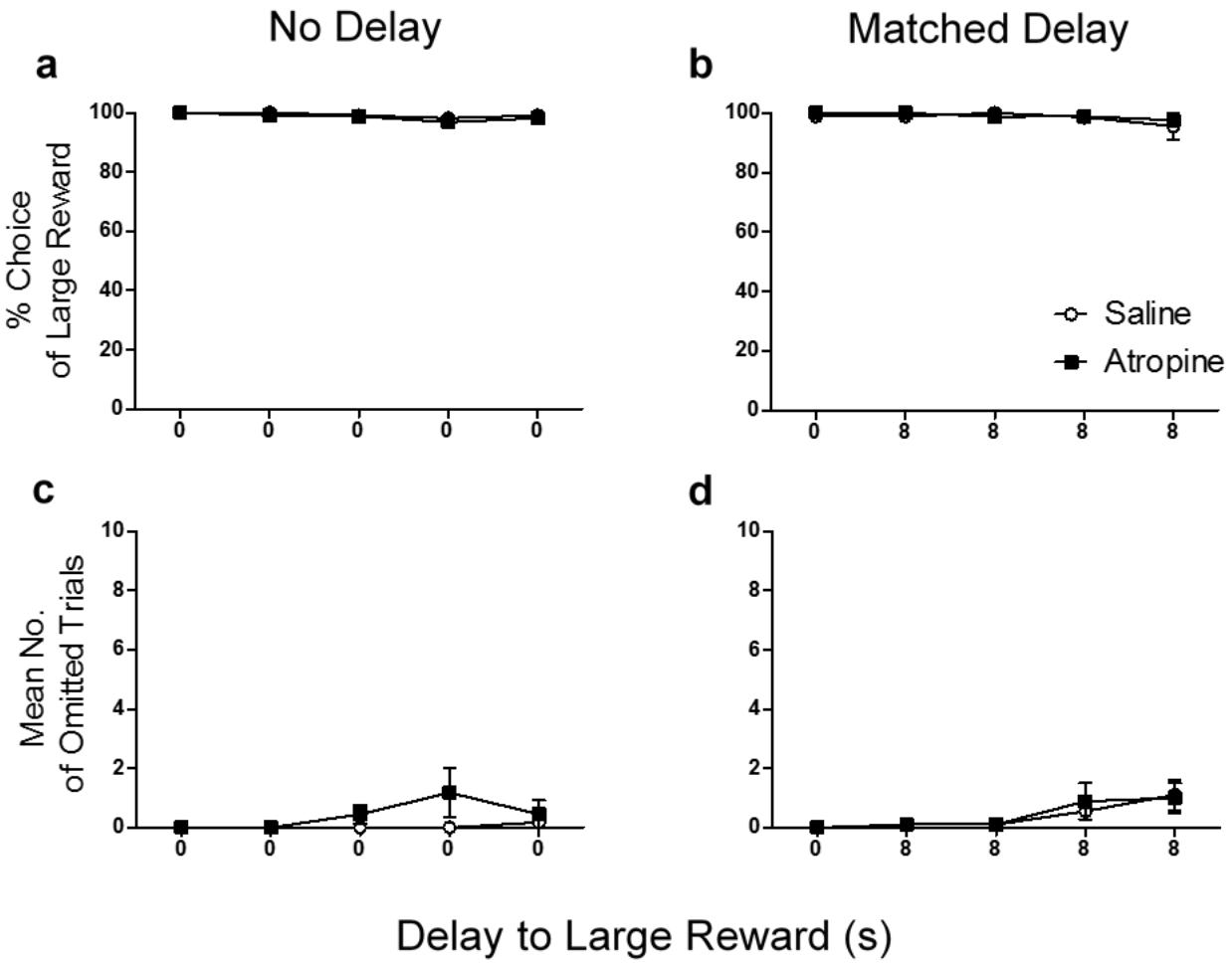


Figure 2.2**.** Effect of atropine on choice behavior during the control tasks. Atropine didn't reduce preference for large reward when the delay to its delivery was held constant at 0s (a) or 8s (b) in blocks 2-5. An increase in omissions was also not observed in either control task following atropine administration (c-d). Data shown are mean ± SEM.

2.3.2 *Experiment 2- timing*

Following Experiment 1, we sought to explain why atropine was only effective at biasing choice in the No Cue version of the delay discounting task. We reasoned that perhaps the uncued delay-to-reinforcement relies more heavily on the engagement of certain cognitive processes. Since valuation of delayed rewards likely requires the ability to represent and discriminate delays as well as reward magnitudes, we first tested whether the increased delay discounting observed in

Experiment 1 was attributable to impairments in interval timing, timing in the seconds to minutes range. Interval timing is an attractive candidate cognitive process because atropine has previously been shown to induce timing imprecision (Meck, 1983) and evidence for a relationship between interval timing and impulsivity can be found across species. Humans with disorders characterized by high levels of delay discounting are known to exhibit dysfunctional timing abilities as well (Allman & Meck, 2012; Berlin et al., 2004; Meck, 2005; Piras et al., 2014; Rubia et al., 2009) and two recent reports found correlations between delay discounting and timing imprecision in rodents (Marshall et al., 2014; J. McClure et al., 2014). Thus, we tested the effect of atropine on rats' ability to perceive and discriminate interval durations using the temporal bisection procedure. More precisely, we used two versions of the temporal bisection procedure that differed based on the presence or absence of signals during the timed intervals in order to appropriately evaluate rats' timing of both types of delays encountered in the two versions of the delay discounting task. The rats in the Empty group were presented unsignalled intervals (similar to No Cue group); whereas the rats in the Filled group were presented signaled intervals (similar to the Cue group). The specific range of interval durations (log steps between 4 and 16s) were chosen to include delays used in the delay discounting task. There were 7 rats assigned to the Empty group and 8 rats assigned the Filled group and comparison of their baselines revealed no difference in PSE (t_{13}=0.3104, ns) or WF (t_{13}=1.503, ns) of both groups. Further, atropine injections changed neither measure of timing in either group (t's <1.206, ns; Figure 2.3b-e), suggesting that a timing deficit was not the cognitive impairment underlying the increased impulsivity seen in the No Cue group in Experiment 1.

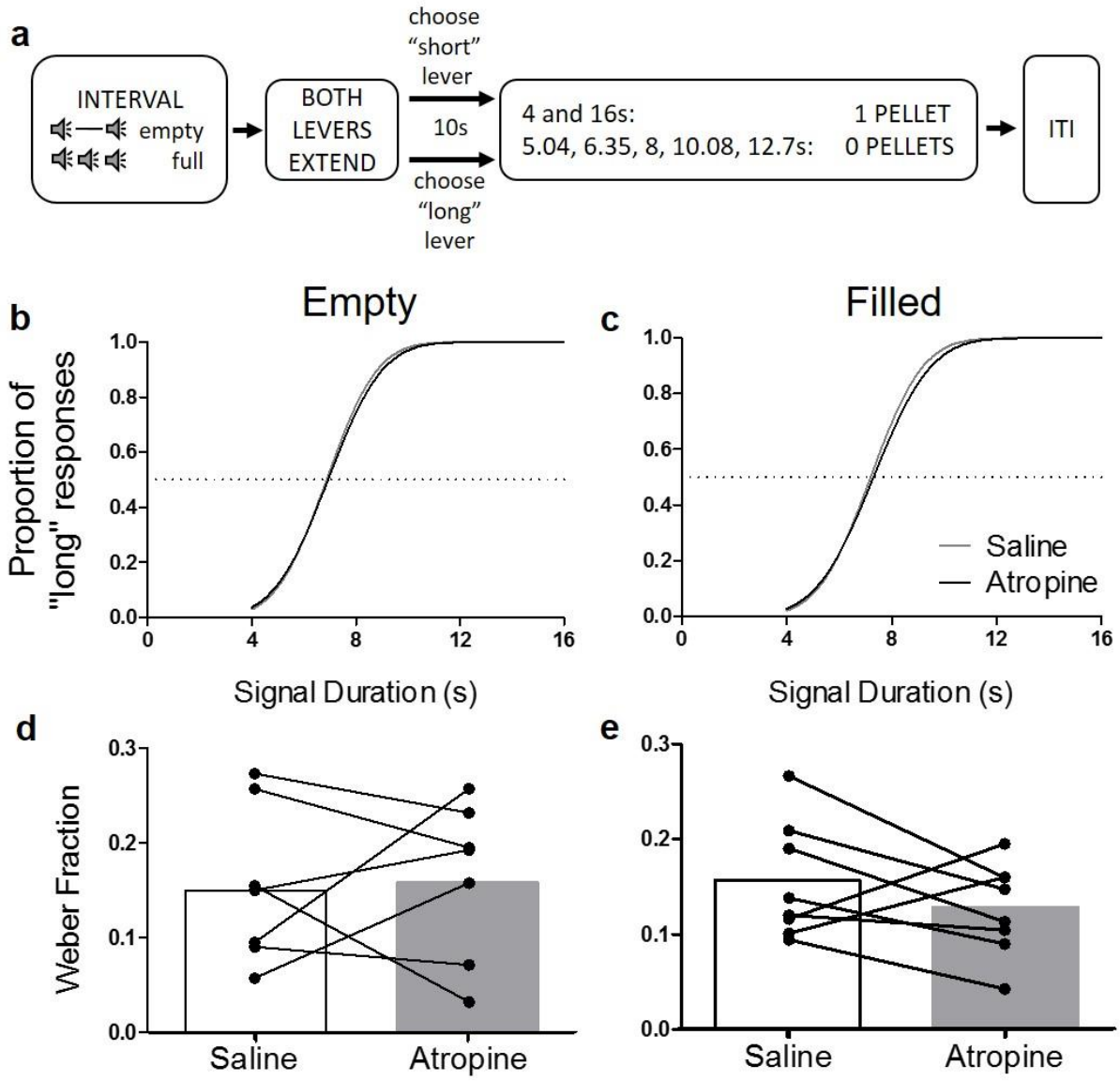


Figure 2.3**.** Effect of atropine on temporal perception during the two versions of the temporal bisection procedure. Detailed schematic of the temporal bisection procedure (a). In both the Empty and Filled group, atropine failed to shift timing accuracy (PSE; b-c) or change timing imprecision (WF; d-e) Data shown in b and c are cumulative normal curve fits of raw data and d and e show individual WF values overlaid over mean values.

2.3.3 *Experiment 3-working memory*

The second cognitive process that could explain the no cue results of Experiment 1 was working memory because atropine has previously been shown to impair working memory in rodents (Heise et al., 1975; Sala et al., 1991) and there is evidence across species that links poor working memory to impulsive decision making. In humans, researchers have observed that working memory and delay discounting are strongly correlated, that they activate overlapping neural clusters in the prefrontal cortex, and that working memory training reduces impulsive decision making (Aranovich, McClure, Fryer, & Mathalon, 2016; Bickel, Yi, Landes, Hill, & Baxter, 2011; Shamosh et al., 2008; Wesley & Bickel, 2014). Rodent researchers have reported a correlation between impulsive choice and poor working memory as well (Renda et al., 2014). Thus, to determine whether the impulsive choice observed in Experiment 1 was related to a working memory deficit, we tested the effect of atropine on rats' ability to correctly remember the location of previous reinforcement over three delays using a delayed alternation task. Specifically, we trained a group of 8 rats to perform a delayed alternation task with reward magnitude (4 pellets) and delays to free-choice trials (4, 8, and 16s delays) chosen to match those used in the delay discounting task in Experiment 1. In total, it took rats 15.5 $\pm$ 1.8 days to reach baseline, at which point they choose the correct arm on nearly 100% of the trials across all three delays. Atropine didn't reduce their accuracy (atropine: $F_{1, 21}$=0.2762, ns; atropine x delay: $F_{2, 21}$=1.605; Figure 2.4). Instead, the rats continued to alternate correctly during free- choice trials preceded by each of the three delays, ruling out atropine induced working memory deficits as an explanation for the No Cue group's impulsivity.

