ROLE OF THE NUCLEUS ACCUMBENS AND ITS DOPAMINERGIC AND GLUTAMATERGIC AFFERENTS DURING DELAY DISCOUNTING

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ABSTRACT

Deirdre Anne Rodeberg: Role of the Nucleus Accumbens and its Dopaminergic and Glutamatergic Afferents during Delay Discounting
(Under the direction of Regina M. Carelli)

Effective decision making depends on an organism's ability to assess available resources and choose the best available option. Organisms must also assess changes to reward value and update their behavior accordingly to keep selecting the best option. Value-based decision making depends on neural pathways including the nucleus accumbens (NAc) and its dopaminergic input from the ventral tegmental area (VTA). A glutamatergic afferent to the NAc core, the prelimbic cortex (PrL), may also be implicated in value-based decision-making, such as delay discounting. However, it is unknown how these dopaminergic and glutamatergic afferents to the NAc mediate value-based decision-making behavior. First, I used optogenetic techniques to examine the causal role of dopaminergic input to the NAc during delay discounting decision making tasks. This experiment revealed that dopamine release in the NAc core does not mediate delay discounting. The next set of experiments shifted focus to the PrL's glutamatergic ennervation of the NAc. Electrophysiological recording of the PrL during our delay discounting task revealed that PrL neurons track the predicted and eventual outcome of preferred rewards, as the value of that reward shifts across blocks. Further, this tracking differentially encoded preferred rewards depending on rats' inherent impulsivity, such that high impulsive rats demonstrated preferential encoding of the small/immediate option. In the next experiment, optogenetic stimulation of the PrL-NAc core pathway revealed that glutamate signaling in the NAc core was not sufficient to mediate delay discounting. Together, these experiments help to characterize of the neural circuits

and mechanisms by which delay discounting behavior is processed within the brain, providing insight into the potential role of the NAc and its dopaminergic and glutamatergic afferents in mediating appropriate decisions.

For my husband, Nathan

“And once the storm is over you won't remember how you made it through, how you managed to survive. You won't even be sure, in fact, whether the storm is really over. But one thing is certain. When you come out of the storm you won't be the same person who walked in. That's what this storm's all about.”

- Haruki Murakami

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PREFACE

This dissertation was prepared within the guidelines set forth by the University of North Carolina at Chapel Hill Graduate School. This dissertation is comprised of a general introduction chapter, 3 chapters of original data, and a general discussion chapter. Each original data chapter includes an introduction, results, and discussion section. All figures and tables referenced in the text are displayed in order at the end of each corresponding section. A complete list of references cited throughout the document can be found at the end. References follow the formatting of The Journal of Neuroscience.

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AAV	Adeno-associated virus
ANOVA	Analysis of variance
CAMK2α	Calmodulin-dependent protein kinase II alpha
ChR2	Channelrhodopsin
DA	Dopamine
EYFP	Enhanced yellow fluorescent protein
FR	Fixed ratio
FSCV	Fast-scan cyclic voltammetry
IACUC	Institutional animal care and use committee
ICSS	Intracranial self-stimulation
IL	Infralimbic cortex
ITI	Inter-trial interval
MSN	Medium spiny neuron
NAc	Nucleus accumbens
NAc core	Nucleus accumbens core
NAc shell	Nucleus accumbens shell
PB	Phosphate buffer
PCA	Principle component analysis
PEH	Peri-event histogram
PFC	Prefrontal cortex
dlPFC	Dorsolateral prefrontal cortex
mPFC	Medial prefrontal cortex

PrL	Prelimbic cortex
SEM	Standard error of the mean
TH	Tyrosine hydroxylase
VTA	Ventral tegmental area

CHAPTER 1

INTRODUCTION

Effective decision making depends on an organism's ability to successfully choose and assign value to resources that will most benefit their survival. To do this, organisms must associate predictive environmental cues with rewarding outcomes. Further, organisms must be able to update these cue-reward associations when outcome value changes and alter their behavioral strategies to obtain the best possible option. Dysfunctional decision making is a symptom of numerous psychological disorders, including drug addiction. For instance, addicts will often impulsively choose a lesser reward, rather than wait for a more rewarding option. Poor decision making negatively affect addicts' social, financial, professional, and personal lives. As such, it is essential to understand the neurobiology of decision making. Characterizing the brain regions and neural circuits involved in decision making may pave the way toward novel therapeutic treatments for drug addiction and other psychiatric disorders.

Decision-making relies on a complex neural circuit that integrates and updates information related to the changing value of reward. Central to this circuit is the nucleus accumbens (NAc), which functions as a "limbic-motor interface" (Mogenson et al., 1980; Alexander et al., 1986). That is, the NAc receives and integrates information from numerous brain regions, including the ventral tegmental area (VTA), prefrontal cortex (PFC), basolateral amygdala and hippocampus, and updates downstream motor structures to influence behavior (Zahm and Brog, 1992, Zahm, 1999). Adding to its complexity, the NAc is divided into two

subsections, the core (NAc core) and shell (NAc shell), which are thought to subserve unique roles in decision making behavior.

Notably, the NAc receives rich dopaminergic input from the VTA, which has been extremely well-characterized for its role in decision making behavior (Cardinal et al., 2001; Cardinal and Howes, 2005; Ghods-Sharifi and Floresco, 2010; Stopper and Floresco, 2011; Stopper et al., 2013). However, it remains unknown if dopaminergic signaling in the NAc is causally linked to different aspects of decision making behavior. Further, while the VTA-NAc circuit has been extensively studied, little is known about how other NAc afferents affect decision making behavior. A notable region of interest is the PFC and its glutamatergic projections to the NAc, which exerts top-down control to drive motivated behavior (Ballard et al., 2011). In the rat, the homologue of the human PFC is divided into two subsections, the prelimbic (PrL) and infralimbic (IL) cortices, which selectively enervate the NAc core and NAc shell, respectively (Krettek and Price 1977; Sesack et al., 1989; Basar et al., 2010; Pinto and Sesack 2000; Brog et al., 1993).

Thus, this dissertation seeks to examine how dopaminergic and glutamatergic afferents to the NAc shape delay discounting behavior. This introductory chapter will provide a review of value-based decision making and the neurobiology behind these processes. Chapter 2 of this dissertation will examine how dopaminergic signaling in the VTA-NAc circuit casually contributes to the processing of delay discounting behavior. Next, Chapters 3 and 4 will examine neural activity in the PrL during delay discounting, and how its glutamatergic input to the NAc core casually influences behavior. Finally, the importance of these findings will be integrated and discussed.

Value-based decision making

To make appropriate decisions, organisms must first assess the resources and options available to them and assign value to these choices. These choices can be simple (selecting a water source; choosing the largest or most nutritious food option) to complex (assessing the risk, effort, or time required to obtain a reward). Organisms must also integrate these values to predict the viability of complex future rewards (i.e., preferring a smaller, easier reward if the risk, effort, or time to obtain a better reward is too high). For instance, an animal may have to decide between consuming smaller, easier prey and spending more time and energy hunting down a larger meal.

Indeed, value-based decision making is a constant and complex process, requiring the synthesis of an organism's internal and external states to make the best possible decision. First, organisms must assign value to all available options based on their internal state (e.g., hunger or thirst) as well as environmental predictive cues. Next, based on this information, the organism must evaluate and compare each available outcome to choose the best reward. After receiving the reward, the organism must then evaluate whether the predicted and actual outcome value resulted in the best possible reward at that time. This comparison results in learning, in which the organism reflects on its choice and can update its behavior to optimize future decisions (Rangel et al., 2008).

Adding to its complexity, value-based decision making is comprised of two discrete, yet interrelated, components. Reward value can be assessed based on objective, or outcome-based, features of reward (i.e., reward magnitude), or more variable subjective components (i.e., impulsivity, risk assessment, willingness to wait for reward) (Saddoris et al., 2015a). For instance, reward magnitude is largely intrinsic, as animals will almost always prefer a larger food

source (assuming equal time/risk/effort to obtain two differently sized rewards). If an organism delays reinforcement or puts in a great amount of effort to obtain a larger reward, it may risk the loss of other food options or exhaust itself before obtaining the reward. On the other hand, organisms can receive guaranteed, yet fewer, rewards when an immediate, smaller option is selected. This subjective decision varies by individual. For instance, in a task where predictive cues signaled equal outcome value but varied risk probability, rats differentially developed a preference for either the risky or safe option (Sugam et al., 2012).

Delay Discounting

One aspect of value-based decision making is delay discounting, in which individuals discount the value of a reward based on the amount of time (the delay) to its receipt (Roesch et al., 2006; Roesch et al., 2007, Roesch and Bryden 2011, Tedford et al., 2015). That is, as the delay to reward increases, the subjective value of that reward decreases. During a typical delay discounting task, individuals are trained to associate cues with either a small, immediate reward, or a large, delayed reward. During the task, the length of the delay to the large reward becomes longer. As such, delay discounting incorporates two components of decision making: delay (subjective) and reward magnitude (objective) (Saddoris et al., 2015a).

Generally, individuals are initially willing to wait for the larger reward when there is a short delay. However, when the delay becomes too long, individuals will shift their preference toward the smaller, immediate reward (Roesch et al., 2006; Roesch et al., 2007; Tedford et al., 2015). In this way, delay discounting serves as a measure of impulsivity, such that it measures the amount of time it takes for individuals to "lose patience" and choose an immediate reward. As the delay to reward increases, a "discounting curve" is calculated based on individuals'

choices (Odum, 2011), with a steeper discounting curve slope reflecting greater impulsivity (Frye et al., 2016, Saddoris et al., 2015b, Moschak and Carelli 2017). Interestingly, delay discounting has been proposed to be a form of "mental time travel," such that individuals may envision future (delayed) events as if they are occurring presently (Boyer 2008). As such, the perceived enjoyment of future rewards may motivate organisms to pursue a delayed, more beneficial outcome (Palombo et al., 2016).

Importantly, heightened delay discounting (increased impulsivity) is a common symptom of substance use disorders (Hoffman et al., 2008; Jentsch and Taylor 1999; Crews and Boettiger 2009; Coffey et al., 2003). That is, addicts will over-value immediate rewards (drugs of abuse) compared to "delayed" rewards such as professional, social, or academic outcomes, failing to reflect on the future implications of their decisions (Bickel et al., 1999; Evenden 1999; Heyman 1996; Poulos et al., 1995; Bechara 2005; Ernst and Paulus 2005). Clinically, drug abusers demonstrate greater impulsivity during delay discounting tasks (Hoffman et al., 2008; Coffey et al., 2003; Jones et al., 2015), and the intensity of drug addiction correlates with the degree of delay discounting (Petry and Casarella, 1999; Vuchinich and Simpson, 1998). Drugs such as cocaine decrease the "breakpoint" at which individuals shift to the small, immediate reward (Jentsch and Taylor 1999; Coffey et al., 2003; Monterosso 2001; Bechara et al., 2002; Dalley et al., 2008), and relapse is often accompanied by a drastic increase in discounting (Heather 1998). These findings are corroborated in rodent models, as prior exposure to cocaine increases impulsivity during decision making tasks involving delay, magnitude, and delay discounting (Roesch et al., 2007; Simon et al., 2007; Mendez et al., 2010; Hernandez et al., 2014; Mitchell et al., 2014). In addition to drug addiction, impulsivity is a characteristic of numerous psychiatric disorders, including attention deficit hyperactivity disorder, schizophrenia, depression, and

borderline personality disorder (Barkley et al., 2001; Heerey et al., 2007; Imhoff et al., 2014; Lawrence et al., 2010).

Interestingly, the relationship between delay discounting and psychiatric disorders appears to be bidirectional. Indeed, as stated above, preclinical and human research demonstrates that heightened delay discounting is often a result of substance abuse (Bickel et al., 1999; Evenden 1999; Heyman 1996; Poulos et al., 1995; Bechara 2005; Ernst and Paulus 2005; Hoffman et al., 2008; Coffey et al., 2003; Jones et al., 2015). However, inherently high impulsivity (heightened delay discounting) may also *predict* future drug use (Jentsch and Taylor, 1999; Winstanley et al, 2010). For instance, preclinical literature demonstrates that high impulsive action predicts cocaine self-administration (Belin et al, 2008; Dalley et al, 2007). Further, drug-naïve animals that exhibited heightened delay discounting acquired drug self-administration faster than less-impulsive subjects (Anker et al., 2009). Animals that displayed higher impulsive choice were also more likely to exhibit drug seeking during extinction and reinstate these behaviors when given access to drug again (Diergaarde et al, 2008; Perry et al, 2008; Economidou et al, 2009). In the human literature, lack of inhibitory control in childhood predicts drug use later in life (Nigg et al, 2006; Tarter et al, 2003; Wong et al, 2010), and specifically stimulant abuse (Ersche et al, 2010). Because of this bidirectional relationship to drug use and its impact on numerous clinical populations, delay discounting is a crucial cognitive process to characterize and understand. Understanding the neural substrates of delay discounting may identify those with a predisposition to impulsivity or may improve current treatments for psychiatric disorders such as drug addiction.

Neurobiology of the nucleus accumbens

Cellular composition of the nucleus accumbens: Value-based decision-making recruits the mesolimbic system, which includes the nucleus accumbens (NAc) (Day et al., 2007, Fields et al., 2007, Clark et al., 2012, Saddoris et al., 2015b). The NAc functions as a "limbic-motor interface" that links environmental cues with rewards and sends the resulting output to downstream motor areas to influence goal-oriented behavior (Mogenson et al., 1980; Alexander et al., 1986). The NAc is composed primarily (~95%) of GABAergic medium spiny neurons (MSNs) which project to downstream structures (Groves, 1983; O'Donnell and Grace, 1993). MSNs possess a soma 10-20 um in diameter (Preston et al., 1980; Gerfen, 1988; O'Donnell and Grace, 1993; Kawaguchi, 1997) and multi-directional dendrites (Preston et al., 1980; Groves, 1983; Gerfen, 1988). MSN axons project to motor areas such as the substantia nigra, ventral pallidum, and lateral hypothalamus to influence behavior (Gerfen, 1988; Kawaguchi, 1997; Zahm, 1999). Adding to their complexity, MSNs possess unique immunohistochemical markers such as enkephalin, dynorphin, substance P, and neurotensin, which determine each neuron's individual output and projection (Meredith 1999).

Dopaminergic input from the VTA further exerts control over MSNs. Most MSNs express either D1-like or D2-like receptors, but rarely both (Bertran-Gonzalez et al., 2008). Specifically, MSNs with D1-like receptors express dynorphin, while MSNs with D2-like receptors express enkaphalin. This discrepancy may indicate that these MSNs project to separate circuits, and strongly indicates that dopamine plays a role in modulating projection pathways from the NAc (LeMoine and Bloch, 1995; Kupchik et al., 2015).

The remaining ~5% of NAc neurons are local interneurons, which serve to inhibit MSNs (Koos and Tepper 1999). NAc interneurons are either GABAergic or cholinergic (Groves, 1983;

Meredith, 1999). GABAergic interneurons are divided into three populations (parvalbumin, calretinin, and somatostatin-neuropeptide Y positive), and exhibit tonic firing activity with brief bursts, compared to MSNs' phasic burst activity. Importantly, the oscillatory behavior between GABAergic interneurons and MSNs helps to mediate MSN activity (Berke et al., 2004). Cholinergic interneurons differ from MSNs in terms of morphology and activity level. They are large (20-50 um diameter cell bodies), exhibit tonic firing rates, and, importantly, supply the NAc with acetylcholine (Kawaguchi et al., 1995; Meredith, 1999).

Nucleus accumbens subregions: structure, function, and connectivity: Critically, the NAc is divided into two subregions, the core (NAc core) and shell (NAc shell), which are anatomically and functionally distinct (Parkinson et al., 1999; Heimer et al., 1991, Zahm and Brog, 1992, Jongen-Relo et al., 1994, Ikemoto, 2007). The NAc core has been implicated in cue-reward associations, goal directed behavior, and reward prediction (Carelli, 2004, Saddoris et al., 2013). Conversely, the NAc shell appears to maintain the valence and novelty of rewards (Kelley, 2004, Zorrilla and Koob, 2013, Castro et al., 2015, Saddoris et al., 2015a). While both the core and the shell receive dopaminergic input from the VTA, the core exhibits higher levels of dopamine transporters (Jones et al,. 1996). As such, differences in dopamine reuptake and synaptic availability may reflect the functional discrepancies between these two subregions. Notably, while the core and shell are functionally unique, they are not anatomically isolated from one another. Direct interconnections between the core and shell exist; specifically, core neurons project to the shell, while the shell's projections to the core are sparse (Van Dongen et al,. 2005).

In addition to their functional differences, the NAc subregions also differ in their afferent and efferent connections. For instance, dopaminergic input from the VTA varies by subregion; generally, the NAc core and lateral NAc shell receive dopaminergic innervation from the lateral

VTA, whereas the medial NAc shell receives medial VTA input (Ikemoto 2007). Dopamine in this circuit serves as a neuromodulator and a "teaching signal" during behavior and cue-reward associations (Schultz et al., 1997; Fiorillo et al., 2003; Tobler et al., 2005; Day et al., 2007). The NAc also receives excitatory, glutamatergic projections from cortical and limbic regions including the mPFC, basolateral amygdala (BLA), and hippocampus (Zahm and Brog, 1992; Brog et al., 1993). While the BLA sends glutamatergic projections to both NAc subregions, the core and shell receive heterogenous excitatory input from distinct prefrontal subregions. Generally, the prelimbic and anterior cingulate cortices project to the NAc core, whereas the infralimbic and orbitofrontal cortices project largely to the NAc shell (Brog et al., 1993; Montaron et al., 1996).

The NAc subregions also feature unique MSN output projections. The core projects primarily to motor-related structures such as the globus pallidus and substantia nigra, which in turn project to premotor cortical areas. In contrast, the shell projects largely to limbic regions such as the lateral hypothalamus, ventral part of the bed nucleus of the stria terminalis and VTA (Zahm and Brog, 1992, Zahm and Heimer, 1993, Corbit et al., 2001). This anatomic distinction appears to reflect different functional properties of the NAc subregions during cue-reward learning and motivated behavior (Saddoris et al., 2012; Ikemoto 2007).

Neurobiology of the prefrontal cortex

Cellular composition: Value based decision making also recruits the prefrontal cortex (PFC) (Ernst and Paulus 2005), which exerts top-down inhibitory control over behavior and assists in storing action-outcome contingencies (Balleine and Dickinson, 1998; Coutureau et al., 2000; Cardinal et al., 2002; Aron et al., 2004; Conway and Fthenaki 2003). The PFC is

composed of ~85% glutamatergic pyramidal projection neurons (Gabbott and Bacon 1996; Gabbott et al., 1997). As defined by Ramon y Cajal (1893): "The pyramidal cell, or psychic cell, possesses specific characteristics … a dendritic shaft and tuft directed toward the cerebral surface; the existence of collateral spines on the dendritic processes…" Indeed, PFC pyramidal neurons are quite complex and arborous, with up to 16 times more spines than those in visual cortices (Elston 2000). Adding to their complexity, layer V mPFC pyramidal neurons possess unique morphology and firing activity depending on the projection target (Brown and Hestrin 2009; Molnar and Cheung 2006; Otsuka and Kawaguchi 2008). GABAergic interneurons comprise the remaining ~15% of the PFC and help to modulate pyramidal cell excitation (Beaulieu 1993).

PFC structure, function, and connectivity: Notably, the PFC is not one homologous structure, and can be compartmentalized into distinct cortices, including medial, dorsolateral, ventromedial, and orbitofrontal (Miller et al., 2001; Siddiqui et al., 2008; Barbas and Pandya 1989; Carmichael and Price 1994; Uylings and Van Eden 1990). These cortices interact to produce complex executive behaviors, such as working memory, attentional processes, and decision making (Barbas 1995; Barbas et al., 2002; Fuster 1997; Miller and Wallace 2003). Specifically, the dorsolateral prefrontal cortex is critical for goal-directed and decision-making behavior, as it is implicated in expected or obtained rewards (Watanabe, 1996; Leon and Shadlen, 1999; Kobayashi et al., 2002; Tsujimoto and Sawaguchi, 2004, Seo et al., 2007), working memory (Barbey et al., 2013), response inhibition (Garavan et al., 1999) and intertemporal choice (McClure et al., 2004).

In the rat, the PFC consists of dorsal (prelimbic; PrL) and ventral (infralimbic; IL) areas (Basar et al., 2010; Heidbreder and Groenewegen 2003; Moorman and Aston-Jones, 2015;

Ongur and Price, 2000). The PrL cortex is the neural correlate of the human dorsolateral PFC (Uylings et al., 2003), and is generally associated with goal-directed behavior and cognition (Moorman and Aston-Jones 2015, Koob 2010). Importantly, the PrL primarily enervates the NAc core, whereas the IL largely enervates the NAc shell (Krettek and Price 1977; Sesack et al., 1989; Basar et al., 2010; Pinto and Sesack 2000; Brog et al., 1993).

The PFC receives input from numerous brain structures, including the raphe nucleus (serotonin), locus coeruleus (noradrenaline), and VTA (dopamine), which serve to modulate neuron activity in the PFC (Steinbusch, 1981; Van Eden et al., 1987; Aston-Jones and Cohen, 2005; Puig et al., 2005; Celada et al., 2013; Chandler et al., 2014). This modulation is cell- and layer-specific, as dopaminergic input from the VTA solely modulates interneurons in Layers V and VI (Kolk et al., 2009, van Schouwenbur et al., 2010, Zhang et al., 2010), and glutamatergic input from the BLA targets layer II pyramidal neurons (Little and Carter 2013).

Conversely, the PFC's glutamatergic pyramidal cells project to a wide range of structures, such as the striatum, amygdala, (hypo)thalamus, and brainstem (Little and Carter 2013; Ernst and Fudge 2009; Heidbreder and Groenewegen 2003). For instance, layer III pyramidal neurons project largely to other cortical structures, while layer V and VI pyramidal neurons project to subcortical structures (Gabbott et al., 2005; Dembrow et al., 2010). Notably, pyramidal neurons of deep layers V and VI innervate both the NAc core and NAc shell with glutamatergic input (Ding et al., 2001), which facilitates decision making by integrating DA-ergic reinforcement signals with environmental stimuli (Russo and Nestler, 2013; Britt et al, 2012; Pine et al., 2010; Stuber et al., 2008) to encode the salience of reward-predictive cues (Hotsenpiller et al., 2001; Everitt and Wolf, 2002).

Specific manipulation of neural circuitry using optogenetics

Optogenetics is a technique that allows researchers to modulate discrete neural pathways *ex* and *in vivo*. This method utilizes light-activated proteins (opsins) that are selectively expressed on neurons via viral vectors or introduced transgenically. Opsins can be activated by external light sources (i.e., a laser) at discrete experimental time points, allowing researchers precise control over neuronal function (Boyden et al., 2005; Aravanis et al., 2007; Zhang et al., 2010; Bernstein and Boyden, 2011; Boyden, 2011; Stuber et al., 2012). Importantly, opsin expression does not alter basic cellular morphology or characteristics (i.e., resting membrane potential) compared to uninfected control neurons, ensuring the validity of this technique (Zhang et al., 2006; Gradinaru et al., 2008; Chow et al., 2010). Neuron activity can be enhanced or reduced depending on the type of opsin used. One well-characterized opsin is channelrhodopsin-2 (ChR2), a blue-light-gated (470 nm) cation channel which allows for rapid neuronal depolarization at physiological frequencies (Nagel et al., 2003; Zhang et al., 2006). Neuron function can also be inhibited via two other light-activated opsins: archaerhodopsin, an outward proton pump (Chow et al., 2010; 2012), and halorhodopsin, an inward chloride pump (Gradinaru et al., 2008; Zhao et al., 2008).

An important milestone in optogenetics research was the development of transgenic rat lines expressing Cre-recombinase in tyrosine hydroxylase neurons (*TH::Cre*$^{+/-}$). When injected with a Cre-inducible adeno-associated virus (AAV) containing a doubly floxed inverted opsin gene (DIO), TH+ neurons expressing Cre-recombinase become “infected” with the opsin gene (Butler 2011). This allows for the selective expression of opsins on TH+ (i.e., dopaminergic and noradrenergic) neurons. For instance, injection of a DIO-Cre AAV linked to ChR2 into the VTA allows for the selective expression of ChR2 on dopaminergic neurons projecting from the VTA

to the NAc, and optical stimulation of infected VTA cell bodies produces dopamine release in the NAc (Witten et al., 2011).

Importantly, however, the use of AAVs to infect neurons is not limited to transgenic animals. Because AAVs infuse their DNA into host cells (Buchschacher 2003), they can be modified to insert opsin genes into neurons containing certain promoters. For instance, to optically excite the PrL-NAc core pathway, one could infuse a ChR2-containing AAV linked to the calmodulin-dependent protein kinase II alpha (CAMK2α) promoter into the PrL, and place optical fibers in the NAc core to deliver light to glutamatergic PrL terminals. This would allow ChR2 expression only on neurons containing the CAMK2α promoter (i.e., glutamatergic pyramidal cells). Notably, for both viral vector and transgenic methods, the majority of optogenetics literature focuses on mice. As such, a continuous effort is being made to determine if similar behavioral effects are reproducible in rats.

