Dissociating Reward Prediction from Action Selection:

Distinct Roles for Nucleus Accumbens Inputs

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THESIS

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DEDICATION

This thesis is dedicated to my wonderful family. Their love, support, and academic curiosity gave me the fortitude to pursue what I love, and has culminated in the proudest moment of my life thus far.

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LIST OF ABBREVIATIONS

μA	microamps
μg	micrograms
μL	microliters
6-OHDA	6-hydroxydopamine
Ag	silver
Ag/AgCl	silver/silver chloride
AMPA	2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid
ANOVA	analysis of variance
AP5	2-amino-5-phosphonopentanoic acid
cAMP	cyclic adenosine monophosphate
CNQX	6-cyano-7-nitroquinoxaline-2,3-dione
CPP	conditioned place preference
CS+	reward-paired conditioned stimulus
CS-	unpaired conditioned stimulus
CV	cyclic voltammogram
DA	dopamine
D-AP5	D(−)-2-Amino-5-phosphonopentanoic acid
DAT	dopamine transporter
DRL	differential reinforcement for low rates of responding
DS	discriminative stimulus
EMG	electromyographic
FSCV	fast-scan cyclic voltammetry

LIST OF ABBREVIATIONS (CONTINUED)

GABA	γ-aminobutyric acid
GNG	Go/NoGo
Go+	Reward-predictive cue that signals animal to respond for reward
GP_e	external segment of globus pallidus
GP_i	internal segment of globus pallidus
HD	Huntington's disease
Hz	hertz
ICSS	intracranial self-stimulation
IL	infralimbic prefrontal cortex
IP	intraperitoneal
kg	kilograms
mg	milligrams
mm	millimeters
ms	milliseconds
MSN	medium spiny neuron
mV	millivolts
nA	nanoamps
NAc	nucleus accumbens
nM	nanomolar
NMDA	*N*-methyl-*D*-aspartate
NoGo+	Reward-predictive cue that signals animal to withhold responding for reward
NoGo-	Cue not predictive of reward that signals animal to withhold responding

ns	non-significant
PD	Parkinson’s disease
PL	prelimbic prefrontal cortex
RPE	reward prediction error
s	seconds
SNpc	substantia nigra pars compacta
STN	subthalamic nucleus
VTA	ventral tegmental area

SUMMARY

Our everyday behavior is aimed at maximizing reward and in order to accomplish this, we must learn from past experiences and adjust our behavior accordingly. Basal ganglia structures in general and the ventral striatum in particular are essential for reward-related learning and goal-directed behavior. However, it remains ambiguous whether ventral striatal components such as the nucleus accumbens and dopamine (DA) signaling subserve learning, directed behavior, or both. Here, I investigated the role of multiple inputs – dopaminergic, glutamatergic, and γ-aminobutyric acid (GABA) – to a primary component of the ventral striatum, the nucleus accumbens (NAc), in reward prediction and goal-directed behavior.

DA has been characterized as a reward signal. However, studies supporting this characterization are confounded as rewards energize behavior. Though most behavioral paradigms lack the ability to dissociate changes in phasic DA resulting from reward-predictive cues versus the actions they energize, the current study utilizes a unique Go+/NoGo+ paradigm utilizes two separate equally reward-predictive cues that require different actions. We contrasted this with a behavioral paradigm (Go+/NoGo-) in which the cues differed not only with respect to the pattern of action required, but also the reward-predictive nature of the cues. Using fast-scan cyclic voltammetry, we examined the role of phasic DA signaling in reward prediction as compared to the selected pattern of action in our behavioral paradigms to dissociate the effects of learned associations from action selection on phasic DA release within in the NAc. Reward predictive cues from both paradigms (Go+ and NoGo+ cues) evoked significant increases in the concentration of DA release in the NAc core. In contrast, the NoGo-, a cue that not predictive of reward availability, failed to elicit changes in the concentration of DA. Thus, phasic DA signaling within the NAc appears to be critical for encoding the reward-predictive nature of cues.

The results from these experiments were contrasted to pharmacological manipulations of the NAc on Go+/NoGo+ performance. The NAc has a rich history of influencing not only goal-directed behaviors, but the selection of certain actions at the expense of others. Numerous inputs from cortical and limbic structures influence the signaling of NAc neurons via the release of a variety of neurotransmitters such as DA, glutamate, and GABA. To ascertain the role DA, glutamate, and GABA within the NAc on various aspects of goal-directed behavior we pharmacologically manipulated the NAc immediately prior to Go+/NoGo+ testing. Pharmacological manipulations of the NAc revealed that while blockade of DA receptors, glutamate NMDA receptors, and activation of GABA receptors reduced Go+ trial performance, blockade of glutamate AMPA receptors selectively impaired NoGo+ performance. Interference with AMPA receptor function within the NAc increased overall responding, indicating that these animals had reduced behavioral inhibition. Taken together, these studies suggest while the NAc itself is important for many aspects of goal-directed behavior, the reward predictive nature of cue appears to be encoded within phasic DA signaling while the ability to behaviorally inhibit a response appears to be governed by a glutamatergic afferent to the NAc.

Chapter I

Introduction

Our everyday behavior is energized by the desire to seek and enjoy rewarding stimuli. In order to accomplish this, we are motivated to learn from past experiences and adjust our behavior accordingly to maximize encounters with these rewards. We are constantly bombarded by rewarding stimuli that entice us to approach and consume. The delicious smell of chocolate beckons us to enter a sweets shop. A sales sign boasting fifty percent off encourages us to enter the store and spend money on items we had no intention of purchasing. At times, we resist adjusting our behavior in response to these reward-predictive cues as we recognize that it is ultimately not in our best interest – that is, we exercise behavioral inhibition. Of course, often we fail to inhibit, which is partly reflected in the rise of obesity and monetary debt. In addition, one core characteristic of some clinical conditions is impaired behavioral inhibition. Addicts continue to approach and consume drugs that offer no nutritive value even when faced with losing job and family. These cues that signal reward availability, whether adaptive or maladaptive, arouse and motivate our behavior directed at one particular goal. Motivated behavior is thought to be governed by an integrated network of structures called the basal ganglia (Graybiel, 1998; Mogenson, Jones, & Yim, 1980). In the sections that follow, I will deconstruct the functional neuroanatomy of the basal ganglia with a strong emphasis on a region of the ventral striatum called the nucleus accumbens (NAc) and the neurotransmitter dopamine (DA).

A. The Basal Ganglia: Anatomy and Function

The basal ganglia represent a circuit of interconnected structures that are thought to play an important role in learning, goal-directed behavior, and voluntary motor behavior (Gerfen, 1992). The primary input structure of the basal ganglia is the striatum which can be subdivided

into dorsal (dorsomedial and dorsolateral) and ventral (NAc and olfactory tubercle) components. The striatum as a whole is critical for voluntary motor behaviors and specifically the formation and expression of motivated behavior (Voorn, Vanderschuren, Groenewegen, Robbins, & Pennartz, 2004). Composed of 90-95% of γ-aminobutyric acid (GABA)-containing medium spiny neurons (MSNs), the striatum is also sparsely populated with small numbers of GABAergic and cholinergic interneurons (Voorn et al., 2004). Its primary afferent connections arise from virtually all areas of the cortex (Gerfen, 1992; Parent & Hazrati, 1995). Additional glutamatergic input is received from subcortical limbic structures such as the hippocampus, amygdala, and thalamus (Carlezon & Thomas, 2009; Kelley, Baldo, Pratt, & Will, 2005; Voorn et al., 2004; Wilson, 2007). The striatum is densely innervated by DA neurons originating in the substantia nigra pars compacta (SNpc) and the ventral tegmental area (VTA; Joel & Weiner, 2000; Voorn et al., 2004). Given its position as the primary input structure of the basal ganglia, the striatum is ideally positioned to synthesize information from numerous cortical and limbic inputs.

The GABAergic MSNs of the striatum form two distinct subpopulations termed “direct” and “indirect” pathways that provide tonic inhibition over downstream motor structures (Wilson, 2007; Wylie, Ridderinkhof, Bashore, & van den Wildenberg, 2010). The direct pathway of MSNs (striatonigral) projects to the internal segment of the globus pallidus (GP_i; entopeduncular nucleus in rodents) in addition to the SNpc and VTA (Carlezon & Thomas, 2009; Gerfen et al., 1990; Wilson, 2007). Direct pathway excitation leads to the release of GABA in the substantia nigra pars reticulata (SNpr) and GP_i, inhibiting these motor output structures. As these structures are themselves GABAergic, excitation of the direct pathway serves to release downstream motor structures from inhibition, thereby increasing motor output (Wylie et al., 2010).

In contrast to the direct pathway which sends efferents directly to the GP_i and SNpr, the indirect pathway of MSNs (striatopallidal) projects to the external segment of the globus pallidus (GP_e; globus pallidus in rodents) and subthalamic nucleus (STN) (Carlezon & Thomas, 2009; Gerfen et al., 1990). Activation of the indirect pathway of MSNs suppresses GABA release in the GP_e. As the GP_e tonically inhibits the glutamatergic STN, inhibition of the GP_e reduced inhibitory tone on the STN and therefore increases STN activity which excites the GABAergic SNpr and serves to suppress motor activity (Wylie et al., 2010).

The importance of the striatum in the selection and initiation of various motor behaviors is clearly demonstrated by diseases of the basal ganglia such as Parkinson's disease and Huntington's chorea. Parkinson's disease (PD) is characterized by the severe degeneration of dopaminergic inputs from the SNpc to the striatum (Hornykiewicz & Kish, 1987). In sharp contrast, Huntington's disease (HD) results from the programmed premature cell death of cholinergic and GABAergic neurons of the striatum (Martin, 1984). Despite disparate origins, PD and HD both result in severe motor impairments. One of the primary symptoms of PD involves a poverty of movement called akinesia. However, it has been proposed that this lack of movement is not due to an actual motor impairment, but in fact may be the result of the patients having difficulty initiating new movements, an impaired ability to switch between tasks once they have started, and poor suppression of conflicting responses (Benecke, Rothwell, Dick, Day, & Marsden, 1987; Harrington & Haaland, 1991; Hayes, Davidson, Keele, & Rafal, 1998; Kropotov & Etlinger, 1999; Wylie et al., 2009). In fact, the more severe a patient's PD is (according to the Unified Parkinson's Disease Rating Scale), the more difficulty there is in switching between different actions (Helmich, Aarts, De Lange, Bloem, & Toni, 2009). These impairments result in difficulty in selecting alternate, and potentially more advantageous, actions

than those recently performed (Hayes et al., 1998; Helmich et al., 2009). Degeneration of dopaminergic neurons projecting to the striatum impairs motivated behavior by interfering with the ability to initiate new actions (Kropotov & Etlinger, 1999). In sharp contrast, patients with HD suffer impaired inhibition of movement. The degeneration of GABAergic neurons of the striatum serves to release downstream motor structures from inhibition resulting in chorea, or uncontrollable dance-like movements (Kropotov & Etlinger, 1999). While PD patients are unable to easily initiate movements, patients with HD display an almost complete inability to inhibit inappropriate movements. Regardless of origin, the impairments resulting from PD and HD suggest a powerful role of the striatum in movement and action selection.

B. The Nucleus Accumbens: Anatomy and Function

The striatum as a whole has often been implicated in behavioral selection and goal-directed behavior, however, this structure is far from homogenous. The dorsal striatum (comprised of the dorsomedial and dorsolateral striatum) is thought to be critical in the stimulus-response learning that leads to habit formation (Faure, Haberland, Condé, & El Massioui, 2005; Wickens, Budd, Hyland, & Arbuthnott, 2007) spatially-guided behavior (Devan & White, 1999; Yin & Knowlton, 2004), behavioral flexibility (Lawrence et al., 1996; Monchi, Petrides, Petre, Worsley, & Dagher, 2001; Owen et al., 1993; Ragozzino, Ragozzino, Mizumori, & Kesner, 2002), and strategy shifting (Monchi et al., 2001; Ragozzino & Choi, 2004; Ragozzino, Ragozzino, et al., 2002). These functions are most likely mediated by the afferent projections to the dorsal striatum from motor, premotor, supplementary and cingulate motor cortical areas, the somatosensory cortex, associative cortical areas such as prefrontal, temporal, posterior parietal and preoccipital cortices, SNpc, as well as frontal eye field and supplementary eye field (Nakano, Kayahara, Tsutsumi, & Ushiro, 2000). In contrast, the ventral striatum (composed of

the NAc core and shell and olfactory tubercle) has been proposed to be more integral in modulating reward and motivated behaviors (for review see: Carlezon & Thomas, 2009; Mogenson, Jones, & Yim, 1980).

While more is known about the structure and function of the dorsal striatum, there are a great number of parallels between dorsal and ventral components. The NAc, the primary component of the ventral striatum, resembles the dorsal striatum in that it is composed of 90-95% of GABA-containing MSNs along with small numbers of GABAergic and cholinergic interneurons (Carlezon & Thomas, 2009; Gerfen, 1992; Meredith, 1999). Traditionally, the NAc is divided functionally and anatomically into distinct core and shell subregions. While both the core and shell receive numerous inputs from limbic structures involved in the regulation of affect and motivation such as the hippocampus, piriform, prelimbic (PL) and infralimbic (IL) prefrontal cortices, and sub-cortical amygdala, hippocampus, and VTA (Carlezon & Thomas, 2009; Ikemoto, 2007; Kelley et al., 2005; Kiyatkin, 2002; Nakano et al., 2000; Salamone, 1996; Voorn et al., 2004), the density of these cortical and sub-cortical afferents differ. The core predominantly receives cortical projections from the prelimbic, anterior cingulate, and dorsal agranular insular cortices, while the shell is cortically innervated by infralimbic, ventral agranular insular, and piriform cortices (Zahm & Brog, 1992; Zahm, 2000). While both the core and shell receive sub-cortical projections from the ventral pallidum and raphe nuclei, the core is also innervated by the subthalamic nucleus while the shell receives numerous projections from the amygdala and hypothalamus (Zahm, 2000). Perhaps more important, the efferents of these regions vastly differ as the core feeds back into conventional basal ganglia circuitry including the ventral pallidum, globus pallidus, and the SNpr, whereas the shell proceeds to innervate other sub-cortical limbic structures such as the lateral hypothalamus, the VTA, and the ventromedial

ventral pallidum (Zahm & Brog, 1992). Despite the similarities in composition, the differences in inputs and outputs of NAc core and shell undoubtedly account for the diverse functions they mediate.

C. The Nucleus Accumbens and Primary Reward

Our everyday behavior is motivated and energized by the desire to seek and enjoy rewarding stimuli. The NAc is critical for the signaling of rewarding stimuli. Pharmacological manipulations of the NAc alter the rewarding value of taste stimuli. Intra-NAc infusions of glutamatergic antagonists or GABA, opioid, and cannabinoid agonists, especially when infused into the NAc shell, have been demonstrated to greatly promote food intake (Kelley et al., 2005; Maldonado-Irizarry, Swanson, & Kelley, 1995; Reynolds & Berridge, 2001; Stratford & Kelley, 1997, 1999; Stratford, Swanson, & Kelley, 1998; Will, Pratt, & Kelley, 2006). Positive hedonic responses to taste stimuli are potentiated following intra-NAc shell infusions of opioid agonists (Peciña & Berridge, 2000; Peciña & Berridge, 2005). Conditioned place preference (CPP) paradigms indirectly investigate reward by pairing a stimulus with a visually distinct part of a chamber during training, and then observing which side of the chamber the animal spends more time in during a drug-free test session. Intra-accumbens infusions of the pharmacological agents amphetamine, NPY, and specific DA D_1 (SKF38393) and D_2 (LY17155, quinpirole) receptor agonists increase the amount of time spent in the infusion-paired chamber (Carr & White, 1983; Hemby, Jones, Justice, & Neill, 1992; Josselyn & Beninger, 1993; Liao, 2008; Papp, Muscat, & Willner, 1993; Schildein, Agmo, Huston, & Schwarting, 1998; White, Packard, & Hiroi, 1991). As evidenced by pharmacological manipulations, the NAc strongly alters the perception of reward and the rewarding qualities of food.

Further evidence of the role of the NAc in primary reward arises from drug self-administration. Lesions of the NAc reduce the rewarding quality of peripherally self-administered drugs of abuse including stimulants and opiates (Kelsey, Carlezon, & Falls, 1989; Roberts, Koob, Klonoff, & Fibiger, 1980). Animals readily respond in operant paradigms to deliver drugs of abuse that modulate DA such as amphetamine and nomifensine directly into the NAc (Carlezon, Devine, & Wise, 1995; Hoebel et al., 1983; Phillips, Howes, Whitelaw, Robbins, & Everitt, 1994; Phillips, Robbins, & Everitt, 1994). Self-administration is not dependent on a direct action on DA as non-dopaminergic drugs, such as morphine, met-enkephalin, and phencyclidine, are also effective at maintaining operant behavior when infused directly into the NAc (Carlezon & Wise, 1996; Goeders, Lane, & Smith, 1984; Olds, 1982). The NAc appears to play a pivotal role in the rewarding quality of self-administered drugs.

Pharmacological manipulations and lesions suggest a strong role for the NAc in signaling rewarding stimuli. However, experiments of this nature fail to reveal the precise function of the NAc in food and drug rewards. Electrophysiological recordings of NAc neurons in awake and behaving subjects allow monitoring of the firing rates of NAc neurons and provide temporal resolution of the time course of changes in NAc signaling. Multiple groups have demonstrated that the firing rate of individual NAc neurons are modulated by the delivery of rewarding taste stimuli (Nicola, Yun, Wakabayashi, & Fields, 2004a, 2004b; Roitman, Wheeler, & Carelli, 2005; Wheeler et al., 2008). The role of the NAc in primary reward is clearly demonstrated in several studies employing intra-oral delivery of rewarding solutions that result in modulations in NAc firing rate (Roitman et al., 2005; Wheeler et al., 2008). These studies allow for the separation of appetitive from consummatory behaviors and focuses directly on the effects of rewarding stimuli on NAc neuronal firing. Typically NAc neurons decrease their firing rate in response to

rewarding taste stimuli (Carlezon & Thomas, 2009; Nicola et al., 2004a, 2004b; Roitman et al., 2005; Taha & Fields, 2006; Wheeler et al., 2008; Wilson & Bowman, 2005). Temporally-precise recordings of neuronal activity using electrophysiology support the NAc as a structure that encodes crucial information about rewards.

D. The Nucleus Accumbens, Goal-Directed Behavior, and Reward-Related Learning

In addition to the rich body of literature implicating the NAc in signaling primary reward, the NAc also influences goal-directed behaviors and reward-related learning. Termed a bridge between motivation and action, the NAc is optimally positioned to integrate cortical and limbic afferents and translate them into motivated behavior (Mogenson et al., 1980). However, the role of the NAc in mediating goal-directed behavior is still controversial. Some studies have demonstrated that electrolytic lesions or inactivation of the NAc increase motor output, non-reinforced lever responding, and responding in motivationally demanding operant paradigms (Bowman & Brown, 1998; Kubos, Moran, & Robinson, 1987; Lorens, Sorensen, & Harvey, 1970; Pulman, Somerville, & Clifton, 2012; Starkstein, Moran, Bowersox, & Robinson, 1988; Stratford & Wirtshafter, 2012; Wirtshafter & Stratford, 2010). However, lesions of the NAc have also been reported to decrease goal-directed behavior, such as animals' motivation to obtain food (Trojniar, Plucinska, Ignatowska-Jankowska, & Jankowski, 2007) and reductions in motivation to work for food despite preserved sensitivity to hedonic value (Balleine & Killcross, 1994). Therefore, lesion studies suggest that assigning a unified theory of NAc function in goal-directed has been challenging.

Electrophysiology, which provides superior temporal resolution to capture changes in NAc firing rate, has demonstrated that individual NAc neurons modulate their firing rate during operant responses for food, water, and drug reward (Carelli & Deadwyler, 1994; Carelli, 2002;

Chang, Paris, Sawyer, Kirillov, & Woodward, 1996; Nicola et al., 2004a; Taha & Fields, 2006). Indeed, NAc neurons appear to encode all aspects of operant responding, with modulations in firing rate evoked by reward-predictive cues (Ambroggi, Ghazizadeh, Nicola, & Fields, 2011; Day, Wheeler, Roitman, & Carelli, 2006; Day, Jones, & Carelli, 2011; Roitman et al., 2005), anticipation of operant responding (Ambroggi et al., 2011; Carelli & Deadwyler, 1994; Carelli, 2002), and immediately following the response during reward delivery (Carelli & Deadwyler, 1994; Carelli, 2002; Day et al., 2006). Though the NAc appears to influence goal-directed behavior, it remains unclear whether the NAc is truly signaling reward or behavioral responding.

Despite the controversial role of the NAc in the execution of goal-directed behaviors, inactivation studies strongly link the NAc to the acquisition of reward-directed behaviors. Kelley and colleagues (1997) inactivated the NAc during a lever-pressing paradigm using the glutamate *N*-methyl-*D*-aspartate (NMDA) receptor antagonist 2-amino-5-phosphonopentanoic acid (AP5). Blockade of NAc NMDA receptors had little to no effect on task performance once the task had already been learned. AP5 also had no effect on already formed stimulus-reward associations in a strictly Pavlovian paradigm. However, AP5 impaired operant responding during the acquisition phase of the lever-pressing task (Kelley et al., 1997). Similarly, blockade of NAc NMDA receptors impaired the acquisition of a food-rewarded radial arm maze task, but had no effect on performance once the task had already been learned (Smith-Roe, Sadeghian, & Kelley, 1999). Electrolytic lesions of the NAc have also been demonstrated to impair the acquisition of place preference, but not the expression of preference once formed (Gremel & Cunningham, 2008). Impairments in goal-directed behavior following the inactivation of the NAc during acquisition but not after the task is learned support the NAc as a structure critical for the learning of reward-related behaviors.

E. The Nucleus Accumbens and Action Selection

As individuals motivated to pursue and engage rewarding stimuli, we easily learn what situations lend themselves to reward. Utilizing what we have learned, we modify our behavior and direct our actions to maximize reward. Given the demonstrated importance of the NAc in reward and goal-directed behavior, it follows that the NAc may also have a role in selecting the behavioral patterns that will result in the most favorable outcome. Indeed, the NAc and striatum as a whole have been proposed to facilitate the selection of one action while inhibiting competing behaviors that would interfere (Hikosaka, Nakamura, & Nakahara, 2006; Mink, 1996; Nicola, 2007; Pennartz, Groenewegen, & Lopes da Silva, 1994; Redgrave, Prescott, & Gurney, 1999).

The importance of the NAc in the selection of behavior is evident in both Pavlovian and operant learning tasks. Approach behavior to Pavlovian reward-predictive cues, such as pecking at the cue in pigeons (Brown & Jenkins, 1968) and approach behavior in rats (Peterson, Ackil, Frommer, & Hearst, 1972) appear to be under the control of the NAc as excitotoxic lesions reduce approach behavior (Cardinal, Parkinson, Lachenal, et al., 2002). Inactivation of the NAc results in increases in premature (Cardinal, Pennicott, Sugathapala, Robbins, & Everitt, 2001) and impulsive responding (Cardinal et al., 2001; Christakou, Robbins, & Everitt, 2004) in addition to perseveration on incorrect responding (Christakou et al., 2004). Differential reinforcement for low rates of responding (DRL) tasks, which require the animal to withhold an operant response for a fixed period of time in order to obtain a reward, are extremely sensitive to manipulations of the NAc. Lesions of the NAc increase responding on DRL tasks despite the fact that increased responding impairs the ability to obtain rewards (Pothuizen, Jongen-Rêlo, Feldon, & Yee, 2005; Reading & Dunnett, 1995). The impaired and impulsive responding that occurs

during goal-directed behavior following lesions suggests that the NAc may exert a great degree of control over normal behavioral selection.

Investigations of the firing rates of NAc neurons reveal that individual neurons exhibit pre-movement (Bowman, Aigner, & Richmond, 1996; Schultz, Apicella, Scarnati, & Ljungberg, 1992) and pre-operant response (Ambroggi et al., 2011; Carelli & Deadwyler, 1994; Carelli, 2002) changes in firing rate following cue presentation. In fact, the firing rates of NAc neurons are tightly correlated with the direction of future movement suggesting that at least a subpopulation of NAc neurons encode information about the selection of actions during goal-directed operant tasks (Taha, Nicola, & Fields, 2007). The accumulated evidence suggests that not only does that NAc contribute to the signaling of rewarding stimuli and goal-directed behavior, but the NAc may instruct behavioral selection to maximize these rewards.