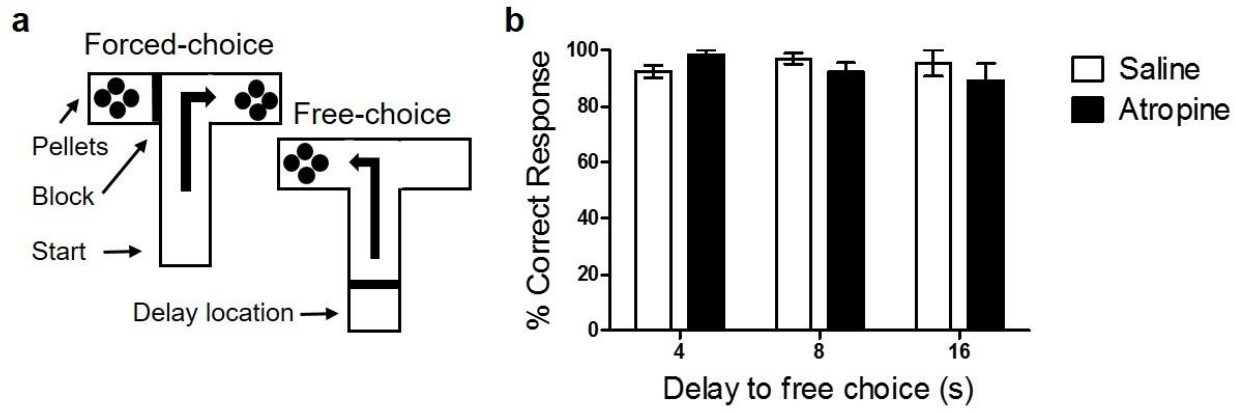
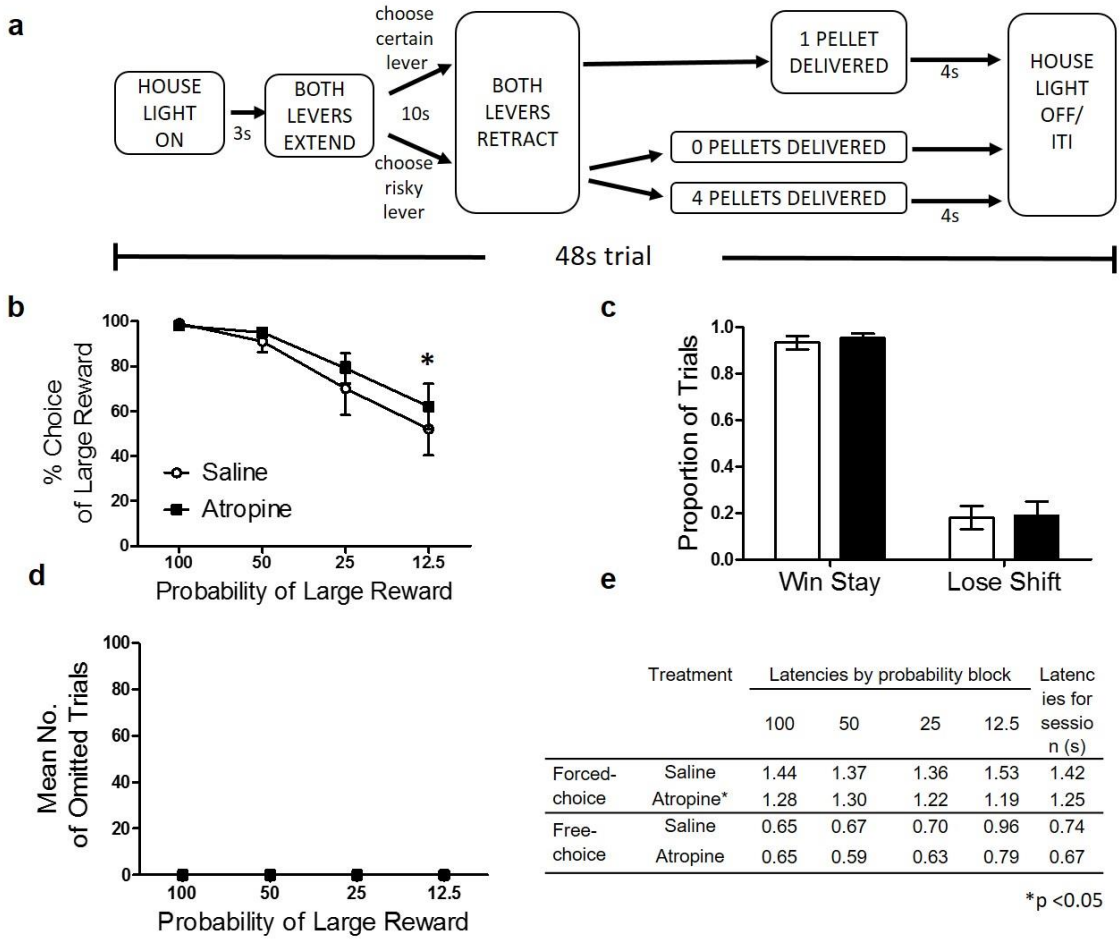


Figure 2.4**.** Effect of atropine on choice behavior during the delayed alternation task. Detailed schematic of delayed alternation task (a). Atropine didn't reduce choice accuracy at any of the delays tested (b). Data shown are mean ± SEM.

2.3.4 *Experiment 4- probability discounting*

In the last experiment, we turned our attention from delay-based decisions toward risk-based decisions. We trained 10 rats in a descending probability discounting task for 52.9 ± 2.4 days prior to drug testing and found that atropine induced risky choice by increasing the choice of the large/risky reward (atropine: $F_{1, 36}$=3.953, p=0.05; atropine x delay: $F_{3, 36}$=0.8385, ns, Figure 2.5b) without causing animals to omit any trials (Figure 2.5d). No change was observed when both rewards were guaranteed (block 1), and post hoc analysis revealed that the increased risky choice was driven by an increased preference for large reward during the 12.5% probability block ($p<0.05$), even though that probability made the large reward the mathematically disadvantageous option. Consistent with our finding in experiment 1, we weren't able to identify changes to choice latencies during free-choice trials following atropine administration ($F_{1, 36}$=2.250, ns), but, surprisingly, rats pressed the levers faster during the forced-choice trials (atropine: $F_{1, 36}$=8.899, $p<0.05$; atropine x delay: $F_{3, 36}$=0.9706, ns; Figure 2.5e). Additionally, in order to probe the underlying cognitive mechanisms of the atropine induced risky choice, we calculated win-stay and lose-shift ratios during drug testing to determine whether the increased choice of the risky reward

was driven by changes in feedback sensitivity as measured by choices following reward (win-stay performance) or no reward (lose-shift performance). Atropine changed neither ratio ($F_{1, 18}$= 0.4645, ns, Figure 2.5c), so we weren't able to attribute the suboptimal choices observed in the probability discounting task to a specific cognitive change.



	Treatment	Latencies by probability block				Latencies for session (s)
		100	50	25	12.5	
Forced-choice	Saline	1.44	1.37	1.36	1.53	1.42
	Atropine*	1.28	1.30	1.22	1.19	1.25
Free-choice	Saline	0.65	0.67	0.70	0.96	0.74
	Atropine	0.65	0.59	0.63	0.79	0.67

*p <0.05

Figure 2.5**.** Effect of atropine on choice behavior during the probability discounting task. Detailed schematic of probability discounting task (a). Atropine caused rats to increase preference for the large/risky reward (b) without changing reward/negative feedback sensitivity (c) or increasing the number of omitted trials (d) or the free-choice trial lever press latencies (e). Data shown are mean ± SEM. * $p<0.05$.

2.4 DISCUSSION

This study provides novel insight into the contribution of muscarinic cholinergic signaling to cost-benefit decision making. Blockade of muscarinic acetylcholine receptors by atropine induced

suboptimal choices (impulsive and risky) in delay and probability discounting tasks. Additionally, atropine's effect on delay discounting disappeared when the delay-to-reinforcement was cued and was neither attributable to timing nor working memory deficits. Together, our data suggest that muscarinic cholinergic signaling supports optimal decisions when choices require valuation of reward options whose costs are not externally signified.

Like Mendez et al (2012), we found that systemic administration of 0.03mg/kg atropine increased choice of the small reward when the delay-to-reinforcement was uncued. The shift in preference was restricted to blocks in which the large reward was delayed and was not accompanied by an increase in the number of omitted trials nor a lengthening of the time taken to perform responses within the task. However, new to this paper, we observed that atropine injections failed to change rats' choices when the delay-to-reinforcement was cued. This is the first reported instance of a neural manipulation only impacting choice in the uncued version of the delay discounting task. The two previous reports that also directly compared effects of treatments on both versions of the delay discounting task observed that systemic pharmacological manipulations changed choices in the two versions in the same and opposite directions and one neural manipulation only changed choice in the cued version (Cardinal et al., 2000; Zeeb et al., 2010).

In order to understand the significance of our selective atropine effect, a consideration of the differences between the two versions of the delay discounting task is necessary. Both versions require animals to make decisions between immediate and delayed rewards, and both are identical at the time of those choices. In the cued version, the cue is only turned on after the choice is made, so it doesn't provide any discriminatory information. Nonetheless, the cue exerts an influence on choice. Rats establish their baseline choices through their accumulated experience with the task

over many sessions; which means that cueing the delay-to-reinforcement changes the response-reward association that is learned (Killeen, 2011).Cued rats learn the delay discounting task faster, as demonstrated by reaching baseline in fewer sessions than their uncued counterparts (Cardinal et al., 2000; Zeeb et al., 2010; 26 vs 28 days to first baseline in the current study). Additionally, it is known that a reward's relative reinforcement value is increased when the delay to reinforcement is cued: cued delayed reinforcement maintains higher response rates than uncued delayed reinforcement (Mazur, 1997; Richards, 1981); and cues act as conditioned reinforcers, increasing the incentive salience of otherwise less valuable rewards (Williams & Dunn, 1991). In fact, evidence from previous delay discounting studies confirms that the cue functions as a conditioned reinforcer within the cued delay discounting task. Pharmacological manipulations of the dopamine (DA) system impacted choice in a manner consistent with their known abilities to potentiate or attenuate the conditioned reinforcing properties of a cue: systemic administration of a DA agonist amphetamine increased choice of the large reward (Cardinal et al., 2000) and intra-orbitofrontal cortex infusion of DA receptor antagonists SCH23390 and eticlopride decreased choice of the large reward (Zeeb et al., 2010). Therefore, it is clear that the cue- an external signal that bridges the delay between the response and the reward- impacts the valuation of delayed rewards by establishing cue-contingent response-reward associations; whereas valuation and choice in the absence of the cue must rely upon a different response-reward association that is contingent on a separate set of processes.

Given that numerous cognitive processes have been associated with muscarinic cholinergic signaling, we sought to identify a process whose disruption by atropine might explain the decreased choice of the large reward in the uncued version of the delay discounting task. As many have previously pointed out, a shift in choice in rodent delay discounting tasks may truly reflect a

change in decision making processes or it may stem from perturbations to more fundamental cognitive processes (Dassen, Jansen, Nederkoorn, & Houben, 2016; Floresco, St Onge, et al., 2008; Fobbs & Mizumori, 2014; Ho et al., 1999; Koffarnus et al., 2013). First, we ruled out the possibility that sustained attention or motor impairments drove the shift in choice since our rats remained able to perform task actions with normal reaction times. Next, we focused on processes that are considered conceptually important for normal valuation- the tracking and integration of reward and delay information. Even though muscarinic antagonists can decrease motivation towards food rewards (Pratt & Kelley, 2004), which is expected to manifest as decreased sensitivity to rewards and a shift in choice away from the large reward (Ho et al., 1999), the rats showed no signs of motivational deficits. Instead, following atropine injections, they continued to discriminate reward magnitudes, as shown by their sustained preference for the large reward throughout the no delay control session. The rats' behavior also lent no credence to the notion that impatience or an inability to consider the complex options was responsible for shifting the preference: rats still preferred the large reward across the matched delay session, even though doing so meant they had to patiently wait for reinforcement and integrate reward and delay information for two complex options.