Examination of neural activity using *in-vivo* electrophysiology

Unlike optogenetics, *in-vivo* electrophysiology is a technique that examines real-time changes in individual neurons' activity during behavior. Arrays containing 8 microwire electrodes are implanted into the brain (e.g., bilaterally into the PrL), allowing for extracellular recording of the cell(s) surrounding each microwire. Specifically, these microwires detect changes in voltage potential surrounding each neuron. Cellular activity is recorded between the 8 active electrodes and an inactive reference electrode, and aligned with discrete task events (e.g., cue presentation or lever press). As detailed in Chapter III, neuronal activity is then filtered and processed to ensure that these characteristics are biologically appropriate, and action potentials (or "spikes") are identified via a thorough sorting process. Further, analysis of waveform

dynamics allow for the identification of different cell types (e.g., pyramidal versus GABAergic neurons in the PrL). Once spikes are sorted, cells can be further classified into non-phasic and phasic categories. Non-phasic cells do not exhibit changes in firing rate to discrete task events, whereas phasic neurons exhibit either increases (excitations) or decreases (inhibitions) to stimuli. As such, *in-vivo* electrophysiology can identify how a particular brain region encodes discrete task events, based on the phasic activity of its neuronal populations.

The neural mechanisms of value-based decision making

Value-based decision-making recruits the mesolimbic system, including the nucleus accumbens (NAc) and its dopaminergic input (Day et al., 2007, Fields et al., 2007, Clark et al., 2012). Dopamine neurons increase activity to cues that predict rewards, and track choice behaviors related to a range of decision making including effort, delay, risk, and delay discounting (Schultz, 1997, Roesch et al., 2007, Day et al., 2011). Rapid DA release in the NAc reflects this pattern of cellular activity (Day et al., 2010, Sugam et al., 2012, Saddoris et al., 2015b). Indeed, increases in transient dopamine release have been measured during cues predicting food, liquid, cocaine and intracranial self-stimulation (Phillips et al., 2003, Roitman et al., 2004, Day et al., 2007, Owesson-White et al., 2008). Further, pharmacological disruption or lesions of mesolimbic circuitry, including the NAc, results in maladaptive decision making, such that animals cannot update behavior to reflect changes in reward value (Cardinal et al., 2001, St Onge and Floresco, 2009, Ghods-Sharifi and Floresco, 2010).

Notably, the core and shell subregions may subserve unique functions with regard to decision making. The NAc core is associated with Pavlovian cue encoding, goal-directed behavior, and selection of rewards of different value (Saddoris et al., 2013). Further, the core

receives input from regions such as the PrL and BLA, which are also linked to value-based decision making and cognitive processes. Further, dopamine release in the core tracked the value of reward-predictive cues, but not the eventual outcome. Specifically, core dopamine release scaled to the subjective preference and value of the reward during risk, effort, delay, and delay discounting tasks (Day et al., 2010; Sugam et al., 2012; Saddoris et al., 2015b). As such, the NAc core may play a role in computing the expected value of each reward, allowing for the selection of the best available option.

Conversely, the NAc shell appears to play a role in reward outcome. Rewarding tastants increase DA release, while aversive tastes decrease DA release in the shell (Roitman et al., 2008; Wheeler et al., 2011). Further, cues predicting large (compared to smaller) reward magnitude elicit greater dopamine cell firing (Tobler et al., 2005, Roesch et al., 2007), which is reflected in distinct dopamine release dynamics to rewards of different magnitudes (Beyene et al., 2010, Wanat et al., 2010; Cacciapaglia et al., 2012; Sackett et al., 2017). Inactivation of the shell reduced sensitivity to reward magnitude (Stopper and Floresco, 2011), but did not disrupt the ability to differentiate costs associated with outcomes (Ghods-Sharifi and Floresco 2010). Further, a history of cocaine disrupted dopamine signaling in the NAc shell and impaired the ability to discriminate between reward magnitudes (Saddoris et al., 2016; 2017). During subjective-based decision-making tasks (i.e., delay, effort, or risk), dopamine release in the NAc shell was released during reward-predictive cues, but did not track the subjective value of the reward (Day et al., 2010; Sugam et al., 2012). However, the reward outcome was the same in these tasks (1 sugar pellet), and as such, the shell may have been encoding the availability of these equal reward outcomes.

Delay discounting and the NAc core: Lesion and pharmacological inactivation studies have linked the NAc core to subjective-based decision making (Cardinal et al., 2001, Cardinal and Cheung, 2005, Cardinal and Howes, 2005, Pothuizen et al., 2005, Hauber and Sommer, 2009, Ghods-Sharifi and Floresco, 2010). In support, rapid dopamine release in the NAc core, but not shell, encodes subjective preference during delay, risk, effort, and delay discounting tasks (Day et al., 2010, Sugam et al., 2012, Saddoris et al., 2015b). Indeed, lesions to the NAc core increase impulsive choice in rats (Cardinal et al., 2001; da Costa Araújo et al., 2009; Feja et al., 2014; Valencia-Torres et al., 2012), and diminish sensitivity to reward delay (Acheson et al., 2006).

However, delay discounting recruits both outcome (reward magnitude) and subjective (willingness to wait) components. Studies have implicated the NAc core in processing optimal choice behavior, rather than impulsivity specifically. DA-ergic antagonist SCH-23390 reduced reward magnitude sensitivity, but glutamatergic antagonist ifenprodil decreased sensitivity to delay (Yates and Bardo 2017). For instance, lesions to the NAc core reduced sensitivity to both reward magnitude and delay during a delay discounting task (Steele et al., 2018). Amphetamine (a dopaminergic agonist) increased the choice of a large delayed reward when delays increased, but decreased this choice when delays decreased, indicating that DA in the NAc core functions to modulate the best available choice, rather than impulsivity (Orsini et al., 2017). Rats' inherent impulsivity may also modulate dopamine activity in the NAc core, as highly impulsive rats exhibited attenuated DA release during cues during the delay discounting task (Moschak and Carelli, 2017).

Delay discounting and the PFC: Delay discounting also recruits the prefrontal cortex (PFC) (Ernst and Paulus, 2005), which exerts top-down inhibitory control over behavior (Aron et

al., 2004; Conway and Fthenaki 2003). Specifically, the human dorsolateral prefrontal cortex (dlPFC) is implicated in impulsive behavior (Cho et al., 2010; Cho et al., 2013; Cho et al., 2012). Drug addicts demonstrate altered dlPFC function reflective of increased impulsive behavior (Hoffman et al., 2008; Monterosso et al., 2007; Boettiger et al., 2007). In the rat, the PFC consists of dorsal (prelimbic; PrL) and ventral (infralimbic; IL) areas (Basar et al., 2010; Heidbreder and Groenewegen 2003; Moorman and Aston-Jones 2015; Ongur and Price 2000). The PrL cortex is the neural correlate of the dlPFC (Uylings et al., 2003), and is generally associated with goal-directed behavior (Moorman and Aston-Jones 2015; Killcross and Coutureau 2003; Balleine and O'Doherty 2010; Tran-Tu-Yen et al., 2009; Smith and Gryabiel 2013). The PrL cortex is also causally implicated in impulsivity, as inactivation of the PrL area induced spontaneous and premature behaviors (Ishikawa et al., 2008; Jonkman et al., 2009; Narayanan et al., 2006), and reduction of D2 receptor mRNA in the PrL increased impulsive choice (Simon et al., 2013). Further, inactivation of the medial PFC, which includes the PrL area, increased impulsive choice in a delay discounting task (Churchwell et al., 2009). As such, the rodent PrL cortex is an ideal area in which to study the neurobiology of delay discounting.

Goals of this dissertation

As discussed above, the NAc is a key brain region that contributes to goal-directed behavior and value-based decision making. Importantly, studies clearly implicate a role of the NAc in reward learning and value (Schultz 1998; Saddoris et al., 2013) and the NAc and its dopaminergic inputs from the ventral tegmental area (VTA) encode delay discounting (Saddoris et al., 2015b; Moschak et al., 2017). Presentations of reward-predictive cues evoke changes in NAc cell firing as well as phasic dopamine (DA) release during delay discounting tasks (Roesch

and Bryden 2011; Kobayashi and Schultz 2008; Saddoris et al., 2015b). The NAc contains two primary subregions, the core and shell that differ in their afferent and efferent connections (Basar et al., 2010), and are believed to subserve different functions (Saddoris et al., 2015b, Parkinson et al., 1999; Heimer et al., 1991, Zahm and Brog, 1992, Jongen-Relo et al., 1994, Ikemoto, 2007). Our lab has shown that DA in the NAc core subregion in rats selectively encodes features of subjective decision making, such as risk, effort, delay, and delay discounting (Saddoris et al., 2015b; Day et al., 2010; Sugam et al., 2012; Day et al., 2011). The NAc core is also causally implicated in impulsive choice, as lesions to the core reduce preference for delayed, large and certain rewards (Pothuizen et al., 2005; Cardinal et al., 2001). Conversely, the shell is implicated in outcome (or objective) decision making (Beyene et al., 2010; Stopper and Floresco 2010; Sackett et al., 2017). However, it remains unclear if NAc dopamine activity is causally linked to discrete aspects of delay discounting. Manipulation of dopamine release in the NAc during delay discounting may elucidate how dopamine mediates and updates information related to changing reward value.

Another important afferent to the NAc is the prefrontal cortex (PFC). In the rat, the PFC consists of dorsal (prelimbic; PrL) and ventral (infralimbic; IL) areas (Basar et al., 2010; Heidbreder and Groenewegen 2003; Moorman and Aston-Jones, 2015; Ongur and Price, 2000). The NAc receives glutamatergic input from pyramidal cells in the PFC, and facilitates decision making by integrating DA-ergic reinforcement signals with environmental stimuli (Russo and Nestler 2013; Britt et al., 2012; Pine et al., 2010; Stuber et al., 2008) to encode the salience of reward-predictive cues (Hotsenpiller et al., 2001; Everitt and Wolf 2002). Importantly, the PrL selectively enervates the NAc core (Krettek and Price 1977; Sesack et al., 1989; Basar et al., 2010; Bro et al., 1993), and studies show an interaction between these two areas during drug

seeking and craving (Ma et al., 2014; Kalivas 2009). As such, the PrL-NAc core pathway is likely a critical neural substrate of value-based decision making. Importantly, glutamatergic signaling from the PrL may aid in updating information in the NAc core with respect to value changes to allow for the animal to change behavior accordingly. Investigation of PrL neuron activity, as well as manipulation of the PrL-NAc core circuit, during value-based decision making may yield further insight into how this glutamatergic pathway regulates goal-directed behavior. Taken together, the proposed studies seek to elucidate the specific roles of the NAc's dopaminergic and glutamatergic afferents during delay discounting behavior.

Specific Aims:

1. **To investigate causal links between NAc core dopamine release and delay discounting.**

Our lab has shown that DA in the NAc core subregion in rats selectively encodes features of subjective decision making, such as risk, effort, delay, and delay discounting (Day et al., 2010; Sugam et al., 2012; Saddoris et al., 2015b). The NAc core is also causally implicated in impulsive choice, as lesions to the core reduce preference for delayed, large and certain rewards (Cardinal et al., 2001, Cardinal and Cheung, 2005, Cardinal and Howes, 2005, Pothuizen et al., 2005, Hauber and Sommer, 2009, Ghods-Sharifi and Floresco, 2010). However, no studies have examined a casual role for NAc core dopamine during delay discounting. Here, I will use optogenetics tools in *TH::Cre*$^{+/-}$ rats to stimulate NAc core dopamine terminals from the VTA during discrete components of our delay discounting task (from Saddoris et al., 2015b). Specifically, I will enhance dopamine release during the presentation of cues during the Forced Choice Long Delay block, and then during the Forced Choice Short Delay block. Importantly, the Short and Long Delay blocks are when rats normally shift their preference from the delayed

large reward to the immediate small reward (the more impulsive choice). Free choice preference for the large/delayed reward (independent of optical stimulation) will indicate if dopamine stimulation during reward-predictive cues casually influences delay discounting choice selection toward the less-impulsive option.

2. To determine how neurons in the PrL encode information about delay discounting.

To understand the neurobiological basis of delay discounting, it is important to investigate how neurons process information about the critical features of this behavior. Specifically, the PrL is implicated in impulsive choice, an aspect of delay discounting (Ishikawa et al., 2008; Jonkman et al., 2009; Narayanan et al., 2006). While no studies have directly examined neural processing in the PrL cortex during this behavior, several studies, including those from our lab, have shown that neuronal activity in one of its key efferents, the NAc, tracks motivated responses and cue-reward associations (Day et al., 2011; Carelli and Deadwyler 1994; Carelli et al., 1999; Carelli and Ijames 2000; Stott and Redish 2014; Roesch et al., 2009; Carelli et al., 2000). For example, work in the Carelli lab has demonstrated unique cell firing patterns in the NAc core to cues predictive of subjective reward costs such as effort, delay, and risk [Day et al., 2011; Sugam et al., 2014). Further, other labs have examined neuronal activity during delay discounting and found that NAc neurons encode information related to cues that predict reward of varying delay and magnitude (Roesch and Bryden 2011, Stott and Redish 2014; Roesch et al., 2009). Importantly, neuronal activity in the PrL also appears to encode cue-associated behavior and reward seeking (West and Carelli, 2014; Mulder et al., 2003; Durstewitz et al., 2010; Sul et al., 2010). In this aim, I will use multi-neuron recording methods to build upon prior work and examine extracellular activity in the PrL cortex during our delay discounting task. As such, this aim will clarify the role of the PrL during discrete aspects of delay discounting.

3. **To assess potential causal links between the PrL-NAc core circuit and delay discounting**

The PrL does not function in isolation but is part of a larger neural circuit including a primary efferent to the NAc core (Krettek and Price 1977; Sesack et al., 1989; Basar et al., 2010; Pinto and Sesack 2000; Brog et al., 1993; Simon et al., 2013; Sesack and Bunney 1989). While numerous studies implicate both the PrL and the NAc core in impulsive choice and delay discounting behavior (Churchwell et al., 2009; Cardinal et al., 2001; Pothuizen et al., 2005; Ishikawa et al., 2008; Jonkman et al., 2009; Narayanan et al., 2006), additional studies are needed to determine if PrL inputs to the NAc core play a causal role in delay discounting behavior. Here, I will infuse ChR2 into the PrL and stimulate glutamate release from terminals in the NAc core via a chronically implanted optical fiber. Critically, this approach is well-established and used previously by others in prefrontal cortical regions (Ma et al., 2014; Ji and Neugebauer 2012; Van den Oever et al., 2013). On test day in well-trained rats, I will optically stimulate (excite) glutamatergic terminals in the NAc core during the presentation of cues during the Forced Choice Long and Short delay blocks. As in Aim 1, the Long and Short Delay blocks are when rats normally shift their preference from the delayed large reward to the immediate small reward (the more impulsive choice). Further, Free Choice behavior will serve as a measure of learned preference for the different options independent of any nonspecific effects of stimulation during Free Choice trials. As such, this aim will investigate a critical role for the PrL-NAc core circuit in delay discounting behavior, specifically during a time of heightened impulsive choice, and examine its causal link to this behavior

CHAPTER 2

OPTICAL STIMULATION OF NAc CORE DOPAMINE RELEASE DOES NOT MODULATE DELAY DISCOUNTING BEHAVIOR

Introduction

Delay discounting is the degree to which the subjective value of a large, future reward is reduced relative to that of a smaller, immediate reward (Roesch et al., 2006; Roesch et al., 2007; Roesch and Bryden 2011; Tedford et al., 2015). Heightened delay discounting occurs when the delayed reward is devalued such that the individual shifts strategies to select the immediate reward (Roesch et al., 2006; Roesch et al., 2007; Tedford et al., 2015). This shift toward an immediate, yet smaller reward indicates a loss of patience and a more impulsive choice strategy (Cardinal et al., 2001; Dellu-Hagedorn, 2006; Pothuizen et al., 2005; Robinson et al., 2009; Winstanley et al., 2006). Impulsivity (heightened delay discounting) is a common symptom of numerous disorders, including ADHD, obesity, and drug addiction (Amlung et al., 2016; Jackson and MacKillop 2016; Bickel et al., 2012; de Wit, 2009). As such, understanding the neural underpinnings of delay discounting may contribute to the development of therapeutics which can improve aberrant impulsivity.

Delay discounting recruits the mesolimbic reward system, including the nucleus accumbens (NAc) and its core subregion (NAc core) (Cardinal et al. 2003; Cardinal 2006; Floresco et al. 2008; Dalley et al. 2008; Basar et al. 2010). Ablation of the NAc core results in a decreased preference for the large, delayed reward (Cardinal et al., 2001; Pothuizen et al. 2005; Bezzina et al. 2007; Bezzina et al., 2008; da Costa Araújo et al. 2009; da Costa Araújo et al.

2010). However, lesions to the NAc core did not alter sensitivity to reward magnitude (Cardinal and Cheung, 2005; Cardinal and Howes, 2005). As such, it is thought that the NAc core promotes the encoding of the subjective value of the delayed reward. Delay discounting was also found to produce neuronal activation in the NAc core as determined by Fos expression (da Costa Araújo et al., 2010).

Delay discounting and impulsivity is modulated by the dopaminergic input from the ventral tegmental area (VTA) to the NAc. Low levels of D2/D3 receptors in the NAc core are correlated with high impulsive action (Dalley et al., 2007). Further, dopamine transporter overexpression and D1 receptor antagonism both lead to increased impulsivity (Adriani et al., 2009; Broos et al., 2012; Koffarnus et al., 2011; van Gaalen et al., 2006). Many drugs of abuse such as amphetamine, methamphetamine, and cocaine function by agonizing dopamine, and notably, high doses of these drugs increase impulsive choice (Cardinal et al., 2000; Orsini et al., 2017). Importantly, NAc core dopamine release tracks the predicted subjective value of rewards during delay discounting (Saddoris et al. 2015b; Moschak et al., 2017). Specifically, dopamine exhibited a "graded and dynamic prediction" toward the large reward as the delay to its receipt increased, and remained the same for the small, immediate reward (Saddoris et al., 2015b). In this way, dopamine functions as a neural "currency" that compares the relative values of two distinct options (Saddoris et al., 2015b; Salzman and Fusi, 2010).

However, it is unknown if NAc core dopamine release is *causally* linked to elements of delay discounting. Previously in our laboratory, dopamine was optically stimulated during "unpreferred" (i.e., delay-predictive or small reward-predictive) cues, in an effort to shift the perceived predicted value of these unpreferred rewards. It was shown that NAc core dopamine release was causally linked to delay, but not magnitude, decision making (Saddoris et al.,

2015b). However, optical stimulation during delay discounting (i.e., that incorporates the integration of delay and magnitude components) was not examined. Previously, cue-evoked NAc dopamine tracked the "preferred" option in delay discounting, such that dopamine during delay-predictive cues declined as the delay to reward increased (Saddoris et al., 2015b). Given these findings, here we hypothesize that elevation of dopamine during the normally less preferred delay-predictive cues will increase the perceived future value of the delayed reward, and will bias rats' choice behavior toward this less-impulsive option. In the current study, we used optogenetics to stimulate dopamine release in the NAc core during cues that predicted the Forced Choice Long Delay (20 s) large reward, to assess whether this dopamine elevation would bias rats' Free Choice behavior toward the long delay (less impulsive) option. Following the long-delay stimulation paradigm, we next stimulated dopamine release during cues that predicted the Forced Choice Short Delay (10 s) large reward, to determine if stimulation would bias Free Choice behavior toward the less-impulsive (delayed) reward during *both* the short delay and subsequent long delay block.

Methods

Animals

Singly-housed *TH::Cre*$^{+/-}$ (n = 9; male n = 3, female n = 6) and littermate control (n = 9; male n = 4, female n = 5) Long Evans rats were approximately 90 to 120 days old, weighing 275-330 g at the start of experiments. Animals were maintained at no less than 85% of pre-experimental body weights by food restriction, except during the post-operative recovery period when food was given ad libitum (Purina Lab Chow). Water was available *ad libitum* throughout the duration of the experiment. Animal procedures were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, and were approved by the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee (IACUC).

Apparatus

Behavioral testing was conducted in 43x43x53 cm Plexiglas chambers housed in sound-blocking boxes (Med Associates, St. Albans, VT) described in detail previously (Saddoris et al., 2011). Briefly, one side of each chamber was equipped with two retractable levers (Coulbourn Instruments, Allentown, PA) 17 cm apart, with a stimulus light 6 cm above each lever. Sucrose pellets (45 mg) were delivered to a food receptacle, which was located equidistantly between the levers. A house light (100 mA) was mounted on the opposite side of the chamber.

Virus Surgery

For all surgical procedures, rats were deeply anesthetized with a ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (10 mg/kg) mixture (i.p.). Following all surgeries, rats

were given an anti-inflammatory medication (meloxicam, 1 mg/kg, s.q.) for two days post-surgery and were allowed access to food and water *ad libitum.*

Rats were first infused with a Cre-induced adeno-associated virus encoding ChR2 with EYFP (AAV5-DIO-ChR2-EYFP) into the VTA (2 injections at AP -5.4 mm, ML ±0.7 mm, DV -8.4 mm from bregma; 2 injections at AP -6.2 mm, ML ±0.7 mm, DV -7.4 mm from bregma; 4 µl total; 1 µl per site using a 2 µl Hamilton syringe). Before infusion, the syringe was held in place for 5 min, and following infusion, held in place for 8 min. The virus was allowed to incubate for at least 8 weeks to permit expression in dopamine neuron terminals.

Training on Delay Discounting Task

Following 1 week recovery from virus surgery, rats were trained to press two distinct levers in which each response was reinforced on a continuous schedule of reinforcement. Reinforced responses resulted in the delivery of a sucrose pellet to a centrally located food cup. Animals were trained to a criterion of 50 presses on each response lever.

Next, rats were trained on the delay discounting task, which was comprised of three trial types. On Forced Choice Delay trials (Figure 1A, left), a cue light was illuminated for 5 seconds followed by extension of two levers. A single press on the associated lever positioned below that cue light resulted in a large reward (three sucrose pellets) delivered after a period of delay. During Forced Choice Immediate trials (Figure 1A, middle), another 5-second cue light signaled that responses on the associated lever resulted in a small (one sucrose pellet) immediate reward. On Free Choice trials (Figure 1A, right), both cue lights illuminated for 5 seconds, signaling that both responses were rewarded based on the contingency of the lever chosen. Importantly, each behavioral session consisted of three blocks of trials: during the first block, the large reward was

presented immediately (no-delay block); in the subsequent block, the delay to large reward was 10 seconds following a lever press (short-delay block); while in the last block, there was a 20-second delay to obtain the large reward (long-delay block). Rats performed 30 trials per block, with 20 Forced Choice (10 of each type) and 10 Free Choice trials. If animals failed to respond within 10 s, both levers retracted and the trial was counted as an omission. Because each trial was a fixed duration (60 s), reward choice did not influence how quickly the rat completed the task (i.e., choosing the small reward did not lead to the next trial quicker).

Once behavior was stable, animals were surgically prepared for optical stimulation of dopamine terminals. Here, optical fibers (200 µm diameter core) coupled to ferrules (2.25 mm diameter, 250 µm bore) were bilaterally implanted over the NAc core (AP +1.4 mm, ML ±2.5 mm (10˚ angle from midline) and DV -6.5 mm from bregma). Optical fibers were secured with dental cement and stainless steel screws.

Optical Stimulation of Dopamine Terminals in the NAc during Delay Discounting Behavior

As demonstrated previously (Saddoris et al., 2015), cue-elicited core dopamine signaling scales preferentially during Forced Choice trials to the large, non-delayed reward on Block 1, and tapers off in Blocks 2 and 3. Thus, increasing dopamine signaling during cue presentation on Short or Long Delay trials may influence how the predicted value of the reward is processed. Subsequently, this altered dopamine signal may bias animals toward preferring the low value (delayed) option when given a choice.

To test this hypothesis, rats' (n = 9 *TH::Cre*$^{+/-}$, n = 9 controls) dopamine terminals were stimulated during the *cue* that predicted the delayed reward option, as described previously (Saddoris et al., 2015; also see Figures 2A and 4A). Here, optical stimulation of NAc core

dopamine terminals was administered during either the 5 s Forced Choice Long Delay or Short Delay cue, which predicted the large but delayed reward option. As in previous studies (Saddoris et al., 2015), no stimulation was given during Free Choice trials, allowing these trials to act as a measure of choice preference independent of stimulation effects. Thus, any alterations to Free Choice behavior resulted from changes in predicted reward value.

Rats underwent 4 total stimulation sessions (20 Hz, 5 ms pulsewidth, 20 mW), two during Long Delay (Block 3; Figure 2A) and two during Short Delay (Block 2; Figure 4A). On sessions between stimulation days, no light was delivered. Therefore, each animal was its own behavioral control during both stimulation and non-stimulation sessions. Stimulation parameters were based on in vivo measurements of dopamine release using FSCV generated by different presentations of laser light on dopamine cells, reported previously (Saddoris et al., 2015). During the stimulation paradigm, rats were connected to patch cables containing an optical fiber (200 μm core, 0.22 NA, ThorLabs). A ferrule connector at the end of the cable was secured to the rat's optical fiber implant with a ceramic sleeve (Precision Fiber Products). These were attached at the other end to an optical commutator (Doric Lenses), which allowed bilateral stimulation of NAc terminals and allowed the animal to move freely. The commutator was connected, via another patch cable, to a 150 mW DPSS 473 nm laser (OEM Laser Systems). Optical stimulation was controlled by a computer running Med PC IV software (Med Associates) that also recorded behavioral events.