F. Dopamine: Anatomy and Function

The NAc receives numerous afferent projections that could provide critical information about not only the rewarding nature of behaviorally-relevant stimuli, but also information about appropriate actions to maximize these rewards. Among these afferent projections are a rich projection of medium aspiny neurons originating in the VTA and releasing DA (Grace, 2008). While less is known about the NAc than dorsal regions of the striatum, there are believed to be parallels between DA neuronal structure in these two regions. DA neurons greatly arborize within the NAc with individual neurons estimated to cover nearly 6% of striatal volume, and more than a million striatal dopaminergic axon terminals estimated in total (Andén, Hfuxe, Hamberger, & Hökfelt, 2009; Doucet, Descarries, & Garcia, 1986; Matsuda et al., 2009). Following the release of DA, it activates any number of five separate classes of g-protein-coupled DA receptors. These receptors are grouped into two categories: D_1-like receptors (D_1

and D_5) and D_2-like receptors (D_2, D_3, and D_4). While all five of these receptor classes are expressed in the NAc, D_1 and D_2 receptors are far more plentiful (Surmeier, Carrillo-Reid, & Bargas, 2011). Receptor binding studies suggest that within the NAc DA has a much higher affinity for D_2 receptors than D_1 receptors (Rice, Patel, & Cragg, 2011; Richfield, Penney, & Young, 1989). DA receptors are not confined to the synaptic cleft, but rather are predominantly located extrasynaptically (Hersch et al., 1995; Sesack, Aoki, & Pickel, 1994; Yung et al., 1995). Termination of DA signaling within the striatum and NAc is primarily the result of reuptake by the DA transporter (DAT). DATs are also located extrasynaptically solely on dopaminergic neurons, and clear DA via an inward flux (Ciliax et al., 1995; Hersch, Yi, Heilman, Edwards, & Levey, 1997; Leviel, 2011; Nirenberg, Vaughan, Uhl, Kuhar, & Pickel, 1996; Rice et al., 2011). Though DA signaling within the striatum and NAc are often considered to be limited by DAT reuptake (Floresco, West, Ash, Moore, & Grace, 2003; Stamford, Kruk, Palij, & Millar, 1988), high frequency burst firing of DA neurons overwhelms the DAT, permits greater diffusion of DA away from release sites, and results in larger increases in DA signaling within the extracelluar space (Garris, Ciolkowski, Pastore, & Wightman, 1994) which allows DA to interact with more distal receptors.

The direct and indirect pathways of NAc neurons uniquely express D_1 and D_2 receptors. The direct pathway of NAc MSNs, which project to the GP_i (entopeduncular nucleus) in addition to the SNpc and VTA, express the DA D_1 receptor (Carlezon & Thomas, 2009; Gerfen et al., 1990; Wilson, 2007). D_1 receptors are coupled with G proteins $G_{\alpha s}/G_{olf}$ which results in the activation of adenylate cyclase, increases in intracellular levels of cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA), and excitation of the striatonigral MSNs (Carlezon & Thomas, 2009; Hervé, Rogard, & Lévi-Strauss, 1995; Surmeier et al., 2011;

Surmeier, Ding, Day, Wang, & Shen, 2007). Therefore, the implication is that DA action at D_1 receptors would lead to excitation of the direct pathway and thus downstream activation of motor behavior.

In contrast, the indirect projection of NAc MSNs, which projects to the GP_e and STN (Carlezon & Thomas, 2009; Gerfen et al., 1990), express DA D_2 receptors. Unlike D_1 receptors, D_2 receptors are coupled to the G-protein $G_{i/o}$ which leads to an inhibition of adenylate cyclase and suppression of transmembrane Ca^{2+} currents which results in reduced excitability of striatopallidal MSNs (Carlezon & Thomas, 2009; Hernandez-Lopez et al., 2000; Surmeier et al., 2007). Thus, activation of D_2 receptors inhibits activity of the indirect pathway, thereby promoting motor activity.

Though the localization of D_1 and D_2 receptors on the direct and indirect pathways of NAc MSNs respectively have been known for a number of years, only recently has a non-pharmacological technique been developed that allows the manipulation of these individual pathways. The development of optogenetics allows for adeno-associated viruses to be injected selectively into striatal MSNs expressing D_1 or D_2 receptors. Using this technique, Kravitz and colleagues (2010) were able to selectively activate direct and indirect pathways using fiber optic light during various aspects of behavior in both wild-type mice and a mouse model of PD. In wild-type mice, activation of the direct pathways of MSNs resulted in an increase in motor output and a reduction in freezing behavior. In contrast, activation of the indirect pathway increased freezing behavior and markedly reduced any type of locomotion. When tested in PD mice, activation of the direct pathway was able to alleviate the locomotor impairments of bradykinesia and freezing (Kravitz et al., 2010). While this work suggests that direct and indirect pathways work in opposition to each other, recent work from Cui and colleagues (2013) found

activation of both pathways immediately before goal-directed movement. This leads to the theory that direct pathway activation may result in promotion of the correct goal-directed behavior while activation of the indirect pathway functions to prevent competing actions. Regardless, selective activation of the direct and indirect pathways via the D_1 and D_2 classes of DA receptors have drastically different effects on motor behavior and suggest that perhaps these two classes of DA receptors may provide insight into the role of DA in action selection.

G. Dopamine and Primary Reward

The dopaminergic projections from the VTA to the NAc have been demonstrated to play key roles in reward, action selection, motivation, and the acute rewarding effects of drugs of abuse (Carlezon & Thomas, 2009; Ikemoto, 2007; Kelley et al., 2005; Kiyatkin, 2002; Salamone, 1996). However, the exact role of NAc DA in motivated behavior remains unclear. Over the last 30 years, DA has been theorized to play a role in pleasure and hedonia (Wise, 1978), "wanting" of food and drug rewards (Berridge & Robinson, 1998), reward-seeking (Ikemoto & Panksepp, 1999), and the motivation of animals to work to obtain rewards (Aberman & Salamone, 1999; Correa, Carlson, Wisniecki, & Salamone, 2002; Salamone, Cousins, & Bucher, 1994). DA has been proposed to mediate numerous functions, and the data appear to support multiple roles for the activity of DA within the NAc.

The notion that DA plays a key role in reward was championed by Wise and colleagues (1978) over thirty years ago. Following training on operant responding and runway traversing for food reward, Wise and colleagues found that administering the DA receptor antagonist pimozide resulted in attenuated responding for food reward. When tested after drug administration, pimozide-treated animals behaved similarly to animals performing in extinction when the food reward is removed, suggesting that DA blockade was reducing the rewarding quality of the food.

While the animals performed as controls on the first day following pimozide treatment, after experiencing the food reward while treated with pimozide, performance steadily decreased as exhibited by reduced operant responding and an increase in running time and latency to begin the runway task (Wise & Schwartz, 1981; Wise, Spindler, deWit, & Gerberg, 1978). Given the similarities in responding between animals with DA blockade and those performing in extinction, these results suggest that the rewarding quality of the food reward had been altered.

Indeed, not only does blocking DA receptors impair the "rewarding quality" of the food, but ingestion of rewarding substances such as food and water leads to increases in extracellular DA in the striatum and NAc as measured by microdialysis (Ahn & Phillips, 1999; Westerink, Kwint, & de Vries, 1997; Westerink, Teisman, & de Vries, 1994; Yoshida et al., 1992). Increases in DA following consumption of rewarding substances and the impairment in reward-directed behaviors following interference with DA signaling suggest that striatal DA is an integral component of rewarding stimuli.

Further support for the role of NAc DA in reward arises from the self-administration of drugs of abuse. Rats will readily self-administer drugs that increase DA release or act as DA agonists in the NAc (Ikemoto, Glazier, Murphy, & McBride, 1997; Ikemoto & Panksepp, 1999). Intravenous self-administration or intraperitoneal injections of drugs that are highly rewarding and frequently abused such as cocaine (Di Ciano et al., 1995; Hurd, Weiss, Koob, And, & Ungerstedt, 1989; Pettit & Justice, 1989; Pontieri, Tanda, & Di Chiara, 1995), methamphetamine (Pereira et al., 2006), amphetamine (Di Ciano et al., 1995; Pontieri et al., 1995), morphine (Pontieri et al., 1995; Steinmiller, Maisonneuve, & Glick, 2003), heroin (Hemby, Martin, Co, Dworkin, & Smith, 1995; Wise, Leone, Rivest, & Leeb, 1995), and alcohol (McBride et al., 1993; Weiss, Lorang, Bloom, & Koob, 1993) lead to increases in the concentration of

extracellular DA in the NAc and other regions of the striatum. 6-OHDA and kainic acid lesions of the striatum block the rewarding quality of these drugs and result in reduced self-administration of drugs of abuse including heroin (Singer & Wallace, 1984) and cocaine (Gerrits & Van Ree, 1996; Pettit, Ettenberg, Bloom, & Koob, 1984; Roberts & Koob, 1982; Zito, Vickers, & Roberts, 1985). Increases in NAc DA following administration of rewarding drugs coupled with the blockade of these effects following lesions of the NAc lend strong support for the role of DA in signaling drug reward.

CPP can lead to valuable insights into the rewarding or aversive properties of a variety of stimuli including food, drugs of abuse, and even sex. Many studies have found that DA agonists such as amphetamine (Carr & White, 1986; Josselyn & Beninger, 1993; Schildein et al., 1998), and specific DA D_1 (SKF38393) and D_2 (LY17155, quinpirole) (Papp et al., 1993; White et al., 1991) receptor agonists increase the amount of time spent in the drug-paired chamber. Just as this research reveals that drugs that serve to increase the concentration of DA lead to a preference for a particular chamber, antagonism of DA receptors with drugs such as alpha-flupenthixol, SCH23390, haloperidol, sulpiride, and eticlopride result in the blockade of CPPs to drugs of abuse such as amphetamine, cocaine, morphine, nicotine, and diazepam (Acquas, Carboni, Leone, & Di Chiara, 1989; Mackey & van der Kooy, 1985; Pruitt, Bolanos, & McDougall, 1995; Spyraki, Fibiger, & Phillips, 1983). Collectively, the modulations in reward-directed behavior following pharmacological manipulation of NAc DA suggest that DA activity contributes to the signaling of rewarding stimuli.

H. Phasic Dopamine and Primary Reward

Despite the knowledge that the dopaminergic projections from the VTA to the NAc play a critical role in primary reward, the firing of dopaminergic neurons is far from uniform and

many previous studies lack the temporal resolution to tease apart the time course of changes in DA signaling. Typically, DA neurons fire action potentials at lower frequencies (3-8 Hz; tonic) in a slow, irregular pattern (Grace & Bunney, 1984). However, periodically DA neurons exhibit brief (<1s) high frequency (20-60Hz) increases in activity (phasic; Grace & Bunney, 1984b; Hyland, Reynolds, Hay, Perk, & Miller, 2002; Schultz, 1998). Using electrophysiology to monitor the firing rate of dopaminergic neurons, phasic changes have been implicated in motivated behavior and reward-related learning (Hyland et al., 2002; Schultz, Dayan, & Montague, 1997). Phasic changes in DA firing rate play a role in signaling affective stimuli (Mirenowicz & Schultz, 1996), motivation (Hyland et al., 2002; Schultz, 1998), and associative learning (Arbuthnott & Wickens, 2007; Pan, Schmidt, Wickens, & Hyland, 2005; Schultz et al., 1997; Schultz, 1998; Wickens, 2008). Specifically, rewarding stimuli trigger phasic *increases* in the firing rate of dopaminergic neurons (Hyland et al., 2002; Mirenowicz & Schultz, 1996; Schultz et al., 1997; Ungless, 2004). The brief, sub-second changes in the firing rate of DA neurons highlighted by electrophysiology suggest that utilizing techniques with the necessary temporal resolution to observe these phasic changes is critical.

Fast-scan cyclic voltammetry (FSCV) monitors extracellular concentrations of DA on a timescale similar to electrophysiology. High frequency (> 30 Hz) electrical stimulation, which mimics burst firing, of DA neurons in the medial forebrain bundle leads to brief increases in the concentration of DA within the striatum (Kawagoe, Garris, Wiedemann, & Wightman, 1992; Suaud-Chagny, Dugast, Chergui, Msghina, & Gonon, 1995). Recent advances have also led to the ability to selectively drive phasic firing of VTA DA neurons utilizing fiber optic stimulation (optogenetics) which similarly results in brief increases in NAc DA (Tsai et al., 2009). Phasic changes in concentration of NAc DA following electrical and optogenetic stimulation suggest

that FSCV has the ability to monitor fluctuations in DA concentration on a similar timescale as electrophysiology.

Using FSCV, fluctuations in DA concentration in the NAc have been observed in response to primary rewarding stimuli that evoke phasic changes in the firing rate of DA neurons (Addy, Daberkow, Ford, Garris, & Wightman, 2010; Beeler et al., 2012; Roitman, Wheeler, Wightman, & Carelli, 2008; Stuber, Roitman, Phillips, Carelli, & Wightman, 2005; Wheeler et al., 2011). In particular, several studies from our lab have recently demonstrated that delivery of rewarding taste stimuli, such as a sucrose solution or sucrose pellet, evokes phasic increases in the concentration of NAc DA within a few hundred milliseconds (Beeler et al., 2012; Roitman et al., 2008). Delivery of sucrose pellets on a random time schedule elicit increases in the concentration of NAc DA (Beeler et al., 2012; Brown, McCutcheon, Cone, Ragozzino, & Roitman, 2011). Indeed, intraoral delivery of rewarding sucrose solutions, which require no action from the animal other than ingestion, also result in increases in the phasic release of DA (Roitman et al., 2008; Wheeler et al., 2011). Fluctuations in phasic DA release are also seen in response to cocaine administration. Non-contingent cocaine administration increases both the frequency and amplitude of NAc phasic DA release (Addy et al., 2010; Stuber et al., 2005).

Further evidence of the role of phasic DA in primary reward arises from intracranial self-stimulation (ICSS) experiments. Olds and Milner (1954) found that rats will readily perform behaviors that result in the delivery of current to their brain via a chronically implanted stimulating electrode. DA has been demonstrated to play a primary role in the rewarding effects of ICSS. Electrode placements in close proximity to DA cell bodies and axons are highly effective in supporting behavior directed towards ICSS (Fibiger, LePiane, Jakubovic, & Phillips, 1987). In fact, effective ICSS electrode placements excite DAergic fibers resulting in phasic DA

release in the striatum (McBride, Murphy, & Ikemoto, 1999) and NAc (Owesson-White, Cheer, Beyene, Carelli, & Wightman, 2008). Administration of DA agonists such as apomorphine facilitate the acquisition of ICSS and increase self-stimulation rates (Liebman & Butcher, 1973). In sharp contrast, DA antagonists such as haloperidol and pimozide or lesions of the DA system using 6-OHDA disrupt ICSS behavior (Fibiger et al., 1987; Fibiger & Phillips, 1974; Liebman & Butcher, 1973; Lippa, Antelman, Fisher, & Canfield, 1973). These reductions in ICSS responding are not simply an indication of impaired locomotor activity as animals continue to respond normally for ICSS if the stimulation current is increased (Ikemoto & Panksepp, 1999). Rather, interference with the DA system appears to reduce the rewarding quality of ICSS. Phasic changes in DA signaling and release following taste stimuli, drug, and stimulation support DA as an integral part of primary reward, but also serve to highlight the importance of utilizing a technique capable of capturing these brief, subsecond changes in DA.

I. Dopamine and Reward-Related Learning

As previously discussed, DA has been theorized to mediate a number of aspects in goal-directed behavior. One particularly prominent theory of the function of DA arose around 15 years ago and postulated that phasic fluctuations in DA are essential in signaling not only rewarding stimuli, but the expectation of reward in what is termed a “reward prediction error” (RPE). RPE is theorized to be a teaching signal to modify expectations, adjust behavior accordingly, and energize associative learning. The delivery of an unexpected reward will elicit a phasic increase in the firing of DA neurons that is time locked to reward presentation (Mirenowicz & Schultz, 1996; Pan et al., 2005; Schultz et al., 1997; Ungless, Magill, & Bolam, 2004; Ungless, 2004). If the delivery of the reward becomes predicted, such as through consistent pairing of a cue with reward delivery, the timing of these phasic events tends to shift

from being time locked to reward delivery, to the presentation of the reward-predictive cue (Pan et al., 2005; Schultz, 1998). However, cues that are not associated with reward delivery evoke markedly reduced, if any, changes in the firing rate of DA neurons (Guarraci & Kapp, 1999; Waelti, Dickinson, & Schultz, 2001). Phasic changes in DA neuronal firing are finely tuned to indicate whether the obtained reward is more than, less than, or as predicted (Bayer & Glimcher, 2005; Schultz et al., 1997; Schultz, 1998). If a reward-predictive cue is presented, and the reward is delivered as expected, the phasic firing of dopaminergic neurons will be time-locked to the presentation of the cue, but the DA neurons will maintain baseline levels of firing when the actual reward is presented because its delivery is fully predicted. If, after presentation of the cue, the reward is omitted, the firing rate of dopaminergic neurons will be suppressed at the time when the reward should have been delivered as the animal received less than expected (Schultz et al., 1997; Schultz, 1998). The pattern of activity of DA neurons support their role in providing targets with a reward prediction error signal which has been hypothesized to drive associative learning (Schultz et al., 1997; Waelti et al., 2001).

The signaling of a RPE by dopaminergic neurons is strongly supported by FSCV recordings demonstrating that fluctuations in DA concentration in the NAc occur in response to stimuli that evoke phasic changes in the firing rate of DA neurons (Brown et al., 2011; Day et al., 2007; Phillips et al., 2003; Roitman et al., 2004; Stuber et al., 2008). In particular, similar to the results found by Schultz and colleagues (1997), unexpected rewards evoke phasic increases in the release of DA within the NAc (Addy et al., 2010; Beeler et al., 2012; Brown et al., 2011; Roitman et al., 2008; Wheeler et al., 2011). Additionally, consistent pairing of a predictive cue with reward delivery results in phasic rises in DA concentration time-locked to the onset of the cue (Day et al., 2007; McCutcheon, Beeler, & Roitman, 2012; Stuber et al., 2008). In sharp

contrast, cues that are not predictive of reward delivery result in markedly reduced changes in phasic DA concentration (Day et al., 2007; Stuber et al., 2008). Modulations in the firing rate and release of DA during reward and reward-predictive cues support DAs participation in a teaching signal about reward prediction.

The role of DA in reward-related learning is further demonstrated during acquisition of goal-directed behaviors. NAc DA lesions or blockade of DA receptors impairs acquisition of approach behavior in appetitive Pavlovian paradigms (Di Ciano, Cardinal, Cowell, Little, & Everitt, 2001; Parkinson et al., 2002). Selective antagonism of D_1 receptors impairs the acquisition, but not the expression once learned, of flavor preference in a Pavlovian paradigm (Touzani, Bodnar, & Sclafani, 2008). While high doses of D_1 receptor antagonists will impair the acquisition and expression of goal-directed behaviors, smaller doses of co-administered D_1 and NMDA receptor antagonists – neither of which has an effect when administered alone – impair the acquisition of a lever pressing paradigm suggesting that the co-activation of D_1 receptors and NMDA receptors within the NAc are critical for acquisition of instrumental learning (Smith-Roe & Kelley, 2000).

Further evidence of the role of DA in reward-related learning arises from an experiment utilizing the novel technique of optogenetics. As mentioned earlier, optogenetics utilizes adeno-associated virus infusion into select populations of neurons in order to render these cell populations light sensitive. Following virus expression, fiber optic lights can be lowered into the brain to directly activate or inhibit these neuronal populations. Tsai and colleagues (2009) directly infected DAergic neurons in the VTA with light-sensitive channels. Phasic activation of the DA neurons not only resulted in increases in phasic DA release in the NAc, but also conditioned a place preference to the chamber in which phasic activation took place (Tsai et al.,

2009). Collectively, this work suggests that DA, specifically phasic DA, greatly influence reward-related learning.

J. Dopamine and Goal-Directed Behavior

Though DA encodes primary rewards and cues that predict reward availability, operant goal-directed behaviors are also critically modulated by DA (Carlezon & Thomas, 2009; Ikemoto, 2007; Kelley et al., 2005; Kiyatkin, 2002; Salamone, 1996). Cues that predict the ability work for food, drug, or stimulation reward elicit increases in the concentration of NAc DA (Brown et al., 2011; Owesson-White et al., 2009, 2008; Phillips, Robinson, et al., 2003; Phillips, Stuber, Heien, Wightman, & Carelli, 2003; Stuber et al., 2005). Indeed, the concentration of DA has been shown to increase in the hundreds of milliseconds immediately preceding an operant response for rewarding food, drug, and stimulation, suggesting that DA may play a role in the initiation of goal-directed behaviors (Phillips, Stuber, et al., 2003; Roitman et al., 2004; Stuber et al., 2005).

Further evidence for the role of DA in goal-directed behaviors arises from administration of DA agonists and antagonists. Manipulations of DA via systemic injections or intra-accumbens infusions normally do not affect the selection of a preferred food source when given free choice (Salamone et al., 1991). However, following administration of a DA antagonist, animals are less willing to work in an operant paradigm to obtain a highly palatable food source when a less preferred food is freely available (Aberman, Ward, & Salamone, 1998; Nowend, Arizzi, Carlson, & Salamone, 2001; Salamone et al., 1991; Salamone, Arizzi, Sandoval, Cervone, & Aberman, 2002). Similarly, selective destruction of the afferent DA pathway to the NAc with 6-OHDA lesions, reduces performance in operant paradigms compared to non-lesioned animals (Aberman & Salamone, 1999; Aberman et al., 1998; Hamill, Trevitt, Nowend, Carlson, & Salamone, 1999;

Ikemoto & Panksepp, 1999; Salamone et al., 1991; Salamone, Wisniecki, Carlson, & Correa, 2001). Given these results, goal-directed operant tasks appear to be remarkably sensitive to manipulations in DA signifying an important function of NAc DA in motivation and goal directed behaviors.

K. Dopamine and Action Selection

Despite the strong role DA has been shown to play in signaling a RPE and goal-directed behavior, a separate line of evidence supports the role of the basal ganglia, and the dopaminergic projections to the NAc and striatum in particular, as critical influences on the selection of certain actions at the expense of others (Robbins & Sahakian, 1983). DA is theorized to influence action selection in general, and has been shown to mediate the selection of and switching between optimal survival strategies during stressful situations (Cools, 1980; Redgrave et al., 1999). Yun and colleagues (2004) found that animals trained to approach and respond during the presentation of a discriminative stimulus (DS) showed impaired and slowed responding following DA blockade in the NAc. Though large doses of DA antagonists can globally suppress motor behavior, this was not the result of a gross motor deficit as the animals were still capable of performing a simple fixed ratio 1 paradigm. Instead, the animals displayed an impaired ability to select the correct action following interference with DA signaling (Yun, Nicola, et al., 2004). Even more revealing, Morris and colleagues (2006) found that the firing rate of midbrain dopaminergic neurons accurately signaled the future action choices of primates during a paradigm aimed at obtaining a probabilistic reward.

Changes in DA concentration are tightly correlated with the operant responses involved in motivated behavior for a variety of reinforcers including food (Roitman et al., 2004), brain stimulation reward (Cheer et al., 2007; Cheer, Heien, Garris, Carelli, & Wightman, 2005), and

drugs of abuse (Phillips, Stuber, Heien, Wightman, & Carelli, 2003). Phasic increases in the concentration of DA within the NAc have been observed immediately preceding operant responses for food (Cacciapaglia, Saddoris, Wightman, & Carelli, 2012; Roitman et al., 2004), drug (Owesson-White et al., 2009; Phillips, Stuber, et al., 2003; Stuber, Roitman, Phillips, Carelli, & Wightman, 2005), and intracranial self-stimulation (Owesson-White et al., 2008) reward. Animals that trained to make an operant responses for intravenous cocaine can be biased towards greater rates of responding during their refractory period following electrical stimulation of the DA cell bodies in the ventral tegmental area (Phillips, Stuber, et al., 2003). The involvement of NAc phasic DA in signaling operant behaviors indicates that not only does phasic DA seem to be involved in signaling a RPE, but DA may also be involved in the selection of actions or switching between actions during operant goal-directed behaviors.

