THE ROLE OF CELLULAR AND CHEMICAL SIGNALING WITHIN THE NUCLEUS ACCUMBENS IN VALUE-BASED DECISION MAKING BEHAVIORS

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ABSTRACT

JONATHAN ADAM SUGAM: The role of cellular and chemical signaling within the nucleus accumbens in value-based decision making behaviors
(Under the direction of Regina M. Carelli)

A critical component of an organism's survival is the ability to secure the necessary resources including food, shelter and mates. In order to make appropriate decisions to do so, animals must weigh the costs and benefits of different courses of action and choose the best available option. Importantly, these costs and benefits are rarely static, and organisms must attend to these changes in order to act appropriately. Multiple lines of research have identified that value-based decision making is mediated by a distributed network of brain nuclei including the nucleus accumbens (NAc) and its innervation from dopamine neurons located in the midbrain. However, the precise way in which this circuitry mediates value-based decision making remains unclear. The first set of experiments detailed in this dissertation used electrophysiological recording techniques to measure neural activity within the NAc during a risky decision making paradigm. These experiments revealed that a subset of NAc neurons tracked the different options available to the animal, displaying selective activity for risk versus safe options. Further, behavioral preferences to take a risk or play it safe were correlated with neural encoding of reward omissions. In the second set of experiments electrochemical procedures were used to evaluate the patterns of dopamine release that signal reward value as animals attend to changes in their environment and adjust their behavior accordingly. In these experiments, animals learned that cues predicted the

availability of a smaller immediate reward or larger rewards delivered after varying delays. NAc dopamine concentration signaled the predicted value of the future outcome, and shifted as the relative value of the rewards changed. The final set of experiments evaluated possible causal links between phasic dopamine release and decision making using optogenetic methods. Animals displayed goal-directed behavior to receive optical stimulation of dopamine terminals, and adjusted their behavior as the intensity of stimulation changed. Further, stimulation of phasic dopamine release was sufficient to shift certain value-based decisions. Together, these experiments provide novel characterizations of the neural circuits and mechanisms by which value-based decisions are processed within the brain, providing insight into the potential role of the NAc and mesolimbic dopamine system in mediating appropriate decisions.

To my wife

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PREFACE

This dissertation was prepared in accordance with guidelines set forth by the University of North Carolina Graduate School. This dissertation consists of a general introduction, three chapters of original data, and a general discussion chapter. Each original data chapter includes a unique abstract, introduction, results, and discussion section. A complete list of the literature cited throughout the dissertation is included at the end. References are listed in alphabetical order and follow the format of The Journal of Neuroscience.

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ANOVA	Analysis of variance
BLA	Basolateral amygdala
ChR2	Channelrhodopsin
CR	Conditioned response
CS	Conditioned stimulus
FR	Fixed ratio
FSCV	Fast-scan cyclic voltammetry
LH	Lateral hypothalamus
MSN	Medium spiny neuron
NAc	Nucleus accumbens
OFC	Orbitofrontal cortex
PCA	Principal component analysis
PEH	Peri-event histogram
PFC	Prefrontal cortex
SEM	Standard error of the mean
SN	Substantia nigra
TH	Tyrosine hydroxylase
US	Unconditioned stimulus
VP	Ventral pallidum
VTA	Ventral tegmental area

CHAPTER 1

INTRODUCTION

The ability for an organism to survive requires the successful location and collection of necessary resources. Importantly, this type of behavior does not occur in isolation, but instead requires organisms to form and maintain associations between predictive cues in the environment and beneficial outcomes. This type of processing is inherently adaptive, and it is thus hypothesized that dysfunctions related to it underlie maladaptive decisions such as increased risk taking, impulsive actions, and drug addiction (Jentsch and Taylor, 1999; Robbins and Everitt, 1999). As such, understanding the mechanisms that underlie normal reward seeking and decision making is becoming increasingly important. Further, understanding how this circuitry may go awry in disorders such as drug addiction, may provide a therapeutic target for ameliorating the negative effects of these pervasive disorders.

Several lines of research have suggested that adaptive goal-directed action and decision making relies on a distributed neural circuit that includes several distinct brain nuclei, each contributing unique features to behavioral output. This dissertation seeks to examine a portion of this network, focusing on the important role of the nucleus accumbens (NAc) and its dopaminergic innervations from the ventral tegmental area (VTA) in appropriate decision making. The NAc is the center of the corticolimbic reward circuit receiving inputs from the prefrontal cortex (PFC), basolateral amygdala (BLA), hippocampus, thalamus, and a dense dopaminergic input from the VTA. In turn, the NAc

integrates this information and impacts behavior through its connections with motor-related regions (Zahm and Brog, 1992a; Zahm, 1999), supporting the theory that the NAc is a critical site of convergence for reward-related decision making. While a large amount of work has contributed to the characterization of NAc signaling and its critical role in decision making (Cardinal et al., 2001; Cardinal and Howes, 2005; Ghods-Sharifi and Floresco, 2010; Stopper and Floresco, 2011; Stopper et al., 2013), the functional contributions of these signals, and how they may be causally linked to controlling appropriate behavioral responding, has not been extensively studied. Therefore, the experiments outlined in this dissertation seek to further characterize the role of NAc cellular signaling and the associated dopaminergic contributions to decision making based on several factors, including subjective value, reward devaluation/impulsive choice, and the causal link between this signaling and appropriate responding. Here, a brief introduction is provided of the exhaustive literature on the role of the NAc, and mesolimbic dopamine system, in reward processing and goal-directed behavior. First, this chapter will provide a review of the overall relevance of the processes that govern learning and choice behavior. Next, the cellular and systems-level mechanisms underlying neural communication within the NAc is discussed, with emphasis on its dopaminergic input from the VTA. Finally, these ideas will be integrated in order to examine theoretical and empirical links between dopamine release in the NAc, NAc neural activity, and reward-related decision making.

Associative learning and decision making

A critical component of an organism's survival is the ability to maintain necessary resources in a highly demanding and constantly changing environment. To do this, organisms have evolved associative learning mechanisms that increase their ability to predict, procure,

and consume rewards. Within this framework there are two general types of associative processes that have developed. The first type of learning is *stimulus-outcome* learning (known as Pavlovian or classical conditioning) in which an organism learns to associate a previously neutral stimulus (conditioned stimulus CS) with a biologically significant outcome such as food (the unconditioned stimulus US). Following several pairings of the CS with the US, the CS gains biological significance and can then influence ongoing behavior towards collecting resources (Pavlov and Anrep, 1927; Dickinson, 1980; Rescorla, 1988b). Pavlovian stimuli are presented non-contingently to the organism such that behavioral actions are not required to produce the outcome. Importantly, this type of learning is dependent on several factors that influence the ability to associate predictive cues with appropriate outcomes including the identity and value of the US, the identity of the predictive CS, the contingency between the CS and US, and the temporal relationship between the CS and US, among other factors (Rescorla, 1968, 1969; Rescorla, 1988b; Rescorla, 1988a). As such, Pavlovian conditioning is not simply a reflex, but instead reflects a complex understanding of the relationship of the motivational state to distinct and important stimuli in the environment.

The second general type of associative processing is *action-outcome* learning (known as operant or instrumental conditioning) in which an organism learns that a behavioral response results in a biologically salient outcome. Importantly, as with stimulus-outcome learning, the outcomes can be either appetitive or aversive, but both function to modulate behavioral responding. As such, the presence of appetitive outcome functions to increase a particular goal-directed action, while the presence of an aversive outcome functions to decrease associated actions (Thorndike, 1898; Skinner, 1938a, 1938b). Again, the pattern and

vigor of responding are dependent on several factors related both to the action and the outcome including, the amount of responding necessary to produce the outcome, the frequency with which the outcome is presented, the identity and value of the outcome, among other factors (Ferster and Skinner, 1957). Importantly, action-outcome responding can be differentiated from habitual responding for outcomes. The performance of habitual actions depends on the association between a predictive stimulus and associated action, regardless of the outcome, while action-outcome responding is mediated by the associations between the action and the consequences of action, and thus rely on separate brain circuits. In particular action-outcome behaviors are dependent on the NAc while habitual responding is dependent on the dorsal striatum (Everitt and Robbins, 2005; Takahashi et al., 2007; Everitt et al., 2008; Dezfouli and Balleine, 2012). Further, unlike habitual responding, action-outcome responses are sensitive to changes in the value of outcomes (Adams, 1982) and are sensitive to changes in the causal relationship between the action and outcome delivery (Dickinson, 1998). Maladaptive behaviors, such as drug addiction, are often characterized as a shift from action-outcome associations to habitual actions (Robbins and Everitt, 1999; Everitt et al., 2001; Everitt and Robbins, 2005; Everitt et al., 2008).

It is important to note that while stimulus-outcome and action-outcome associations are distinct processes, they rarely occur in isolation. For example, organisms may be presented with situations in which reward paired stimuli (referred to as a discriminative stimulus) predict the opportunity to make a behavioral response for a certain outcome. In this situation, organisms learn that the particular environmental stimuli signal if a behavior will be reinforced (Jones et al., 2010b; Ambroggi et al., 2011). Further, cues paired with rewards following behavioral actions can also gain motivational significance and function to promote

behavioral responding on their own (termed conditioned reinforcers), such that animals will perform operant actions for this cue delivery, even in the absence of the primary reward itself (Zimmerman, 1957). Numerous studies have also shown that Pavlovian cues can potentiate operant responding, even when there is no association between the cue and the response, a behavioral effect known as Pavlovian-to-instrumental transfer (PIT) (Estes, 1948; Rescorla and Solomon, 1967; Saddoris et al., 2011). In a PIT task, animals are first trained that a cue predicts a positive outcome. Next, animals are separately trained that a behavioral response also leads to reinforcement. When animals are engaged in behavioral responses, presentation of the reward paired cue functions to invigorate responding, increasing behavioral response rates, suggesting that the cue also holds some motivational value.

Ongoing learning mechanisms that enable organisms to obtain food, mates, and shelter are clearly adaptive. However, rarely in an environment are organisms presented situations in which only simple stimulus-outcome or response-outcome situations are in effect. Instead, organisms must learn to evaluate the costs and benefits of action selection, a process that requires both stimulus-outcome and response-outcome learning (Green and Myerson, 2004; Cardinal, 2006; Phillips et al., 2007; Rangel et al., 2008). Cost-benefit decision making is a multistep process in which organisms evaluate several different aspects of the environment to make appropriate choices. First, organisms formulate representations of potential courses of action based on internal need states and external predictive stimuli. Next, the organism assigns a value to each possible course of action based on these internal and external representations, and chooses the best available option. Finally, the organism evaluates the outcome of the action to determine if this was the correct choice/behavioral response. Comparisons of the outcome received and the predicted results of action

performance results in learning (i.e. representations of future actions are updated to optimize future choices) (Rangel et al., 2008). This type of value-based decision making can be modeled in humans and animals by exposing organisms to situations in which there is a choice between rewards of different value. For example, humans (Coffey et al., 2003; Green and Myerson, 2004; Hariri et al., 2006; Prévost et al., 2010) and animals (Cardinal et al., 2001; Roesch et al., 2007; Roesch et al., 2009; Day et al., 2010) show similar patterns of choice behavior based on the time the organism spends waiting for the reward, choosing the larger option less often as the delay to reward increases. Similar patterns of discounting are also seen when organisms must choose between rewards based on reward cost and risk (St Onge and Floresco, 2008; Floresco and Whelan, 2009; Simon et al., 2009; Day, 2010; Day et al., 2010; Gan et al., 2010; Prévost et al., 2010; Sugam et al., 2012). These results demonstrate that organisms use cost-benefit analysis to guide selection between actions to maximize resources, even when both actions are rewarded.

Nucleus accumbens circuitry

Cellular and chemical composition of the nucleus accumbens: In order to learn stimulus outcome-associations and make appropriate decisions, the brain requires a circuit that can track environmental stimuli and reward presentations, link these events together, and make connections with motor output areas. The NAc is uniquely situated within this type of network to integrate reward related information and promote appropriate behavioral output. At the cellular level, the NAc is comprised primarily (~95%) of GABAergic medium spiny neurons (MSNs) that send their projections out of the NAc to downstream structures (Groves, 1983; O'Donnell and Grace, 1993). MSNs are defined by a medium sized soma (about 10-20μm in diameter) (Preston et al., 1980; Gerfen, 1988; O'Donnell and Grace, 1993;

Kawaguchi, 1997) with a large radially projecting dendritic tree (about 250μm in diameter) (Preston et al., 1980; Groves, 1983; Gerfen, 1988). These cells have axons that project from the NAc to areas such as the substantia nigra, ventral pallidum, and lateral hypothalamus to influence behavior (Gerfen, 1988; Kawaguchi, 1997; Zahm, 1999). Importantly, the NAc is not a homogeneous structure as MSNs have specific characteristics that define a complex circuitry. For example, immunohistomchemical markers reveal that MSNs contain enkephalin, dynorphin, substance P, and neurotensin, and the specific type of marker predict the separate pathway and output structure projections of each MSN (Meredith, 1999). Further control of this specific circuitry comes from the dense dopaminergic projection from the VTA. The majority of MSNs express either D1-like or D2-like receptors, with very few expressing both (17% coexpress both in the NAc shell and 6% coexpress both in the NAc core) (Bertran-Gonzalez et al., 2008). D1-like labeled MSNs expressing dynorphin and D2-like labeled MSNs expressing enkephalin, and thus may represent separate projection systems. As such dopamine may be playing a specific function in modulating certain projection pathways from the NAc (Le Moine and Bloch, 1995).

MSNs are unique neurons in that they have a bistable potential and thus exist in two potential states. In the “down state” the resting membrane potential for MSNs is ≈ -77mV while the resting membrane potential is ≈ -54 mV in the “up state” (O'Donnell and Grace, 1993; Wilson and Kawaguchi, 1996). Therefore, MSNs are more likely to fire action potentials when they are in the up state. Importantly, activation of D1 receptors functions to maintain neurons in their up state, thus increasing the likelihood that they will fire an action potential. In support, pharmacological inactivation of phasic dopamine release in the NAc preferentially reduces excitatory responses, suggesting that dopamine functions to increase

the likelihood of burst firing of MSNs (Cacciapaglia et al., 2011), classifying dopamine as a key neuromodulator of neuronal function in the NAc (Goto and Grace, 2005).

The other 5% of neurons in the NAc are considered local circuit or interneurons and are of two main types: cholinergic interneurons or GABAergic neurons (Groves, 1983; Meredith, 1999). The cholinergic interneurons are much larger in size (35 μm diameter soma) with radially emanating dendrites, and dendrites that are mostly devoid of spines. Importantly, these cholinergic interneurons can be differentiated from classic MSNs based both on morphology and firing rate. The baseline firing rate for MSNs is typically 1-3Hz exhibiting phasic bursts of activity while cholinergic interneurons display tonic firing rates and are the source of acetylcholine within the NAc (Kawaguchi et al., 1995; Meredith, 1999). GABAergic interneurons make up the rest of the neurons within the NAc. These neurons comprise at least three different populations; parvalbumin, calretinin, or somatostatin and neuropeptide Y positive populations and are differentiated from MSNs based on their tonic activity with brief high frequency bursts. Further, the oscillatory behavior between these GABAergic interneurons and MSNs is critical for mediating normal MSN activity (Berke et al., 2004).

Afferent and efferent connections of the nucleus accumbens: The NAc has been proposed to be critical for associative learning and goal-directed actions because it is the integration center of much of the reward-related processing of the corticolimbic circuitry within the brain (Figure 1.1). The NAc receives glutamatergic afferent projections that carry reward related information from the basolateral amygdala (BLA) (Zahm and Brog, 1992a; Wright and Groenewegen, 1996), prefrontal cortex (PFC) (Zahm and Brog, 1992a; Wright and Groenewegen, 1996), orbitofrontal cortex (OFC) (Wright and Groenewegen, 1996) and

hippocampus (Brog et al., 1993), as well as thalamic regions (Finch, 1996; MacAskill et al., 2012) Further, the NAc receives a dense dopaminergic input from the VTA (approximately 85% of VTA dopamine neurons project to the NAc) (Fields et al., 2007). In turn, the NAc sends efferent projections to nuclei that organize motor behavior including the ventral pallidum and subthalamic nucleus (Nauta et al., 1978).

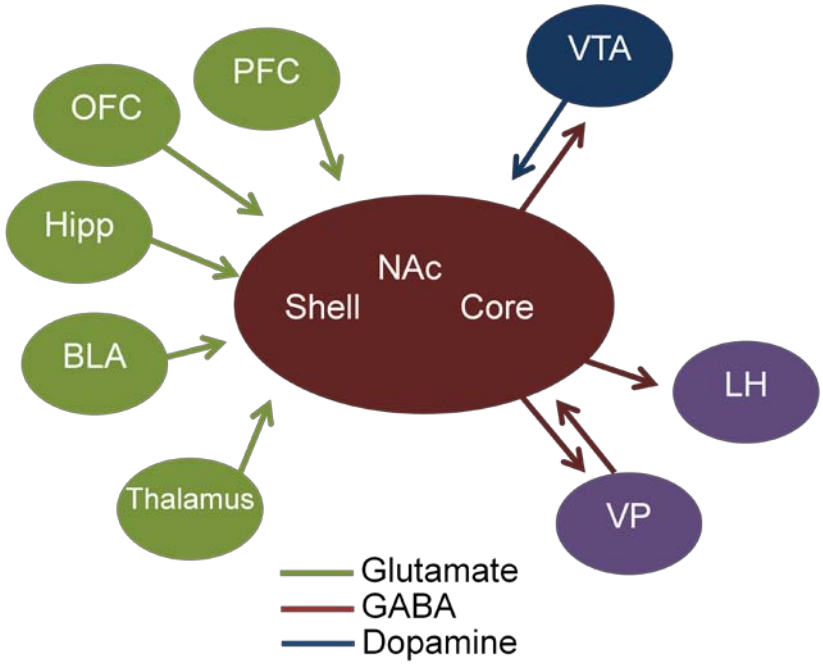

Figure 1.1 Simplified schematic of afferent and efferent connections of the NAc. Green arrows denote glutamate projections, red arrows denote GABA projections, and blue arrows denote dopamine projections. Note, these are not indicative of precise anatomical location or degree of projections.

The connectivity of the NAc supports its role as an integration zone of reward-related information that functions to promote behavioral output, and thus has been classified as a "limbic-motor integrator" (Mogenson et al., 1980). Subsequent studies have shown that the synaptic connections between corticolimbic regions, dopamine projections and MSNs support this theory. For example, dopaminergic projections from the VTA have been shown to synapse on MSNs in the NAc that also receive direct input from the hippocampus (Totterdell and Smith, 1989), while terminals from PFC afferants onto MSNs are also

modulated by VTA activity (Brady and O'Donnell, 2004). Thus, NAc neurons are in a prime position to coordinate associative learning from afferents with action selection in downstream motor targets.

Structural and functional subregions of the nucleus accumbens: the core and shell: Importantly, the NAc is not a homogenous structure, but instead has been shown to be comprised of two important subregions: the core and shell (Ikemoto, 2007) which are separated both physically and functionally. For example, the core is critical for cue-outcome associations, reward learning, and goal-directed behaviors (Cardinal et al., 2002; Kelley, 2004; Day et al., 2007). In contrast, the shell is critical for encoding reward valence (Wheeler et al.; Corbit et al., 2001; Bassareo et al., 2002) and integrating emotional and limbic information with stimulus-outcome associations. Core and shell subterritories receive different projections patterns from cortical and limbic regions. For example, the NAc core receives the majority of its prefrontal input from the prelimbic region and lateral OFC, while the NAc shell receives input from the infralimbic cortex and more medial lateral OFC (Berendse et al., 1992; Wright and Groenewegen, 1996). Importantly, both regions receive dense dopaminergic input from the VTA, but exhibit differential levels of dopamine transporters, with higher levels in the NAc core (Jones et al., 1996). As such, phasic dopamine burst events result in different time courses of dopamine action which may result in the functional differences between the core and shell. Further, these regions are dissociable in their output projections such that the core is similar to basal ganglia circuitry, projecting through the ventral pallidum (dorsolateral district) subthalamic nucleus, and substantia nigra. In turn, these outputs project via the motor thalamus to premotor cortical areas. In contrast, the shell projects to subcortical limbic regions including the lateral hypothalamus, ventral

pallidum (ventromedial district) and VTA (Zahm, 1999). These dissociable afferent and efferent projection patterns suggest that the core and shell differentially participate in reward related behaviors (Saddoris et al., 2012).

The nucleus accumbens and goal-directed action

Given the functional connectivity of the NAc as a corticolimbic motor integration center, early studies sought to inactivate or lesion the NAc to evaluate how disruptions of this portion of the circuit affected motivated responding. This early work found an important role for the NAc in contributing to appetitive and consummatory phases of reward responding (Stratford and Kelley, 1997; Berridge and Robinson, 1998; Kelley, 2004). In particular, studies found that infusion of $GABA_A$ and $GABA_B$ agonists into the NAc shell induced feeding behaviors (likely through connections with the lateral hypothalamus) (Stratford and Kelley, 1997; Stratford and Kelley, 1999; Kelley, 2004) while administration of glutamate antagonists into the NAc (specifically the AMPA antagonist DNQX) also functioned to stimulate feeding behaviors (Stratford et al., 1998; Kelley, 2004). This data suggests that inhibition of NAc activity, and thus a disinhibition of downstream motor areas, was critical for the initiation of food related foraging behaviors. Interestingly, these early studies on the role of NAc activity in food intake found that the NAc shell was predominantly involved in these consummatory phases of reward delivery, again suggesting both a structural and functional division of the NAc core and shell. Recent evidence using optogenetic techniques has provided further evidence that activity of mesolimbic dopamine neurons are also a driving force of reward consumption behaviors (van Zessen et al., 2012).

In order to evaluate a precise role of NAc cellular activity during reward related responding, researchers have used a technique known as *in vivo* electrophysiology. With this

technique, researchers have been able to record NAc neural activity in an intact animal as the animal performs reward related behaviors, therefore giving functional insight into how the NAc is critically involved in encoding reward related behaviors. When examining NAc neural activity during reward consumption, researchers first found that NAc neurons show decreases in firing during this period (Roitman et al., 2005; Taha and Fields, 2005a, 2006; Wheeler et al., 2008; Ambroggi et al., 2011), suggesting the functional link between NAc activity and the earlier pharmacological studies that showed that decreasing neural activity in the NAc induced feeding. This suggests that NAc neurons function to "gate" foraging or consummatory behaviors. Specifically reducing neural activity within the NAc releases motor areas from strong GABAergic inhibitions, releasing the "gate" on reward consumption behaviors. In support of this theory, another study found that individual ventral pallidum neurons (the downstream target of NAc projections) show increases in firing rate during reward consumption (Tindell et al., 2006).

In order to promote appropriate foraging behavior, it is necessary to have a circuit that can differentiate the type of reward, rather than just promoting general consumption behavior. As such, evidence from our lab and others shows that NAc neurons encode specific aspects of reward processing. For example, while neurons encode appetitive rewards with increased inhibitions, the presentation of aversive stimuli induce increased excitatory responses of NAc neurons (Roitman et al., 2005; Wheeler et al., 2008). Further, NAc neurons encode specific aspects of rewarding stimuli, as they exhibit differential activity for drug versus natural rewards (Carelli and Deadwyler, 1994; Carelli et al., 2000; Carelli, 2002; Carelli and Wondolowski, 2003) as well as distinguish rewarding outcomes based on reward palatability (Taha and Fields, 2005b). Further, in order to be implicated in associative

learning and decision making processes, NAc neural encoding not only needs to track reward deliveries, but also the predictive stimuli associated with these rewards. In support, electrophysiological recordings have shown that NAc neurons also display increased and/or decreased cell firing to cues that predict future rewards (Carelli, 2000, 2004; Nicola et al., 2004a; Roitman et al., 2005; Day et al., 2006; Jones et al., 2010a; Saddoris et al., 2011), suggesting that the NAc is able to encode the association between reward predictive cues and positive outcomes. Further, ventral striatal neurons display differential activity to cue presentation based on the value of future outcomes (Schultz et al., 1992; Cromwell and Schultz, 2003; Kim et al., 2009; Roesch et al., 2009; Day et al., 2011), suggesting an ability to track specific features associated with cue presentations. Finally, NAc neurons also show phasic activity during the execution of behavioral responses (Carelli, 2002; Hollander and Carelli, 2005; Day et al., 2011; Saddoris et al., 2011), supporting a direct role for the NAc as a limbic-motor integrator.

Electrophysiological studies provide evidence that the NAc is involved in encoding information about reward related responding and associative learning, however this signaling may be functioning to either encode associative learning mechanisms to drive reward-related responding, or this signaling could be a result of the execution of motivated behaviors. In order to analyze this, researchers have begun to manipulate NAc circuitry during behavioral responding to evaluate the direct role of NAc signaling in behavior. Early evidence suggested that the NAc itself, nor the dopaminergic projections to the NAc, was critical for well learned simple goal-directed actions, as animals would press levers on an FR1 schedule to obtain rewards, even after lesion of the NAc (Sokolowski and Salamone, 1998; Corbit et al., 2001). While the NAc circuitry does not appear critical for the expression of simple goal-directed

actions, an intact NAc circuitry is necessary for learning the associations between cues and outcomes, actions and outcomes, and changes in reward value (Sokolowski and Salamone, 1998; Corbit et al., 2001; Cardinal et al., 2002; Cardinal and Cheung, 2005). Therefore, the NAc appears to be uniquely involved in associative processing of more complex situations rather than driving simple goal-directed actions. As such, researchers have begun to investigate how the NAc is implicated in behaviors in which organisms must use these complex associations, such as in value-based decision making. As discussed above, models of decision making expose subjects to cues that predict outcomes of different value, and animals are allowed to make choices between these different options. Damage to the NAc has repeatedly been shown to disrupt the ability of animals to show normal decision making in these experiments. For example, animals with lesions or temporary inactivation of the NAc were impaired in decisions based on both reward delay, effort and probability, choosing smaller, certain, immediate rewards much more often, even when this was the less advantageous option (Cardinal et al., 2001; Cardinal and Cheung, 2005; Cardinal and Howes, 2005; Floresco et al., 2007; Ghods-Sharifi and Floresco, 2010; Stopper and Floresco, 2011). Further, inactivation of the NAc shell resulted in the inability to choose the appropriate response when evaluating rewards of different magnitude, choosing the larger reward less often then controls (Stopper and Floresco, 2011). This evidence suggests the NAc plays a critical role in the association of reward related cues and behaviors in appropriate reward seeking, especially when presented with several options of different value.

The mesolimbic dopamine system

Anatomy of the VTA: Decades of research on the mesolimbic dopamine system have shown that this system provides a “learning signal” that follows associative learning principals to

guide appropriate behaviors (Schultz et al., 1997; Schultz, 1998; Waelti et al., 2001), and has recently been shown to be causally linked to cue-outcome learning (Stuber et al., 2008; Tsai et al., 2009; Zellner et al., 2009; Zellner and Ranaldi, 2010). The mesolimbic dopamine projections originate from dopamine cell bodies in the VTA which is ventral to the red nucleus and medial to the dopamine rich substantia nigra (SN). As the VTA lacks clear borders, it can be distinguished from the SN based on both the projection patterns of the dopamine neurons and a unique afferent projection from the lateral hypothalamic area (Nauta, 1958; Ikemoto, 2007). The VTA and SN comprise two of the main projection sites of dopamine neurons, and importantly provide two distinct circuits of dopamine projections. The SN comprises the nigro-striatal pathway of dopamine release and sends the majority of its projections to the dorsal striatum (caudate and putamen). Conversely, the VTA projects to diverse brain targets including the PFC, amygdala, hippocampus, ventral pallidum and NAc. Importantly, the majority of dopamine neurons from the VTA project to the NAc core and shell (Anden et al., 1964; Swanson, 1982; Fields et al., 2007; Ikemoto, 2007). Dopamine neurons comprise the majority of the VTA, while GABAergic neurons comprise a smaller population of cells that function to regulate dopaminergic cellular activity within the VTA (Kalivas et al., 1990; Olson and Nestler, 2007). Further, the VTA receives inputs back to dopamine neurons from the hypothalamus, dorsal raphe, NAc, pallidum, and amygdala (Watabe-Uchida et al., 2012).

Release patterns of mesolimbic dopamine neurons: Mesolimbic dopamine neurons display two general types of firing and release patterns. Under basal conditions, dopamine neurons fire at a relatively low firing rate (2-4Hz) that result in low levels of dopamine release in terminal regions, known as a “tonic” firing pattern. Under certain situations, dopamine

neurons will show increased burst like activity which results in significant increases in dopamine release, known as “phasic” activity (Grace and Bunney, 1984; Grace, 1991; Chergui et al., 1993; Grace, 2000). Importantly, this phasic activation of dopamine neurons results in a robust, although transient, increase in dopamine concentration (Garris et al., 1994; Garris et al., 1999; Brady, 2004). Phasic burst firing of dopamine neurons and subsequent terminal release is dependent on glutamatergic activity within the VTA, specifically resulting from stimulation of NMDA receptors (Chergui et al., 1993; Sombers et al., 2009). NMDA receptor antagonism within the VTA results in the elimination of phasic release events within the NAc while not disrupting tonic baseline levels (Sombers et al., 2009). Importantly, phasic dopamine release is not confined to the synaptic cleft and is able to spill over into outlying areas, supporting the role of dopamine as a volume transmitter such that phasic release of dopamine can modulate relatively large territories of neural tissue (Rice and Cragg, 2008). Importantly, phasic dopamine release within the terminal region is highly variable across the microenvironment, suggesting heterogeneity in dopamine release (Venton et al., 2003).

Several factors have been shown to regulate dopamine release in downstream target regions. First, previous dopamine cellular activity and release has a dynamic relationship with subsequent release, and as such, the history of dopamine release can alter subsequent release (Montague et al., 2004). Further, the projections from other brain regions to dopamine terminal regions function to regulate dopamine release. Previous work from our lab has shown that inactivation of the BLA significantly reduces cue-evoked phasic dopamine release while not altering electrically stimulated release, suggesting a terminal modulation mechanism (Jones et al., 2010b). Conversely, enhanced glutamate transmission

in the NAc can also increase dopamine release, presumably through presynaptic mechanisms (Imperato et al., 1990; Howland, 2002). The duration of dopamine release at the level of the NAc is tightly regulated by the presence of dopamine transporters, which function to terminate dopamine signaling through reuptake mechanisms (Cragg and Rice, 2004). Dopamine transporters are highly expressed in both the NAc core and shell, although with greater density in the NAc core (Jones et al., 1996), supporting differential patterns of dopamine transmission within these regions. Dopamine transporters are also the site of action for several drugs of abuse, including cocaine and amphetamine, and blockade of dopamine transporters by these drugs functions to increase phasic dopamine levels and increases the time to dopamine reuptake (Addy et al., 2010).

Dopamine receptors: Once released from the neuron, dopamine can function at one of two different classes of G-protein receptors, "D_1-like" (D_1 and D_5) and "D_2-like" (D_2, D_3, and D_4) receptors (Kebabian and Calne, 1979). D_1-like receptors are coupled to G_s proteins that function to increase intracellular levels of cyclic adenosine monophosphate (cAMP) resulting in a host of intracellular signaling functions. Alternatively, D_2-like receptors are coupled to $G_{i/o}$ proteins that function to decrease intracellular levels of cAMP by inhibiting production (Girault J, 2004; Snyder, 2011). Although the two classes of dopamine receptors have divergent effects, several properties about their location and function enable dopamine signaling to be a very dynamic process. As previously discussed, MSNs in the NAc have been shown to express only one subtype of dopamine receptor (Bertran-Gonzalez et al., 2008), and therefore dopamine can exert very specific effects on each MSN. It is presently unknown exactly which cell populations within the NAc selectively express D_1-like or D_2-like receptors, however this suggests that there may be separate signaling pathways from the

NAc to output regions based on receptor expression. Further, the different subtypes of receptors have a different affinity for dopamine, with D_2-like receptors having a much higher affinity than D_1-like receptors (Richfield et al., 1989; Missale et al., 1998). Therefore, in the presence of low levels of dopamine, such as during baseline tonic release periods, D_2-like receptors are much more likely to be activated. In contrast, high concentration phasic burst events of dopamine release are much more likely to activate D_1-like receptors. Both the anatomical and functional organization of dopamine receptors allow for the mesolimbic dopamine system to function in a highly dynamic manner.