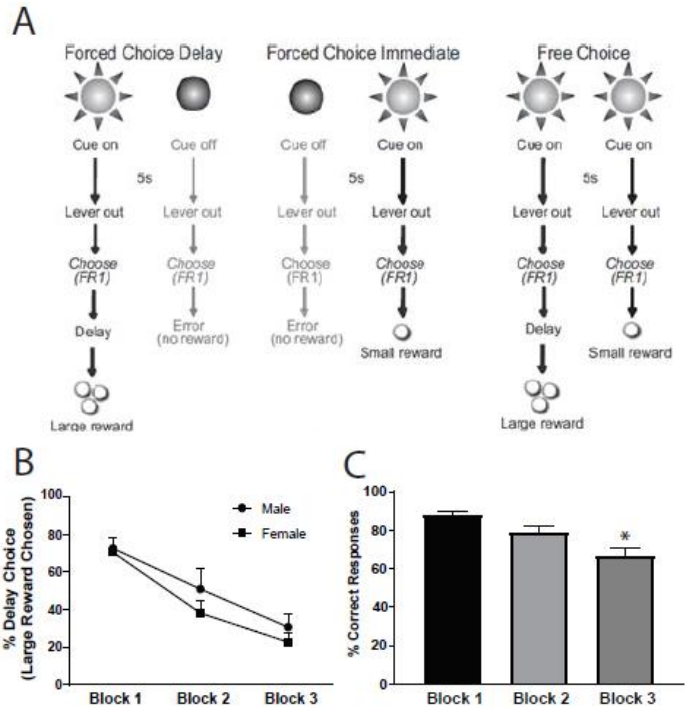

Figure 2.1. Delay discounting task and behavior. **A.** Schematic of delay discounting task. **B.** Baseline percent delay choice (large reward chosen) in male and female rats. **C.** Baseline accuracy (percent correct responses) across all rats.

Intracranial Self Stimulation: Immediately following completion of the delay discounting stimulation paradigm, rats were given access to food *ad libitum* for one week. Then, rats were trained to lever press for optical stimulation of the NAc core (Figure 6A). Here, a houselight illuminated the chamber and a cue light over the right lever indicated the active lever. Animals could lever press for a 5 s bilateral optical stimulation (20 Hz, 5 ms pulsewidth, 20 mW). During the 5 s stimulation period, the cue light extinguished and the lever retracted for 20 s. Animals underwent a total of five 30-minute sessions. Following the fifth session, animals underwent a single 75 min extinction session. Here, lever presses did not result in optical stimulation, and were recorded in 15 minute blocks. Optical self-stimulation was considered "extinguished" after either two 15 minute blocks of no responses or after 75 minutes. Immediately after reaching

extinction criteria, animals received a single 5 s “priming” stimulation, signaling the opportunity to lever press for optical stimulation (20 Hz, 5 ms pulsewidth, 20 mW) for a 45 min reinstatement session.

Histology

Animals were transcardially perfused with physiological saline and a 4% paraformaldehyde solution. Once removed, brains were post-fixed in a 20% sucrose-paraformaldehyde solution. Brains were sectioned coronally (40 μm) and mounted on slides coverslipped with Fluoromount-G mounting medium (Southern Biotech) to determine optical fiber placement and ChR2 expression in the NAc and VTA. Slides were imaged on a confocal microscope to evaluate ChR2 expression and optical fiber placement. A subset of slices was stored in 0.1M PB for immunohistochemistry to verify ChR2-Th co-expression. Slices were washed in Triton-X (0.3% solution in phosphate-buffered saline (PBST) and 0.1M PBS. Slices then were blocked with 1% normal goat serum (Vector Laboratories) with TH polyclonal antibody raised in rabbit (1:1000, Abcam) in 0.3% PBST for 24 hours at room temperature. Then, slices were washed and incubated for 1 hour at room temperature in goat anti-rabbit secondary antibodies conjugated to Alexa Fluor 594 (1:200, Jackson Immunoresearch Laboratories). Next, slices were washed with 0.1M PBS, mounted onto microscope slides, and coverslipped with Fluoromount-G mounting medium (Southern Biotech). Slides were imaged on a confocal microscope to evaluate ChR2 expression, ChR2-Th co-expression, and optical fiber placement. Rats with misplaced optical fibers or poor viral expression were excluded from analysis.

Data Analysis

A Two-Way ANOVA was performed on Free Choice preference to determine sex differences in delay discounting choice behavior. Following this analysis, male and female rats were combined for the remainder of all analyses. Analysis of baseline behavior during the delay discounting task included examination of correct responses, number of errors, and free choice preference. To determine whether rats reliably acquired the task, we evaluated the number of errors and correct responses during Forced Choice trials. One-Way ANOVA were used to compare accuracy (percentage rewarded trials) and percentage errors during Forced Choice trials, as well as free choice preference.

To confirm that laser stimulation did not alter the ability to perform the task, the percentage of correct responses on forced trials was compared across blocks, across stimulation and non-stimulation sessions, and between genetic groups using a Three-Way ANOVA with Bonferroni's test for multiple comparisons. Further, to evaluate if stimulation of dopamine terminals during the delayed option on Forced Choice trials was sufficient to shift behavioral responding during Free Choice trials, we compared response allocation and response latency on Free Choice trials across blocks, across stimulation and non-stimulation sessions, and between genetic groups using a Three-Way ANOVA with Bonferroni's test for multiple comparisons.

For optical self-stimulation tests, we evaluated behavioral responding across days using a Two-Way ANOVA with Bonferroni's test for multiple comparisons to determine if there was a significant increase in lever press behavior in the *TH::Cre*$^{+/-}$ animals compared to controls. Paired t-tests were used to compare rats' extinction and reinstatement behavior to their last day of self-stimulation.

All analysis were considered significant at $\alpha=0.05$. Statistical and graphical analyses were performed using GraphPad Prism 6.0 for Windows (GraphPad Software, La Jolla, CA).

Results

Baseline Delay Discounting Behavior

We first compared male and female rats' baseline free choice preference to examine any sex differences in impulsive choice (i.e., choosing the large delay reward less) (Figure 1B). There was a significant effect of block ($F_{2, 30} = 22.37$, $p < 0.0001$), and a Bonferroni's post-hoc test indicated that all rats discounted the value of the large delay reward ($p < 0.05$). Importantly, there was no effect of sex on delay discounting ($F_{1, 15} = 1.275$, $p = 0.2766$) or a block x sex interaction ($F_{2, 30} = 0.2122$, $p = 0.8100$). As such, male and female rats were combined for the remainder of analyses. All rats accurately discriminated the different reward values during the delay discounting task (Figure 1C). On Forced Choice Trials, rats' accuracy was dependent on the delay to reward receipt ($F_{2, 34} = 21.19$, $p < 0.0001$), and made more errors in Block 3 compared to Blocks 1 and 2 ($p < 0.0001$). During Free Choice trials, rats' preference for the large reward decreased as the delay to large reward receipt increased across blocks ($F_{2, 34} = 27.99$, $p < 0.0001$).

Optical Stimulation of Terminal Dopamine during Forced Choice Long Delay Cues does not alter Choice Behavior

First, we stimulated NAc core dopamine terminals during Forced Choice Long Delay cues (Figure 2A). We examined if optical stimulation cues altered rats' accuracy across blocks, between genetic groups, and between stimulation sessions (Figure 2B, stimulation days; Figure 2C, non-stimulation days). There was a significant effect of block ($F_{2, 32} = 26.636$, $p < 0.0001$) and session ($F_{1, 16} = 7.758$, $p = 0.013$). This indicated that there was a difference in accuracy between stimulation days and non-stimulation sessions, though this effect was largely driven by lower accuracy in controls during stimulation sessions. A Bonferroni post-hoc test on the block

main effect revealed that all rats made more errors on Block 3 compared to Block 1 and 2 ($p < 0.05$), regardless of genetic group or stimulation session. There were no other significant main effects or interactions ($F < 3.142$, $p > 0.05$).

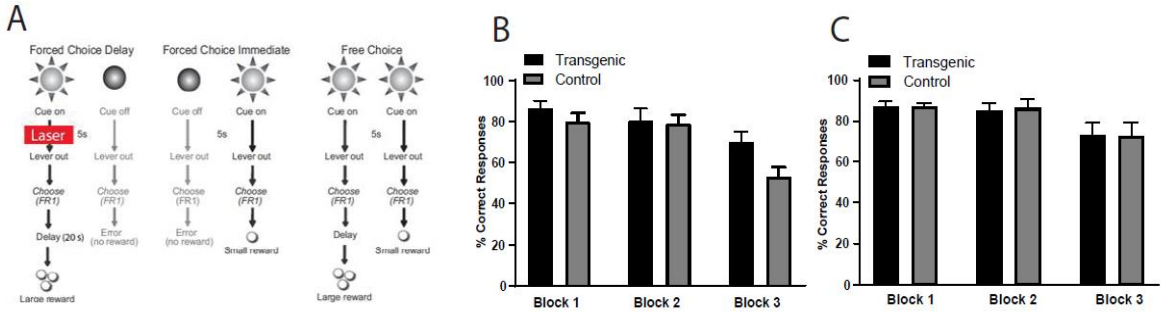

Figure 2.2. Schematic of Long Delay stimulation task design and accuracy on Forced Choice trials. **A.** Long Delay stimulation task schematic. Rats received optical stimulation during the 5 s cue that predicted the Forced Choice Long Delay cue. Free Choice trials were then used as a measure of choice bias, independent of stimulation. **B.** Accuracy (percent correct responses) between transgenic and control rats during stimulation sessions. **C.** Accuracy (percent correct responses) between transgenic and control rats during non-stimulation sessions.

We next examined choice selection on Free Choice trials across blocks, between genetic groups, and within stimulation sessions (Figure 3A). There was a significant effect of block ($F_{1.401,\ 22.421} = 68.33$, $p < 0.0001$), indicating that all rats discounted the value of the large delay reward regardless of genetic group or stimulation sessions ($p < 0.05$ for all block comparisons). There was a significant session x group interaction ($F_{1,16} = 4.583$, $p = 0.049$) and a significant block x session x group interaction ($F_{2,32} = 4.668$, $p = 0.017$). However, this effect was driven by a significant interaction during Block 2 between group and session ($F_{1,\ 16} = 9.14$, $p = 0.0081$). Importantly, there was no effect during Block 3, when we stimulated dopamine terminals during Forced Choice trials ($F_{1,\ 16} = 0.06531$, $p = 0.8015$). There were no other significant main effects

or interactions ($F < 1.347$, $p > 0.05$). As such, there was no effect of optical stimulation on Long Delay choice preference.

We next examined if there was an effect of stimulation on Free Choice reaction time. For both delay and immediate choice, there was a significant effect of block (Delay: $F_{2,32} = 21.478$, $p < 0.0001$, Figure 3B (stimulation sessions shown); Immediate: $F_{2, 32} = 6.596$, $p = 0.004$, Figure 3C (stimulation sessions shown)). For Delay Choice, reaction time increased across all blocks ($p < 0.05$), and for Immediate Choice, reaction time was fastest during Block 1, but no different during Block 2 and 3 ($p < 0.05$). There were no other main effects or interactions for response latency (Delay: $F < 3.374$; Immediate: $F < 3.077$; $p > 0.05$). As such, there was no significant effect of stimulation on Free Choice reaction time.

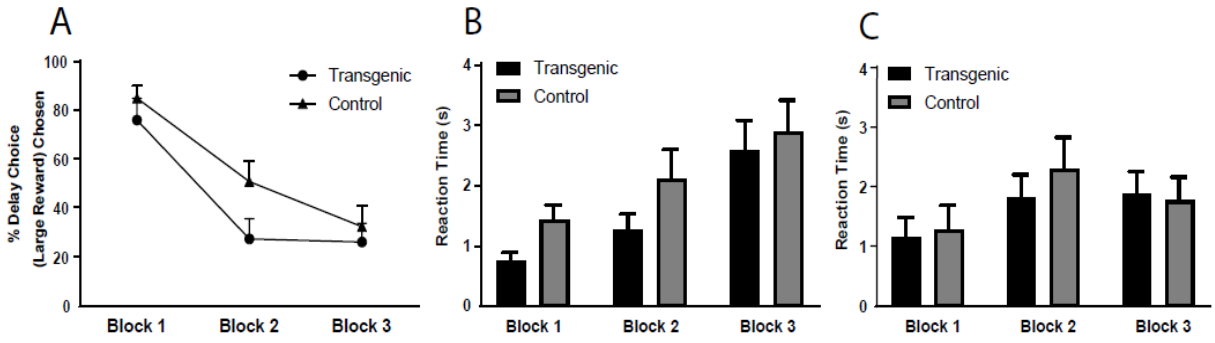

Figure 2.3. Free choice behavior and reaction time during Long Delay stimulation paradigm. **A.** Percent delay choice (large reward) chosen across blocks between transgenic and control groups. Data from stimulation sessions shown. **B.** Reaction time for Free Choice delay selection between transgenic and control groups on stimulation sessions. **C.** Reaction time for Free Choice immediate selection between transgenic and control groups on stimulation sessions.

Optical Stimulation of Terminal Dopamine during Forced Choice Short Delay Cues does not alter Choice Behavior

Following the Long Delay stimulation paradigm, we then stimulated dopamine terminals during Forced Choice Short Delay cues (Figure 4A). As in the Long Delay experiment, we examined correct responses (accuracy) during Forced Choice trials across blocks, between genetic groups and between stimulation sessions (Figure 4B, stimulation sessions; Figure 4C, non-stimulation sessions). There were significant main effects of block ($F_{2, 32} = 14.207$, $p < 0.0001$) and session ($F_{1, 16} = 6.415$, $p = 0.022$), indicating a difference in accuracy between stimulation days and non-stimulation days, and a Bonferroni post-hoc test on the block main effect revealed that rats made more errors on Block 3 compared to Blocks 1 and 2 ($p < 0.05$). There were no other significant main effects or interactions ($F < 2.25$, $p > 0.05$).

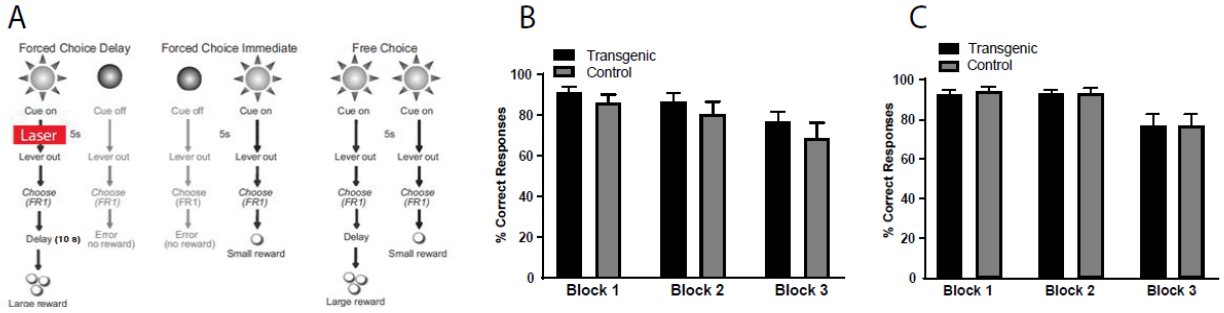

Figure 2.4. Schematic of Short Delay stimulation task design and accuracy on Forced Choice trials. **A.** Short Delay stimulation task schematic. Rats received optical stimulation during the 5 s cue that predicted the Forced Choice Short Delay cue. Free Choice trials were then used as a measure of choice bias, independent of stimulation. **B.** Accuracy (percent correct responses) between transgenic and control rats during stimulation sessions. **C.** Accuracy (percent correct responses) between transgenic and control rats during non-stimulation sessions.

We next examined Free Choice behavior across blocks, between groups, and within stimulation sessions (Figure 5A). We found an effect of block ($F_{2, 32} = 97.115$, $p < 0.0001$), but no other significant effects or interactions ($F < 2.757$, $p > 0.05$). As in the Long Delay

stimulation experiment, all rats discounted the value of the large delay reward regardless of genetic group or stimulation day ($p < 0.05$ for all block comparisons), but optical stimulation during Forced Choice Short Delay cues was not sufficient to bias subsequent Free Choice behavior.

We also examined reaction time during Free Choice behavior. There was a significant main effect of block **(**Delay: $F_{1.314,\ 21.027} = 20.692$, $p < 0.0001$, Figure 5B (stimulation session shown); Immediate: $F_{1.495,\ 23.914} = 13.684$, $p < 0.0001$, Figure 5C (stimulation session shown). A Bonferroni post-hoc test showed that reaction time increased across blocks for both delay and immediate selections ($p < 0.05$). When rats chose the immediate choice, there was a significant main effect of session ($F_{1,\ 16} = 10.132$, $p = 0.006$), indicating a difference between stimulation and non-stimulation days. There were no other effects or interactions for Free Choice reaction times (Delay: $F < 3.286$; Immediate: $F < 2.475$; $p > 0.05$).

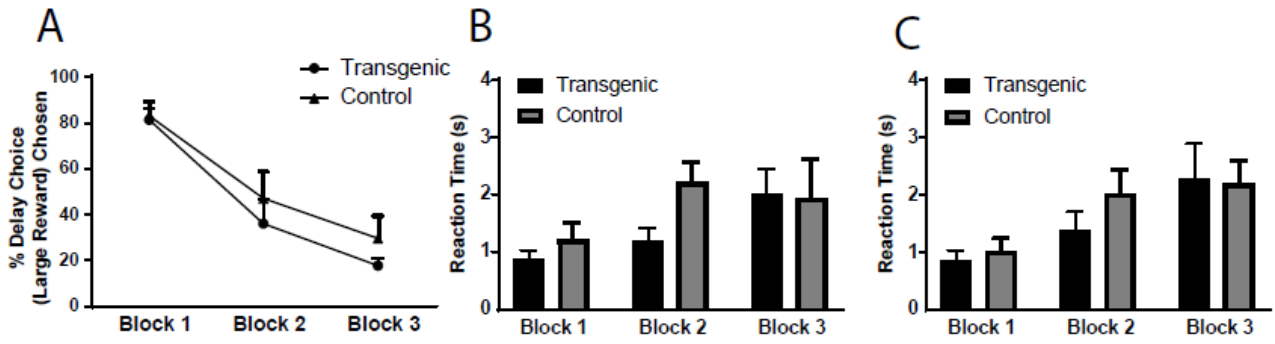

Figure 2.5. Free choice behavior and reaction time during Short Delay stimulation paradigm. **A.** Percent delay choice (large reward) chosen across blocks between transgenic and control groups. Data from stimulation sessions shown. **B.** Reaction time for Free Choice delay selection between transgenic and control groups on stimulation sessions. **C.** Reaction time for Free Choice immediate selection between transgenic and control groups on stimulation sessions.

Optical Self-stimulation of Dopamine Terminals in the NAc core

One week following the delay discounting paradigm, rats underwent 5 self-stimulation sessions in which they were allowed to lever press for 5 s optical stimulation in the NAc core, followed by an extinction and reinstatement session (Figure 6A). We found a main effect of genetic group ($F_{1, 17} = 13.13$, $p = 0.0021$), a main effect of session ($F_{10, 170} = 22.37$, $p < 0.0001$) and a significant group x session interaction ($F_{10, 170} = 5.20$, $p < 0.0001$), indicating *TH::Cre*$^{+/-}$ animals responded significantly more than controls during self-stimulation sessions. *TH::Cre*$^{+/-}$ rats extinguished responding, with significantly fewer presses at the end of extinction compared to the final training session ($t = 4.654$, $p = 0.0012$). When laser stimulation was resumed following extinction, *TH::Cre*$^{+/-}$ rats rapidly reinstated lever pressing to pre-extinction levels ($t = 1.703$, $p = 0.1228$). Optical fiber tip locations within the NAc core are shown in Figure 7A, and representative co-localization of ChR2 and tyrosine hydroxylase is shown in Figure 7B.

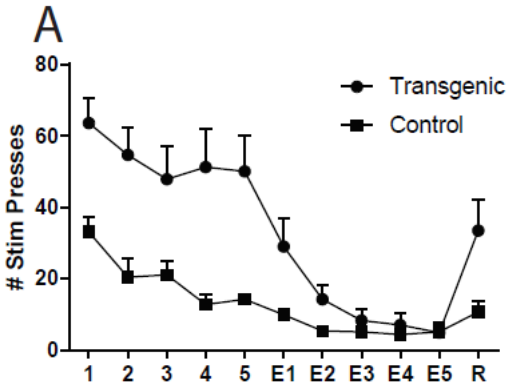

Figure 2.6. Optical self-stimulation, extinction, and reinstatement. **A.** Lever press responses for 5 s optical stimulation (20 Hz, 5 ms pulsewidth, 20 mW) for all animals in both groups. Laser was active on days 1-5. Following the last day of self-stimulation, rats underwent an extinction session during which laser was off (E1-E5), followed by a reinstatement session (R) in which presses were reinforced again with optical stimulation.

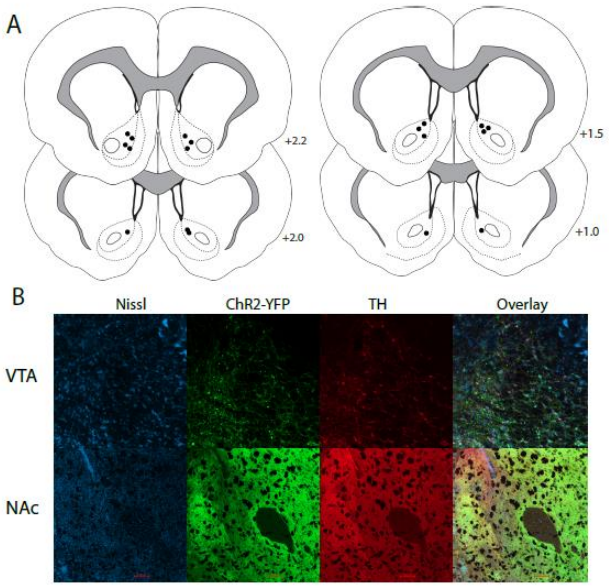

Figure 2.7. Histology. **A.** Optical fiber tip placements in transgenic animals. **B.** Top: Representative co-localization of TH and ChR2-EYFP expression in VTA cell bodies and projection neurons. Bottom: Representative co-localization of TH and ChR2-EYFP expression in NAc dopamine terminals.

Discussion

Our lab has previously reported that NAc core dopamine release tracks reward-predictive cues during delay discounting (Saddoris et al., 2015b). Initially, dopamine release was highest to cues signaling the large, more preferred option when there was no delay between obtaining the large (3 pellet) versus small (1 pellet) food pellet reward. However, as the delay to the large reward increased, dopamine signaling decreased in concentration and no longer scaled to reward predictive cues, while remaining the same for the Small/Immediate option. As such, dopamine release during predictive cues tracked the preferred value of reward and was modulated by increasing delay costs as the task progressed.

However, it remained unknown how dopamine signaling causally influences delay discounting behavior. The current study assessed if rapid dopamine signaling during delay-predictive cues causally influences delay discounting behavior. Here, we used optogenetics in *TH::Cre*$^{+/-}$ rats to manipulate NAc core dopamine release during Forced Delay cues of our delay discounting task. We examined Free Choice behavior in the absence of stimulation to test if elevation of dopamine was sufficient to increase selection of the delayed (i.e., less impulsive) option. We found that optical stimulation during either Forced Long Delay or Forced Short Delay cues was not sufficient to bias subsequent rats' preference toward these delayed reward options when given a choice in the absence of stimulation. These findings indicate that rapid dopamine signaling in the NAc core tracks, but is not causally linked to, value-based predictive strategies in delay discounting.

The current findings are surprising, as the NAc core is strongly linked to delay discounting behavior. Lesions to the NAc core alter delay discounting behavior, such that rats increase preference for small, immediate rewards (i.e., increase impulsive choice) (Cardinal and

Howes 2005; Cardinal et al., 2003; Cardinal et al., 2001; Bezzina et al., 2007; Galtress and Kirkpatrick, 2010; Pothuizen et al., 2005). NAc core lesions also impair learning of instrumental responses to delayed reinforcement (Cardinal and Cheung 2005), and diminish sensitivity to changes in delay (Acheson et al., 2006). Further, as discussed above, NAc core dopamine dynamically tracks reward-predictive cues during delay discounting, functioning as a "neural currency" that encodes the shift in reward value (Saddoris et al., 2015b). However, despite this extensive literature, the current findings indicate that elevation of dopamine signaling during delay-predictive cues is not sufficient to bias choice behavior toward a less-impulsive option.