L. Behavioral Paradigms to Dissociate Reward Prediction from Action Selection

The NAc and its afferents have a rich history of involvement in multiple aspects of goal-directed behavior including the signaling of primary reward and reward-predictive cues, but also the selection of certain behaviors at the expense of others and promotion of behavioral approach. Numerous studies have claimed that these afferents are exclusively involved in either reward-prediction or action selection, however the fact remains that these studies are in fact confounded as both rewards and reward-predictive stimuli tend to encourage approach and interaction. As both the NAc and phasic DA signaling have been shown to play a role in action selection, the possibility remains that modulations in NAc activity and phasic DA release in particular following the presentation of an unexpected reward or reward-predictive cue are not the result of a reward prediction signal, but are instead due to the selection and generation of an action. Even cues presented in Pavlovian conditioning, which require no overt response from the animal in

order for reward to be delivered, generate motor responses (Brown & Jenkins, 1968; Peterson et al., 1972). Reward and reward-predictive cues modulate the firing rate of NAc neurons (Ambroggi et al., 2011; Day et al., 2006; Day et al., 2011; Roitman et al., 2005). However, these same reward-predictive cues also encourage approach and interaction while cues that are not predictive of reward delivery do not (Ambroggi et al., 2011; Day et al., 2006). Thus, the role of the NAc and its afferents in motivated behavior is unclear as the paradigms used to investigate them lack the ability to disentangle reward prediction from action selection.

In order to examine the role of the NAc and its afferents on goal-directed behavior, the current work utilizes two unique Go/NoGo (GNG) paradigms to dissociate the effects of reward/reward-predictive cues from those of action selection on phasic DA signaling. While all GNG paradigms reward correct responses on Go trials, our first Go+/NoGo+ paradigm is unique from other GNG paradigms in its treatment of NoGo responses. Some alternative versions of GNG tasks choose to attach punishing stimuli to incorrect NoGo responding such as the delivery of a foot shock or bitter quinine solution (Mulder, Nordquist, Örgüt, & Pennartz, 2003; Setlow, Schoenbaum, & Gallagher, 2003). Still others have no programmed response when the animals successfully inhibit on NoGo trials (Anker, Zlebnik, Gliddon, & Carroll, 2009; Bouret & Sara, 2004; Kalenscher et al., 2005; Kay, Krysiak, Barlas, & Edgerton, 2006; Villa, Tetko, Hyland, & Najem, 1999). In the Go+/NoGo+ paradigm used here, animals were trained to discriminate between two separate sets of cues that predicted the ability to engage in certain behaviors, both of which resulted in reward if performed correctly, making this a symmetrical Go+/NoGo+ paradigm. Following the presentation of a set of reward-predictive cues (Go+), animals were trained to make an operant response within four seconds to receive a sucrose pellet reward. When presented with a second set of cues (NoGo+), the animals were trained to refrain from

making an operant response on the same lever in order to obtain a sucrose reward. Therefore, after training there were two cues equally predictive of reward, however they required different responses to obtain this reward. Correct trials (responses following Go+/withholding response following NoGo+ cues) were rewarded with a 45 mg sugar pellet and incorrect trials (withholding response following Go+/press following NoGo+ cue) were punished with a timed pause in the experiment (time out; see Figure 1.1).

Additionally, to further assess disentangle the effects of reward-prediction and the selection of different patterns of action on phasic DA signaling, we created a second paradigm (Go+/NoGo-) in which the cues differed not only in the pattern of actions engaged in, but also in the reward-predictive nature of the cues. Animals were trained to discriminate between two visually distinct sets of cues. The first set of cues (Go+) signaled the ability to respond for a sucrose pellet reward. Failing to respond following Go+ cues resulted in a 40s time out period. A second set of cues was never paired with reward delivery (NoGo-) and responding following these cues once again resulted in a time out period. While no reward was obtained from correctly withholding responding following the NoGo- cue, incorrect responding during the NoGo- still resulted in the same time out punishment (see Figure 1.2). In combination with our Go+/NoGo+ paradigm, this task allowed us to separate out the effects of reward prediction and action selection, as signaled by the cues, on phasic DA release.

M. <u>Experimental Aims</u>

The goal of the current experiments was to dissociate the role of the NAc and its afferents with respect to reward-prediction and the selection of different patterns of action using our novel Go+/NoGo+ and Go+/NoGo- paradigms. First, using FSCV we assessed the role of phasic DA release real-time within the NAc core on various aspects of Go+/NoGo+ responding. We

hypothesized that if DA activity within the NAc signals reward prediction, there would be no difference in phasic DA release to the Go+ and NoGo+ cues as they both signal reward availability. However, if DA signaling influences the pattern of actions being selected, we would see differential signaling to the Go+ and NoGo+ cues as an operant response is required to obtain the reward following a Go+ cue, while the NoGo+ cue requires the withholding of an operant response. Our symmetrical Go+/NoGo+ paradigm provided the opportunity for the first time to disentangle the effects of reward prediction and action selection on phasic DA release within in the NAc.

Second, we monitored changes in phasic DA release in the NAc core in real time during Go+/NoGo- responding. If DA activity within the NAc signals reward prediction, there would be an increase in phasic DA release to the Go+ cues, but no change in phasic DA release to the NoGo- cue as it does not signal reward availability. If DA signaling influences the pattern of actions being selected, we would also see differential signaling to the Go+ and NoGo+ cues as different patterns of action are required to avoid the time out period. This paradigm, coupled with Go+/NoGo+, provides us with the tools to evaluate changes in phasic DA release during different aspects of goal-directed behavior.

In addition to receiving a dense dopaminergic projection from the VTA, the NAc receives numerous other afferent inputs from both glutamatergic and GABAergic sources. As the NAc has been repeatedly shown to play a role in the signaling of rewards and in the execution of goal-directed behaviors, we sought to tease apart the role of these inputs in mediating Go+/NoGo+ behavior. By pharmacologically manipulating dopamine, GABA, and glutamate activity within the NAc core we aimed to assess the role of these inputs in the mediation of operant responding and behavioral inhibition.

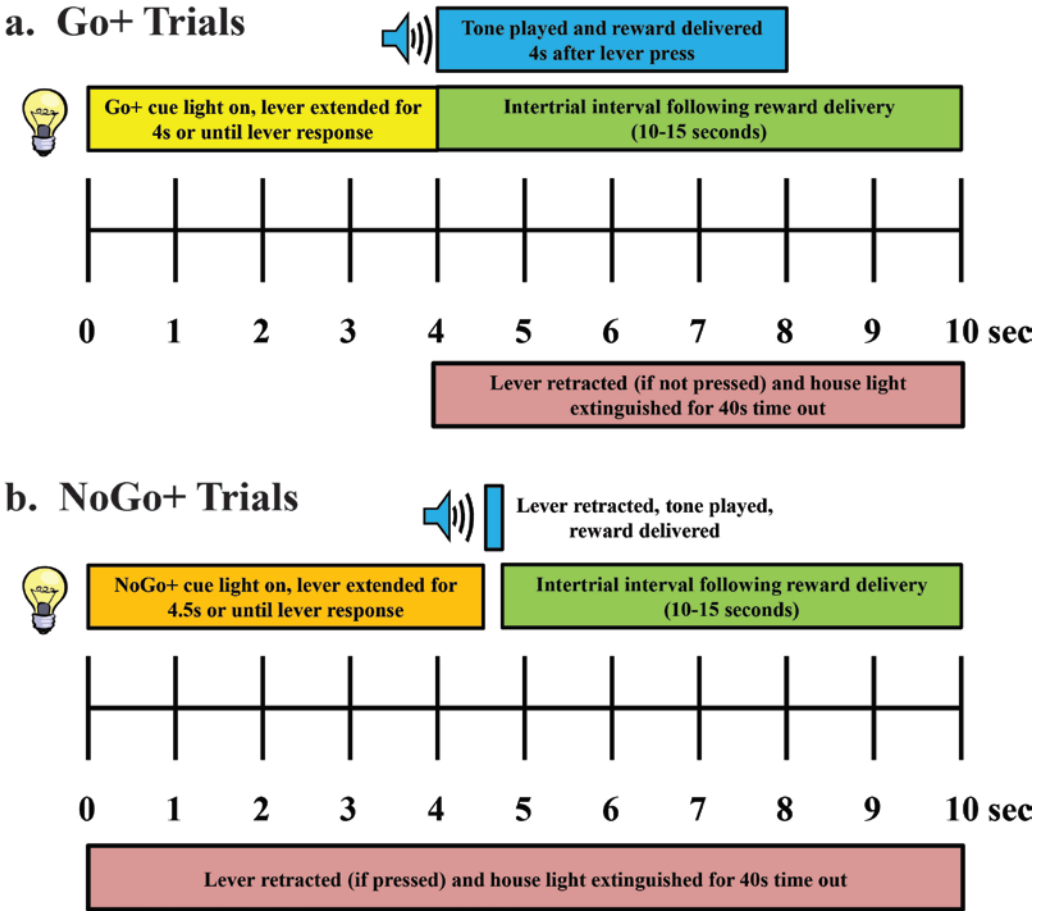

Figure 1.1: A schematic diagram of the Go+/NoGo+ behavioral task. Animals were pseudorandomly presented with one of two trial types (Go+ or NoGo+). Go+ trials (a) were associated with a cue light presentation and lever extension. Operant responses within 4s led to lever retraction, and a tone and reward delivery 4 seconds later. NoGo+ trials (b) were associated with a spatially distinct cue light but the same lever extension. Withholding operant responses for 4.5s led to immediate lever retraction, tone and reward delivery. Following rewarded trials there was a 10-15s inter-trial interval. Improper responses on either trial (withholding following Go+ cue or responding following NoGo+ cue) were punished with a 40s timeout in which the house lights were extinguished.

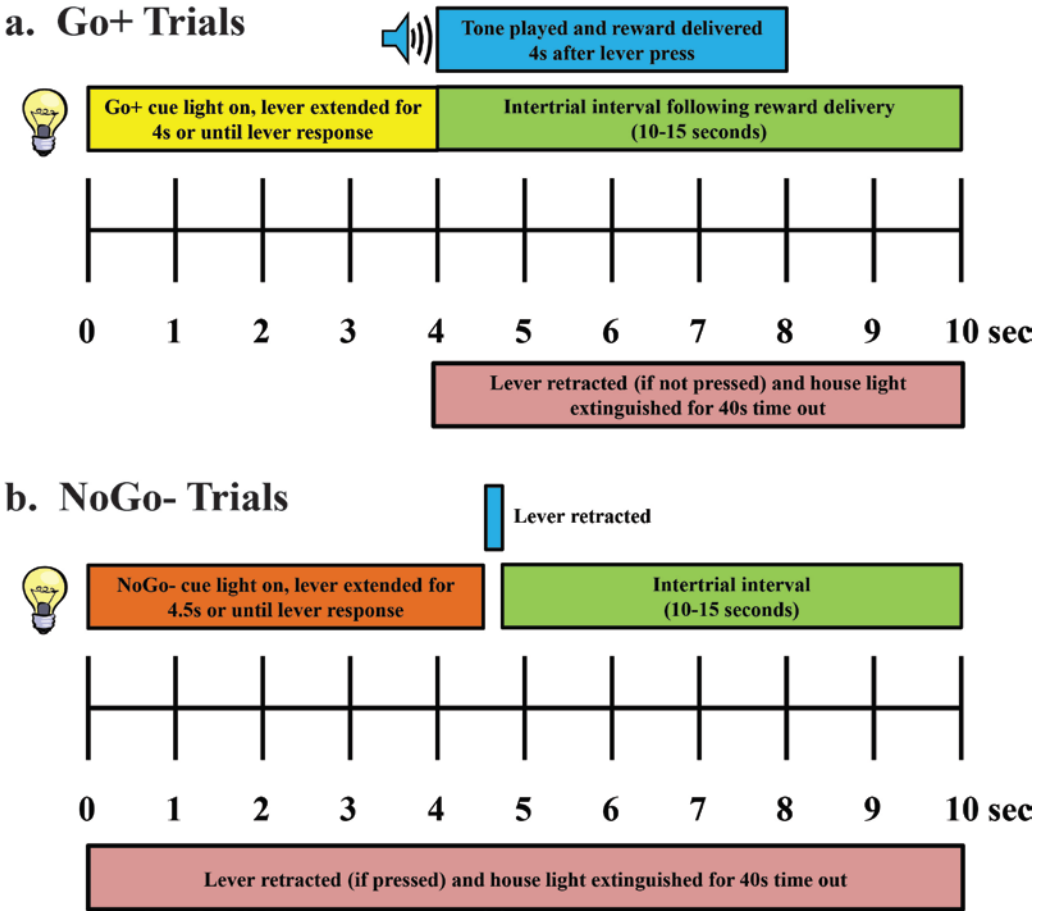

Figure 1.2: A schematic diagram of the Go+/NoGo- behavioral task. Animals were pseudorandomly presented with one of two trial types (Go+ or NoGo-). Go+ trials (a) were associated with a cue light presentation and lever extension. Operant responses within 4s led to lever retraction, and a tone and reward delivery 4 seconds later. Following the reward was a 10-15s inter-trial interval. NoGo- trials (b) were associated with a spatially distinct cue light but the same lever extension. Withholding operant responses for 4.5s led to immediate lever retraction and the start of the inter-trial interval. Improper responses on either trial (withholding following Go+ cue or responding following NoGo- cue) were punished with a 40s timeout in which the house lights were extinguished.

Chapter II

Nucleus Accumbens Phasic Dopamine Responses to Symmetrical Go+/NoGo+ and Asymmetrical Go+/NoGo- Paradigms

A. Introduction

The NAc and specifically phasic DA signaling within the NAc have been shown to be involved in signaling reward and reward-predictive cues. Numerous studies have demonstrated that rewarding stimuli trigger phasic increases in the firing rate of dopaminergic neurons (Hyland et al., 2002; Mirenowicz & Schultz, 1996; Schultz et al., 1997; Ungless, 2004). Similarly, work from our lab has demonstrated that the delivery of rewarding taste stimuli, such as sucrose solutions or sugar pellets, result in an increase in the phasic release of DA in the NAc within a few hundred milliseconds (Brown et al., 2011; McCutcheon, Beeler, et al., 2012; Roitman et al., 2008). Phasic DA release is thought to encode cues that signal reward availability and fluctuations in NAc DA concentration occur in response to stimuli that evoke phasic changes in the firing rate of DA neurons (Brown et al., 2011; Day et al., 2007; Phillips, Robinson, et al., 2003; Roitman et al., 2004; Stuber et al., 2008). Cues paired with reward delivery in Pavlovian paradigms come to elicit phasic increases in the concentration NAc DA (Day et al., 2007). Similar to purely Pavlovian paradigms, cues that predict the ability to make operant responses for reward result in phasic rises in DA concentration time-locked to the onset of the cue (Brown et al., 2011).

However, the role of phasic DA in signaling the reward-predictive nature of these cues is confounded by the fact that both rewards and reward-predictive stimuli tend to encourage approach and interaction. As phasic DA signaling has also been shown to play a role in other aspects of goal-directed behavior and action selection, the possibility remains that modulations in

phasic DA release following the presentation of reward or reward-predictive cues are not the result of a reward prediction signal, but are instead due to the generation of an approach behavior.

Even cues presented in Pavlovian conditioning which require no response from the animal in order to obtain the reward, generate motor responses (Brown & Jenkins, 1968; Peterson et al., 1972). Waelti and colleagues (2001) found that reward-predictive cues (CS+) not only result in phasic increases in the firing rate of DA neurons, but also generate anticipatory behavior when the animals know a reward is forthcoming. Cues not paired with reward delivery (CS-) did not modulate the firing rate of DA neurons or generate anticipatory motor behavior. Similarly, reward-predictive cues result in robust increases in phasic DA release in the NAc in addition to generating approach behavior aimed at the cue. Cues not paired with reward delivery (CS-) result in attenuated changes in phasic DA release compared to the CS+ in addition to generating less approach (Day et al., 2007). It remains unclear whether the reduced firing rate of DA neurons and phasic release of DA to the CS- is because the cue does not predict reward, or if it results from a lack of approach behavior. Cues are frequently employed to signal the opportunity to engage in an operant response to obtain a food, drug, or electrical stimulation reward (Brown et al., 2011; Cacciapaglia et al., 2012; Owesson-White et al., 2008; Phillips, Stuber, et al., 2003; Roitman et al., 2004; Stuber et al., 2005). However, these experiments are all confounded by the fact that observed changes in DA cannot be conclusively tied to the reward-predictive cues nor to the operant responses that follow them. The inability of current behavioral paradigms to elucidate the roles of the NAc and phasic DA signaling in motivated behavior emphasizes the importance of utilizing a paradigm that has the ability to separate out the effects of reward and reward-predictive cues from those of the pattern of action being selected.

To elucidate whether changes in phasic DA release are related to the reward-predictive nature of cues or the behavioral pattern of action being selected, we utilized a novel Go+/NoGo+ paradigm. Animals were trained to discriminate between two separate sets of cues that instructed differential sets of behaviors, both of which resulted in reward if performed correctly. One set of cues, termed Go+ cues, signaled that animals needed to make an operant response to receive a sugar pellet reward. In contrast, NoGo+ cues indicated animals needed to refrain from making an operant response on the same lever in order to obtain a sugar pellet reward. This paradigm yielded two cues that were equally predictive of reward, but required different responses to obtain.

A second paradigm (Go+/NoGo-) was employed to further dissociate reward prediction from the behavioral pattern of actions selected. While similar to the Go+/NoGo+ paradigm, a fundamental difference is that in addition to the Go+ cue, a second non-reward-predictive cue, the NoGo-, signaled that the animals needed to withhold responding in order to avoid a timeout. While no reward was obtained from correctly withholding responding following the NoGo- cue, incorrect responding during the NoGo- still resulted in the same timeout punishment. In combination with the Go+/NoGo+ paradigm, this task allowed us to dissociate the correlation of phasic DA with reward prediction versus the selection of distinct patterns of action. The Go+ cues in both paradigms were reward predictive cues that required an operant response to obtain reward. The NoGo-, like the NoGo+ cue, required the inhibition of action, HOWEVER there was no reward following the NoGo-.

We recorded changes in phasic DA release in the NAc core in real time using FSCV while animals performed the Go+/NoGo+ and Go+/NoGo- paradigms. The NAc core was selected as our recording site as our lab previously demonstrated that reward-predictive cues

evoke phasic DA release in the core, but not the shell, of the NAc (Brown et al., 2011). If DA activity within the NAc signals reward prediction, there will be no difference in phasic DA release to the Go+ and NoGo+ cues as both signal the opportunity to receive a reward. Additionally, when recording during the Go+/NoGo- paradigm there will be a difference in phasic DA signaling to Go+ and NoGo- cues as only one is predictive of reward delivery. However, if DA signaling is correlated with distinct patterns of action, there will be differential signaling to the Go+ and NoGo+ cues as an operant response is required to obtain the reward following a Go+ cue, while the NoGo+ cue requires the withholding of an operant response. Similarly, there will be differential signaling to Go+ and NoGo- cues as only one cue requires an operant response. Thus, our symmetrical Go+/NoGo+ paradigm and asymmetrical Go+/NoGo- paradigms permits, for the first time, the opportunity to dissociate NAc phasic DA signaling in response to reward prediction versus the pattern of actions selected.

B. Experimental Methods

1. Subjects

Male Sprague-Dawley rats (n = 37; Charles River Laboratories, Portage, MI) weighing approximately 300-400 g were used for these experiments. Animals were individually housed and maintained on a 12 h/12 h light/dark cycle in a temperature (22°C) and humidity (30%) controlled environment. All experiments were conducted between 8:00 am and 6:00 pm. Animals received ad libitum access to water and were maintained at no less than 90% of free feeding weight during experimentation (10-20 g/day; LabDiet) based on task performance in addition to consuming approximately 5-7 grams of sugar (45mg each Bio-Serve Precision Pellets; Frenchtown, NJ) during daily training and testing sessions. All animals were treated

according to the guidelines recommended by the Animal Care Committee at the University of Illinois at Chicago.

2. Apparatus

Each fast-scan cyclic voltammetry chamber was a standard experimental operant chamber (Med Associates, Inc.; St. Albans, VT) equipped with two levers, two cue lights, a pellet dispenser, a white noise generator, a tone generator, and a house light in addition to a removable headstage attached to an electric swivel (Crist Instrument Company, MD, USA) to permit free movement throughout the chamber.

3. Go+/NoGo+ Task

After a one week acclimation period, animals were food restricted to approximately 90% of their free feeding weight. Food restricted animals were given approximately 20 45mg sucrose pellets for one day in their home cages. For the following two days animals were placed in the operant chamber. Sucrose pellets were delivered to the food receptacle on a variable interval schedule (every 60, 90, or 120s) for 45 minutes in order to train the animal that sucrose pellets were available (magazine training). Following magazine training, animals were shaped to an active lever on a fixed ratio 1 schedule. The active lever was alternated on a daily basis to encourage responding on both levers. Stable operant responding on the active lever – defined as 100 sucrose pellets obtained on two consecutive days within 30 minutes – led to animals being shifted to the Go+/NoGo+ training programs. Go+/NoGo+ training took place in three distinct phases.

Phase 1. The goal of phase 1 of Go+/NoGo+ training was for the animal to respond on the Go+ lever within 4 seconds of cue presentation and withhold responding on the NoGo+ lever for 4.5 seconds. Phase 1 training began with assigning a “Go+” lever (right or left). On

approximately 75% of the 150 trials, a cue light positioned over the Go+ lever was illuminated and the Go+ lever simultaneously extended into the chamber. Animals were assigned a “Go+” lever (right or left). Operant responses on these trials were rewarded with a sucrose pellet while failures to press resulted in a 40 second timeout. On the remaining 25% of the trials, a cue light positioned over the spatially distinct “NoGo+” lever was illuminated and the NoGo+ lever simultaneously extended into the chamber. Responses on these trials were punished with a 40 second time out, however correct withholding of responding had no programmed response. Trials were separated by an average inter-trial interval of 12.5 seconds, and ranged between 10 and 15 seconds. At the beginning of phase 1 training, the Go+ lever was extended for 15 seconds, and the NoGo+ lever only 2 seconds. Lever extension times were adjusted daily based on performance until animals responded on the Go+ lever within 4 seconds and successfully avoided responding on the NoGo+ lever for 4.5 seconds. Failure to respond on the Go+ lever 20 times during the behavioral session resulted in session termination. Following two consecutive days of successful completion of 150 trials with the Go+ lever extended for 4 seconds and NoGo+ lever extended for 4.5 seconds, animals were advanced to phase 2 of training.

Phase 2. The goal of phase 2 of Go+/NoGo+ training was for the animals to learn that correctly withholding an operant response on NoGo+ trials resulted in reward. An auditory white noise cue was randomly paired with either the Go+ or NoGo+ lever (and associated cue light), and a tone was paired with pellet delivery. Correct withholding of responses on the NoGo+ lever was reinforced with a sucrose pellet during this phase. Failure to respond on the Go+ lever 20 times during these behavioral sessions resulted in session termination. Following two consecutive days of successful completion of 150 trials, animals were advanced to phase 3 of training.

Phase 3. The goal of phase 3 of Go+/NoGo+ training was to shift animals to a one lever task in which they responded on the lever following Go+ cues and withheld responding at least 50% of the time following NoGo+ cues. Animals were shifted from a two lever task to a single lever (the Go+ lever), however cue lights and white noise remained associated with the same trial types. During this phase animals learned to press the lever only following the Go+ cues and to withhold lever responding during the NoGo+ cues. Successful completion of Go+/NoGo+ training was defined as completion of 150 trials (meaning no more than 19 errors on Go+ trials), and successful withholding of responding on greater than 50% of NoGo+ trials. Following two consecutive days of meeting these criteria, animals were placed on ad libitum food in preparation for surgery.

Post-Operative Training. Following recovery of pre-surgery body weight, animals were food restricted to 90% of free-feeding body weight and retrained on phase 3 of the Go+/NoGo+ paradigm. After two days of successful completion of phase 3 criteria, animals were deemed ready for testing.

4. <u>Go+/NoGo- Task</u>

After a one week acclimation period, animals were food restricted to approximately 90% of their free feeding weight. Food restricted animals were given approximately 20 45mg sucrose pellets for one day in their home cages. For the following two days animals were placed in the operant chamber. Sucrose pellets were delivered to the food receptacle on a variable interval schedule (every 60, 90, or 120s) for 45 minutes in order to train the animal that sucrose pellets were available (magazine training). Following magazine training, animals were shaped to an active lever on a fixed ratio 1 schedule. The active lever was alternated on a daily basis to encourage responding on both levers. Stable operant responding on the active lever – defined as

100 sucrose pellets obtained on two consecutive days within 30 minutes – led to animals being shifted to the Go+/NoGo- training programs. Go+/NoGo- training took place in three distinct phases.