Synaptic actions of dopamine within the nucleus accumbens: MSNs in the NAc receive glutamatergic synaptic inputs from several cortical and limbic regions as discussed above. Importantly, dopamine neurons projecting from the VTA synapse onto the necks and spines of MSNs and are located adjacent to these glutamatergic synapses (Voorn et al., 1986; Groves et al., 1994), suggesting a critical role in the modulation of these glutamatergic inputs. Several *in vitro* and *in vivo* studies have confirmed that dopamine release does not have direct excitatory or inhibitory actions but instead function to modulate incoming glutamatergic activity from areas such as the PFC, specifically functioning to dampen the effect of glutamatergic activity from the PFC (Brady and O'Donnell, 2004; Goto and Grace, 2005). As such, one effect of dopamine may be to "gate" glutamatergic inputs in the NAc, such that only the strongest inputs can control NAc output (Floresco et al., 2001). Further, MSNs have been shown to go through extensive plasticity, displaying both long term potentiation (LTP) and long term depression (LTD) through numerous mechanisms and projection inputs (Russo et al., 2010). Importantly, dopamine release within the NAc has been shown to be critical for inducing both LTP (Calabresi et al., 2000; Fasano et al., 2013)

and LTD (Thomas et al., 2000; Ishikawa et al., 2013), and is dependent on which dopamine receptors are activated. This evidence suggests that dopamine release within the NAc can function to modulate specific synaptic connections, interacting with specific glutamatergic inputs depending on where dopamine neurons synapse, thus providing a dynamic system for the mediation of synaptic plasticity within specific target regions of the NAc.

Role of mesolimbic dopamine activity in associative learning and decision making

Several decades of research have tried to determine the precise role of mesolimbic dopamine activity in reward related behavior and have demonstrated that the blockade of dopamine receptors produced a decrease in goal-directed behavior for food and other rewards (Wise et al., 1978b; Wise et al., 1978a). Specifically, rats still worked for rewards with dopamine antagonists on board, however responding decreased across time. This suggested that dopamine functions to encode the "pleasurable" aspects of reward seeking. One of the leading preliminary hypotheses for dopamine function was the "anhedonia hypothesis" of reward. Proposed by Roy Wise in 1982, this hypothesis suggested that dopamine was the "rewarding" neurotransmitter and as such, dopamine release signaled rewards and this pleasure signal is what promoted goal-directed behaviors. Organisms would work to obtain rewards because the reward receipt "felt good" as a result of increased dopamine release (Wise, 1982; Wise, 2008). These findings initially led to the suggestion that dopamine release in the NAc mediates the hedonic or "pleasure" aspects of rewarding stimuli, and, in turn, that both natural and drug rewards could be defined by this common path of activation. Further, this hypothesis suggested that neurological diseases that decrease dopamine release in the brain are associated with decreases in pleasure as a result of the decreased dopamine release. However, this hypothesis has been questioned based on several

lines of evidence. For example, research has shown that dopamine mediates the "wanting" or how much an animal will work for a reward, but not how much an animal "likes" the reward. Specifically, dopamine depletions do not disrupt orofacial responses for hedonic rewards, suggesting that dopamine does not mediate the appetitive valence, hedonic value, or simply the "pleasure" associated with rewards (Berridge et al., 1989). Further, aversive stimuli have also been shown produce increases in dopamine release in the NAc and dorsal striatum, suggesting that the mesolimbic dopamine circuit is also important for aversive responding (Badrinarayan et al., 2012; Budygin et al., 2012; Lammel et al., 2012). Further, dopamine antagonists as well as lesions of the NAc disrupted reward related responding only when effort requirements were high, but not in simple response-outcome situations (Salamone et al., 2001; Salamone et al., 2002; Salamone et al., 2005). Taken together, these findings suggest that dopamine does not simply signal the hedonic or "pleasurable" aspects of reward-related behaviors, but instead supports a more complex role for the mesolimbic dopamine system in goal-directed behaviors.

Since the original "anhedonia" hypothesis, several lines of research have led to many different hypotheses of dopamine function. One of the most influential hypotheses has come from electrophysiological recordings of mesolimbic dopamine neurons in both rats and monkeys. In a seminal study from Schultz and colleagues (Schultz et al., 1997), dopamine neurons were recorded in the midbrain of monkeys while the animals were learning cue-outcome associations. It was found that dopamine neurons exhibit brief increases in activity when rewards are presented unexpectedly. However, when the animal learns that a CS predicts the reward delivery, signaling of dopamine neurons shifts to the reward predictive cue, such that dopamine neurons increase firing rate during cue presentation rather than

during the reward period. Further, when a reward is unexpectedly omitted, there is a reduction in neural activity (Schultz et al., 1997). Schultz and colleagues believed that this dopamine signaling functioned as a "teaching signal" (Schultz et al., 1997) and as such this signaling is consistent with contemporary learning theory (Mirenowicz and Schultz, 1994; Waelti et al., 2001; Pan et al., 2005). Work from our laboratory has shown that this dopamine signal is transmitted to the terminal regions in the NAc, as electrochemical recordings of dopamine release in the NAc show similar patterns of activity to dopamine neural recordings during Pavlovian learning paradigms (Day et al., 2007). According to this hypothesis, activation of dopamine neurons during unexpected reward presentations signals an error in ongoing reward predictions. As cues come to predict future outcomes, the dopamine signaling shifts to these cues and acts as a predictor of future outcomes and no longer signals the reward delivery because this does not constitute a violation of reward predictions. By computing the difference between expected and actual outcomes, dopamine neurons are hypothesized to play a key role in reward-related learning. In support of its role in learning cue-outcome associations, pharmacological blockade of dopamine activity blocks the acquisition of Pavlovian learning (Di Ciano et al., 2001; Dalley et al., 2002; Zellner et al., 2009; Zellner and Ranaldi, 2010), while stimulation of phasic dopamine release is sufficient to promote associative learning (Tsai et al., 2009).

Recent research has begun to examine how the prediction error signaling of dopamine neurons may be implicated in more complex decision making behaviors rather than simple stimulus-outcome associations. Studies have shown that perturbations of the mesolimbic dopamine circuitry including the terminal region of the NAc disrupt value-based decisions based on reward effort, delay, and risk. Specifically, blocking the activity of DA transmission

through receptor antagonists or dopamine lesion biases animals towards emitting more "impulsive" responses that lead to less desirable rewards, but require less effort (Sokolowski and Salamone, 1998; Salamone et al., 2001; Salamone et al., 2002; Ishiwari et al., 2004; Mingote et al., 2005), shorter latency to reward (Floresco et al., 2007) or higher probability of reinforcement (St Onge and Floresco, 2008; St. Onge et al., 2010). Further, dopamine neurons display dynamic encoding of reward value displaying increased activation for higher value rewards based on reward probability, delay, and magnitude (Fiorillo et al., 2003; Tobler et al., 2005; Kobayashi and Schultz, 2008). Value signaling of dopamine activity has also been observed in situations when animals are actively making decisions between two options, showing increased activity and dopamine release for the more valuable option (Roesch et al., 2007; Day et al., 2010; Sugam et al., 2012). This type of processing by dopamine neurons is hypothesized to be critical for decision making as it functions to broadcast information about reward value to striatal circuits that enable animals to maximize behaviors (Roesch et al., 2007; Day et al., 2010).

Using this framework, value-based decision making can be explained through basic utility functions as a "cost-benefit analysis." Thus animals must evaluate the behavioral costs that discount reward value to determine if a behavior is beneficial. In order to do this, animals have an intrinsic "threshold" for behavior such that options that fall below the "cost threshold" are deemed worthwhile, while options that fall above the cost threshold are deemed not worthwhile and will be rejected. It has been hypothesized that value encoding by the dopamine system is used to monotonically set the "cost threshold" (or breakpoint) beyond which the net outcome is no longer worthwhile. With this model, phasic dopamine activity in the NAc core signals information about the value of future rewards, and if this

prediction is below the cost threshold the behavior is deemed worthwhile and the animal will perform the task. Conversely, options that are predicted to have low value will evoke much less dopamine release, and thus this signal of future value will fall above the cost threshold and will be deemed not worthwhile (Phillips et al., 2007). This model can also be applied to a situation in which animals are given concurrent choices with different values. Rather than serving as a threshold for performing a behavior or not, the dopamine system can be functioning to compare how two separate behaviors relate to the cost threshold and thus inform animals of which option is more worthwhile to guide choice behaviors. In support, dopamine neural activity and terminal release encode information about the best available option when animals are given a concurrent choice of options with different explicit value, irrespective of what the animal actually chooses (Roesch et al., 2007; Day et al., 2010; Sugam et al., 2012), thereby functioning to bias animals towards more valuable options. Thus, dopamine release in the NAc may play a key role in the evaluation of different responses and thus functions to promote appropriate action selection.

Goals of this dissertation

As reviewed above, the NAc and its dopaminergic afferents are critical for associative learning, goal-directed behaviors, and appropriate decision making. With the advances in electrophysiological and electrochemical recording techniques as well as optogenetics to manipulate specific circuitry, real time characterization of NAc signaling during behavioral tasks have allowed for novel insights regarding the role of NAc in decision making behaviors. Previous investigations from this laboratory and others have shown that NAc neurons exhibit time-locked phasic changes in activity during the presentation of reward paired cues (Nicola et al., 2004a; Jones et al., 2010a; Day et al., 2011; Saddoris et al., 2011),

operant responses for rewards (Carelli et al., 2000; Carelli, 2002), and signal specific information during value-based decision making (Roesch et al., 2009; Day et al., 2011). Further, dopamine release within the NAc tracks cues that predict rewarding outcomes (Beyene et al., 2010; Day et al., 2010; Jones et al., 2010b; Sugam et al., 2012), operant responses for drug and natural rewards (Phillips et al., 2003b; Roitman et al., 2004), and is causally linked with behavioral conditioning (Tsai et al., 2009). However, little is known about how this NAc activity and dopamine signaling is implicated in more complex decision making behaviors based on subjective value, or as the value associated with reward predictive stimuli changes. Further, little is known about how dopamine release in the NAc terminals is causally linked with goal-directed behaviors and value-based decision making. The proposed studies seek to elucidate the specific role of NAc signaling and dopamine release in complex value-based decision making paradigms requiring animals to evaluate multiple aspects of reward value to make appropriate decisions.

Specific Aims:

1. **To examine NAc cell firing during a risky decision making task.** A large body of evidence suggests the NAc and associated dopaminergic input is critical for decision making based on risk, as disruptions of this system result in impairments in risky decision making (Cardinal and Howes, 2005; Adriani et al., 2009; Simon et al., 2011; Stopper and Floresco, 2011; St. Onge et al., 2012). Previous work from our lab has shown that phasic dopamine encodes the subjective value of future rewards, such that phasic dopamine scales to cues that signal the preferred option to “take a risk” or “play it safe” (Sugam et al., 2012), and this signaling may contribute to differential signaling within the NAc during a risky decision making. Further, prior work has evaluated how NAc neurons encode decisions based on

extrinsic reward factors (Roesch et al., 2007; Day et al., 2010); however, little is known on how the NAc encodes the subjective or intrinsic value of behavioral responding. This aim will advance the existing literature by using a risky decision making task in which individual preferences determine if the risky or safe reward is more valuable. Individual NAc neurons will be monitored using in vivo electrophysiology to assess if NAc neurons differentially encode task related information about risk taking behaviors, including cue responding, behavioral choices, and reward deliveries.

2. To examine rapid dopamine release in the NAc during a delay discounting task.

Phasic dopamine signaling in the NAc has been implicated in goal-directed behaviors for both food and drug rewards (Phillips et al., 2003b; Roitman et al., 2004). Further, phasic dopamine signaling encodes the value associated with reward predictive cues (Fiorillo et al., 2003; Tobler et al., 2005) including during complex decision making (Roesch et al., 2007; Day et al., 2010; Sugam et al., 2012). These previous studies evaluated dopamine signaling in well trained animals in which the task remained constant for each session. However, an organism's environment is not a static system; instead the availability of resources and the cues that signal these resources are always changing. In order to adapt to these changes and promote survival, organisms must update cue-outcome associations. Dopamine release in the NAc has been implicated in the process of updating reward value as dopamine depletions disrupt reward devaluation (Lex and Hauber, 2010). To date, no work evaluating dopamine release in the NAc has studied how this signaling changes as reward value changes. Here, I will use a delay discounting task paired with fast-scan cyclic voltammetry to detect dopamine signaling to cues that predict different reward options as well as behavioral response and

reward delivery. As such, this study will clarify the role of phasic NAc dopamine release in encoding reward value as options change.

3 and 4. To determine if optical stimulation of dopamine terminals in the NAc is sufficient to promote motivated behavior, and guide value-based decision making.

Phasic dopamine release has been previously shown to be necessary for appropriate cue-outcome learning (Yun et al., 2004; Nicola et al., 2005; Zellner et al., 2009) while stimulation of VTA dopamine neurons is sufficient to promote behavioral conditioning and support motivated behavior (Phillips et al., 2003b; Tsai et al., 2009; Witten et al., 2011). Importantly, all of the previous research evaluating the causal relationship between dopamine neural activity and behavior focused on the cell body region of the VTA. While the majority of mesolimbic dopamine projections from the VTA synapse in the NAc, there are also projections to other structures including the PFC and BLA (Fields et al., 2007). Therefore, it is necessary to determine the relationship between terminal stimulation of dopamine neurons in the NAc and its relationship with simple motivated behaviors. In the first part of these studies I will build upon prior work (Witten et al., 2011) and determine if phasic dopamine release in the NAc is sufficient to promote a simple goal-directed behavior, a nosepoke operant response for optical stimulation. The second part of this aim will determine the causal link between the value encoding of the dopamine system and decision making by selectively stimulating dopamine release in the NAc during cue presentation of a complex value-based decision making task to determine for the first time if there is a causal link between value-based dopamine release and appropriate decision making.

CHAPTER 2

NUCLEUS ACCUMBENS NEURONS ENCODING OF REWARD RELATED INFORMATION TRACKS RISK TAKING BEHAVIOR

ABSTRACT

In order to make appropriate decisions, organisms must evaluate the risks and benefits of action selection. The nucleus accumbens (NAc) has been shown to be critical for this processing, and is necessary for appropriate risk-based decision making behavior. However, it is not clear how NAc neurons encode this information to promote appropriate behavioral responding. Here, rats (n=17) were trained to perform a risky decision making task in which discrete visual cues predicted the availability to respond for a smaller certain (safer) or larger uncertain (riskier) reward. Electrophysiological recordings were made in the NAc core and shell to evaluate neural activity during task performance. Animals exhibited individual differences in risk-taking behavior. Electrophysiological analysis indicated that NAc neurons selectively encoded cues that predicted safe versus risk options, displaying differential phasic activity for each cueytpe. However, this selective encoding was not related to behavioral preferences as there were no differences in the populations of selective encoding across the risk preferring, safe preferring animals, and nonpreferring animals. Instead, neural encoding of reward outcomes was correlated with behavioral preferences. Specifically, safe preferring rats displayed a greater proportion of excitations to reward omissions in the NAc core, compared to risk preferring rats, suggesting a possible connection between reward omission and aversion. Interestingly, we found the opposite relationship in

the NAc shell with risk preferring rats showing a greater proportion of excitations. These results suggest that NAc neural activity during outcome evaluations may function to bias future risk-based decisions.

INTRODUCTION

Organisms must learn appropriate behaviors to secure the necessary resources for survival such as food, shelter, and mates. This behavior requires complex cost benefit decisions in which organisms evaluate potential risks and benefits of different courses of actions (Green and Myerson, 2004; Cardinal, 2006; Phillips et al., 2007; Rangel et al., 2008; Saddoris et al., 2013). Impairments in appropriate cost-benefit decision making is associated with several psychiatric disorders including drug and gambling addiction (Avanzi et al., 2004; Redish, 2004; Dodd et al., 2005; Schultz, 2011; Chang et al., 2012), as well as more complex disorders such as schizophrenia (Chang et al., 2012). As such, there is a growing interest in understanding how the brain encodes normal decision making, and how changes in this signaling may result in disordered decision making.

Recent evidence suggests that the NAc is part of a distributed neural network that regulates risky decision making and is essential for appropriate behavioral choices. Risky decision making has been modeled in humans and animals by using modified gambling paradigms in which organisms choose between smaller certain rewards (safe option) and larger more uncertain rewards (risk option). Similar to humans, animals evaluate both the size of the reward and the probability of delivery when making appropriate decisions, and decrease responding for larger rewards as the probability decreases (Green and Myerson, 2004; Cardinal and Howes, 2005; St Onge and Floresco, 2008; Floresco and Whelan, 2009; St. Onge et al., 2010; St. Onge and Floresco, 2010; Stopper and Floresco, 2011; St. Onge et al., 2012; Sugam et al., 2012; Stopper et al., 2013). Disruptions of NAc circuitry result in specific dysfunctions in risky decision making. Specifically, lesions of the NAc result in increased risk aversion, such that lesioned rats chose smaller certain reinforcers more than

controls. Interestingly, lesioned animals avoided the risky lever, even when choosing it was more advantageous (Cardinal and Howes, 2005). Further, inactivation of the NAc biased animals away from larger rewards particularly when they were more uncertain (Stopper and Floresco, 2011). These observations suggest that NAc activity is critical for the evaluations of risks and making appropriate choices, and aberrations in this circuitry result in dysfunctional behaviors.

The NAc is the center of a larger corticolimbic circuit that receives input from the prefrontal cortex (PFC), basolateral amygdala (BLA) and a dense dopaminergic input from the ventral tegmental area (VTA). Importantly, each of these areas have been shown to be critical for appropriate decision making (Winstanley et al., 2004; Cardinal, 2006; Floresco and Whelan, 2009; St. Onge and Floresco, 2010). Our lab in particular has previously shown that the dopaminergic projections to the NAc are critical for encoding the subjective value associated with risk taking behavior (Sugam et al., 2012) which preferentially activates D1 receptors to promote appropriate choices (Stopper et al., 2013). The NAc functions to integrate this dopaminergic value signal with the signaling from cortical and limbic areas and impacts behavior through connections with motor areas (Zahm and Brog, 1992b; Zahm, 1999). This connectivity supports the role of the NAc as a "limbic motor interface" (Mogenson et al., 1980) and is therefore a candidate site for the mediation of risky decision making behaviors. In support, ventral striatal neurons display differential activity to cue presentation based on the value of future outcomes (Schultz et al., 1992; Cromwell and Schultz, 2003; Kim et al., 2009). NAc neurons also differentially encode reward predictive cues as well as behavioral responding and outcome evaluation when animals are actively

making decisions based on several factors including effort, delay, and magnitude (Roesch et al., 2009; Day et al., 2011).

Previous research examined how NAc neurons encode explicit reward value based on external factors such as the size of reward or the effort required to obtain it (Roesch et al., 2009; Day et al., 2011). However, many decisions involve subjective or intrinsic evaluations of reward value based on individual factors such as risk tolerance. Indeed, there is evidence that this type of subjective value is encoded in the human ventral striatum (Kable and Glimcher, 2007). Further, studies indicate that NAc function is critical for subjective decision making and is linked to impulsivity, risk taking behavior, and drug addiction (Cardinal et al., 2002; Cardinal and Howes, 2005; Dalley et al., 2007; Mendez et al., 2010). However, it is unclear how NAc neurons encode risk-taking behavior, and if NAc neural activity is related to risk predictive cues, behavioral responses, outcomes, or individual risk attitudes. Here, we collected electrophysiology data during the performance of a risky decision making task (Sugam et al., 2012) to assess whether NAc neurons encode subjective value associated with risky decision making or prediction errors related to unexpected reward deliveries or omissions.

METHODS

Animals

Male Sprague Dawley rats (*n*=17, Harlan Sprague Dawley, Indianapolis, IN), aged 90-120d and weighing 275-350g were used as subjects and were individually housed with a 12/12-h light/dark cycle. All experiments were conducted between 8:00am and 5:00pm. Animals were maintained at no less than 85% of pre-experimental bodyweights by food restriction (~10-15g of Purina laboratory chow each day in addition to approximately 1g of sucrose consumed during behavioral sessions) except during the post-operative recovery period when food was given *ad libitum*. All procedures were approved by the UNC Institutional Animal Care and Use Committee.

Surgery

Prior to the start of behavioral training, rats underwent surgery for implantation of microelectrode recording arrays into the NAc core and shell. Surgery was conducted under anesthesia with ketamine (100mg/kg) and xylazine (20mg/kg) using established procedures routinely used in the Carelli laboratory (Carelli et al., 1993; Carelli and Deadwyler, 1994; Carelli and Wondolowski, 2003; Roitman et al., 2005). Custom designed electrode arrays (NB Labs, Denison, TX) were stereotaxically guided into the core (AP: +1.7; ML: +/- 1.3 relative to bregma; DV: -6.5 relative to skull surface) and shell (AP: +1.7; ML +/- 0.8 relative to bregma; DV:-6.5 relative to skull surface) of the NAc. Each array consisted of eight microwires (50 µm diameter) arranged in a 2 X 4 bundle that measure ~1.5 mm anteroposterior and ~0.75 mm mediolateral. Ground wires for each array were inserted into the brain remote to electrode arrays. The arrays and ground wires were anchored to the skull

via stainless steel screws and dental acrylic. All animals were allowed at least five post-operative recovery days before beginning training on the behavioral task.

Behavioral Training

Following recovery from surgery, rats were trained on a risk-based decision making task developed in our laboratory (Sugam et al., 2012). Rats received at least 25 training sessions on the risky decision making task prior to electrophysiology recordings. All training was conducted in 43 X 43 X 53cm Plexiglas chambers housed in sound-attenuated cubicles (Med Associates, St Albans, VT). One side of the chamber had 2 retractable levers (Coulbourn Instruments, Allentown, PA) 17cm apart, with a stimulus light 6cm above each lever. A white noise speaker (80db) was located 12cm above the floor on the opposite wall. A houselight (100mA) was mounted 6cm above the speaker. Sucrose pellets (45 mg) were delivered to the food receptacle located equidistantly between the levers. Initially, lever pressing behavior in all rats was reinforced on a continuous schedule of reinforcement on two levers, such that every response on either lever resulted in the delivery of a 45mg sucrose pellet to a centrally located food receptacle. Rats could make 50 presses on each lever for a maximum of 100 reinforcers per session. Once rats reached stable responding (50 presses on each lever; 5 training sessions) daily training began on the risk-based decision making task in which rewards were contingent on operant responses in 90 discrete trials per session. Importantly, each trial was initiated randomly (without replacement) after a variable time interval with an average of 30 seconds between trials. Distinct cue lights were illuminated (5 seconds) before lever extension and levers were available for 15 seconds unless the response requirements were completed. Upon completion of the appropriate requirement the lever was retracted and the behavior was rewarded. There were 60 forced trials in which one cue light

was presented alone and a response on the corresponding lever was reinforced with 45mg sucrose pellets. Responses on the non-cued lever were counted as "errors" (houselight extinguished and no reward delivered). The number of errors was used as a behavioral measure of discrimination between the two different response options. For the first 10 training sessions each lever was reinforced on a fixed ratio 1 (FR1) schedule with one sucrose pellet. This was done to allow animals to fully learn the predictive associations of the cue lights before the reward contingencies were altered. Furthermore, this ensured that there would be no bias in response allocation as a result of differential learning between the two levers. For the next 15 sessions one lever (counterbalanced across animals) was designated the "risky lever" and was reinforced on 50% of the trials with 2 sucrose pellets while the other lever, designated the "safe lever" remained on the original contingency of 1 sucrose pellet 100% of the time (Figure 2.1). During each behavioral session there were also 30 choice trials in which both cue lights were illuminated and both levers were active such that the rat was rewarded based on the contingency of reinforcement for the lever chosen. Response allocation on choice trials was used to determine the subjective value associated with each response option. Animals were considered to have a behavioral preference if they displayed 60% responding for the preferred lever during choice trials. Electrophysiological activity of NAc neurons was recorded during the final behavioral session. A subset of animals (n=8) did not display a behavioral preference during the first recording session and therefore continued training until a behavioral preference developed (from 3-8 additional training sessions). Animals were then recorded when a stable behavioral preference developed.

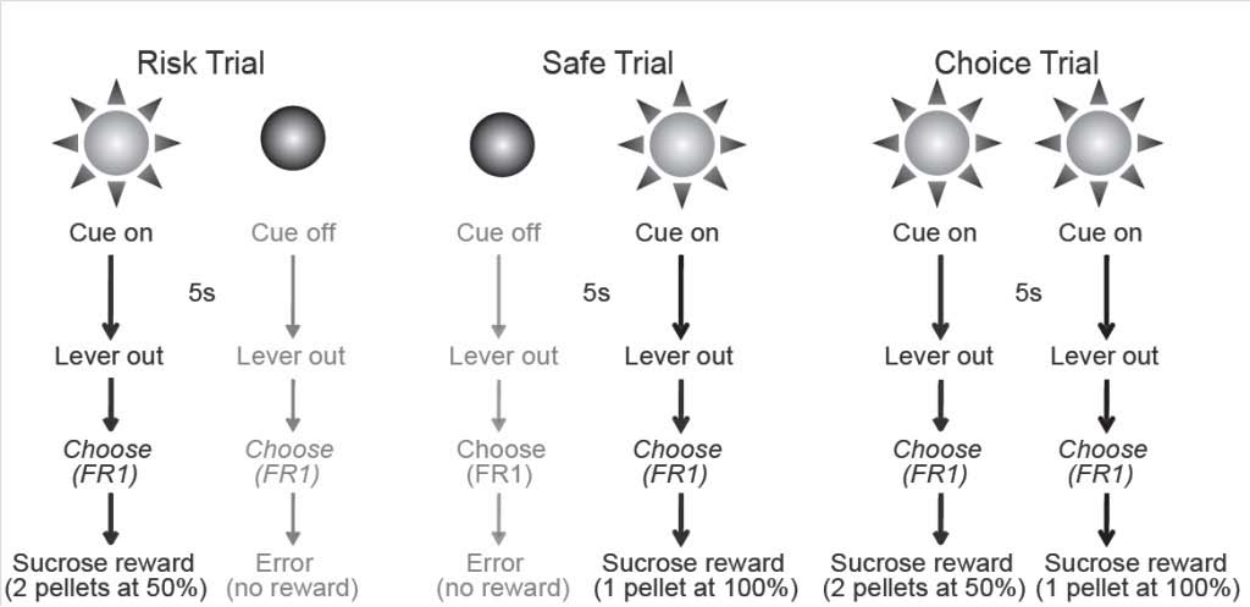

Figure 2.1 Schematic representation of risky decision making task. On Risk trials (left panel), a cue light was presented for 5s and was followed by the extension of two response levers. A single lever press on the lever positioned below the cue light led to a 2 sucrose pellet reward presented on 50% of lever presses. Responding on the other lever did not produce reward delivery and terminated the trial. On Safe trials (middle panel) the other cue light was presented for 5s before lever extension. Here presses on the associated lever resulted in 1 sucrose pellet while presses on the other lever did not produce reward and terminated the trial. On choice trials (right panel) both cue lights were presented for 5 seconds, and animals could select either option.

Electrophysiological recordings

The procedure for extracellular recording in the NAc during behavior is routinely used in the Carelli laboratory and is described in detail elsewhere (Carelli et al., 1993; Carelli and Deadwyler, 1994; Hollander and Carelli, 2005). Briefly, before the start of the session, the rat was connected to a flexible recording cable attached to a commutator (Crist Instrument Company, Inc.) which allows for virtually unrestrained movement within the chamber. The headstage of each recording cable contains 16 miniature unity-gain field effect transistors. Online isolation and discrimination of neuronal activity was accomplished using

commercially available neurophysiological system (MAP System, Plexon, Inc., Dallas TX). Criteria for identifying different neurons on a single wire have been described in detail elsewhere (Chang et al., 1994; Nicolelis et al., 1997; Carelli et al., 1999). Briefly discrimination of individual waveforms corresponding to a single cell was accomplished using template analysis procedures provided by the neurophysiological software system. The template analysis procedure involves taking a 'sample' of the waveform and building a template of that extracellular waveform. Subsequent neurons that match this waveform are included as the same cell. Principal component regression of waveform data was conducted using the Offline Sorter Program (Plexon, Inc) to further separate waveforms recorded from the same microwire.

Neural Analysis

Analysis of neural activity collected during behavioral sessions had two main goals. First, we identified if neurons exhibited increases (excitations) or decreases (inhibitions) in activity relative to the behavioral events. Secondly, we evaluated if the response patterns of neurons were sensitive to differences in risk versus safe options. Cells that showed significant differences (either excitation or inhibition) following the onset of a behavioral event (e.g., cue presentation) were considered phasic for that event. Cells with a baseline firing rate of less than 0.1 Hz were excluded from analysis to ensure the ability to detect both excitations and inhibitions. Differences in firing were calculated by performing a 2-way analysis of variance (ANOVA) for each cell using the mean firing rate for each trial in each analysis period similar to previous reports (Roesch et al., 2009; Saddoris et al., 2011). Cellular activity was analyzed over 5 time epochs within each given trial: the 10s baseline period was compared to the first 2.5 seconds following cue onset (Cue period), the 2.5

seconds prior to lever press (Prepress period), and two 2.5 second epochs during the reward period (Early Reward and Late Reward periods) (Figure 2.3A). In each analysis, a single cell was analyzed with bin as a repeated-measures factor (e.g., baseline bin vs. cue bin), and trial type (e.g., risk vs. safe; reward omission vs. large reward vs. small reward) as an independent-measures factor. To determine the type of phasic encoding, post-hoc Tukey tests were completed. We then determined the type of phasic encoding by comparing the firing during the bins: *phasic* cells showed significantly different firing during the effect period (cue, prepress, reward) compared to the baseline while *nonphasic* cells showed no differences between the effect periods or baseline bins. *Selective* cells were classified as cells that were phasic to only one trial type and/or cells that were phasic to both trial types that showed significantly different firing rates during each the trial types (e.g., risk vs. safe cue).

Encoding was evaluated as the percentage of the population of cells that encoded a particular event (e.g., risk cue) on each session and in each region (core or shell). Population analysis was conducted separately for cells in the nonpreferring, risk preferring, and safe preferring groups. Differences in the frequency or proportion of neuronal responses across different trial types, subregions, or reward preferences were examined using chi square analysis. All analyses were considered significant at $\alpha=0.05$. Statistical and graphical analyses were conducted in Graphpad Prism 4 (Graphpad software, Inc.) and STATISTICA (StatSoft, Tulsa, OK).

Histology

Upon completion of the experiment, rats were deeply anesthetized with a ketamine/xylazine mixture (100 mg/kg and 10 mg/kg, respectively). To mark the placement of electrode tips, a 13.5μA current was passed through each microwire electrode for 5s.

Transcardial perfusions were then performed using physiological saline and a 10% formalin mixture containing potassium ferricyanide (3%), which reveals a blue dot reaction product corresponding to the location of each electrode tip. Brains were then removed, post-fixed using a 10% formalin solution, and frozen. After postfixing and freezing, 30 μm coronal brain sections were mounted on microscope slides. The specific placement of the electrode tips in the NAc core or shell were verified using a standard stereotaxic atlas (Paxinos and Watson, 2005). Neurons recorded on electrodes placed outside of the NAc core or shell were excluded from all analyses.

RESULTS

Individual Differences in Risky Decision Making Behavior

Several behavioral measures were used to verify that animals had acquired the task (Figure 2.1) and could discriminate between cues to guide behaviors. First, animals showed a significant decrease in the percentage of errors on risk and safe trials ($F_{(24,384)}$=8.076, P<0.0001) with a significant reduction in errors by session 5 compared to session 1 (Tukey's HSD test, P<0.05 for sessions 5-25). During the recording session, the number of errors was not significantly different from 0 for any of the groups or trial types (Figure 2.2A; one sample *t*-test, comparison with theoretical mean of 0%, P>0.1 for all analyses).Further, across all three groups there was no significant differences in the number of reward pellets received for both cues ($F_{(5,48)}$=0.6769, P=0.643), and the number of pellets received was not significantly different from the maximum possible (Figure 2.2B; one sample *t*-test, comparison with a theoretical mean of 30, P>0.15 for all comparisons). This data indicates that rats were able to use the cues to guide ongoing behaviors and select the appropriate response option that would be rewarded.

On free choice trials, both cues were presented signaling that both levers were active, and the animal was rewarded based on the contingency of the lever chosen. During session 25, the majority of rats (n=9) displayed a clear preference for the risk or safe lever (defined as 60% or greater presses on the preferred lever; Figure 2.2D). Rats that did not show a preference on session 25 (n=8; $t(7)$=0.6831, P=0.516, comparison to a theoretical mean of 50%) were included as part of the nonpreferring group and were given additional training sessions until a behavioral preference developed (from 1 to 8 more sessions) On recording, of all of the rats, 59% displayed a risk preference, showing significantly more presses on the

risk lever than chance ($t(11)=9.299$, $P<0.001$) while the remaining animals showing a safe preference($t(6)=6.367$, $P=0.0007$, compared to chance).