In addition to choice paradigms, we used two other behavioral tasks to directly assess the contributions of interval timing and working memory. Chronic administration of atropine can cause rats to overestimate the length of intervals in two different interval timing tasks (Meck et al., 1987; Meck, 1983), and it's expected that longer or more imprecisely timed delays cause preferences to shift away from the large reward in a delay discounting task(Cui, 2011; Ho et al., 1999; Marshall et al., 2014; J. McClure et al., 2014; Namboodiri, Mihalas, Marton, & Shuler, 2014; Takahashi, 2009). Yet, acute administration of atropine failed to induce timing deficits:

neither the accuracy nor precision of timing was affected in the temporal bisection procedure. Satisfied that the ability to track reward and delay information was still intact under atropine, we investigated a final cognitive process that might be particularly important for delay-based decision making in the absence of a conditioned reinforcer-working memory. There is no consensus on the mechanism by which working memory contributes to delay discounting (Renda et al., 2014; Shamosh et al., 2008; Wesley & Bickel, 2014), but one possibility is that working memory maintains an active representation of the response (choice) during the delay or the delay/rewards/values recently experienced which then contribute to forming or updating the response-reward association that is used for valuation. Nonetheless, we found no evidence of a deficit: rats injected with atropine continued to use their working memory to accurately alternate in the delayed alteration task.

It's important to acknowledge a couple of caveats when interpreting these results. First, it's possible that other tasks would be more effective at assessing the form of timing or working memory that is engaged during the uncued version of the delay discounting task. In fact, there's evidence to suggest that environmental stimuli present during delay periods on the maze may act as de-facto conditioned reinforcers and better approximate the cued rather than uncued conditions; making it advisable to determine whether atropine reduces performance accuracy in an operant chamber-based working memory task instead (Zeeb et al., 2010). Second, the reason we didn't observe any attention (Mirza & Stolerman, 2000; Robbins et al., 1998), motor, motivational (Pratt & Kelley, 2004), or working memory (Heise et al., 1975; Sala et al., 1991) deficits is likely because our dose (0.03 mg/kg) was much lower than the doses used previously. With those facts in mind, the data show that muscarinic cholinergic signaling contributes to delay-based decisions when delays to reinforcement are not cued. In support of this conclusion, Tian et al. (2016) recently

demonstrated that stimulating muscarinic cholinergic receptors caused the opposite effect. Highly impulsive rats increased choice of delayed rewards during the uncued version of the rodent delay discounting task when injected systemically with the muscarinic agonist oxotremorine.

Our data also demonstrate that muscarinic cholinergic signaling is necessary for probabilistic decision making. Systemic administration of 0.03mg/kg atropine increased choice of the large, risky reward while sparing choice of the large reward when it was guaranteed in block 1. There was no increase in the number of omitted trials or the length of response latencies. Although inconsistent with Mendez et al (2012), which reported a flattening of the curve following the same dose of atropine; the result is complimentary to Silveira et al. (2015), which found that the muscarinic antagonist scopolamine increased choice of the riskiest option (in terms of probability of reward) with the shortest punishment in a rodent gambling task that requires rats to weigh reward gains and losses. It also lends further mechanistic support to previously reported capacity of acetylcholine to signal uncertain outcomes (Yu & Dayan, 2005) by indicated that such signals might impact choice through muscarinic receptors.

The fact that atropine shifted choice of the large reward in the opposite direction during the probability discounting tasks is surprising but not unprecedented. Both forms of cost-benefit decision making rely on overlapping, but distinct neural circuits whose disruption (lesion or inactivation of structure or systemic injection of drug) has been shown to cause dissociable effects (increased delay discounting and decreased probability discounting, vice versa, etc.) before (Floresco, St Onge, et al., 2008; Fobbs & Mizumori, 2014). Additionally, the divergent atropine effect might reveal that muscarinic cholinergic signaling is only necessary when costs are not cued, a shared feature of the uncued delay discounting task and probability discounting task but not the cued delay discounting task.

2.5 CONCLUSION

Our data not only confirm that muscarinic cholinergic signaling mediates two forms of cost-benefit decision making but also suggests that muscarinic cholinergic signaling is selectively engaged when decisions require valuation of reward options whose costs are not externally signified. However, the neural and cognitive mechanisms by which the muscarinic cholinergic system exerts its selective influence are still unknown and open to further investigation. One testable hypothesis is that the cholinergic system affects cost-benefit decision making through interactions with the DA systems. The case seems strongest for delay-based decisions for two reasons: one, only our systemic atropine effect on uncued delay discounting mirrored previously reported effects of systemic administration of dopamine antagonist on delay and probability discounting; and two, Tian et al. (2016) found that co-administration of the D1R antagonist SCH23390 and oxotremorine inhibited the impulsive choice observed with oxotremorine alone (Cardinal et al., 2000; St. Onge, Chiu, & Floresco, 2010; Tian et al., 2016).

One of the goals of using rodent models of cost-benefit decision making is to inform our understanding of human decision making and possibly identify cognitive and neurobiological therapeutic targets. Since many neuropsychiatric conditions linked to suboptimal decision making are also accompanied by cholinergic dysfunction, information that clarifies the relationship between the cholinergic system and decision making is likely clinically relevant. In order to apply our observations to humans, we needed to identify human decision making situations or frames that are analogous to the cued and uncued decisions that our tasks model. We found examples in the human literature that show that human subjects also exhibit different levels of delay discounting depending on whether or not the delayed option is associated with cues, specifically verbal episodic cues or conditioned contextual cues (Dixon & Holton, 2009; Peters & Büchel,

2010). Thus, a second testable hypothesis of our data is that the impulsivity that accompanies cholinergic dysfunction in humans might be alleviated by introducing cues during important decisions.

Chapter 3. THE ROLE OF THE NUCLEUS BASALIS AND INTRA-STRUCTURAL MUSCARINIC SIGNALING IN COST-BENEFIT DECISION MAKING

3.1 INTRODUCTION

Cost-benefit decision making is a complex behavior that depends on a cortico-limbic striatal circuit comprised of the orbitofrontal cortex (OFC), basolateral amygdala (BLA), nucleus accumbens (NAc), hippocampus (HPC), and dopamine (DA) system. In Chapter 2, we showed that endogenous cholinergic signaling via muscarinic receptors is required for optimal cost-benefit decision making in rodents. While that study implicates the involvement of the cholinergic system, it doesn't establish where in the decision circuit (at which structure/s), the muscarinic cholinergic signal exerts its influence. In order to begin to understand the mechanism by which the muscarinic cholinergic system is engaged during cost-benefit decision making, we chose to investigate the effect of blocking cholinergic signaling by, first, inactivating one source of cholinergic output (nucleus basalis magnocellularis, NBM) and second by using the antagonist atropine to block muscarinic signaling within the OFC, BLA, or NAc core.

Among the many sources of Acetylcholine (ACh) in the brain, the NBM is the only one able to directly influence the majority of the cost-benefit decision circuit. Rodent NBM neurons, which are predominantly but not exclusively cholinergic, innervate the entire cortical mantle and the amygdala. By activating pre- and postsynaptic muscarinic and nicotinic ACh receptors (m/nAChRs), cholinergic NBM neurons are able to modulate target structures through many means, including altering the presynaptic release of DA (Hedrick & Waters, 2010; Mesulam, Mufson, Warner, & Levey, 1983; Picciotto et al., 2012). Early lesion and inactivation studies suggested that cholinergic NBM neurons supported learning and memory; but the use of selective

ACh immunotoxins revealed: 1) that learning and memory behaviors are actually the purview of noncholinergic NBM neurons and 2) that cholinergic NBM neurons are instead critical for sustained attentional processes, maintenance of arousal and sleep states, and interval timing (Everitt & Robbins, 1997; McGaughy, Everitt, Robbins, & Sarter, 2000; G L Wenk, 1997). With those diverse neuromodulatory and behavioral roles in mind and the knowledge that NBM degeneration is linked to conditions characterized by suboptimal decision making (Alzheimer's disease and aging), we predicted that temporary inactivation of the NBM would recapitulate the delay and probability discounting effects reported in Chapter 2.

Additionally, in order to directly locate the critical site(s) of muscarinic cholinergic signaling during delay-based (or intertemporal) decisions, we sought to establish whether injections of atropine into the OFC, BLA, or NAc core would be sufficient to induce impulsive choice like that observed followed systemic atropine administration during the uncued delay discounting task (Chapter 2). Not only will such data provide insight into whether local muscarinic cholinergic neurotransmission is required for normal cost-benefit decision making, but it also has the potential to inform our understanding of the neural processes that occur within structures of the decision circuit and guide choices during cost-benefit decision making.

3.2 Materials and Methods

3.2.1 *Subjects*

33 Long-Evans rats, weighing between 250-300g upon arrival (Simonsen Labs, Gilroy, California, USA), were housed individually in Plexiglas cages. The rats took part in 2 separate experiments: 23 rats in Experiment 1 participated in the delay and probability discounting task (No Cue group, n=7; Cue group, n=7; and probability discounting, n=9 with a subset of 4 rats also participating in ascending) and 10 rats in Experiment 2 that only participated in the uncued delay discounting task.

Every rat had unrestricted access to water and was food-restricted to 80-83% of their free-feeding body weight to ensure motivation to work for food rewards. Prior to the start of behavior training, they were exposed to 10 sugar pellets (Test Diet, 45mg) in their cages for 3 days to accustom them to the new food rewards. Behavioral training and testing took place during the light cycle of a 12h light/dark schedule (lights on 0700-1900) during 5 to 6 days per week. Procedures for this study were reviewed and accepted by the Institutional Animal Care and Use Committee at the University of Washington.

3.2.2 *Surgery*

Following a week-long acclimation period in the colony room, rats underwent cannulation surgery. All rats were anesthetized with isoflurane (4% mix with oxygen at a flowrate of 1L/min) in an induction chamber, and moved to a stereotaxic frame where isoflurane was delivered through a nosecone to maintain anesthesia throughout the surgery. After their heads were secured in place, their skulls were exposed and leveled horizontally and small burr holes were drilled for anchoring screws and 28-gauge guide cannula, which were ultimately secured in place with dental acrylic. The exact location of the bilateral cannula implants differed within and between experiments. In Experiment 1, 14 rats received cannula in the NBM (1.5mm posterior, $\pm$ 2.8 lateral from midline, 6.2 ventral from the dural surface); whereas in Experiment 2, 4 rats received cannula in both the OFC (3 mm anterior, $\pm$ 3.2 lateral from midline, 4.0 ventral from the dural surface) and BLA (2.80mm posterior, $\pm$ 5 lateral from midline, 7.5 ventral from the dural surface) and a separate group of 6 rats received cannula in the NAc core (1.5 mm anterior, $\pm$ 1.8 lateral from midline, 6.0 ventral from the dual surface). Finally, 33-gauge dummy cannula were inserted into each guide to ensure they remained unclogged throughout the week-long recovery, which included daily handling, and subsequent behavioral training.

3.2.3 *Behavioral Apparatus*

All of the behavioral testing was performed in 7 standard rat operant chambers (30.5 x 31.8 x 29.2cm; Med Associates, St. Albans, VT, USA) enclosed in sound attenuating boxes with fans that masked external noise and supplied ventilation. One wall of each chamber contained two retractable levers, one on either side of a central food cup into which sucrose pellet rewards were delivered by pellet dispenser and over which an infrared photo beam was located to record nosepokes. Cue lights and a traylight were also located above the two levers and food cup, respectively. In contrast, the opposite wall of each chamber was equipped with a single houselight and a speaker system. All experimental data were recorded by computers connected to the chambers through the MED-PC interface and software.

3.2.4 *Delay Discounting Task*

Pre-training: Prior to training on the delay discounting task, rats underwent two pre-training procedures designed to teach them to lever press for rewards and familiarize them with the task structure. In the first, they were trained to press both levers, one after the other, for pellets using a fixed-ratio (FR) 1 schedule. Once they met the criterion of 60 presses in 30 min on each lever, they continued to the retractable lever training, which was a 96 trial-long simplified version of the full delay discounting task. During the retractable lever training, the trial start was signaled by the illumination of the houselight and traylight every 50s. Rats learned to nosepoke above the food cup within 10s of the trial start in order to prevent the lights from being extinguished and the trial being counted as an omission. When they nosepoked in a timely manner, the traylight was turned off, a single lever was extended, and they again had 10s to respond before the lever was retracted and the trial recorded as omitted. However, if they responded within the window, they were immediately rewarded with a single pellet and the traylight was again turned on until the pellet

was collected (or the 10s collection period ended). Finally, the houselight was switched off for the remainder of the time left in the 50s long trial, the inter-trial interval (ITI). Trials were presented in pairs, with each pair consisting of random orders of left and right lever trials. Rats were trained to a stable baseline performance allowing no more than 1 omitted trials for 3 consecutive sessions.