Phase 1. The goal of phase 1 of Go+/NoGo- training was for the animal to respond on the Go+ lever within 4 seconds of cue presentation and withhold responding on the NoGo- lever for 4.5 seconds. Phase 1 training began with assigning a "Go+" lever (right or left). On approximately 75% of the 150 trials, a cue light positioned over the Go+ lever was illuminated and the Go+ lever simultaneously extended into the chamber. Animals were assigned a "Go+" lever (right or left). Operant responses on these trials were rewarded with a sucrose pellet while failures to press resulted in a 40 second timeout. On the remaining 25% of the trials, a cue light positioned over the spatially distinct "NoGo-" lever was illuminated and the NoGo- lever simultaneously extended into the chamber. Responses on these trials were punished with a 40 second time out, however correct withholding of responding had no programmed response. Trials were separated by an average inter-trial interval of 12.5 seconds, and ranged between 10 and 15 seconds. At the beginning of phase 1 training, the Go+ lever was extended for 15 seconds, and the NoGo- lever only 2 seconds. Lever extension times were adjusted daily based on performance until animals responded on the Go+ lever within 4 seconds and successfully avoided responding on the NoGo- lever for 4.5 seconds. Failure to respond on the Go+ lever 20 times during the behavioral session resulted in session termination. Following two consecutive days of successful completion of 150 trials with the Go+ lever extended for 4 seconds and NoGo- lever extended for 4.5 seconds, animals were advanced to phase 2 of training.

Phase 2. The goal of phase 2 of Go+/NoGo- training was to white noise with either the Go+ or NoGo- cue, and the tone with sucrose pellet delivery. An auditory white noise cue was

randomly paired with either the Go+ or NoGo- lever (and associated cue light), and a tone was paired with pellet delivery. Failure to respond on the Go+ lever 20 times during these behavioral sessions resulted in session termination. Following two consecutive days of successful completion of 150 trials, animals were advanced to phase 3 of training.

Phase 3. The goal of phase 3 of Go+/NoGo- training was to shift animals to a one lever task in which they responded on the lever following Go+ cues and withheld responding at least 50% of the time following NoGo- cues. Animals were shifted from a two lever task to a single lever (the Go+ lever), however cue lights and white noise remained associated with the same trial types. During this phase animals learned to press the lever only following the Go+ cues and to withhold lever responding during the NoGo- cues. Successful completion of Go+/NoGo- training was defined as completion of 150 trials (meaning no more than 19 errors on Go+ trials), and successful withholding of responding on greater than 50% of NoGo- trials. Following two consecutive days of meeting these criteria, animals were placed on ad libitum food in preparation for surgery.

Post-Operative Training. Following recovery of pre-surgery body weight, animals were food restricted to 90% of free-feeding body weight and retrained on phase 3 of the Go+/NoGo- paradigm. After two days of successful completion of phase 3 criteria, animals were deemed ready for testing.

5. Electrodes

The goal of these studies was to measure phasic DA signaling during the performance of each of the tasks described above. Phasic DA signaling was measured using fast scan cyclic voltammetry at carbon fiber electrodes that are fashioned in house. Carbon fiber microelectrodes were constructed as previously described (Heien et al., 2005). A single 5-μm diameter carbon

fiber was aspirated into a glass capillary and pulled in a vertical micropipette puller. Each electrode was examined under an optical microscope to determine if there was a good seal between the carbon fiber and the glass, and then cut to a length of 50-100 μm with a scalpel. Electrodes were loaded into custom-designed micromanipulators (University of Illinois at Chicago Engineering Design Shop) which allowed them to be raised and lowered in micrometer increments. Following fabrication, electrodes were soaked in isopropyl alcohol until use (~2-12 hours).

6. Surgery

On the day prior to surgery, animals were removed from the food restriction regime and given ad libitum access to food. Animals were prepared for voltammetric recording as previously described (Day et al., 2007; Ebner, Roitman, Potter, Rachlin, & Chartoff, 2010; Jones et al., 2010; Brown et al., 2011). Animals were anesthetized with ketamine hydrochloride (100 mg/kg body weight, intraperitoneal) and xylazine hydrochloride (10 mg/kg body weight, intraperitoneal (IP)). Following anesthesia, hair was removed from the animals' heads prior to placement in a stereotaxic frame (Kopf Instruments; Tujenga, CA). The scalp was scrubbed with Betadine and alcohol swabs before a midline incision was made from anterior to posterior. After the skin and membranes were retracted, the skull was leveled between bregma and lambda.

A guide cannula (Bioanalytical Systems; West Lafayette, IN), extending 2.5mm into the brain, was placed dorsal to the NAc core (1.3 mm anterior, 1.5 mm lateral, -2.5mm ventral from bregma). A metal obdurator, cut to extend approximately 1 mm past the end of the cannula, was inserted to occupy the lumen of the cannula until recording. A silver/silver chloride (Ag/AgCl) reference electrode was placed contralateral to the guide cannula in the left forebrain. Stainless

steel skull screws and dental cement were used to secure the guide cannula and reference electrode to the skull.

During surgery, the obdurator was removed and a micromanipulator containing a carbon fiber electrode was inserted into the guide cannula. The electrode was then lowered into the NAc core. A bipolar stimulating electrode (Plastics One, Inc.; Roanoke, VA) was positioned dorsal to the VTA (-5.2 mm posterior, 1.0 mm medial, 7.00 mm ventral) and lowered in 0.2 mm increments until electrically evoked (60 pulses, 60 Hz, 120 µA, 4 ms/phase) DA release was detected via the carbon fiber electrode (see below for details). After optimizing evoked DA release, the stimulating electrode was cemented, the carbon fiber electrode retracted, and the micromanipulator removed and replaced with the obdurator (Day et al., 2007; Hafizi, Kruk, & Stamford, 1990; Heien et al., 2005; Kuhr & Wightman, 1986; Lu, Peters, & Michael, 1998; Roitman et al., 2004; Schultz, 2007; Sunsay & Rebec, 2008; Wightman et al., 1988). Animals were placed under a heat lamp until ambulatory, given subcutaneous fluids, and then returned to their home cages. Post-operative pain was managed by subcutaneous administration of Rimadyl (2.5-5.0 mg). Animals recovered when they reached pre-surgery body weight (~2 days) at which time they were returned to a restricted diet.

7. Fast Scan Cyclic Voltammetry Recordings

Fast-scan cyclic voltammetry (FSCV) procedures used here were performed as previously described (Brown et al., 2011; Ebner et al., 2010; McCutcheon, Beeler, et al., 2012; McCutcheon, Ebner, Loriaux, & Roitman, 2012) and allowed the real-time identification and quantification of extracellular concentrations of electroactive species such as DA. FSCV was performed by altering the voltage of the carbon fiber microelectrode. Specifically, the carbon fiber is held at -400mV relative to the Ag/AgCl reaction at the reference electrode. Periodically,

the voltage is increased at a rate of 400V/s to +1.3V and then decreased to -0.4V (Roitman et al., 2008). This triangle waveform (scan) was repeated at 10Hz. A single triangle waveform generates current due to oxidation and reduction of functional groups that make up the carbon fiber microelectrode (background). This background is highly stable from scan to scan and thus can be subtracted from measurements to reveal moment-to-moment changes in neurochemicals. In the experiments described below, the background (average current at each voltage over 1s) was selected during the 5s baseline recording period before cue presentation on each trial.

Chemical species that are electroactive across the scan will oxidize and reduce at specific voltages and the resultant current can be detected and measured at the surface of the carbon fiber recording electrode (see Figure 2.1a). Changes in current are transduced through the headstage, and recorded on a computer using software written in LabView (National Instruments) (Heien, Johnson, & Wightman, 2004; Hermans, Keithley, Kita, Sombers, & Wightman, 2008; Robinson, Venton, Heien, & Wightman, 2003). Dopamine is electroactive within the scan and is identified by the potentials at which it oxidizes and reduces. At ~0.6V DA undergoes a conformational change into dopamine-*o*-quinone, shedding two electrons in the process which is detected as oxidative current at the carbon fiber electrode. Dopamine-*o*-quinone reduces back to dopamine at ~-0.2V which, again, is detected as a change in current at the electrode surface (see Figure 2.1a). These REDOX reactions at specific voltages can be visualized by plotting the observed current changes against the applied voltage, called a cyclic voltammogram (see Figure 2.1b). This cyclic voltammogram, or CV, serves as the identification signature for dopamine. The magnitude of the current due to DA oxidation is directly proportional to its concentration at the electrode surface (see Figure 2.1c; Heien et al., 2004). Thus, using FSCV, dopamine can be identified and its concentration quantified with high temporal resolution.

8. Go+/NoGo+ Experimental Procedure

On the day of testing, animals were placed in the operant chamber. The obdurator was removed from the guide cannula and a micromanipulator containing a carbon-fiber microelectrode was inserted and locked into place. Animals were connected to a removable headstage via the stimulating, recording, and reference electrodes. The headstage contained the necessary electrical components for application of voltage changes, measurement of resultant changes in current at the electrode surface as well as the delivery of current via the stimulating electrode (see above for details).

The carbon fiber recording electrode was lowered down into the NAc core and allowed to equilibrate for 40 minutes (applied scan rate of 60Hz for 30 minutes followed by 10Hz for 10 minutes) to minimize drift in the background. Following equilibration, dopamine was evoked by stimulating the VTA (24 pulses, 60Hz, 120 μA, 4 ms/pulse) to verify that the recording electrode was in a location capable of measuring DA. Following successful electrical stimulation of DA release, approximately 15 Go+/NoGo+ trials were presented to monitor for changes in phasic DA release in response to task stimuli. If changes were observed in response to task cues, the animals were presented with 150 trials (approximately 112 Go+ trials and 38 NoGo+ trials) while changes in phasic DA signaling were monitored in response to all task cues. If the probe Go+/NoGo+ trials failed to elicit any changes in phasic DA signaling, the recording electrode was lowered 0.3 mm and further probe trials were presented. All electrochemical data were then relayed through the headstage, digitized and recorded on a computer using programs written with LabView software (National Instruments; Heien et al., 2004; Hermans et al., 2008; Robinson et al., 2003). Immediately following voltammetric recordings of Go+/NoGo+ task performance, a series of electrical stimulations (10-24 pulses, 30-60Hz, 120μA, 4 ms/pulse) were taken to use

for principle component analysis (PCA). Training sets were constructed from cyclic voltammograms for dopamine and pH to allow for principal component regression on data collected during the behavioral session as previously described (Brown et al., 2011; Day et al., 2007; Ebner et al., 2010; Heien et al., 2004; McCutcheon, Beeler, et al., 2012; McCutcheon, Ebner, et al., 2012). At the end of the experiment, the carbon fiber electrode was removed from the guide cannula and the obdurator replaced. Animals were disconnected from the headstage and returned to the home cage.

9. Go+/NoGo- Experimental Procedure

Animals were prepared for voltammetric recording as previously described in section 8 (page 42). Approximately 15 Go+/NoGo- trials were presented to monitor for changes in phasic DA release in response to task stimuli. If changes were observed in response to task cues, the animals were presented with 150 trials (approximately 112 Go+ trials and 38 NoGo- trials) while changes in phasic DA signaling were monitored in response to all task cues. If the probe Go+/NoGo- trials failed to elicit any changes in phasic DA signaling, the recording electrode was lowered 0.3 mm and further probe trials were presented. Immediately following voltammetric recordings of Go+/NoGo- task performance, a series of electrical stimulations (10-24 pulses, 30-60Hz, 120μA, 4 ms/pulse) were taken to use for principle component analysis (PCA). Training sets were constructed from cyclic voltammograms for dopamine and pH to allow for principal component regression on data collected during the behavioral session as previously described (Brown et al., 2011; Day et al., 2007; Heien et al., 2004; McCutcheon, Beeler, et al., 2012; McCutcheon, Ebner, et al., 2012). At the end of the experiment, the carbon fiber electrode was removed from the guide cannula and the obdurator replaced. Animals were disconnected from the headstage and returned to the home cage.

10. Data Analysis

Principal Component Analysis (PCA): Post-session stimulations were used to develop a training set for PCA (as described above). Five to 10 background-subtracted cyclic voltammograms for dopamine and extracellular pH were used in the training set for each recording session. The background-subtracted cyclic voltammograms from the training set were reduced by PCA to approximately 3-9 factors, which captured 99.9% of the variance in the training set. These results were used with regression analysis to calculate DA concentration and pH evoked on individual trials of the behavioral paradigms. Changes in current resulting from behaviorally relevant stimuli were converted to concentration based upon calibration factors (1 nA = 66.6 nM for dopamine, and 1 nA = 0.0958 units for pH).

To examine differences in phasic dopamine evoked by behavioral cues, data files were cut to 15 seconds, with 5 seconds before and 10 seconds after the behaviorally relevant stimulus (ex: Go+ cue, NoGo+ cue, NoGo- cue, signal of end of time out). Backgrounds were selected for each individual trial in the 5 seconds before stimulus onset at a location where dopamine was minimally present. PCA analysis was performed on each of these files to extract a dopamine trace for each trial, by ascribing the amount of current attributable specifically to dopamine and a snapshot of the background subtracted color plot was recorded. For each rat, trials of each type (e.g. Go+) were averaged across a behavioral session. Two distinct epochs within the average dopamine concentration traces were examined for further analysis: a baseline epoch (average of 5 seconds prior to cue onset) and a cue epoch (average of 1s after cue onset). We compared epochs across trial types using a two way repeated-measures analysis of variance (ANOVA). Significant differences were followed up with Tukey's HSD post-hoc test. Changes in DA evoked by the cue signaling the end of time out were analyzed using a paired Student's t-test,

Additionally, response latency, defined as the time between cue presentation and operant response, was measured and compared using a paired Student's t-test. Statistical analyses were carried out using Statistica 10 (StatSoft, Inc.; Tulsa, OK) software with a significance level of 0.05.

11. Histological Verification of Electrode Placement

Following completion of the experiment, animals were injected with a lethal dose of sodium pentobarbital (100 mg/kg). Recordings in the NAc core were verified by lowering a stainless steel electrode (A-M Systems #571500, Sequim, WA, USA) into the NAc to the depth where experimental recordings were made. An electrolytic lesion (0.5 nA, 4s) was made. Following the lesions, animals were transcardially perfused with 0.9% phosphate buffered saline followed by a 4% formalin solution. Brains were extracted and stored in 4% formalin before being mounted and frozen in a -20°C cryostat (LEICA CM1850). Coronal sections (50 µm) through the NAc were made and examined for the location of the electrolytic lesion. Brain slices were mounted on gelatin-subbed slides, coverslipped using Permount (Fisher Scientific), and examined under a light microscope (VistaVision). Placements were verified within the NAc using the stereotaxic atlas of Paxinos and Watson (2005).

C. Go+/NoGo+ Results

1. Electrode Placement Verification in the Nucleus Accumbens Core

Electrode placements for all successful recordings (n=6) are shown in Figure 2.2. Voltammetric recordings were confined to the NAc core and located between 1.68 and 2.28mm anterior to bregma. Placements were located between 1.2 to 2.2mm lateral to the midline and from 6.2 to 6.7mm ventral to the surface of the brain.

2. Animals Learn to Accurately Perform the Go+/NoGo+ Paradigm

Rats (n=6) were trained on a symmetrical Go+/NoGo+ paradigm where two cues equally predicted the availability of reward; however, different behavioral patterns were required to obtain these rewards. Following the presentation of a Go+ cue, animals were required to respond on the lever in order to obtain a sucrose reward. However, following the spatially distinct NoGo+ cue, animals were required to withhold responding on the same active lever in order to obtain the sucrose reward. On the day of voltammetric recording animals performed this task with great accuracy, responding following 85.28±6.5% of Go+ cues and withholding responding following 77.32±5.4% of NoGo+ cues. There was no difference in behavioral performance (percent correct) on Go+ trials as compared to NoGo+ trials, $t(5) = 0.93$, *ns*. Response latency (the time between cue presentation and operant response) was evaluated to determine if errors of commission took place faster or slower than correctly performed operant responses. Animals correctly responded following Go+ cues after 1.25±0.24s and incorrectly responded following NoGo+ cues after 0.79±0.10s, but there were no differences in reaction time between trial types, $t(5) = 1.55$, *ns*.

3. Nucleus Accumbens Phasic Dopamine Increases in Response to Go+ and NoGo+ Cues

Comparison of All Go+ and NoGo+ Trials. To dissociate changes in phasic DA evoked reward-predictive cues and the behavioral pattern of action selected, DA concentration traces were aligned to the onset of the Go+ and NoGo+ cues (Time = 0) and representative trials demonstrating an increase in phasic dopamine signaling to both Go+ and NoGo+ cues are presented in Figure 2.3. Go+ (n = 495 trials) and NoGo+ (n = 200 trials) trials were averaged for each animal (Figure 2.4a), separated by trial type, and two distinct behavioral epochs were calculated: baseline epoch (5s prior to cue presentation) and cue epoch (1s following cue

presentation). A two-way ANOVA was calculated examining the effects of trial type (Go+ vs NoGo+) and behavioral epoch (baseline vs cue) on phasic DA release in the NAc core. There was no main effect of trial type on phasic DA concentration with similar amounts of DA evoked by both Go+ and NoGo+ cues, $F(1, 5) = 0.03$, *ns*. Both cues evoked a significant increase in phasic DA as compared to baseline, $F(1, 5) = 11.41$, $p < 0.05$. However, there was no interaction in the effects of trial type and behavioral epoch on phasic DA concentration, $F(1, 5) = 1.02$, *ns* (see Figure 2.4b).

Comparison of Correctly Performed Go+ and NoGo+ Trials. In order to further evaluate the changes in dopamine evoked by Go+ and NoGo+ cues, trials were further separated in correctly (n = 429 trials) and incorrectly (n = 153 trials) performed trials. Trials in which the animal correctly responded following the Go+ cue (Go+ Cue Correct) or correctly withheld following the NoGo+ cue (NoGo+ Cue Correct) were compared to directly evaluate trials in which the cues were equally reward predictive but the animals performed different behavioral patterns. Changes in phasic dopamine evoked during correctly performed trials were aligned to cue presentation (Figure 2.5a). A two-way ANOVA was calculated examining the effects of trial type (Go+ vs NoGo+) and behavioral epoch (baseline vs cue) on phasic DA release in the NAc core. There was no main effect of trial type on phasic DA concentration with similar amounts of DA evoked by both Go+ and NoGo+ cues, $F(1, 5) = 0.13$, *ns*. Both cues evoked a significant increase in phasic DA as compared to baseline, $F(1, 5) = 12.28$, $p < 0.05$. However, there was no interaction in the effects of trial type and behavioral epoch on phasic DA concentration, $F(1, 5) = 0.21$, *ns* (see Figure 2.5b).

Comparison of Randomly Selected Subset of Trials to Equalize Trial Number. In order to further evaluate the changes in dopamine evoked by Go+ and NoGo+ cues, a subset of correctly

performed Go+ and NoGo+ trials were selected for additional analysis. As previously described (see page 34), 75% of trials during a Go+/NoGo+ behavioral sessions are Go+ trials. As Go+ trials are oversampled relative to NoGo+, the previously presented results may be skewed. Therefore, an equal number of trials (n=15) of each type per animal were randomly selected for a total of 90 Go+ trials and 90 NoGo+ trials. Changes in phasic dopamine evoked during correctly performed trials were aligned to cue presentation (Figure 2.6a). A two-way ANOVA was calculated examining the effects of trial type (Go+ vs NoGo+) and behavioral epoch (baseline vs cue) on phasic DA release in the NAc core. There was no main effect of trial type on phasic DA concentration with similar amounts of DA evoked by both Go+ and NoGo+ cues, $F(1, 5) = 0.06$, *ns*. Both cues evoked a significant increase in phasic DA as compared to baseline, $F(1, 5) = 13.49$, $p < 0.05$. However, there was no interaction in the effects of trial type and behavioral epoch on phasic DA concentration, $F(1, 5) = 0.02$, *ns* (see Figure 2.6b).

4. Go+ Cues, Regardless of Future Behavioral Action, Elicit Increases in Nucleus Accumbens Phasic Dopamine

Trials in which the animal correctly responded following the Go+ cue (Go+ Cue Correct; n = 429 trials) or incorrectly withheld (Go+ Cue Error; n = 66 trials) were compared to directly to evaluate differences phasic dopamine release to the same cue on trials in which the animal executed different behavioral patterns. Changes in phasic dopamine evoked by Go+ cues were aligned to cue presentation (Figure 2.7a). A two-way ANOVA was calculated examining the effects of Go+ trial performance (correct vs error) and behavioral epoch (baseline vs cue) on phasic DA release in the NAc core. There was no main effect of trial performance on phasic DA concentration with similar amounts of DA evoked by correctly and incorrectly performed Go+ trials, $F(1, 5) = 0.02$, *ns*. Cues on both correctly and incorrectly performed Go+ trials evoked a

significant increase in phasic DA as compared to baseline, $F(1, 5) = 22.08$, $p < 0.05$. However, there was no interaction in the effects of Go+ trial performance and behavioral epoch on phasic DA concentration, $F(1, 5) = 0.01$, *ns* (see Figure 2.7b).

5. NoGo+ Cues, Regardless of Future Behavioral Action, Elicit Increases in Nucleus Accumbens Phasic Dopamine

Trials in which the animal correctly withheld responding following the NoGo+ cue (NoGo+ Cue Correct; n = 153 trials) or incorrectly responded (NoGo+ Cue error; n = 47 trials) were compared to directly to evaluate differences phasic dopamine release to the same cue on trials in which the animal executed different behavioral patterns. Changes in phasic dopamine evoked by NoGo+ cues were aligned to cue presentation (Figure 2.8a). A two-way ANOVA was calculated examining the effects of NoGo+ trial performance (correct vs error) and behavioral epoch (baseline vs cue) on phasic DA release in the NAc core. There was no main effect of trial performance on phasic DA concentration with similar amounts of DA evoked by correctly and incorrectly performed NoGo+ trials, $F(1, 5) = 0.00$, *ns*. Cues on both correctly and incorrectly performed NoGo+ trials evoked a significant increase in phasic DA as compared to baseline, $F(1, 5) = 12.05$, $p < 0.05$. However, there was no interaction in the effects of NoGo+ trial performance and behavioral epoch on phasic DA concentration, $F(1, 5) = 0.07$, *ns* (see Figure 2.8b).

6. Cues That Predict the End of a Time Out Elicit Increases in Nucleus Accumbens Phasic Dopamine

While every trial is preceded by an inter-trial interval of 10-15s, incorrectly performed Go+ and NoGo+ trials were punished with a 40s time out from the behavioral session in which all lights within the chamber were extinguished. After 40s, the houselight was re-illuminated,

providing a salient cue for the end of the time out period. To evaluate the influence of the end of the timeout on phasic dopamine signaling in the NAc core, changes in phasic dopamine were aligned to the re-illumination of the houselight following a time out (Figure 2.9a). Two behavioral epochs were calculated: baseline (5s prior to cue presentation) and cue (1s following cue presentation). Using a paired Student's t-test, changes in phasic dopamine from baseline to cue epoch were evaluated. Re-illumination of the houselight following a timeout period resulted in an increase in phasic dopamine signaling from baseline to cue epoch (Figure 2.9b; $t(5) = 6.24$, $p < 0.05$).

D. Go+/NoGo- Results

1. Electrode Placement Verification in the Nucleus Accumbens Core

Electrode placements for all successful recordings (n=5) are shown in Figure 2.10. Voltammetric recordings were confined to the NAc core and located between 1.80 and 2.16mm anterior to bregma. Placements were located between 1.5 to 1.8mm lateral to the midline and from 6.4 to 6.7mm ventral to the surface of the brain.

2. Animals Learn to Accurately Perform the Go+/NoGo- Paradigm

Rats (n=5) were trained on an asymmetrical Go+/NoGo- paradigm where two cues differentially predicted the availability of reward, but still required similar patterns of action as the previously described Go+/NoGo+ paradigm. Following the presentation of a Go+ cue, animals were required to respond on the lever in order to obtain a sucrose reward. However, following the spatially distinct NoGo- cue, animals were required to withhold responding on the same active lever in order to avoid a 40s timeout. No reward was associated with responding following NoGo- cues. On the day of voltammetric recording animals performed this task with great accuracy, responding following 97.38±1.3% of Go+ cues and withholding responding

following 78.66±6.7% of NoGo- cues. There was no difference in behavioral performance on Go+ and NoGo- trials, $t(4) = 2.46$, *ns*. Response latency (the time between cue presentation and operant response) was evaluated to determine if errors of commission took place faster or slower than correctly performed operant responses. Animals correctly responded following Go+ cues within 0.75±0.03s and incorrectly responded following NoGo- cues within 1.04±0.10s, but there were no differences in reaction time between trial types, $t(4) = 2.37$, *ns*.