One possible reason why NAc core dopamine release is not causally linked to delay discounting is the complexity of our delay discounting task. Previously, optical stimulation of the NAc core during "nonpreferred" reward-predictive cues was sufficient to bias delay (subjective), but not magnitude (objective) choice (Saddoris et al., 2015b). In each task, one aspect of reward value was kept constant. For instance, in the delay task, reward magnitude remained at 1 sucrose pellet, whereas in the magnitude task, large or small rewards were delivered immediately (i.e., no delay). As such, optical manipulation of dopamine was sufficient to bias a simple subjective (but not objective) decision making process. However, the current study's delay discounting task manipulated both delay and magnitude values simultaneously, such that the value of the large magnitude reward decreased due to increasing delay costs. We stimulated dopamine terminals during delay-predictive cues which predicted a reward that dynamically shifted in value across the task. As such, manipulation of dopamine terminal release during delay cues may not have been sufficient to "overcome" the increased value assigned to the small, immediate reward during Blocks 2 and 3.

Further, stimulation of *only* the VTA-NAc core circuit may not have been sufficient to shift choice behavior, as numerous brain regions other than the NAc core contribute to delay discounting behavior. The prefrontal cortex, including the medial (mPFC) and dorsolateral (dlPFC) regions, is heavily involved in delay discounting. Activity in the dorsolateral prefrontal cortex is correlated with subjective value of delayed rewards (Peters and Buchel 2011; Kim et al., 2008, Weber and Huettel 2008). Further, addicts demonstrate heightened delay discounting (i.e., increased impulsive choice), and have lessened dlPFC activity compared to controls during delay discounting tasks (Hoffman al., 2008; Monterosso et al., 2007; Wang et al., 2017). Conversely, stimulation of the dlPFC (He et al., 2016; Cho et al., 2013) and mPFC (Cho et al., 2015) decreases impulsive choice. Another brain region involved in delay discounting is the amygdala, which may play a role in increasing the value of smaller, immediate rewards (Everitt et al., 1999) as a function of the individual's current emotional state (Bechara, 2005; Morrison and Salzman, 2010; Gupta et al., 2011). Amygdala activation was associated with individual delay discounting preferences, such that increased amygdala activity predicted steeper discounting (i.e., more impulsive behavior) (Hoffman et al., 2008; Pine et al., 2010). As such, future studies may be able to bias delay discounting choice behavior via manipulation of multiple circuits, including NAc core, prefrontal and amygdala regions.

It is important to note that the lack of choice bias was not due to failure of the optogenetics technique. One week following the delay discounting stimulation task, rats underwent five 30-minute sessions in which they could self-administer optical stimulation. The last session was followed by an extinction session, in which lever presses did not result in stimulation. Afterward, a reinstatement session allowed rats to resume pressing for optical stimulation. Notably, transgenic rats self-stimulated extinguished responding, and reinstated

lever pressing, whereas controls did not. We were able to replicate this finding from Saddoris et al. (2015b), indicating that optical stimulation was sufficient to produce robust dopamine release in NAc core terminals and to drive goal-directed behavior.

In conclusion, the data presented in this aim indicate that optical stimulation of dopamine release in the NAc core during delay-predictive cues was not sufficient to bias delay discounting choice behavior toward the less impulsive option. Previously, NAc core dopamine release dynamically tracked the preferred cue during our delay discounting task (Saddoris et al., 2015b). However, the current study indicates that this dopamine release is not causally linked to delay discounting. Importantly, rats lever pressed for optical stimulation, indicating that dopamine release is occuring in NAc core terminals and this release is sufficient to drive goal-directed behavior. This work contributes to the understanding of optogenetic technique and the overall role of dopamine release in the NAc during delay discounting.

CHAPTER 3

PRELIMBIC CORTICAL NEURONS TRACK PREFERRED REWARD VALUE AND REFLECT IMPULSIVE CHOICE DURING DELAY DISCOUNTING BEHAVIOR

Introduction

Delay discounting is a decision-making process in which the subjective value of a reward decreases based on the amount of delay to its receipt (Roesch et al., 2006; Roesch et al., 2007; Roesch and Bryden 2011; Tedford et al., 2015). When the delay becomes too long, individuals will shift their preference from a large delayed reward to a smaller, immediate one. In this way, delay discounting serves as an index of impulsivity, such that it measures the amount of time it takes for individuals to "lose patience" and choose an immediate reward.

Importantly, heightened delay discounting (increased impulsivity) is a common symptom of substance use disorders (Hoffman et al., 2008; Jentsch and Taylor 1999; Crews and Boettiger 2009; Coffey et al., 2003). That is, addicts will over-value immediate rewards (drugs of abuse), failing to reflect on the future negative consequences of their decisions (Bickel et al., 1999; Evenden 1999; Heyman 1996; Poulos et al., 1995; Bechara et al., 2005; Ernst and Paulus 2005). Clinically, drug abusers demonstrate greater impulsivity during delay discounting tasks (Hoffman et al., 2008; Coffey et al., 2003; Jones et al., 2015). For example, drugs such as cocaine decrease the "breakpoint" at which individuals shift to the small, immediate reward (Jentsch and Taylor 1999; Coffey et al., 2003; Monterosso et al., 2001; Bechara et al., 2002; Dalley et al., 2008). These findings are corroborated in rodent models, as prior exposure to cocaine increases impulsivity during decision making tasks involving delay, magnitude, and

delay discounting (Roesch et al., 2007; Mitchell et al., 2014; Mendez et al., 2010). In addition to drug addiction, impulsivity is a characteristic of numerous psychiatric disorders, including attention deficit hyperactivity disorder, schizophrenia, depression, and borderline personality disorder (Barkley et al., 2001; Heerey et al., 2007; Imhoff et al., 2014; Lawrence et al., 2010).

Delay discounting recruits several brain regions, including the prefrontal cortex (PFC). Specifically, the human dorsolateral prefrontal cortex (dlPFC) is implicated in impulsive behavior (Cho et al., 2010; Cho et al., 2012; Cho et al., 2013), and drug addicts demonstrate altered dlPFC function reflective of increased impulsive behavior (Hoffman et al., 2008; Monterosso et al., 2001; Boettiger et al., 2007). Notably, the dlPFC is activated during delay discounting (Weber and Huettel, 2008; Xu et al., 2009; Liu et al., 2012), and neurons in the dlPFC change activity based on changing (discounted) values (Kim et al., 2008). Further, disruption of the left dlPFC via rTMS led to more impulsive choices during a delay discounting task (Figner et al., 2010).

The rodent prelimbic cortex (PrL) is considered to be the homologue of the human dlPFC (Uylings et al., 2003), and is heavily interconnected with other brain regions implicated in delay discounting, such as the nucleus accumbens (NAc) core (Cardinal et al., 2001; Cardinal and Howes 2005) and basolateral amygdala (BLA) (Winstanley et al. 2009; Churchwell et al., 2009). The PrL is generally associated with goal-directed behaviors (Moorman and Aston-Jones 2015; Killcross and Coutureau 2003; Balleine and O'Doherty 2010; Tran-Tu-Yen et al., 2009; Smith and Graybiel 2013), and is causally implicated in impulsivity, as inactivation of the PrL induced spontaneous and premature behaviors (Ishikawa et al. 2008; Jonkman et al., 2009; Narayanan et al., 2006). Further, inactivation of the medial PFC, which includes the PrL area, increased impulsive choice in a delay discounting task (Churchwell et al., 2009). Further, individual

differences in impulsivity may be reflected in PrL function, as high PrL D2 mRNA expression predicted a higher preference for large, delayed rewards (i.e., a less impulsive choice) (Simon et al., 2013).

However, it remains unknown how neurons in the PrL specifically encode information related to delay discounting and impulsive choice. Here, we used electrophysiological recording techniques in male and female rats to examine PrL neuronal responses during discrete elements of a delay discounting task that varied subjective costs (delay to reward) across the session. Rats could choose between a small reward (1 sugar pellet) available immediately versus a large reward (3 sugar pellets) available after either no delay (0 s), a short delay (10 s), or a long delay (20 s). We examined neuronal activity during cues that predicted the availability of either the small/immediate or large/delay reward, as well as during the responses for reward, and reward delivery. We found that PrL neuron populations were phasic to cue presentations, lever presses, and reward delivery. Notably, phasic neuron populations were selectively phasic to either large/delay, small/immediate, or both trial types. These "selective neurons" tracked preferred cue-outcome associations and reward value during Free Choice trials. Further, these tracking dynamics differed based on rats' impulsivity, with high impulsive rats demonstrating more small/immediate-selective neurons as the task progressed. Taken together, the findings indicate a unique role of PrL neurons in tracking preferred reward outcome during delay discounting and impulsive choice.

Methods

Animals

Singly-housed male (n=10) and female (n=9) Long Evans rats were approximately 90 to 120 days old, weighing 275-330 g at the start of experiments. Animals were maintained at no less than 85% of pre-experimental body weights by food restriction, except during the post-operative recovery period when food was given ad libitum (Purina Lab Chow). Water was available *ad libitum* throughout the duration of the experiment. Animal procedures were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, and were approved by the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee (IACUC).

Apparatus

Behavioral testing was conducted in 43x43x53 cm Plexiglas chambers housed in sound-blocking boxes (Med Associates, St. Albans, VT) described in detail previously (Saddoris et al., 2011). Briefly, one side of each chamber was equipped with two retractable levers (Coulbourn Instruments, Allentown, PA) 17 cm apart, with a stimulus light 6 cm above each lever. Sucrose pellets (45 mg) were delivered to a food receptacle, which was located equidistantly between the levers. A house light (100 mA) was mounted on the opposite side of the chamber.

Surgical Procedures

Rats were deeply anesthetized with a ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (10 mg/kg) mixture (i.p.), and were given an anti-inflammatory medication (meloxicam, 1 mg/kg, s.q.) prior to surgery and again for two days post-surgery. Microwire

electrode arrays consisting of 8 microwires (50 um in diameter, described previously in Carelli et al., 2000) were bilaterally implanted in the prelimbic cortex (AP +2.7, ±ML 0.6, DV -4.0 from bregma) and secured in place with dental cement and stainless steel screws. Following surgery, rats were allowed to recover for 1 week and had access to food and water *ad libitum* during this time.

Behavioral Procedures

All behavioral experiments were conducted at least 1 week post surgery, and rats underwent similar pretraining before beginning each behavioral task. Here, rats were trained to press two distinct levers in which each response was reinforced on a continuous schedule of reinforcement. Reinforced responses resulted in the delivery of a sucrose pellet to a centrally located food cup. Animals were trained to a criterion of 50 presses on each response lever.

Next, rats were trained on the delay discounting task, comprised of three trial types. On Forced Choice Delay trials (Figure 1A, left), a cue light was illuminated for 5 seconds followed by extension of two levers. A single press on the associated lever positioned below that cue light resulted in a large reward (three sucrose pellets) delivered after a period of delay, described below. During Forced Choice Immediate trials (Figure 1A, middle), another 5-second cue light signaled that responses on the associated lever resulted in a small (one sucrose pellet) immediate reward. On Free Choice trials (Figure 1A, right), both cue lights illuminated for 5 seconds, signaling that both responses were rewarded based on the contingency of the lever chosen. Each behavioral session consisted of three blocks of trials: during Block 1, the large reward was presented immediately (no-delay block); in Block 2, the delay to large reward was 10 seconds following a lever press (short-delay block); while in Block 3, there was a 20-second delay to

obtain the large reward (long-delay block). Rats performed 30 trials per block (20 Forced Choice (10 of each type) and 10 Free Choice trials). If animals failed to respond within 10 s, both levers retracted and the trial was counted as an omission. Because each trial was a fixed duration (60 s), reward choice did not influence how quickly the rat completed the task (i.e., choosing the small reward did not lead to the next trial quicker).

Once trained on the task (4-6 weeks), rats underwent electrophysiological recording to determine PrL neuronal activity during discrete task events.

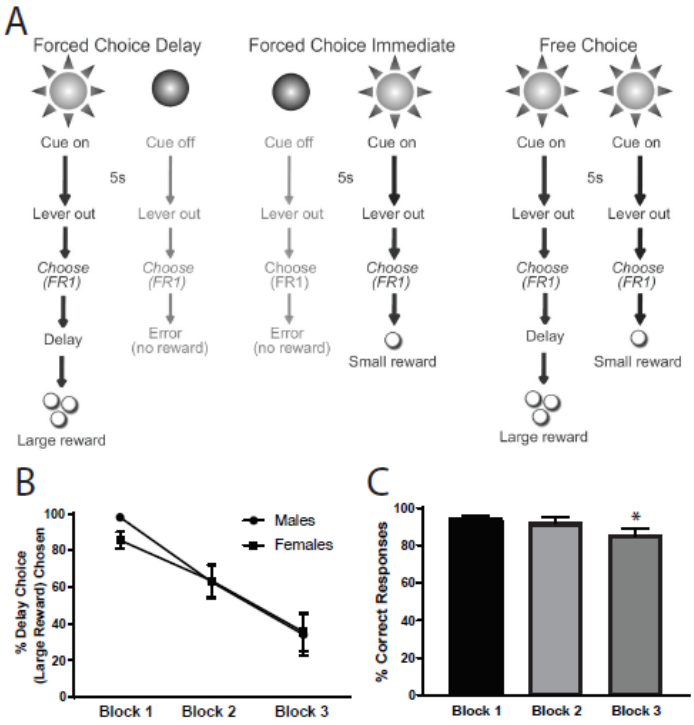

Figure 3.1. Delay discounting task and behavior. **A.** Schematic of delay discounting task. **B.** Baseline percent delay choice (large reward chosen) in male and female rats. **C.** Baseline accuracy (percent correct responses) across all rats.

Electrophysiological Recordings

Electrophysiological procedures have been described in detail previously (Day et al., 2011; West et al., 2014). Before the start of each session, the subject was connected to a flexible

recording cable attached to a commutator (Med Associates), which allowed virtually unrestrained movement within the chamber. The head stage of each recording cable contained 16 miniature unity-gain field effect transistors. Neurons were recorded differentially between each active and the inactive (reference) electrode from the permanently implanted microwires. The inactive electrode was examined before the start of the session to verify the absence of neuronal spike activity and served as the differential electrode for other electrodes with cell activity. Online isolation and discrimination of neuronal activity was accomplished using a commercially available neurophysiological system (multichannel acquisition processor (MAP) system; Plexon). Multiple window-discrimination modules and high-speed analog-to-digital signal processing in conjunction with computer software enabled isolation of neuronal signals based on waveform analysis. The neurophysiological system incorporated an array of digital signal processors (DSPs) for continuous spike recognition. The DSPs provided a continuous parallel digital output of neuronal spike events to a Pentium computer. Another computer processed operant chamber input and output (Med Associates) and sent digital outputs corresponding to each event to the MAP box to be time stamped along with the neural data. Discrimination of individual waveforms began by setting a threshold level (well above background noise) for each wire. Units detected had to display peak voltage at least 20% greater than baseline. Individual waveforms corresponding to a single cell were discriminated using template analysis procedures and time–voltage boxes provided by the neurophysiological software system (MAP system; Plexon). Cell recognition and sorting was finalized after the experiment using the Offline Sorter program (Plexon). This allowed neuronal data to be further assessed based on the principle component analysis of the waveforms, cell firing characteristics such as autocorrelograms and interspike interval distribution to ensure that putative cells showed biologically appropriate firing

refractory periods, and crosscorrelograms to ensure that multiple cells recorded on the same wires showed firing independently of each other. Waveform and spontaneous firing rates were examined to identify putative glutamatergic pyramidal neurons in the PrL and exclude GABAergic interneurons from analysis (Moorman and Aston-Jones, 2015; Devilbiss et al., 2017).

Determining phasic response patterns of PrL neurons

First, neurons that exhibited increased or decreased activity relative to three behavioral events including cue presentation, lever press responses, and reward delivery were characterized. Changes in neuronal firing patterns relative to each behavioral event were analyzed by constructing peri-event histograms (PEHs) and raster displays (bin width, 250 ms) surrounding each event using commercially available software (Neuroexplorer for Windows version 4.034, Plexon, Inc). For this analysis, each cell was examined for changes in activity relative to cue onset (0 to 5 s following cue presentation), following the lever press (0 to 2.5 s after response completion), and/or following reward delivery (0 to 2.5 s after reward delivery). Here, individual units were categorized as showing either a decrease (inhibition) or an increase (excitation) in firing rate compared to baseline (i.e., termed 'phasic') or no difference from baseline (termed 'nonphasic'). Specifically, cells were classified as phasic if during one of these epochs the firing rate was greater than or less than the 95% confidence interval projected from the baseline period (10 to 0 s before cue onset or 10 to −2.5 s before a lever press) for at least one 250 ms time bin. This confidence interval was selected such that only robust responses were categorized as excitatory or inhibitory following established procedures (Day et al., 2011; West et al., 2014). Some neurons in this analysis exhibited low baseline firing rates, and the 95% CI included zero.

Where this was the case, inhibitions were assigned if $e_0 > 2*b_0$ (where e_0 = the number of consecutive 0 spikes/s time bins during the event epoch and b_0 = the maximal number of consecutive 0 spikes/s time bins during the baseline period). Units that exhibited both excitations and inhibitions within the same epoch were classified by the response that was most proximal to the event in question, unless the most proximal response was ongoing when the event occurred. Importantly, the above analysis was completed separately for Forced Choice Large/Delay and Small/Immediate trial types, as well as during Free Choice trials when rats eventually chose the Large/Delay or Small/Immediate reward. This allowed determination as to how many neurons responded to each cue, lever press, and reward event in each block and trial type. However, the resultant categories of neuronal response profiles were not mutually exclusive. For example, a neuron could potentially exhibit an excitation to the Forced Large/Delay cue and an inhibition to the Forced Small/Immediate, or an excitation to both Forced Large/Delay and Small/Immediate cues.

Phasic Neurons Selective to Task Events

Phasic neurons were further characterized as "selective" to discrete task events (cue, press, or reward) during either Forced or Free Choice trials. These selective phasic neurons were classified as one of three types. The first type was "large/delay selective." These neurons were phasic (either excitatory or inhibitory) during either the cue, lever press, or reward delivery during Forced Choice large/delay trials, or when rats chose the large/delay option during Free Choice and were non-phasic during small/immediate trials. The second type was "small/immediate selective." These neurons were phasic during task events (cue, press, reward delivery) on Force Choice small/immediate trials, or when rats chose the small/immediate option

during Free Choice trials, and were non-phasic during large/delay trials. Finally, other neurons were selective to "both" trial types. That is, these neurons were phasic during both large/delay and small/immediate trials, regardless of rats' Free Choice selection. Importantly, because Forced and Free Choice trials were analyzed separately, selective neuron populations were not mutually exclusive to Forced or Free trial types. For example, a neuron selective to Forced Choice Large/Delay trials could also be selective to *Free* Choice Large/Delay trials.

Data Analysis

First, to determine if sex differences existed during the task, percent delay choice chosen across blocks (discounting curve) was analyzed using a 2-Way ANOVA (block x gender). Because there were no differences in any measure between sexes, all rats were combined for further analyses. Overall accuracy (delay and immediate trials combined) and percent delay choice chosen across blocks (discounting curve) were analyzed using a 1-Way ANOVA.

Rats were separated into high impulsive (n = 9, HI) and low impulsive (n = 9, LI) groups using a median split on average discounting score (the average of delay choice across blocks). Percent correct responses (accuracy) on Forced Choice trials were analyzed using a 1-Way ANOVA for HI and LI rats. Percent delay choice chosen across blocks (discounting curve) was analyzed using a 2-Way ANOVA (block x impulsivity trait).

Differences in the frequency or proportion of neuronal responses across different trial types were examined using Chi-squared or Fisher's exact test. For population activity, the firing rate of each cell was normalized by a Z-score transformation (using baseline mean and standard deviation) to reduce the potential influence of baseline differences in this analysis. Peak cell

firing was analyzed using t-tests. All analyses were considered significant at $\alpha = 0.05$. Statistical and graphical analysis was conducted in Graphpad Prism 4 (Graphpad software, Inc.).

Histology

Upon completion of the experiment, rats were deeply anesthetized with a ketamine and xylazine mixture (100 mg/kg and 10 mg/kg, i.p., respectively). In order to mark the placement of electrode tips, a 13.5 μA current was passed through each microwire electrode for 5 seconds. Brains were removed and placed into a formalin solution with 20% sucrose and 3% potassium ferricyanide, after which 40 μm coronal brain sections were sliced and mounted on slides. The addition of potassium ferricyanide allowed for a blue reaction corresponding to the location of individual electrode tips, which was assessed by visual examination of successive coronal sections. Placement of an electrode tip within the PrL was determined by examining the relative position of observable reaction product to visual landmarks and anatomical organization of the rodent mPFC represented in a stereotaxic atlas (Paxinos and Watson, 2005).

Results

Behavior

Animals reliably acquired the delay discounting task. We first examined if any gender differences existed for task accuracy or choice preference. There was no significant difference in accuracy ($F_{1, 17}$) = 0.3102, p = 0.5848, data not shown) or delay choice (discounting curve) ($F_{1,17}$) = 0.1442, p = 0.7089; Fig 1B) between males and females. As such, for the remainder of this analysis, males and females were combined. All rats responded accurately to Forced Choice trials and made significantly more errors on Block 3 Forced Choice trials ($F_{2, 36}$ = 4.499, p = 0.0180; Fig 1C).

Task-Related Neuronal Activity

A total of 125 individual PrL neurons were recorded from 19 animals (n = 10 male, n = 9 female) during behavioral performance. Waveform analysis revealed that 115 neurons were putative pyramidal (i.e., glutamatergic) neurons, and the remaining 10 were excluded from analysis.

Task cues evoked changes in firing rate in a large population of PrL neurons. Of the 115 neurons, 96 cells (83.5%) exhibited phasic changes in firing rate during at least one Forced Choice cue presentation and 88 (76.5%) exhibited changes during at least one Free Choice cue presentation. These phasic neurons were classified as cue-activated (Figure 2A) or cue-inhibited (Figure 2B). During Forced Choice cue presentations, there was no significant difference between the percentage of phasic neurons across blocks for either large/delay trials or small/immediate trials (p > 0.05 for all analyses; data not shown). However, during the Free Choice cue presentations that preceded rats' choice of the Large/Delay option, PrL neurons

exhibited dynamic changes in percent phasic neurons across blocks, shown in Figure 2C, top. Here, significantly more phasic neurons were observed during Block 1 as compared to later blocks ($X^2 = 16.34$, $p = 0.0003$). There was no difference between phasic percentage during Free Choice cue presentations preceding the small/immediate option ($X^2 = 0.42$, $p = 0.8106$; Figure 2C, bottom). These results indicate that phasic PrL neurons preferentially encode the best available predicted option in the *absence* of delay (i.e., preferring a large magnitude versus small magnitude option). However, once the delay to receipt increases, the general population of phasic PrL neurons do not differentially encode or track cue presentation.

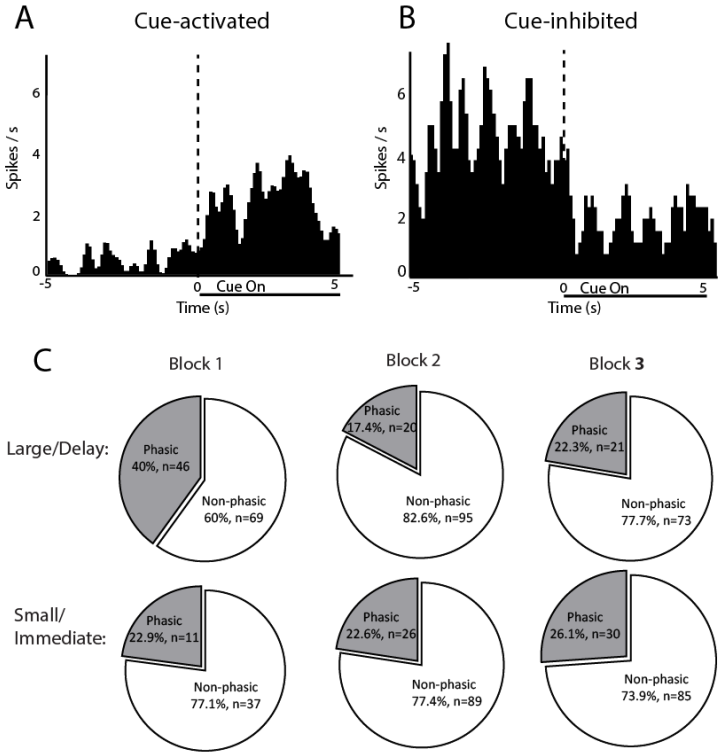

Figure 3.2. PrL neurons respond to cue presentations. **A.** Peri-event histogram (PEH) of a representative cue-activated neuron. Data are aligned to cue onset (time 0, dashed line); cue duration indicated by horizontal lines below PEHs here and in B. B. PEH of a representative cue-inhibited neuron. C. Pie charts illustrating proportion of neurons that exhibited phasic responses during Free Choice cue presentations, based upon whether the rat subsequently chose the large/delay reward (top row) or small/immediate reward (bottom row).