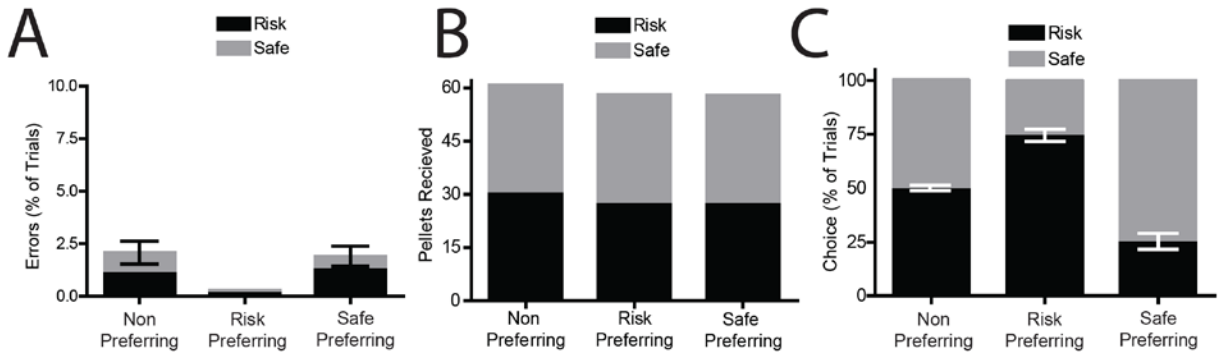

Figure 2.2 Individual differences in risky decisions making. (A) Percentage of errors on Risk and Safe trials were not significantly different from 0 ($P>0.1$ for all comparisons), demonstrating behavioral discriminations between cues. (B) Reward pellets received during Risk and Safe trials. All groups of animals received maximum amounts of rewards (30 pellets for each trial type). (C) Response allocation on choice trials (as a percentage of choice) during recording sessions. 3 groups of rats were observed: non preferring rats chose both options equally ($P=0.516$), risk preferring rats chose the risk lever significantly more than chance ($P<0.0001$), safe preferring rats chose the safe lever significantly more than chance ($P=0.0007$).

Overview of nucleus accumbens neural activity during risky decision making

A total of 339 NAc neurons were recorded from 17 rats during behavioral performance ($n=118$ neurons from nonpreferring rats, $n=142$ neurons from risk preferring rats, $n=79$ neurons from safe preferring session). Of these, 286 (84%) exhibited significant modulation in firing rate during at least one task event. This amount of neural activity is similar to previous reports of NAc neural recordings during value-based decision making tasks (Day et al., 2011). 130 neurons (38%) showed significant changes in firing rate during cue presentations (29% were phasic during risk trials and 28% during safe trials, Table 2.1) shown for a representative neuron in Figure 2.3B (Top). 225 cells (66%) exhibited changes in firing rate during the prepress period (55% of cells were phasic during risk trials and 53% during safe trials) illustrated for one neuron in Figure 2.3B (Middle). Finally, 244 cells (72%)

encoded information during the reward periods (early or late), as shown for one neuron in Figure 2.3B (Bottom). The distribution of NAc activity by trial type and during the reward period can be found in Tables 2.1 and 2.2.

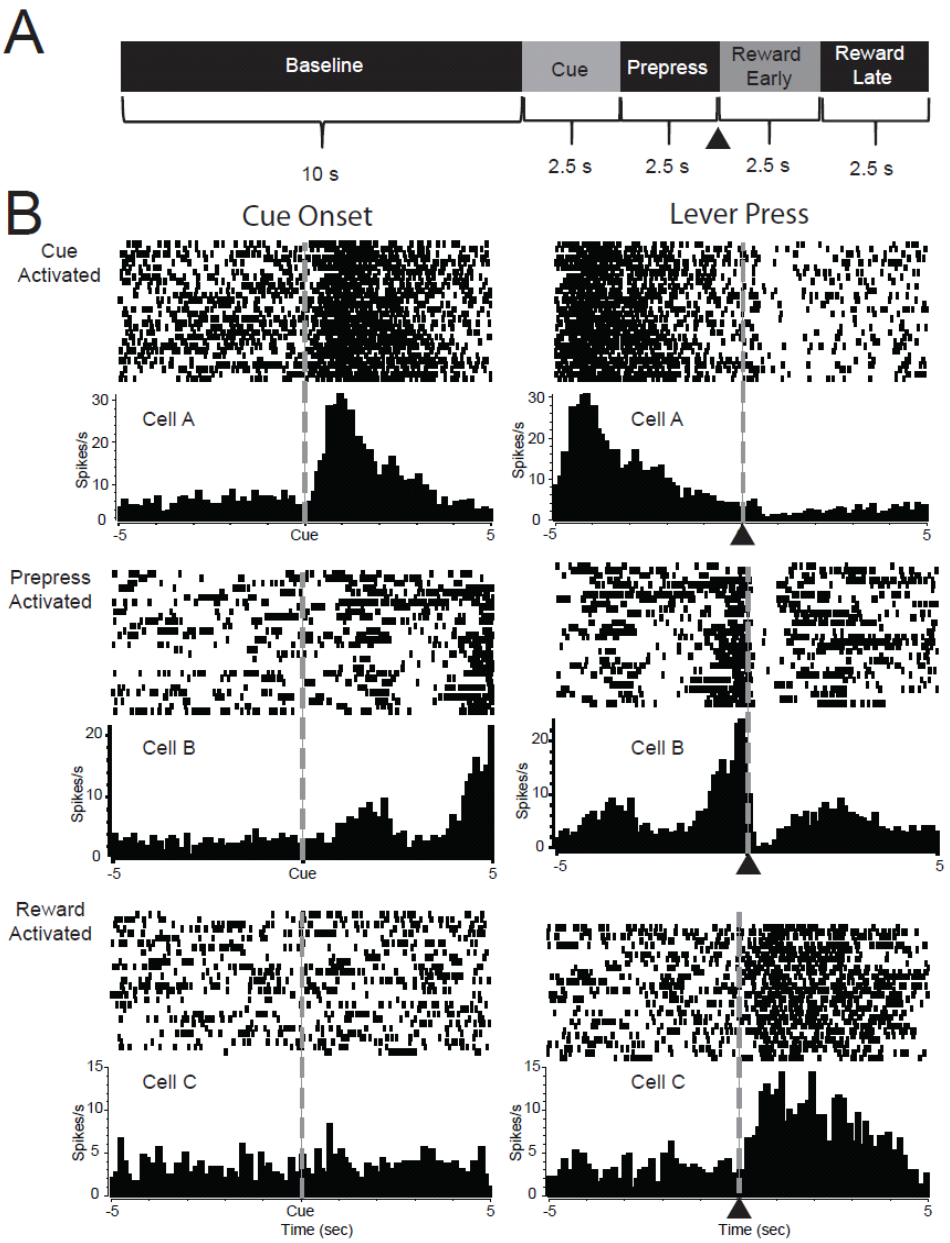

Figure 2.3 NAc neurons are activated during different components of the risky decision making task. (A) Schematic representation of neuronal data analysis. Neural activity during each time epoch was compared to a 10 second baseline period. Following cue onset, the first 2.5 seconds analyzed were classified as cue related activity, the second 2.5 seconds (prior to the lever press) were classified as prepress activity. Following the lever press (denoted by ▲) the first 2.5 seconds were classified as the reward early period; the second 2.5s were classified as the reward late period. (B) Peri-event histogram (PEH) and raster plots of representative NAc neurons activated during each of the time epochs. Each cell is aligned to cue onset (left panel) and lever press (denoted by ▲ ; right panel). Cell A (Top) is an example of a cue-activated cell showing increased activity immediately after cue onset with no phasic activity following the cue period. Cell B (Middle) is an example of a prepress activated cell, showing increased activation prior to the lever press. Cell C (Bottom) is an example of reward activation, showing increased neural activity following the lever press during the reward periods.

Table 1

Distribution of NAc Cellular Activity By Trial Type

Total Cells (*n* = 339)	**Event**	
	Cue	**Prepress**
Risk Trial	98/339 (29%)	187/339 (55%)
Excitation	*38/98 (39%)*	*58/187 (31%)*
Inhibition	*60/98 (61%)*	*129/187 (69%)*
Safe Trial	96/339 (28%)	178/339 (53%)
Excitation	*29/96 (30%)*	*52/178 (29%)*
Inhibition	*67/96 (70%)*	*126/178 (71%)*

Table 2

Distribution of NAc Cellular Activity During Reward Period

Total Cells (*n* = 339)	**Reward**	
	Early	**Late**
Large Reward	99/339 (29%)	111/339 (33%)
Excitation	*40/99 (40%)*	*39/111 (35%)*
Inhibition	*59/99 (60%)*	*72/111 (65%)*
Small Reward	124/339 (37%)	128/339 (38%)
Excitation	*47/124 (38%)*	*53/128 (41%)*
Inhibition	*77/124 (62%)*	*75/128 (59%)*
Omission	80/339 (24%)	107/339 (32%)
Excitation	*39/80 (49%)*	*74/107 (69%)**
Inhibition	*41/80 (51%)*	*33/107 (31%)**

** signifies $p<0.05$ compared to early omission period*

Cue activated neurons selectively encode risk versus safe options but not behavioral preferences

NAc neurons encode information about cues that predict rewarding outcomes (Nicola et al., 2004a; Day et al., 2006; Jones et al., 2010a; Saddoris et al., 2011), and selectively encode value related information during decision making (Roesch et al., 2009; Day et al., 2011). Therefore, here we examined how NAc cells encode information about risk versus safe options, and if encoding was related to each animal's individual behavioral preference. Of the neurons that were activated (i.e., phasic) during risk trials, 61% showed decreases in activity during the cue period. Likewise, during safe trials 70% of cells were inhibited by the cue (see Table 2.1). This majority of inhibitory activity is consistent with the notion that NAc neurons primarily encode rewarding outcomes with decreases in firing rate (Roitman et al., 2005; Taha and Fields, 2006; Roitman et al., 2008; Wheeler et al., 2008). Further, there were no significant differences in the percentages of cells that displayed phasic activity across the three groups of animals, such that 36% of cells responded during cue presentations in the risk preferring group, 39% in the safe preferring group and 44% in the non preferring group ($\chi^2(2)=1.44$, $P=0.49$).

A substantial proportion of cue-activated neurons exhibited either cue-selective excitations (e.g., greater activity for one cue compared to the other and greater activity than baseline) or cue-selective inhibitions (e.g., lower firing rate for one cue compared to the other and lower activity than baseline). We classified these responses into three separate types: "risk selective", "safe selective", and "both risk/safe" (e.g., firing rates that were not significantly different from each other but were different from baseline) (Figure 2.4A). The majority of cue-activated cells, the majority (77 cells, 58%) displayed cue selective encoding (i.e., risk or safe selective). Interestingly, there were no significant differences in the

percentages of neurons that were risk selective, safe selective, or both risk/safe in the three groups of rats ($\chi^2(4)=2.65$, $P=0.618$), suggesting that NAc activity during cue presentations similarly encoded risk versus safe options in the three groups (Figure 2.4B). Further, there was no difference in the percentage of cells that were risk selective versus safe selective within each group of rats, indicating that neuronal responses were not modulated by the subjective value associated with each cue type. There were also no differences in the distribution of responses between the core and shell of the NAc during the cue period, nor were selective responses more concentrated in one region than another. As such, NAc core and shell data is presented together (Fisher's exact test P>0.05 for all comparisons).

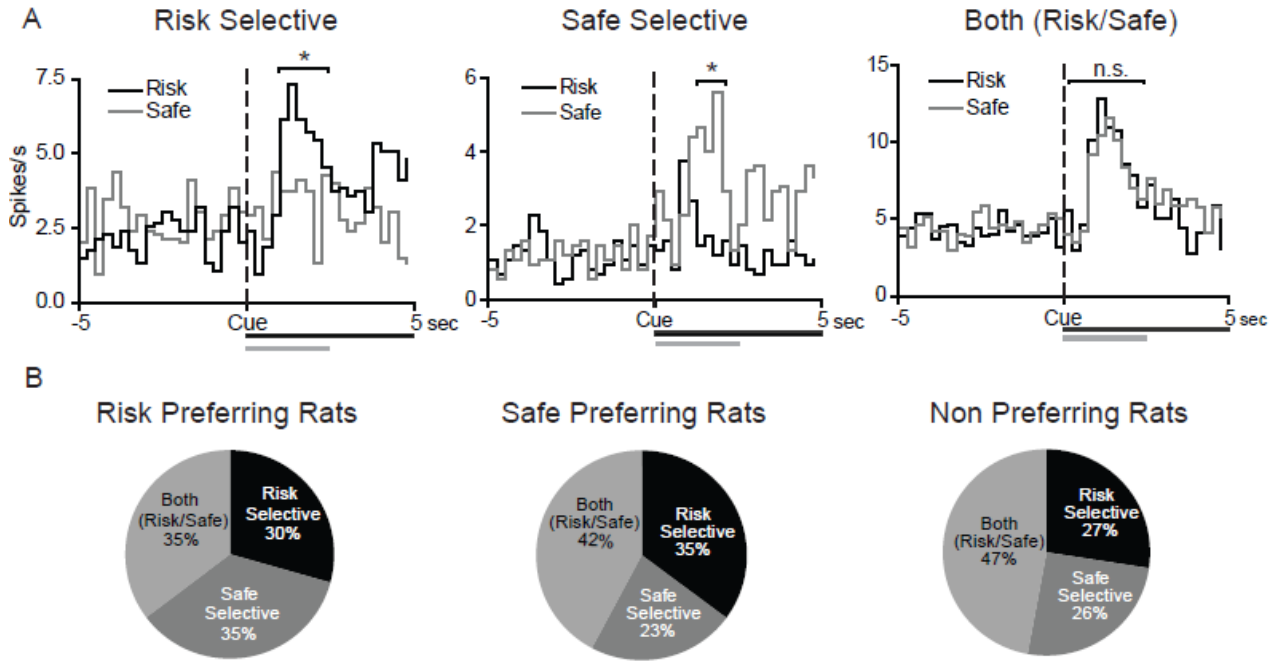

Figure 2.4 NAc neurons display cue-selective encoding for safe versus risk cue presentations. (A) PEHs of representative risk selective (left panel), safe selective (middle panel) and both (risk/safe) (right panel) cue activated NAc neurons during the risky decision making task. Data are aligned to cue onset (black bar/dashed line). Grey bar signifies 2.5s cue effect period that is analyzed. Time bins during the effect period in which neural activity is significantly different is signified by * ($P<0.05$). (B) Percentages of phasic cells that display the three different types of encoding in the risk preferring (left panel), safe preferring (middle panel), and non preferring (right panel) rats. There were no differences in the population encoding of cue selectivity across the three groups of rats ($P=0.6$).

Response activated neurons selectively encode risk versus safe options but not behavioral preferences

Previous research has shown that the NAc is critical for appropriate action selection (Cardinal et al., 2001; Cardinal and Howes, 2005; Cardinal, 2006; Phillips et al., 2007; Stopper and Floresco, 2011). We examined NAc neural activity during the 2.5s prior to lever press in our task, allowing for a functional measure of action selection processing within the NAc during risky decision making. A significantly greater proportion of NAc cells (229 cells 68%) were phasic during the prepress period compared to the cue period (χ^2=52.25, P<0.0001), supporting the role of the NAc in the processing of action selection. Similar to the cue period, the majority of prepress activated cells displayed phasic inhibitions (70% for both risk and safe levers; See Table 2.1). There were no significant differences between the three groups in the number of cells that were phasically activated during the prepress period (70% in the risk preferring group, 75% in the safe preferring group 67% in the nonpreferring group; $\chi^2(2)$=1.35, P=0.509), suggesting that the three groups are not differentially encoding response behaviors.

Similar to neural activity during the cue period, we found several populations of phasically active cells. A subset of cells showed selective activation prior to presses on the risk lever, another subset prior to presses on the safe lever, and a third group were similarly activated prior to both lever presses (Figure 2.5A). Of prepress activated cells, the majority (140 cells, 59%) displayed prepress selective encoding. While this encoding suggests that the NAc may be critical for action selection, it appears that NAc neural activity does not encode the subjective value associated with response selection. Specifically, there were no significant differences in the percentages of cells that were risk selective, safe selective or both risk/safe in the three groups (Figure 2.5B, χ^2 (4) =1.28, P=0.864). As during the cue

period, similar percentages of cells were risk selective versus safe selective within each group of rats suggesting that the NAc does not differentially encode action selection based on individual preferences. Again there were no differences in the distribution of responses between the core and shell of the NAc during the prepress period, nor were selective responses more concentrated in one region than another so NAc core and shell data is presented together (Fisher's exact test P>0.05 for all comparisons).

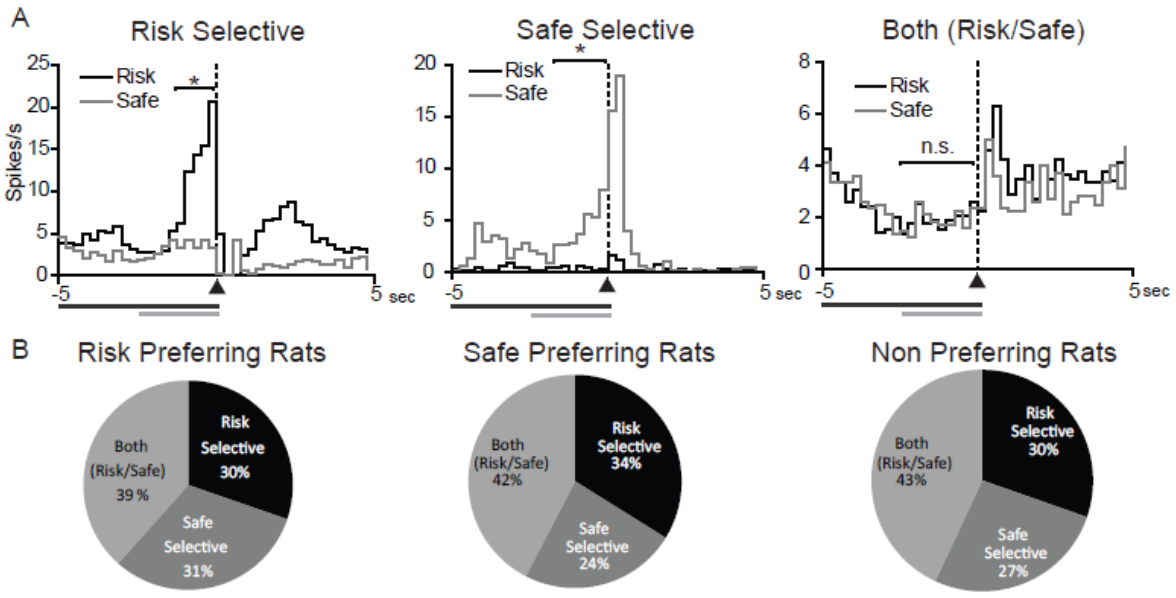

Figure 2.5 NAc neurons display prepress selective encoding for safe versus risk lever presses. (A) PEHs of representative risk selective (left panel), safe selective (middle panel) and both (risk/safe) (right panel) prepress activated NAc neurons during the risky decision making task. Data are aligned to lever press onset (▲ /dashed line). Black bar signifies cue onset. Grey bar signifies 2.5s prepress effect period that is analyzed. Time bins in which neural activity is significantly different during the effect period is signified by * (P<0.05). (B) Percentages of phasic cells that display the three different types of encoding in the risk preferring (left panel), safe preferring (middle panel), and non preferring (right panel) rats. There were no differences in the population encoding of prepress selectivity across the three groups of rats (P=0.864).

Accumbens neural activity encodes unexpected reward deliveries and omissions

For all neurons (regardless of preference), we first compared how the NAc encodes unexpected reward presentations and omissions. We found that similar percentages of neurons (roughly 30%) were phasic during large reward presentations, small reward presentations, and reward omissions (Table 2.2). Further, a larger percentage of cells displayed inhibitions compared to excitations during reward presentations. During omissions we found a larger percentage of excitations compared to inhibitions. Work in our lab and others has suggested that neurons in the NAc primarily encode rewarding outcomes with decreases in firing, while aversive outcomes are typically encoded as increases in firing (Roitman et al., 2005; Taha and Fields, 2006; Roitman et al., 2008). The current data support this finding, and extend the interpretation, such that reward omissions may be encoded as aversive events by the NAc. There was also a significant increase in the percentage of cells that encoded reward omissions from the early to late period (80 neurons (24%) to 107 neurons (33%), χ^2=4.992, *P*=0.026). Further, there was a significant shift in the ratio of cells that encoded excitations versus inhibitions. During the early period 49% of phasic cells encoded excitations while 51% encoded inhibitions. During the late reward period, 69% encoded excitations while 31% of phasic cells encoded inhibitions (χ^2=7.143, *P*=0.0075). This suggests that omission of rewards may induce a negative affective state, particularly later during the reward period when animals are normally consuming the reward.

Neural responses to reward omissions and deliveries encode risk preferences

Previous research has shown that there is a direct correlation between neural encoding of loss aversion in the ventral striatum and behavioral responses in a risky decision making paradigm in humans (Tom et al., 2007). Here, we first evaluated the relationship between

neural responding in the NAc core and shell to reward omissions and how this activity was correlated with behavioral preferences. First, there were no significant differences in the percentages of cells that were phasic during the omission period in either the NAc core ($\chi^2(2)=0.42$, $P=0.81$) or NAc shell ($\chi^2(2)=0.72$, $P=0.698$) across the three groups. Cells responded with either an increase in activity during the reward period (excitation, Figure 2.6A left) or a decrease in activity during this period (inhibition, Figure 2.6A right).

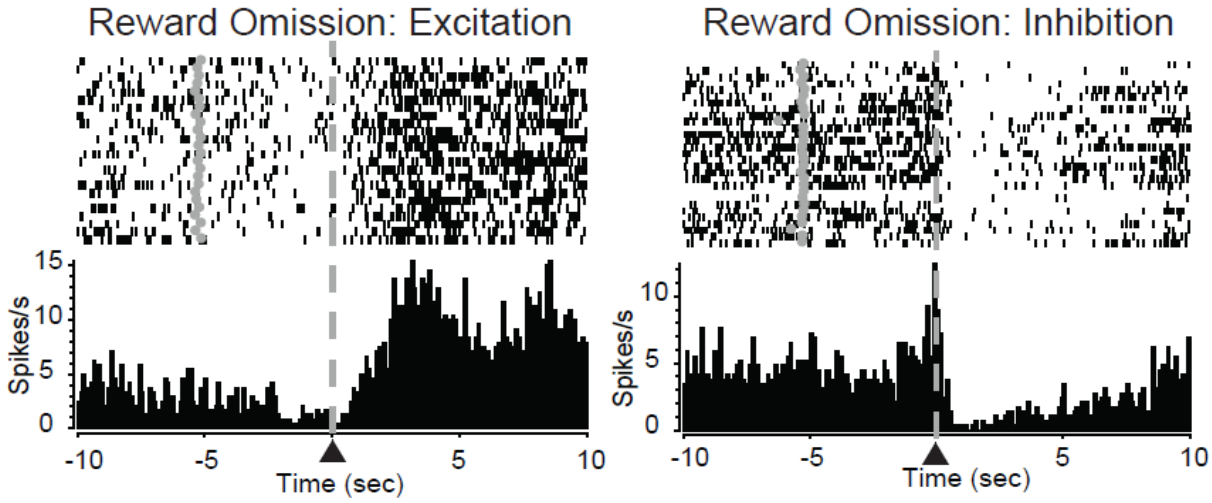

Figure 2.6 Reward omission processing in the nucleus accumbens. PEH and raster plot from representative reward omission activated NAc neurons. Data aligned to lever press (▲/dashed line). Grey circles in rasters denote cue onset. A subset of NAc neurons showed increased activity following lever press during the time period in which the animal expected the reward (left panel). Another subset of neurons exhibited decreases in activity following lever press during the time period in which the animal expected the reward (right panel).

Interestingly, however, we found a relationship between the type of phasic activity in response to reward omissions and risk preferences in both the NAc core and shell. In the NAc core, there was a significant difference in the ratio of excitations versus inhibitions between the groups (P=0.0177, Fisher's exact test). Specifically, of phasic cells, there were significantly greater amounts of excitations in the safe preferring compared to the risk preferring group ($P<0.05$, Fisher's exact test; Figure 2.7A). We then analyzed each animal

individually to determine the relationship of excitation/inhibition ratio and risk preference. Importantly, we only included animals in this analysis with at least 5 cells recorded in the NAc core to ensure that animals with low numbers of cells did not bias our results. We found a negative correlation between the percentage of phasic cells that were excitatory and preference for the large risky lever (r^2=.33, *P*=0.02; Figure 2.7B). That is, risk preferring rats exhibited less excitatory activity compared to safe preferring rats. As aversive outcomes are encoded with increases in excitations (Roitman et al., 2005; Wheeler et al., 2008), the current data suggest a differential processing of reward omission, a seemingly aversive event, between safe and risk preferring rats, such that increased encoding of aversion related to reward omission is associated with an increase in safe preferences.

Interestingly, we found a trend towards the opposite relationship in the NAc shell. Such that the safe preferring group had fewer excitations compared to risk preferring rats (*P*=.08, Fisher's exact test, Figure 2.7C). When we compared the percentage of excitations with each animals risk preference, we found an increase in the percentage of excitatory cells and greater risk preferences in the NAc shell (r^2=0.20, *P*=0.063; Figure 2.7D). By evaluating the percentage of inhibitory and excitatory cells in the core and shell together, we found that the risk preferences could be separated. Risk preferring rats had a lower percentage of excitations in the core and a higher percentage in the shell. Instead, safe preferring rats had a higher percentage of excitations in the core and a lower percentage of excitations in the shell.

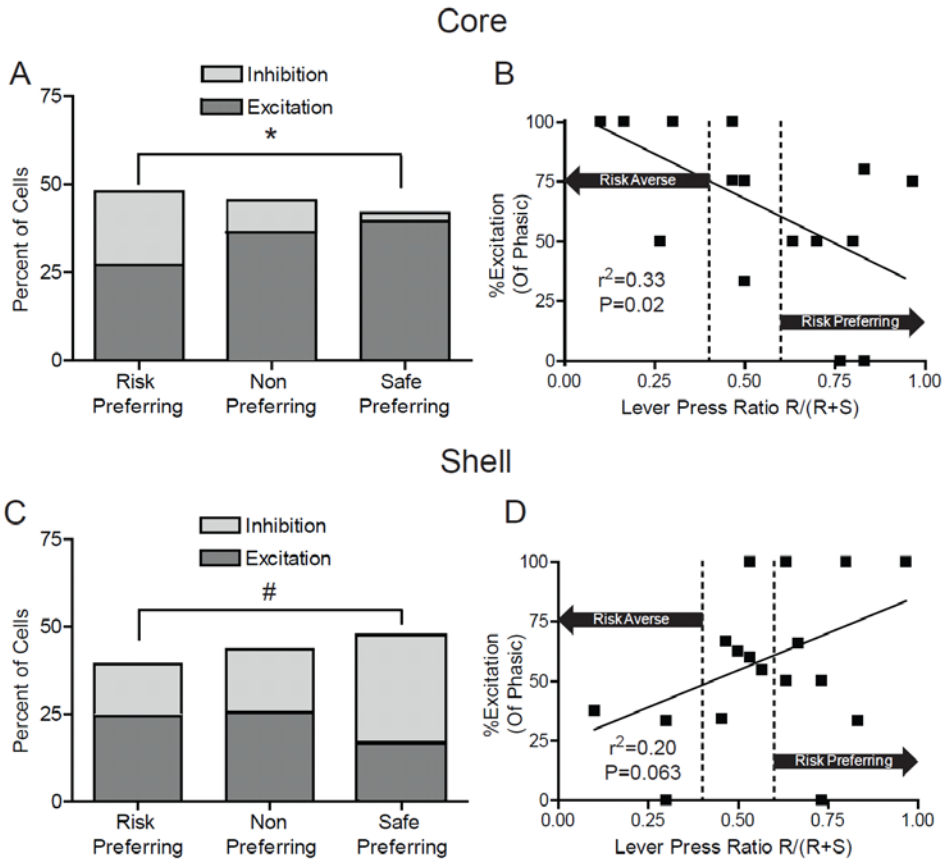

Figure 2.7 Individual risk preferences related to reward omission processing. (A) Percentages of NAc core cells that exhibit excitations versus inhibitions during reward omission period in each of the three groups. There was no significant difference in the amount of cells that were phasic during reward omissions; however, safe preferring rats had a significantly greater percentage of cells that exhibited excitations versus inhibitions compared to risk preferring animals. (B) Percentage of phasic cells that display excitations in the NAc core plotted against individual risk preferences. The x-axis is lever press behavior during free choice trials showing the risk preference of each animal. Dotted lines indicate the criteria for a significant preference (defined as pressing the preferred lever 60% of the time). The ratio of lever pressing was determined by dividing the number of presses on the risk lever during free-choice trials by the total number of presses on free-choice trials (Risk/(Risk+Safe)). A ratio greater than 0.6 indicates that an animal is risk-prone while a ratio of less than 0.4 indicates an animal is risk-averse. Area in between the dotted lines indicate no preference. The y-axis is the percentage of phasic cells that showed significant excitations during the reward omission period. Only animals with at least 5 cells recorded in each region were included in the analysis as to not bias results due to low cell counts (C) Reward omission processing in the NAc shell. There was no significant difference in the amount of cells that were phasic during reward omissions; however, risk preferring rats had a greater percentage of cells that exhibited excitations versus inhibitions compared to safe preferring animals. (D) Percentage of phasic cells that display excitations in the NAc shell plotted against individual risk preferences. Conventions follow from B. *$P<0.05$ #$P<0.1$

Electrode placement

A total of 272 wires (16 per animal) were implanted bilaterally and aimed at the NAc and shell. Of these, we confirmed 98 wires were implanted in the NAc core and 113 wires in the NAc shell (Figure 2.8). Data from electrodes located outside of the NAc were excluded from analysis. We recorded 142 neurons from the risk preferring group (46 neurons in the core and 96 in the shell), 79 neurons from the safe preferring group (43 neurons in the core and 36 in the shell) and 118 neurons in the non preferring group (44 neurons in the core and 74 neurons in the shell).

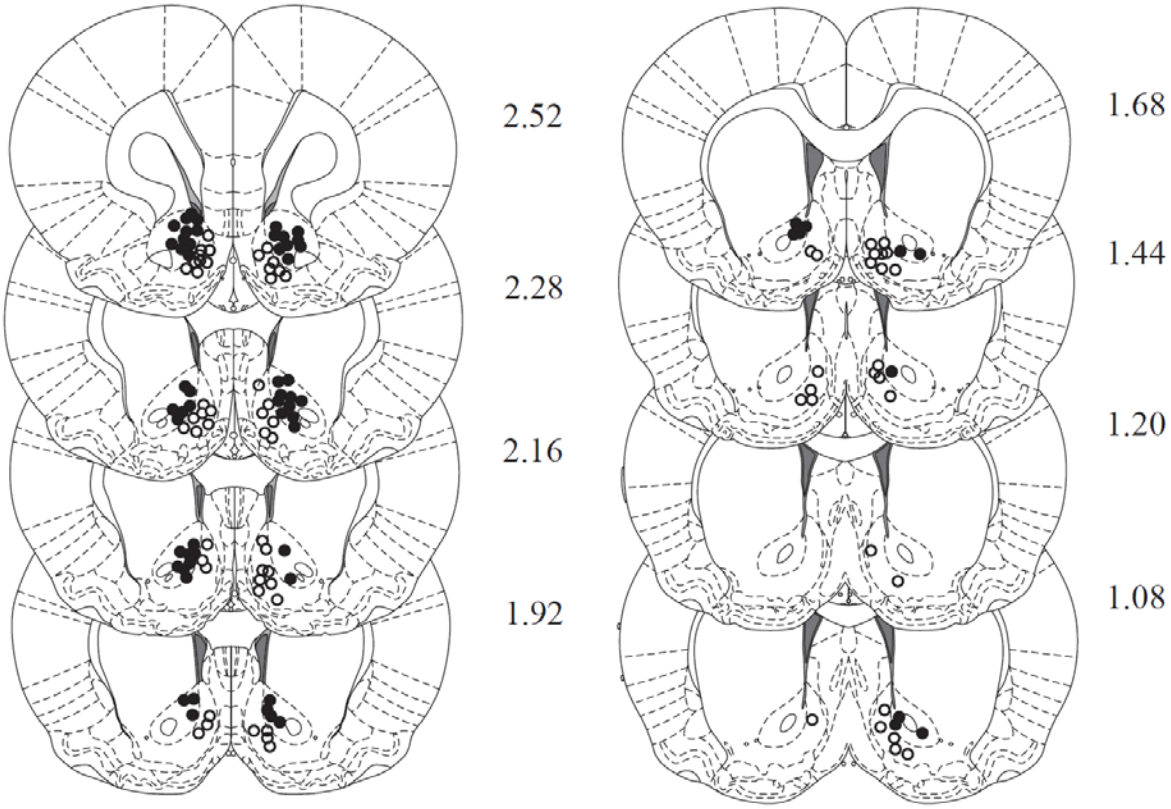

Figure 2.8 Histological verification of recording array wires in the NAc core and shell. Marked locations are limited to electrodes that contributed to data presented here. Filled circles indicate electrode locations in the NAc core, and open circles indicate electrode locations in the NAc shell. Numbers to the right indicate anteroposterior coordinates rostral to bregma (mm).