3. Nucleus Accumbens Phasic Dopamine Selectively Increases in Response to Go+ and Not NoGo- Cues

Comparison of All Go+ and NoGo- Trials. To dissociate changes in phasic DA evoked reward-predictive cues and the behavioral pattern of action selected, DA concentration traces were aligned to the onset of the Go+ and NoGo- cues (Time = 0) and representative trials demonstrating an increase in phasic dopamine signaling to the Go+ cue, but not NoGo- cue, are presented in Figure 2.11. Go+ (n = 516 trials) and NoGo- (n = 156 trials) trials were averaged for each rat (Figure 2.11a), separated by trial type, and two distinct behavioral epochs were calculated: baseline epoch (5s prior to cue presentation) and cue epoch (1s following cue presentation), and Reward Epoch (4.5-5.5s following cue presentation). A two-way ANOVA was calculated examining the effects of trial type (Go+ vs NoGo-) and behavioral epoch (baseline vs cue) on phasic DA release in the NAc core. There was no main effect of behavioral epoch on phasic DA concentration with similar amounts of DA evoked on average during baseline and cue epochs, $F(1, 4) = 2.15$, *ns*. There was a significant main effect of trial type as Go+ trials evoked more phasic DA release than NoGo- trials, $F(1, 4) = 22.70$, $p < 0.01$. These results were moderated by a significant interaction of the effects of trial type and behavioral epoch on phasic DA concentration, $F(1, 4) = 59.90$, $p < 0.01$. While Go+ cues evoked a

significant increase in the concentration of DA from baseline, there was no change in phasic DA release on NoGo- trials ($p < 0.01$; see Figure 2.11b).

Comparison of Correctly Performed Go+ and NoGo- Trials. In order to further evaluate the changes in dopamine evoked by Go+ and NoGo- cues, trials were further separated in correctly (n = 501 trials) and incorrectly (n = 123 trials) performed trials. Trials in which the animal correctly responded following the Go+ cue (Go+ Cue Correct) or correctly withheld following the NoGo- cue (NoGo- Cue Correct) were compared to directly evaluate trials in which the cues differed with respect to reward prediction and also with respect to the behavioral patterns evoked. Changes in phasic dopamine evoked during correctly performed trials were aligned to cue presentation (Figure 2.12a). A two-way ANOVA was calculated examining the effects of trial type (Go+ vs NoGo-) and behavioral epoch (baseline vs cue) on phasic DA release in the NAc core. There was no main effect of behavioral epoch on phasic DA concentration with similar amounts of DA evoked on average during baseline and cue epochs, $F(1, 4) = 1.67$, *ns*. There was a significant main effect of trial type as Go+ trials evoked more phasic DA release than NoGo- trials, $F(1, 4) = 122.48$, $p < 0.001$. These results were moderated by a significant interaction of the effects of trial type and behavioral epoch on phasic DA concentration, $F(1, 4) = 43.68$, $p < 0.01$. While Go+ cues evoked a significant increase in the concentration of DA from baseline, there was no change in phasic DA release on NoGo- trials ($p < 0.01$; see Figure 2.12b).

Comparison of Randomly Selected Subset of Trials to Equalize Trial Number. In order to further evaluate the changes in dopamine evoked by Go+ and NoGo- cues, a subset of correctly performed Go+ and NoGo- trials were selected for additional analysis. As previously described (see page 36), 75% of trials during a Go+/NoGo- behavioral sessions are Go+ trials. As Go+ trials are oversampled relative to NoGo-, the previously presented results may be skewed.

Therefore, an equal number of trials (n=15) of each type per animal were randomly selected for a total of 75 Go+ trials and 75 NoGo- trials. Changes in phasic dopamine evoked during correctly performed trials were aligned to cue presentation (Figure 2.13a). A two-way ANOVA was calculated examining the effects of trial type (Go+ vs NoGo-) and behavioral epoch (baseline vs cue) on phasic DA release in the NAc core. There was no main effect of behavioral epoch on phasic DA concentration with similar amounts of DA evoked on average during baseline and cue epochs, $F(1, 4) = 5.10$, *ns*. There was a significant main effect of trial type as Go+ trials evoked more phasic DA release than NoGo- trials, $F(1, 4) = 73.72$, $p < 0.01$. These results were moderated by a significant interaction of the effects of trial type and behavioral epoch on phasic DA concentration, $F(1, 4) = 33.16$, $p < 0.01$. While Go+ cues evoked a significant increase in the concentration of DA from baseline, there was no change in phasic DA release on NoGo- trials ($p < 0.05$; see Figure 2.13b).

4. Go+ Cues, Regardless of Future Behavioral Action, Elicit Increases in Nucleus Accumbens Phasic Dopamine

Trials in which the animal correctly responded following the Go+ cue (Go+ Cue Correct; n = 501 trials) or incorrectly withheld (Go+ Cue Error; n = 15 trials) were compared to directly to evaluate differences phasic dopamine release to the same cue on trials in which the animal executed different behavioral patterns. Changes in phasic dopamine evoked by Go+ cues were aligned to cue presentation (Figure 2.14a). A two-way ANOVA was calculated examining the effects of Go+ trial performance (correct vs error) and behavioral epoch (baseline vs cue) on phasic DA release in the NAc core. There was no main effect of trial performance on phasic DA concentration with similar amounts of DA evoked by correctly and incorrectly performed Go+ trials, $F(1, 4) = 0.09$, *ns*. Cues on both correctly and incorrectly performed Go+ trials trended

towards evoking a significant increase in phasic DA as compared to baseline, $F(1, 4) = 5.72$, $p = 0.07$. However, there was no interaction in the effects of Go+ trial performance and behavioral epoch on phasic DA concentration, $F(1, 4) = 0.37$, *ns* (see Figure 2.14b).

5. NoGo- Cues, Regardless of Future Behavioral Action, Do Not Elicit an Increase in Nucleus Accumbens Phasic Dopamine

Trials in which the animal correctly responded following the NoGo- cue (NoGo- Cue Correct; n = 123 trials) or incorrectly withheld (NoGo- Cue Error; n = 33 trials) were compared to directly to evaluate differences phasic dopamine release to the same cue on trials in which the animal executed different behavioral patterns. Changes in phasic dopamine evoked by NoGo- cues were aligned to cue presentation (Figure 2.15a). A two-way ANOVA was calculated examining the effects of NoGo- trial performance (correct vs error) and behavioral epoch (baseline vs cue) on phasic DA release in the NAc core. There was no main effect of trial performance on phasic DA concentration with similar amounts of DA evoked by correctly and incorrectly performed NoGo- trials, $F(1, 4) = 2.33$, *ns*. Similarly, there was no difference in the phasic DA evoked by the two behavioral epochs, suggesting that the NoGo- cue consistently failed to increase phasic DA release, $F(1, 4) = 0.02$, *ns*. There was no interaction in the effects of NoGo- trial performance and behavioral epoch on phasic DA concentration, $F(1, 4) = 2.03$, *ns* (see Figure 2.15b).

6. Cues That Predict the End of a Time Out Elicit Increases in Nucleus Accumbens Phasic Dopamine

While every trial is preceded by an inter-trial interval of 10-15s, incorrectly performed Go+ and NoGo- trials were punished with 40s time outs from the behavioral session in which all lights within the chamber were extinguished. After 40s, the houselight was re-illuminated,

providing a salient cue for the end of the time out period. To evaluate the influence of the end of the timeout on phasic dopamine signaling in the NAc core, changes in phasic dopamine were aligned to the re-illumination of the house light following a time out (Figure 2.17a). Two behavioral epochs were calculated: baseline (5s prior to cue presentation) and cue (1s following cue presentation). Using a paired Student's t-test, changes in phasic dopamine from baseline to cue epoch were evaluated. Re-illumination of the houselight following a timeout period resulted in an increase in phasic dopamine signaling from baseline to cue epoch (Figure 2.17b; $t(4) = 4.69$, $p < 0.05$).

E. Discussion

Previous studies have established a critical role for phasic DA signaling in reward and reward-prediction. Unexpected rewards and reward-predictive cues evoke phasic increases in both the firing rate (Mirenowicz and Schultz, 1996; Schultz et al., 1997; Hyland et al., 2002; Ungless, 2004) and the concentration of DA within a region of the ventral striatum called the NAc (Roitman et al., 2004; Day et al., 2007; Roitman et al., 2008; Stuber et al., 2008; Brown et al., 2011; McCutcheon et al., 2012). However, these studies are fundamentally confounded as rewards and reward-predictive stimuli also generate approach and consummatory behaviors (Waelti, Dickinson, & Schultz, 2001; Day et al., 2007). To tease apart the role of phasic DA signaling in goal-directed behavior, we employed a novel Go+/NoGo+ paradigm in which two distinct visual cues equally signal the availability of reward, but the behavioral pattern required to obtain the reward differs. This paradigm was contrasted to a Go+/NoGo- paradigm in which the same behavioral patterns were executed, however the cues were no longer equally reward predictive.

1. Selection of the Nucleus Accumbens Core, A Region Sensitive to Reward-Predictive Cues

Several reasons underlie the selection of the NAc core as the striatal subregion of interest in this experiment. While data from several electrophysiology studies fail to dissociate neurons from the SNpc and VTA and suggest that all DA neurons fire heterogeneously to rewarding and aversive stimuli (Ljungberg, Apicella, & Schultz, 1992; Schultz et al., 1997), these results have been questioned. Multiple studies have identified separate populations of DA neurons within the SNpc and VTA that differ with respect to molecular properties and projection target (Lammel et al., 2008). In particular, DA neurons located in the medial posterior VTA, not traditionally labeled as DAergic based on higher than normal firing rates and reduced DA reuptake capacity, have been identified as projecting to the medial prefrontal cortex, NAc core, and NAc shell. Another population of DA neurons consistently identified as DAergic and located in the lateral VTA and SNpc project primarily to the NAc shell and dorsal striatum (Lammel et al., 2008). In addition to the heterogeneous physiological properties and projection targets of midbrain DA neurons, additional work supports multiple populations of DA neurons that differentially encode rewarding and aversive stimuli (Brischoux, Chakraborty, Brierley, & Ungless, 2009; Guarraci & Kapp, 1999; Matsumoto & Hikosaka, 2009; Ungless et al., 2004) suggesting that DA signaling is not as homogeneous as originally proposed.

Monitoring of changes in DA concentration in various striatal subregions corroborates release to different aspects of rewarding stimuli. Reward signals are not broadcast in a uniform manner, but rather selectively encode reward in different striatal subregions. The NAc was selected for this experiment for its role in integrating motivational information from limbic structures and translating this motivation into action. The NAc not only signals reward (Nicola et

al., 2004a, 2004b; Roitman et al., 2005; Wheeler et al., 2008), but is also involved in the execution of goal-directed behaviors (Carelli & Deadwyler, 1994; Carelli, 2002; Chang, Paris, Sawyer, Kirillov, & Woodward, 1996; Nicola et al., 2004a; Taha & Fields, 2006). As we are interested in teasing apart the role of phasic DA in reward-predictive cues and goal-directed behavior, the NAc is the ideal striatal subregion to investigate.

The NAc is traditionally subdivided into core and shell subregions (Záborszky et al., 1985). The NAc shell presents a slightly controversial picture as to function. Possessing what has been termed a "hedonic hotspot," the shell is thought to mediate the hedonic value of rewarding taste stimuli (Peciña & Berridge, 2000; Peciña & Berridge, 2005). Additionally, the shell is thought to play a strong role in innate and unconditioned behaviors such as feeding, and when inactivated results in voracious feeding behavior (Basso & Kelley, 1999; Stratford & Kelley, 1997, 1999; Stratford & Wirtshafter, 2011, 2012b; Wirtshafter, Covelo, Salija, & Stratford, 2012). While some have found that the NAc shell encodes rewarding stimuli with increases in phasic dopamine (Aragona et al., 2009; Roitman et al., 2008; Wheeler et al., 2011), others have failed to find any changes in phasic DA release in response to primary food rewards (Brown et al., 2011). This may reflect that phasic DA in the NAc shell encodes *novel* food and drug rewards, but this response habituates upon repeated exposure (Bassareo & Chiara, 1999; Bassareo, Musio, & Di Chiara, 2011). Therefore, differences in phasic DA release in the NAc shell to primary reward may reflect that animals in several of the previous experiments had very limited experience with the reward prior to testing (Roitman et al., 2008; Wheeler et al., 2011) while others had more extensive training (Brown et al., 2011). Discrepancies have also been found with regards to the effect of reward-predictive cues on phasic DA release within the NAc shell. Work from our own lab and others have failed to find any changes in NAc shell phasic DA

signaling to reward-predictive cues (Aragona et al., 2009; Brown et al., 2011). However, there remain other groups that have detected changes in phasic DA release in the NAc shell in response to both food- and brain stimulation-predictive cues (Beyene, Carelli, & Wightman, 2010; Cacciapaglia et al., 2012; Wanat, Kuhnen, & Phillips, 2010).

The NAc core, the area immediately surrounding the anterior commissure, has been demonstrated to play a strong role in goal-directed behavior. Reward-predictive cues elicit greater changes in NAc core neuronal firing rate and inactivation of these neurons impairs responding to these cues (Ambroggi et al., 2011). DA signaling within the NAc core is important for encoding information about rewards and reward-associated stimuli (Day et al., 2007; Di Ciano & Everitt, 2001; Fuchs, Evans, Parker, & See, 2004; Jones et al., 2010; Parkinson, Olmstead, Burns, Robbins, & Everitt, 1999; Parkinson et al., 2002). The NAc core may additionally mediate the level of effort exerted in motivationally challenging tasks (Sokolowski & Salamone, 1998). Lesions and inactivation of the NAc core have been demonstrated to interfere with the acquisition of Pavlovian and operant learning (Ito, Robbins, & Everitt, 2004; Kelley, 2004; Kelley et al., 1997). Given the well-established role of the NAc core in goal-directed behavior and signaling reward-predictive cues, this particular subregion of the NAc was ideal for investigating the role of phasic DA in reward-prediction versus the selection of different patterns of action in our Go+/NoGo+ paradigm.

2. Reward Predictive Cues Elicit an Increase in Nucleus Accumbens Core Phasic DA Release

Using FSCV to record changes in phasic DA signaling during task performance, we found that the equally reward-predictive Go+ and NoGo+ cues evoked similar increases in phasic DA release within the NAc core. Similar to previous studies, Go+ cues, which required

approach and an operant response to obtain a sucrose pellet reward, evoked increases NAc core phasic DA (Brown et al., 2011; Cacciapaglia et al., 2012). However, as previously detailed, the role of phasic DA is unclear as increases may encode the reward-predictive nature of the cues or the selected pattern of actions to perform in response to cues. To dissociate the role of phasic DA in reward-prediction versus the selection of distinct patterns of action, trials in which the animal incorrectly withholds following Go+ cues were also examined. Increases in phasic DA release to the reward predictive Go+ cue remain even when the animal fails to approach and engage in an operant response, suggesting that regardless of the pattern of actions executed, reward-predictive cues evoke similar increases in phasic DA release. Further support of the role of phasic DA in reward-prediction arise from evaluation of NoGo+ trials in which animals are required to withhold operant responding in order to obtain sucrose rewards. Similar dissociation of NoGo+ trials reveals that there were no differences in phasic DA evoked by the NoGo+ cue on correct or error trials. The NoGo+ cue, regardless of behavioral performance, evoked significant increases in the concentration of NAc core DA release. Taken individually, the reward-predictive Go+ and NoGo+ cues elicit increases in the phasic release of DA in the NAc core regardless of the pattern of actions selected.

Further support of the role of NAc core in reward-prediction arises from direct comparisons of phasic DA evoked by Go+ and NoGo+ cues during Go+/NoGo+ performance. There were no differences in phasic DA release during Go+ and NoGo+ trials as both cues evoked increases in DA release. These findings remain even when the traces are separated to compare only correct Go+ and NoGo+ trials, a situation when the cues are truly asymmetrical with respect to the actions evoked. Random selection of 15 correct trials of each type, in order to avoid biasing results by the unequal number of Go+ cues presented, also support phasic DA is

signaling cues predictive of reward availability. Go+ and NoGo+ correct trials are composed of equally reward predictive cues, but very different patterns of action are evoked by these cues in that these Go+ cues are initiating an operant response while these NoGo+ cues are resulting in the withholding of a response. Yet, despite these differences in the pattern of actions evoked, these two reward-predictive cues elicit similar increases in phasic DA signaling within the NAc core. Though this work appears to merely provide support for studies demonstrating increases in DA signaling to reward-predictive cues (Cacciapaglia, Wightman, & Carelli, 2011; Day et al., 2007; Jones et al., 2010; McCutcheon, Beeler, et al., 2012; Roitman et al., 2004; Stuber et al., 2008), the current experiments actually contribute a critical piece of information. Namely, responses to reward-predictive cues are present despite execution of different patterns of action supporting that DA is not encoding approach behavior. However, in order to truly verify that the changes in DA evoked by the Go+ and NoGo+ cues signal reward availability, it is necessary to compare a distinct behavioral paradigm in which animals perform the same behavioral patterns of action, however the cues differ with respect to their ability to predict reward.

3. Cues That Do Not Predict Reward Availability Fail to Drive Dopamine Signaling

Utilizing our Go+/NoGo- paradigm, we trained animals to perform similar patterns of action as our Go+/NoGo+ paradigm. However, while animals were still required to withhold responding following NoGo- cues, these cues never led to reward delivery. In this way, we dissociated changes in DA due to the pattern of behavior during NoGo cues from the reward-predictive nature of the cue itself. Similar to the Go+/NoGo+ paradigm, the Go+ trended towards eliciting an increase in the concentration of phasic DA within the NAc. Due to larger variability in the data, and larger variability in the scarce incorrectly performed Go+ trials, this data approached but did not fully reach statistical significance. However, this trend supports the

previously acquired data that cues that predict reward availability elicit increases in phasic DA release. Evaluation of the NoGo- cue, which did not predict reward availability, revealed that these cues consistently failed to drive phasic DA release regardless of behavioral performance. These results are in concordance with the theory that cues not predictive of reward fail to drive phasic DA signaling regardless of the pattern of actions executed (Brown et al., 2011; Day et al., 2007; Waelti et al., 2001).

Further support of the role of NAc core in reward-prediction arises from direct comparisons of phasic DA evoked by Go+ and NoGo- cues during Go+/NoGo- performance. While Go+ cues evoke increases in phasic DA, NoGo- cues failed to elicit changes in phasic DA release. These results corroborate the findings of numerous studies reporting that changes in the firing rate and phasic release of DA are evoked primarily in response to cues predicting reward availability (Brown et al., 2011; Day et al., 2007; Pan et al., 2005; Schultz, 1998), and DA signaling remains largely unchanged following cues not predictive of reward delivery (Brown et al., 2011; Day et al., 2007; Guarraci & Kapp, 1999; Waelti et al., 2001). These findings remained after the data were further distilled to examine both all correctly performed trials and a subset of correctly performed trials. NoGo- cues consistently failed to drive phasic DA release within the NAc core in direct contrast to the DA evoked by the reward-predictive Go+ cues

4. Nucleus Accumbens Phasic Dopamine Signals Earliest Predictor of Reward Availability

Throughout both behavioral paradigms animals would make occasional mistakes, either failing to respond on Go+ trials, or incorrectly executing operant responses following NoGo+ or NoGo- cues. Following these errors in behavior, the animal was put into time out in which the houselights were extinguished and the animal was unable to continue with the behavioral paradigm until 40 seconds had elapsed. At the end of this time out period, the houselights were

re-illuminated and the inter-trial interval began to advance the animal to the next trial. As observed in both of our behavioral paradigms, the end of the time out period, as signaled by the illumination of the houselights, was effective in evoking a very strong phasic DA response within the NAc core.

Increases in NAc DA concentration following house light illumination may reflect that this is a salient event for these animals. Past work suggests that DA neurons increase their firing rate in response to novel and otherwise salient sensory stimuli that cause orienting responses (Horvitz, Stewart, & Jacobs, 1997; Ljungberg et al., 1992; Schultz et al., 1992; Schultz, 1998; Steinfels, Heym, Strecker, & Jacobs, 1983; Strecker & Jacobs, 1985). However, while novel salient stimuli reliably evoke increases in the firing rate of DA neurons, evidence also suggests that these responses decay over time with repeated presentation (Schultz, 1998). While at the time of testing animals perform both tasks accurately making few mistakes and therefore experiencing few time out periods, they had extensive training for approximately six weeks prior to testing. Throughout the training period errors are frequent, and therefore time outs were a regular part of their experience. As a result, it is unlikely that at the time of voltammetric testing the houselight illumination was still a novel cue.

Perhaps a more encompassing explanation for the increase in the concentration of phasic DA in response to the illumination of the houselights is that DA encodes cues that are predictive of reward. Specifically, DA signaling tracks the earliest predictor of reward availability (Pan et al., 2005). If there are multiple predictors of reward within a short amount of time, DA will signal the earliest predictor of reward. However, multiple predictors that are separated in time by more than a few seconds will all elicit increases in DA signaling (Schultz, Apicella, & Ljungberg, 1993). As the end of the time out period signals the reinstatement of the program and

therefore the ability to once again work for food rewards, it is likely that the illumination of the house lights is in fact another reward-predictive cue. The re-illumination of the house light is followed by the inter-trial interval (10-15 seconds) before another behaviorally relevant cue is presented. Because of the long time interval between these reward-predictive cues, both the illumination of the house light and the trial cue elicited robust increases in the concentration of DA within the NAc core.

5. Phasic Dopamine Activity Within the Nucleus Accumbens Core Does Not Encode a Motor Plan

As previously discussed, our results suggest that phasic DA activity within the NAc core encodes behavioral cues that are predictive of reward delivery, rather than the upcoming pattern of actions to execute. However, there are substantial discrepancies in the literature as to the role of DA in motor behavior and the planning of behavioral responses. Pharmacological manipulations of DA have a wide impact on goal-directed behavior and high doses of DA antagonists are theorized to reduce willingness to exert effort in behavioral paradigms when lower effort options are available (Aberman, Ward, & Salamone, 1998; Nowend, Arizzi, Carlson, & Salamone, 2001; Salamone et al., 1991; Salamone, Arizzi, Sandoval, Cervone, & Aberman, 2002). Similarly, destruction of afferent DA pathways to the NAc with 6-OHDA lesions reduce performance in operant paradigms (Aberman & Salamone, 1999; Aberman et al., 1998; Hamill, Trevitt, Nowend, Carlson, & Salamone, 1999; Ikemoto & Panksepp, 1999; Salamone et al., 1991; Salamone, Wisniecki, Carlson, & Correa, 2001). Yun and colleagues (2004) examined the effects of infusing DA antagonists into the NAc on discriminative stimulus (DS) task performance. Animals were trained that responding during a DS cue led to subsequent reward delivery, however responding during a non-associated stimulus (NS) or on an inactive

lever never led to reward. Blockade of DA receptors in the NAc impaired and slowed responding. They interpreted these results as DA antagonists impairing the animals' ability to select the correct action during goal-directed behavior (Yun, Nicola, et al., 2004).

While these results seem to suggest that DA within the NAc could be involved in generating correct responses in operant paradigms, there remains a flaw in this line of thinking. As described in Chapter I (see page 16), DA neurons fire action potentials at lower frequencies (3-8 Hz; tonic) in a slow, irregular pattern (Grace & Bunney, 1984), but also exhibit brief high frequency increases in activity (20–60 Hz; phasic) often accompanied by bursts in firing (Grace & Bunney, 1984b; Hyland, Reynolds, Hay, Perk, & Miller, 2002; Schultz, 1998). DA activity is far from homogenous and therefore the possibility remains that DA released during tonic firing may play a very different role in behavior than the phasic activity that we recorded during our task. As blockade of DA receptors and lesioning DA producing neurons will interfere with *both* tonic and phasic activity, these studies are perhaps unable to tease apart the role of phasic release in the NAc core on different components of goal-directed behavior.

Electrophysiology affords precise temporal resolution of the changes in the firing rates of DA neurons to behaviorally relevant stimuli. Using this technique, the relationship between tonic/phasic neuronal activity and goal-directed behavior can be examined. Multiple studies by Schultz and colleagues (Ljungberg et al., 1992; Schultz, 1986) have examined DA neuronal activity during goal-directed behavior in primates while simultaneously looking at electromyographic (EMG) recordings of muscle activity. DA neuronal responses were generally time-locked to the cues instructing behavioral responses rather than the onset of muscle movement according to EMG activity (Ljungberg et al., 1992; Schultz, 1986). Furthermore, changes in DA neuronal activity remained even on trials in which the animals incorrectly did not

make a motor response (Ljungberg et al., 1992). DA neuronal firing was not correlated with spontaneous mouth movements, but was related to reward ingestion (Schultz, 1986). These results support that DA neuronal activity is not explicitly tied to the planned pattern of behavioral actions. The firing rate of DA neurons has also been examined with respect to behavioral switching, or the shifting of attention from one task to another. Similar to results previously reported by Schultz and colleagues, the firing rate of DA neurons is more consistent with encoding reward-prediction rather than facilitating the switching of attention and efforts to another task (Wilson & Bowman, 2006).