Another subset of neurons demonstrated changes in firing rate following lever press. Of 115 neurons, 86 (74.8%) exhibited changes following Forced Choice lever presses, and 62 (53.9%) exhibited changes following Free Choice lever presses. Phasic neurons were classified as press-activated (Figure 3A) or press-inhibited (Figure 3B). On *Forced* Choice blocks, there was no significant difference between the percentage of phasic neurons during the lever press across blocks for either Large/Delay or Small/Immediate trials ($p > 0.05$ for all analyses; data not shown). Likewise, during Free Choice Large/Delay blocks, there was no difference in phasic lever press related neurons across blocks ($X^2 = 3.02$, $p = 0.2209$; Figure 3C, top). However, during Free Choice Small/Immediate trials, there were more phasic neurons to the press during Block 3 compared to the other blocks ($X^2 = 8.92$, $p = 0.0115$; Figure 3C, bottom row). This finding indicates that during goal-oriented behavior (i.e., lever press), PrL neurons dynamically track the 'preferred' *action* as delay to reward increases, but only for the small/immediate option. Importantly, this suggests that PrL neurons function to update (i.e., increase) the value of initially nonpreferred actions, and promote goal-oriented behavior toward this outcome.

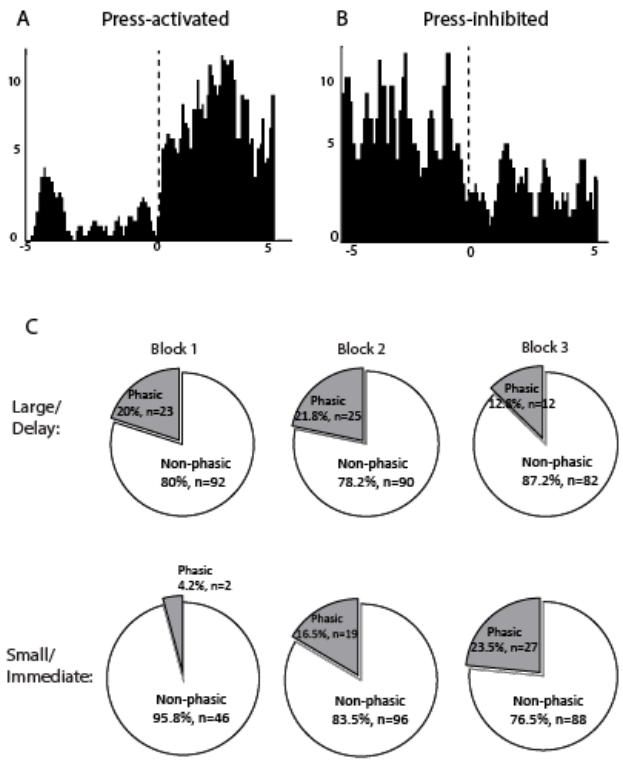

Figure 3.3. PrL neurons respond to lever press. **A.** PEH showing a representative press-activated neuron. Data are aligned to lever press (time 0, dashed line) here and in B. B. PEH of a representative press-inhibited neuron. C. Pie charts illustrating the proportion of neurons that exhibited phasic responses during Free Choice lever presses for either the large/delay reward (top row) or small/immediate reward (bottom row).

Finally, a third subset of neurons exhibited firing rate changes following reward delivery, with 83 out of 115 (72.2%) exhibiting changes following Forced Choice reward delivery and 66 (57.4%) exhibited changes following Free Choice reward delivery. Phasic neurons were classified as reward-activated (Figure 4A) or press-inhibited (Figure 4B). Similar to cue and lever press responsive cells, there was no significant differences across blocks on Forced Choice trials ($p < 0.05$ for all analyses; data not shown). In contrast, on Free Choice Small/Immediate reward delivery, there were more phasic neurons during Block 3 compared to the other blocks ($X^2 = 8.92$, $p = 0.0115$; Figure 4C), but no difference in Free Choice Large/Delay reward

delivery across blocks (X^2 = 3.45, p = 0.1781; Figure 4C). Thus, similar to cue and lever press encoding, phasic PrL neuron activity dynamically shifts in reward processing across blocks for Free Choice, but not Forced Choice, trials. Similar to our lever press findings, this may indicate that phasic PrL neurons preferentially assign greater value to the small/immediate reward outcome as the delay to large reward receipt increases. Further, these neurons appear to promote the selection and consumption of smaller rewards once the delay to the large option becomes intolerable.

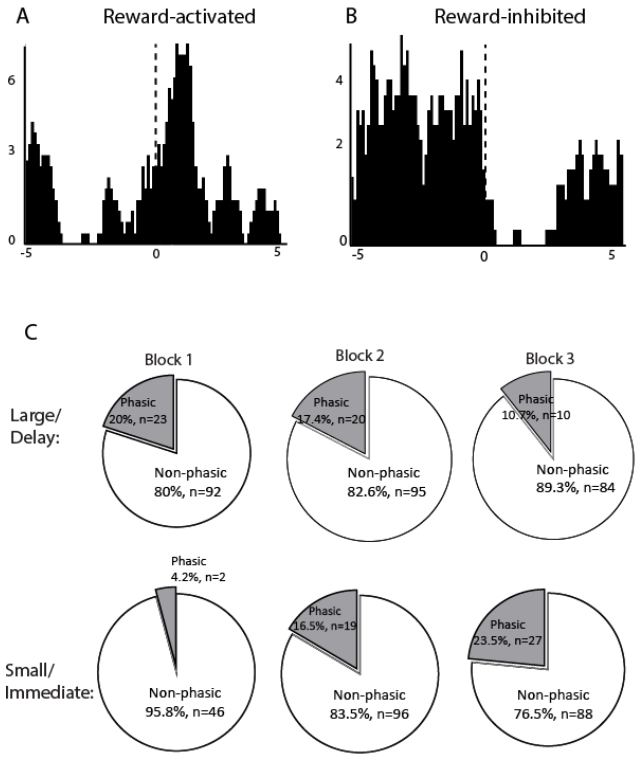

Figure 3.4. PrL neurons respond to reward delivery. **A.** PEH showing a representative reward-activated neuron. Data are aligned to reward delivery (time 0, dashed line) here and in B. B. PEH of a representative reward-inhibited neuron. C. Pie charts illustrating the proportion of neurons that exhibited phasic responses during Free Choice reward delivery for either the large/delay reward (top row) or small/immediate reward (bottom row).

Examination of Strength of the Neural Signal to Cues, Lever press and Rewards

The above analysis shows population neural responses wherein we combined excitatory and inhibitory neurons into 'phasic' cells, illustrated in pie charts. Here, neurons with a phasic change in activity were further divided into subgroups based on whether firing rate *increased* or *decreased* during a given epoch (cue, lever press or reward). A complete analysis of neuronal response counts by task, event, and response direction are shown in Table 1 (Forced Choice trials) and Table 2 (Free Choice trials). To examine if differences existed in the strength of the neural signal, we compared peak activity during the 5 s cue period prior to lever extension, as well as during lever press or reward delivery epochs in each block of the delay discounting task. A similar analysis was completed for minimal (trough) firing rates for inhibitory neurons. Population-level comparisons did not yield significant differences in maximal peak increases in firing rate for excitations (paired t-tests, $p > 0.05$ for all comparisons) or minimal trough activity (inhibitions) during any epoch (paired t-tests, $p > 0.05$ for all comparisons).

	Cue		Lever Press		Reward	
Trial Type	EXC	INH	EXC	INH	EXC	INH
Block 1						
Small/Immediate	28 (24.3)	7(6.1)	16 (13.9)	14(12.2)	16 (13.9)	14(12.2)
Large/Delay	37 (32.2)	8 (7.0)	21 (18.3)	9(7.8)	21 (18.3)	10 (8.7)
Block 2						
Small/Immediate	23 (20.0)	12(10.4)	15(13.0)	6(5.2)	15(13.0)	6(5.2)
Large/Delay	29(25.2)	7(6.1)	17(14.8)	13(11.3)	18(15.7)	7(6.1)
Block 3						
Small/Immediate	24 (20.9)	10(8.7)	19(16.5)	7(6.1)	19(16.5)	7(6.1)
Large/Delay	24 (20.9)	11(9.6)	16 (13.9)	15(13.0)	14(12.2)	7(6.1)

Table 3.1. Percent phasic neurons during Forced Choice task events. Numbers expressed as count (percentage of total).

	Cue		Lever Press		Reward	
Trial Type	EXC	INH	EXC	INH	EXC	INH
Block 1						
Small/Immediate	11(22.9)	0(0)	2(4.2)	0(0)	2(4.2)	0(0)
Large/Delay	37(32.2)	9(7.8)	16 (13.9)	7(6.1)	16 (13.9)	7(6.1)
Block 2						
Small/Immediate	25(21.7)	1(0.9)	17(14.8)	2(1.7)	17(14.8)	2(1.7)
Large/Delay	16 (13.9)	4(3.5)	19(16.5)	6(5.2)	20(17.4)	0(0)
Block 3						
Small/Immediate	19(16.5)	11(9.6)	21 (18.3)	6(5.2)	21 (18.3)	6(5.2)
Large/Delay	19(20.2)	2(2.1)	10(10.6)	2(2.1)	10(10.6)	0(0)

Table 3.2. Percent phasic neurons during Free Choice task events. Numbers expressed as count (percentage of total).

Distinct PrL Neurons Selectively Encode Information Related to Discounted Choice

A substantial proportion of PrL neurons exhibited event-selective (cue, press, reward) excitations or inhibitions *across* large/delay versus small/immediate trials. Examples of cue-selective activity are shown for representative excitatory neurons in Figure 4. We classified these responses into three separate types. “Large/delay selective” neurons exhibited a phasic response during the large/delay cue, press, or reward (Figure 4A). “Small/immediate selective” neurons exhibited a phasic response during the small/immediate cue, press, or reward (Figure 4B). “Both selective” neurons demonstrated phasic responses during both large/delay and small/immediate cues, press, and reward delivery (4C). During Free Choice trials, when both cue lights were presented, we labeled “large/delay” cue-selective neurons when the rat chose the large/delay option, and “small/immediate” cue-selective neurons when the rat chose the

small/immediate option. Thus, during Free Choice trials, cue-selective neurons were phasic during the time when the rat was deciding to make its choice.

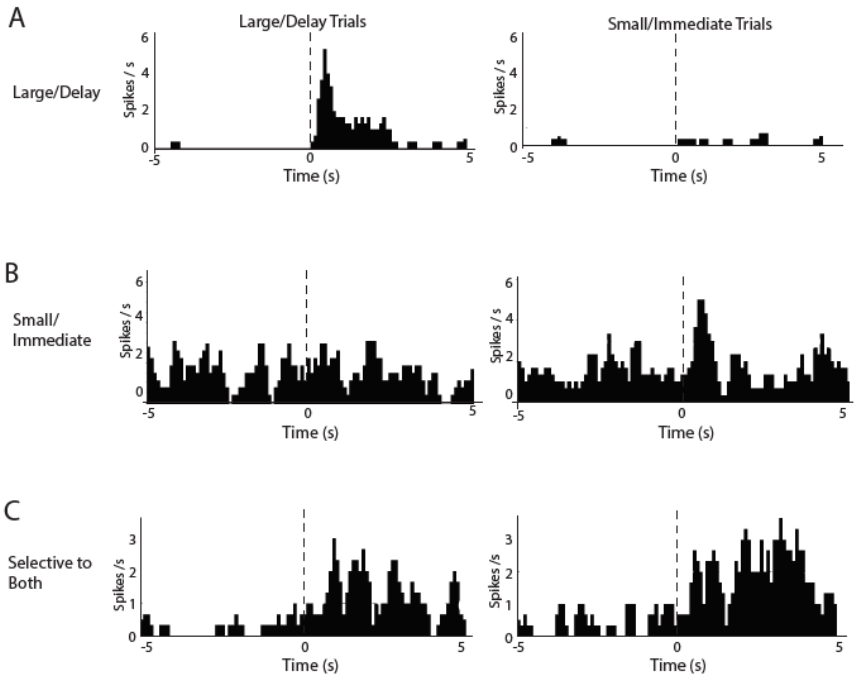

Figure 3.5. Subsets of PrL cue-responsive neurons exhibit distinct properties of neural coding. **A.** PEH of a representative large/delay selective neuron during cue presentation (left, phasic) and the same neuron during small/immediate cue presentation (right, nonphasic). Data are aligned to cue presentation (time 0, dashed line); duration indicated by horizontal line below PEHs here and in B and C. B. PEH of a representative small/immediate selective neuron during cue presentation (right, phasic) and the same neuron during large/delay cue presentation (left, nonphasic). C. PEH of a representative neuron selective to both large/delay (left) and small/immediate (right) cue presentation.

Notably, the percentage of Free Choice cue-selective phasic neuron populations shifted from "large/delay preferring" to "small/immediate preferring" across blocks ($X^2 = 30.03$, $p < 0.0001$, Figure 6A). The same phenomenon was observed in Free Choice press- and reward-selective populations, with neurons shifting from "large/delay" to "small/immediate" selective across blocks (Press: $X^2 = 31.96$, $p < 0.0001$, Figure 6B; Reward: $X^2 = 30.1$, $p < 0.0001$, Figure 6C). Interestingly, Forced Choice phasic selective neuron populations did not track "preferred"

cues, presses, or reward deliveries across blocks. (Cue: $X^2 = 1.48$; Press: $X^2 = 4.38$; Reward: $X^2 = 6.31$; $p > 0.05$ for all analyses; Fig. 6 D-F.) These findings indicate that PrL neurons reflect the changes in both predicted and outcome reward value as delay to reward increases in the Free Choice (but not Forced Choice) trials. That is, these neurons tracked and updated the "preferred" option in each Free Choice block when given a choice between an immediate and delayed reward.

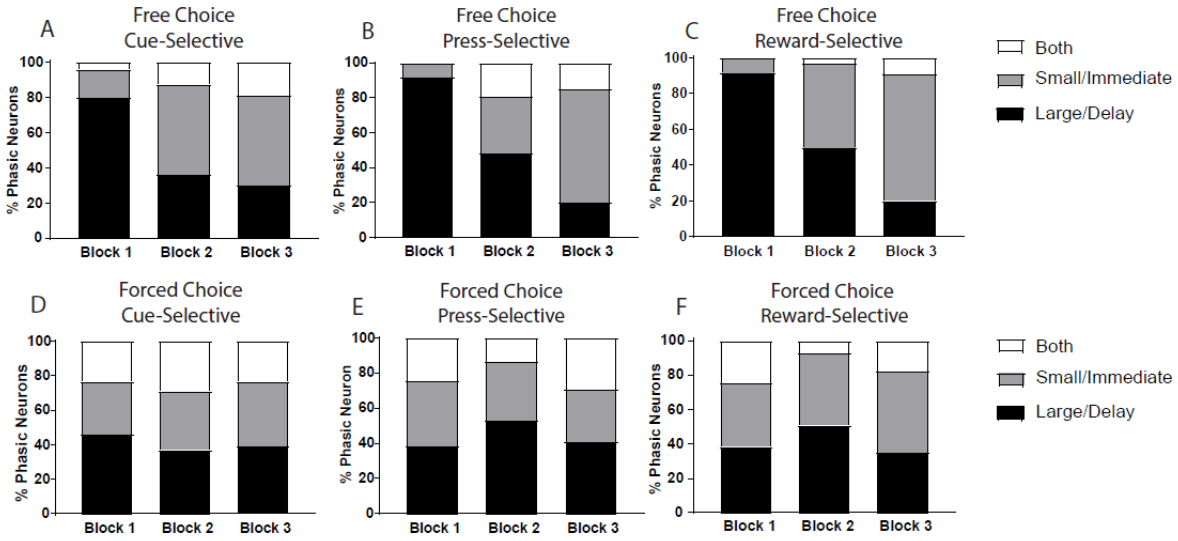

Figure 3.6. Proportions of event-related (cue, press, reward) selective neurons dynamically decline across Free Choice (top) but not Forced Choice (bottom) blocks. **A.** Proportion of phasically active selective neurons to Free Choice cue presentation. **B.** Proportion of phasically selective neurons to Free Choice lever press. C. Proportion of phasically selective neurons to Free Choice reward delivery. D. Proportion of phasically active selective neurons to Forced Choice cue presentation. **E.** Proportion of phasically selective neurons to Forced Choice lever press. F. Proportion of phasically selective neurons to Forced Choice reward delivery.

Differences in Behavior in High versus Low Impulsive Rats

We next separated rats into high impulsive (n = 9, HI) and low impulsive (n = 9, LI) groups using a median split on average discounting score (the average of delay choice across blocks; Figure 7A). HI and LI rats significantly differed in delay choice behavior across blocks ($F_{2,32}$ = 47.27, p < 0.0001) and between impulsivity traits ($F_{1,16}$ = 59.3, p < 0.0001), with a significant interaction between impulsivity and block ($F_{2,32}$ = 6.775, p = 0.035). Both HI (Figure 7B) and LI rats (Figure 7C) were less accurate on Block 3 trials ($F_{2,32}$ = 4.205, p = 0.0239). However, there was no effect of impulsivity ($F_{1,16}$ =1.235, p = 0.2828) or a block x impulsivity interaction ($F_{2,32}$ = 0.9905, p = 0.3825) on accuracy. As such, impulsivity did not alter accuracy on Free Choice trials.

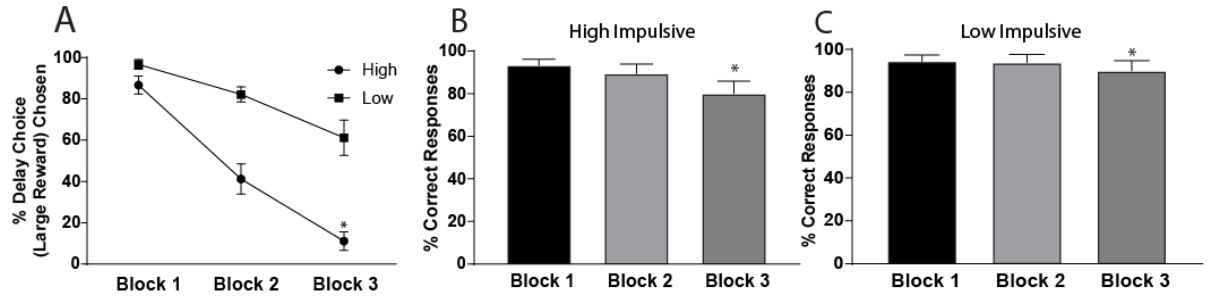

Figure 3.7. Differentiation of rats' impulsivity. **A.** Baseline percent delay choice (large reward chosen) in high versus low impulsive rats. **B.** Accuracy (percent correct responses) in high impulsive rats on recording day. C. Accuracy (percent correct responses) in low impulsive rats on recording day.

Selective Neuron Population Dynamics Differ between High and Low Impulsive Rats

During Free Choice cue presentations, both HI and LI rats' selective neuronal populations shifted from "large/delay selective" to "small/immediate selective" across blocks (HI: p = 0.0013, Figure 8A; LI: p = 0.0006, Figure 8B; Fisher's exact). This indicated that the proportion of selective neurons reflected all rats' *eventual*, preferred Free Choice option. In

Block 1, there was no difference in selective neuron proportions *between* HI and LI rats ($p > 0.05$, Fisher's exact). However, there was a difference between HI and LI rats in Block 2 ($p = 0.0457$, Fisher's exact) and Block 3 ($p = 0.0037$, Fisher's exact). These findings indicated that HI rats (Figure 8A) exhibited significantly more "small/immediate" cue-selective neurons than LI rats (Figure 8B) in Blocks 2 and 3.

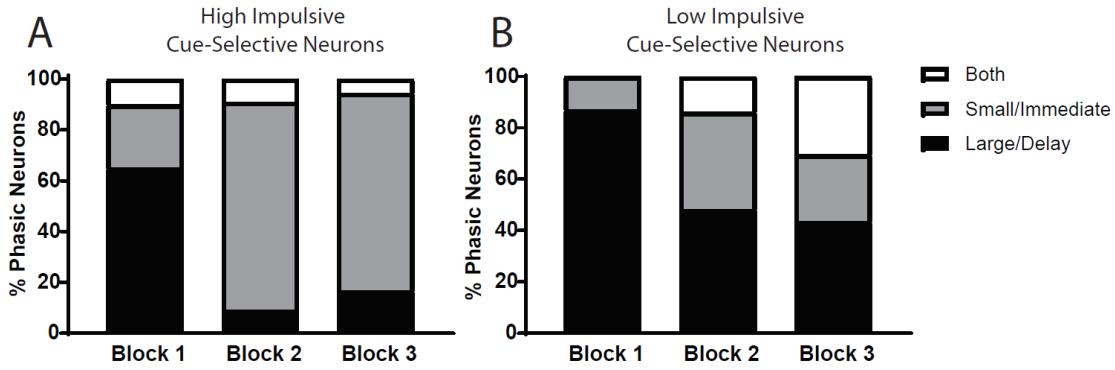

Figure 3.8. Proportions of cue-selective phasic neurons across blocks in high versus low impulsive rats. **A.** Proportion of phasically selective neurons to Free Choice cue presentation in high impulsive rats across blocks. **B.** Proportion of phasically selective neurons to Free Choice cue presentation in low impulsive rats across blocks.

During Free Choice lever presses, both HI and LI rats' selective neuronal populations also shifted from "large/delay selective" to "small/immediate selective" across blocks (HI: $p = 0.0004$, Figure 9A; LI: $p = 0.0016$, Figure 9B; Fisher's exact). However, there was no difference *between* HI and LI rats' selective neuron proportions in any block ($p > 0.05$, Fisher's exact). This indicated that while the proportion of selective neurons reflected all rats' preferred Free Choice lever press selection, there was no difference between HI and LI rats' neuron populations as delay to reward increased. As such, inherent impulsivity did not seem to play a role in how the PrL tracks animals' action-oriented behavior (i.e., pressing the lever).

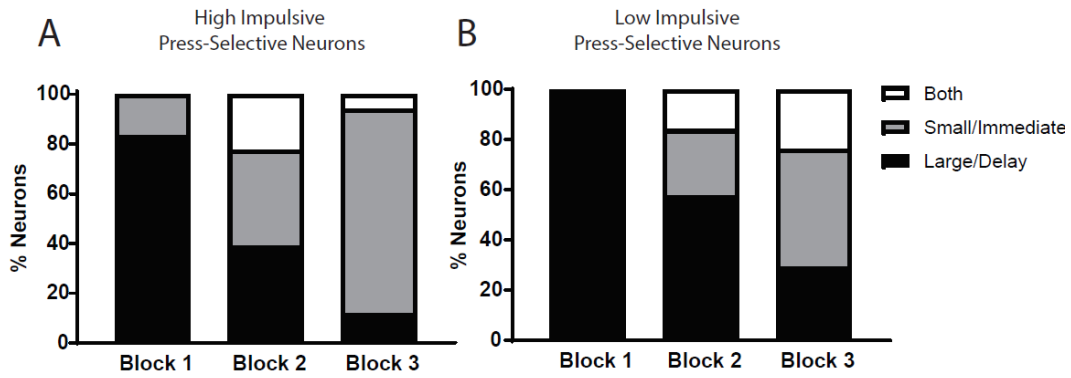

Figure 3.9. Proportions of press-selective phasic neurons across blocks in high versus low impulsive rats. **A.** Proportion of phasically selective neurons to Free Choice lever press in high impulsive rats across blocks. **B.** Proportion of phasically selective neurons to Free Choice lever press in low impulsive rats across blocks.

During Free Choice reward delivery, both HI and LI rats' selective neuronal populations shifted from "large/delay selective" to "small/immediate selective" across blocks (HI: $p = 0.00002$, Figure 10A; LI: $p = 0.001$, Figure 10B; Fisher's exact). This indicated that the proportion of selective neurons reflected all rats' preferred Free Choice outcome. There was no difference *between* HI and LI rats' selective neuron proportions in Block 1 ($p = 0.22$, Fisher's exact) and a nearly significant trend in Block 2 ($p = 0.06$, Fisher's exact). During Block 3, HI rats demonstrated significantly more "small/immediate selective" neurons than LI rats ($p = 0.007$, Fisher's exact). These findings indicated that HI rats (Figure 10A) exhibited significantly more "small/immediate" reward-selective neurons than LI rats (Figure 10B) when the delay to receipt was largest. Taken together, these results indicate that inherent impulsivity drives how the PrL tracks both the predicted (cue, Figure 8) and outcome (reward, Figure 10) value of preferred rewards during delay discounting, but not the press for reward (Figure 9).

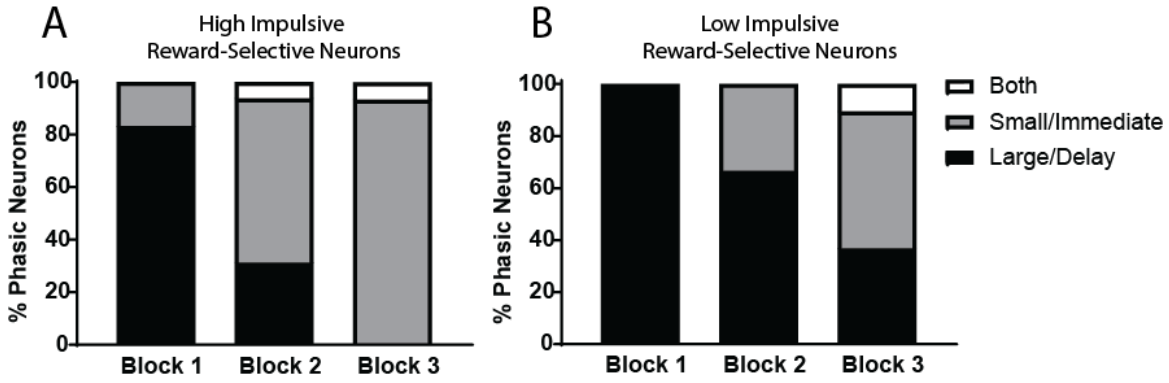

Figure 3.10. Proportions of reward-selective phasic neurons across blocks in high versus low impulsive rats. **A.** Proportion of phasically selective neurons to Free Choice reward delivery in high impulsive rats across blocks. **B.** Proportion of phasically selective neurons to Free Choice reward delivery in low impulsive rats across blocks.