Side preference test: After pre-training, rats were tested for their side bias using a 1 session task described by Haluk and Floresco (2009). In total, rats were presented with 7 trials that were structured similarly to the retractable lever training, except that both levers were inserted simultaneously and rats had to press each lever once in order to move onto the next trial. The trial began by rewarding rats for a press to either lever and then proceeded until the opposite lever was pressed and rewarded (with presses to the same lever turning the houselight off and not being rewarded). The first lever pressed (left or right) in every trial was recorded and counted, so that we could determine each rat's preferred side (the lever they chose four or more times) and assign that lever as their small reward lever to ensure that their ultimate choice preference for the large reward lever during the delay discounting task was due to the task rather than side bias.

Delay discounting task: The full delay discounting task used the same lights schedule and response windows as the retractable lever training (described above), but it differed from that task by presenting rats with a series of choices between two levers associated with different rewards and delays. Sessions were 50 min long, consisting of 5 blocks of 12 trials that included 2 forced-choice trials during which only one lever was extended (one trial per lever) and 10 free-choice trials during which both levers were extended simultaneously. Pressing one lever always resulted in the immediate delivery of a small reward (1 pellet), while pressing the other lever resulted in the

delivery of a large reward (4 pellets) after a delay that increased with each block (0, 4, 8, 16, 32s). Lever assignments were based on the side preference test and counterbalanced within groups. Two different versions of the delay discounting task were used. In the Cue version, the stimulus light above the large reward lever was illuminated during the delay, signaling the duration of the delay; whereas the cue light remained off in the No Cue version. For every session, choice behavior for each delay block (percentage of large reward choices made during completed free-choice trials) was recorded to generate delay discounting curves. The number of omitted trials (omissions) and the time it took rats to perform different aspects of the task was also recorded for every session. This included the time it took to initiate the trial with a nosepoke (nosepoke latency); time it took to choose a lever (choice latency), and the time it took to start consuming pellets once delivered (collection latency). Drug testing started as soon as individual rats reached criterion, which was stable baseline performance for 3 consecutive sessions as analyzed by ANOVA (see 'Data Analysis' section for further details of ANOVA structure). (See Figure 3.1a for task schematic)

No delay task: Following the delay discounting task, rats with cannula in their NAc core were tested in a variant of the delay discounting task designed to assess reward magnitude discrimination and satiety. In this control version of the delay discounting task, the only task feature that was changed was the delay to the large reward: instead of the delay increasing with each block, it was held constant at 0s throughout the entire session. Rats were trained until they exhibited stable performance for 3 consecutive session and then drug testing began.

3.2.5 *Probability Discounting Task*

Pre-training: Two pre-training procedures were used to train rats to lever press for rewards in a timely manner. First, they were trained under a FR-1 schedule to a criterion of 60 presses in 30

min to press both levers, one after the other, for pellets. Then, they were trained on a 90 trial, 50s task that started with the illumination of the houselight and the insertion of one of two levers into the chamber. Rats learned to press the lever within 10s because failure to do so resulted in the lever being retracted, the houselight extinguished, and the trial counted as an omission. When they pressed the lever within the window, the lever retracted and a single pellet was delivered immediately and the houselight was switched off 4s later and the ITI started for the remainder of the trial. Each trial lasted 40s with no more than two consecutive presentations of the same lever. Rats were trained to a strict criterion of 89 or 90 successful trials over 3 consecutive sessions.

Side preference task: After pre-training, rats were tested for their side bias using a 1 session task described above for the delay discounting task.

Probability discounting task: The full probability discounting task used the same light schedule and response window as the pre-training (described above) but presented rats with a series of choices between two levers associated with different reward sizes and probability of reward. Sessions were 48 minutes long, consisting of 5 blocks of 18 trials that included 8 forced-choice trials during which only one lever was extended (one trial per lever) and 10 free-choice trials during which both levers were extended simultaneously. Pressing one lever (small/certain lever) always resulted in the delivery of 1 pellet, while pressing the other lever (large/risky lever) resulted in delivery 4 pellets. Two versions of the task were used that differed in whether the probability of large reward increased or decreased over the session. In the descending version, the probability of large reward systematically decreased (100, 50, 25, 12.5%); whereas in the ascending version, the probability systematically increased (12.5, 25, 50, 100%). Lever assignments were based on the

side preference test and counterbalanced. After every session, choice behavior for each probability block (percentage of large reward choices made during completed free-choice trials) and choice latencies were recorded and probability discounting curves generated. Drug testing started as soon as individual rats reached criterion, which was stable baseline performance for 3 consecutive sessions as analyzed by ANOVA (see 'Data Analysis' section for further details of ANOVA structure). To clarify whether changes in risky choice were driven by changes in feedback sensitivity, we determined how choice of the large/risky option was influenced by receiving or not receiving reinforcement on preceding trials. Specifically, we calculated the proportion of trials during which rats chose the large/risky lever after choosing it and being rewarded on the preceding trial (win-stay ratio) and the proportion of trials during which the rat chose the small/certain lever after not receiving reinforcement for their risky choice on the preceding trial (lose-shift ratio). (See Figure 3.1b for task schematic).

3.2.6 *Drugs*

All drugs were purchased from Sigma-Aldrich (Oakville, ON, Canada) and administered through microinfusions. In Experiment 1, the two microinfusions rats received were either 0.9% saline or a mixture of $GABA_A$ agonist muscimol and $GABA_B$ agonist baclofen in saline (delay discounting: 0.0436mmol/L muscimol and 0.438 mmol/L baclofen in 0.5µL, based on (Muir, Everitt, & Robbins, 1994); probability discounting: 25ng muscimol and baclofen in 0.2µL), delivered according to a counterbalanced design and punctuated with retrain days between the two injections. In Experiment 2, rats received microinfusions of saline and three doses of the muscarinic antagonist atropine (0.1, 0.3, and 1.0 µg/side in 0.5µL) in an order counterbalanced according to a Latin square design and separated by single retrain days between infusions. Finally, for the no delay control task, drug testing took place over two days: saline was infused into the

NAc core on the first day while the most effective dose of atropine (1.0µg/side) atropine was infused on the second day.

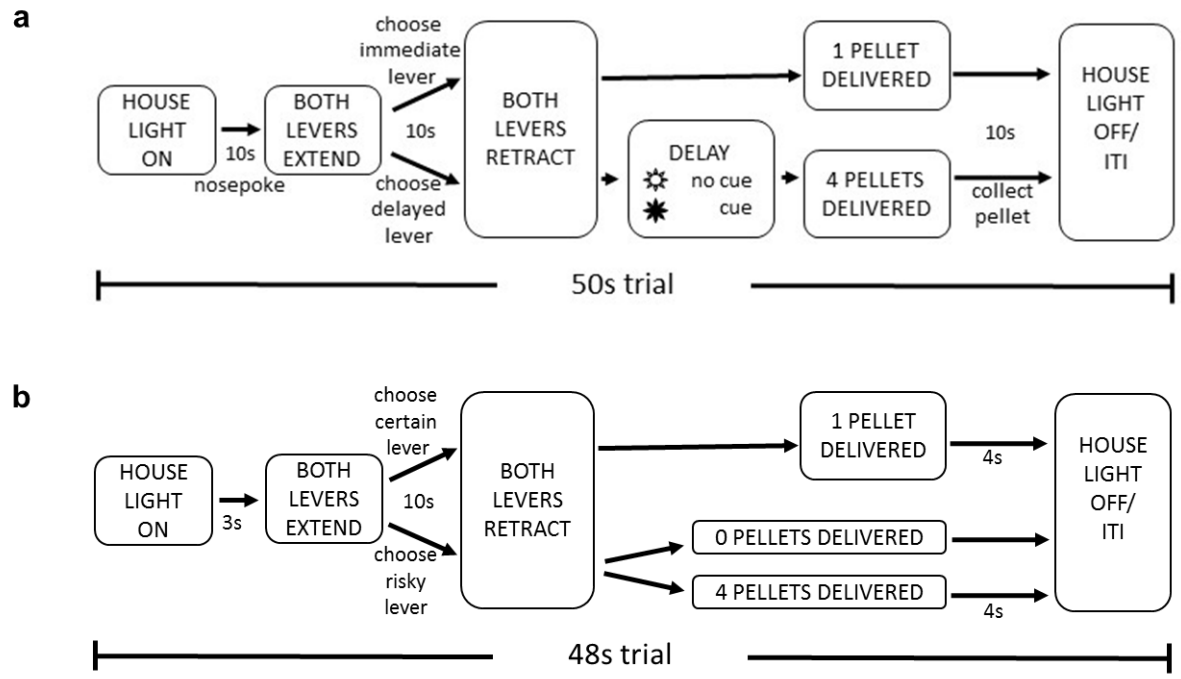


Figure 3.1**.** Detailed schematics of the delay and probability discounting tasks (a-b). The delays presented during blocks 1-5 of the delay discounting task are 0, 4, 8, 16, 32 s; and the probabilities of large reward delivery in blocks 1-4 of the descending probability discounting task and ascending probability discounting task were 100, 50, 25, 12.5% and 12.5, 25, 50, 100%, respectively.

3.2.7 *Microinfusion Procedure*

On drug testing days, rats were gently restrained, their dummies removed, and two 33-gauge stainless steel injectors (Plastics One, Roanoke, VA, USA), which extended 1mm beyond the guide cannula, were inserted into the guide cannula and the volume of drug corresponding with the target site was infused at a rate of 0.25 µL/ min using a dual channel infusion pump (KD Scientific) with 10µL syringes (Hamilton) connected to the cannula through PE20 polyethylene tubing. Following infusions, the injector was left in place for a minute to allow the drug to diffuse in the local vicinity

before the injection cannula were removed, the dummies replaced, and the rats returned to their cages for 30 minutes before the day's behavioral session. One or two days before the first microinfusion was performed, a mock infusion was conducted in order to control for any initial mechanical damage done by the injector.

3.2.8 *Data Analysis*

All statistical analyses and curve fitting were conducted in Graph Pad. Stable baseline performance in discounting tasks was determined using two-way repeated-measures ANOVA (day x delay/ probability) and was defined by 1) a main effect of delay and 2) the absence of a main effect or interaction for day. For all experiments, reported baselines were calculated by averaging across the 3 stable days assessed for criterion. Analyses of choice behavior and response latencies for discounting tasks was also conducted using two-way repeated-measures ANOVA (NBM inactivation/ dose x delay/probability) with Bonferroni post hoc tests used when warranted. In contrast, we used a two-tailed paired t-test to analyze the effects of NBM inactivation on the feedback sensitivity (win-stay and lose-shift) during the probability discounting task. In all cases, p values <0.05 were considered significant. The main effect of delay and probability on choice was always significant for discounting tasks and saline injections did not significantly changed behavior when compared to baseline in any of the experiments, so neither will be reported further.

3.2.9 *Histology*

Rats were given an overdose of sodium pentobarbital and transcardially perfused with 0.9% saline and 10% formalin. Their brains were removed and postfixed in 10% formalin-30% sucrose solution at 4°C for 72h. Next, we froze the brains, cut them into 40µm coronal sections with a freezing microtome, mounted the sections on gelatin-coated slides, and stained them with cresyl

violet. Finally, we confirmed placement of the injector tips using light microscopy and the standardized sections of the rat brain in Paxinos and Watson (1998). Only behavioral data from rats with properly localized bilateral cannula were included in the data analysis. Depictions of the location of injector tips in the four target sites is provided in Figure 3.2.