DISCUSSION

NAc activity has been implicated in cue outcome learning (Day et al., 2006), reward related responding (Schultz et al., 1992; Nicola et al., 2004a; Nicola et al., 2004b; Taha and Fields, 2006; Wheeler et al., 2008; Jones et al., 2010a; Saddoris et al., 2011), and decision making (Roesch et al., 2009; Day et al., 2011). Here, we evaluated the role of NAc activity during risky decision making. As with previous reports using similar tasks (Roitman and Roitman, 2010; Sugam et al., 2012), we found that rats displayed individual preferences for safe versus risky options (risk preferring, safe preferring, and nonpreferring rats). In all groups of rats, NAc neurons exhibited phasic patterns of activity (excitations or inhibitions) relative to all aspects of the task (cue presentation, prepress period, and reward deliveries/omissions). Further, NAc neurons displayed selective activation, signifying differential encoding of risk versus safe options during each of these time periods. Somewhat unexpectedly, there were no differences in the percentages of cells that selectively encoded risk versus safe options during the cue or prepress period in the three groups. This suggests that NAc activity may not signal the subjective or intrinsic value associated with the different options, but instead may signal the expected value. The expected value is the prediction of future out comes based on the computation of several factors of reward value including the probability and magnitude. In support, we found that neural responding to reward outcomes was related to individual differences in risk taking behavior.

Consistent with its role in associative learning, electrophysiological studies have shown that NAc neurons are selectively activated by reward-related stimuli (Nicola et al., 2004a; Roitman et al., 2005; Day et al., 2006; Wheeler et al., 2008; Jones et al., 2010a; Saddoris et al., 2011). Importantly, neural encoding of reward predictive cues is dependent on the learned association between the predictive cue and associated outcome (Nicola et al.,

2004a; Day et al., 2006; Saddoris et al., 2011) Further, striatal neurons differentially encode cues that are predictive of rewards that require effort (Day et al., 2011), reward delay and magnitude (Schultz et al., 1992; Cromwell and Schultz, 2003; Roesch et al., 2009), the location of upcoming rewards (Taha and Fields, 2006), and reward valence (appetitive versus aversive) (Setlow et al., 2003; Roitman et al., 2005; Taha and Fields, 2005a; Wheeler et al., 2008). As such, we expected to see differences in cue related neuronal activity for the risk versus safe options based on risk preferences. Specifically, if the NAc encoded information about subjective value we expected that the three groups would differentially encode the risk versus safe cues, such that risk preferring rats have significantly greater percentage of selective cells encode the risk option, safe preferring rats have significantly greater percentage of selective cells encode the safe option, and nonpreferring rats have the greatest percentage of cells similarly encode both. Indeed we found that the majority of phasic neurons differentially encoded risk versus safe options, suggesting that the NAc does track these differences. Surprisingly, we found that there were similar proportions of cells that encoded the risk versus safe options in each of the three groups. Importantly, previous studies that showed that NAc neurons encode information about reward value during cue presentations (Schultz et al., 1992; Cromwell and Schultz, 2003; Roesch et al., 2009; Day et al., 2011) used rewards with different explicit value (i.e., situations in which choosing one reward is more advantageous than another) such as rewards of different size, delay, or effort. In these tasks, the expected value of one option is always more advantageous than the other option and therefore the expected and subjective value are conflated with one another.

Instead of encoding subjective value represented by the cue, NAc neurons may be encoding the expected value of different options. In support, fMRI studies in humans have

shown that ventral striatal activity was correlated with the expected value associated with different cues based on the probability and magnitude of reward delivery. However, this value signaling was not correlated with individual risk attitudes, suggesting a primary role in encoding expected values irrespective of behavioral preferences (Tobler et al., 2007). Further, neurons in the dorsal striatum have been shown to encode information about subjective value during decision making behaviors (Samejima et al., 2005), while optical stimulation of dorsal striatal neurons prior to behavioral responses can modulate subjective value related decisions (Tai et al., 2012). As such, the dorsal versus ventral striatum may send competing value related information (subjective versus expected value) to movement related output structures to promote appropriate decision making (Nicola, 2007). The present findings are consistent with a role of the NAc in encoding expected value as the population encoding of risk versus safe options was similar for risk preferring, safe preferring, and nonpreferring animals.

The NAc has been described as a site of limbic motor integration (Mogenson et al., 1980), and is therefore critically involved in the action selection portion of behavioral choices. In support, we show that the largest proportion of phasic responding was observed during the prepress period. Further, a majority of the phasic cells were inhibitory during this period. Previous reports suggest that NAc neurons are predominantly inhibitory during goal-directed behaviors, playing a role in "gating" actions, releasing motor areas from strong GABAergic inhibitions (Taha and Fields, 2005a; Taha et al., 2007), keeping motor systems engaged and ready for reward delivery (Day et al., 2011). As the percentages of inhibitions were similar for both lever presses, this suggests that rats similarly expected rewards following both safe and risk lever responding in the current task. Further, there were no

differences across the three groups in the selectivity of responding, such that similar populations of neurons were risk selective and safe selective in risk, safe, and nonpreferring rats. Again, this suggests that NAc neural activity during the prepress period encoded information on the expected value of lever presses rather than subjective value.

Our findings on the role of the NAc in processing information about expected value of action performance are consistent with prior studies. Previous work has shown that the NAc is not necessary for goal-directed behaviors that do not require expected value discriminations, such that animals are still able to press levers for reward following NAc inactivation or lesion (Cardinal et al., 2001; Cardinal and Howes, 2005; Floresco et al., 2008; Ghods-Sharifi and Floresco, 2010; Stopper and Floresco, 2011). However, when animals are given choices that require complex expected value computations, disruptions of NAc activity result in specific deficits on choice behavior. In particular, when given the choice between a small certain versus large uncertain reward, lesion (Cardinal and Howes, 2005) and inactivation (Stopper and Floresco, 2011) of the NAc result in risk averse behavior, choosing the small certain reward even when it is less advantageous. Further, when evaluating reward delays or effort, animals also choose the smaller lower effort or immediate reward compared to the delayed or higher effort reward following NAc activity disruption (Cardinal et al., 2001; Ghods-Sharifi and Floresco, 2010). This suggests that animals are unable to calculate these more complex expected value associations appropriately (based on risk, delay, or effort) and therefore shift behaviors towards the option that does not require expected value calculations: the small certain, low effort, immediate reward (even when this behavior is less advantageous). This supports the current findings that NAc neurons encode the expected

value of action selection, and disruptions of this signaling result in maladaptive decision making.

NAc neurons are critical for encoding information about rewarding situations (Nicola et al., 2004b; Roitman et al., 2005; Taha and Fields, 2005b; Krause et al., 2010) to promote feeding behaviors. Importantly, appetitive rewards are encoded by increased inhibitions while aversive events are encoded by increased excitations (Roitman et al., 2005; Wheeler et al., 2008). In the current study we found a similar pattern of processing such that during large and small reward presentations (both appetitive events) ~60% of phasic neurons displayed inhibitory processing. While increased inhibitions appear to release motor related areas from inhibition to promote feeding (Taha and Fields, 2005b; Krause et al., 2010; Ambroggi et al., 2011), it is hypothesized that increased excitations during aversive events may function to inhibit behaviors to avoid future negative consequences and thus may signal the animal is experiencing a negative affective state (Roitman et al., 2005; Wheeler et al., 2008). In support, in the current data set, we found increased excitations compared to inhibitions during the reward omission period. Further, there was a significant increase in the percentage of phasic cells that showed excitations from the early to late reward periods (the time period in which the animal is evaluating the outcome and learns that the reward is not presented). It is therefore possible that reward omission in our task evokes a negative affective state that is encoded by NAc neurons. Further, in the NAc core there was a significant correlation of the percentage of phasic cells that displayed excitations and risk aversion, such that animals that had greater percentage excitation were more risk averse. From this we hypothesize that reward omissions are more aversive to these animals and therefore they are more likely to avoid this response and display a safe preferring phenotype.

Interestingly, we found the opposite pattern of responding in the NAc shell, an area that is normally linked with encoding reward valence (Roitman et al., 2008; Wheeler et al., 2011). Specifically, we found an increased percentage of neurons exhibiting excitatory activity during reward omissions in the risk preferring compared to safe preferring rats. A key difference between normal reward aversion tasks and the data reported here is that in our task the aversive event is reward omission, rather than the presentation of an aversive stimulus. The fact that there is no reward present may alter the way the NAc shell processes this information. Further, reward omission processing may function again as a gating signal within the NAc. For risk preferring rats, reward omissions may not be as aversive, and therefore increased excitations function to inhibit reward seeking behaviors. In contrast, in safe preferring rats, reward omissions appear highly aversive and thus the increased inhibitions during reward omissions may function to instruct the animal to continue seeking for rewards in a safe manner.

The NAc is embedded in a larger corticolimbic neural circuit that has been linked with decision making and risk taking behavior including the prefrontal cortex (PFC), basolateral amygdala (BLA), orbitofrontal cortex (OFC), and mesolimbic dopamine projections (Winstanley et al., 2004; Ghods-Sharifi et al., 2009; Hauber and Sommer, 2009; Day et al., 2010; Gan et al., 2010; Roitman and Roitman, 2010; St. Onge and Floresco, 2010; St. Onge et al., 2012; Sugam et al., 2012; Stopper et al., 2013). The OFC provides a direct projection to the NAc (Berendse et al., 1992; Wright and Groenewegen, 1996) and may be involved in the NAc neural encoding of risky decision making observed here. Previous work has shown that there is differential responding to reward omissions and presentations in OFC that is related to risk preference. (Roitman and Roitman, 2010). This glutamatergic projection

from the OFC may be driving the NAc neural responding observed during reward periods here. Finally, the mesolimbic dopamine projections to the NAc encode the value associated with cues that predict different rewards (Fiorillo et al., 2003; Tobler et al., 2005; Roesch et al., 2007; Kobayashi and Schultz, 2008; Day et al., 2010; Gan et al., 2010), and importantly, encode the subjective value during risky decision making (Sugam et al., 2012). Taken with the NAc neural activity, this provides a complete neural network for the prediction of reward value (through the mesolimbic dopamine projection) and the evaluation of outcomes (through the NAc neural activity) based on risk preferences. Importantly, understanding how these systems interact during risky decision making will provide new insights into the mechanism of appropriate risk taking behaviors, as well as provide a candidate region of interest for disorders characterized by aberrant decisions and risk taking such as drug and gambling addiction.

CHAPTER 3

ROLE OF PHASIC NUCLEUS ACCUMBENS DOPAMINE IN DELAY DISCOUNTING

ABSTRACT

To promote survival, organisms must evaluate the costs and benefits of different courses of action. However an organism's environment is not static. Instead, the availability of resources and the cues that signal these resources are always changing, such that organisms must update cue-outcome associations and adjust behaviors accordingly. Dopamine transmission within the nucleus accumbens (NAc) has been implicated in cue-outcome learning and value-based decision making. Further, this circuitry is necessary for adapting behaviors when the value of future outcomes change. Here, we evaluated how the mesolimbic dopamine system encodes changes in reward value as animals learn to shift behavior from lower value to higher value options. We monitored dopamine concentration in the NAc core on a rapid time scale using fast-scan cyclic voltammetry (FSCV) during a delay discounting task. Rats (n=7 with 8 recording locations) were trained to associate distinct visual cues with responses that predicted smaller immediate or larger delayed rewards. Importantly, the delay to large reward increased during the session, thus devaluing the large reward. Animals were able to successfully discriminate between the cues and adjust their behavior accordingly; the rats showed decreased responding on the larger delayed lever as the delays to reward increased. Reward predictive cues evoked increases in phasic dopamine concentrations that scaled with the value of the reward, such that there was higher dopamine

release for cues that predicted the large immediate reward compared to the small reward. As the delay to the large reward increased, dopamine signaling decreased to the cues predicting the large reward. As such, there was a shift in the relative dopamine release to each cue type within each session. Specifically dopamine concentration was higher to the cue signaling the large reward during early trials but as the delay increased, dopamine concentration was higher to the smaller reward. These findings are consistent with previous reports that phasic dopamine release encodes the value associated with reward predictive cues, but also indicates that dopamine release updates in real time as the reward value changes.

INTRODUCTION

Learned associations between cues in the environment and positive outcomes are critical for making appropriate decisions. In order to maximize resources, organisms must evaluate the costs and benefits of different actions and choose the most valuable option available (Green and Myerson, 2004; Cardinal, 2006; Phillips et al., 2007; Rangel et al., 2008). This type of decision making engages a specific network of nuclei including the nucleus accumbens (NAc) and its dopaminergic input (Roesch et al., 2007; Roesch et al., 2009; Day et al., 2010; Day et al., 2011; Sugam et al., 2012). Subsecond dopamine release within the NAc is believed to modulate reward-seeking behaviors including those involving food and cocaine (Phillips et al., 2003b; Roitman et al., 2004), and track decisions based on effort, delay and risk (Day et al., 2010; Sugam et al., 2012). Importantly, the mesolimbic dopamine system functions as a prediction signal, displaying increased phasic activation to cues that reliably predict reward delivery (Mirenowicz and Schultz, 1994; Schultz et al., 1997; Waelti et al., 2001; Stuber et al., 2008; Tsai et al., 2009; Zellner and Ranaldi, 2010). This encoding provides a mechanism for the brain to track reward related information to mediate appropriate resource seeking behaviors. Phasic dopamine signaling of reward predictive cues is not only sufficient to promote behavioral conditioning (Tsai et al., 2009), but is also necessary for reward related learning (Sombers et al., 2009; Zellner et al., 2009).

In order to be implicated in cost benefit decision making, dopamine signaling must differentiate between cues of different value to promote appropriate behavioral responding. In support, dopamine neurons show increased activity to reward paired cues based on several reward value factors including reward delay, probability, magnitude, and expected value (Fiorillo et al., 2003; Tobler et al., 2005; Roesch et al., 2007; Kobayashi and Schultz, 2008;

Schultz, 2010). Further, dopamine release within the NAc encodes information related to reward costs, delays, and subjective value (Day et al., 2010; Gan et al., 2010; Sugam et al., 2012). However, dopamine signaling of reward value appears to be restricted to the NAc core (Day et al., 2010; Sugam et al., 2012; Saddoris et al., 2013). As such, this circuit provides a mechanism to encode the most valuable option available to the animal to bias decisions and promote adaptive behaviors. Finally, the mesolimbic dopamine projections to the NAc are necessary for appropriate value-based decision making as lesions or inactivation of it result in maladaptive choices (Cardinal et al., 2001; Salamone et al., 2001; Salamone et al., 2002; Cardinal and Howes, 2005; St Onge and Floresco, 2008; Ghods-Sharifi and Floresco, 2010; St. Onge et al., 2010; Stopper and Floresco, 2011; Stopper et al., 2013).

Rarely in a natural environment do cue-outcome associations remain static. Instead an organism's environment, the availability of resources in that environment, and the cues that predict these resources constantly change. In order to adapt to these changes, organisms must be able to update encoding of cue-outcome associations. Delay discounting models this type of situation. In these tasks, animals are presented with the choice between a small immediate reward and a larger delayed reward. Importantly, the delay to the large reward increases as the session continues, requiring the animal to shift behavior over time to maximize resources (Cardinal et al., 2001; Winstanley et al., 2004; Perry et al., 2005; Perry et al., 2008; Setlow et al., 2009). Importantly, the NAc and associated dopamine projections are critical for this type of decision making behavior. For example, lesions of the NAc induce increased responding for the small immediate reward (Cardinal et al., 2001), while administration of drugs that alter dopamine function result in aberrant delay discounting (Floresco et al., 2007; Simon et al., 2007; Setlow et al., 2009; Mendez et al., 2010). Further, neural recordings of putative

dopamine neurons in the ventral tegmental area (VTA) show that these neurons are able to update when reward values are reversed (Roesch et al., 2007). Further, dopamine neurons encode information about cues that predict rewards following periods of delay, and this signaling is correlated with delay discounting (Kobayashi and Schultz, 2008). Finally the mesolimbic dopamine system and its terminal region, the NAc, are critical for appropriate reward devaluation learning (Lex and Hauber, 2010; Singh et al., 2010). As both the NAc and its dopamine input are implicated in updating cue-outcome associations as reward values are changed, it provides a unique circuit for the ability of an organism to adapt to its ever changing environment and make appropriate decisions. To date, no work evaluating dopamine release in the NAc has studied how phasic dopamine signaling changes as reward value changes. Here, we developed a delay discounting task in which animals were presented with reward predictive cues that signaled the availability to respond for a small immediate reward versus a large delayed reward. Importantly, early in the session, the large reward was presented immediately. Later in the task, the delay to large reward significantly increased. This design allows for the analysis of how dopamine signals the value associated with each reward predictive cue and if there is a shift in dopamine signaling as the relative value of the reward predictive cues change. Using FSCV, we measured phasic dopamine signaling in the NAc core during the delay discounting task.

METHODS

Animals

Male Sprague Dawley rats (*n*=7 rats with 8 recording locations, Harlan Sprague Dawley, Indianapolis, IN), aged 90-120d and weighing 275-350g were used as subjects and were individually housed with a 12/12-h light/dark cycle. All experiments were conducted between 8:00am and 5:00pm. Animals were maintained at no less than 85% of pre-experimental bodyweights by food restriction (~10-15g of Purina laboratory chow each day in addition to approximately 1g of sucrose consumed during behavioral sessions) except during the post-operative recovery period when food was given *ad libitum*. All procedures were approved by the UNC Institutional Animal Care and Use Committee.

Behavioral Training

Rats were initially magazine trained in which they received 10 deliveries of a single 45mg sucrose pellet to a centrally located food receptacle over a 30 min session. Next, rats were trained to press two distinct levers in which responses were reinforced on a continuous schedule of reinforcement. Reinforced responses resulted in the delivery of a sucrose pellet to the food cup situated equal distance between the levers. A maximum of 100 reinforcers (50 per lever) were available per session. Once rats reached stable responding (50 presses on each lever; typically 5 training sessions) daily training on the delay discounting task began (Figure 3.1). Our task varies slightly from the previously described risk task (see chapter 2 pg 33-35), and is modeled after work by Setlow and colleagues (Simon et al., 2007; Setlow et al., 2009; Mendez et al., 2010). Each delay discounting session consisted of 3 blocks of 30 discrete trials .The first 20 trials of each block were intermixed immediate or delayed trials

(10 trials of each). For Delay trials (Figure 3.1, left) a single cue light was illuminated over one lever for 5s, followed by extension of both levers. Responses (FR1) on

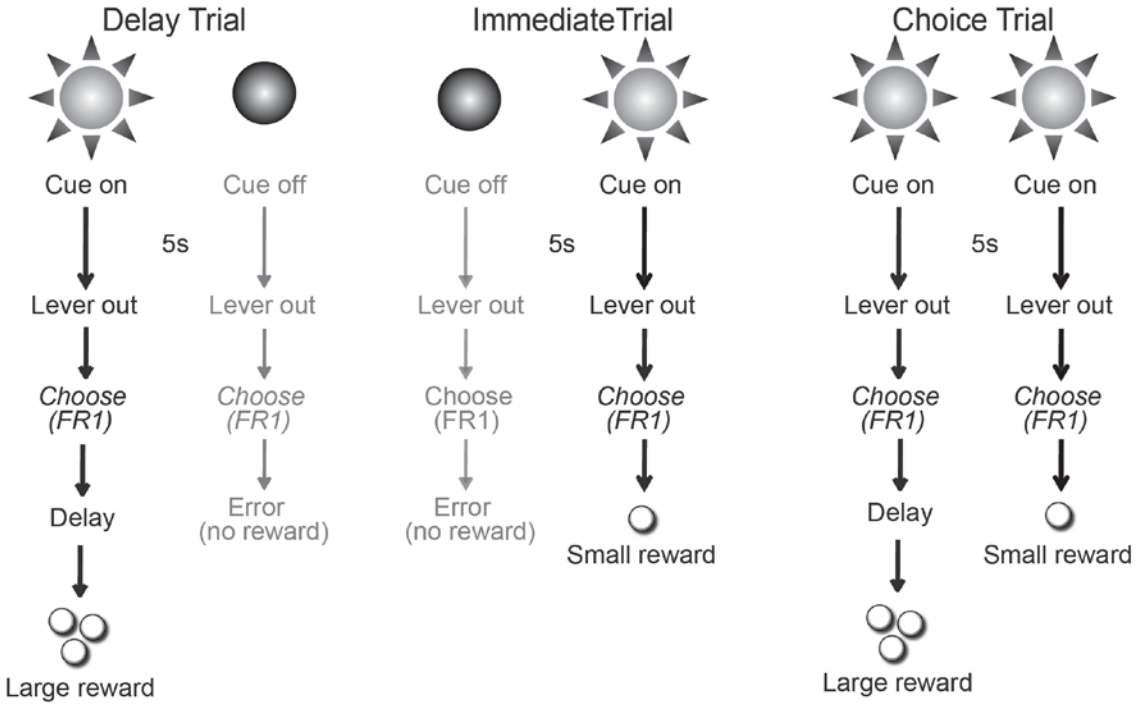

Figure 3.1 Schematic representation of delay discounting task. On delay trials (left panel), a cue light was presented for 5s and was followed by the extension of two response levers. A single lever press on the lever positioned below the cue light led to a 3 sucrose pellet reward presented after a period of delay. Responding on the other lever did not produce reward delivery and terminated the trial. On Immediate trials (middle panel) the other cue light was presented for 5s before lever extension. Here, presses on the associated lever resulted in 1 sucrose pellet delivered immediately while presses on the other lever did not produce reward and terminated the trial. On choice trials (right panel) both cue lights were presented for 5s, and animals could select either option. During the first block of the sessions (10 trials of each type) the delay to large reward was 0s. During the second block the delay was 10s and during the third block the delay was 20s.

the lever below the illuminated cue light were rewarded with three sucrose pellets delivered after a delay. Lever presses on the other lever were not rewarded and counted as an error. The number of errors served as a behavioral measure of discrimination between the immediate and delayed cues. If the animal did not respond on either lever within 10s both

levers retracted and the trial was counted as an omission. Delay trials were critical since they informed the rat as to which delay was imposed during the upcoming free choice trials of that block. For Immediate trials (Figure 3.1, middle) the cue light above the second lever was illuminated for 5s, then both levers were extended into the chamber. Lever presses (FR1) on the lever under the illuminated cue light within 10 s were rewarded with a single sucrose pellet. Lever presses on the other lever were not rewarded and counted as an error. The next 10 trials within each block were Choice trials (Figure 3.1, right) in which both cue lights simultaneously illuminated for 5 s, and both levers were extended for 10 s. Once either lever was pressed, both levers retracted and the animal was rewarded based on the contingency of reinforcement for that block and chosen lever. Again, if the animal did not respond within 10 s the levers retracted and the trial was counted as an omission. Choice trials served as a measure of an animal's overall sensitivity to the changes in reward delay across the session. During the first block/session, the delay to the large reward was 0s. In subsequent blocks, the delay to large reward was increased to 10s and 20s. Importantly, each trial was of fixed duration (60 s) so that reward choice did not influence how quickly a rat can complete the task, and choosing the smaller immediate reward did not lead to the next trial more quickly. Rats were trained for 25 sessions until stable behavior was observed. Following 25 training sessions, all rats were be prepared for electrochemical recording in the NAc core as described below. After recovery, animals underwent additional training sessions until behavior reached the presurgery baseline (at least 5 sessions).

Surgery

Rats were surgically prepared for voltammetric recordings as previously described (Phillips et al., 2003a; Sugam et al., 2012). Briefly, animals were anesthetized with a

ketamine hydrochloride (100mg/kg) and xylazine hydrochloride (10mg/kg) mixture (intramuscular) and placed in a stereotaxic frame. A guide cannula (Bioanalytical Systems, West Lafayette, IN) was positioned dorsally to the NAc core (1.3 mm anterior, 1.3mm lateral from bregma). An Ag/AgCl reference electrode was placed contralateral to the stimulating electrode in the left forebrain. The bipolar stimulating electrode (Plastics 1 Inc., Roanoake, VA) was placed dorsally to the VTA (5.2 mm posterior, 1.0 mm lateral from bregma and 7 mm ventral from brain surface). Stainless steel skull screws and dental cement were used to secure all items. The bipolar stimulating electrode was lowered in 0.2 mm increments until physical responses to electrical stimulation diminished indicative of proper electrode placement. The stimulating electrode was then fixed with dental cement.

Fast-Scan Cyclic Voltammetry

Following surgery, animals were allowed one week to recover to presurgery body weight. Food restriction was then resumed to increase motivation during behavioral performance. On test day, a new carbon-fiber electrode, housed in the micromanipulator, was lowered into the NAc core and was used to measure dopamine changes during task performance. The potential of the carbon-fiber electrode was held at -0.4V versus the Ag/AgCl reference electrode. The carbon-fiber and Ag/AgCl electrodes were connected to a head-mounted voltammetric amplifier attached to a commutator (Crist Instrument Company, Hagerstown, MD). Prior to recording, the carbon fiber electrode was allowed to equilibrate for 20-30 minutes in the brain to minimize current drift.

Voltammetric recordings were made every 100 ms by applying a triangular waveform that drives the potential to +1.3V and back to -0.4V at a scan rate of 400V/s. Application of the triangular waveform results in the oxidation and reduction of chemical species that are

electroactive within this potential range (including dopamine), producing a measurable change in current at the carbon-fiber. Specific analytes (including dopamine) were verified by plotting this change in current against the applied potential to produce a cyclic voltammogram (Heien et al., 2004; Heien et al., 2005). The stable contribution of current produced by oxidation and reduction of surface molecules on the carbon-fiber was removed by using a differential measurement (i.e., background subtraction) between a time when such signals were present but dopamine was not. For data collected during the behavioral session, this background period was obtained during the baseline window (10 s prior to cue onset). Following equilibration, dopamine release was electrically evoked by stimulating the VTA using a range of stimulation parameters (2-24 biphasic pulses, 20-60 Hz, 120μA, 2 ms per phase) to make sure that the carbon fiber electrode was placed close to dopamine release sites and to create a training set for principal component analysis. Animals then underwent task performance and electrochemical recordings were made continuously with 100 ms temporal resolution. A second computer and software system (Med Associates Inc) controlled behavioral events and sent digital outputs for each event to the voltammetry recording computer to be time stamped along with the electrochemical data. Following termination of the behavioral session, VTA stimulation was repeated to verify the stability of the electrode and ensure that the location of the electrode still supported dopamine release.

Signal Identification and Separation

After in vivo recordings, dopamine release evoked by VTA stimulation was used to identify naturally occurring dopamine transients. Stimulation of the VTA leads to two well-characterized electrochemical events: an immediate but transient increase in dopamine and a delayed but longer lasting basic pH shift. To separate these signals a training set was

constructed from representative, background-subtracted cyclic voltammograms for dopamine and pH, as previously described (Heien et al., 2004; Heien et al., 2005). The background period was obtained at the minima for the dopamine signal 5 s before event onset. This training set was used to perform principal component regression on data collected during the behavioral session. Principal components were selected such that at least 99% of the variance in the training set was accounted for by the model. All data presented here fit the resulting model at the 95% confidence level.

Data Analysis

All behavioral events occurring during training and electrochemical recordings were available for analysis. To determine if animals reliably acquired the delay discounting task we evaluated the number of errors and the number of omitted responses during the recording sessions. Further, we examined the ability of the animals to adjust behaviors as delays increased by using a repeated measures ANOVA to compare responses during choice trials for the large delayed reward across the three blocks of the session.

Changes in extracellular dopamine concentration were assessed by aligning dopamine concentration traces to each behavioral event. Increases or decreases in NAc dopamine concentration from baseline for the cue presentations and were evaluated separately for each cue type (across both block and type of cue) using a two-way repeated measures ANOVA with Dunnett's correction for multiple comparisons. This analysis compared the baseline mean dopamine concentration (10s prior to cue onset) to each data point (100 msec bin) obtained within 5s following the task event. The effect of cue type (large versus small) and reward delay (no delay block, short delay block, long delay block) were evaluated with a 2-way repeated measures ANOVA that compared peak changes in dopamine levels following

each event, with Tukey's correction for multiple post-hoc comparisons. The effect of large reward delivery on dopamine signaling was evaluated using a 1-way repeated measures ANOVA comparing baseline to peak levels. A 1-way repeated measures ANOVA with Tukey's post hoc test was used to evaluate the differences in the time to reach peak dopamine concentration following reward delivery for each delayed reward. All analyses were considered significant at $\alpha = 0.05$. Statistical and graphical analysis was performed using Graphpad Prism (Graphpad Software, Inc) and STATISTICA (Statsoft, Tulsa, OK).

Histology

Upon completion of the experiment, rats were deeply anesthetized with a ketamine/xylazine mixture (100 mg/kg and 10 mg/kg, respectively). In order to mark the placement of the electrode tip, a 150 to 250 µA current was be passed through a stainless steel electrode for 5 seconds using established procedures (Roitman et al., 2004; Day et al., 2007). Animals were decapitated and brains removed and postfixed in 10% formalin. After post fixing and freezing, brains were sliced at the level of the NAc at 30 µm coronal sections and mounted on microscope slides. The specific position of electrodes was assessed by visual examination of successive coronal sections in comparison to visual landmarks and anatomical organization of the NAc represented in a stereotaxic atlas (Paxinos and Watson, 2005).

RESULTS

Behavior during the delay discounting task

Animals were able to discriminate between cue presentations during recording sessions, displaying error rates significantly below chance levels (10.4% ± 2.554%, $t(7)=15.5$, $P<0.0001$), suggesting that when no alternatives were available, animals were capable of overcoming delays to obtain rewards. In total, animals received rewards on 88 ± 2.3% of all trials, suggesting that they reliably performed the task. Further, response allocation on choice trials support that animals were attending to both the magnitude and delay of response options; rats displayed a significant decrease in choices of the large lever as delays increased (Figure 3.2; $F_{(2,7)}= 21.68$, $P<0.001$). When the large reward was presented immediately, rats chose this option significantly greater than chance ($t(7)=7.519$, $P<0.001$), pressing the lever 89 ± 5.2% of the time, and thus displaying a strong preference for the large immediate option. As the delay to the large reward increased to 10 s, animals displayed a significant decrease in responses on the large lever ($P<0.05$), choosing it 39 ± 10.3% of trials which was not different from chance performance ($t(7)=1.097$, $P=0.31$). Finally, animals displayed a shift to a preference for the smaller immediate option as the delay to large reward increased to 20 s. In this case, they choose the large delayed option only 20 ± 8.0% of the time, which was significantly below chance levels ($t(7)=3.742$, $P<0.01$).

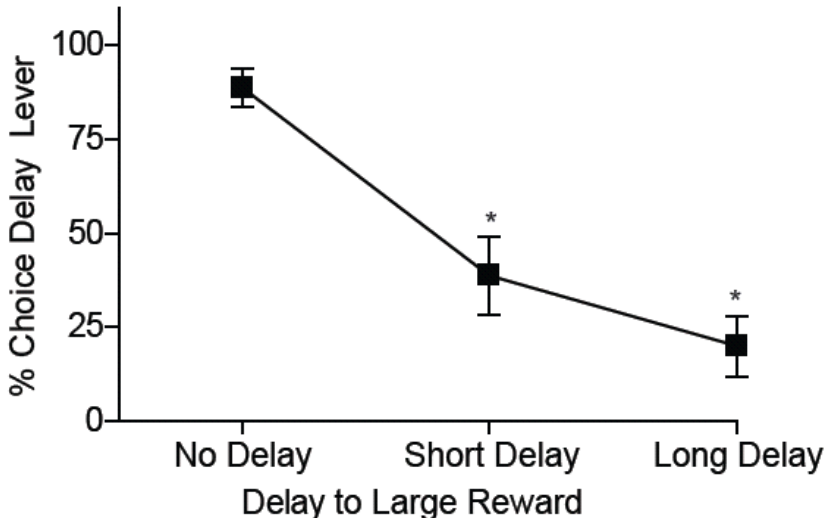

Figure 3.2 Choice behavior during delay discounting task. Response allocation on free choice trials during the three different blocks of the task. During the first block the delay to large reward was 0 s, during the second block the delay to large reward was 10 s, and during the final block the delay to large reward was 20 seconds. Responses for the large delayed reward decreased across the three blocks of trials. *P<0.05 compared to no delay block. Data are mean ± SEM.