For all Forced Choice task events (cue, press, reward), there was no shift in selective neuron populations across blocks in HI or LI rats ($p > 0.05$, Fisher's exact, data not shown). Further, for all Forced Choice task events, there was no difference *between* HI and LI rats' selective neuron populations within any block ($p > 0.05$, Fisher's exact, data not shown). As such, rats' impulsivity had no influence on selective neurons during Forced Choice trials.

Histological Reconstruction of Electrode Placements

A total of 304 microelectrodes (16 per animal) were implanted bilaterally and aimed at the PrL. On test days, 140 neurons were recorded across 130 electrodes. Neurons in the infralimbic cortex ($n = 15$) were excluded from analysis. Of the remaining 125 PrL neurons, waveform analysis revealed that 115 were putative pyramidal (i.e., glutamatergic) neurons, and the remaining 10 were excluded from analysis. Electrode tip placements in the PrL are shown in Figure 11.

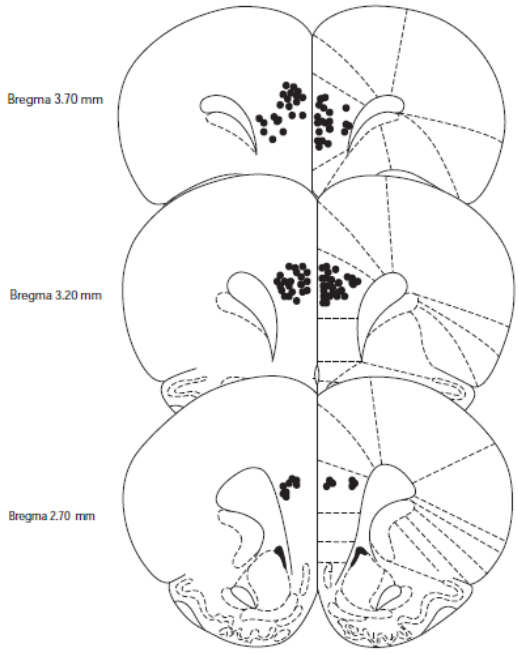

Figure 3.11. Electrode tip placement in the PrL.

Discussion

The current study assessed how PrL pyramidal neurons encode and track delay discounting behavior. Here, we used *in-vivo* electrophysiology techniques to record neuronal activity in the PrL during discrete elements of our delay discounting task (i.e., cue presentation, lever press, and reward delivery). PrL neurons exhibited phasic responses to cue presentations, lever presses, and reward delivery during Forced and Free Choice trials. We found that distinct phasic neuron populations selectively tracked Free Choice (but not Forced Choice) task events across blocks, indicating that the PrL recruits distinct cellular groups that encode shifts in preferred reward value. Notably, highly impulsive rats experienced a greater shift in the proportion of small/immediate cue-selective neurons as delay to reward increased. The results indicate that PrL neurons track preferred choice in delay discounting, and that neural activity in this region may influence impulsive choice.

Distinct PrL neurons were selectively phasic to discrete task events and trial types. That is, unique neuron groups were activated during task events on either large/delay, small/immediate, or both trial types. The proportion of these selective neuronal populations shifted across blocks, from more large/delay selective neurons in Block 1 to more small/immediate selective neurons in Block 3. Notably, this shift occurred only in Free Choice trials, in which rats were given a choice between two options that shifted in value across blocks. Importantly, Free Choice trials present both cue lights simultaneously for 5 s, allowing rats to choose the preferred option, and so we classified cue-selective neurons based on rats' eventual choice. For example, when rats chose the large/delay option, phasic neurons during the preceding cue period were labeled as "large/delay" selective. Not only did selective neuron populations

track the predicted value of reward during cue presentation, but they also tracked preferred choice selection (lever press) and reward outcome (reward delivery).

Similarly, in a different delay discounting task, VTA neural activity encoded the perceived value of the best available option (Roesch et al., 2007). NAc core dopamine release also tracked cues that predicted the preferred reward option during Free Choice behavior, though this effect was not present in the Long Delay block (Saddoris et al., 2015). Notably, the current study found no shift in selective neuron populations during Forced Choice trials, which presented a selection "rule" rather than a choice. Conversely, VTA neural activity (Roesch etal., 2007) and NAc core dopamine release (Saddoris et al., 2015b) *did* track preferred reward value during Forced Choice cue presentations. While the aforementioned studies were examining dopamine's role in delay discounting, here we find that putative PrL glutamatergic neurons only encode Free Choice behavior.

We also found that this shift is dependent on inherent impulsivity. Rats that were more impulsive (e.g., a steeper shift toward the small/immediate option) also showed a greater shift in the proportion of small/immediate neurons across blocks compared to low impulsive rats. Notably, this shift occurred in cue-activated neurons, indicating that the PrL may contribute to differences in impulsive choice by differentially encoding *predicted* preferred reward values. High impulsive rats also had more small/immediate selective neurons during Block 3 reward delivery, indicating that the PrL may also contribute to how reward outcome is processed in high versus low impulsive individuals. Further, these neuronal differences were reflected in rats' behavioral responses, as high impulsive rats dramatically shifted preference toward the small/immediate option during Free Choice trials, whereas low impulsive rats equally preferred both options during Free Choice trials. Taken together, neuronal activity in the PrL reflected

impulsive behavior and predicted inherent impulsivity toward both anticipated and eventual reward outcomes.

This experiment was the first to examine neuronal activity in the PrL during delay discounting. However, the PrL has been previously shown to play a role in the neural circuitry controlling delay discounting and impulsive choice. Inactivation of the PrL produces spontaneous and premature behaviors (Ishikawa et al. 2008; Jonkman et al., 2009; Narayanan et al., 2006), and increased impulsive choice (i.e., small/immediate reward selection) during delay discounting (Churchwell et al., 2009). The PrL also selectively enervates the nucleus accumbens (NAc) core) with glutamatertic input (Krettek and Price 1977; Sesack et al., 1989; Basar et al., 2010; Pinto and Sesack 2000; Brog et al., 1993). The NAc core has been extensively implicated in delay discounting, as lesions increase impulsive choice (Cardinal et al., 2001; Pothuizen et al. 2005; Bezzina et al. 2007, 2008; da Costa Araújo et al. 2009, 2010). Further, dopamine release in this region tracks preferred reward value during Force Choice trials (Saddoris et al., 2015b; Moschak and Carelli, 2017). However, it is unclear how PrL glutamatergic input contributes to delay discounting. The data presented here may begin to uncover how PrL neuronal activity influences the NAc core during delay discounting behavior.

The findings may suggest that more impulsive individuals have different PrL physiology than low impulsive individuals. Previously, individual differences in impulsivity have been shown to play a role in the neurobiology of delay discounting, although most rodent studies have focused on the NAc core. Inactivation of the NAc core decreased delay discounting in low impulsive rats (Moschak and Mitchell, 2014), while high impulsive rats exhibited less dopamine release in this region during delay discounting compared to less impulsive rats (Moschak and Carelli 2017). Both systemic administration of amphetamine and *in vitro* electrical stimulation of

NAc elicit less DA release in high impulsive individuals (Diergaarde et al., 2008; Zeeb et al., 2016). Conversely, less impulsive rats demonstrate more dopamine release than high impulsive rats (Diergaarde et al., 2008). In the PrL, the number of D2 receptors was correlated with greater preference for the large/delay reward (Simon et al., 2013), and activation of these receptors impairs rats' ability to shift reward preference during delay discounting (St. Onge et al., 2011). The current study demonstrates that high versus low impulsive individuals differ in how PrL neuron populations encode predicted reward value and eventual outcome. As such, individual differences in PrL physiology may contribute to impulsive choice during delay discounting.

It is essential to understand the neurobiology of impulsivity, as this behavior appears to have a bidirectional relationship with disorders such as drug addiction. Heightened delay discounting is often a result of substance abuse, but inherently high impulsivity may also *predict* future drug use (Bickel et al., 1999; Evenden 1999; Heyman 1996; Poulos et al., 1995; Bechara 2005; Ernst and Paulus 2005; Hoffman et al., 2008; Coffey et al., 2003; Jones et al., 2015; Jentsch and Taylor, 1999; Winstanley et al, 2010). Drug abusers demonstrate greater impulsivity during delay discounting tasks (Hoffman et al., 2008; Coffey et al., 2003; Jones et al., 2015), and the intensity of drug addiction correlates with the degree of delay discounting (Petry and Casarella, 1999; Vuchinich and Simpson, 1998). Further, individual impulsivity levels during delay discounting predicted the severity of cocaine self-administration in rats (Anker et al., 2009).

Our results indicate that the PrL encodes delay discounting information based on inherent impulsivity. These findings have clinical relevance, as the PrL (and its human correlate, the dlPFC) are highly implicated in impulsivity and drug addiction. Drug addicts demonstrate altered dlPFC function reflective of increased impulsive behavior (Hoffman et al., 2008; Monterosso et

al., 2007; Boettiger et al., 2007). The PrL demonstrated enhanced encoding of cocaine-associated stimuli following abstinence (West et al., 2014). Further, lesions to the PrL reduced drug seeking (Di Pietro et al., 2006), and inhibition of this region blocked cocaine reinstatement (Capriles et al., 2003; Di Pietro et al., 2006; McFarland and Kalivas, 2001). Conversely, activation of the PrL-NAc core pathway increased cocaine reinstatement behavior (McGlinchey et al., 2016). As such, understanding the neural underpinnings of delay discounting may identify impulsive individuals at risk for psychiatric disorders, or may improve treatments for diseases such as drug addiction.

The current findings reveal an essential role of the PrL during delay discounting. PrL neurons are phasically active to discrete task events, including cue presentation, lever press, and reward delivery. Specifically, these phasic neurons formed unique subgroups that were selectively phasic to the large/delay, small/immediate, or both trial types. These selective neuron populations tracked the shift in preferred reward value as the task progressed. Further, this tracking was dependent on inherent impulsivity, such that high impulsive rats demonstrated a greater shift in small/immediate selective neurons across blocks. The findings indicate a role for the PrL in encoding impulsive choice as a function of individual impulsivity. As such, further investigation of this brain region is necessary to understand the neural underpinnings of delay discounting.

CHAPTER 4

OPTICAL STIMULATION OF NAc CORE GLUTAMATE RELEASE DOES NOT MODULATE DELAY DISCOUNTING BEHAVIOR

Introduction

Delay discounting measures the decreasing value of rewards based on the delay to their receipt (Roesch et al., 2006; Roesch et al., 2007; Roesch and Bryden 2011; Tedford et al., 2015). Once the delay becomes too long to tolerate, individuals shift their choice allocation toward a smaller, immediate reward (Roesch et al., 2006; Roesch et al., 2007; Tedford et al., 2015). Because delay discounting measures the amount of time it takes for individuals to "lose patience" and choose an immediate reward, it is often used as a measure of impulsivity.

Delay discounting recruits the mesolimbic reward system, including the nucleus accumbens core (NAc core) and its dopaminergic input from the ventral tegmental area (VTA) (Cardinal et al. 2003; Cardinal 2006; Floresco et al. 2008; Dalley et al. 2008; Basar et al. 2010). Extensive literature has linked the NAc core and its dopaminergic input to delay discounting. For instance, lesions to the NAc core increase impulsive choice (i.e., decreases preference for the large, delayed reward) (Cardinal et al., 2001; Pothuizen et al. 2005; Bezzina et al. 2007, 2008; da Costa Araújo et al. 2009, 2010). Further, NAc core dopamine release tracks the predicted subjective value of rewards during delay discounting (Saddoris et al. 2015b; Moschak et al., 2017).

However, in addition to dopamine, the NAc core receives glutamatergic input from numerous brain regions, including the prefrontal cortex (Krettek and Price 1977; Sesack et al.,

1989; Basar et al., 2010; Pinto and Sesack 2000; Brog et al., 1993). While less understood than dopamine, glutamatergic activity in the NAc core may also contribute to delay discounting behavior. Indeed, aberrant glutamate activity contributes to impulsivity disorders such as ADHD (Jensen et al., 2009; Miller, Pomerleau, Huettl, Gerhardt, & Glaser, 2014; Perlov et al., 2007) and substance abuse (Ben-Shahar et al., 2012; Griffin, Haun, Hazelbaker, Ramachandra, & Becker, 2014). Interestingly, antagonism of N-methyl-D-aspartate (NMDA) glutamate receptors produces differential effects on delay discounting, e.g., ketamine and memantine increase delay discounting, while MK-801 decreases impulsive choice (Cottone et al., 2013; Floresco, Tse, & Ghods-Sharifi, 2008; Higgins et al., 2016; Yates, Batten, Bardo, & Beckmann, 2015).

However, it is unknown if NAc core glutamate release is *causally* linked to elements of delay discounting. Previously in our laboratory, *dopamine* was optically stimulated during "unpreferred" (i.e., delay-predictive or small reward-predictive) cues, in an effort to shift the perceived predicted value of these unpreferred rewards. It was shown that NAc core dopamine release was causally linked to delay, but not magnitude, decision making (Saddoris et al., 2015b). Further, Aim 1 of this dissertation demonstrated that optically-evoked dopamine release was not causally linked to delay discounting behavior. However, optical stimulation of glutamate during delay discounting was not examined. Because glutamate activity in the NAc core is linked to impulsivity and delay discounting behavior, we hypothesize here that elevation of glutamate during delay-predictive cues will bias rats' choice behavior toward this less-impulsive option. In the current study, we used optogenetics to stimulate glutamate release in the NAc core during forced cues that predicted the delayed, large reward during a delay discounting task, and examined if this shifted free choice behavior in the absence of stimulation.

Methods

Animals

Singly-housed male (n = 8) and female (n= 6) Long Evans rats were approximately 90 to 120 days old, weighing 275-330 g at the start of experiments. Animals were maintained at no less than 85% of pre-experimental body weights by food restriction, except during the post-operative recovery period when food was given ad libitum (Purina Lab Chow). Water was available *ad libitum* throughout the duration of the experiment. Animal procedures were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, and were approved by the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee (IACUC).

Apparatus

Behavioral testing was conducted in 43x43x53 cm Plexiglas chambers housed in sound-blocking boxes (Med Associates, St. Albans, VT) described in detail previously (Saddoris et al., 2011). Briefly, one side of each chamber was equipped with two retractable levers (Coulbourn Instruments, Allentown, PA) 17 cm apart, with a stimulus light 6 cm above each lever. Sucrose pellets (45 mg) were delivered to a food receptacle, which was located equidistantly between the levers. A house light (100 mA) was mounted on the opposite side of the chamber.

Virus Surgery

For all surgical procedures, rats were deeply anesthetized with a ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (10 mg/kg) mixture (i.p.). Following all surgeries, rats

were given an anti-inflammatory medication (meloxicam, 1 mg/kg, s.q.) for two days post-surgery and were allowed access to food and water *ad libitum.*

Prior to any behavioral training, rats were infused with either a CAMKIIa-promoter virus containing ChR2 (AAV5:CaMKII::hChR2(H134R)-EYFP) or a control virus (AAV5:CaMKII::eYFP) in the PrL (AP +2.5 mm, ML ±0.6 mm, DV -4.0mm from skull; 1 µl total; 0.5 µl per site using a 2 µl Hamilton syringe). Before infusion, the syringe was held in place for 5 min, and following infusion, held in place for 8 min. The virus was allowed to incubate for at least 6 weeks to permit expression in NAc core glutamate neuron terminals.

Training on Delay Discounting Task

Following 1 week recovery from virus surgery, rats were trained to press two distinct levers in which each response was reinforced on a continuous schedule of reinforcement. Reinforced responses resulted in the delivery of a sucrose pellet to a centrally located food cup. Animals were trained to a criterion of 50 presses on each response lever.

Next, rats were trained on the delay discounting task, which was comprised of three trial types (also described in Aims 1 & 2). On Forced Choice Delay trials (Figure 1A, left), a cue light was illuminated for 5 seconds followed by extension of two levers. A single press on the associated lever positioned below that cue light resulted in a large reward (three sucrose pellets) delivered after a period of delay. During Forced Choice Immediate trials (Figure 1B, middle), another 5-second cue light signaled that responses on the associated lever resulted in a small (one sucrose pellet) immediate reward. On Free Choice trials (Figure 1C, right), both cue lights illuminated for 5 seconds, signaling that both responses were rewarded based on the contingency of the lever chosen. Each behavioral session consisted of three blocks of trials: during the first

block, the large reward was presented immediately (no-delay block); in the subsequent block, the delay to large reward was 10 seconds following a lever press (short-delay block); while in the last block, there was a 20-second delay to obtain the large reward (long-delay block). Rats performed 30 trials per block (20 Forced Choice (10 of each type) and 10 Free Choice trials). If animals failed to respond within 10 s, both levers retracted and the trial was counted as an omission. Because each trial was a fixed duration (60 s), reward choice did not influence how quickly the rat completed the task (i.e., choosing the small reward did not lead to the next trial quicker).

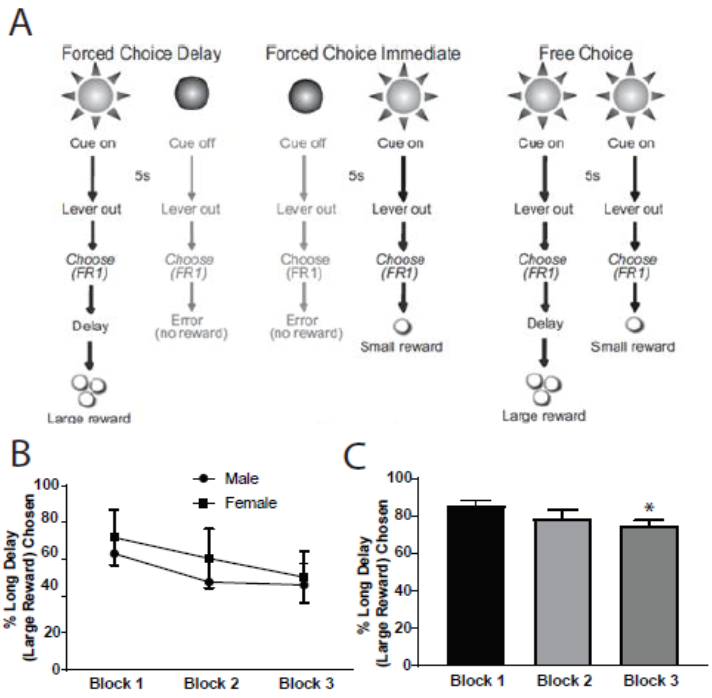

Figure 4.1. Delay discounting task and behavior. **A.** Schematic of delay discounting task. **B.** Baseline percent delay choice (large reward chosen) in male and female rats. **C.** Baseline accuracy (percent correct responses) across all rats.

As demonstrated previously (Saddoris et al., 2015), cue- and reward-elicited core dopamine signaling scales preferentially during Forced Choice trials to the large, non-delayed

reward on Block 1, and tapers off in Blocks 2 and 3. Since dopamine may function to modulate glutamate signaling in the NAc that in turn, influences behavioral output (Berridge and Robinson, 1998; Kapur, 2003; Berridge, 2007; Palmiter, 2007; Robbins and Everitt, 2007), I hypothesized that increasing glutamatergic signaling during cue presentation on Low Forced trials may influence how the predicted value of the reward is processed. Subsequently, this altered glutamate signal may then bias animals toward preferring the low value (delayed) option when given a choice.

To test this hypothesis, rats' (n = 8 experimental (n = 5 male, n = 3 female); n = 6 controls (n = 3 male, n = 3 female)) glutamate terminals were stimulated during the *cue* that predicted the lower value option, as described previously (Saddoris et al., 2015; also see Figure 2A and 4A). Here, optical stimulation of NAc core glutamate terminals was administered during the 5 s Low Forced *cues* that predicted the large but delayed reward option. As in previous studies (Saddoris et al., 2015), no stimulation was given during Free Choice trials, allowing these trials to act as a measure of choice preference independent of stimulation effects. Thus, any alterations to Free Choice behavior resulted from changes in either predicted (cue) or outcome (reward) value.

Rats underwent 4 total stimulation sessions (20 Hz, 5 ms pulsewidth, 20 mW), two during Block 3 (Long Delay) and two during Block 2 (Short Delay). On sessions between stimulation days, no light was delivered. Therefore, each animal was its own behavioral control during both stimulation and non-stimulation sessions. During the stimulation paradigm, rats were connected to patch cables containing an optical fiber (200 μm core, 0.22 NA, ThorLabs). A ferrule connector at the end of the cable was secured to the rat's optical fiber implant with a ceramic sleeve (Precision Fiber Products). These were attached at the other end to an optical

commutator (Doric Lenses), which allowed bilateral stimulation of NAc terminals and allowed the animal to move freely. The commutator was connected, via another patch cable, to a 150 mW DPSS 473 nm laser (OEM Laser Systems). Optical stimulation was controlled by a computer running Med PC IV software (Med Associates) that also recorded behavioral events.

Intracranial Self Stimulation: Immediately following completion of the delay discounting stimulation paradigm, rats were given access to food *ad libitum*. Then, rats were trained to lever press for optical stimulation of NAc core glutamatergic terminals. Here, a houselight illuminated the chamber and a cue light over the right lever indicated the active lever. Animals could lever press for a 5 s bilateral optical stimulation (20 Hz, 5 ms pulsewidth, 20 mW). During the 5 s stimulation period, the cue light extinguished and the lever retracted for 20 s. Animals underwent a total of three 30-minute self-stimulation sessions. Following the third session, animals underwent a single 75 min extinction session. Here, lever presses did not result in optical stimulation, and were recorded in 15 minute blocks. Optical self-stimulation was considered "extinguished" after either two 15 minute blocks of no responses or after 75 minutes. Immediately after reaching extinction criteria, animals received a single 5 s "priming" stimulation, signaling the opportunity to lever press for optical stimulation (20 Hz, 5 ms pulsewidth, 20 mW) for a 45 min reinstatement session.

Histology

1.5 h prior to sacrifice, rats underwent brief (1 min) optical stimulation of the PrL-NAc core pathway to induce the expression of c-fos in PrL cell bodies and NAc core terminals. Following sacrifice, brain slices were stained for c-fos using antibodies available in the Carelli laboratory (SC-52 (1:2000, Santa Cruz) and ABE457 (1:10,000, EMD Millipore)). Both of these

antibodies have been validated in our laboratory using transgenic FosLacZ rats that co-express beta galactosidase when Fos is elevated (>95% of Fos and beta galactosidase co-expression). To verify viral infusion targets and c-fos expression, the PrL and NAc core were imaged using a confocal microscope, in which both the virus EYFP tag and c-fos expression can be seen. Optical fiber placement in the NAc core was also verified. Rats with improper viral expression or misplaced optical fibers were excluded from the study.

Data Analysis

A Two-Way ANOVA was performed on Free Choice preference to determine sex differences in delay discounting choice behavior. Following this analysis, male and female rats were combined for the remainder of all analyses. Analysis of baseline behavior during the delay discounting task included examination of correct responses, number of errors, and free choice preference. To determine whether rats reliably acquired the task, we evaluated the number of errors and correct responses during Forced Choice trials. One-Way ANOVA were used to compare accuracy (percentage rewarded trials) and percentage errors during Forced Choice trials, as well as free choice preference.

To confirm that laser stimulation did not alter the ability to perform the task, the percentage of correct responses on forced trials was compared between groups and between stimulation and non-stimulation sessions using a Three-Way ANOVA. Further, to evaluate if stimulation of dopamine terminals during the delayed option on Forced Choice trials was sufficient to shift behavioral responding during Free Choice trials, we compared response allocation and response latency on Free Choice trials between stimulation and non-stimulation sessions and groups using a Three-Way ANOVA.

For optical self-stimulation tests, we evaluated behavioral responding across days using a two-way repeated measures ANOVA with Bonferroni's multiple comparison test to determine if there was a significant increase in lever press behavior in the experimental animals compared to controls.

All analyses were considered significant at $\alpha=0.05$. Statistical and graphical analysis were performed using GraphPad Prism 6.0 for Windows (GraphPad Software, La Jolla, CA).

Results

Baseline Delay Discounting Behavior

We first compared male and female rats' baseline free choice preference to examine any sex differences in impulsive choice (i.e., choosing the large delay reward less) (Figure 1B). There was a significant effect of block ($F_{2,24} = 3.991$, $p = 0.032$), and a Bonferroni's post-hoc test indicated that all rats discounted the value of the large delay reward ($p < 0.05$). Importantly, there was no effect of sex on delay discounting ($F_{1,12} = 0.2655$, $p = 0.6157$) or a block x sex interaction ($F_{2,24} = 0.1874$, $p = 0.8303$). As such, male and female rats were combined for the remainder of analyses. All rats accurately discriminated the different reward values during the delay discounting task (Figure 1C). On Forced Choice Trials, rats' accuracy was dependent on the delay to reward receipt ($F_{2,26} = 5.982$, $p = 0.0073$), and made more errors in Block 3 compared to Block 1 ($p < 0.05$). During Free Choice trials, rats' preference for the large reward decreased as the delay to large reward receipt increased across blocks ($F_{2,26} = 4.447$, $p = 0.0218$).