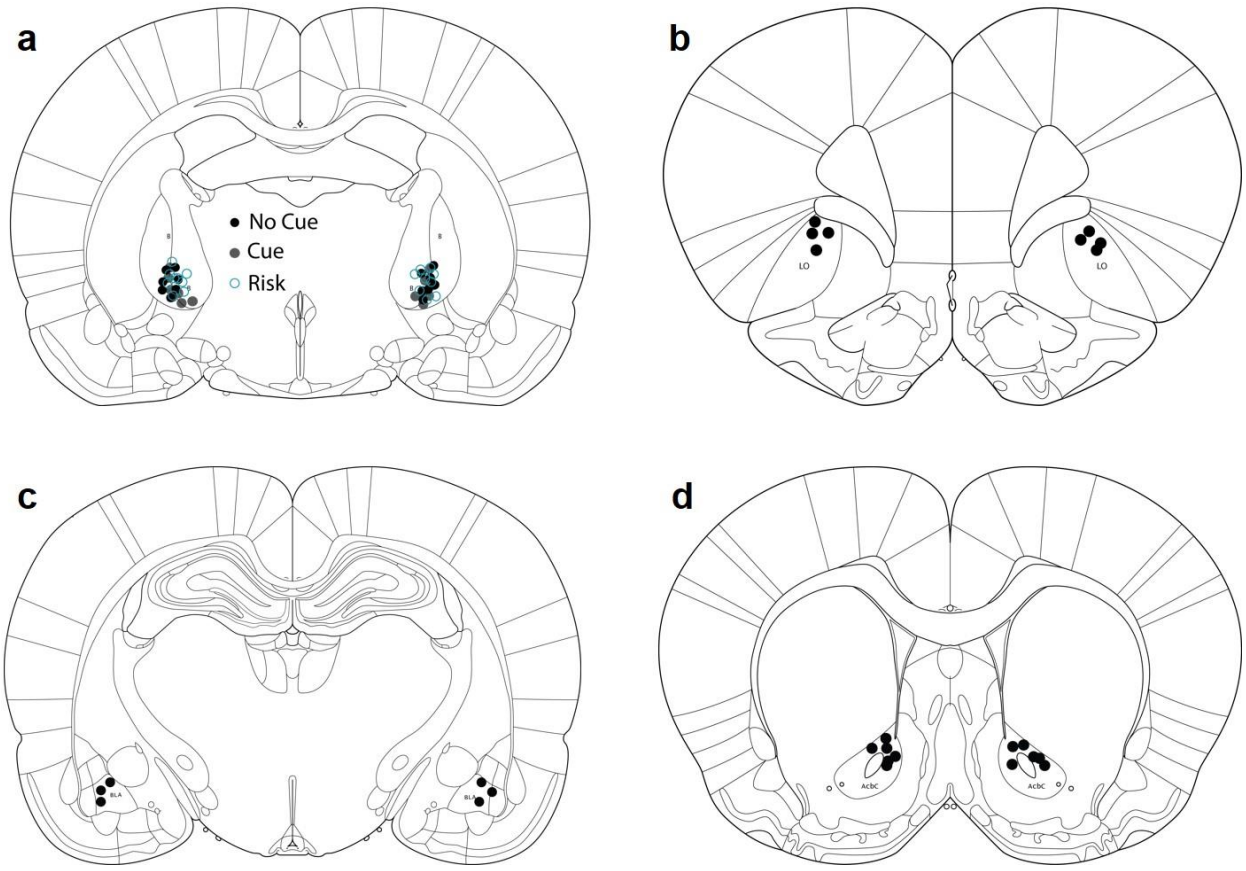


Figure 3.2**.** Location of the injector tips in the NBM (a); the OFC (b); the BLA (c); and NAc core (d).

3.3 RESULTS:

3.3.1 *Experiment 1*

3.3.1.1 NBM inactivation during delay discounting

A total of 14 rats were trained in the delay discounting task. Even though the 7 rats in the No Cue group (28.9 $\pm$ 3.66 days) and 7 rats in the Cue group (29.7 $\pm$ 2.5 days) took a similar number of

days to reach stable baseline, the Cue group displayed a lower basal level of large reward choices than the No Cue group. A non-repeated measure ANOVA showed that there was no group x delay interaction ($F_{4, 30}$=0.7396, not significant [ns]) but that there was a main effect of group ($F_{1, 30}$=7.519, $p<0.05$). Microinfusions of muscimol and baclofen had no main effect on choice in either version of the delay discounting task (No Cue group NBM inactivation: $F_{1, 30}$=2.480, ns; Cue group NBM inactivation: $F_{1,30}$= 0.3046, ns; Cue group NBM inactivation x delay: $F_{4,30}$ = 0.2266, ns; Figure 3.3 3); but there was a significant NBM inactivation by delay interaction in the No Cue group ($F_{4,30}$ = 3.398, $p<0.05$) that post hoc analysis revealed was driven by a significant decrease in the 8s block ($p<0.01$). Animals in both groups also showed no increase in omissions or latencies to collect large and small rewards (No Cue group NBM inactivation: F's <0.3081, ns; Cue group NBM inactivation: F's <0.1630, ns). However, the Cue group did display significantly lengthened nosepoke latencies (NBM inactivation: $F_{1, 30}$=14.68, $p<0.001$; NBM inactivation x delay: $F_{4, 30}$=0.2029, ns), even though that measure of response latency was a left unchanged in the No Cue group (NBM inactivation: $F_{1, 30=}$ 0.005960, ns; NBM inactivation x delay: $F_{4, 30}$=0.1539, ns).

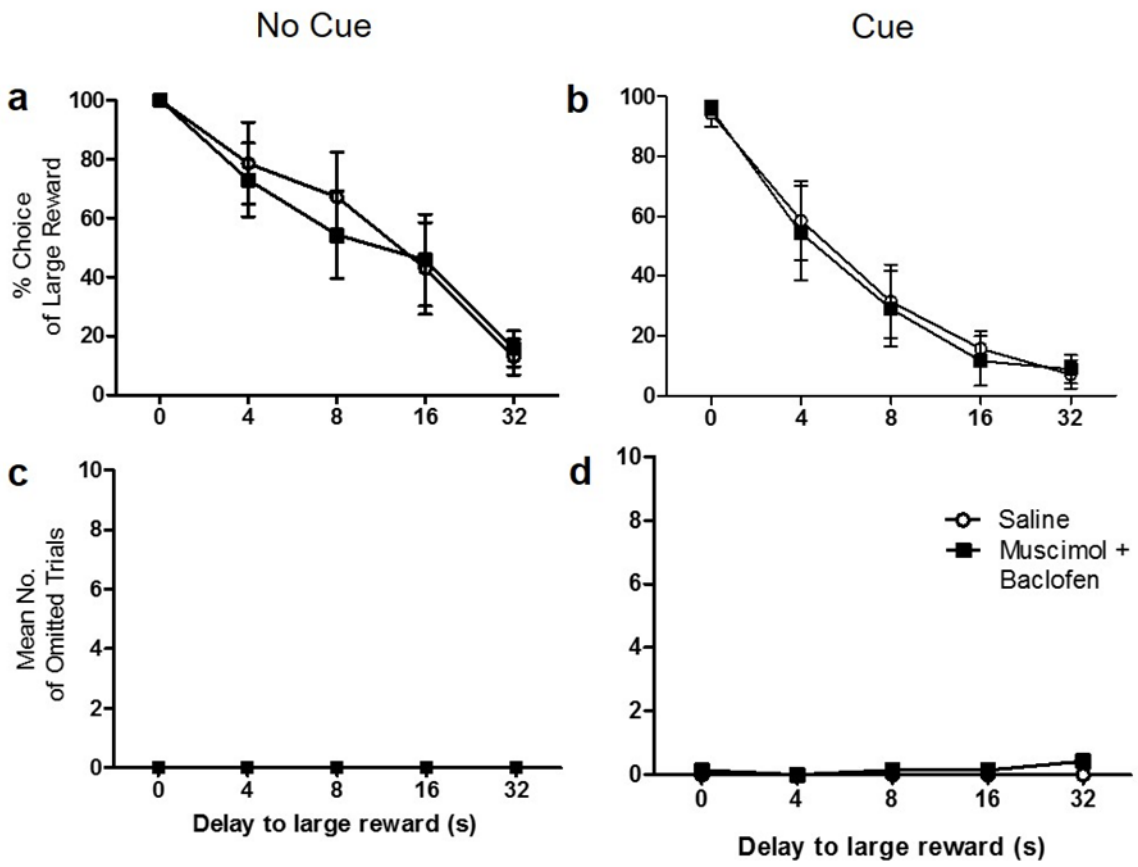


Figure 3.3. Effect of NBM inactivation on choice behavior during the two versions of the delay discounting task. NBM inactivation fails to alter choice and the number of omissions in both the No Cue (a, c) and Cue (b, d) groups. Data shown are mean ± SEM.

3.3.1.2 NBM inactivation during probability discounting

First, we trained 9 rats in a descending probability discounting task for 30.3 ± 2.5 days, at which point we found that microinfusions of muscimol and baclofen in the NBM decreased choice of the large/risky reward (NBM inactivation: $F_{1,32}$=16.20, p< 0.0005; NBM inactivation x probability: $F_{3,32}$= 1.108, ns; Figure 3.4a). While NBM inactivation didn't affect the choice of the large reward when guaranteed in the 100% block, post hoc analysis revealed that the shift in choice was driven by changes during the 50% and 25% probability blocks (p<0.05). The change was also accompanied by an increase in the number of omissions (NBM inactivation: $F_{1, 32}$= 6.149, p<0.05;

NBM inactivation x probability: $F_{3, 32}$= 0.1348, ns; Figure 3.4c) and a slowing of the free-choice trial lever press latencies (NBM inactivation: $F_{1, 32}$=6.564, $p<0.05$; NBM inactivation probability: $F_{3, 32}$= 0.09397, ns). The forced-choice lever press latencies, however, remained unaffected (NBM inactivation: F=2.742, ns; NBM inactivation probability: $F_{3, 32}$= 01910, ns). In order to determine whether the reduction in risky choice induced by NBM inactivation was accompanied by a significant change in feedback sensitivity, we calculated win-stay and lose-shift ratios. Lose-shift (t_8= 2.575, $p<0.05$), but not win-stay ratios (t_8= 1.345, ns), were increased by NBM inactivation, indicating that the risk-averse choices were caused by an increased sensitivity to negative feedback, or loss (Figure 3.4).

Given that some neural manipulations have caused differential effects on probability discounting that depended on whether the probability of the large, risky reward increased or decreased over the session (St. Onge et al., 2010; St. Onge & Floresco, 2010), we decided to train a subset of the rats in the ascending version of the task. Only 4 rats successful learned the ascending probability discounting task, and it took them 36.3 $\pm$ 4.6 days to reach baseline. In contrast to the result observed during the descending version, NBM inactivation failed to significantly change their choice. But, visual inspection of the data indicates that there was a trend toward a reduction similar to that seen in the descending version (NBM inactivation: $F_{1, 12}$= 1.520, ns; NBM inactivation x probability: $F_{3, 12}$= 0.4427, ns; Figure 3.4b). Rats in the ascending version also exhibited no significant change to the number of omitted trials ($F_{1,12}$= 3.103, ns; $F_{3,12}$=0.2991, ns; Figure 3.4d) nor measures of feedback sensitivity (lose-shift: t_3= 0.3861, ns; win-stay: t_3= 0.5357, ns; Figure 3.4f). Additional rats will need to be run to assess significance. But, if their data corroborates the trend, it will lend additional support to the conclusion that NBM inactivation leads to risk aversion irrespective of how the probabilities change during a session.