Despite the research suggesting that DA is not encoding upcoming motor patterns, Morris and colleagues (2006) report that DA neuronal activity encodes future action choice within 122ms after cue presentation. Though their results appear to conflict with those previously described, Morris and colleagues recorded solely from neurons in the SNpc. As these neurons project primarily to the dorsal striatum, these results suggest that phasic DA activity within the dorsal striatum may be important for encoding the selected pattern of actions to execute. The dorsal striatum, and the dorsomedial striatum in particular, is thought to be critical for behavioral flexibility, or the ability to shift from one pattern of behavior to another during reward-related learning (Kimchi & Laubach, 2009; Ragozzino, Jih, & Tzavos, 2002; Ragozzino, Mohler, Prior, Palencia, & Rozman, 2009; Ragozzino, Ragozzino, et al., 2002) and strategy shifting (Ragozzino & Choi, 2004; Ragozzino, Ragozzino, et al., 2002). Indeed, neuronal activity within the dorsal striatum increases within 3s of self-initiated movements and in response to trigger stimuli provided that a movement follows (Romo, Scarnati, & Schultz, 1992; Schultz & Romo, 1988). Therefore, while phasic DA within the NAc is important for encoding cues that signal reward

availability, phasic DA activity within the dorsal striatum may be potentially important for facilitating behavioral switching and flexibility.

Further evidence that NAc phasic DA is not encoding planned motor patterns arises from a Pavlovian paradigm. Animals were trained that a CS+ was associated with reward delivery, while a CS- was not-associated with reward delivery. Animals quickly learned to dissociate between these two cues and inevitably began to respond on a lever associated with the CS+ cue. While approach and engagement with the lever is not required for reward delivery, approach behavior in response to Pavlovian reward-predictive cues are observed in many autoshaping and sign tracking paradigms (Brown & Jenkins, 1968; Peterson et al., 1972). However, these approach behaviors make it difficult to ascertain that changes in phasic DA are related to the cue and not to ensuing motor responses. Day and colleagues (2007) found no relationship between the magnitude of NAc core DA release and the behavioral vigor, or number of lever presses the animal engaged in following the cue. Despite the fact that the animal engaged in numerous operant responses following cue presentation, phasic DA was time-locked to the onset of the cue and did not remain elevated throughout the entire cue presentation when the lever was being pressed (Day et al., 2007). This suggests that phasic DA was not encoding the ongoing decision to repeatedly make operant responses, but instead was signaling the presence of a cue associated with reward delivery.

6. Conclusion

While other studies hint that NAc core phasic DA encodes cues predictive of reward delivery rather than the pattern of action selected to execute, the current study is the first to explicitly test this using two behavioral paradigms designed to dissociate the effects of reward-prediction from approach behavior. Collectively, the results from our Go+/NoGo+ and

Go+/NoGo- paradigms suggest that phasic DA within the NAc core is encoding a reward-prediction signal rather than the upcoming pattern of actions to be executed. All reward-predictive cues, regardless of behavioral response, elicited increases in the concentration of NAc core DA while our sole non-reward-predictive cue (NoGo-) failed to drive DA release. Regardless of behavioral response, phasic DA continued to signal cues associated with reward availability. Additionally, we found that a cue signaling the end of the time out period, and therefore return to behavioral trials, became another reward-predictive cue that also evoked DA release within the NAc core. Taken together, the dopaminergic projection from the VTA appears to be encoding information about which cues are behaviorally significant for reward and transmitting this information to the NAc core where it is integrated with other aspects of goal-directed behavior in order to evoke behavioral responses.

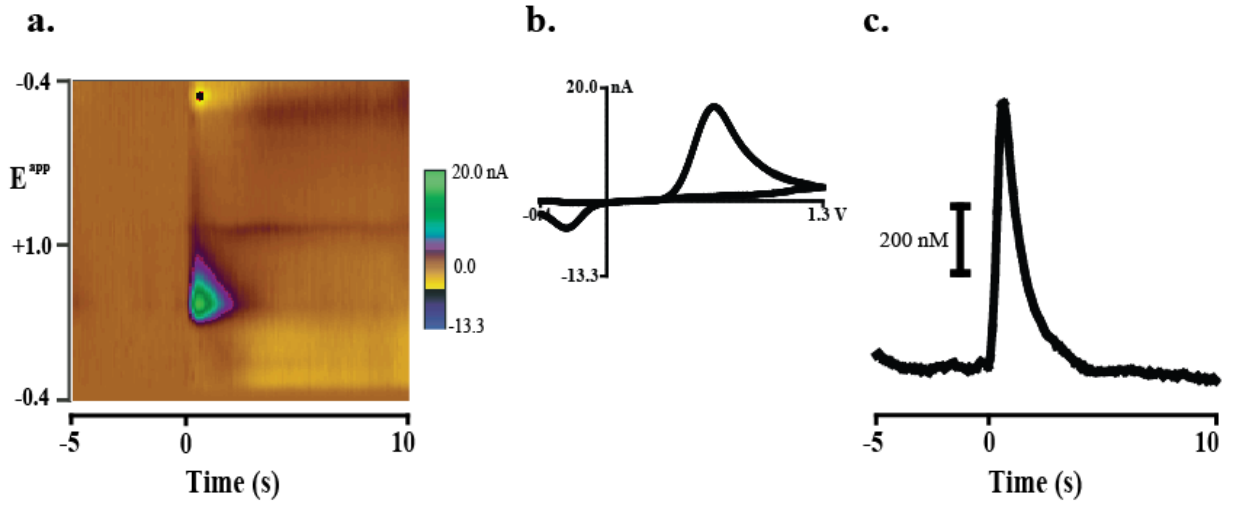

Figure 2.1: Representative example of an increase in NAc dopamine release in response to electrical stimulation (Time=0) of the VTA/SNpc (24p, 60Hz, 120µA, 4ms/phase, monophasic). The color plot indicates changes in current as a function of electrode potential and time. Time is on the abscissa, the applied electrode potential is on the ordinate, and the current changes are encoded in color (a). Stimulation of dopamine neurons (time = 0) evoked current at several applied potentials along the triangular waveform. Cyclic voltammograms at time = 0.5 s after stimulation (b). Voltage is on the abscissa (negative and positive going scans), and change in current is on the ordinate. Current changes at the time of stimulation are due to the presence of dopamine at the recording electrode, identified by its oxidation (~0.6 V) and reduction (~-0.2 V; on the negative going scan) potentials. The identification of dopamine on this cyclic voltammogram matched identically with previous work using FSCV to measure exogenous dopamine in a flow cell system (Heien et al., 2004). Dopamine concentration increases in response to electrical stimulation of the VTA/SNpc (c). Dopamine concentration is directly proportional to the oxidative current at 0.6 V (1 nA= ~66 nM dopamine). Time is the abscissa and dopamine concentration is the ordinate.

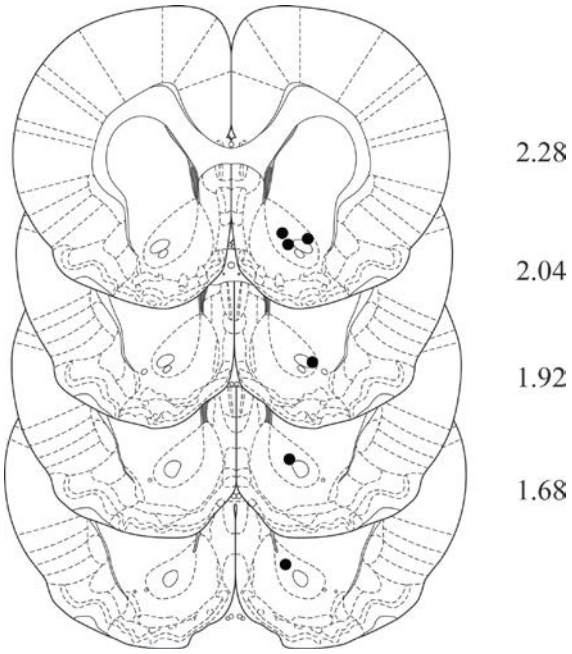

Figure 2.2: Location of carbon fiber recording electrodes examining phasic dopamine release during the symmetrical Go+/NoGo+ task. Each histological image is paired with a number which represents the distance in mm anterior from bregma. Brain histological images were adapted from the sterotaxic atlas of Paxinos & Watson (1998).

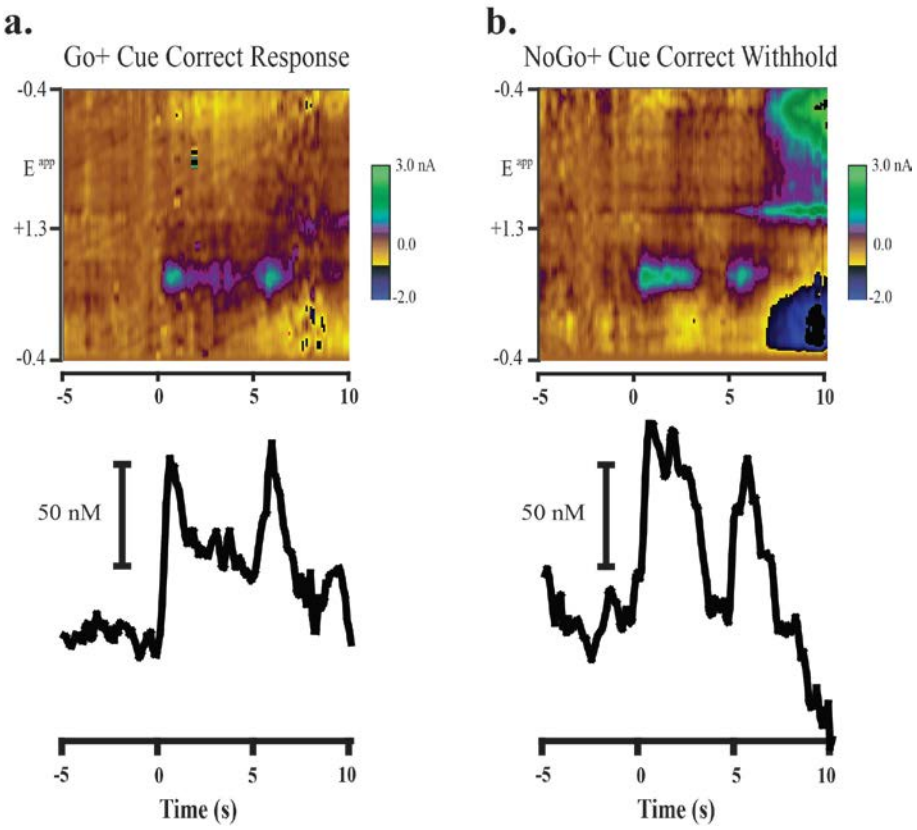

Figure 2.3: Representative examples of changes in NAc phasic dopamine signaling in response to a Go+ cue (a) in which the animal correctly responded and NoGo+ cue (b) in which the animal correctly withheld responding. Top: Color plots indicate changes in current due to dopamine oxidation in NAc core. Time is the abscissa, the electrode potential is the ordinate, and current changes are encoded in color. Bottom: Dopamine concentration as a function of time is extracted from the color plots above. Cue onset at time = 0s for all graphs.

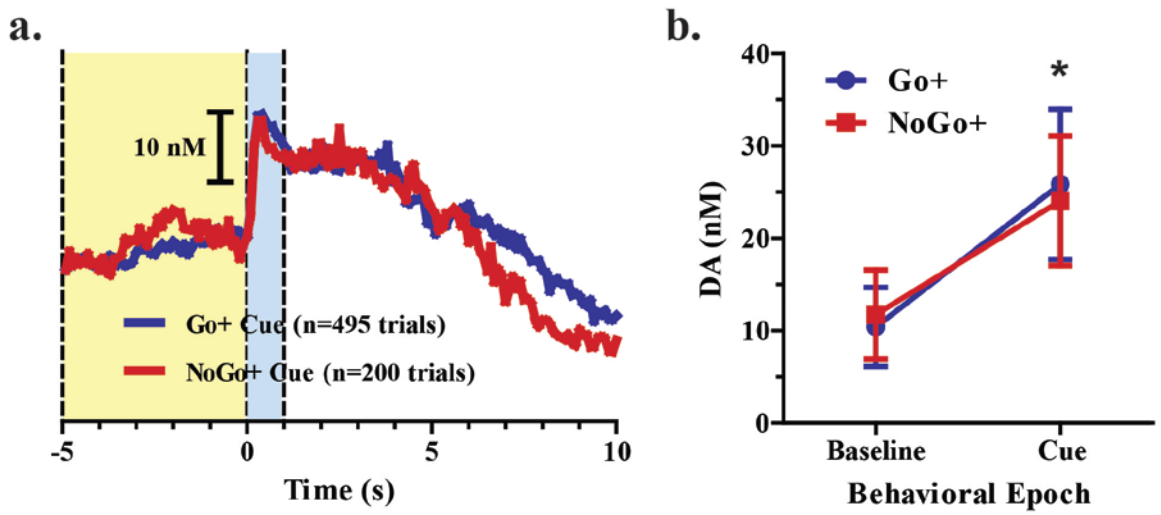

Figure 2.4: Changes in phasic dopamine signaling following the presentation of Go+ and NoGo+ cues regardless of the action performed are shown aligned to the presentation of the cue (a; Time 0). While there was no main effect of trial type, there was a main effect of behavioral epoch on the concentration of NAc core DA. There was a similar increase in phasic DA from baseline to cue epochs following both trial types (b; $*p < 0.05$). There was no interaction between trial type and behavioral epoch on NAc core phasic DA concentration.

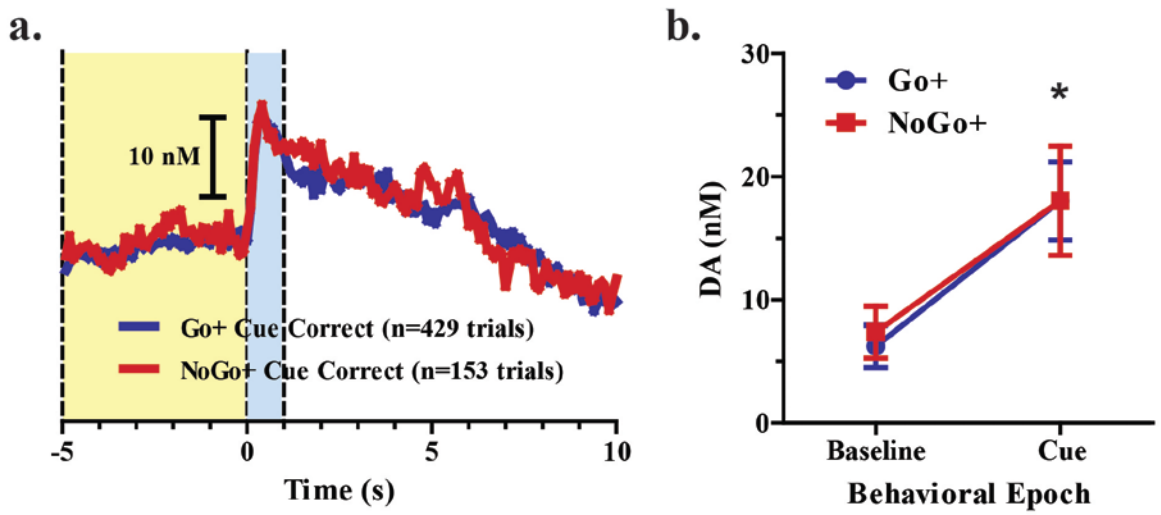

Figure 2.5: Changes in phasic dopamine signaling following the presentation of Go+ and NoGo+ cues in which the animal correctly responded following the Go+ cue and correctly withheld responding following the NoGo+ cue are shown aligned to the presentation of the cue (a; Time 0). While there was no main effect of trial type, there was a main effect of behavioral epoch on the concentration of NAc core DA. There was a similar increase in phasic DA from baseline to cue epochs following both trial types (b; $*p < 0.05$). There was no interaction between trial type and behavioral epoch on NAc core phasic DA concentration.

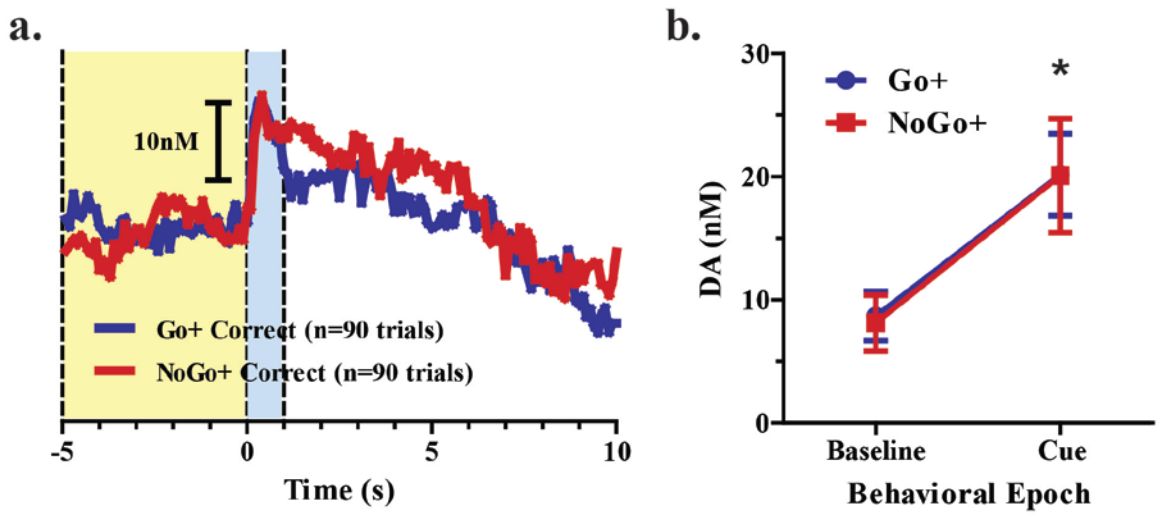

Figure 2.6: In order to equalize the number of correctly performed Go+ and NoGo+ trials, 15 trials of each type were randomly selected per animal for a total of 90 correctly performed Go+ trials and 90 correctly performed NoGo+ trials. Changes in phasic dopamine signaling following the presentation of these Go+ and NoGo+ cues are shown aligned to the presentation of the cue (a; Time 0). While there was no main effect of trial type, there was a main effect of behavioral epoch on the concentration of NAc core DA. There was a similar increase in phasic DA from baseline to cue epochs following both trial types (b; $*p < 0.05$). There was no interaction between trial type and behavioral epoch on NAc core phasic DA concentration.

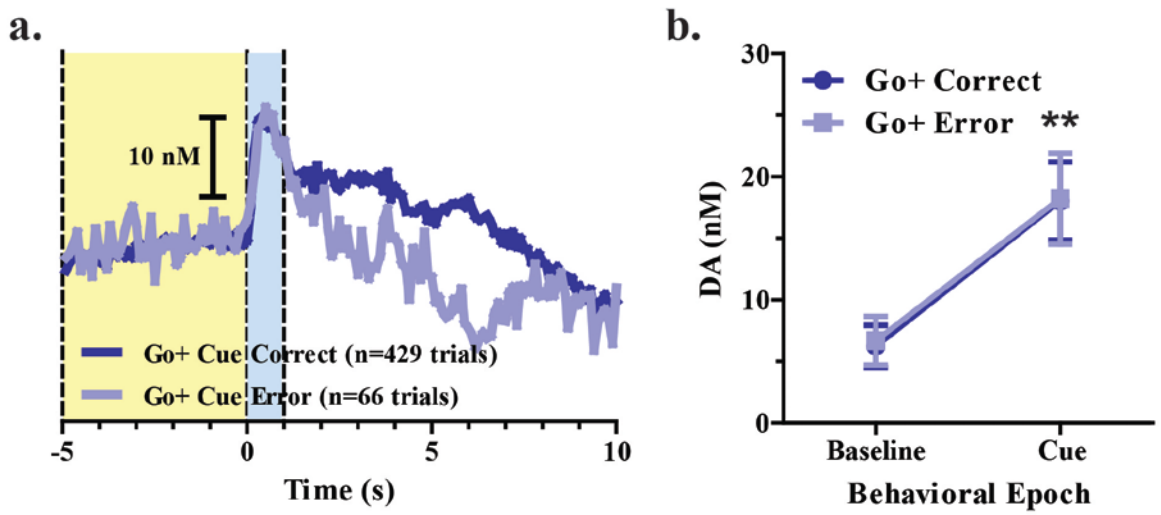

Figure 2.7: Changes in phasic dopamine signaling following the presentation of Go+ cues in which the animal correctly responded (Correct) or incorrectly withheld responding (Error) are shown aligned to the presentation of the cue (a; Time 0). While there was no main effect of trial performance, there was a main effect of behavioral epoch on the concentration of NAc core DA. There was a similar increase in phasic DA from baseline to cue epochs following both correct and error Go+ trials (b; $^{**}p < 0.01$). There was no interaction between trial type and behavioral epoch on NAc core phasic DA concentration.

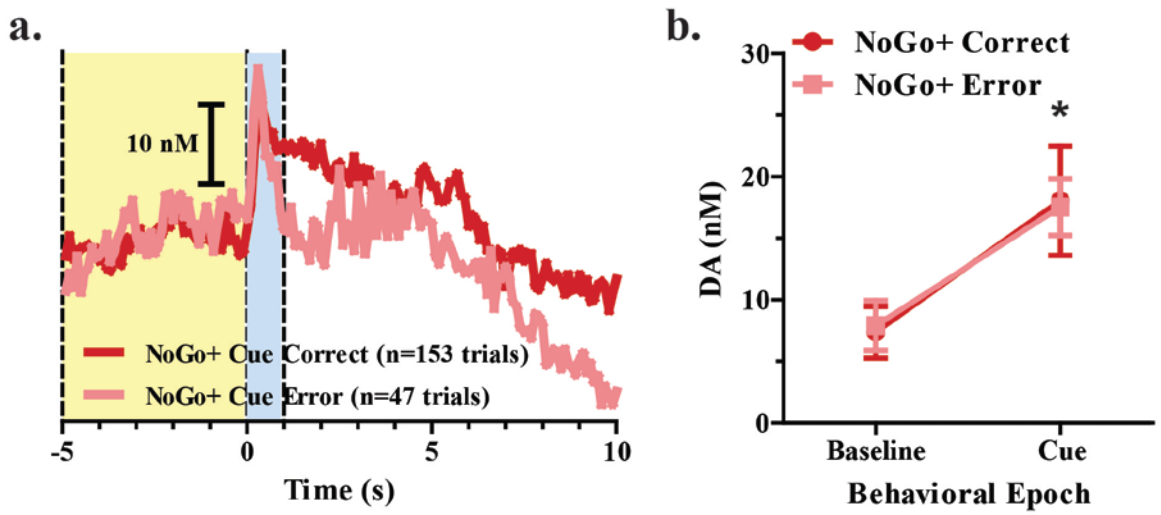

Figure 2.8: Changes in phasic dopamine signaling following the presentation of NoGo+ cues in which the animal correctly withheld responding (Correct) or incorrectly responded (Error) are shown aligned to the presentation of the cue (a; Time 0). While there was no main effect of trial performance, there was a main effect of behavioral epoch on the concentration of NAc core DA. There was a similar increase in phasic DA from baseline to cue epochs following both correct and error NoGo+ trials (b; $*p < 0.05$). There was no interaction between trial type and behavioral epoch on NAc core phasic DA concentration.