Cue-evoked dopamine within the NAc core reflect relative reward values

Reward predictive cues in all trial types and across all reward delays evoked the largest increase in phasic dopamine release in the NAc core, consistent with previous reports (Day et al., 2007; Day et al., 2010; Sugam et al., 2012). A separate repeated measures ANOVA was evaluated for each trial type within each block and revealed that cue presentation significantly increased dopamine concentration above baseline (P<0.0001 for all analyses). However, the relative amplitude of cue-evoked dopamine release varied depending on the type of cue (forced small versus forced large reward) presented and the delay associated with the reward. Group changes in dopamine concentration, time locked to cue onset, are shown in Figures 3.3 A, B, C across the three different delay periods. A 2-way repeated measures ANOVA on forced trials showed a main effect of both cue type (small versus large $F_{(1,14)}$=8.39, P=0.05) and block of behavioral session ($F_{(2,28)}$=33.35, P<0.0001) demonstrating that there are both relative differences in dopamine signaling between cues

predictive of differentially valued outcomes, as well as shifts in the total amount of dopamine signaling to reward predictive cues as delays to obtain the larger reward are increased. Importantly, there was also a significant interaction between cue type and reward delay ($F_{(2,28)}$=9.48, P<0.001), indicating that dopamine signaled the value associated with each cue type and this signaling changed with increasing delays. Specifically, during the first block of trials, in which both the large and small reward were presented immediately following behavioral responses (Figure 3.3A), the cue presentations that predicted the large reward evoked significantly greater dopamine release than cues that predicted the small reward (P<0.001). However, increasing the delay to the large reward correspondingly resulted in a significant decrease in dopamine signaling for the large reward cue despite the fact that the magnitude of the large reward (3 pellets) remained the same in all blocks (Figure 3.3B). Specifically, there was significantly less dopamine release for the large-reward cue during the short delay (10s) compared to the no delay (P<0.05) and a further decrease in dopamine for the large reward cue when the delay increased from the short delay to the long delay (20s), (P<0.05, Figure 3.3C). Interestingly, there was no change in dopamine signaling for cues that predicted the small immediate reward across the 3 blocks of the task (Figure 3.3 A, B, C; P>0.05 for all comparisons). These findings are summarized in Figure 3.3 D in which peak dopamine concentration is shown during a 5s period following cue onset for each of the cue types across each of the reward delays. Interestingly, there was a linear relationship between reward delay and peak dopamine release as dopamine signaling decreased by 33% as the delay shifted from 0s to 10s. Doubling the delay to 20s resulted in a 61% decrease in peak dopamine signaling for the 0s versus 20s block. (Figure 3.3D, black trace)

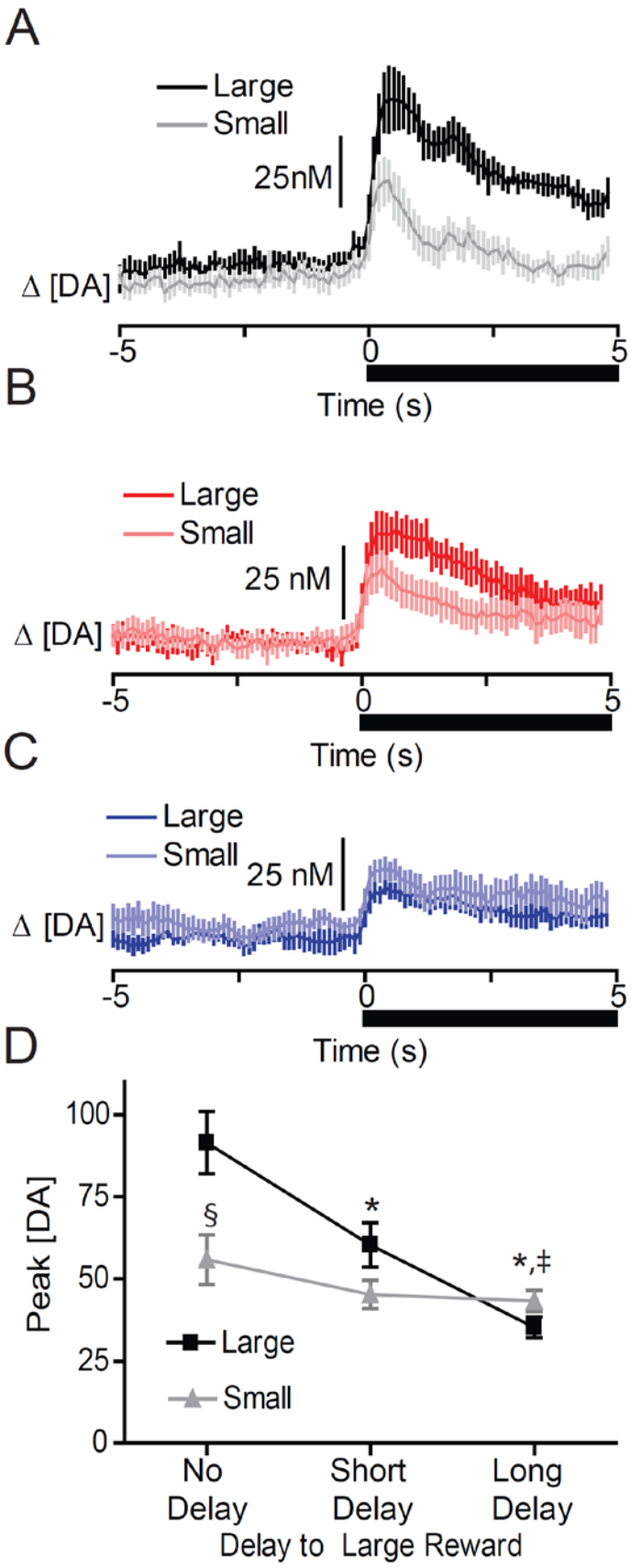

Figure 3.3 Dopamine release encodes the relative value of cue presentations during delay discounting. (A) Dopamine concentration aligned to cue onset (black bar, time 0s) on forced large versus forced small reward trials during the first block of the task in which there was no delay to the large reward. Cue presentation led to significantly greater dopamine release for the large immediate versus small immediate reward. (B) Dopamine concentration aligned to cue onset on forced large versus forced small reward trials during the second block of the task when the delay to the large reward was short (10s). Conventions follow from A. (C) Dopamine concentration aligned to cue onset on forced large versus forced small reward trials during the third block of the task when the delay to large reward was long (20s). Conventions follow from A. (D) Peak dopamine concentration during a 5s period following cue onset for each of the cue types across each of the reward delays. There was a significant reduction in dopamine release for the forced large delayed cue and no change in dopamine for the forced small immediate cue. *P<0.05 for comparisons to large reward during the no delay block. ‡ P<0.05 for comparison of large short delay versus large long delay. §P<0.05 for comparison of small versus large reward during no delay block. All data are mean ± SEM.

In order to determine if there was a shift in dopamine signaling as the relative value of each option shifted, we evaluated the differences in dopamine signaling between the large versus small option across each block of trials. We have previously shown that cues that predict higher value reward evoke higher increases in peak dopamine release (Day et al., 2010; Sugam et al., 2012). As such, we predicted that this value encoding biases responses towards the more valuable option. Therefore, we expected that the differences in peak dopamine release for the large versus small option would track the relative value associated with each option to bias appropriate responding. We found that, during the first block, rats displayed a strong behavioral preference for the large reward option over the small reward, suggesting that the relative value of the large reward was much higher than the small reward. However, as the delays increased animals shifted their preference away from the large delayed reward in favor of the small immediate reward suggesting a shift in the relative value of each option (Figure 3.4A). We evaluated if this shift in behavior was related to differences in dopamine release dynamics by comparing behavior preference to the difference in peak dopamine release (difference score defined as (peak dopamine large cue) – (peak dopamine small cue); Figure 3.3A,B,C) for each animal during each trial block. Figure 3.4A shows the relationship between the difference scores in dopamine release and delay discounting behavior. During the no delay block, rats strongly preferred the large reward, and also displayed the largest significant difference in cue-evoked dopamine for small versus large options (35.5 ± 4.4 nM, $t(7)=8.097$, $P<0.001$ significantly different from a theoretical mean of 0nM). During the short delay block, cues predicting the large reward evoked significantly less dopamine release than during the no delay block, and as such there is a significant reduction in the difference score between the first block and second block ($P<0.05$). In this

short delay block, cues predicting the large delayed reward still evoked higher dopamine signaling than small immediate cues during this block, as the difference in peak concentration between the cues was still greater than 0, ($t(7)=2.57$, $P=0.04$). As the delays increased to 20s for the large reward, there was a further relative shift in dopamine signaling such that during the long delay block when animals showed a preference for the small immediate option, they correspondingly showed a relative negative difference in peak dopamine concentration (-8.0 ± 3.24 nM). Indeed, this negative relative difference was due to greater dopamine release during the small immediate cue compared to the large delayed cue (difference versus 0nM $t(7)=2.447$, $P=0.04$) and was accompanied by a behavioral shift towards a preference for the small immediate option.

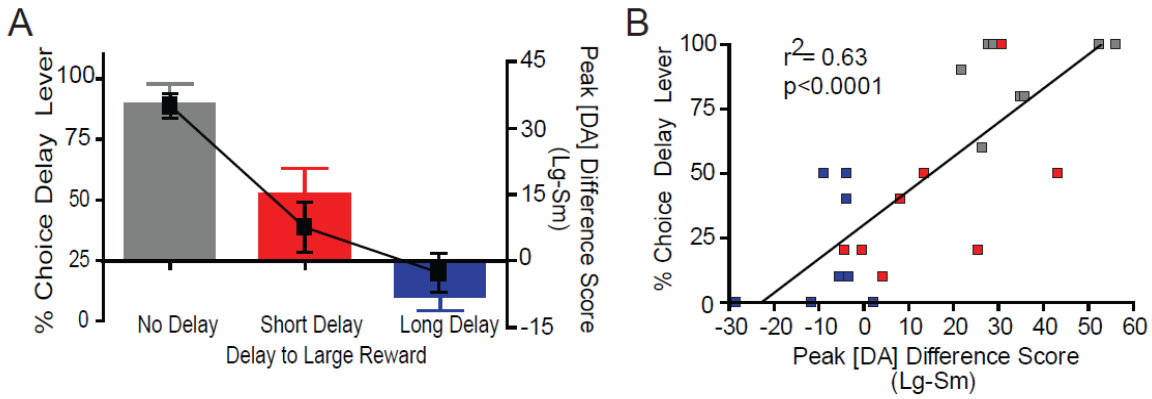

Figure 3.4 Differential dopamine release is correlated with response allocation in delay discounting. (A) Response allocation during choice trials for the large delay lever during each of the three blocks compared to difference scores in peak dopamine release for cues that predict large versus the small option. Data are mean ± SEM (B) Correlation of response choices and dopamine difference score for each animal during each of the three blocks of the task. Grey dots are behavior during the no delay block, red dots during the short delay, and blue dots during the long delay block.

In order to evaluate if there was a direct relationship between this relative value encoding of cue-evoked dopamine release and choice behaviors, we correlated each individual animals' difference in peak dopamine signaling between the cues with their choice

behaviors during each block of the task (Figure 3.4B). There was a significant correlation between differences in relative dopamine signaling during cues on the force trials and subsequent response allocation on choice trials (r^2=0.63, P<0.001), suggesting a strong association between the strength of behavioral preferences and the value encoding by the dopamine system. Specifically, the stronger the behavioral preference, the larger the difference in cue-evoked dopamine release during the task.

Dopamine signaling during choice cues tracks the value of options available

Following the analysis of forced cue signaling, we next evaluated dopamine signaling as rats were given the option to choose the small immediate versus large delayed reward (i.e., during choice trials). Similar to the forced trials, choice cues evoked significant increases in dopamine release during cue presentation (Figure 3.5A, repeated measures ANOVA P<0.05 for all comparisons of cue period versus baseline). There was a significant decrease in cue-evoked dopamine signaling across the three blocks of trials as the value of available rewards decreased (Figure 3.5B, $F_{(2,14)}$=9.75, P=0.002). Further, dopamine release scaled with the best option available. Post hoc analysis determined that peak dopamine signaling during choice cue presentation was not significantly different from dopamine signaling of the preferred option during each forced block (P>0.05 for comparisons to large reward during the no delay block, comparisons to the small reward during the short and long delay blocks). This data is consistent with previous reports of other decision making behaviors (Day et al., 2010; Sugam et al., 2012), and suggests that dopamine signals the best available option to the animal during choice trials, likely biasing responses towards maximizing resources.

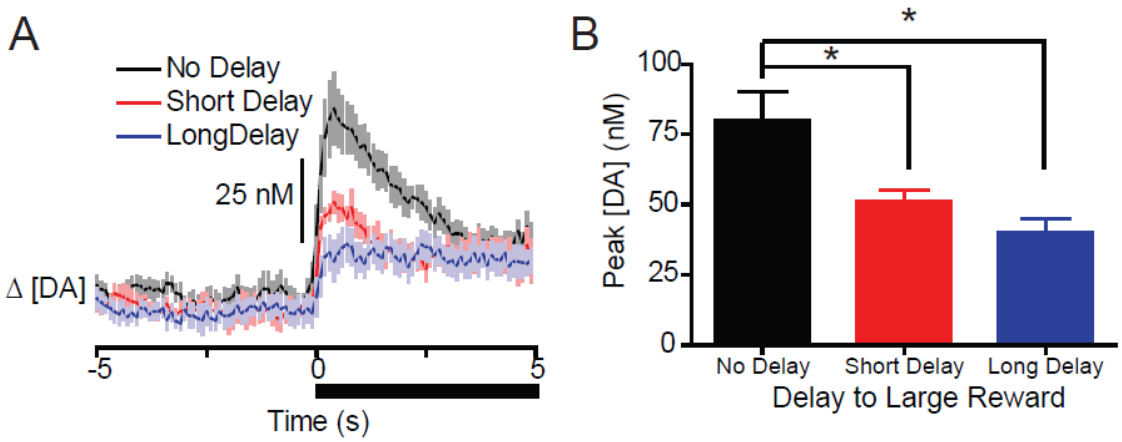

Figure 3.5 Dopamine signaling during choice trials on the delay discounting task. (A) Dopamine concentration aligned to cue onset (black bar, time 0s) on choice trials during each of the three blocks of the task. (B) Peak dopamine concentration during choice trials (during 5s period immediately after cue onset). Choice cues during the no delay block evoked significantly greater dopamine than choice trials during the short or long delay blocks. There was no difference in dopamine signaling during the short and long delay blocks. Data are mean ± SEM. *P<0.05.

Dopamine signaling during delayed reward delivery

Previous work from Schultz and colleagues (Kobayashi and Schultz, 2008) has shown that delayed reward presentation results in increased phasic dopamine activity during the reward delivery. They postulate that reward delays begin to decouple the cue-outcome association, and as such, reward delivery is somewhat uncertain resulting in increases in dopamine activity to reward delivery, compared to situations in which the cue-outcome association is more predictable (Schultz et al., 1997; Kobayashi and Schultz, 2008). Our behavioral task enabled an examination of whether dopamine release in the NAc encodes reward deliveries following extended delays. In a subset of animals (n=5), we evaluated dopamine signaling following large reward presentation during each of the three blocks of trials. Interestingly, reward delivery evoked increases in dopamine signaling during each of the three blocks of trials, as evidenced by a significant increase in dopamine signaling compared to baseline periods (Figure 3.6A, P<0.05 for all comparisons). However, as the delay to the large reward

increased, there was a significant increase in the time to peak dopamine release following reward delivery (Figure 3.6B, $F_{(2,14)}$=47.58, P<0.001). This suggests that there may be a decoupling of the cue-outcome association as reward delays increased, such that animals were not able to attend to or predict the reward deliveries as well during the longer delays and were thus 'surprised' by reward delivery on long delay trials.

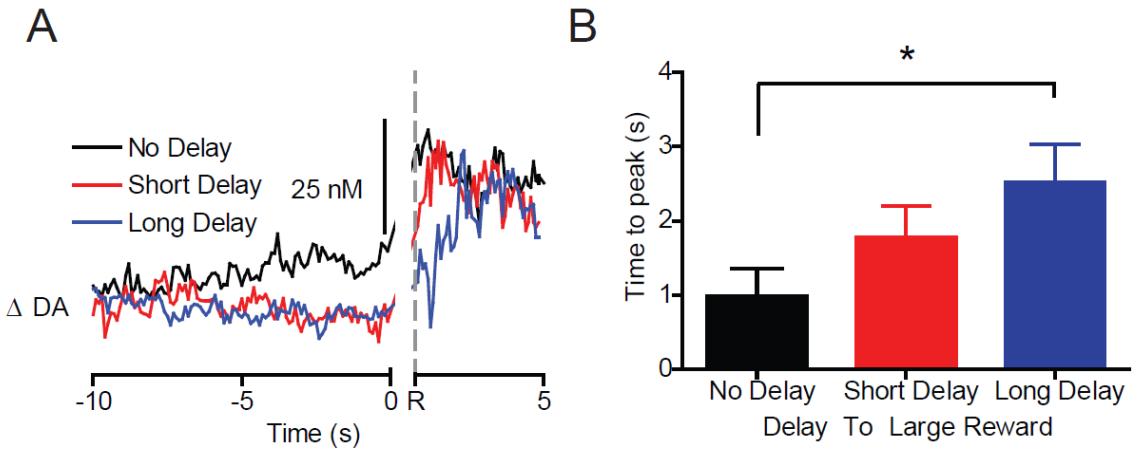

Figure 3.6 Dopamine release to delayed reward delivery. (A) Dopamine release time locked to large reward delivery (R) during the three blocks of trials. Rewards evoked increases in dopamine release compared to baseline levels (-10 prior to cue onset). (B) Latency to peak dopamine release following reward delivery. There was a significant increase in the time to peak dopamine release for the large reward delivery across the three blocks of trials. Data are mean ± SEM. *P<0.05.

Histology

There were a total of 8 recording locations from 7 animals. All electrode tips terminated in the NAc core. Figure 3.7 shows verification of recording locations.

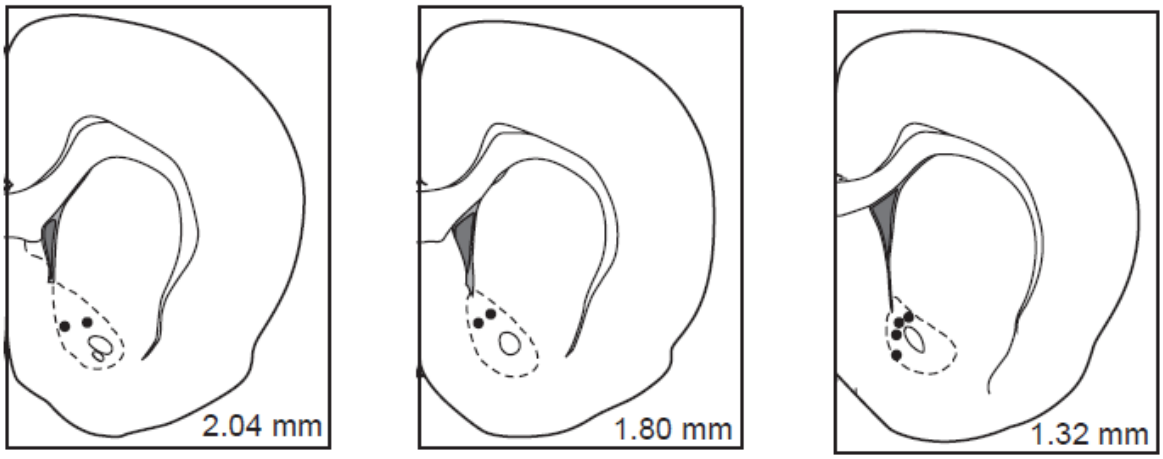

Figure 3.7 Anatomical distribution of electrode recording sites in the nucleus accumbens. Coronal sections show electrode tip locations for 8 recording locations (black dots). Numbers indicate anterior posterior coordinates rostral to bregma.

DISCUSSION

The present data further support and extend existing knowledge on the role of phasic dopamine signaling in encoding reward values and mediating appropriate decision making. Dopamine neural activity has previously been shown to encode several features of upcoming reward such that dopamine activity is increased for cues that predict rewards that are more immediate, more probable, higher subjective value, and lower effort (Fiorillo et al., 2003; Tobler et al., 2005; Kobayashi and Schultz, 2008; Schultz, 2010). One prevailing theory of this signaling is that phasic dopamine activity functions to perform cost-benefit analyses by signaling the overall utility of behavioral options (Phillips et al., 2007). Higher utility options, such as larger more immediate rewards, evoke higher dopamine release and thus functions to bias animals towards the most valuable option available. Recent evidence has shown that this value signal is transmitted to the NAc core, as phasic dopamine release tracks reward value based on effort, delay, and risk (Day et al., 2010; Sugam et al., 2012). Further, multiple studies have implicated dopamine signaling in value-based decision making involving shifts in behavioral responding (Koffarnus et al., 2011; Stopper et al., 2013). However, to date no studies have investigated whether this signaling encodes changes in reward value in real time, and if these changes are associated with shifts in decision making.

In the present study, dopamine release was recorded in the NAc core while animals performed a delay discounting task. Importantly, this task allowed for the assessment of whether cues predictive of rewards of different value based on combinations of reward size and delay affected patterns of dopamine release. Further, as this task forced animals to shift behavioral responding as the reward contingencies changed, we were able to evaluate how dopamine signaling shifts in relationship to behavioral changes. We found that the

presentation of reward predictive cues resulted in phasic increases in dopamine release during each block of the task. Importantly, this dopamine signaling tracked the relative value of response options. Early during the session, dopamine signaling was greater for the more valuable large reward presented immediately compared to cues predicting smaller immediate rewards. As this large reward was devalued by increasing delays, there was a relative shift in dopamine signaling that was commensurate with a relative shift in reward seeking behavior, such that during the final block of the session, small immediate cues evoked higher dopamine release than large delayed cues, and animals strongly preferred the small immediate option.

The data presented here suggest that phasic dopamine release directly follows the cost-benefit analysis hypothesis of dopamine signaling such that this signal functions to determine the overall utility of available behavioral options to bias behaviors towards maximizing resources. Importantly, in order to be advantageous, information about reward utility must be prospective, or available to the organism prior to behavioral choices being made. In the present task, the cues presented to the animal instructed which response would be rewarded, and how valuable this response option was. We found that cues that predicted large immediate rewards, (i.e. rewards with high economic utility and thus are advantageous choices for animals), resulted in the highest increases in phasic dopamine release. Further, the absolute identity of each reward predictive cue remained constant throughout the session (one cue always predicted the large reward and one predicted the small reward), however the relative value associated with each cue-outcome association shifted. Concurrently we found a relative shift in dopamine signaling that was correlated with this shift in behavioral responding, suggesting that this cue-evoked utility signal is related to appropriate behavioral responses.

The shifts in behavioral responding observed here could result from one of two options. First, animals could be shifting behavior as a result of an increase in value of the small immediate reward because the large delayed reward was decreasing in value. Alternatively, animals could be shifting behavioral responses simply because the value of the large reward decreases over time while there is no change in the value associated with the small immediate reward. Importantly, behaviorally, both of these options are identical and appear as a relative shift in reward value such that the small immediate reward is more valuable as the delay to large reward increases, and therefore response allocation shifts towards the small immediate option. Importantly, previous research on value encoding of the mesolimbic dopamine system presented situations in which one option was always more valuable the other, and therefore how dopamine signals tracks differences in the relative value of future options previously could not be disambiguated (Fiorillo et al., 2003; Tobler et al., 2005; Kobayashi and Schultz, 2008; Day et al., 2010; Sugam et al., 2012).The data presented here supports the second option. Specifically, we found that increasing reward delays decreased phasic dopamine release to cues that predicted the large reward, while there were no changes in dopamine release for cues predicting the small reward. This suggests that the shift in behavioral responding was the result of the decrease in value associated with the large reward, rather than an increase in value of the small reward. Further, we found that there was a significant effect of reward block on dopamine signaling, such that the total amount of cue evoked dopamine release decreased throughout the session. This was a direct result of the decrease in dopamine signaling for the large delay reward and no change in signaling for the small immediate reward. This data suggests that while the relative value of response options shifted throughout the behavioral session, dopamine signaling also tracked

the overall net benefit of both response options. The overall decrease in dopamine signaling across the blocks suggests that although the animals preferred the small immediate response option during the last block of the session, the overall value of reward options at the end of the session was significantly worse than the overall value of reward options during the first block of the session.

Choice trials served two functions in the delay discounting task. First, these trials functioned to determine each animal's individual behavioral preference, such that animals showed a significant shift away from the large delayed option during choice trials as the delays increased. Second, dopamine signaling during choice trials was recorded to evaluate how dopamine release encoded information about the value of two concurrent choices. We found that on these choice trials, cue-evoked dopamine release was highly similar to dopamine signaling of the preferred response option. Specifically, choice cue-evoked dopamine release was highest during the first block of the task, and was not significantly different from signaling of the large immediate option. Further, as the delays increased there was a correlated decrease in dopamine signaling such by the end of the session choice cue-evoked dopamine was not significantly different from the preferred small immediate option. This suggests that although the actual behavioral choice had not yet be made, dopamine release was signaling the better of the two options available or reflected the intention of the animal to choose the more valuable option. Previous reports on dopamine signaling during value-based decision making support the current findings, showing that dopamine release functions to encode the most valuable option available which functions to bias responses to maximize resources (Roesch et al., 2007; Day et al., 2010; Sugam et al., 2012).

The ability to evaluate the value associated with different response options is clearly adaptive, as organisms must choose the most valuable options available to maximize resources. However, when behavioral choices are extremely biased in one direction or another this type of behavior may be detrimental to an organism and lead to maladaptive choices. Impulsivity is one of these behavioral characteristics that can be beneficial in certain situations, however when taken to an extreme level, this behavior can be detrimental. Impulsivity is defined as an action without foresight, however in behavioral economics impulsivity is observed as an inability to withhold responding for a certain outcome or the inability to wait for delayed gratification (Winstanley et al., 2006). Delay discounting, such as the model used here, is one of the most widespread animal models of impulsive choice (Winstanley et al., 2006), as it is able to evaluate whether or not animals are able to wait for the delayed gratification of a larger reward. In this model, more impulsive animals shift responding to the small immediate option early on, and are thus unable to wait for the large reward. Conversely, less impulsive animals are able to wait for the larger rewards and thus it takes longer delays to shift behavioral responding. By using this behavioral model, we were able for the first time to evaluate if there was a link between the dopamine signaling and impulsive choice behaviors. We found that there was a direct correlation between the differential dopamine release and choice behaviors, suggesting there may be a direct link between phasic dopamine release and impulse control. Specifically, more impulsive individuals may display differential value encoding in the mesolimbic dopamine system that may function to bias impulsive versus nonimpuslive responses.

The current data provide a system to evaluate the critical role of phasic dopamine signaling in normal delay discounting behaviors. However, this type of behavior has been

repeatedly linked to maladaptive behaviors such as drug addiction. In fact there is a direct correlation with cocaine addiction in humans and increased impulsive choices (Coffey et al., 2003). Animal models of impulsivity have further suggested that impulsive choice behavior predicts the propensity to take drugs (Dalley et al., 2007; Belin et al., 2008). Conversely, previous experience with drugs of abuse also increase subsequent impulsive choice behaviors (Simon et al., 2007; Setlow et al., 2009; Mendez et al., 2010), suggesting a complex relationship between impulsivity and drug addiction. Recent evidence has suggested that normal NAc and mesolimbic dopamine function are critical for normal impulse control, and as such aberrations in this system may be related to the dysfunctional impulse control behaviors observed in drug addiction. Cardinal and colleagues provided one of the first studies of the critical role of the NAc in impulsive choice, as lesion of the NAc core resulted in increased impulsive choices in a delay discounting model while lesion of the anterior cingulate cortex and medial prefrontal cortex had no effects (Cardinal et al., 2001). Interestingly, modulation of dopamine signaling also impacts impulsive choices, as pharmacological blockade of dopamine receptors results in increased impulsive choices (Floresco et al., 2007), and this appears to be modulated through a D1 receptor mechanism (Koffarnus et al., 2011). As phasic dopamine functions to predominantly activate D1 receptors (Richfield et al., 1989), it is possible that appropriate phasic dopamine signaling within the NAc core is critical for normal impulse control. The data presented here support this hypothesis, as the percent choice of the large delayed lever was directly correlated with dopamine release patterns. Lower percentages of response on the larger delayed lever, indicative of increased impulsive choice, was associated with a specific pattern in dopamine release (higher dopamine signaling for the small immediate compared to large delayed

reward). Therefore, imbalances in phasic dopamine signaling may be related to maladaptive impulse control and associated disorders, such as drug addiction. In support, rats with a propensity for increased impulsive actions and increased subsequent drug taking showed a significant reduction in dopamine D2/D3 receptor availability in the NAc (Dalley et al., 2007). As such, animals with increased impulsivity have an imbalance in dopamine receptor function, as a reduction in D2/D3 receptors would result in a relative increase in D1 receptor activity. This suggests a critical role for phasic dopamine release in normal impulse control while imbalances in this system may result in disease states such as increased impulsivity and drug taking.

CHAPTER 4

OPTOGENETIC STIMULATION OF PHASIC DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS AND VALUE-BASED DECISION MAKING

ABSTRACT

Dopamine transmission within the nucleus accumbens (NAc) has been critically implicated in reward related behaviors and in encoding the value of options available during choices. As such, it has been hypothesized that the NAc biases responses towards the most valuable option available. However, exactly how the value encoding of phasic dopamine release mediates goal-directed behaviors is currently unknown. Here we used new optogenetic technology to precisely control dopamine release in the NAc to evaluate the role of phasic dopamine in general goal-directed responding and value-based decision making. In order to evaluate if dopamine release in the NAc was sufficient to promote goal-directed behaviors we trained rats to make an operant response (nosepoke) for optical stimulation of dopamine terminals expressing channelrhodopsin (ChR2) in the NAc, which resulted in phasic patterns of dopamine release. We found that rats learned to nosepoke for laser stimulation, and this behavior was tightly coupled to laser onset as rats decreased responding when the laser was turned off and quickly reinstated behavior when the laser stimulation was reinitiated. Further, rats attended to the intensity of laser stimulation as they decreased responding when laser intensity was decreased. In order to evaluate how cue-evoked dopamine release functions to mediate decision making behaviors, rats were trained to associate discrete cues with the opportunity to respond for smaller immediate and larger

delayed rewards. Animals were able to successfully discriminate between the cues and adjust their behaviors accordingly, showing decreased responding on the large delayed lever as reward delays increased or reward size decreased. Animals then underwent test sessions in which we paired optical stimulation of dopamine terminals with cues that predicted lower value options based on reward delay and magnitude. Interestingly, optical stimulation of dopamine release increased responding for a lower value delayed reinforcer during choice trials, but did not affect decisions based on reward magnitude. This suggests that phasic dopamine release is critical for appropriate decision making; however, there is a dissociation of value encoding in the NAc between reward delay and magnitude.

INTRODUCTION

Decades of research have suggested that the mesolimbic dopamine system, particularly the projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) are critical for mediating goal-directed behaviors and appropriate decision making (Baldo et al., 2002; Kelley, 2004; Roitman et al., 2004; Phillips et al., 2007; Schultz, 2010; Witten et al., 2011). A critical component in appropriate behavioral choices is the ability to associate cues with positive outcomes and assign appropriate value to those cues, in order to adjust behaviors according to the value of options available (Green and Myerson, 2004; Cardinal, 2006; Phillips et al., 2007; Rangel et al., 2008). Previous studies have shown that dopamine signaling is necessary for learning cue-outcome associations (Zellner et al., 2009; Zellner and Ranaldi, 2010), and is implicated in goal-directed behaviors and complex decision making (Phillips et al., 2007; Rangel et al., 2008; Clark et al., 2012).

A working hypothesis for the specific role of dopamine signaling in decision making is that phasic dopamine release within the NAc functions to set a “cost-threshold” for which behaviors are evaluated. More valuable options evoke higher dopamine release, such that dopamine signaling predicts that this value falls below the cost threshold and is thus deemed worthwhile. Conversely, lower value options evoke much less dopamine, and thus this signaling predicts that the value falls above the cost threshold and is thus deemed not worthwhile (Phillips et al., 2007). In support, dopamine neural firing and dopamine release in the NAc encode the value of cues associated with future reward based on reward probability, magnitude, delay, cost, and expected value (Schultz et al., 1997; Fiorillo et al., 2003; Day et al., 2007; Tobler et al., 2007; Day et al., 2010; Gan et al., 2010; Sugam et al., 2012) which is hypothesized to bias animals towards the best option available. Further, perturbations of the

mesolimbic dopamine circuitry result in maladaptive decision making based on reward costs, delay, and risk (Cardinal et al., 2001; Cardinal and Howes, 2005; St Onge and Floresco, 2008; Ghods-Sharifi and Floresco, 2010; St. Onge et al., 2010; Stopper and Floresco, 2011).

Lesion or inactivation studies are only able to determine if a certain structure is necessary for a given behavior, however researchers cannot determine the temporal relationship between signaling and behavior. The electrophysiological and electrochemical data suggest that there is a correlation between phasic dopamine signaling and reward value encoding, however these studies are unable to determine the causal relationship between the two. Several studies have suggested a causal relationship between mesolimbic dopamine neural activation in promoting goal-directed actions as electrical stimulation of the VTA is sufficient to promote behavioral responding (German and Bowden, 1974; Fibiger et al., 1987; Cheer et al., 2007; Beyene et al., 2010; Wheeler et al., 2011). However, the VTA is a heterogeneous structure, and as such this goal-directed behavior may not be dependent on stimulation of the dopamine neurons (Margolis et al., 2006). Further, it is not clear from electrochemistry or electrophysiology studies if changes in dopamine signaling are causally linked with decision making behavior.