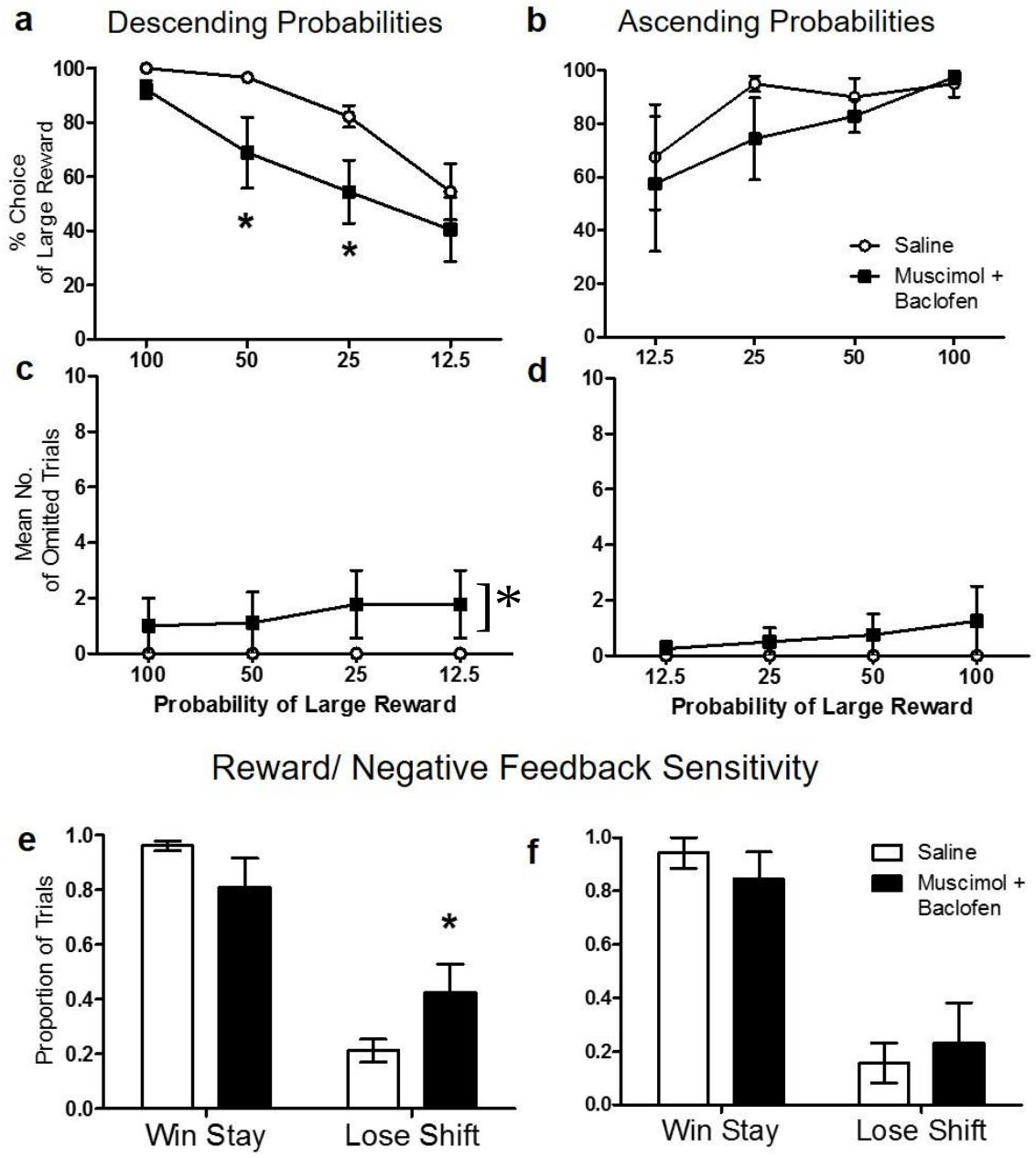


Figure 3.4**.** Effect of NBM inactivation on choice behavior during the two versions of the probability discounting task. In the descending probability discounting task, NBM inactivation induced risk aversion (a), increased the number of omitted trials (c), and increased sensitivity to loss (e). On the other hand, NBM inactivation in the ascending probability task failed to change choice (b), omissions (d), and feedback sensitivity (f). Data shown are mean $\pm$ SEM.* $p<0.05$.

3.3.2 *Experiment 2*

3.3.2.1 Intra-OFC or intra-BLA infusion of atropine during delay discounting

A total of 4 rats with cannula in both the OFC and BLA were trained in the uncued delay discounting task. They were first trained for 30.0 $\pm$ 5.4 days prior to OFC infusions, and then for an additional 8.3 $\pm$ 2.3 days to reestablish baseline prior to BLA infusions. While all 4 OFC cannula were correctly placed (Figure 3.2b), only 3 rats had cannula in the BLA (Figure 3.2c). Microinfusions of atropine in neither structure changed choice of the large, delayed reward (OFC dose: $F_{3, 45}$=1.094, ns; OFC doses delay: $F_{12, 45}$=0.9543, ns; BLA dose: $F_{3, 30}$=2.599, ns; BLA dose x delay: $F_{12, 30}$=1.037, ns; Figure 3.5a, b). Atropine infusions also failed to increase the number of omitted trials. However, there were effects of dose on response latencies following intra- OFC and intra-BLA infusions of atropine. When injected in the OFC, atropine dose interacted with delay to increase nosepoke latency in the early delay blocks and decrease nosepoke latency in the later delay blocks (OFC dose: $F_{3, 45}$= 0.5663, ns; OFC dose x delay: $F_{12, 45}$=2.724, $p<0.05$). Alternatively, when infused in the BLA, atropine decreased nosepoke latencies (BLA dose: $F_{3, 30}$= 4.438, $p<0.05$; BLA dose x delay: $F_{12, 30}$= 0.8198, ns), with the strongest effect at the highest dose (saline vs 1.0µg/side: $F_{1, 10}$=1.75, $p<0.05$). Additional rats should be run to confirm these findings; but assuming they stand, this data suggests that blockade of local muscarinic signaling in neither the OFC nor the BLA alone is sufficient to recapitulate the effect of blocking muscarinic signaling with atropine throughout the entire brain.

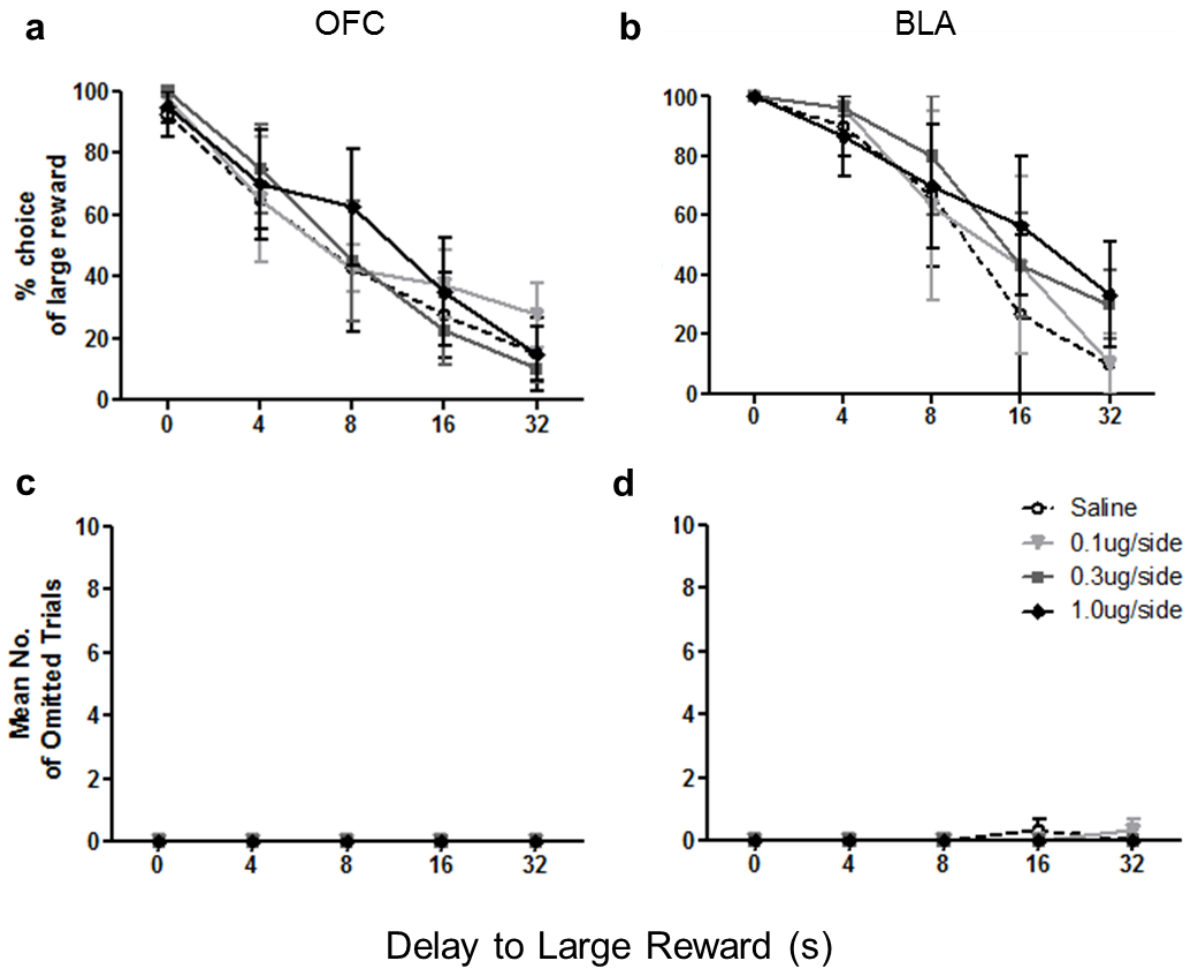


Figure 3.5. Effect of intra- OFC and intra-BLA infusion of atropine on choice behavior during the No Cue version of delay discounting task. Neither choice nor omissions were changed by atropine infusions into the OFC (a, c) or BLA (b, d). Data shown are mean ± SEM.

3.3.2.2 Intra-NAc core infusion of atropine during delay discounting

In contrast to the intra-OFC and intra-BLA infusions, there was a significant main effect of dose when atropine was infused into the NAc of 6 rats trained in the uncued version of the delay discounting task (trained for 28.2 ± 1.9 days; dose: $F_{3, 75}$= 6.839, $p<0.0005$, dose x delay: $F_{12, 75}$= 1.273, ns; Figure 3.6a). Atropine infusions increased the choice of the large, delayed reward in blocks 2-5 without altering choice of the immediately available large reward in block 1. When the impact of each dose was investigated separately, there was a significant effect for both the middle

and highest doses (saline vs. 0.3µg/side: $F_{1, 25}$= 9.323, $p<0.05$; saline vs. 1.0µg/side: $F_{1,25}$= 13.78, $p< 0.005$), with the greatest shifts in choice occurring in the 8 and 16s blocks for the middle dose and the 8s block for the highest dose (saline vs. 0.3µg/side: $p<0.05$; saline vs. 1.0µg/side: $p< 0.01$). Despite the robust effect on choice, no effect was observed for the response latencies nor the number of omissions (Figure 6c) following atropine infusion, suggesting the change in choice couldn't be attributed to changes in motor, attention, and/or motivation deficits just by analyzing the task performance (F's<2.33, ns).

A priori, we decided to perform the no delay control because there are discrepancies about the satiety effects of muscarinic blockade within the NAc. While the infusion of the muscarinic antagonist scopolamine into the NAc has been shown to decrease pressing for food, injection of either AF64A, a cholinergic cell toxin, or the M1 receptor antagonist pirenzepine in the NAc increased bar pressing for food on the first day after the lesion, which is also consistent with the oft-reported increase in NAc cholinergic tone that accompanies satiety (Hoebel, Avena, & Rada, 2007). Thus, even though we observed increased preference for the large reward in blocks 2-5, we still assessed the impact of atropine on the performance of rats in a control task in which the delays to the large reward were fixed at 0s throughout all 5 blocks of the session. All 6 rats were trained for 4.3 $\pm$ 0.2 days, and at baseline they exhibited near 100% preference for the large reward over the small reward. Infusions of the largest atropine dose (1.0µg/side) didn't reduce their preference for the large reward in any block of the session (1.0µg/side: $F_{1, 25}$ =3.456, ns; 1.0µg/side x delay: $F_{4, 25}$= 0.5901, ns; Figure 3.6b) nor did it increase the number of omitted trials (1.0µg/side: $F_{1, 25}$ =1.00 ns; 1.0µg/side x delay: $F_{4, 25}$= 1.00, ns; Figure 6d). As a result, we didn't find any evidence of increased satiety nor were we able to attribute the decreased discounting to an inability to

perceive the difference between the two rewards (Cardinal, Pennicott, Lakmali, Robbins, & Everitt, 2001; Ho et al., 1999).