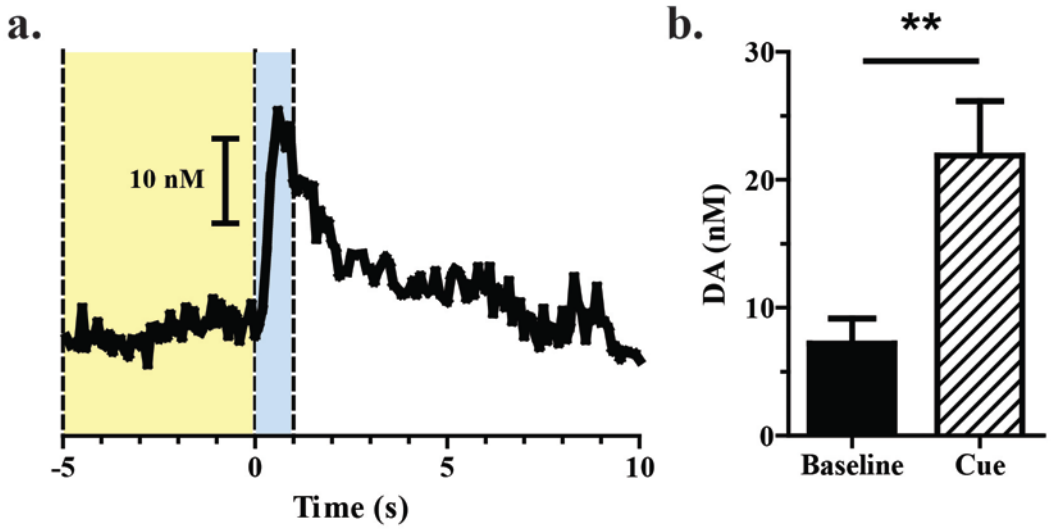

Figure 2.9: Changes in phasic dopamine signaling following the re-illumination of the houselight (Time = 0) after a time out during the Go+/NoGo+ paradigm (a). House light re-illumination evoked a significant increase in phasic dopamine from baseline to cue epoch (b; ** $p < 0.01$).

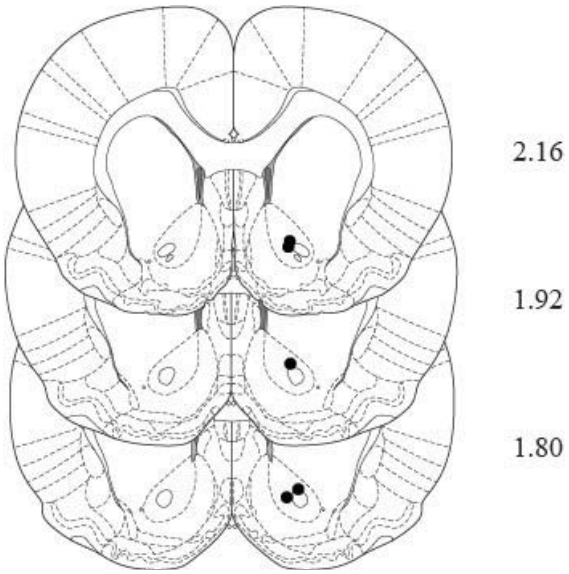

Figure 2.10: Location of carbon fiber recording electrodes examining phasic dopamine release during the asymmetrical Go+/NoGo- task. Each histological image is paired with a number which represents the distance in mm anterior from bregma. Brain histological images were adapted from the sterotaxic atlas of Paxinos & Watson (1998).

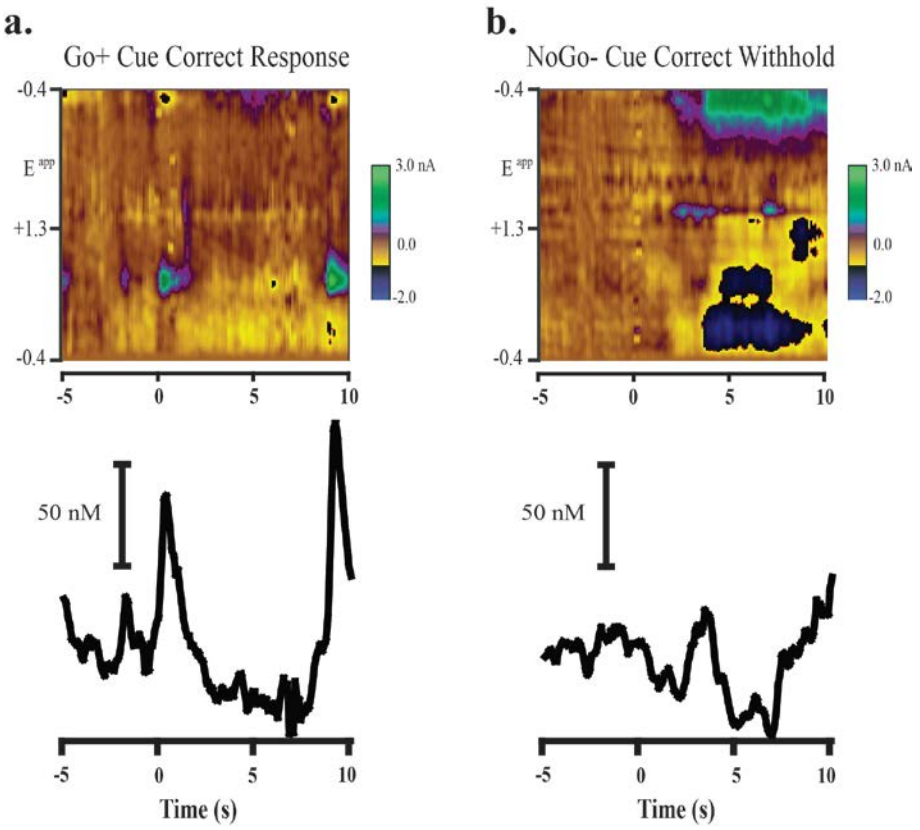

Figure 2.11: Representative examples of changes in NAc phasic dopamine signaling in response to a Go+ cue (a) in which the animal correctly responded and NoGo- cue in which the animal correctly withheld responding. Top: Color plots indicate changes in current due to dopamine oxidation in NAc core. Time is the abscissa, the electrode potential is the ordinate, and current changes are encoded in color. Bottom: Dopamine concentration as a function of time is extracted from the color plots above. Cue onset at time = 0s for all graphs.

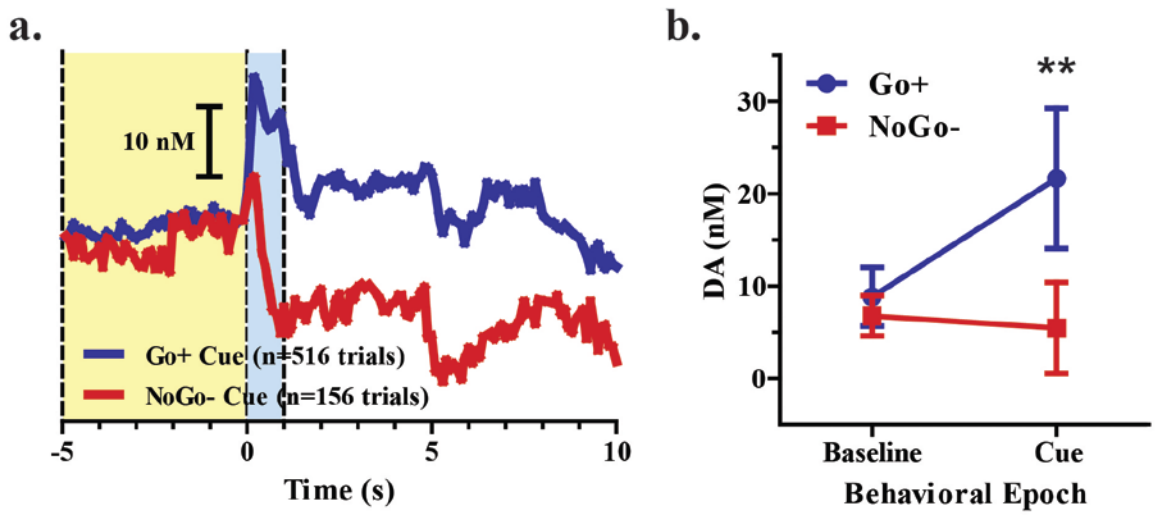

Figure 2.12: Changes in phasic dopamine signaling following the presentation of Go+ and NoGo- cues regardless of the action performed are shown aligned to the presentation of the cue (a; Time 0). While there was no main effect of behavioral epoch, there was a main effect of trial type on the concentration of NAc core DA. There were greater levels of DA evoked on Go+ trials than NoGo- trials ($p < 0.01$). Additionally, there was a significant interaction between trial type and behavioral epoch with the Go+ cue, but not the NoGo+ cue, evoking a significant increase in the concentration of DA as compared to baseline (b; $^{**}p < 0.01$).

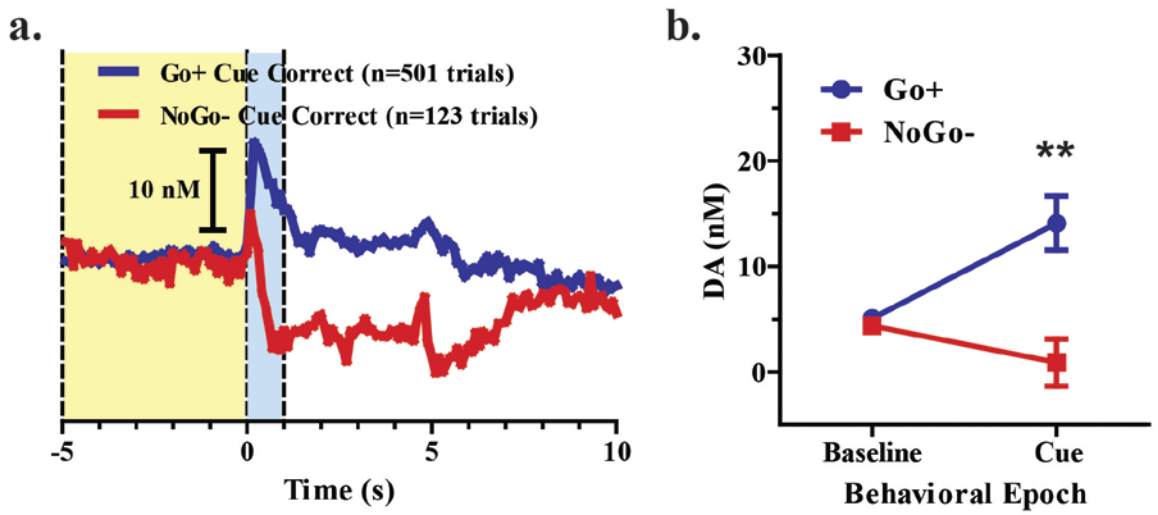

Figure 2.13: Changes in phasic dopamine signaling following the presentation of Go+ and NoGo- cues in which the animal correctly responded following the Go+ cue and correctly withheld responding following the NoGo- cue are shown aligned to the presentation of the cue (a; Time 0). While there was no main effect of behavioral epoch, there was a main effect of trial type on the concentration of NAc core DA. There were greater levels of DA evoked on Go+ trials than NoGo- trials ($p < 0.001$). Additionally, there was a significant interaction between trial type and behavioral epoch with the Go+ cue, but not the NoGo+ cue, evoking a significant increase in the concentration of DA as compared to baseline (b; $^{**}p < 0.01$).

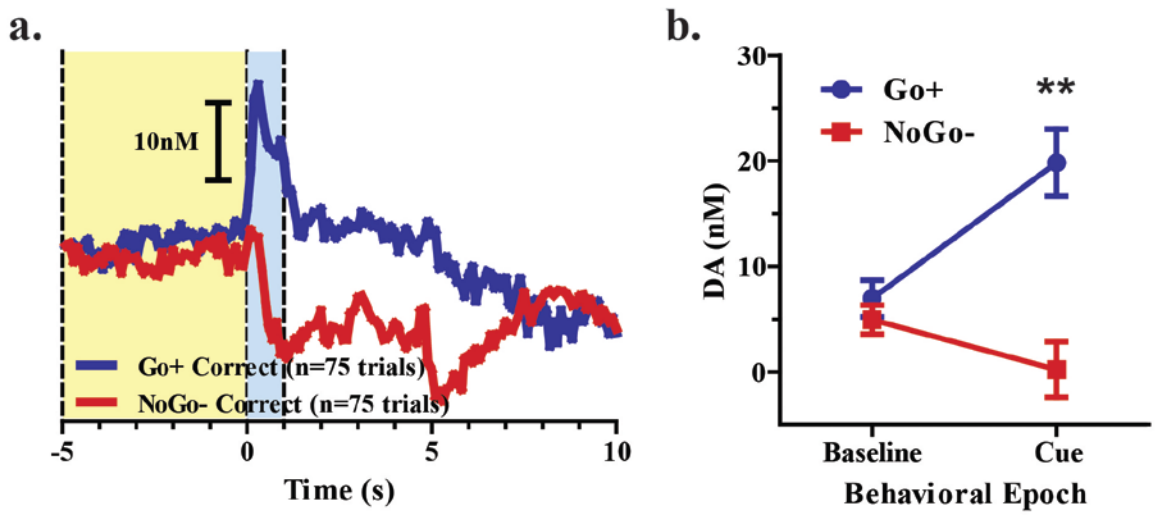

Figure 2.14: In order to equalize the number of correctly performed Go+ and NoGo- trials, 15 trials of each type were randomly selected per animal for a total of 75 correctly performed Go+ trials and 75 correctly performed NoGo- trials. Changes in phasic dopamine signaling following the presentation of these Go+ and NoGo- cues are shown aligned to the presentation of the cue (a; Time 0). While there was no main effect of behavioral epoch, there was a main effect of trial type on the concentration of NAc core DA. There were greater levels of DA evoked on Go+ trials than NoGo- trials ($p < 0.01$). Additionally, there was a significant interaction between trial type and behavioral epoch with the Go+ cue, but not the NoGo+ cue, evoking a significant increase in the concentration of DA as compared to baseline (b; $^{**}p < 0.01$).

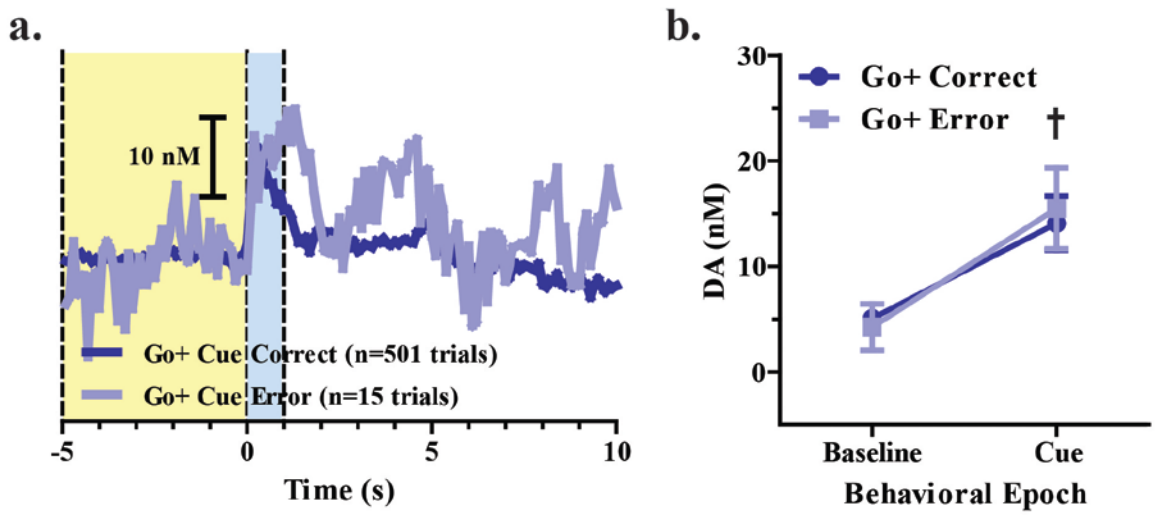

Figure 2.15: Changes in phasic dopamine signaling following the presentation of Go+ cues in which the animal correctly responded (Correct) or incorrectly withheld responding (Error) are shown aligned to the presentation of the cue (a; Time 0). While there was no main effect of trial performance, there was a trend towards a main effect of behavioral epoch on the concentration of NAc core DA. There was a similar increase in phasic DA from baseline to cue epochs following both correct and error Go+ trials (b; † p = 0.07). There was no interaction between trial performance and behavioral epoch on NAc core phasic DA concentration.

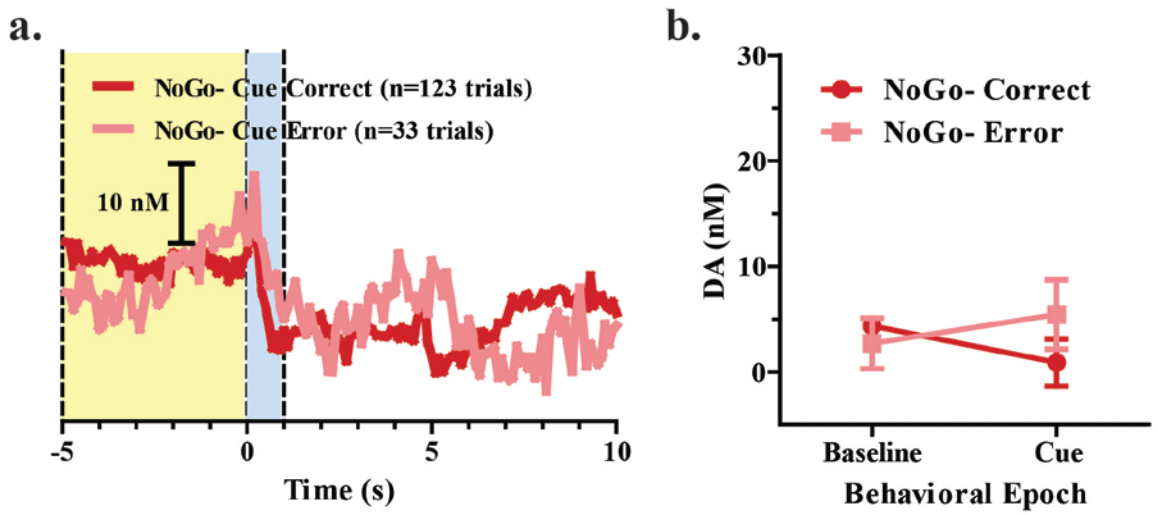

Figure 2.16: Changes in phasic dopamine signaling following the presentation of NoGo- cues in which the animal correctly withheld responding (Correct) or incorrectly responded (Error) are shown aligned to the presentation of the cue (a; Time = 0). There was no main effect of trial performance or behavioral epoch on the concentration of NAc core DA. Similarly, there was no interaction between trial performance and behavioral epoch on NAc core phasic DA concentration.

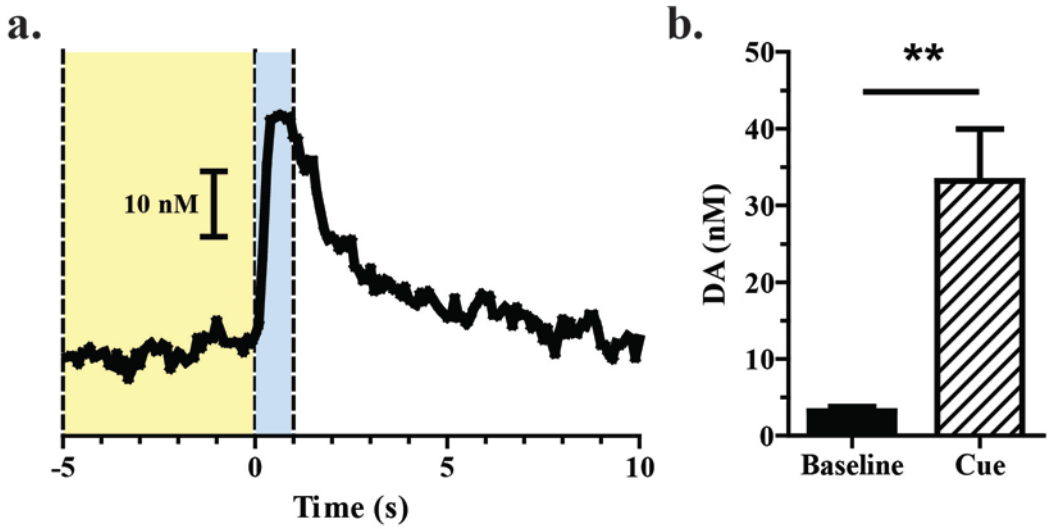

Figure 2.17: Changes in phasic dopamine signaling following the re-illumination of the houselight (Time = 0) after a time out during the Go+/NoGo- paradigm (a). House light re-illumination evoked a significant increase in phasic dopamine from baseline to cue epoch (b; ** $p < 0.01$).

Chapter III

Pharmacological Manipulations of the NAc Influence Action Selected

A. Introduction

The NAc, speculated to be the bridge between motivation and action (Mogenson, Jones, & Yim, 1980), has long been associated not only with encoding rewarding properties of salient stimuli, but also with behaviors directed at obtaining these rewards. As our data from Chapter II supports, the NAc receives information about the reward-predictive nature of behavioral stimuli via a dense afferent phasic DA projection from the VTA. However, both reward and the selection of actions are regulated by vast neural networks, integrating information not only from specific neurotransmitter systems such as DA, but also from distinct brain regions. The NAc has been firmly established as a structure that is important for not only for encoding reward, but also goal-directed behavior and the selection of certain behaviors at the expense of others. However, it remains unclear which afferent projections to the NAc are essential for encoding which behavioral response will be executed.

While our earlier work supports phasic DA as encoding the reward-predictive nature of the cues, dopamine has action within the NAc beyond phasic activity. The direct and indirect pathways of NAc neurons uniquely express different types of dopamine receptors which are hypothesized to have very different actions on behavior. Activation of the direct D_1 pathway has been demonstrated to increase motor output and reduce freezing behavior while activation of the indirect D_2 pathway increases freezing behavior and reduces behavioral output (Kravitz et al., 2010). Several behavioral studies from Fields and colleagues have attempted to ascertain the role of dopaminergic modulation of the direct and indirect pathways of NAc MSNs on the selection of behavior in a discriminative stimulus (DS) task. Animals were trained to lever press in the

presence of a DS that predicted the availability of reward and were also presented a non-reward associated stimulus (NS) that never predicted the availability of reward. Inactivation of the direct pathway of the NAc with the D_1 receptor antagonist SCH23390 resulted in a reduction in overall responding during both DS and NS cues. In sharp contrast, inactivation of the indirect pathway with the D_2 receptor antagonist raclopride caused no changes in responding on either the DS/NS paradigm or FR1 (Yun et al., 2004). This suggests a potential role for DA receptors within the NAc in mediating the selection of actions in operant goal-directed behavior. If DA signaling is important for action selection through selective activation of D_1 and D_2 type receptors, blockade of D_1 receptors should lead to a reduction in goal-directed behavior whereas blockade of D_2 receptors may release downstream motor structures from inhibition and result in an increase in goal-directed behavior.

In addition to the actions of specific DA receptors within the NAc, DA appears to modulate the excitability of NAc MSNs to glutamatergic limbic inputs (O'Donnell, Greene, Pabello, Lewis, & Grace, 1999). Through numerous glutamatergic projections from limbic structures such as the amygdala, hippocampus, medial prefrontal cortex, and thalamus (Selemon and Goldman-Rakic 1985; Haber, Kunishio, Mizobuchi, & Lynd-Balta, 1995; Groenewegen, Wright, Beijer, & Voorn, 1999) the NAc is thought to play a role in the selection and inhibition of actions (Alexander, DeLong, & Strick, 1986; Robbins & Brown, 1990; Mink & Thach, 1993; Mink, 1996; Hikosaka, 1998; Redgrave et al., 1999; Aron, Durston, Eagle, Logan, Stinear, & Stuphorn, 2007). Support for this theory arises from a discriminative stimulus paradigm in which animals are rewarded for responding during a discriminative stimulus (DS) cue, whereas there is no reward associated for responding during a non-associated stimulus (NS) or on the inactive lever. Temporary inactivation of the NAc with glutamate antagonists 6-cyano-7-

nitroquinoxaline-2,3-dione (CNQX) and AP5 effected little change or a mild reduction in responding during the DS cue. However, intra-NAc CNQX and AP5 resulted in an increase in inappropriate responding during the presentation of a NS (Yun et al., 2004; Ambroggi et al., 2011) suggesting that blockade of glutamate signaling within the NAc may release downstream motor structures from tonic inhibition. Further evidence for the role of the NAc in the selection of different patterns of action arises from a paradigm in which animals were trained to self-administer alcohol prior to undergoing extinction training. Blockade of 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid (AMPA) receptors within the NAc increases responding for alcohol in extinction (Millan & McNally, 2011). Increases in inappropriate responding following blockade of NAc glutamate signaling suggests that glutamate released by limbic inputs may contribute to the selection of appropriate patterns of behavior.

Regardless of whether DA and glutamate influence the signaling of action selection within the NAc, a large amount of evidence suggests that global inactivation of the NAc results in changes in activity and operant behavior. General inactivation of the NAc with GABA agonists such as baclofen and muscimol increase motor activity (Wong, Eshel, Dreher, Ong, & Jackson, 1991) and increase both reinforced and non-reinforced operant responding for food and drug reward (Yoon et al., 2009; Wirtshafter & Stratford, 2010; Stratford & Wirtshafter, 2012). In addition, inactivation of the NAc interferes with Pavlovian conditioned approach and increases responding during cues not paired with reward delivery (Blaiss & Janak, 2009). The increases in non-rewarded responding following inactivation of the NAc strongly indicate that this region is tonically inhibiting downstream motor structures and exerting control over action selection during both Pavlovian and operant tasks.