Optical Stimulation of Terminal Glutamate during Forced Choice Long Delay Cues does not alter Free Choice Behavior

First, we stimulated NAc core glutamate terminals during Forced Choice Long Delay cues (Figure 2A). We examined if optical stimulation during these cues altered rats' accuracy across blocks, between genetic groups, and between stimulation sessions (Figure 2B, stimulation days; Figure 2C, non-stimulation days). There was a main effect of block ($F_{1.420, 17.04} = 10.908$, $p = 0.002$) and session ($F_{1,12} = 4.911$, $p = 0.047$). A Bonferroni post-hoc test on the block main effect revealed that all rats made more errors on Block 3 compared to Block 1 and 2 ($p < 0.05$), regardless of genetic group or stimulation session. There were no other significant main effects or interactions ($F < 1.068$, $p > 0.05$).

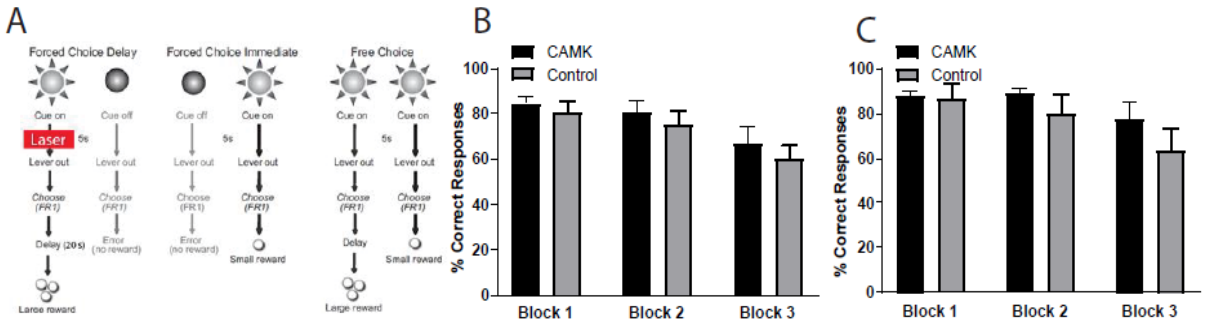

Figure 4.2. Schematic of Long Delay stimulation task design and accuracy on Forced Choice trials. **A.** Long Delay stimulation task schematic. Rats received optical stimulation during the 5 s cue that predicted the Forced Choice Long Delay cue. Free Choice trials were then used as a measure of choice bias, independent of stimulation. **B.** Accuracy (percent correct responses) between experimental (CAMK) and control rats during stimulation sessions. **C.** Accuracy (percent correct responses) between experimental (CAMK) and control rats during non-stimulation sessions.

We next examined choice selection on Free Choice trials across blocks, between genetic groups, and within stimulation sessions (Figure 3A). There was a main effect of block ($F_{1.384, 16.607} = 16.85$, $p < 0.001$), indicating that rats were able to discount the value of the large reward across blocks ($p < 0.05$). There was also a session x group interaction ($F_{1,12} = 5.779$, $p = 0.049$), however, further analysis revealed no effects of session or group ($p > 0.05$ for all analyses). There were no other significant main effects or interactions ($F < 1.746$, $p > 0.05$). As such, there was no effect of optical stimulation on Long Delay free choice preference.

We next examined if there was an effect of stimulation on Free Choice reaction time. For both delay and immediate choice, there was a significant effect of block (Delay: $F_{2,24} = 16.138$, $p < 0.001$; Immediate: $F_{2,24} = 7.696$, $p = 0.004$), indicating that reaction time increased across blocks ($p < 0.05$). There were no other significant main effects or interactions for response

latency (Delay: $F < 1.909$; Immediate: $F < 1.691$; $p > 0.05$). As such, there was no significant effect of stimulation on Free Choice reaction time.

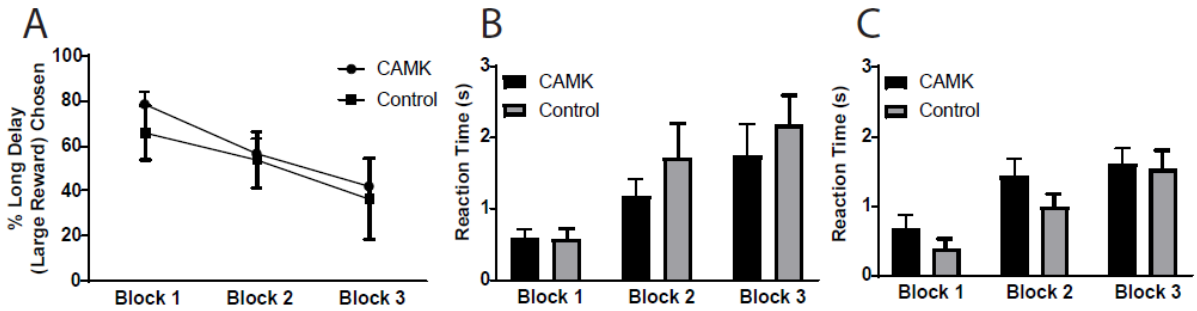

Figure 4.3. Free choice behavior and reaction time during Long Delay stimulation paradigm. **A.** Percent delay choice (large reward) chosen across blocks between experimental (CAMK) and control groups. Data from stimulation sessions shown. **B.** Reaction time for Free Choice delay selection between experimental (CAMK) and control groups on stimulation sessions. **C.** Reaction time for Free Choice immediate selection between experimental (CAMK) and control groups on stimulation sessions.

Optical Stimulation of Terminal Glutamate during Forced Choice Short Delay Cues does not alter Free Choice Behavior

Following the Long Delay stimulation paradigm, we then stimulated glutamate terminals during Forced Choice Short Delay cues (Figure 4A). As in the Long Delay experiment, we examined correct responses (accuracy) during Forced Choice trials across blocks, between genetic groups and between stimulation sessions (Figure 4B, stimulation sessions; Figure 4C, non-stimulation sessions). There was a main effect of block ($F_{2, 16} = 4.082$, $p = 0.037$), indicating that rats made more errors during Block 3 ($p < 0.05$). There were no other significant main effects or interactions ($F < 2.407$, $p > 0.05$).

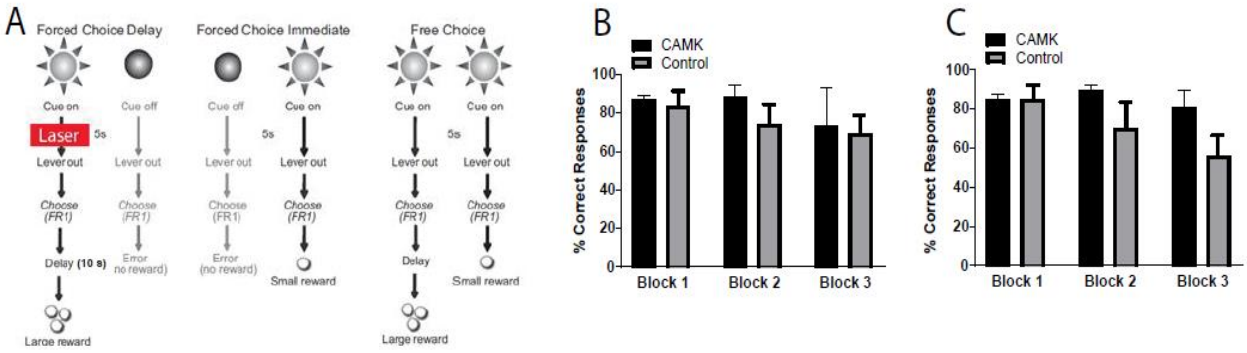

Figure 4.4. Schematic of Short Delay stimulation task design and accuracy on Forced Choice trials. **A.** Short Delay stimulation task schematic. Rats received optical stimulation during the 5 s cue that predicted the Forced Choice Short Delay cue. Free Choice trials were then used as a measure of choice bias, independent of stimulation. **B.** Accuracy (percent correct responses) between experimental (CAMK) and control rats during stimulation sessions. **C.** Accuracy (percent correct responses) between experimental (CAMK) and control rats during non-stimulation sessions.

We next examined Free Choice behavior across blocks, between groups, and within stimulation sessions (Figure 5A). We found a main effect of block ($F_{1.373,\ 9.614} = 14.495$, $p = 0.001$), such that all rats were able to discount the value of reward ($p < 0.05$). There were no other significant main effects or interactions ($F < 3.661$, $p > 0.05$).

We also examined reaction time during Free Choice behavior. During trials where rats chose the large, delayed reward, there was a block x session x group interaction ($F_{1.562,\ 12.498} = 8.427$, $p = 0.007$). However, within any block, there were no significant session or group effects or interactions ($p > 0.05$ for all analyses). There were no other significant main effects or interactions (Delay: $F < 3.455$; Immediate: $F < 3.470$; $p > 0.05$).

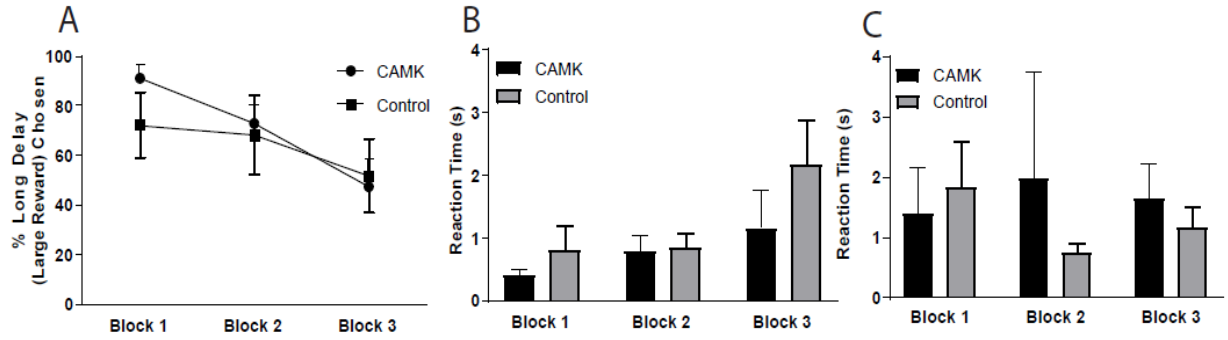

Figure 4.5. Free choice behavior and reaction time during Short Delay stimulation paradigm. **A.** Percent delay choice (large reward) chosen across blocks between experimental (CAMK) and control groups. Data from stimulation sessions shown. **B.** Reaction time for Free Choice delay selection between experimental (CAMK) and control groups on stimulation sessions. **C.** Reaction time for Free Choice immediate selection between experimental (CAMK) and control groups on stimulation sessions.

Optical self-stimulation of glutamatergic terminals in the NAc core

One week following the delay discounting paradigm, rats underwent 3 self-stimulation sessions in which they were allowed to lever press for 5 s optical stimulation in the NAc core, followed by an extinction and reinstatement session (Figure 6A). We found no difference between experimental and control animals ($F_{1,11}$ = 0.3546, p = 0.5636) or any session x group interaction ($F_{8,96}$ = 0.3526, p = 0.9423). There was a main effect of session ($F_{8,88}$ = 16.54, p < 0.0001), indicating that all rats' self-stimulation responses decreased over sessions (p < 0.05). These results indicate that stimulation of glutamatergic terminals was not inherently rewarding.

Histology and verification of optogenetic technique using c-Fos quantification

Optical fiber tip locations within the NAc core are shown in Figure 7A. Further, there was significantly more c-Fos (a bio marker indicative of neuronal activation) present in PrL cell bodies in experimental compared to control rats (t_{12} = 4.957, p = 0.0003; Figure 7B). A

representative image of ChR2 and c-Fos co-expression in experimental and control animals is shown in Figure 7C.

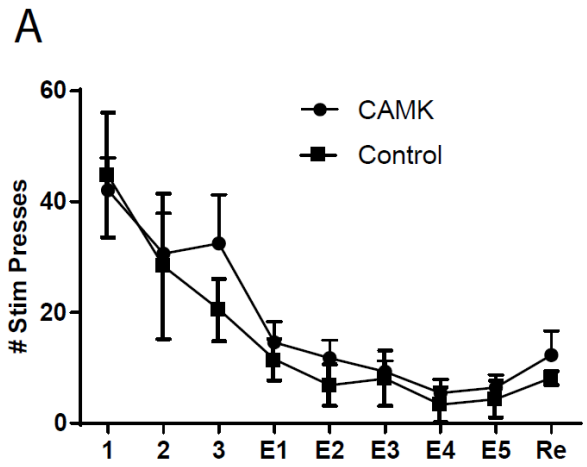

Figure 4.6. Optical self-stimulation, extinction, and reinstatement. **A.** Lever press responses for 5 s optical stimulation (20 Hz, 5 ms pulsewidth, 20 mW) for all animals in both groups. Laser was active on days 1-3. Following the last day of self-stimulation, rats underwent an extinction session during which laser was off (E1-E5), followed by a reinstatement session (R) in which presses were reinforced again with optical stimulation.

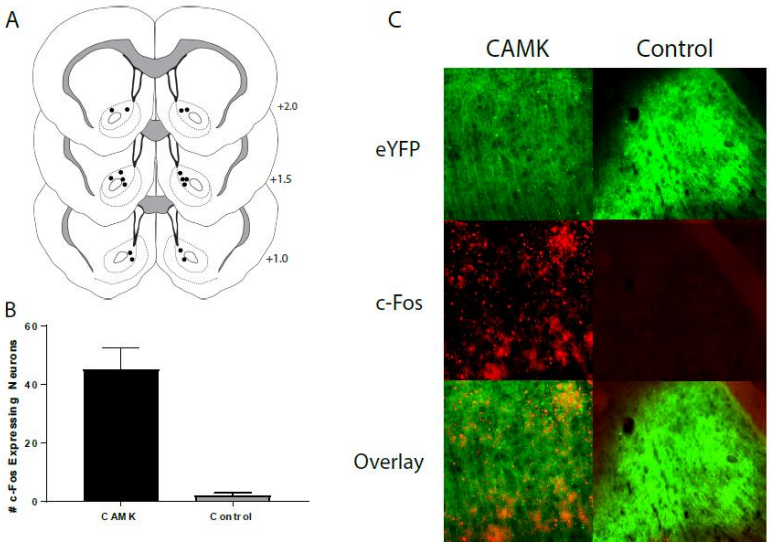

Figure 4.7. Histology. **A.** Optical fiber tip placements in experimental animals. **B.** Quantification of c-Fos in PrL cell bodies. **C.** Left: Representative co-localization of c-Fos and ChR2-EYFP expression in PrL cell bodies in experimental (left) and control rats (right; eYFP only, no c-Fos present).

Discussion

The PrL sends glutamatergic projections to the NAc core (Krettek and Price 1977; Sesack et al., 1989; Basar et al., 2010; Pinto and Sesack 2000; Brog et al., 1993). This glutamate signaling may contribute to impulsive choice and delay discounting, as aberrant glutamate activity contributes to impulsivity disorders such as ADHD (Jensen et al., 2009; Miller, Pomerleau, Huettl, Gerhardt, & Glaser, 2014; Perlov et al., 2007). Further, delay discounting recruits both the PrL, as demonstrated in Aim 2, as well as the NAc core (Cardinal et al., 2001; Pothuizen et al. 2005; Bezzina et al. 2007, 2008; da Costa Araújo et al. 2009, 2010). However, it is unknown if PrL-NAc core glutamate signaling alone mediates aspects of delay discounting behavior.

The current study assessed if glutamate release during delay-predictive cues causally influences delay discounting behavior. Here, we used optogenetics in rats to manipulate NAc core glutamate release during Forced Delay cues of our delay discounting task. We examined Free Choice behavior in the absence of stimulation to test if elevation of glutamate was sufficient to increase selection of the delayed (i.e., less impulsive) option. We found that optical stimulation during either Forced Long Delay or Forced Short Delay cues was not sufficient to bias subsequent rats' preference toward these delayed reward options when given a choice in the absence of stimulation. These findings indicate that glutamate signaling by itself in the NAc core is not causally linked to value-based predictive strategies in delay discounting.

As stated in Aim 1, the NAc core is strongly linked to delay discounting behavior. Lesions to the NAc core alter delay discounting behavior, such that rats increase preference for small, immediate rewards (i.e., increase impulsive choice) (Cardinal and Howes 2005; Cardinal et al., 2003; Cardinal et al., 2001; Bezzina et al., 2007; Galtress and Kirkpatrick, 2010; Pothuizen

et al., 2005). Also, NAc core lesions impair learning of instrumental responses to delayed reinforcement (Cardinal and Cheung 2005) and diminish sensitivity to changes in delay (Acheson et al., 2006). Notably, this literature is largely focused on *dopamine* release in the NAc core, which functions as a "neural currency" that tracks changes in reward value (Saddoris et al., 2015b). However, the role of glutamate is less understood.

In the NAc core, both dopamine and glutamate afferents synapse onto medium spiny neurons (Sesack and Pickel 1990). Specifically, dopamine synapses connect to dendritic spines beneath glutamatergic synapses. Dopamine functions as a neuromodulator of glutamatergic signals in the NAc (Chuhma et al., 2004; Grace et al., 2007; Schultz, 2007; Surmeier et al., 2007). However, glutamate can also modulate NAc dopamine release (Chesselet, 1984; Surmeier et al., 2007). Notably, dopamine inhibits glutamate neurotransmission via D1 and D2 receptors (Pennartz et al., 1992; Nicola et al., 1996; Harvey and Lacey, 1997; Li and Kauer, 2004; Ortinski et al., 2012, O'Donnell and Grace, 1994; Brady and O'Donnell, 2004), and this inhibition is caused by both endogenous dopamine (Harvey and Lacey, 1996; Brady and O'Donnell, 2004) and drugs of abuse (Nicola et al., 1996; Li and Kauer, 2004; Wang et al., 2012). This dopaminergic modulation of glutamatergic signaling may contribute to motivational behaviors and the processing of salient environmental stimuli (Berridge and Robinson, 1998; Kapur, 2003; Berridge, 2007; Palmiter, 2007; Robbins and Everitt, 2007).

In Aim 1, dopamine release itself in the NAc core was not sufficient to mediate delay discounting. Here, we find that glutamate only is also unable to casually influence delay discounting. These experiments suggest that manipulation of one particular circuit (the dopaminergic VTA-NAc core path or the glutamatergic PrL-NAc core path) is not sufficient to alter this complex behavior. The NAc core receives glutamatergic inputs from numerous brain

regions, including the hippocampus (Kelley et al., 1982; Groenewegen et al., 1987; Brog et al., 1993), amygdala (Stuber et al., 2011), basolateral amygdala (Kelley et al., 1982; McDonald, 1991; Brog et al., 1993) and ventral subiculum, in which glutamatergic afferents modulate NAc dopamine activity (Blaha et al., 1997; Taepavarapruk et al., 2000). Further, goal-directed behaviors and reward-predictive strategies likely rely on a synthesis of dopamine, glutamate, and other neurotransmitter activity from a range of inputs. As such, the "single-pathway" optogenetic approach utilized in the current aim may not be appropriate to examine how neurotransmission mediates delay discounting behavior.

It is important to note that the lack of choice bias was not due to failure of the optogenetics technique. One week following the delay discounting stimulation task, rats underwent three 30-minute sessions in which they could self-administer optical stimulation. The last session was followed by an extinction session, in which lever presses did not result in stimulation. Afterward, a reinstatement session allowed rats to resume pressing for optical stimulation. Notably, optical stimulation of glutamatergic terminals was not sufficient to drive this goal-directed behavior. These findings replicate those of Britt et al. (2012), which found similar results in mice. However, our optical technique was verified by measuring post-stimulation levels of c-Fos. c-Fos is a proto oncogene which is elevated following neuronal activity (Herrera and Robinson 1996). One and a half hours prior to sacrifice, brief (1 min) optical stimulation was applied to NAc core glutamate terminals to activate c-Fos. In rats expressing ChR2 (i.e., experimental animals), there were elevated levels of c-Fos in PrL cell bodies compared to those of controls. This finding indicates that optical stimulation of ChR2-containing NAc core glutamate terminals was sufficient to activate these PrL-NAc core projecting neurons.

In conclusion, the data presented in this aim indicate that optical stimulation of glutamate release in the NAc core during delay-predictive cues was not sufficient to bias delay discounting choice behavior toward the less impulsive option. Previously, NAc core dopamine release dynamically tracked the preferred cue during our delay discounting task (Saddoris et al., 2015b). However, Aim 1 indicated that dopamine release is not causally linked to delay discounting. Adding to these findings, the current aim indicates that glutamate release in the NAc core is *also* not causally linked to delay discounting as well. Thus, the role of glutamate in delay discounting behavior remains an important topic for further investigation.

CHAPTER 5

GENERAL DISCUSSION

The studies outlined in this dissertation were designed to investigate the neurobiology of delay discounting, with a specific focus on the nucleus accumbens (NAc) core subregion and its afferents. Chapter 2 used optogenetics to stimulate dopamine terminals in the NAc core during Forced Choice delay-predictive cues of our delay discounting task, to elevate the perceived value and bias Free Choice behavior toward the delayed (less impulsive) option. However, there was no effect of optical stimulation of NAc core dopamine on choice behavior. As such, Chapter 2 demonstrated that the dopaminergic input from the VTA to the NAc core is not causally linked to delay discounting behavior. Chapter 3 shifted focus to the prelimbic cortex (PrL), a glutamatergic afferent to the NAc core. This experiment examined neuronal activity in the PrL during elements of our delay discounting task (cue, press, and reward delivery). Here, the data revealed that distinct, event-selective neuronal populations encode rats' preferred Free Choice selection during delay discounting. Interestingly, this tracking of preferred choice is contingent on rats' inherent impulsivity. As such, Chapter 3 revealed an essential role of the PrL in delay discounting behavior, and unique neural encoding during this task related to impulsivity. Chapter 4 sought to expand these findings by optically stimulating PrL glutamatergic terminals in the NAc core during delay-predictive cues. Like in Chapter 2, we sought to shift Free Choice behavior toward the less-impulsive, delayed choice. However, this study also found no effect of

optical stimulation on choice behavior, and as such, no causal link between glutamate release and delay discounting. A summary of each experiment is listed below.

Summary of Experiments

Examination of causal links between NAc core dopamine release and delay discounting behavior

This study examined if dopamine release is causally linked (i.e., mediates) delay discounting choice behavior. Transgenic *TH:Cre*$^{+/-}$ rats and their littermate controls received optical stimulation of VTA-NAc core dopamine terminals during the 5 s Forced Choice cues that predicted the Long (20s) and Short (10s) Delays during our task. Free Choice behavior was then examined in the absence of stimulation to determine if this elevation in dopamine was sufficient to bias rats' choice behavior toward the delayed (less impulsive) option. However, optical stimulation had no effect on rats' behavior, indicating that, in this task, dopamine release was not sufficient to mediate delay discounting. Optogenetic technique was verified via an optical self-stimulation paradigm, in which rats could lever-press for stimulation of dopamine release, followed by an extinction and reinstatement session. Transgenic rats reliably self-stimulated dopamine terminals, and extinguished and reinstated this behavior, compared to controls. As such, dopamine release was sufficient to drive goal-directed behavior, but not delay discounting. The overall findings of this chapter are surprising, as NAc core dopamine preferentially tracks the "preferred" value of delay discounting cues (Saddoris et al., 2015b). It likely that, in addition to dopaminergic signaling, other circuits or neurotransmitters interact with the VTA-NAc core pathway to influence delay discounting choice behavior.

Neurons in the PrL encode preferred options during delay discounting behavior and are uniquely influenced by impulsivity

This study used *in-vivo* electrophysiology to examine neuronal activity in the PrL during our delay discounting task. PrL neurons responded phasically to cue presentations, lever presses, and reward delivery during the task. Further, in Free Choice trials, we found that distinct neuronal populations exhibited selective phasic activity to either the large/delay, small/immediate, or both reward options. These selective phasic neurons tracked preferred reward value in each block, such that the proportion of these neurons shifted from greater large/delay selective in Block 1, to more small/immediate selective in Block 3. Importantly, this tracking neural signaling occurred during Free Choice cue presentation, lever press, and reward delivery, indicating that PrL neurons track the predicted and eventual reward outcome. Additionally, we divided rats into high and low impulsive groups, and discovered that these selective neuron populations differentially tracked preferred Free Choice rewards based on inherent impulsivity. High impulsive rats demonstrated a steeper shift in the proportion of small/immediate selective neurons compared to low impulsive rats. Notably, these findings were only significant during Free Choice trials, where the rat was presented with a choice between two options that shifted in value across blocks. These findings indicate that neuron subgroups in the PrL track preferred reward value during delay discounting, and this neuronal encoding is dependent on individual impulsivity.