Recent research using optogenetic techniques has allowed for dissection of this circuitry, targeting of specific neural types including dopamine neurons and an examination of its causal role in behavior. Using this approach, research has shown that stimulation of dopaminergic cell bodies is sufficient to promote goal-directed behaviors (Tsai et al., 2009; Witten et al., 2011). Further, optical stimulation of the NAc itself is rewarding as animals show a conditioned place preference for the optically stimulated side (Airan et al., 2009). These studies have provided evidence that phasic stimulation of the mesolimbic dopamine

system is sufficient for goal-directed behaviors and cue-outcome associations, however, mesolimbic dopamine neurons project to a wide variety of structures, including the NAc (Fields et al., 2007), and as such it is not clear that these behaviors are dependent on signaling in the NAc. Here, we paired this optogenetic technique to temporally and spatially control dopamine release with several tests of goal-directed behaviors to evaluate the causal link between phasic dopamine release and motivated actions. First, we trained animals to perform an operant response (nosepoke) for optical stimulation of dopamine terminals in the NAc to evaluate if phasic dopamine release in the NAc itself was sufficient to promote goal-directed actions. In order to evaluate the role of value prediction signaling of the dopamine system, we trained animals to associate reward predictive cues with the opportunity to respond for smaller immediate and larger delayed rewards. During test sessions we stimulated dopamine release in the NAc during a low value cue presentation which is hypothesized to signal that the associated reward is very high in value. We evaluated if rats were attending to this dopamine signaling by evaluating behavior during choice trials in which rats could choose between the two options. If phasic dopamine signaling is sufficient to mediate appropriate decision making, then we should be able to shift behavioral choices towards lower value options by stimulating dopamine release during cues that predict these lower value options, and for the first time display a direct causal link between phasic dopamine signaling and value-based decision making.

METHODS

Animals

Male Long-Evans rats (in house bred) approximately 90 to 120 days old weighing 300 to 350 grams were used as subjects and were individually housed with a 12/12-h light/dark cycle. Two groups of rats were used: in TH::Cre$^{(+/-)}$, all tyrosine hydroxylase (TH)-expressing (i.e. catecholaminergic) neurons co-expressed a Cre-linked marker while TH::Cre$^{(-/-)}$ (controls) do not co-express a Cre-linked marker in TH-expressing neurons (Witten et al., 2011). All experiments were conducted between 8:00am and 5:00pm. Animals were maintained at no less than 85% of pre-experimental bodyweights by food restriction (~10-15g of Purina laboratory chow each day in addition to approximately 1g of sucrose consumed during behavioral sessions) except during the post-operative recovery period when food was given *ad libitum*. All procedures were approved by the UNC Institutional Animal Care and Use Committee.

Surgery

Prior to the start of behavioral training, rats underwent surgery for infusion of channelrhodopsin (ChR2) into the VTA and implantation of optical fibers into the NAc. Surgery was conducted under anesthesia with ketamine (100 mg/kg) and xylazine (20 mg/kg). Rats were infused with a Cre-dependent adeno-associated viral construct encoding channelrhodopsin (ChR2) with EYFP (AAV-DIO-ChR2-EYFP), into the VTA (4 injections: AP -5.4 mm, ML ±0.7 mm, DV -8.4 mm; and AP -6.2 mm, ML ±0.7 mm, DV -7.4 mm from skull at Bregma, 1 µl per site infused ~0.5 µl/min with a 2µl Hamilton syringe). The syringe was held in place for an additional 15 min before removal. To stimulate fibers arising from these cells, optical fibers (200 µm diameter core) coupled to ferrules (2.25 mm diameter,

250µm bore) (Sparta et al., 2012) were chronically implanted over the NAc bilaterally at AP +1.8 mm, ML ±2.5 mm and DV -6.9 mm from skull at bregma, and angled in the ML plane away from the midline 10̊. Optical fibers were held in place with dental cement. The virus was given at least 8 weeks to be taken up and expressed in the terminals in the NAc before behavioral stimulation experiments were conducted (Witten et al., 2011). Animals were allowed at least 5 days of recovery before training on the behavioral task was initiated. For voltammetry experiments, animals were injected with virus but no optical fibers were implanted and animals were given at least 8 weeks for virus expression in the terminals before anesthetized voltammetry recording sessions.

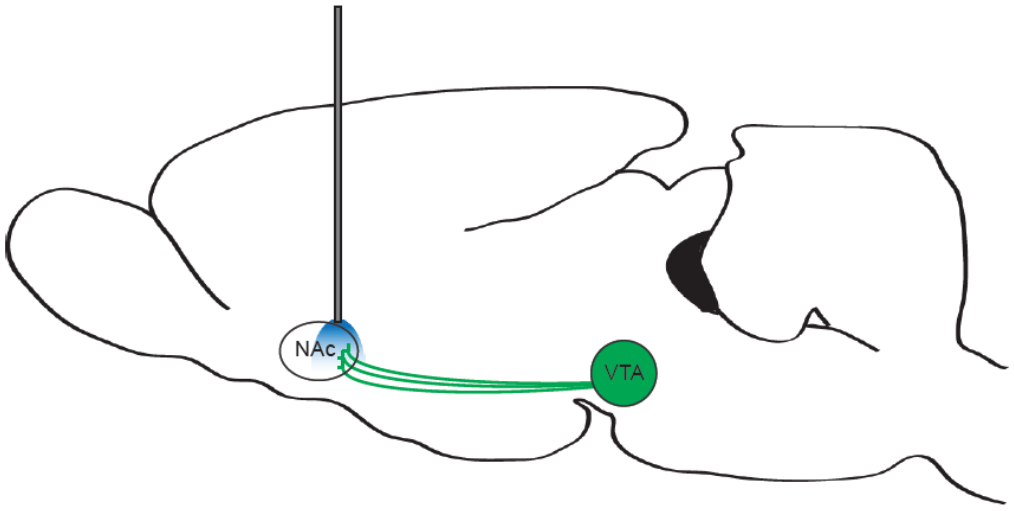

Figure 4.1 Schematic representation of optogenetic technique. The VTA was bilaterally injected with a Cre-dependent ChR2 virus, and bilateral optical fibers were aimed at the NAc. Stimulation sessions were conducted at least 8 weeks following surgery to allow for expression of ChR2 in the terminal region of the NAc.

Fast-scan cyclic voltammetry

Prior to any behavioral manipulations using optogenetic techniques it was necessary to verify that injections of ChR2 into transgenic animals resulted in expression of the opsin

protein and stimulation of the terminal region in the NAc resulted in dopamine release. To do this, we performed fast-scan cyclic voltammetry in anesthetized TH::Cre$^{(+/-)}$ animals that expressed ChR2 to verify that both cell body and terminal stimulation of dopamine neurons was sufficient to evoke dopamine release. Electrochemical procedures were similar to to those reported in Chapter 3 (see pages 69-70 for details). Briefly, animals were anesthetized with urethane (ip) (Sigma-Aldrich, St. Louis, MO) and prepared for electrochemical recordings. To verify that cell body stimulation in the VTA was sufficient to promote dopamine release we coupled an optical fiber to the electrical stimulation probe and lowered them into the VTA. A carbon fiber electrode was lowered into the NAc. We electrically stimulated (60Hz 24 biphasic pulses, 120µA, 2 ms per phase) the VTA first to verify that the recording electrode was in a location that supported dopamine release. We then optically stimulated (20 mW, 20Hz, 40 pulses) cell bodies while recording dopamine release in the NAc. In another animal, we coupled a carbon fiber electrode to an optical fiber and lowered them into the NAc to verify that dopamine terminal stimulation was sufficient to promote release in vivo, as this had previously only been shown in vitro (Tsai et al., 2009; Witten et al., 2011). Dopamine release was recorded while we optically stimulated terminals at varying intensities (20mW, 20Hz, 5-100 pulses) to verify that there was an intensity dependant relationship between optical stimulation and dopamine release.

Signal identification and separation

Dopamine was identified and separated from electrochemical data using methods identical to those described in chapter three (see chapter three, pages 70-71 for details).

Behavioral Apparatus

Behavioral sessions were conducted in 25 × 25 × 30 cm chambers (MED Associates) which were comprised of two clear Plexiglas walls in the front and rear, and two stainless-steel walls on the left and right side of the chamber. The floor grid of the chamber was comprised of evenly-spaced stainless-steel bars (0.5 cm diameter, 1.5 cm apart). On the left wall a centrally-located houselight was positioned 1 cm below the Plexiglas ceiling. On the right wall, 5 cm below the ceiling, two cue lights were spaced 14 cm apart. Directly under the cue lights were two retractable response levers. An illuminated nosepoke hole (2.5 cm diameter) was located 1 cm above the floor grid in the middle of the left wall, and a recessed foodcup was located on the opposite wall positioned equidistant between response levers. During optical stimulation sessions, rats were connected to patch cables with optical fiber (200 µm core, 0.22 NA, Doric Lenses), encased in a durable plastic covering. These cables terminated with a ferrule connector (Precision Fiber Products) that were secured to the rat's optical fiber implant with a fitted ceramic sleeve (Precision Fiber Products), and were attached at the other end to an optical commutator (Doric Lenses). This commutator allowed for bilateral stimulation of NAc terminals and provided unrestrained movement for the animal. The commutator was connected via a second optical patch cable to a 150 mW DPSS 473 nm laser (OEM Laser Systems). Optical stimulation was controlled by a computer running Med PC IV (Med Associates) software, which also recorded behavioral events.

Intracranial self stimulation of dopamine terminals in the nucleus accumbens

In order to determine if optical stimulation of dopamine terminals in the NAc was reinforcing, animals were trained to lever pres for optical stimulation. Two groups of animals (TH::Cre$^{(+/-)}$, n=10 , and littermate controls, n=9) were infused with ChR2 into the VTA and allowed at least 8 weeks for ChR2 expression in the terminal region of the NAc. During each

session, a houslight illuminated the chamber and a single white LED lamp recessed in the rear of the nosepoke receptacle indicated that entries would be rewarded. During the first 5 *acquisition* sessions, a single nosepoke resulted in a 5s bilateral optical stimulation (20mW 20Hz). During this 5s stimulation period, the light in the nosepoke hole extinguished signifying that any further nosepokes during this period would not be reinforced. Both rewarded nosepokes and total nosepokes were recorded. Animals were allowed to respond for stimulation over 30 minute behavioral sessions. Following the 5 acquisition sessions, the laser was switched off, and animals were able to respond during one 75 minute *extinction* session. During this session, all behavioral events were identical to acquisition, however nosepoke responses were not reinforced. Nosepokes were recorded every 15 minutes during the extinction session. Animals were considered extinguished following either two 15 minute periods of no responses or following 75 minutes. Directly following extinction, the laser was turned back on and one 5s "priming" stimulation was delivered. Animals were then allowed to nosepoke for optical stimulation during a 45 minute *reinstatement* session. Following reinstatement, the intensity of the laser was decreased to a 1s stimulation (20mW 20Hz) while all other parameters of the task remained identical. Rats were then allowed to nosepoke for this lower level 1s stimulation over a 30 minute behavioral session. This final behavioral session was included to evaluate if animals were able to differentiate lower versus higher levels of dopamine release in the NAc.

Decision Making Task

Following recovery from surgery animals (n=23 Delay testing: TH::Cre$^{(+/-)}$ n=11, control n=12; n=16 Magnitude testing: TH::Cre$^{(+/-)}$ n=7, control n=9;) began training on an operant decision making task to evaluate the causal relationship between value related

dopamine signaling and decision making. Importantly, task training lasted at least 8 weeks, such that stimulation sessions occurred after ChR2 was expressed in the terminal region of the NAc. Animals first underwent magazine training in which 30 sucrose pellets were delivered to the centrally located foodcup in one 45 minute training session. Following magazine training, animals were trained to press levers in which every response on either lever resulted in the delivery of a 45mg sucrose pellet to a centrally located food receptacle. Rats began training on one lever (counterbalanced across animals) until 50 responses were made. Following acquisition, animals were trained to press the other lever until 50 presses were made. Once rats learned to lever press, they were trained in the discrete trial task to learn to associate each cue light with a specific response. During this training session there were two forced trial types. During forced left trials, the left cue light was illuminated for 5s prior to both levers extending, to signal that the left lever was active. Levers were available for 10s unless response requirements were completed in which case the levers retracted and the reward was delivered. During left forced trials the animal was required to press the left lever to obtain reinforcement. Conversely, during right forced trials the right cue light illuminated and the animal was required to press the right lever to obtain the reward. Presses on the incorrect lever were counted as errors. There were 35 trials of each type within each session. Animals were trained on this task for at least 15 sessions and until reaching behavioral criterion (85% accuracy). Following discrete trial training animals begin training on the decision making task (Figure 4.2). In this task one lever was designated the small immediate lever and one lever was designated the large delay lever. These lever assignments remained constant for each animal across training days but were counterbalanced across animals. Within a given session there were 3 trial types, immediate, delay, and choice.

During immediate trials (20 trials) the cue light was illuminated for 5s followed by lever extension for 10s. Presses on the correct lever resulted in a single sucrose pellet delivered immediately. Presses on the non signaled lever were counted as errors. During delay trials (20 trials), the other cue light was illuminated for 5s followed by lever extension. If the correct lever was chosen rats were rewarded with the reward after a specified delay. Again presses on the non signaled lever were counted as errors and went unrewarded. Importantly, the magnitude and delay of the larger reinforcer remained constant throughout the entire training session, but varied across sessions. The magnitude of reinforcement ranged from 1, 2, or 3 pellets while the delay to reinforcer varied from 0, 10, 20, 40, or 60 s. Finally, during choice trials (30 trials), both cue lights illuminated for 5s followed by lever extension and responses were rewarded based on the contingency of the lever chosen. Choice trials provided a behavioral readout of response preference for each rat on each day. During the first 20 trials of each session, only immediate and delay trials were presented (10 of each type) to ensure that the animals learned the reward contingency for each session prior to any choice trials. This task was set up to be a delayed discounting task across training sessions, and allowed us to specifically manipulate decisions based on reward magnitude and delay across different training sessions. Further, this task required that animals' update information about reward value on a daily basis, and as such, were always "learning" the new task rules. Animals were trained such that they experienced each delay and magnitude pairing and choice behavior showed that they were attending to the value of the large delayed reinforcer. Following this training, animals underwent the stimulation tests. Each stimulation test occurred during separate training sessions. To test how dopamine signaling is causally linked with reward value, terminal dopamine release was optically stimulated during cues that

predicted lower value options. During delay based decision testing, the delay to reinforcement was set at 10 seconds and the value of the large reinforce was 1 pellet, such that animals were choosing between one pellet immediately and one pellet after a 10 second delay. Rats were allowed to follow the same training as before, however during forced cues that predicted the delay option, optical stimulation of dopamine terminals was administered during the 5s cue period. Animals were then allowed to make behavioral choices without stimulation. Magnitude based decision testing was conducted similarly to delay based testing, however instead of receiving one pellet after a 10 second delay, animals received two pellets delivered immediately for pressing the large delay lever. As such animals were evaluating the choice between two pellets immediately or one pellet immediately. Optical stimulation therefore occurred during forced cues that predicted the one pellet option. Importantly, stimulation only occurred during forced cue presentations and not during choice cue presentations, behavioral responses, or reward delivery. Therefore, any alterations in behavior resulted from changes in the predicted reward values as a result of the stimulation. The number of lever presses on choice trials was compared between sessions in which stimulations occurred and sessions with the same reward contingencies and no laser stimulation to evaluate if stimulation during cue presentation increased the likelihood of choosing the associated lever during free choice trials.

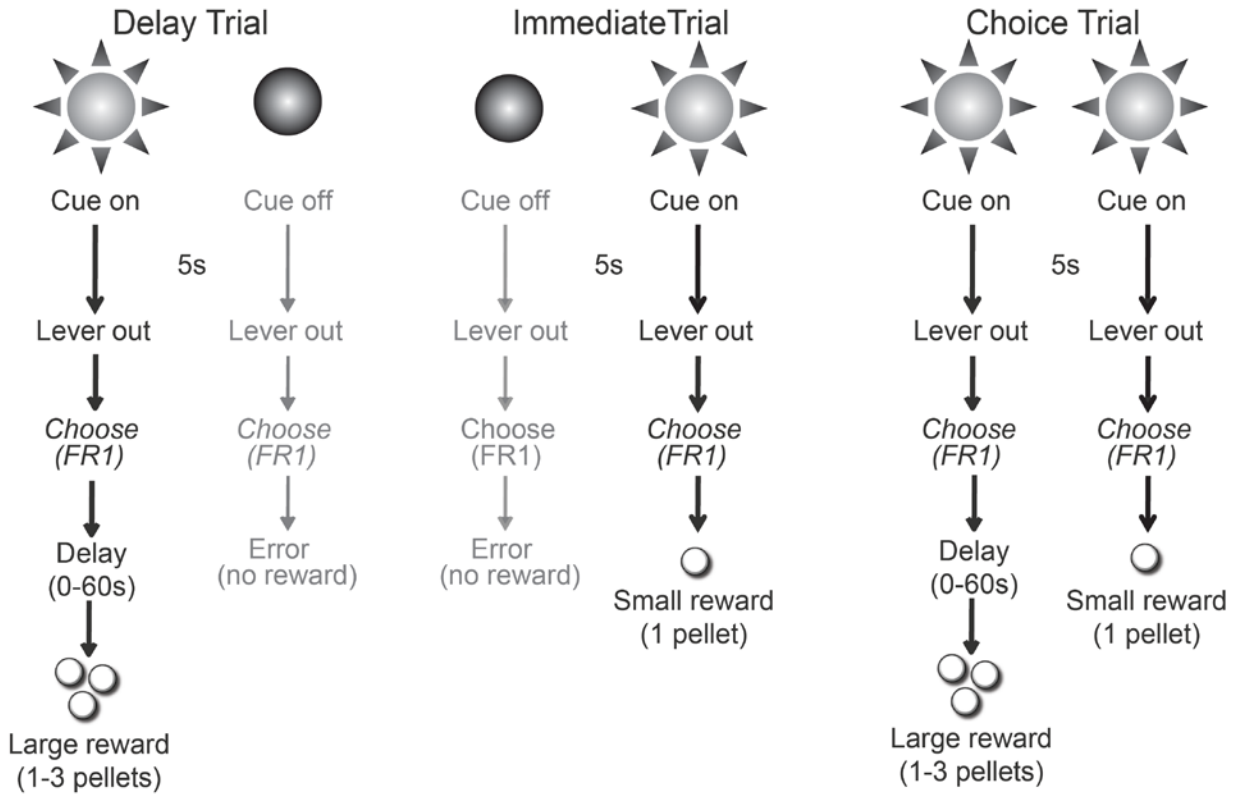

Figure 4.2 Schematic representation of decision making task. On delay trials (left panel), a cue light was presented for 5s and was followed by the extension of two response levers. A single lever press on the lever positioned below the illuminated cue light led to a 1-3 sucrose pellet reward following a delay (0-60s). Responding on the other lever did not produce reward delivery and terminated the trial. On immediate trials (middle panel) the other cue light was presented for 5s before lever extension. Here presses on the associated lever resulted in 1 sucrose pellet delivered immediately while presses on the other lever did not produce reward and terminated the trial. On choice trials (right panel) both cue lights were presented for 5s and animals could select either option. Importantly the delay and magnitude of reinforcement for the delay lever was held constant during each behavioral session and was changed between behavioral sessions

Histology

Following completion of experimental procedures rats were deeply anesthetized with a ketamine/xylazine mixture (100 mg/kg and 10 mg/kg, respectively) and perfused transcardially with physiological saline and a 4% paraformaldehyde solution. Brains were then removed, post-fixed, and frozen. Brains were sectioned coronally at 30 µm with half of the sections mounted on slides to verify optical fiber placement with a light microscope and

the other half placed in 0.1M PB for immunohistochemistry to verify ChR2 expression. Free floating sections were washed in Triton-X (0.5% solution in Phosphate Buffered Saline, (PBST)) and 0.1M PBS. Sections then were incubated with 10% normal donkey serum (Jackson Immunoresearch Laboratories) in 0.1% PBST for 60 minutes. Sections were then incubated for 72 hours at 4°C in TH polyclonal antibody raised in sheep (1:500, Abcam). Sections were then washed and incubated for 2 hours at room temperature in donkey anti-sheep secondary antibodies conjugated to Alexa Fluor 647 (1:800, Jackson Immunoresearch Laboratories). Sections were then washed and incubated for 60 minutes in 2% NeuroTrace (435/455 nm, Invitrogen LifeTech). Next, sections were washed and mounted onto microscope slides in phosphate-buffered water and coverslipped with Vectashield mounting medium (Vector Laboratories). Sections were visualized on a confocal microscope to quantify virus expression.

Data Analysis

In order to evaluate if optical stimulation of dopamine terminals in the NAc was sufficient to promote goal-directed behaviors, we tested if rats would nosepoke for optical stimulation. We evaluated behavioral responding across days using a 2 way repeated measures ANOVA to determine if there was a significant increase in nosepoke behavior in the TH::Cre$^{(+/-)}$ animals compared to controls as they learned the contingency of the task. Further, we then made specific planned comparisons of responding on the final session of training to the extinction session, reinstatement session, and 1s stimulation using *t*-tests.

For the decision making task, behavioral analysis during training and test sessions included examination of overall response rates and allocation, number of errors committed, number of aborts committed, and preference between both levers. During training on the

decision making task, the response preferences were tracked for each magnitude and delay pairing to analyze the across day delay discounting behavior. Responses for larger versus smaller rewards and immediate versus delayed rewards were compared across days using a repeated measures ANOVA. Appropriate post hoc tests were completed to ensure that rats were appropriately attending to the reward properties. Further, we compared responding between the two groups to confirm that there were no differences in baseline responding during task performance. To evaluate if stimulation of dopamine terminals during lower value options was sufficient to shift behavioral responding we compared response allocation on choice trials during stimulation versus nonstimulation sessions and across both groups of rats. Further, the percentage of correct responses on forced trials was compared between stimulation versus nonstimulation sessions to confirm that laser stimulation did not alter the ability to perform the task. Finally, we correlated responses during nosepoke behaviors and changes in choice behavior during decision making with virus expression.

RESULTS

Temporal and spatial specificity of dopamine release in the NAc terminal region

In order to precisely modulate dopamine release in the NAc, we used a genetic line of rats expressing Cre recombinase in TH neurons (*TH::Cre*). Injection of a Cre-dependent virus in structures containing dopaminergic cell bodies (VTA) resulted in highly specific ChR2 expression in catecholamine neurons. Specifically, ChR2 was seen to be highly coexpressed with TH in cell bodies in the VTA (Figure 4.3A) and dopamine varicosities in the terminal region of the NAc (Figure 4.3B). Further, within the VTA and NAc, opsin expression was confined to TH+ cell bodies and processes, and no expression was observed in non dopaminergic cells. We also verified that 8 weeks was sufficient to allow for full spread of ChR2 to terminals in NAc.

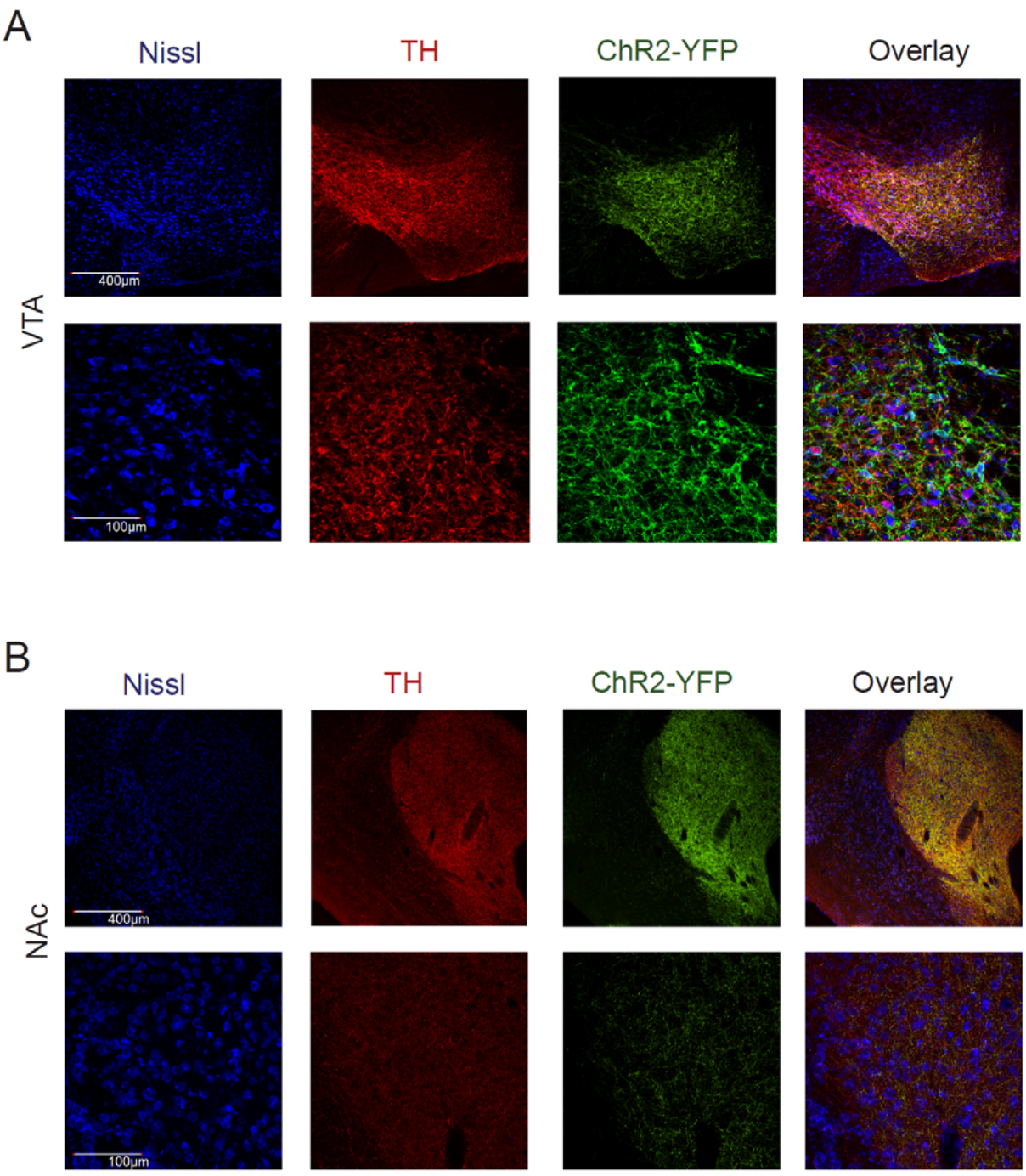

Figure 4.3 Channelrhodopsin expression in dopaminergic cells. (A) TH staining and ChR2-YFP expression in coronal slices display colocalization in cell bodies and projection neurons of the VTA. (Bottom) High-magnification view of ChR2-YFP expression and neurotrace staining in TH^{+} VTA cell bodies after injection of Cre-dependent virus in the VTA of a $TH::Cre^{(+/-)}$ (B) TH staining and ChR2-YFP expression in coronal slices display colocalization in dopamine varicosities in the terminal region of the NAc. (Bottom) High magnification view of ChR2-YFP expression and neurotrace staining in TH^{+} fibers after injection of Cre-dependent virus in the VTA of a $TH::Cre^{(+/-)}$. Importantly expression is not seen in the cell bodies in the terminal region of the NAc.

Prior to any behavioral manipulations, we also verified that optical stimulation of ChR2 expressing cells was sufficient to induce dopamine release in vivo, particularly optical stimulation of the terminal region. Previous research with this line of rats had shown that optical stimulation of terminals in the NAc was sufficient to produce dopamine release *in vitro*, however this had not been confirmed *in vivo* (Witten et al., 2011). Electrical stimulation of the medial forebrain bundle has been repeatedly used as a model of phasic dopamine release during reward related learning (Montague et al., 2004; Cheer et al., 2007; Owesson-White et al., 2008). As such, we sought to confirm that optical stimulation of dopamine neurons resulted in similar patterns of release as electrical stimulation and therefore support the role of using optical stimulation to mimic phasic dopamine release. To accomplish this we recorded dopamine release in the NAc of an anesthetized rat while we optically and electrically stimulated cell bodies in the same location in the VTA. We first confirmed that optical stimulation of dopamine cell bodies was sufficient to promote dopamine release in TH::Cre$^{(+/-)}$ rats expressing ChR2 (Figure 4.4A). Dopamine release was time-locked to laser onset, and peaked at laser offset. Further, dopamine release was stable across stimulations, such that the mean peak dopamine release for the 3 stimulation trains in Figure 4.4A was similar for each stimulation pulse (521nM ± 19nM). Next, the pattern of dopamine release in response to optical stimulation was compared to that of electrical stimulation, showing similar patterns of dopamine release dynamics and reuptake (Figure 4.4B). In order to be functionally relevant for our current task design, we also needed to characterize dopamine release resulting from terminal stimulation in the NAc *in vivo*. Similar to cell body stimulation, terminal stimulation resulted in a significant increase in dopamine release time-locked to stimulation onset, which peaked at stimulation offset (Figure 4.4C).

This is also evidenced by the fact that the time to peak increased for each of the stimulation parameters, as longer duration stimulation trains resulted in an increase in the latency to peak (Figure 4.4D, top). Further, we found that there was a linear relationship between stimulation intensity and dopamine release (r^2=0.8254, P<0.0001, Figure 4.4D bottom), with the highest levels of release associated with the greatest stimulation intensity. As seen in the dopamine traces in Figure 4.4D, dopamine increases at laser onset (time 0s), and peaks when the laser is turned off (0.05, .25, 1, 3, 5 seconds following stimulation onset), displaying precise spatial and temporal control of phasic dopamine signaling.

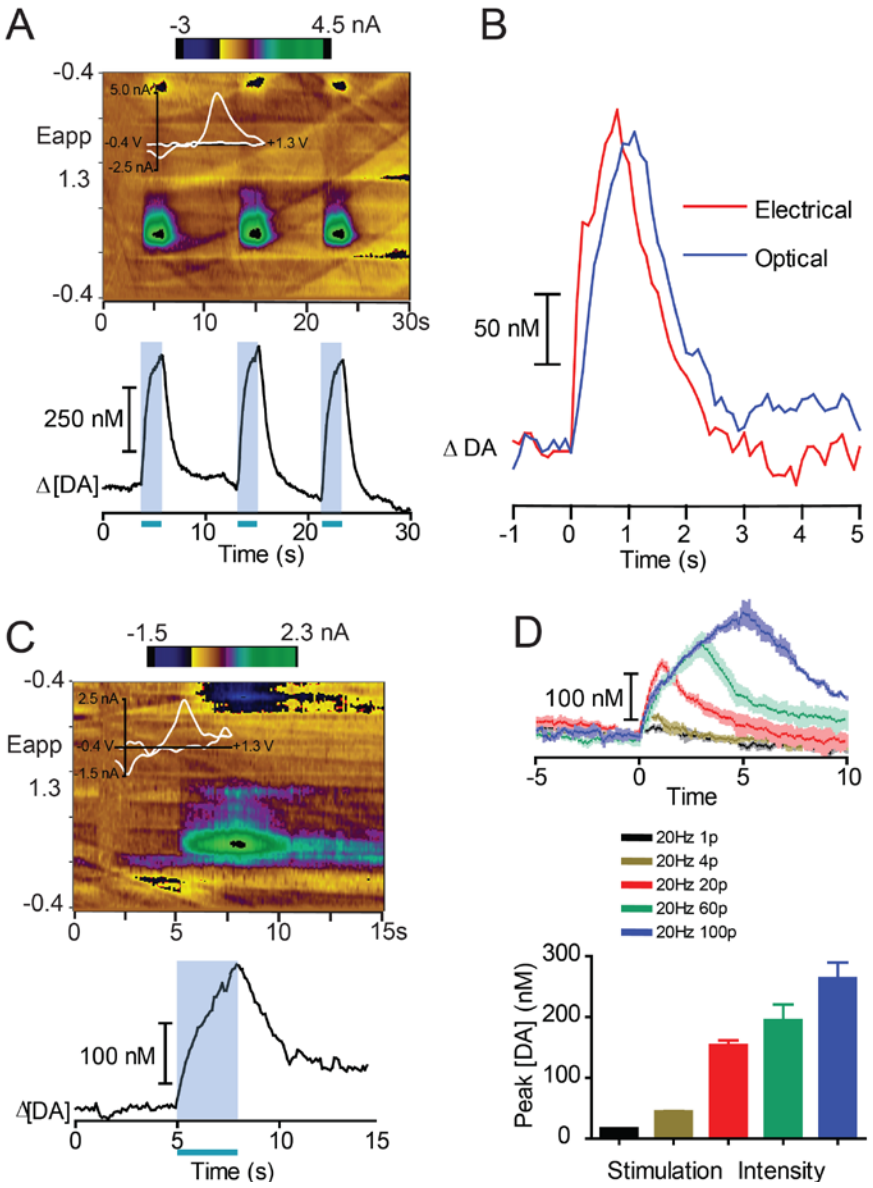

Figure 4.4 Optical stimulation of dopamine neurons promotes phasic dopamine release. (A) Optical stimulation of dopamine cell bodies in the VTA is sufficient to promote phasic dopamine release in the NAc. (Top) Three-dimensional representation of electrochemical data during 20Hz 40p 20mW optical stimulation and corresponding dopamine concentration trace (bottom). Cyclic voltammogram confirming that the signal measured is dopamine shown in inset (top). Optical stimulation denoted by blue bars underneath graph. Optical stimulation evoked significant increases in dopamine release time locked to laser onset and offset. (B) Optical stimulation (blue trace) of dopamine cell bodies evoked similar patterns of dopamine release as electrical stimulation (red trace). Stimulation was presented at time 0s. Release patterns and decay of dopamine were similar for optical and electrical stimulation. (C) Optical stimulation of dopamine terminals in the NAc was sufficient to promote phasic dopamine release, conventions follow from A. (D) Amount of phasic dopamine released was dependent on the stimulation intensity. Timecourse and pattern of dopamine release for different stimulation intensities (top) and peak dopamine release as a function of stimulation intensities (bottom). Error bars are SEM of peak dopamine release for several stimulations.