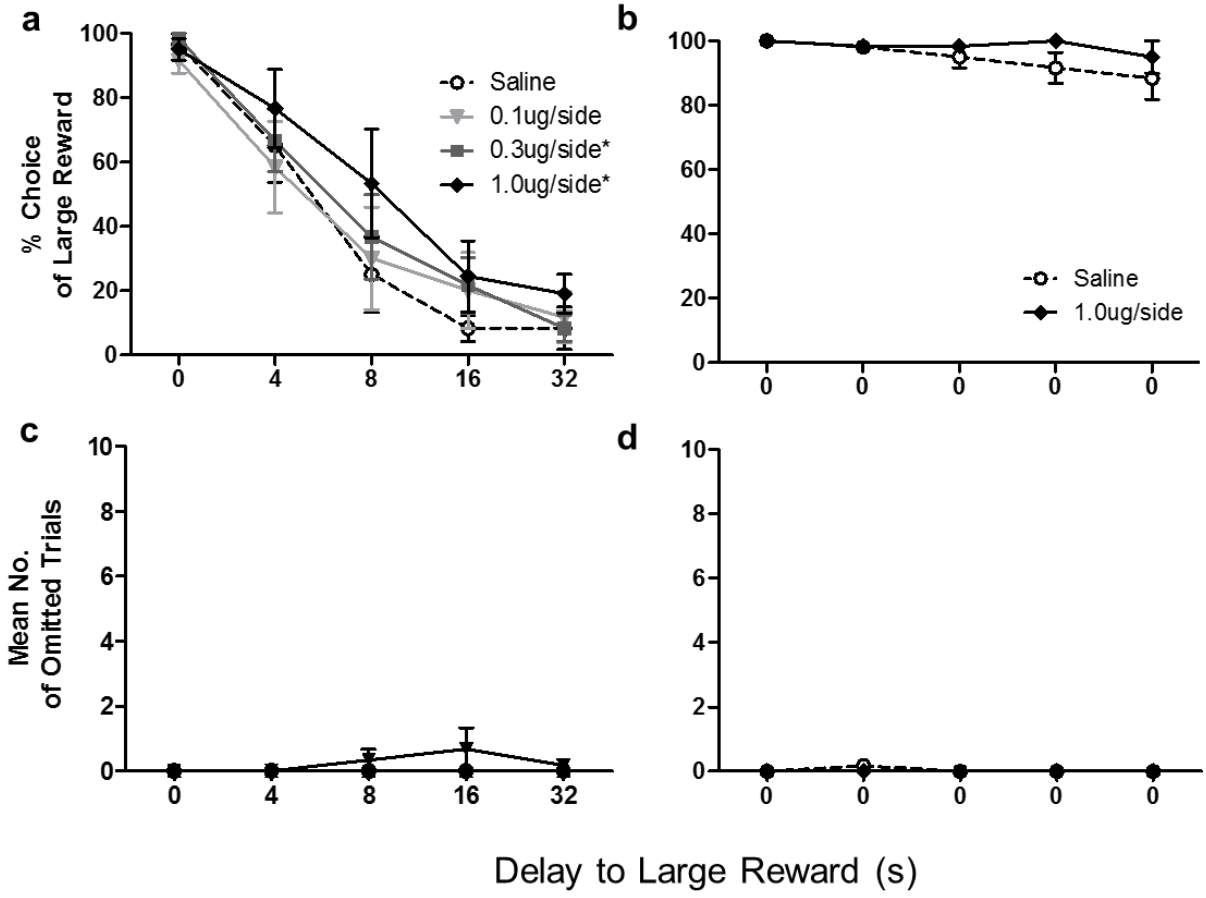


Figure 3.6**.** Effect of intra- NAc core infusion of atropine on choice behavior during the No Cue version of the delay discounting task. Atropine infusions increased choice of the large reward (a) without changing reward discrimination (b) or increasing the number of omitted trials (c, d). Data shown are mean ± SEM. * $p<0.05$.

3.4 DISCUSSION

The present data reveal previously uncharacterized roles for the NBM and muscarinic cholinergic signaling in the mediation of cost-benefit decision making. Temporary inactivation of the NBM increased risk aversion in the probability discounting task, but failed to impact choice during either

version of the delay discounting task. Further, local blockade of muscarinic receptors in the NAc core, but not the OFC or the BLA, increased choice of the delayed reward in the uncued version of the delay discounting task. Together, these data provide novel insight into where and how forebrain acetylcholine contributes to valuation of costly options and inspire numerous future hypotheses and investigations.

3.4.1 *The Nucleus Basalis promotes risky choices*

When a mixture of muscimol and baclofen was infused into the NBM, rats exhibited decreased choice of the large, risky reward that was restricted to blocks in which delivery of the large reward was probabilistic. The increased risk aversion was accompanied by a modest increase in the number of omitted trials, with rats going from not making any omissions to omitting about 1.2 trials per block. Since that's well within the number of omissions others have reported following saline injections, the change is likely too small to indicate that deficits were responsible for the shift in choice (Cardinal et al., 2000; Mendez et al., 2012). However, the lengthening of lever press latency during the free-choice trials was similar to that which has been reported previously for the NBM and might reflect an attentional deficit that impacted valuation (Dekker, Connor, & Thal, 1991). Additionally, we found that the effect was driven by a selective enhancement of the lose-shift behavior: inactivating the NBM rendered rats more likely to shift to the small, certain lever following an unrewarded risky lever choice. While our data is notable because it is the first to demonstrate that the NBM plays a critical role in promoting risky choices, it also raises many intriguing questions about the neural mechanism of NBM involvement.

The first question to consider is whether the two subpopulations of NBM neurons work independently or in concert during risk-based choices. Even though cholinergic and noncholinergic neurons are intermixed throughout the NBM and send overlapping projections to

regions and layers of the cortex and amygdala, there's an abundance of evidence to suggest that they have dissociable roles during complex behaviors (Chandler & Waterhouse, 2012; Gritti et al., 2006; Zaborszky et al., 2015). Specifically, cholinergic neurons are critical during attentional tasks, especially those requiring greater attentional effort; while noncholinergic NBM neurons are critical during learning and memory tasks and some attentional tasks (Everitt & Robbins, 1997; Hasselmo & Sarter, 2011; Hasselmo, 2006; McGaughy et al., 2000; Wenk, 1997). Dissimilarities in how the two subpopulations process information have also been revealed through functional characterization of their signaling properties during cue detection tasks. During such tasks, noncholinergic NBM neurons responded to reward and punishment- predicting cues with short latency bursts, while cholinergic NBM neurons exhibited phasic responses to the primary rewards and punishments they encountered (Hangya, Ranade, Lorenc, & Kepecs, 2015; Lin & Nicolelis, 2008). The noncholinergic signal was interpreted as a 'motivational salience' signal a because the strength of the bursts scaled with perceptual certainty, i.e. stronger burst to tones that were more easily distinguished and successfully acted upon, and decision speed (Avila & Lin, 2014; Lin & Nicolelis, 2008). In contrast, the cholinergic signal was described as a 'reinforcement surprise' signal because the phasic cholinergic responses scaled with unexpected reinforcement that stemmed from either perceptual uncertainty or valence surprise (Hangya et al., 2015). The latter finding also lent support to the theoretical proposal by Yu and Dayan (2005) that ACh broadcasts a perceptual uncertainty signal that boosts cue learning and attention. Taken together, the literature supports the hypothesis that both subpopulation are responsible for supporting risky choices and also indicates that further study might reveal a common mechanism in the NBM by which perceptual uncertainty and economic risk are encoded.

Another question worthy of consideration is why NBM inactivation biased choice in the opposite direction from systemic administration of the muscarinic antagonist atropine, as reported in Chapter 2? We know that NBM inactivation can only reduce cholinergic projections to the cortex and amygdala, and thus, spares muscarinic cholinergic signaling in other sites. Since the critical sites of muscarinic signaling that normally *discourage risky* choices are still unknown, it's possible that NBM inactivation disrupts a separate site that is instead responsible for *promoting* risky choices. We predict that site is the mPFC and more specifically nicotinic signaling in the mPFC based on the following two pieces of evidence. First, stimulation of nAChRs in the mPFC increased DA release, which suggests that reducing NBM activation of mPFC nAChRs would decrease DA release (Drew, Derbez, & Werling, 2000). Meanwhile, a second study showed that intra-mPFC infusion of the D1 receptor antagonist induced risk aversion and increased lose-shift behavior (Drew et al., 2000; St. Onge et al., 2011). Thus, our full hypothesis is that NBM inactivation leads to reduced nicotinic signaling in the mPFC that then diminishes D1 activation and causes increased risk aversion and lose-shift behavior.

Unlike the effects observed for the probability discounting task, no significant shift in preference was observed when a mixture of muscimol and baclofen was infused into the NBM during two versions of the delay discounting task. A closer look at the data and protocols suggests that the absence of an effect following NBM inactivation might stem from the drug dose used. The dose of the drug mixture infused in the NBM of rats performing the delay discounting task was less concentrated than the drug mixture infused in the NBM of rats performing the probability discounting task, which increased omissions, decreased choice latency, and was shown to be effective in another experiment performed in our lab (Baker, Oh, Kidder, & Mizumori, 2015). As such, the absence of an effect in both versions of the delay discounting task is more likely due to

the drug dose and not reflective of the fact that the NBM output is truly unnecessary for intertemporal choices. Therefore, it is still necessary to replicate the delay discounting experiments with the effective dose in order to see whether the NBM contributes to more than one form of cost-benefit decision making.

3.4.2 *Muscarinic signaling in the Nucleus Accumbens core discourages choice of delayed reward*

In order to isolate the critical site(s) of muscarinic cholinergic signaling that encourages delayed choices when the delay-to-reinforcement is uncued, three doses of the muscarinic antagonist atropine were infused into the OFC, BLA, or NAc core. The three sites were chosen because inactivation of each has previously been shown to increase delay discounting (Cardinal et al., 2001; Mobini et al., 2002; Winstanley, 2004). While we failed to observe any change to choice following atropine infusion into the OFC or BLA, we observed increased choice of the large reward when atropine was infused into the NAc core. The shift in preference toward the large reward, observed across all delay blocks, was not accompanied by changes to other aspects of task performance, suggesting that blocking muscarinic cholinergic signaling in the NAc core spared attention and motor performance while selectively increasing valuation of the delayed option.

Since there is some confusion in the literature regarding the role of muscarinic cholinergic signaling in the NAc during feeding and satiety, a control task was performed to establish the impact of intra-NAc core infusion of atropine on reward discriminability and satiety during a variant of the delay discounting task in which both rewards are available immediately. The confusion stems from the fact that even though the selective M1 antagonist pirenzepine blocks the endogenous NAc ACh satiety effect and increases feeding, scopolamine, a broad acting muscarinic antagonist more similar to atropine, actually decreases feeding behavior when injected into the

NAc (Hoebel et al., 2007). Given that preference for the large reward remained throughout the session following infusion of 1.0µg/side atropine into the NAc core, it seems atropine impacted feeding behavior differently from scopolamine by neither increasing satiety nor disrupting reward discrimination. Thus, the increased choice of the large reward reveals that endogenous muscarinic signaling in the NAc is responsible for mitigating pursuit of delayed rewards and raises the possibility that it does so by tempering motivation for the large reward via M1 signaling.

The fact that the effect of blocking intra-NAc muscarinic signaling was opposite from the effect of blocking systemic muscarinic signaling with atropine, suggests that the two effects are driven by different mechanisms. Blocking muscarinic signaling throughout the entire brain had the net effect of shifting choice away from the delayed reward and indicated that endogenous muscarinic signaling normally promotes choice of delayed rewards via an interaction with the DA system. Specifically, it was suspected that the behavioral effect of systemic muscarinic blockade was driven by decreasing the overall level of DA receptor activation. Indeed, because local blockade of muscarinic signaling in the NAc can also decrease DA release, it was expected that intra-NAc atropine infusions would recapitulate the systemic effect (Zhou et al., 2003). However, in light of the fact that others have shown that directly modifying DA signaling in the NAc core alone, via 6-hydroxydopamine or D1/D2 agonists and antagonists, doesn't change choice and thus indicates that DA signaling in the NAc is actually not required for normal delay discounting, our data instead reveals a novel, DA-independent mechanism by which delayed choice can be discourage d through endogenous muscarinic signaling in the NAc core (Winstanley et al., 2005; Yates, 2014). To gain a more complete idea of the neural mechanism responsible for the choice bias, it will be important to determine whether the blockade of muscarinic signaling in the NAc core altered choice by an indirect mean, modifying the glutamatergic inputs from other regions

involved in intertemporal choice, or by a direct mean- modulating the activity level of NAc medium spiny neurons.