Examination of causal links between the PrL-NAc core circuit and delay discounting behavior

Experiment 3 (Chapter 4) used the same optogenetics task as in Chapter 2 to examine if *glutamate* signaling in the PrL-NAc core pathway is causally linked to delay discounting. Optical

stimulation of NAc core glutamate terminals occurred during Force Choice Delay cues (Long and Short Delay). Free Choice behavior was examined in the absence of stimulation to assess if glutamatergic signaling was sufficient to bias rats' choice toward the less impulsive (delayed) option. However, as in Chapter 2, glutamatergic elevation was not sufficient to bias choice behavior, indicating no causal role in delay discounting. Further, while rats did not self-stimulate for glutamate release (consistent with Britt et al., 2012), the optogenetic technique was verified by the presence of c-Fos in PrL cell bodies in experimental (ChR2) rats, indicating that these neurons were activated (i.e., released glutamate) during the task. These findings indicate that while the PrL tracks and encodes information related to "preferred" reward value in delay discounting (as seen in Chapter 3), its glutamatergic input to the NAc core alone isn't sufficient to drive this behavior. Further, taken together with Chapter 2, optical manipulation of single pathways (VTA-NAc DA pathway or PrL-NAc core glutamatergic circuit) may not be sufficient to drive complex goal-directed and motivated behavior.

General discussion and relevance of findings

Aberrant delay discounting and neural circuitry in drug addiction

Heightened delay discounting (greater impulsive choice) is a symptom of drug addiction, such that addicts have a lower "breakpoint" at which they switch to the small, immediate option and over-value more immediate actions and rewards. Interestingly, delay discounting and drug addiction share a bidirectional relationship. Heightened delay discounting (more impulsive choice) is observed in those suffering from substance abuse (Bickel et al., 1999; Evenden 1999; Heyman 1996; Poulos et al., 1995; Bechara 2005; Ernst and Paulus 2005; Hoffman et al., 2008; Coffey et al., 2003; Jones et al., 2015). Drug addicts demonstrate heightened preference for

immediate over delayed rewards (Madden et al., 1997; Bickel et al., 1999; Kirby et al., 1999; Bickel and Marsch, 2001; Heil et al., 2006). However, inherently high impulsivity may also *predict* future drug use (Jentsch and Taylor, 1999; Winstanley et al, 2010). For example, in preclinical models, high impulsive action and heightened discounting predicts drug self-administration (Belin et al, 2008; Dalley et al, 2007, Anker et al., 2009). Further, high impulsive animals were more likely to exhibit drug seeking during extinction and reinstate these behaviors when given access to drug again (Diergaarde et al, 2008; Perry et al, 2008; Economidou et al, 2009). In humans, impulsivity in childhood predicts drug use, including stimulant abuse, later in life (Ersche et al, 2010, Nigg et al, 2006; Tarter et al, 2003; Wong et al, 2010). As such, understanding the neural underpinnings of delay discounting may identify impulsive individuals at risk for psychiatric disorders, or may improve treatments for diseases such as drug addiction by minimizing impulsive actions directly toward continued drug use.

Notably, drug addiction recruits the NAc and associated regions, the same learning/reward-seeking circuits that are activated during delay discounting. Indeed, drug-associated stimuli and self-administration activate NAc neurons and alter DA dynamics within this region (Carelli and Deadwyler, 1994; Peoples et al., 1997; Carelli et al., 1999; Carelli et al., 2000; Nicola and Deadwyler, 2000; Peoples et al., 2004; Hollander and Carelli, 2007). Most drugs of abuse increase DA levels in the NAc (Phillips et al., 2003b; Aragona et al., 2009; Di Chiara and Imperato, 1988; Cheer et al., 2007b). Further, stimulation of midbrain dopamine neurons is also sufficient to promote drug seeking (Phillips et al., 2003). The role of NAc glutamate is less understood, although drug-elicited elevation of DA may also potentiate its glutamatergic synapses (Wolf and Ferrario, 2010; Dobi et al., 2011; Luscher and Malenka, 2011; Pascoli et al., 2012; Wolf and Tseng, 2012). Because the NAc is responsible for encoding cue-

outcome associations and promoting the learning of motivational behaviors, it is believed that drugs of abuse "hijack" this system to promote the seeking and taking of more drug.

Like the NAc, the PrL (and its human homologue, the dlPFC), is another brain region implicated in drug addiction. Drug addiction is thought to produce a "hypofrontality" effect in prefrontal regions, caused by a decrease in cerebral blood flow, diminished neural transmission, and alterations to PFC neurons (Goldstein and Volkow 2002; Sun and Rebec 2006; Lidow and Song 2001). Further, drug addicts demonstrate altered dlPFC function reflective of increased impulsive behavior (Hoffman et al., 2008; Monterosso et al., 2007; Boettiger et al., 2007). Following abstinence from cocaine, neurons in the PrL exhibit enhanced encoding of cocaine-associated stimuli compared to saline controls (West et al., 2014). Further, lesions to the PrL reduced drug seeking (Di Pietro et al., 2006), and inhibition of this region blocked cocaine reinstatement (Capriles et al., 2003; Di Pietro et al., 2006; McFarland and Kalivas, 2001). Conversely, activation of the PrL-NAc core pathway increased cocaine reinstatement behavior (McGlinchey et al., 2016). Taken together, both the PrL and NAc are implicated in the maintenance of drug addiction. Further, aberrant delay discounting is both a predictor *and* symptom of drug abuse, and recruits both these brain regions. As such, studying the neurobiology of the PrL and NAc during delay discounting may lead to the development of preventative or palliative treatments for addiction.

PrL physiology and impulsivity: a predictor of drug abuse?

As discussed above, impulsivity may predict future drug abuse, and the PrL is implicated in delay discounting and drug addiction. Further, the results of Chapter 3 suggest that individuals' PrL physiology may differ depending on inherent impulsivity. Here, high versus low

impulsive individuals differed in how PrL neuron populations encode predicted reward value and eventual outcome. Specifically, high impulsive rats showed a greater percentage of neurons that preferentially encoded the small, immediate option when they had a choice between that and the delayed option, compared to low impulsive rats. Further, this shift in neuronal proportions occurred during Free Choice cue presentations, when the rats were *deciding* which reward they wanted to choose. High impulsive rats also differed in the number of small, immediate selective neurons during reward delivery in the long delay block, indicating that the PrL of high impulsive individuals may also track preference for the *receipt* of an immediate reward compared to a very delayed reward.

These findings suggest that individual differences in PrL neuron activity may predict or indicate future impulsive choice, and as such, may provide insight into one's future risk for drug addiction. Specifically, the greater proportion of small/immediate selective neurons in high impulsive rats could indicate a lack of prefrontal top-down control over behavior. That is, these small/immediate neurons may be promoting the selection of the immediate over delayed option when given a choice between the two options. This finding adds to a growing body of literature identifying biomarkers of future drug abuse, though these studies largely focused on dopamine receptors. The level of dopamine autoreceptors in the VTA were correlated with trait impulsivity (Buckholtz et al., 2010), and D2 receptor availability predicted both impulsivity and drug abuse (Jentsch and Pennington 2014; Volkow et al., 2002). Specifically, in the PrL, the number of D2 receptors was correlated with greater preference for the large/delay reward (Simon et al., 2013), and activation of these receptors impairs rats' ability to shift reward preference during delay discounting (St. Onge et al., 2011). The current findings suggest that PrL neural activity could serve as a biomarker for heightened impulsivity. Here, neuronal activity in the PrL reflected rats'

impulsivity toward preferred reward outcomes. High impulsive rats (e.g., a steeper shift toward the small/immediate option) also showed a greater shift in the proportion of small/immediate neurons across blocks compared to low impulsive rats. Notably, this shift occurred in cue- *and* reward-activated neurons, indicating that the PrL of high impulsive individuals may differentially or aberrantly encode the preferred value of predicted and eventual rewards.

Interactions between dopamine and glutamate in the NAc

The two optogenetic studies presented in Chapters 2 and 4 examined a potential causal role of dopamine and glutamate neurotransmission in the NAc core in delay discounting. In both studies, NAc core dopamine or glutamate terminals were optically stimulated during cues that predicted a long delay or short delayed option. Notably, as delay to reward increases, rats will begin to shift their choice preference toward the smaller, immediate (i.e., more impulsive) option. Because neurotransmission in the NAc encodes cue-outcome associations during decision making (Day et al., 2010; Sugam et al., 2012; Sackett et al., 2017; Saddoris et al., 2015b), we hypothesized that transient elevation of either dopamine or glutamate during delay-predictive cues would increase the perceived value of the subsequent delayed reward. As such, we theorized that this artificial shift in value would be sufficient to shift animals' choice behavior during Free Choice trials toward the delayed (i.e., less impulsive) option, rather than the small, immediate option. Previously, stimulation of NAc core dopamine terminals during cue presentation was sufficient to bias delay, but not magnitude, decision making (Saddoris et al., 2015b). Further, elevation of dopamine during reward delivery during a magnitude discounting task was sufficient to bias choice behavior (Schlep et al., 2017). To date, no studies have examined how glutamate release mediates decision making behavior. However, in the current

study, elevation of neither dopamine nor glutamate alone was sufficient to mediate delay discounting choice behavior.

One issue with this approach is that we manipulated dopamine and glutamate release independently of each other during our task. *In-vivo,* dopamine and glutamate share complex interactions within the NAc core to drive goal-directed and motivated behavior. Both dopamine and glutamate afferents synapse onto medium spiny neurons in the NAc (Sesack and Pickel 1990). Importantly, dopamine modulates glutamatergic signals in the NAc (Chuhma et al., 2004; Grace et al., 2007; Schultz, 2007; Surmeier et al., 2007) by inhibiting glutamate neurotransmission via D1 and D2 receptors (Pennartz et al., 1992; Nicola et al., 1996; Harvey and Lacey, 1997; Li and Kauer, 2004; Ortinski et al., 2012, O'Donnell and Grace, 1994; Brady and O'Donnell, 2004). Both endogenous dopamine (Harvey and Lacey, 1996; Brady and O'Donnell, 2004) and drugs of abuse (Nicola et al., 1996; Li and Kauer, 2004; Wang et al., 2012) are sufficient to drive inhibition of glutamate. This modulation may contribute to motivational behaviors and the processing of salient environmental stimuli (Berridge and Robinson, 1998; Kapur, 2003; Berridge, 2007; Palmiter, 2007; Robbins and Everitt, 2007). Conversely, glutamate activity also modulates NAc dopamine release (Chesselet, 1984; Surmeier et al., 2007). *Ex-vivo* application of glutamate (Bowyer et al., 1991; Clow and Jhamandas 1989; Jhamandas and Marien 1987; Roberts and Anderson 1979; Roberts and Sharif 1978) and NMDA (Jones et al., 1987; Marien et al., 1983) increased striatal dopamine release, as did *in-vivo* application of NMDA (Carrozza et al., 1992; Keefe et al., 1992; Martinez-Fong et al., 1992; Taber et al., 1996). However, other studies suggest dopamine release is *inhibited* via activation of striatal iGlu receptors or low concentrations of NMDA (Iravani and Kruk 1996; Morari et al., 1996; Taber et al., 1996; Wu et al., 2000). It is generally thought that NMDA receptors interact with D1

receptors to produce excitatory control over striatal neurons (Cepeda and Levine 1998; Cepeda et al., 1998; Harvey and Lacey 1997; Scott et al., 2002), whereas the interaction of NMDA and D2 receptors produces inhibitory control (Zheng et al., 1999; Kotecha et al., 2002; Marti et al., 2002). Taking this complex interaction into account, it is likely that stimulation of *only* dopamine or glutamatergic pathways in Chapters 2 and 4 was not sufficient to shift rats' behavior.

Another explanation for these results may stem from the interaction of neurotransmitter release dynamics and the amount of training rats received. Indeed, by the time optical stimulation was applied, rats were extremely well-trained on the delay discounting task. As such, manipulation of neurotransmission during this well-learned task may not have been sufficient to shift behavior. Rather, it may be that *new* learning of cue-outcome associations is more susceptible to change via dopaminergic manipulation. Indeed, well-learned stimuli elicited dopamine release in the dorsal striatum, a region associated with habitual learning, whereas stimuli during early learning elicited dopamine release in the NAc (Yin and Knowlton 2006; Willuhn et al., 2012). Thus, while NAc core dopamine signaling tracked reward value during a well-learned delay discounting task (Saddoris et al., 2015b), optical manipulation of dopamine *or* glutamate during learned cue-outcome associations was not able to influence this trained behavior. Notably, delay discounting also recruits the dorsal striatum, as lesions to this area increased impulsive choice (heightened discounting) (Tedford et al., 2015). As such, future directions may consider stimulating dorsal striatum dopamine terminals during delay-predictive cues, as manipulation of dopamine signaling in this region may be sufficient to modulate the "learned" cue-outcome associations.

The neural circuitry of delay discounting

The above discussion focused on the glutamatergic and dopaminergic afferents to the NAc. However, there is likely a larger explanation for why the optogenetic manipulations in Chapters 2 and 4 did not mediate delay discounting. Delay discounting is a complex behavior in which the value of a reward changes subjectively as the task progresses. Further, this shift in preferred reward value is dependent on individual differences in subjectivity, impulsivity, and neurobiology. Individuals need to update selection strategies as the rules change in each block, and must weigh the subjective value of rewards. As such, it has been proposed that delay discounting recruits at least three neural circuits that process and encode changes in rules, reward values, and preferred choices (Peters and Buchel 2011).

First, delay discounting recruits "cognitive control" brain regions, including the prefrontal cortex (PFC) (Peters and Buchel 2010, Prevost et al., 2010). The medial PFC exerts top down control over impulse control (Peters and Buchel 2011) and helps to encode the subjective value of reward in delay discounting (Sripada et al., 2011; Peters and Buchel 2010). Disruption of the lateral PFC increased preference for the small, immediate reward over a large, delayed option (Figner et al., 2010). The dorsolateral PFC is also recruited (McClure et al., 2007; Kim et al., 2008; Civai et al., 2016; Ballard and Knutson 2009). As demonstrated in Chapter 2, the rat homologue of the dlPFC, the PrL, tracks the preferred reward value across blocks, and this tracking is dependent on individuals' inherent impulsivity.

Second, delay discounting relies upon memory and "prospection" regions such as the hippocampus and amygdala (Peters and Buchel 2011). The hippocampus allows individuals to evaluate the future value of rewards (Johnson and Redish, 2007; Johnson et al., 2007). Lesions to the hippocampus impair the ability to discriminate magnitude in delay discounting (Bett et al.,

2015; Peters and Buchel, 2010), and increases discounting in rats (Cheung and Cardinal, 2005; Mariano et al., 2009; Rawlins et al., 1985). However, activation of the hippocampus may encourage the selection of the small, immediate reward (Cheung and Cardinal, 2005; Mariano et al., 2009; McHugh et al., 2008). The amygdala is also implicated in delay discounting, as activation in this region is correlated with the difference in subjective value of reward (Sripada et al., 2011; Peters and Buchel 2010). Further, in impulsive individuals, activity in the amygdala was higher during the receipt of immediate versus delayed reward (Ludwig et al.,2015).

Lastly, the mesolimbic reward ("valuation") system tracks the changes in subjective reward value as the delay to receipt increases (Peters and Buchel 2011). The striatum is activated when choosing an immediate over delayed reward (McClure et al. 2004), and the magnitude of this activation predicts impulsive choice during delay discounting (Hariri et al. 2006). As discussed above, the NAc and its dopaminergic input from the VTA is implicated in delay discounting (Saddoris et al., 2015b; Cardinal et al., 2001; McClure et al., 2007; Peters and Buchel 2009; Cardinal et al., 2001; Galtress and Kirkpatrick 2010; Roesch et al., 2007). The basolateral amygdala (BLA) is another brain region in the reward system that is recruited during delay discounting. Disruptions of the basolateral amygdala increase impulsive choice (Winstanley et al., 2004; Cardinal et al., 2004; Churchwell et al., 2009). BLA neurons have also been shown to differentiate between delayed versus immediate rewards (Roesch et al., 2010; Roesch et al., 2012; Schoenbaum et al., 1998).

In the studies presented in Chapters 2 and 4 of this dissertation, we optically stimulated terminals in the NAc from single circuits – either the VTA-NAc core or PrL-NAc core. However, stimulation of these individual circuits was not sufficient to mediate a complex behavior like delay discounting. Indeed, the NAc receives numerous inputs from the

aforementioned executive, memory, and reward structures (Zahm and Brog, 1992; Wright and Groenewegen, 1996), and integrates this influx of information to produce motivated behavior and representations of preferred reward value. As such, it may be necessary to take a global, holistic approach to understanding the neurobiology of delay discounting, rather than manipulating single pathways.

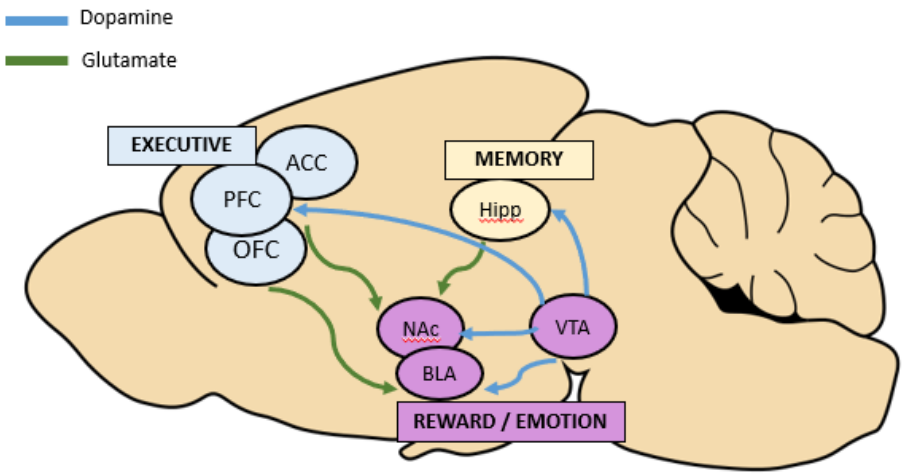

Figure 5.1. Simplified schematic of delay discounting neurobiology. Green arrows denote glutamate projections, and blue arrows denote dopamine projections. This schematic is not representative of precise anatomical location or degree of projections.

To date, few studies have examined the interactions between these brain regions during delay discounting. Existing fMRI studies have largely examined the role of frontal regions, perhaps due to the difficulty of measuring subcortical structures in human subjects. Decreased integrity in fronto-striatal circuitry is associated with steeper discounting and less self-control (Diekhof and Gruber 2010; Peper et al., 2013). Further, neural activity during delay discounting, specifically during periods of self-control, was correlated between dorsolateral and ventromedial prefrontal regions (Hare et al., 2009). Greater discounting was also associated with increased connectivity between the anterior cingulate and hippocampus (Peters and Buchel 2010). Further

exploration of fronto-striatal-hippocampal interaction is necessary to fully understand the neural mechanisms of delay discounting.

Future directions

The three experiments described above were designed to examine the neural underpinnings of delay discounting. However, further experiments are necessary to expand upon these findings and determine the precise neurobiology of this behavior. Indeed, while Chapter 3 demonstrated that the PrL is a promising region to examine the neurobiology of delay discounting, Chapters 2 and 4 revealed further questions and issues about the neural pathways that mediate delay discounting. A brief discussion is presented below to address future experiments that may clarify the neurobiology of delay discounting.

Effect of chronic cocaine on PrL encoding of delay discounting behavior

Chapter 3 suggests that neuronal activity in the PrL tracks and encodes delay discounting behavior. Further, this encoding is dependent on inherent impulsivity. Previously, our lab demonstrated that cocaine did not alter either choice behavior or dopamine activity in the NAc core during delay discounting (Moschak and Carelli 2017). However, cocaine did alter how PrL neurons encoded drug-related stimuli and seeking behavior (West et al., 2014). Still, it remains unknown how a history of cocaine self-administration affects how PrL neurons encode delay discounting behavior. To examine this, two groups of rats could be fitted with intrajugular catheters and microelectrode arrays implanted bilaterally into the PrL. Rats could then be trained on the delay discounting task, and their baseline impulsivity and behavioral responses could be recorded. After task acquisition, one group of rats could be trained to self-administer cocaine for

14 days, while the other group could be saline-yoked controls trained to respond to water. Following completion of self-administration, rats could then be retrained on the delay discounting task. Changes in behavioral selection and impulsive choice could be recorded. Further, PrL neuron activity could be examined to determine if cocaine alters this brain region's ability to track and encode delay discounting behavior. Specifically, we could examine if cocaine alters the "shift" in selective neuron populations that we observe in Chapter 3. Further, we could determine if cocaine differentially alters how the PrL in high versus low impulsive rats encode delay discounting. However, we may not observe behavioral effects of cocaine on delay discounting behavior. While some studies have shown effects of cocaine on delay discounting (Roesch et al., 2007; Simon et al., 2007; Anker et al., 2009b; Mendez et al., 2010; Mitchell et al., 2014), others demonstrate no effect (Moschak and Carelli 2017; Broos et al., 2012), indicating a complex interaction between cocaine and this behavior. However, if we see no behavioral effect of cocaine, but do see changes in PrL neural activity, it may indicate that cocaine-induced alterations to PrL neurons are not sufficient to mediate delay discounting. This may shed light on the functional output of hypofrontality, the dampening of frontal regions during addiction. Alternatively, we could conduct further experiments that separate the delay discounting task into two separate delay and magnitude tasks. Here, we could examine how cocaine alters behavior and PrL firing during subjective versus objective decision making tasks.

Causal role of glutamate signaling in subjective versus objective decision-making tasks

Chapter 4 suggests that glutamate signaling in the PrL-NAc core path does not mediate delay discounting. However, this task combined two aspects of value-based decision making: subjective (delay) and objective (reward magnitude) components. Previously, our lab

demonstrated that optical stimulation of NAc dopamine terminals during delay, but not magnitude, decision making was sufficient to shift choice behavior toward the unpreferred option (Saddoris et al., 2015b). These results indicated that dopamine release in the NAc is causally linked to subjective, but not objective, decision making. However, the role of glutamate in these types of value-based decision making has not been examined. To determine if glutamate signaling in the NAc mediates subjective versus objective aspects of value-based decision making, rats could be trained on a delay or magnitude decision making task similar to that described previously (Day et al., 2010; Sackett et al., 2017; Saddoris et al., 2015b). We could then stimulate glutamate terminals in the NAc during cues that predict the delayed reward, or cues that predict the low magnitude reward, and examine rats' behavior during Free Choice trials, to determine if glutamate plays a causal role in subjective versus objective decision making.

Global examination of brain regions during delay discounting

A major takeaway from Chapters 2 and 4 is that optical manipulation of one neural pathway (i.e., VTA-NAc core, PrL-NAc core) is not sufficient to drive a complex goal-directed behavior like delay discounting. Indeed, delay discounting recruits a number of brain regions that share complex interactions. As such, a series of experiments could be conducted to examine coherence and communication between brain regions during delay discounting. Multineuron recording electrode arrays could be implanted in two brain regions, and the relationship between each regions' cellular activity could be analyzed during discrete elements of the task. First, we could extend the findings of Chapter 3 and examine the relationship between PrL and NAc core cell firing during delay discounting. The basolateral amygdala (BLA) is another prime candidate

to further examine during delay discounting, as disruption of the BLA and its connection to the mPFC heightens discounting (Churchwell et al., 2009; Ghods-Sharifi et al., 2009), and BLA neurons can differentiate between delayed versus immediate rewards (Roesch et al., 2010; Roesch et al., 2012; Schoenbaum et al., 1998). As such, we may be able to discover a relationship between BLA and NAc cell firing during the task. The coherence between regions may be further dependent on rats' impulsivity levels, as seen in Chapter 3, indicating a global neural effect of inherent impulsivity. Further, we could compare the neural activity between pathways to determine if the magnitude or patterns of neural activity differs across circuits.

Concluding remarks

Delay discounting occurs when the value of a large reward diminishes as the delay to its receipt increases. As such, delay discounting is a measure of impulsivity, as it measures the "breakpoint" when individuals shift preference toward a more immediate, yet smaller option. Delay discounting recruits the NAc core, which receives dopaminergic input from the VTA and glutamatergic tone from the PrL. The experiments detailed above examined how dopaminergic and glutamatergic signaling in the NAc core mediates delay discounting behavior, as well as how the PrL, an afferent to the NAc core, encodes aspects of our delay discounting task. The data presented here suggest that the PrL contributes to the encoding and tracking of "preferred" choices in delay discounting behavior, such that distinct neuronal populations preferentially and selectively track preferred choice as reward value changes. Further, in more impulsive animals, the PrL preferentially encoded the smaller, immediate choice compared to less impulsive animals. These results indicated a discrepancy in how this brain region encodes delay discounting depending on inherent impulsivity. However, the optogenetic studies described

above found no causal relationship between either dopamine or glutamate release in the NAc core and delay discounting. These findings may indicate that a larger neural circuit, incorporating executive, memory, and reward systems, is globally responsible for driving delay discounting behavior. Heightened delay discounting is a symptom of drug addiction, as addicts over-value the immediate, impulsive option. As such, understanding the neurobiology of delay discounting behavior is essential for developing therapeutics that could prevent and treat drug addiction and other aberrant disease states.

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