Optical stimulation of dopamine terminals in the nucleus accumbens is sufficient to promote goal-directed actions

Extensive research has suggested that activation of the mesolimbic dopamine pathway emanating from the VTA is reinforcing (Cheer et al., 2007; Beyene et al., 2010; Wheeler et al., 2011). As the majority of these neurons synapse in the NAc, it has been hypothesized that dopamine release within the NAc functions to mediate this type of behavioral responding. However, until recently, it was not possible to specifically stimulate this portion of the pathway to determine if this dopamine release is in itself sufficient to promote goal-directed responding. To evaluate this, TH::Cre$^{(+/-)}$ and littermate controls expressing ChR2 in the NAc were given the opportunity to nosepoke for a 5s train of light pulses in the NAc (20Hz, 100p 5msec pulse duration) delivered on an FR1 schedule. Figure 4.5A shows cumulative nosepoke responding across the five initial training sessions for a representative TH::Cre$^{(+/-)}$ animal. First, we found that animals rapidly learned to respond in the nosepoke hole for optical stimulation, and showed consistent behavior across the training session. Further, the latency to the first nosepoke decreased across training sessions, as evidenced by a delay to the first nosepoke during session 1 and no delay during session 5 (Figure 4.5A). Across all animals there was a significant reduction in the latency to first nosepoke for TH::Cre$^{(+/-)}$ group for session 1 to session 5 ($t(9)=2.452$, $P=0.037$). Further, the cumulative activity plot shows that across all 5 sessions, nosepoke behavior occurred at a constant steady rate for the entire half hour following the first nosepoke. Further, the slope of the cumulative activity plot is steeper for session 5 compared to session 1, suggesting that as this animal learned the task the rate of nosepoking increased, allowing for greater total reinforced responses during the session. Following acquisition, we turned off the laser and allowed animals to undergo extinction and then reinstatement. Figure 4.5B shows the

cumulative activity for a representative rat for the fifth training session, extinction, and reinstatement. We found that responding rapidly extinguished when the laser was turned off, and was reinstated when the laser was turned on. Interestingly, the slope of the cumulative activity plot for this representative animal was similar for reinstatement and the fifth training session, suggesting that the rat reinstated to the same rate of activity compared to the preextinction session. Figure 4.5C shows the data for all training and test sessions for the two groups of animals. We compared nosepoke behaviors between both groups (TH::Cre$^{(+/-)}$ and controls) during training, extinction, reinstatement, and 1s pulse sessions using a 2 way repeated measures ANOVA. We found a main effect of group ($F_{(1,17)}$= 8.264, P=0.011), a main effect of Session ($F_{(11,17)}$=7.935, P<0.0001) and a significant interaction ($F_{(11,17)}$=5.258, P<0.0001), suggesting that TH::Cre$^{(+/-)}$ and controls performed different levels of nosepoke behaviors across training sessions. We further probed these differences by looking at specific timepoints of interest. First, we found there was a significant increase in nosepoke behavior from session 1 to session 5 for the TH::Cre$^{(+/-)}$ subjects (t(9)=3.555, P=0.0062), suggesting animals were learning the contingency of the response-outcome association. There was also a significant decrease in the number of nosepokes for the control animals when comparing session 1 to session 5 (t(8)=3.606, P=0.0069), suggesting that behavior early on was exploratory and as rats learned that the nosepoke response had no consequence, response rates decreased to low levels. Further, TH::Cre$^{(+/-)}$ animals responded significantly more than control animals during session 5 displaying 191.0 ± 53.66 responses compared to 18.89 ± 8.525 responses (t(17)=3.004, P=0.008). Interestingly, there was no correlation between the TH::Cre$^{(+/-)}$ nosepoke behavior and ChR2 expression in the NAc (r^2=0.0123, P=0.761).

In order to determine that rats were responding to obtain response-contingent optical stimulation, rather than just nonspecific increases in arousal or activity, we tested the effects of discontinuing laser stimulation on response behaviors. TH::Cre$^{(+/-)}$ rats rapidly learned to extinguish responding when the laser was turned off, showing significantly fewer responses at the end of extinction compared to the final training session ($t(9)=3.676$, $P=0.0051$), and responding during extinction was not significantly different from 0 (one sample t-test with a theoretical mean of 0, $P=.1914$). Further, when laser stimulation was resumed, rats rapidly reinstated the behavior such that they made similar numbers of responses during the preextinction and reinstatement sessions, (preextinction: 191.0 ± 53.66 responses, reinstatement: 141.3 ± 46.29 responses, $t(9)=1.591$, $P=0.146$). Important for the current studies, we evaluated whether animals were able to differentiate the intensity of the stimulation and adjust behavior accordingly. To do this, animals were allowed one final session to respond for 1s (20Hz 20p 20mW) optical stimulation. As shown in Figure 4.4D, the intensity of the stimulation is directly correlated with the amount of phasic dopamine release. We hypothesized that lower levels of dopamine release would be less reinforcing and thus animals would decrease responding compared to the higher level 5s stimulation as seen in previous reports of cell body stimulation (Beyene et al., 2010; Witten et al., 2011). We found that rats reliably responded for the 1s stimulation, however the responding was significantly blunted compared to the 5s stimulation period during the final day of training ($t(9)=3.308$, $P=0.0091$). This suggests that animals were in fact attending to the intensity of dopamine release in the terminal region and adjusted behaviors accordingly.

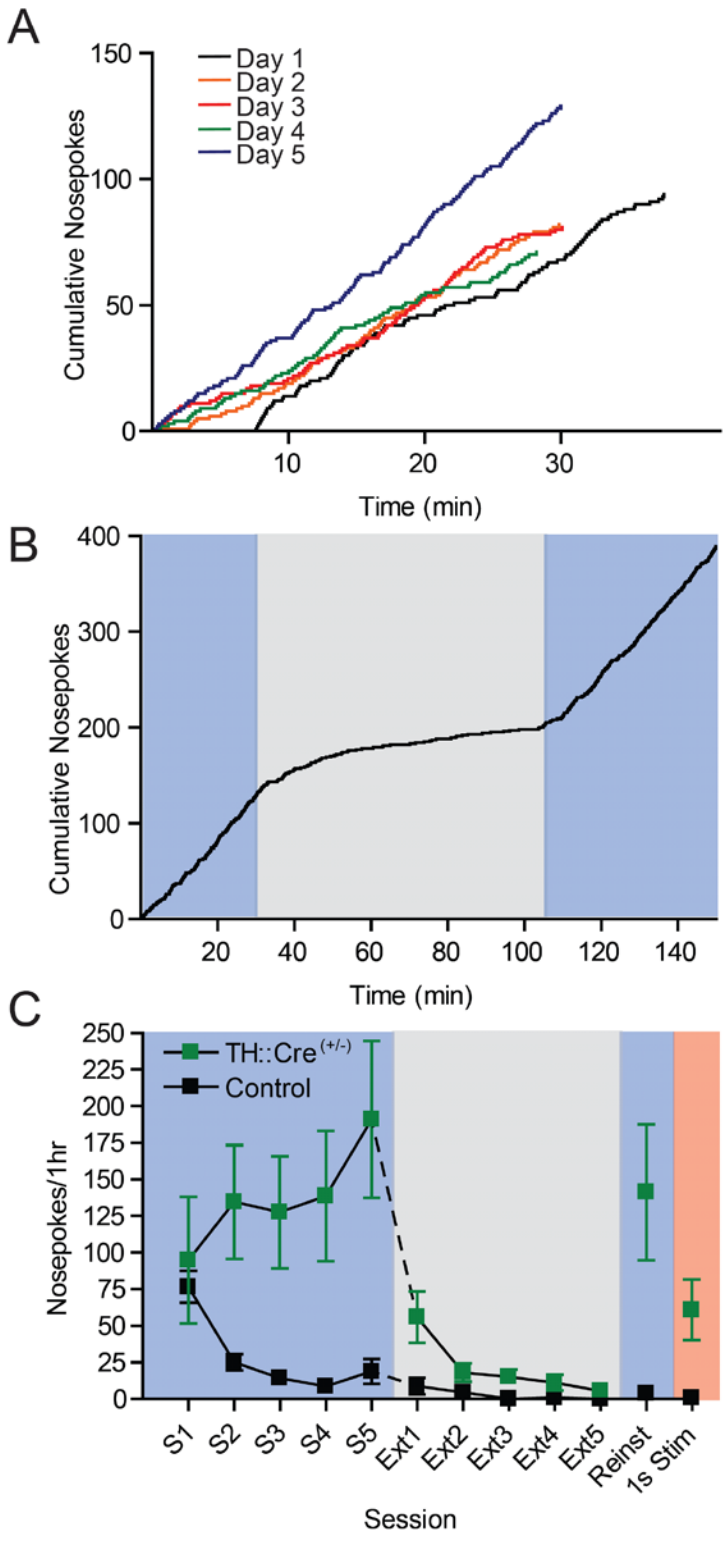

Figure 4.5 Optical stimulation of dopamine terminals in the NAc is sufficient to promote goal-directed behavior. (A) Cumulative activity graph from a representative animal for the number of active (i.e. rewarded) nosepokes across the 5 training sessions. Nosepokes were rewarded with 5s optical stimulation for 30 minutes following the first nosepoke. (B) The final training session, extinction, and reinstatement of nosepoke responding in a representative animal. Area shaded in blue signifies when the laser was active and nosepokes were rewarded with stimulation. Gray area signifies when the laser was off and nosepokes were unrewarded. (C) Nosepoke behavior for all animals in both groups. Data shown as the rate of nosepokes per hour. Blue area signifies when the laser was active and nosepokes were rewarded while gray area signifies when the laser was off and nosepokes were unrewarded. The 75 minute extinction session was broken up into 5 separate 15 minute blocks. Red area signifies when responding was rewarded with a 1s optical stimulation rather than 5 s. Data shown is mean ± SEM.

Stimulation of terminal dopamine release modulates delay but not magnitude based decision making

To evaluate the causal link between the value signaling of phasic dopamine release in the NAc (Day et al., 2010; Gan et al., 2010; Sugam et al., 2012) and appropriate responding we developed a dynamic decision making task to evaluate if optical stimulation of dopamine release during lower value cues was sufficient to modulate choice behaviors. Prior to training on the decision making task, we trained animals to learn that lever responses on the correct lever following a discrete cue presentation would be rewarded with a sucrose pellet. Both TH::Cre$^{(+/-)}$ and control animals were able to learn the task, showing a significant increase in accurate responding across training sessions (Figure 4.6). A 2-way repeated measures ANOVA shows a main effect of session ($F_{(14,420)}$=65.624, P<0.0001), with no effects of

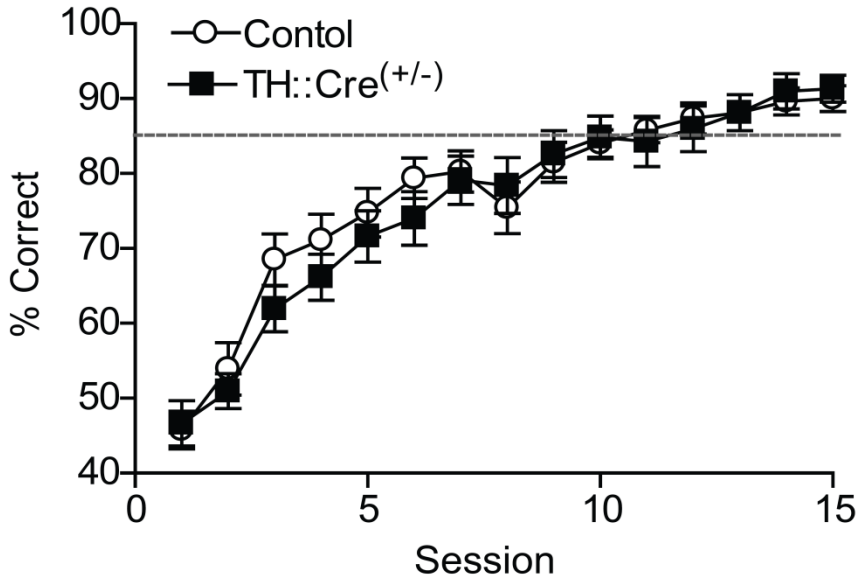

Figure 4.6 Lever press training for TH::Cre$^{(+/-)}$ versus control rats. Both groups showed significant increases in correct responding across behavioral training sessions. Both groups reached criterion by session 10. On the final training session (session 15) animals pressed the correct lever significantly greater than the behavioral criterion of 85% (P<0.05 for both groups). Grey dotted line shows behavioral criterion. Data shown are mean ± SEM.

group ($F_{(1,30)}$=0.072, *P*=0.79) or group by session interaction ($F_{(14,420)}$=0.91, *P*=0.547) showing that both groups similarly learned the lever press contingencies. Further, we considered lever press behavior to be well learned when rats pressed the correct lever 85% of the time. By session 15 both control and TH::Cre$^{(+/-)}$ rats pressed the correct lever significantly greater than 85% of the time (Controls: 90% ± 1.76% accuracy, *t*(16)=2.84, *P*<0.05; TH:: Cre$^{(+/-)}$: 90.92% ± 1.97 accuracy, *t*(13)=3.005, *P*<0.05; difference from theoretical mean of 85%).

Following the acquisition of lever pressing, training was initiated on the modified delay discounting task in which animals were given the option to press for a small immediate reward versus a larger delayed reward. Both groups showed preferences for larger and more immediate rewards, decreasing responding on the large delayed lever as both the delay and magnitude of reward decreased (Figure 4.7). This is shown by a main effect of Delay, $F_{(4,248)}$ =115.6, $P < 0.0001$, a main effect of Magnitude, $F_{(2,62)} = 4.03$, $P = 0.022$, and an interaction of Magnitude X Delay, $F_{(8,248)} = 3.32$, $P = 0.0012$. Posthoc analysis indicated that rats preferred the large reward to the small reward at delays of 0s, 10s and 40s. Rats decreased their preference for either reward across time, showing significant decreases in large/delay choices between 0s and 10s delay, between 10s and 20s delay, and between 40s and 60s delays (*P*<0.05 for all comparisons). Indeed, a linear contrast on the delay effect was significant, $F(\psi_{lin}; 1, 62) = 583.4$, $p < 0.0001$, and accounted for over 96% of the delay effect. However, there was no main effect of Group, $F_{(1,62)} = 2.0,7$ $P = 0.155$, or an interaction of GroupXMagnitude, $F_{(2,62)} = 0.01$, $P = 0.99$, GroupXDelay, $F_{(4,248)} = 0.41$, $P = 0.80$, or GroupXMagnitudeXDelay, $F_{(8,96)} = 0.93$, $P = 0.49$, indicating that there were no differences between the genetic strains of rats in choice behaviors and the ability to attend to both

magnitudes and delays to reward. Further, planned comparisons found that rats preferred the larger rewards more than the single reward when there was no delay to reinforcement, as they were significantly above chance (50% responding) for both the 3 pellet, $P < 0.01$, and 2 pellet, $P < 0.05$, option in both groups. In neither group was the 1 pellet option different than chance at 0s delay. In contrast, rats preferred the small immediate reward when delays were 20s or longer. In both conditions, for all magnitude values (except TH::Cre$^{(+/-)}$, 3pellet, 40s delay), rats chose the large delayed reward significantly less than chance $P<0.05$ (20s delay), $P<0.01$ (40s delay), $P<0.002$ (60s delay).

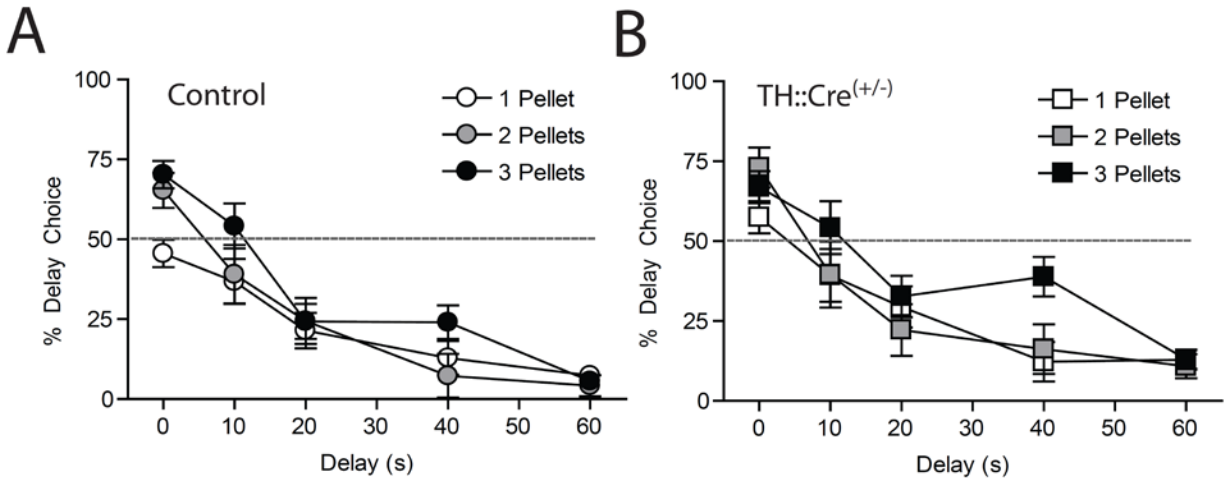

Figure 4.7 Delay and Magnitude Discounting for TH::Cre$^{(+/-)}$ and control groups. (A) Discounting behavior for control animals, showing the percent of presses on the larger delayed lever plotted against the delay to reward. Animals discriminated between reward magnitudes, pressing significantly more than chance for the larger reward when no delay was imposed and decreased responding as the delays increased. (B) Discounting behavior for TH::Cre $^{(+/-)}$ animals, conventions follow from A. There were no differences between groups in delay discounting behavior. Data shown are mean ± SEM

Using this type of training, we were then able to test how phasic dopamine release in the NAc is causally linked to several aspects of reward value, including the delay to reward and reward magnitude. During test sessions, animals underwent the same behavioral

paradigm as training sessions, such that they were presented with cues that predicted the opportunity to respond for higher value versus lower value rewards based on reward delay or magnitude (separate test sessions for each). Further, animals were presented with choice trials to evaluate behavioral preferences for each reward type. Importantly, during test sessions, optical stimulation only occurred during forced trial cue presentation and not choice trials, thus serving to instruct animals of the value associated with each option to drive choice behavior. Therefore any differences observed during behavioral choice would be the result of the learned value of each option, rather than other nonspecific effects of stimulation. During delay test sessions, optical stimulation had no effect on the accuracy of behavioral performance on forced trials in either group as animals pressed the correct lever ~85% of the time (Figure 4.8A). Further, animals performed similarly on forced trials during the laser stimulation session and sessions in which the laser was turned off and no laser stimulation was given. This is shown by a 2 way repeated measures ANOVA in which there were no main effects or interactions of group or stimulation (Group: $F_{(1,21)}$ =0.55, P= 0.47; Stimulation: $F_{(1, 21)}$ = 1.39, P = 0.26; Group X Stimulation: $F_{(1, 21)}$ = 1.06, P = 0.31). Animals were also able to attend to reward delays during the no laser sessions, showing a preference for the small immediate reward in both groups (responding less than 50% on the delay lever; Figure 4.8B). Optical stimulation during cues predicting the opportunity to respond for a small delayed reward significantly altered later behavior at the time of choice. Specifically, TH::Cre$^{(+/-)}$ rats significantly increased responses for the delayed lever during free choice trials, suggesting that they have associated this response option with higher value. Using a repeated measures ANOVA, a significant interaction of Group X Stimulation, $F_{(1,21)}$ = 7.95, P = 0.010, indicated that rats in the TH::Cre$^{(+/-)}$ group significantly increased their preference

for the delayed lever compared to both controls ($P < 0.001$) and to themselves in the non-stimulated version of the task ($P< 0.02$). Specifically, TH::Cre$^{(+/-)}$ rats showed a 50% increase in choice behavior, choosing the delayed lever 31% of the time without stimulation and 45% of the time with stimulation. Interestingly, similar to the nosepoke behavior, there was no relationship between decision making effects and ChR2 expression ($r^2= 0.008$, $P = 0.784$).

During separate test sessions, we also evaluated whether optical stimulation of dopamine release during cues that predict rewards of lower magnitude was sufficient to alter choice behaviors. Again, we found no effect of stimulation on forced trials with animals performing the task accurately during both stimulation and no stimulation sessions, as indicated by no main effects or interactions of group or stimulation (Group: $F_{(1, 14)} = 0.05$, $P = 0.82$; Stimulation: $F_{(1,14)} = 0.71$, $P = 0.41$; Group X Stimulation: $F_{(1,14)} = 2.56$, $P = 0.13$; Figure 4.8C). Further, unlike the stimulation effect in the delay condition, stimulation during magnitude sessions had no effect on subsequent choice trials (Figure 2.8D). There were no main effects or interactions on choice preference due to group, $F_{(1, 14)} = 0.24$, $P = 0.63$, stimulation, $F_{(1, 14)} = 1.02$, $p = 0.33$, or an interaction of group X stimulation, $F_{(1, 14)} = 0.01$, $P = 0.92$. This suggests that there is a dissociation in the encoding of reward value in the NAc based on the different characteristics of reward including reward delay and magnitude.

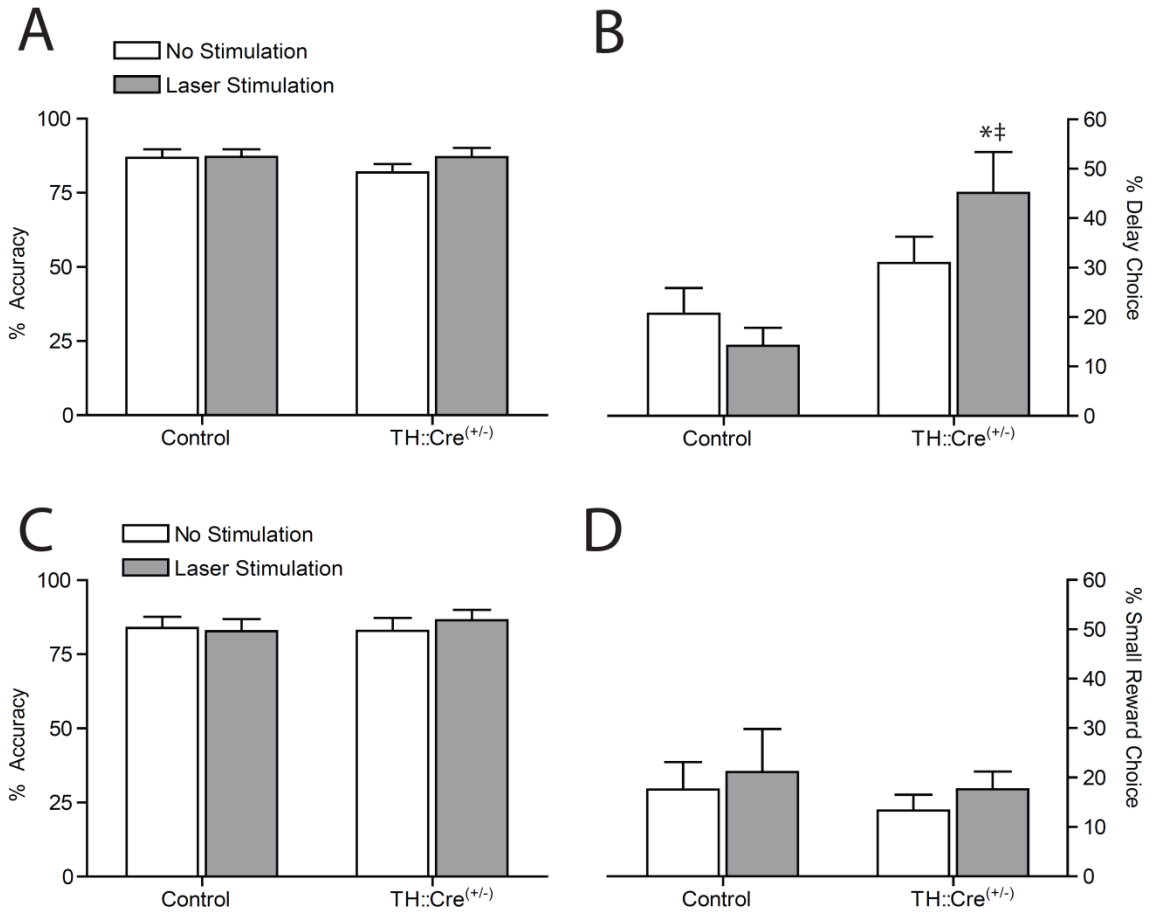

Figure 4.8 Optical stimulation of dopamine terminals modulates delay but not magnitude based decision making. (A) Percent accuracy on forced trials in control and TH::Cre$^{(+/-)}$ animals during delay based decision making sessions. No significant differences between groups or across sessions. (B) Percent choice of the small delayed lever for both control and TH::Cre$^{(+/-)}$ animals during delay based decision task. TH::Cre$^{(+/-)}$ animals showed a significant increase in delay choices during stimulation sessions compared to no stimulation sessions and control animals. (C) Percent accuracy on forced trials in control and TH::Cre$^{(+/-)}$ animals during magnitude based decision making sessions. No significant differences between groups or across sessions. (D) Percent choice of the lower magnitude lever for both control and TH::Cre$^{(+/-)}$ animals during magnitude based decision task. There were no significant effects of optical stimulation during magnitude session. Data shown is mean ± SEM. *P<0.05 for within subject effects. ‡P<0.05 for between subjects effect

Histology

Only animals with optical fibers confirmed within the boundaries of the NAc were included in behavioral analysis (Figure 2.9). TH::Cre$^{(+/-)}$ animals were all confirmed to show expression of ChR2 within the NAc terminal region, while controls did not show ChR2

expression. We calculated the spread of light through brain tissue using online calculators (www.optogenetics.org) and found that based on the light required to activate ChR2 (Boyden et al., 2005) we activated an area of about 1 mm^3 around the optical fiber. Due to the size of the core and shell of the NAc and the location of our optical fibers, we were activating dopamine release in both regions of the NAc in our behavioral experiments.

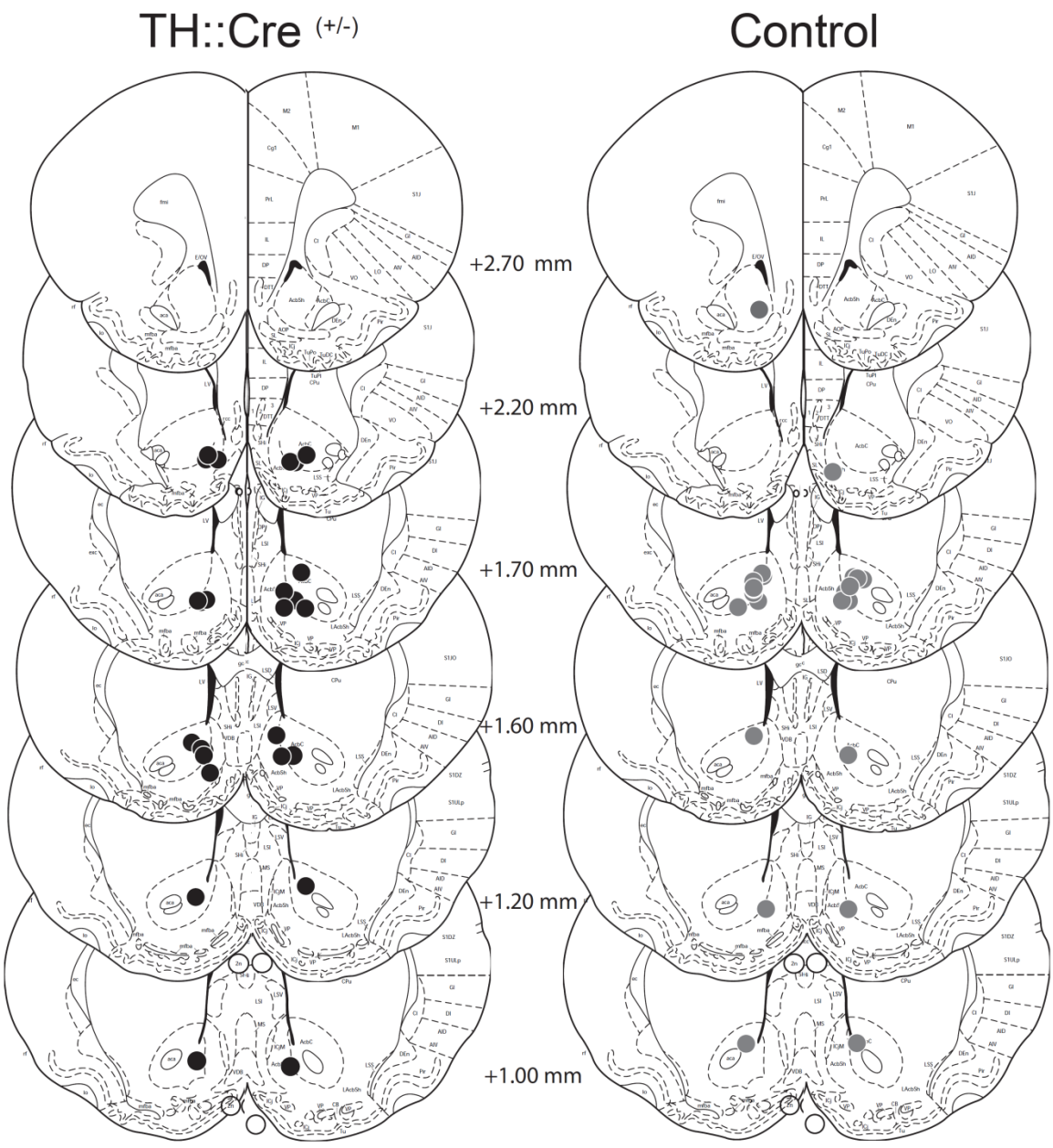

Figure 4.9 Optical fiber location tips for all subjects. Acceptable locations in both the TH::Cre$^{(+/-)}$ (black circles, left) and control (gray circles, right) groups were in the core and shell of the NAc.

DISCUSSION

Phasic dopamine release in the NAc has previously been implicated in promoting goal-directed actions (Cheer et al., 2007; Owesson-White et al., 2008; Wheeler et al., 2011) and signaling the value of future rewards based on several factors including reward cost, delay, and subjective value (Day et al., 2010; Gan et al., 2010; Sugam et al., 2012), It is hypothesized that this value signaling functions to bias animals towards the more valuable option when making choices. Here we used a genetic line of rats (TH::Cre$^{(+/-)}$) to investigate the causal link between phasic dopamine signaling and goal-directed actions. Using optogenetic technology, for the first time, we were able to specifically isolate and probe the mesolimbic dopaminergic projections to the NAc with both spatial and temporal precision.

Using this technique, we advanced previous research (Tsai et al., 2009; Witten et al., 2011), showing that stimulation of dopamine terminals in the NAc alone was reinforcing and therefore sufficient to promote reward seeking behavior. Specifically, animals vigorously responded for stimulation of dopamine terminals in the NAc, and adjusted responding as the intensity of stimulation changed. As we confirmed that dopamine release within the NAc itself was reinforcing, our next experiment sought to evaluate how this signaling controlled decision making. We have shown for the first time that there is a direct causal link between the value encoding of phasic dopamine activation in the NAc and appropriate decision making. Specifically, by stimulating dopamine release during forced trials which instructed animals of the value associated with appropriate responding, we were able to manipulate choice behaviors. Importantly, no stimulation occurred during choice trial cue presentations or any response behaviors; therefore, the shift in behavior observed during test sessions was due to an alteration in the predicted value that has become associated with each option. We

have shown that phasic dopamine release is causally related to appropriate delay based decision making. Further, we have shown that there is dissociation in different aspects of decision making as we found no effects on magnitude based decisions.