3.5 Conclusion

Together, our data defines the involvement of the NBM and intra-structural muscarinic cholinergic signaling in cost-benefit decision making. Experiment 1 demonstrates for the first time that the NBM is required for risk-based decisions. While our data identifies two cognitive roles for the NBM, attention and promotion of risky choices by minimizing the influence of loss, it is insufficient to identify specific neural mechanisms by which the NBM is engaged. However, when considered along with other findings in the literature, there is evidence to support three mechanistic hypotheses. The first hypothesis is that both cholinergic and noncholinergic subpopulations of the NBM play a role in risk-based choices; the second is that cholinergic NBM neurons contribute via activation of nicotinic, rather than muscarinic, receptors in the mPFC; and the third hypothesis is that cholinergic and noncholinergic NBM neurons have the capacity to code for economic or value-based uncertainty in addition to perceptual uncertainty. Interestingly, the data can also be interpreted for translational relevance. Although correlations have separately linked age/Alzheimer's disease to NBM hypofunction and risk aversion, NBM hypofunction has never been directly linked to risk aversion (Deakin, Aitken, Robbins, & Sahakian, 2004; Doyon et al., 2013; Smith & Booze, 1995; Tryon, King, Long, Rapp, & Mizumori, 2015; Wenk, 1997; Wenk, 1995; Zhang, Ji, & Mei, 2000). Thus, our data represents the first direct, causal link between the two and could lead to a possible treatment for age–related decision deficits. In addition to discovering a novel role for the NBM in risk-based decision making, in Experiment 2 we clarified the role of muscarinic cholinergic signaling in the brain. We found that muscarinic cholinergic signaling in the NAc core, not the OFC or BLA, is required to discourage choice of delayed

rewards via a DA-independent process that likely relies upon M1 receptors. Lastly, while we dismissed the veracity of the null effects observed for NBM inactivation, we recognize that it's possible the null effect is real and might actually indicate, along with our OFC and BLA data, that cholinergic activation of muscarinic receptors in the OFC and BLA from the NBM is not necessary for normal delay discounting when the delay-to-reinforcement is uncued. While our data is notable for presenting new roles for the NBM and muscarinic signaling in cost-benefit decision making, it is noteworthy for pinpointing number of novel theoretical questions, some of which we describes, that will likely push the field forward in interesting directions.

Chapter 4. GENERAL DISCUSSION

For my thesis, I choose to investigate the cognitive and neural contribution of muscarinic cholinergic signaling to cost-benefit decision making. My choice of thesis topic was based on three facts. First, that diseases associated with cholinergic dysfunction are accompanied by suboptimal decisions; second, that the cholinergic system is anatomically and functionally positioned to modulate the entire cortico-limbic striatal circuit involved in decision making; and finally, that muscarinic receptors were implicated in delay- and risk- based decisions. To achieve my goal, I used systemic and intra-structural injections of atropine or a muscimol/baclofen cocktail to determine the effects of muscarinic receptor blockade and/or NBM inactivation on delay and probability discounting. Although I successfully identified several novel neural contributions of muscarinic cholinergic signaling and the NBM to delay- and risk-based decision making, I was not able to reveal many cognitive contributions. Yet, I still recommend that other researchers mimic my approach and investigate cost-benefit decisions with an eye toward both neural and cognitive processes. In this concluding chapter, I will summarize what I learned from my data as well as present the testable hypotheses and avenues of future study inspired by my research.

4.1 IMMEDIATE CONCLUSIONS

4.1.1 *Brain wide muscarinic cholinergic signaling promotes choice of delayed rewards when the delay-to-reinforcement is uncued and discourages choice of risky rewards.*

In chapter 2, I re-examined the effects of atropine on delay and probability discounting, paying special attention to the implications of non-choice task variables. Blockade of muscarinic acetylcholine receptors by atropine induced suboptimal choices (impulsive and risky) in delay and probability discounting tasks. Additionally, atropine's effect on delay discounting disappeared

when the delay-to-reinforcement was cued and was neither attributable to timing nor working memory deficits. *Together, our data suggest that muscarinic cholinergic signaling mediates two forms of cost-benefit decision making and is selectively engaged when decisions require valuation of reward options whose costs are not externally signified.*

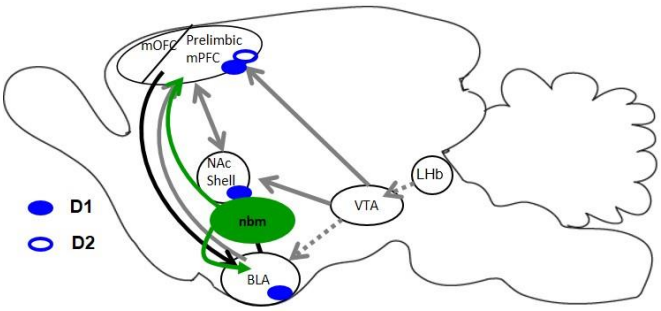
4.1.2 *The Nucleus basalis promotes risky choices and mitigates sensitivity to loss, but might not contribute to choice of delayed rewards.*

In chapter 3, I assessed whether the NBM was required for delay and probability discounting by inactivating it with a muscimol and baclofen mixture. While NBM inactivation failed to shift choice in either version of the delay discounting task, it induced risk aversion and increased the sensitivity to loss in the probability discounting task. I suspect that the absence of an effect in both versions of the delay discounting task is due to the drug dose and not reflective of the fact that the NBM output is truly unnecessary for intertemporal choices. *Further, in light of the contradiction with the atropine result, our data suggests that nicotinic signaling, rather than muscarinic cholinergic signaling, in the prefrontal cortex might be responsible for promoting risky choices.* (Summarized in Figure 4.1).

4.1.3 *Local muscarinic cholinergic signaling in the nucleus accumbens core discourages choice of delayed rewards.*

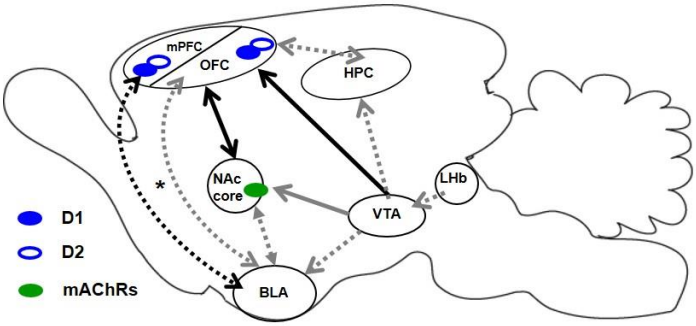
Also in Chapter 3, I determined whether infusion of atropine into the OFC, BLA, or NAc core would be sufficient to induce impulsive choice during the uncued delay discounting task. I found that muscarinic cholinergic signaling in the NAc core, not the OFC or BLA, is required to discourage choice of delayed rewards via a DA-independent process that likely relies upon M1 receptors. *Despite the fact that intra-NAc infusion of atropine didn't shift choice in the same direction as the systemic injection, the NAc should still be regarded as a critical site of*

muscarinic cholinergic signaling during intertemporal decisions; one that discourages rather than promotes choice of delayed rewards. (Summarized in Figure 4.2).



Structure/ Connection	Probability Discounting		DA manipulation		Probability Discounting	
	D	A			D	A
mPFC	↑	↓	Flupenthixol	--	↓	↓
mOFC	↓	↓	Amphetamine	+	↑	↓
NAc shell	↓		D1 antagonist in mPFC	--	↓	
BLA	↓		D2 antagonist in mPFC	--	↑	
BLA→NAc	↓		D1 antagonist in NAc	--	↓	
mPFC → BLA	↑		D1 antagonist in BLA	--	↓	↓
LHb	Indifference		DA lesion in mPFC or NAc	--	NE	
ACh Manipulation			Atropine	--	↑	
			NBM		↓	↓

Figure 4.1**.** Updated circuit representation (Left) and table (Right) indicating the structures and receptors required for normal probability discounting performance. Based on my thesis research, I can add the NBM to the circuit diagram and the NBM and atropine to the table (indicated in green).



Structure/ Connection	Delay Discounting	DA manipulation		Delay Discounting
OFC	↓ ↑ NE	Flupenthixol / D1 antagonist	--	↓
mPFC	Flattens curve	Amphetamine	+	↑
BLA	↓	DA lesion in OFC	--	↓
Nac core	↓	D1/D2 antagonist in OFC	--	↓
HPC	↓	D1/D2 antagonist in mPFC	--	↓
OFC → NAc	↓	DA lesion in NAc	--	NE
LHb	Indifference	D1/D2 agonist/ antagonist in NAc	+ --	NE
ACh Manipulation		Atropine	--	↓ NE
		Oxotremorine	+	↑
		mAChR antagonist in NAc core	--	

Figure 4.2**.** Updated circuit representation (Left) and table (Right) indicating the structures and receptors required for normal delay discounting performance. Based on my thesis research, I can add NAc mAChRs to the circuit diagram and whole brain atropine and intra-NAc core atropine to the table (indicated in green).

4.2 TESTABLE HYPOTHESES

4.2.1 Hypothesis: The muscarinic cholinergic system promotes choice of delayed rewards through interactions with the DA systems via the D1 receptor.

Experiment: Co-administer atropine and the D1 agonist SKF 3883 and observe whether the atropine induced impulsivity is diminished by the D1 agonist.

4.2.2 Hypothesis: Introducing temporal or contextual cues to human patients with cholinergic dysfunction will decrease their delay discounting.

Experiment: Use the same protocols previously used to associate delayed options with verbal episodic cues or conditioned contextual cues (Dixon & Holton, 2009; Peters & Büchel, 2010) and assess whether they improve discounting in patients with cholinergic dysfunction.

4.2.3 Hypothesis: The NBM is required for both versions of the delay discounting task.

Experiment: Inactivate the NBM using the effective dose of the muscimol/baclofen mixture during both versions of the delay discounting task.

4.2.4 Hypothesis: Cholinergic and noncholinergic NBM output is responsible for promoting risky choices.

Experiment: Use optogenetic or DREADD technologies to selectively inhibit the activity of cholinergic or noncholinergic NBM neurons during the probability discounting task.

4.2.5 Hypothesis: NBM output promotes risky choice via activation of nAChRs in the mPFC.

Experiment: Simultaneously inactivate the NBM and block nicotinic signaling in the mPFC (intra-mPFC infusion of the nicotinic antagonist mecamylamine) during the probability discounting task.

4.2.6 Hypothesis: NBM neurons are capable of encoding economic or value-based uncertainty as well as perceptual uncertainty.

Experiment: Identify and record cholinergic and noncholinergic neurons' responses to cues and rewards during the probability discounting task.

4.2.7 Hypothesis: There is a causal relationship between NBM hypofunction and risk aversion in aged individuals.

Experiments: First, determine whether the degree of NBM hypofunction is related to the degree of risk aversion within the same, aged individuals (rodents and humans). Second, determine whether NBM deep brain stimulation, which has been shown to improve cognitive symptoms in Alzheimer's and Parkinson's disease patients, can also reduce risk aversion in aged subjects.

4.2.8 Hypothesis: Muscarinic cholinergic signaling discourages choice of delayed rewards via M1 receptor activation.

Experiment: Infuse the M1 receptor antagonist pirenzepine into the NAc core during the uncued delay discounting task.

4.3 Future Directions

4.3.1 Gain a comprehensive understanding of the differences between the cued and uncued versions of the delay discounting task by determining whether other neural manipulations also selectively impair one version of the delay discounting task.

4.3.2 Incorporate measures of timing or delay perception as routine controls in delay discounting experiments.

4.3.3 Continue to explore the relationship between cholinergic modulation of choice and cholinergic modulation of other complex behaviors.

4.3.4 Consider whether cholinergic signaling interacts with other fundamental choice variables, including basal levels of choice, animal's sex, age, vendor, etc.

4.3.5 In order to address the role of learning and memory, assess whether cholinergic signaling is equally important for choices of costly options that are *not* well-learned by the animals (adjusted delay task; task with fixed risks; tasks with delays or risks intermixed).

4.3.6 Continue to locate the critical site(s) of muscarinic cholinergic signaling during delay discounting and probability discounting by injecting atropine into other areas in the intertemporal and risk-based decision circuits.

4.3.7 Similarly, assess whether stimulation of muscarinic cholinergic signaling in target sites can modify choice during delay and probability discounting tasks.

4.3.8 Consider the role of specific muscarinic cholinergic receptors, i.e. M1 type vs M2 type, both globally and locally within target structures.

4.3.9 Record specific changes in task encoding that accompany muscarinic blockade within specific structures in the brain, e.g. determine how NAc cue-responses are changed by blocking muscarinic signaling in the NAc core.

4.3.10 Finally, determine whether the muscarinic cholinergic system is also required for a third form of cost-benefit decision making- effort-based decision making.

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