Our data support the idea that phasic dopamine signaling encodes the value of rewards associated with operant responding and that this signaling functions to modulate appropriate behaviors. For the self stimulation study, one of two outcomes was expected when the intensity of the stimulation was altered. First, animals could have been attending to the lower level stimulation and increased responding, to overcome the lower levels of stimulated release and thus increase overall levels of dopamine tone. This pattern of behavior would be consistent with drug taking, such that animals increase responding for lower doses of drug in order to maintain certain brain-cocaine and thus brain-dopamine levels (Oleson and Roberts, 2012). Alternatively, as the intensity of the stimulation decreased responding could decrease, as the reinforcement is no longer as rewarding. We found that animals diminished responding as the intensity of the stimulation decreased, and discontinued responding all together when the laser stimulation was terminated. These results support the prevailing theory that dopamine release in the NAc mediates appropriate choices by signaling the value of options to bias decisions towards more valuable actions. Specifically, dopamine encodes the expected utility of reward related behaviors, with higher costs, lower probability, and longer delays having lower perceived utility and thus evoke less dopamine release deeming associated behaviors less worthwhile (Phillips et al., 2007). Following this theory, as dopamine release decreased, the behavioral responses became less worthwhile, resulting in decreased response rates. Further, with no dopamine release, when the laser was off, the behavior was not worthwhile at all and was thus inhibited.

This cost-benefit signaling theory can also be applied to situations in which animals can make concurrent choices between two valuable options, as in our decision making task. Previous work from our lab and others has shown that phasic dopamine release scales with cues that predict the value of future rewards, showing higher release for more valuable or more preferred options. Further, when given a free choice, dopamine scales to the most valuable option, regardless of what the animal chooses (Day et al., 2010; Gan et al., 2010; Sugam et al., 2012). Dopamine thus is predicted to contribute to value-based decision making by broadcasting the overall utility of actions to striatal circuits that control motivated behaviors biasing responding towards actions that will maximize resources. The current data supports this hypothesis, showing that value signaling of mesolimbic dopamine release biases choices towards higher value options. Specifically, we were able to bias responses towards a lower value delayed reward by stimulating dopamine release during cues that predicted the value of the upcoming reward. As such, this signaling predicted that the value of the delayed reward was high, and therefore animals shifted responding towards the delayed reward, even though this option was less advantageous.

Dopamine is a key neuromodulator of NAc function, and has specific roles in mediating appropriate NAc function. Phasic dopamine release preferentially activates D1-like receptors as these receptors are of low affinity and require larger burst release events to be activated (Missale et al., 1998). As the activation of D1 receptors facilitates neuronal activity within the NAc (O'Donnell and Grace, 1993; Wilson and Kawaguchi, 1996), it is hypothesized that phasic dopamine release may function to increase the overall excitability of NAc neurons. Importantly, evidence from human literature shows that cues that predict high value options based on reward probability or delay recruit larger amounts of ventral striatal

activity (Kable and Glimcher, 2007; Tobler et al., 2007). By stimulating phasic dopamine release during lower value cues, we may be modulating a larger population of NAc neurons, increasing the proportion of NAc neurons that can be activated. Therefore, phasic dopamine signaling may function to bias responses towards higher value options by recruiting larger portions of NAc circuitry to promote behavioral outputs. By stimulating phasic dopamine release during lower value options, we have artificially recruited a larger portion of the circuit to drive behavioral responses.

Stimulation during magnitude based decision making provided evidence that not all characteristics of reward value are equal and processed the same way. We were able to shift delay based decisions but not magnitude based decisions suggesting that there is a dissociation in reward value encoding within the NAc. In support of this theory, previous research has shown that the NAc is not necessary for magnitude discriminations, as lesion or inactivation of the NAc does not impair the ability to choose larger rewards over smaller rewards, while impairing the ability to choose rewards based on probability and effort (Cardinal and Howes, 2005; Ghods-Sharifi and Floresco, 2010). Further, dopamine specific manipulations within the NAc also do not alter magnitude based discriminations (Stopper et al., 2013), suggesting that NAc dopamine release may not be involved in magnitude based decisions. Reward magnitude discriminations are relatively simple analyses of reward value, and thus may not require calculations of expected value and utility, a key role of the NAc and associated dopamine system, and therefore may rely on separate circuitry.

An alternative interpretation for the divergent effects on delay versus magnitude may be related to the strength of the preference. Previous research from our lab has also suggested that there is a correlation between differential dopamine release for risk versus safe cues, and

risk preference (Sugam et al., 2012). As such, a stronger risk preference is thus associated with greater dopamine release to risk versus safe cues. In our current findings, the preference for the larger reward compared to the smaller reward was stronger than that of the immediate compared to the delayed reward. Therefore, the differential dopamine signaling for the larger reward versus smaller reward may be much greater, and therefore as such, this preference may be harder to modulate through dopamine stimulation.

While we were able to train rats to self administer optical stimulation and modulate decision making behaviors through dopamine activation, there are several limitations that may have affected the results. First, we found that animals nosepoked for optical stimulation of dopamine terminals significantly less than previous reports with cell body stimulation (Witten et al., 2011). This decrease may be related to the fact that only the NAc portion of the mesolimbic dopamine circuit was activated, while cell body stimulation activates the entire circuit. While this may explain some of the decrease in the current results, the majority of dopamine cells from the VTA synapse within the NAc (Fields et al., 2007), and therefore responding should be similar. Instead, one of the limitations of using optogenetics in rats is the size of the structure being activated. Optogenetic modulation of mammalian behavior has previously been performed almost exclusively in the mouse. Rats have much larger brains and therefore may require more light to activate the entirety of the structure. The VTA is a much smaller structure than the NAc, and therefore cell body stimulation may be activating significantly more of the projections to the NAc than terminal stimulation, resulting in an increase in operant responding. As the NAc is much larger than our calculations of the area of light spread (www.optogenetics.org for calculator; (Lebedev et al., 2008)), we were not able to activate the entire structure through optical stimulation. Since dopamine encoding of

reward value appears to be critical for mediating appropriate choices based on reward delays, the ability to modify behaviors when only manipulating a portion of the circuit provides strong evidence that phasic dopamine release mediates delay based decision making.

Another important factor to consider when evaluating the effectiveness of terminal stimulation of dopamine release on reward related behaviors and decision making is the anatomical specificity of dopamine stimulation. Due to the location of the optical fibers, size of the NAc, and spread of light, we were most likely activating parts of both the core and shell of the NAc. Further, previous work from our lab and others has shown that dopamine release in the NAc core encodes information about reward delays (Day et al., 2010) and is necessary for appropriate delay based decision making (Cardinal et al., 2001). This data suggests a critical role of the NAc core in the effects observed here. Conversely, work from Berridge and colleagues suggests that there are hotspots in the NAc shell associated with increased wanting, "desire," or increased fearful responding and avoidance, "dread," that form a rostral-caudal gradient. These behaviors of desire and dread are controlled through AMPA receptor blockade (Faure et al., 2008), and importantly, dopamine modulates these responses. Specifically inhibition of dopamine receptor activity blocks the ability of AMPA receptor blockade to induce intense feeding (desire) or fearful behaviors (dread) (Richard and Berridge, 2011; Berridge and Kringelbach, 2013). As such, the shift in behavioral responding observed here may have been from a combination of both the delay encoding in the core and desire versus dread encoding in the shell. Future studies will need to be conducted to further differentiate the specific role of the core versus shell in the NAc.

Another important aspect to consider in regards to the current results is that the dopamine system dynamically encodes predictions and errors. Studies on the prediction error

signaling of dopamine activity have repeatedly shown that when animals receive unexpected reward omissions, there is a decrease in dopamine neural activity and dopamine release in the NAc (Schultz et al., 1997; Schultz, 1998; Waelti et al., 2001; Fiorillo et al., 2003; Sugam et al., 2012). This reduction in activity is interpreted as an outcome that is worse than expected, updating future predictions to instruct the animal that this cue-outcome association is less valuable. In the current decision making task, we are artificially increasing the value associated with the specific delayed stimulus; however we did not manipulate dopamine during reward delivery. Once the animal performed the operant response and received the delayed reward, this reward was of lower value than the animal predicted based on the cue evoked dopamine signaling. Therefore, during our stimulation sessions, dopamine may be encoding a negative prediction error during each of the delay trials which decreases the value associated with the response, thus decreasing the ability to shift behavior as strongly.

The data presented here supports the theory that phasic dopamine signaling within the NAc encodes the value of future options, and this signaling biases decisions towards choosing the most valuable option available. The NAc is embedded in a larger corticolimbic reward circuit and receives inputs from regions such as the orbitofrontal cortex (OFC), medial prefrontal cortex (PFC), basolateral amygdala (BLA) and hippocampus that each play important roles in modulating learning about actions and outcomes as well as value-based decision making (Burns et al., 1996; Winstanley et al., 2004; Ramirez and Savage, 2007; Ghods-Sharifi et al., 2009; St. Onge and Floresco, 2010; St. Onge et al., 2012). For example, perturbations of BLA circuitry result in animals shifting responses towards lower effort, higher probability, and more immediate rewards, suggesting that the BLA is critical for overcoming response costs (Winstanley et al., 2004; Ghods-Sharifi et al., 2009). Importantly,

previous research on the structure of the corticolimbic reward circuit has shown that dopamine projections from the VTA synapse on medium spiny neurons of the NAc adjacent to glutamatergic synapses from these cortical and limbic regions (Groves et al., 1994). This arrangement functions to modulate incoming activity from these cortical and limbic areas, such as dampening the effect of the glutamatergic activity from the PFC (Brady and O'Donnell, 2004; Goto and Grace, 2005). As such, one effect of dopamine may be to "gate" glutamatergic inputs in the NAc, such that only the strongest inputs can control NAc neurons (Floresco et al., 2001). While the current study displayed a critical role for phasic dopamine signaling in biasing appropriate choices, future work must further dissect this circuitry to investigate how these systems interact. For example, we may be able to further enhance or alter choices by coincidently modulating dopamine and glutamatergic inputs.

Given both the significant shift in decision making as well as the limitations in optogenetics listed above, the current data suggests a critical role of mesolimbic dopamine signaling in appropriate decision making. Further, this data suggests that alterations in phasic dopamine signaling alone are sufficient to promote behavioral responding and alter subsequent choices. As such, imbalances in this signaling may result in aberrant decision making processes such as increased risk taking behavior, impulsive choice, and drug addiction. In support, evidence from human literature has shown that there is a positive correlation between dopamine transporter levels (responsible for reuptake of dopamine) and impulsivity (Costa et al., 2013). Further, rodent studies have repeatedly shown that there is a direct correlation between impulsivity and drug taking behavior, such that animals with increased impulsivity also show increased drug taking behaviors (Perry et al., 2005; Belin et al., 2008; Perry et al., 2008). The current results therefore provide a direct link between

dopamine signaling and decision making, and therefore may provide a therapeutic target for ameliorating disorders involving maladaptive choices such as impulse control and drug addiction.

CHAPTER 5

GENERAL DISCUSSION

Summary of experiments

The studies described in the previous chapters were designed to expand our understanding of the role of dopamine and nucleus accumbens (NAc) signaling in value-based decision making. Further, we sought for the first time to evaluate the causal mechanisms between value encoding of phasic dopamine release and appropriate decision making. Taken together, the results demonstrate that NAc neurons process specific information about the expected value and outcome of action performance, while NAc dopamine signaling encodes information about the relative value of outcome associations, updates with changes in these associations, and is causally linked to appropriate action selection based on reward delays. A brief summary of each experiment is presented below.

NAc neurophysiology during risky decision making

The study described in chapter two examined how NAc neurons encode information about risk-based decision making. This experiment employed a task in which animals were presented with cues that predicted the ability to respond for smaller certain versus larger uncertain rewards. We found that NAc neurons selectively encoded cues that predicted risk versus safe options, displaying differential phasic activity for each cue type. However, there

were no differences in the amount of selective activity in any of the groups (risk preferring, safe preferring, or non preferring). This finding suggests that populations of NAc neural activity may be encoding the expected value of predicted options rather than the subjective value of those options Future studies could determine if there are more subtle differences in cue encoding between groups related to the type and time course of the neural firing pattern, (e.g., excitations versus inhibitions). Regardless, NAc neurons differentially encoded reward outcomes which correlated with individual risk preferences. Specifically, safe preferring animals displayed a greater percentage of phasic excitations compared to inhibitions during reward omissions in the NAc core, suggesting a possible connection between reward omission and aversion. Conversely, the opposite pattern was observed in the NAc shell with risk preferring animals displaying greater amounts of excitations. These results suggest a specific role for NAc neurons in encoding reward evaluations which may function to bias future behaviors towards more preferred outcomes.

Rapid dopamine signaling during delay discounting behavior

The experiments reported in chapter three provide one of the first characterizations of rapid NAc dopamine release during decision making in which the value of rewards are altered during a single behavioral session. Animals were trained on a delay discounting task in which distinct cues predicted the availability of a small reward delivered immediately or a large reward delivered after a period of delay. Importantly, the delay to the large reward increased throughout the session, thus devaluing this reward. Cue–evoked dopamine release scaled with the value of associated rewards as previously described (Day et al., 2010; Gan et al., 2010; Sugam et al., 2012), showing increased dopamine for the large reward early in training compared to the smaller reward. Interestingly, dopamine signaling dynamically

encoded changes in reward value, significantly decreasing cue-evoked dopamine levels for the large reward as the delay increased. This resulted in a shift in the relative peak dopamine signaling that followed the shift in behavioral responding from the large delayed reward to the small immediate reward. These results establish that NAc dopamine release encodes the relative value associated with future outcomes and confirms that dopamine release updates with changes in reward value to promote appropriate shifting of behavior.

Phasic dopamine release in the NAc mediates goal-directed responding and decision making

The results in chapter four used a novel technique, optogenetic control of neural circuits in rats, to demonstrate that phasic NAc dopamine release is causally related to goal-directed actions and delay based decision making. Specifically, stimulation of dopamine release within the NAc was sufficient to promote goal-directed action, as animals expressing ChR2 in the NAc displayed significant nosepoke responses to control laser onset. Further, animals were able to attend to the magnitude of dopamine release, such that they showed a decrease in responding for lower intensity stimulation and ceased responding when laser stimulation was terminated. Next animals were trained that cues predicted the option to respond for a small immediate or large delayed reward. Phasic dopamine release was stimulated during cues that predicted lower value delayed or lower magnitude options to evaluate the causal link between value-based dopamine signaling and decision making. Interestingly, animals displayed a significant shift in responding when dopamine release was stimulated during cues that predicted delayed reinforcers but not lower magnitude reinforcers. The results of these experiments show that phasic dopamine signaling within the NAc is reinforcing, and this signal is sufficient to mediate appropriate decision making, specifically decisions that evaluate reward delays.

General discussion and relevance of findings

Although the unique implications of each study are discussed individually following each original data chapter, these findings also have further implications for how the mesolimbic dopamine system and associated NAc cellular activity function in vivo, and how this function may relate to value-based decision making, learning, and psychiatric disorders such as drug addiction. Therefore, these topics are addressed below.

Phasic dopamine signaling and NAc cellular activity

The results presented above characterize a particular role for NAc dopamine release as well as NAc cellular activity in several models of value-based decision making. Specifically, we have shown that dopamine signaling tracks the predicted value of future outcomes, shifts as reward value changes, and are causally linked with biasing responses towards the most valuable option available. Importantly, phasic dopamine release does not occur in a vacuum, but exerts its effects via post synaptic changes in cellular activity at MSNs. Interestingly, the first study showed that NAc neurons encoded specific information about cue-outcome associations; however this signaling was not correlated with behavioral preferences. This data exemplifies one of the key issues that arise from studies of the mesolimbic dopamine system, which is how phasic dopamine release impacts MSN output, and how this is functionally relevant. The specific action of phasic dopamine activity on NAc neurons has been a contentious question as there is evidence that dopamine functions to both inhibit and excite MSNs (Nicola et al., 2000). Instead, the precise function of phasic dopamine release may depend on a range of factors including the dopamine receptors expressed, the amount of dopamine released, and the coincident afferent inputs to the cell (Nicola et al., 2000). As this data suggests, dopamine does not function to directly drive post

synaptic signaling, but instead, functions as a neuromodulator (O'Donnell et al., 1999; O'Donnell, 2003). Through this mechanism, dopamine functions primarily to augment the ability of afferent inputs, such as glutamatergic projections from the PFC or BLA, to elicit action potentials (Nicola et al., 2000).

Recently, technological advances have started to untangle the roles of dopamine and MSN activity through the simultaneous recording of both subsecond dopamine release and cell firing at the same carbon fiber electrode (Cheer et al., 2005; Cheer et al., 2007; Owesson-White et al., 2009; Cacciapaglia et al., 2011). Through the use of this "combined" technique, these studies have shown that in several behavioral responses (sucrose seeking, intracranial self stimulation, cocaine seeking) phasic changes in neural activity occur in the same locations as phasic dopamine release. These results confirm that phasic dopamine likely plays a key role in driving MSN activity, through direct neural excitability changes or prolonged changes in the ability of afferents to influence firing rates.

As the activity of NAc neurons does not always reflect the pattern of dopamine signaling, further research has begun to evaluate the specific role of phasic burst release of dopamine on cellular activity during reward seeking behaviors. Recent evidence has shown that phasic dopamine events are dependent on the activation of NMDA receptors in the VTA (Sombers et al., 2009), as pharmacological blockade of this circuitry reduces phasic events while not altering tonic levels of dopamine. Using this technique, Cacciapaglia and colleagues showed that disruption of phasic dopamine release abolishes task related cellular excitations in the NAc while leaving inhibitions unaltered (Cacciapaglia et al., 2011). This supports the hypothesis that there are microcircuits within the NAc that may differentially respond to phasic dopamine release. Specifically, as discussed in the introduction, MSNs in

the NAc differentially express D1 versus D2 receptors (Bertran-Gonzalez et al., 2008), and these cells may also send divergent projections to output structures (Meredith, 1999). Further, D1 receptors primarily exist in a low affinity state, and therefore, it is hypothesized that phasic dopamine functions to selectively activate D1 receptors (Richfield et al., 1989). Therefore, phasic dopamine release may be activating a unique subcircuit within the NAc. Specifically, task related excitatory neurons may primarily express D1 receptors, which are responsive to phasic dopamine release, and thus may send specific and divergent projections to output structures compared to D2 expressing neurons (Meredith, 1999; Sesack and Grace, 2010). In support, previous studies have revealed that there are microcircuits in the NAc for encoding drug versus nondrug rewards (Carelli et al., 2000; Carelli and Wondolowski, 2003). Further, previous research has also shown that pharmacological manipulation of D1 receptor activity disrupts decisions based on reward delay and risk, while D2 receptor modulation did not affect decision making (Koffarnus et al., 2011; Stopper et al., 2013). The results presented here suggest a specific role of phasic dopamine release in modulating appropriate decision making which may function to activate specific microcircuits within the NAc.

Role of phasic dopamine release in reward learning and value-based decision making

As discussed in the introduction, decades of research have tried to elucidate the role of phasic dopamine signaling in reward related behaviors. One model has shown that dopamine signaling functions to link cues and positive outcomes to drive appropriate responding (Schultz et al., 1997; Waelti et al., 2001). As such, dopamine signaling complies with the temporal difference learning model which follows that learning occurs as a result of differences in predicted versus expected outcomes (Rescorla and Wagner, 1972; Schultz et al., 1997; Waelti et al., 2001; Redish, 2004; Pan et al., 2005). At the center of the temporal

difference model is the idea that environmental stimuli and contexts are not randomly associated with future outcomes, but instead can be used to predict future rewards. Within this framework, when an organism is presented with a reward predictive cue, the organism makes a prediction of the future outcome (the expected value of a response, ΣV). When an organism receives exactly what was predicted, there is no new learning and the predictive association is maintained. However, the critical component to these temporal difference models is when there are differences in the predicted outcome, and the actual outcome, which results in a prediction error (termed ΔV). During learning situations, this discrepancy between predicted and actual outcomes functions to promote learning by updating stimulus outcome associations. As such, this model predicts that early in learning ΣV is low while ΔV is high, and as such learning rates are high. Conversely, as learning increases, ΣV increases and ΔV decreases. Learning rates comply with these changes, such that learning rates are high early on and begin to slow and reach peak as reward predictions become well learned. Importantly, other factors such as cue salience and reward value modulate the rate and total amount of learning (Rescorla and Wagner, 1972; Rescorla, 1988b).

In a seminal series of experiments, Schultz and colleagues determined that mesolimbic dopamine activity complies with these learning theories (Schultz et al., 1997; Schultz, 1998; Waelti et al., 2001). Specifically, these studies showed that when monkeys were presented with cues that predicted juice rewards, there were increases in phasic dopamine activity corresponding to ΣV signaling, while unexpected reward presentations and omissions resulted in increases and decreases in dopamine activity corresponding to ΔV. Recent research has shown that this signaling is transferred to the terminal region in the NAc (Day et al., 2007). Data presented in chapter three are clearly applicable to these models and

show that dopamine signaling encode predictions and outcomes, even in complex decision making situations. Specifically, dopamine signaling increased to reward predictive cues, and scaled with the prediction of each cue. Cues that predicted large rewards delivered immediately resulted in greater phasic dopamine release than cues that predicted smaller rewards delivered immediately, suggesting a direct connection of dopamine signaling and ΣV. Further, as the predicted value associated with the large delayed cue decreased (and as such ΣV decreased), there was a correlated decrease in dopamine signaling. This provides a candidate mechanism for reward learning and decision making. In support pharmacological and optogenetic studies have shown that phasic dopamine signaling is both necessary (Stuber et al., 2008; Zellner et al., 2009) and sufficient (Tsai et al., 2009) to promote behavioral conditioning. Further, the observations in chapter two, that NAc neurons are phasically and differentially responsive to reward predictive cues also indicate that this information can be incorporated into NAc output, as seen in previous studies (Setlow et al., 2003; Nicola et al., 2004a; Day et al., 2006).

The results presented here further allow for the application of temporal difference signaling to more complex decision making situations. Based on the temporal difference learning theory, dopamine could be functioning to mediate responding in one of two ways. First, dopamine's role in learning may produce differential learning rates between high value and low value options, as higher value reward presentations evoke larger ΔV and thus produce increased learning rates, and this greater learning for one option over the other may mediate choices. Conversely, when both cues have been fully learned and the predictive value of one cue is higher than the other, dopamine signaling will be higher for the more valuable cue (as ΣV is higher), and thus bias decisions towards the more valuable option. The

data presented here support this second option, such that there was greater dopamine release for higher value options, and stimulation of dopamine release biased responses towards options with higher phasic dopamine signaling. Further, in both tasks, animals were trained that responses on both levers resulted in equivalent rewards prior to any decision making training, removing potential differences in learning rates for each option. Further, in the optogenetics study, there were no differences in accuracy during stimulation versus nonstimulation sessions, suggesting that there are no differences in learning rates. As such, this supports the theory that dopamine signaling encodes the predicted value of available options and this prediction biases responses towards maximizing resources. Further, the combination of the value encoding observed in the mesolimbic dopamine system and differential responding of NAc neurons to reward outcomes observed in chapter two provides a complete system within one brain structure for specific predictions and outcome evaluations based on the predicted value of choices to drive appropriate responding.

Corticolimbic reward circuitry and decision making

The data presented here suggest a critical role for the NAc and associated dopamine signaling in promoting appropriate behavioral choices by biasing animals towards the most valuable option available. However, animals do not always perform optimally and sometimes choose the less advantageous option. In particular, in the tasks here, animals still sampled the nonpreferred or less advantageous option during a subset of choice trials. One mechanism by which this occurs may lie in the unique neuroanatomical arrangement of the NAc. The NAc is embedded in a larger corticolimbic reward circuit as it also receives dense projections from the orbitofrontal cortex (OFC), prefrontal cortex (PFC), basolateral amygdala (BLA), and hippocampus, which are also implicated in value-based decision making (Cardinal, 2006).

Importantly, each of these regions has been shown to be critical for modulating NAc activity (O'Donnell et al., 1999; Ambroggi et al., 2008; Jones et al., 2010a), and have dissociable contributions to appropriate decision making. For example, perturbations of BLA circuitry increase impulsive choices (choices of the small immediate option, (Winstanley et al., 2004)), decrease risk taking behaviors, and bias animals away from effortful responses (Ghods-Sharifi et al., 2009), suggesting a unique role for the BLA in the ability to overcome costs to obtain rewards. In contrast, inactivation of the OFC reduced impulsive choices (Winstanley et al., 2004), and increased risk taking behavior (Stopper et al., 2012), suggesting that OFC activity is responsible for updating the value of options in response to devaluations. Finally, inactivation of the PFC resulted in the inability to shift responses as reward risk increased or decreased, suggesting a critical role in updating value representations to make appropriate choices (St. Onge and Floresco, 2010). As the NAc receives inputs from each of these structures (Zahm and Brog, 1992a; Wright and Groenewegen, 1996), this data supports the unique role of the NAc as the integration center for appropriate goal-directed actions and behavioral choices. Further, this suggests that each neural substrate of the corticolimbic circuit plays a different role in mediating appropriate decision making, and the transfer of information between these structures and the NAc is critical for appropriate choices. As such, this connectivity provides a mechanism for circumstances in which organisms do not choose the best available option, as signaling from the PFC or BLA may override the value signaling of the mesolimbic dopamine system to bias responses towards less valuable options as seen here and previously in decision making tasks (Day et al., 2010; Sugam et al., 2012).

Decision making gone awry: phasic dopamine signaling and drug addiction

The data presented here suggest the mesolimbic dopamine system and NAc activity is critical for adaptive reward seeking and decision making behavior. However, this circuit has also been implicated in maladaptive behaviors such as drug addiction. Specifically, drug addiction involves the same brain regions as natural reward seeking and associative learning, such that drug seeking behavior evokes phasic dopamine release in the NAc. Further, stimulation of midbrain dopamine neurons is also sufficient to promote drug seeking (Phillips et al., 2003a) while drug paired stimuli evoke increases in phasic dopamine release (Phillips et al., 2003b; Aragona et al., 2009). Importantly, many addictive substances, including cocaine, alcohol, heroin, and nicotine all function to increase dopamine levels within the NAc (Di Chiara and Imperato, 1988). Further, addictive drugs produce changes in the mesolimbic dopamine neurons that result in increased excitability of these neurons to rewards and reward predictive cues (Jones and Bonci, 2005). As discussed above, the relationship between dopamine release during reward paired cues versus reward delivery is critical for promoting appropriate learning and goal-directed actions. Due to the pharmacological actions of drugs of abuse, it is hypothesized that these drugs elicit increased dopamine release to both the cues and rewards regardless of the prediction, thus always signaling a positive prediction error, or a reward that was better than expected (Redish, 2004; Schultz, 2011). This would result in situations in which drug reinforcers are always increasing the predicted value of drug paired cues, biasing decisions in favor of drug seeking behavior (Redish, 2004). The mesolimbic dopamine system therefore is critical for encoding reward value that mediates appropriate value-based decision making, however imbalances in this encoding may result in maladaptive decisions related, for example, to drug addiction.

Future directions

The experiments described in the preceding chapters comprise initial experiments designed to begin investigations of the role of the NAc and dopamine release in value-based decision making. However, the results left many questions unanswered and generated new questions that will provide the basis for future research. Here, a brief discussion of future experiments that will further clarify the role of the NAc and dopamine systems in value-based decision making is presented.

Corticolimbic modulation of NAc activity and dopamine release during decision making

As described above, the NAc receives afferent inputs from several cortical and limbic structures including the PFC and BLA which are regions that are critical for value-based decision making (Winstanley et al., 2004; Ghods-Sharifi et al., 2009; St. Onge and Floresco, 2010). However, it is presently unclear how these afferents may differentially contribute to the NAc cellular activity and phasic dopamine signaling described in chapters two and three. Recent studies have suggested that inactivation of the PFC or BLA attenuates cue evoked responses in the NAc (Ambroggi et al., 2008; Ishikawa et al., 2008; Jones et al., 2010a) while BLA inactivation also attenuates cue evoked dopamine release (Jones et al., 2010b). While these studies show there is a relationship between cue related signaling in the NAc and these corticolimbic afferents, it is unclear how these inputs drive the differential activity observed during value-based decision making. To test this, NAc neurons and NAc dopamine release could be recorded during similar decision making tasks to the ones reported here. In combination, the specific projections from the BLA or PFC to the NAc could be inactivated via optogenetic techniques during each task related event while neural and chemical recordings are performed in the NAc. Specifically, each region should be inactivated unilaterally such that behavioral responding remains intact and the importance of each input

to the NAc could be tested during normal decision making. These studies would permit the investigation of how different NAc afferents contribute to the specific encoding observed here.

Effects of chronic cocaine on dopaminergic encoding of delay discounting behavior

The data presented in chapter three suggest that there is a link between impulsive choice and dopamine signaling during delay discounting, as there was a direct correlation between dopamine release and choice behaviors. Increased impulsive choices have been repeatedly linked with drug addiction, such that human drug users exhibit increased impulsive choices during delay discounting compared to nondrug users (Coffey et al., 2003; Crews and Boettiger, 2009). Animal studies have suggested that drug taking behavior results in increased impulsivity as rats with a history of cocaine experience show increased impulsive choice scores (Simon et al., 2007; Setlow et al., 2009; Xie et al., 2012). While appropriate dopamine signaling is critical for normal delay discounting behavior (Koffarnus et al., 2011), how phasic dopamine signaling in the NAc is altered during delay discounting following cocaine experience, as well as how this is related to increases in impulsivity is currently unknown. To test this, two groups of rats could be trained on the delay discounting task, to evaluate baseline impulsivity scores. Following this training, the first group of rats could be trained to self-administer cocaine while the second group of rats would be saline yoked controls and could be trained to respond for water to control for the acquisition of the new operant. Animals could then be retrained on the delay discounting task to evaluate changes in impulsive choice. Once delay discounting behavior following cocaine exposure is stable, animals could be recorded using fast-scan cyclic voltammetry to evaluate dopamine signaling during behavioral performance. Dopamine signaling could then be compared

between the saline and cocaine groups to evaluate how a history of cocaine alters impulsive choices and the associated dopamine signaling.

Causal mechanisms of phasic dopamine release and other value-based decisions

The data presented in chapter four suggest there are dissociations in reward processing in NAc dopamine release, such that optical stimulation was sufficient to modulate delay but not magnitude based decisions. Previous research from our lab has suggested that phasic dopamine release in the NAc encodes information related to reward effort and risk/subjective value (Day et al., 2010; Sugam et al., 2012). Further, evidence from human and animal work suggests that there is a dissociation between delay discounting and risk taking behaviors (Green and Myerson, 2004; Simon et al., 2009; Mendez et al., 2010), suggesting that there may be differences in the ways in which the mesolimbic circuitry mediates this behavior. To test the causal role of dopamine signaling in other value-based decisions, rats could be trained on a task similar to the task described in chapter four, however instead of altering the reward delay and magnitude, the reward probability or effort could be manipulated. Optogenetic techniques could then be used to stimulate dopamine release during lower value options based on effort and reward risk to evaluate the causal relationship between phasic dopamine release and these types of value-based decisions.

Concluding remarks

The ability to procure and use environmental resources, such as food, shelter, and mates, is a clearly adaptive function that is necessary for survival, and this behavior is evident in the smallest of animals up through humans. This ability is mediated by a highly conserved and diverse network of brain nuclei including the NAc and mesolimbic dopamine system. The experiments described in this dissertation reveal how patterns of activity within

this circuitry mediate goal-directed actions and complex decision making. Specifically, the data presented here suggest that this system encodes information about the value associated with reward predictive cues and their outcomes, and this value signaling is critical for driving appropriate behavioral responses. While the data in the current dissertation provide evidence of the importance of this circuitry in an intact system to promote appropriate behaviors, decades of research have demonstrated that the NAc and associated dopamine system are critically involved and altered in numerous human disease states including drug addiction, schizophrenia, impulsive choice/attention deficit hyperactivity disorder, obesity, and depression (Breier et al., 1997; Kienast and Heinz, 2006; Nestler and Carlezon Jr, 2006; Dalley et al., 2007; Scheres et al., 2007; Volkow et al., 2007; Volkow et al., 2009; Johnson and Kenny, 2010). Importantly, perturbations in this circuitry are correlated with these disease states and specific impairments in appropriate decision making. Therefore, understanding how this neural circuit operates to promote appropriate responding will provide a therapeutic target for future treatments to ameliorate many of the maladaptive behaviors associated with these complex disorders.

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