In order to assess the role of the NAc in the selection of different patterns of action, we undertook a series of pharmacological manipulations to dissociate the role of NAc afferents in goal-directed behavior. To ascertain the role of the NAc as a whole in Go+/NoGo+ performance, we globally inactivated the NAc with the $GABA_A$ agonist muscimol and $GABA_B$ agonist baclofen. If the NAc as a whole is important for maintaining behavioral inhibition during behavior, inactivation of the NAc should result in a loss of inhibition in the form of increased responding during NoGo+ trials. To further separate to function of NAc direct and indirect pathways in goal-directed behavior, we selectively manipulated DA signaling utilizing the D_1 receptor antagonist SCH23390 and D_2 receptor antagonist raclopride. The indirect pathway is hypothesized to inhibit downstream motor structures when activated. As D_2 receptor activation blocks indirect pathway activity, blockade of DA D_2 receptors should result in an increase in indirect pathway activity, and therefore further inhibition of downstream motor structures. Activation of the direct pathway functions to activate downstream motor structures, therefore blockade of D_1 receptors with SCH23390 should lead to a reduction in overall operant responding. In addition, we selectively inactivated glutamatergic inputs to the NAc with the AMPA antagonist CNQX and NMDA antagonist D-AP5. If glutamatergic inputs to the NAc are important for maintaining behavioral inhibition during behavior, inactivation of NAc glutamate activity should result in a loss of inhibition in the form of increased responding during NoGo+ trials. Taken together, these studies will provide greater insight into the role of select NAc inputs in reward prediction and action selection.

B. Experimental Methods

1. Subjects

Male Sprague-Dawley rats (n = 39; Charles River Laboratories, Portage, MI) weighing

approximately 300-400 g were used for these experiments. Animals were individually housed and maintained on a 12 h/12 h light/dark cycle in a temperature (22°C) and humidity (30%) controlled environment. All experiments were conducted between 8:00 am and 6:00 pm. Animals received ad libitum access to water and were maintained at no less than 90% of free feeding weight during experimentation (10-20 g/day; LabDiet) based on task performance in addition to consuming approximately 5-7 grams of sucrose (Bio-Serve Precision Pellets; Frenchtown, NJ) during daily training and testing sessions. All animals were treated according to the guidelines recommended by the Animal Care Committee at the University of Illinois at Chicago.

2. Apparatus

All experiments took place in standard experimental operant chambers equipped with two levers, two cue lights, a pellet dispenser, head entry sensors, an audio generator, and a house light (Med Associates, Inc.; St. Albans, VT).

3. Go+/NoGo+ Task

Training on the Go+/NoGo+ Task progressed as described in Chapter II (see page 34.)

4. Surgery

On the day prior to surgery the animals were removed from the food restriction regime and given ad libitum access to food. Animals were anesthetized with ketamine hydrochloride (100 mg/kg body weight, intraperitoneal) and xylazine hydrochloride (10 mg/kg body weight, IP). Following anesthesia, the hair was removed from the subjects' heads and they were placed in a stereotaxic frame (Kopf Instruments; Tujenga, CA). The scalp was scrubbed with Betadine and alcohol swabs and a midline incision is made from anterior to posterior. After the skin and membranes were retracted, the skull was leveled between bregma and lambda.

Bilateral microinjection guide cannulae (22 gauge, PlasticsOne, Inc; Roanoke, VA) were implanted dorsal to the NAc core (1.6 mm anterior, 3.1 mm lateral, 6.4 mm ventral from bregma at a 10° angle). Stainless steel skull screws and dental cement were used to secure the infusion cannulae to the skull. Dummy cannulae (28 gauge, PlasticsOne, Inc; Roanoke, VA) were inserted into the infusion cannulae and screwed into place to protect the cannula from obstruction. Animals were placed under a heat lamp until ambulatory, given subcutaneous fluids, and then returned to their home cages. Post-operative pain was managed by subcutaneous administration of Rimadyl (2.5-5.0 mg). Animals recovered when they reached pre-surgery body weight (~2 days) at which time they are put back on a restricted diet.

5. Drugs

Dopamine Antagonists. To block dopamine activity at the D_1 receptor, the D_1 receptor antagonist R(+)-SCH-23390 hydrochloride (Sigma-Aldrich; St. Louis, MO) was dissolved in physiological saline at concentrations of 0.2, 1.0, 4.0 μg/μL. SCH23390 was infused in a volume of 0.5μL for a total of 0.1, 0.5, and 2.0μg per side. Previous experiments have demonstrated these doses effective in modulating goal-directed behavior (Koch, Schmid, & Schnitzler, 2000; Nowend et al., 2001; Yun, Wakabayashi, Fields, & Nicola, 2004). To selectively block dopamine D_2 receptors, the D_2 antagonist S(−)-Raclopride (+)-tartrate salt (Sigma-Aldrich; St. Louis, MO) was dissolved in physiological saline at concentration of 4, 12, and 20 μg/μL. Raclopride was infused in volume of 0.5μL for a total of 2, 6, and 10μg per side. Previous experiments have demonstrated these doses effective in modulating goal-directed behavior (Nakajima, 1989; Wolterink et al., 1993; Yun, Wakabayashi, et al., 2004).

GABA Agonists. In order to inactivate the NAc core, the $GABA_A$ agonist muscimol and the $GABA_B$ agonist baclofen (Sigma-Aldrich; St. Louis, MO) were each dissolved in a vehicle of

physiological saline at a concentration of 62.5, 250, 500ng/μL. Muscimol and baclofen were then combined in equal parts for a concentration of 31.25, 125, and 250 ng of each drug per μL of solution. This mixture of muscimol and baclofen was infused in a volume of 0.5μL for a total of 15.625, 61.25, and 125ng of each drug per side. Previous experiments have demonstrated these doses effective in modulating goal-directed behavior (Stopper & Floresco, 2011).

Glutamate Antagonists. In order to block afferent glutamate activity to the NAc core, the glutamate NMDA receptor antagonist D(−)-2-Amino-5-phosphonopentanoic acid (D-AP5; Tocris; Ellisville, MO) was dissolved in physiological saline at a concentration of 1.0, 2.0, and 4.0μg/μL. D-AP5 was later infused in a volume of 0.5μL for a total of 0.5, 1.0, and 2.0μg per side. To block glutamate activity at AMPA receptors, 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX; Sigma-Aldrich; St. Louis, MO) was dissolved in a vehicle of 50% dimethyl sulfoxide (DMSO) plus 50% distilled water at concentrations of 0.5, 1.0, and 2.0μg/μL. CNQX was infused in a volume of 0.5μL for a total of 0.25, 0.5, and 1.0μg per side. Previous experiments have demonstrated these doses effective in modulating goal-directed behavior (Yun, Nicola, et al., 2004; Yun, Wakabayashi, et al., 2004).

6. Experimental Procedure

Four separate groups of animals were utilized for behavioral testing: SCH23390/Raclopride (n=6), Muscimol/Baclofen (n=8), D-AP5 (n=7), CNQX (n=18). The order of drug infusion for each group was randomized using a Latin Square design. Animals received drug infusions every three days with two days of normal Go+/NoGo+ performance in between. Three days prior to the beginning of testing, animals were prepared for a test intracranial microinjection. Five minutes prior to the test session, the dummy cannulae were removed and 28 gauge injector cannulae (extending 1mm below the tip of the cannulae) were

inserted into the guides. Each injection cannulae was connected by polyethylene tubing to a 10uL syringe (Hamilton Company, Reno, NV). To ensure that any effects observed on the first test day of testing were not due to the initial acute damage caused by the injection cannulae extending 1mm beyond the guide cannulae, animals received a test infusion of 0.9% isotonic saline. Following saline testing, animals performed two days of standard Go+/NoGo+ training to ensure they maintained baseline levels of performance. On drug testing days, animals were once again prepared for intracranial microinjection. Bilateral drug infusions were made simultaneously at a rate of 0.25μL/minute. Injectors were kept in place for one minute following infusion completion to ensure diffusion of the drug out of the cannulae, before being gently removed and the dummy cannulae replaced. Animals were immediately placed into the behavioral chamber, and the behavioral session started. Following completion of the session, animals were returned to their home cages. The following two days after drug testing were drug-free training days on the Go+/NoGo+ paradigm in which the animal was required to complete 150 trials with less than 20 errors on Go+ trials. Reaching criterion, animals were given another day of pharmacological testing.

7. Data Analysis

Behavioral Performance. To determine the effects of pharmacological manipulation (SCH23390, raclopride, muscimol/baclofen, D-AP5, CNQX) of the NAc core on performance in the Go+/NoGo+ paradigm, a two-way (trial type: Go+ vs NoGo+ by drug treatment) within-subjects analyses of variance (ANOVAs) was calculated for each pharmacological manipulation. Significant main effects and interactions were further explored using Tukey's post-hoc comparisons.

Reaction Time. To determine the effects of pharmacological manipulation (SCH23390, raclopride, muscimol/baclofen, D-AP5, CNQX) of the NAc core on reaction time in the Go+/NoGo+ paradigm, a two-way (trial type: Go+ vs NoGo+ by drug treatment) within-subjects ANOVAs was calculated for each pharmacological manipulation. Significant main effects and interactions were further explored using Tukey's post-hoc comparisons. If there were insufficient NoGo+ errors, and therefore insufficient NoGo+ reaction times, only Go+ reaction times were evaluated using a one-way ANOVA (drug treatment). A significant effect was further explored using Tukey's post-hoc comparison.

Reward Seeking. To determine the effects of pharmacological manipulation (SCH23390, raclopride, muscimol/baclofen, D-AP5, CNQX) of the NAc core on reward seeking behavior, the percentage of Go+ and NoGo+ cues that were followed by a head entry within 10 seconds were calculated. A two-way (trial type: Go+ vs NoGo+ by drug treatment) within-subjects ANOVAs was calculated for each pharmacological manipulation and significant main effects and interactions further explored using Tukey's post-hoc comparisons.

Statistical analyses were carried out using Statistica 10 (StatSoft, Inc.; Tulsa, OK) software with a significance level of 0.05.

8. Histological Verification of Cannulae Placement

Following completion of the experiment, animals were injected with a lethal dose of sodium pentobarbital (100 mg/kg). Microinjection sites were marked with a 0.5μL infusion of black India Ink prior to transcardial perfusion with 0.9% phosphate buffered saline followed by a 4% formalin solution. Brains were extracted and stored in 4% formalin before being mounted and frozen in a -20°C cryostat (LEICA CM1850). Coronal sections (50 μm) through the NAc were made and examined for the location of the injector tips and spread of India Ink. Brain slices

were mounted on gelatin-subbed slides, coverslipped using Permount (Fisher Scientific), and examined under a light microscope (VistaVision). Placements were verified within the NAc using the stereotaxic atlas by Paxinos and Watson (2005).

C. Results

1. Microinjection Cannulae Were Located in the Nucleus Accumbens Core

Histology for DA D_1 Receptor Blockade. Cannulae placements for microinjections blocking dopamine D_1 receptors are shown in Figure 3.1. Infusion locations were located in nucleus accumbens core and along the border between the core and ventral nucleus accumbens shell. Placements were located between 1.44 and 2.52mm anterior to bregma. Placements were located between 1.5 to 1.8mm lateral to the midline and from 6.4 to 6.7mm ventral to the surface of the brain.

Histology for DA D_2 Receptor Blockade. The same group of animals was used for blockade of DA D_2 receptors with raclopride as the previously described SCH23390 experiment. Cannulae placements for this group are shown in Figure 3.1.

Histology for $GABA_A$ and $GABA_B$ Receptor Activation. Cannulae placements for all muscimol/baclofen microinjections are shown in Figure 3.2. Infusion locations were located in nucleus accumbens core and along the border between the core and ventral nucleus accumbens shell. Placements were located between 0.60 and 2.52mm anterior to bregma, 1.4 to 2.1mm lateral to the midline and from 6.6 to 7.0mm ventral to the surface of the brain.

Histology for Glutamate NMDA Receptor Blockade. Cannulae placements for microinjections of the glutamate NMDA receptor antagonist D-AP5 are shown in Figure 3.3. Infusion locations were located in nucleus accumbens core and along the border between the core and ventral nucleus accumbens shell. Placements were located between 1.68 and 2.52mm

anterior to bregma, 1.4 to 1.9mm lateral to the midline, and from 6.6 to 7.3mm ventral to the surface of the brain.

Histology for Glutamate AMPA Receptor Blockade. Cannulae placements for microinjections of the glutamate AMPA receptor antagonist CNQX are shown in Figure 3.4. Infusion locations were located in nucleus accumbens core and along the border between the core and ventral nucleus accumbens shell. Placements were located between 1.44 and 2.52mm anterior to bregma. Placements were located between 1.5 to 1.8mm lateral to the midline and from 6.4 to 6.7mm ventral to the surface of the brain.

2. Blockade of Dopamine D_1 Receptors With SCH23390 Impairs Go+ Responding

SCH23390 Impairs Go+ Responding. In order to assess the impact of blockade of dopamine D_1 receptors in the NAc core on Go+/NoGo+ behavioral performance, SCH23390 was infused immediately prior to behavioral testing and behavioral performance on Go+ and NoGo+ trials was monitored. Data were expressed as the percentage of trials that were performed correctly (responding following Go+ cues and withholding responding following NoGo+ cues). To determine the effects of D_1 receptor blockade on Go+/NoGo+ behavioral performance, a 2 (trial type: Go+, NoGo+) X 4 (drug treatment: 0, 0.1, 0.5, 2.0μg SCH23390) repeated-measures analysis of variance (ANOVA) was conducted. Behavioral performance varied as a function of trial type, $F(1, 5) = 90.95$, $p < 0.001$. NoGo+ trials were completed with greater accuracy than Go+ trials. Additionally, behavioral performance varied as a function of drug treatment, $F(3, 15) = 12.57$, $p < 0.001$. Follow-up analysis using Tukey's HSD revealed that while behavioral performance following treatment with vehicle and 0.1μg SCH23390 were equivalent, animals treated with both 0.5μg and 2.0μg SCH23390 performed substantially worse on the Go+/NoGo+ paradigm. A significant interaction between trial type and drug treatment moderated these

results, $F(3, 15) = 13.11$, $p < 0.001$. *Post hoc* analyses using Tukey's HSD revealed that Go+ performance varied as a function of drug treatment. Performance on Go+ trials following treatment with 0.1μg, 0.5μg, and 2.0μg were impaired as compared to performance following vehicle treatment ($p < 0.05$). In contrast, NoGo+ performance did not differ across the different drug treatments (see Figure 3.5a).

SCH23390 Increases Go+ Trial Reaction Time. To assess the impact of blockade of dopamine D_1 receptors in the NAc core on reaction time, the amount of time between cue presentation and behavioral response was monitored for both correctly performed Go+ trials and incorrectly performed NoGo+ trials. Reaction time data for NoGo+ trials were not analyzed as very few NoGo+ trials were associated with responses (errors). As a result, only reaction time data for Go+ trials were further analyzed. To determine the effects of D_1 receptor blockade on Go+ reaction time, a one way (drug treatment: 0, 0.1, 0.5, 2.0μg SCH23390) repeated-measures analysis of variance (ANOVA) was calculated. Reaction time (s) varied as a function of drug treatment, $F(3, 6) = 14.98$, $p < 0.01$. Follow-up analysis using Tukey's HSD revealed that animals treated with 0.1, 0.5, and 2.0μg SCH23390 had significantly slower reaction times on Go+ trials than animals treated with vehicle (see Figure 3.5b; $p < 0.01$).

SCH23390 Impairs Reward-Seeking Following Go+ Cues. To assess the impact of blockade of dopamine D_1 receptors in the NAc core on reward seeking, the amount of time between cue presentation and a head entry into the reward port was monitored for Go+ and NoGo+ trials. This analysis allows for the assessment of reward-seeking behavior even in the absence of behavioral responding. To determine the effects of D_1 receptor blockade on Go+/NoGo+ reward seeking behavior, a 2 (trial type: Go+, NoGo+) X 4 (drug treatment: 0, 0.1, 0.5, 2.0μg SCH23390) repeated-measures analysis of variance (ANOVA) was calculated.

Reward seeking behavior (as measured by percentage of trials in which the animal made a head entry within 10s of the cue) varied as a function of trial type, $F(1, 5) = 21.35$, $p < 0.01$. NoGo+ trials were more likely to be followed by a head entry than Go+ trials. Additionally, reward seeking varied as a function of drug treatment, $F(3, 15) = 15.00$, $p < 0.001$. Follow-up analysis using Tukey's HSD revealed that while reward seeking following treatment with vehicle, 0.1μg, and 0.5μg SCH23390 were equivalent, animals treated with 2.0μg SCH23390 entered the reward port on significantly fewer trials ($p < 0.001$). A significant interaction of trial type and drug treatment moderated these results, $F(3, 15) = 4.52$, $p < 0.05$. *Post hoc* analyses using Tukey's HSD revealed that animals entered the reward port following cue presentation on a similar number of Go+ and NoGo+ trials following treatment with vehicle, 0.1μg, and 0.5μg SCH23390. However, following treatment with 2.0μg SCH23390, animals entered the reward port following the NoGo+ cue more often than the Go+ cue (see Figure 3.5c; $p < 0.01$).

3. Blockade of Dopamine D_2 Receptors With Raclopride Impairs Go+ Responding

Raclopride Impairs Go+ Responding. In order to assess the impact of blockade of dopamine D_2 receptors in the NAc core on Go+/NoGo+ behavioral performance, raclopride was infused immediately prior to behavioral testing and behavioral performance on Go+ and NoGo+ trials was monitored. Data was expressed as the percentage of trials that were performed correctly (responding following Go+ cues and withholding responding following NoGo+ cues). To determine the effects of D_2 receptor blockade on Go+/NoGo+ behavioral performance, a 2 (trial type: Go+, NoGo+) X 4 (drug treatment: 0, 2, 6, 10μg raclopride) repeated-measures analysis of variance (ANOVA) was calculated. Behavioral performance (as measured by percentage of trials completed correctly) varied as a function of trial type, $F(1, 5) = 159.68$, $p < 0.001$. NoGo+ trials were completed with greater accuracy than Go+ trials. Additionally,

behavioral performance varied as a function of drug treatment, $F(3, 15) = 27.426$, $p < 0.001$. Follow-up analysis using Tukey's HSD revealed that animals treated with 2, 6, and 10μg of raclopride performed less accurately on the Go+/NoGo+ paradigm than vehicle treated animals ($p < 0.001$). A significant interaction between trial type and drug treatment moderated these results, $F(3, 15) = 27.93$, $p < 0.001$. *Post hoc* analyses using Tukey's HSD revealed that Go+ performance varied as a function of drug treatment. Performance on Go+ trials following treatment with 2, 6, 10μg were impaired as compared to performance following vehicle treatment ($p < 0.001$). In contrast, NoGo+ performance did not differ across the different drug treatments (see Figure 3.6a).

Raclopride Does Not Affect Go+ Trial Reaction Time. To assess the impact of blockade of dopamine D_2 receptors in the NAc core on reaction time, the amount of time between cue presentation and behavioral response was monitored for both correctly performed Go+ trials and incorrectly performed NoGo+ trials. Reaction time data for NoGo+ trials were not analyzed as very few NoGo+ trials were associated with responses (errors). As a result, only reaction time data for Go+ trials were further analyzed. To determine the effects of D_2 receptor blockade on Go+ reaction time, a one way (drug treatment: 0, 2, 6, 10μg raclopride) repeated-measures analysis of variance (ANOVA) was calculated. Reaction time (s) did not vary as a function of drug treatment, $F(3, 3) = 1.36$, *ns*, indicating that raclopride did not impact reaction time (see Figure 3.6b).

Raclopride Impairs Reward-Seeking Following Go+ Cues. To assess the impact of blockade of dopamine D_2 receptors in the NAc core on reward seeking, the amount of time between cue presentation and a head entry into the reward port was monitored for Go+ and NoGo+ trials. To determine the effects of D_2 receptor blockade on Go+/NoGo+ reward seeking

behavior, a 2 (trial type: Go+, NoGo+) X 4 (drug treatment: 0, 2, 6, 10μg raclopride) repeated-measures analysis of variance (ANOVA) was calculated. Reward seeking behavior varied as a function of trial type, $F(1, 5) = 277.03$, $p < 0.001$. NoGo+ trials were more likely to be followed by a head entry than Go+ trials. Additionally, reward seeking varied as a function of drug treatment, $F(3, 15) = 15.78$, $p < 0.001$. Follow-up analysis using Tukey's HSD revealed that animals treated with 2, 6, 10μg demonstrated impaired reward seeking as compared to vehicle treated animals ($p < 0.05$). A significant interaction of trial type and drug treatment moderated these results, $F(3, 15) = 6.11$, $p < 0.01$. *Post hoc* analyses using Tukey's HSD revealed that animals entered the reward port following cue presentation on a similar number of Go+ and NoGo+ trials following treatment with vehicle. However, following treatment with 2, 6, and 10μg of raclopride, animals entered the reward port following the NoGo+ cue more often than the Go+ cue (see Figure 3.6c; $p < 0.05$).

4. Activation of $GABA_A$ and $GABA_B$ Receptors Impairs Go+ Responding

Muscimol/Baclofen Microinjections Impair Go+ Responding. In order to assess the impact of activation of $GABA_A$ and $GABA_B$ receptors in the NAc core on Go+/NoGo+ behavioral performance, muscimol and baclofen were infused immediately prior to behavioral testing and behavioral performance on Go+ and NoGo+ trials was monitored. Data was expressed as the percentage of trials that were performed correctly (responding following Go+ cues and withholding responding following NoGo+ cues). To determine the effects of $GABA_A$ and $GABA_B$ receptor activation on Go+/NoGo+ behavioral performance, a 2 (trial type: Go+, NoGo+) X 4 (drug treatment: 0, 15.63, 62.5, 125ng muscimol/baclofen) repeated-measures analysis of variance (ANOVA) was calculated. Behavioral performance (as measured by percentage of trials completed correctly) varied as a function of trial type, $F(1, 7) = 14.55$, $p <$

0.001. NoGo+ trials were completed with greater accuracy than Go+ trials. Additionally, behavioral performance varied as a function of drug treatment, $F(3, 21) = 16.91$, $p < 0.001$. Follow-up analysis using Tukey's HSD revealed that animals treated with 125ng of muscimol/baclofen performed less accurately on the Go+/NoGo+ paradigm than vehicle, 15.63, and 62.5ng treated animals. There was not a significant interaction between trial type and drug treatment, $F(3, 21) = 5.92$, $p < 0.01$. While there is no difference in NoGo+ performance across the doses of muscimol/baclofen, activation of GABA receptors dose dependently decreased Go+ trial performance as compared to vehicle, specifically at 62.5ng and 125ng of each drug per side (see Figure 3.7a).

Muscimol/Baclofen Dose Dependently Increases Go+ Trial Reaction Time. To assess the impact of $GABA_A$ and $GABA_B$ receptor activation in the NAc core on reaction time, the amount of time between cue presentation and behavioral response was monitored for both correctly performed Go+ trials and incorrectly performed NoGo+ trials. Reaction time data for NoGo+ trials were not analyzed as very few NoGo+ trials were associated with responses (errors). As a result, only reaction time data for Go+ trials were further analyzed. To determine the impact of $GABA_A$ and $GABA_B$ receptor activation on Go+ reaction time, a one way (drug treatment: 0, 15.63, 62.5, 125ng muscimol/baclofen) repeated-measures analysis of variance (ANOVA) was calculated. Reaction time (s) varied as a function of drug treatment, $F(3, 18) = 5.51$, $p < 0.01$. Follow-up analysis using Tukey's HSD revealed that animals treated with 125ng muscimol/baclofen had significantly slower reaction times on Go+ trials than animals treated with vehicle (see Figure 3.7b; $p < 0.01$).

Muscimol/Baclofen Impairs Reward-Seeking Behavior. To assess the impact of activation of $GABA_A$ and $GABA_B$ receptors in the NAc core on reward seeking, the amount of time

between cue presentation and a head entry into the reward port was monitored for Go+ and NoGo+ trials. To determine the effects of $GABA_A$ and $GABA_B$ receptors on Go+/NoGo+ reward seeking behavior, a 2 (trial type: Go+, NoGo+) X 4 (drug treatment: 0, 15.63, 62.5, 125ng muscimol/baclofen) repeated-measures ANOVA was calculated. Reward seeking behavior varied as a function of trial type, $F(1, 7) = 13.51$, $p < 0.01$. NoGo+ trials were more likely to be followed by a head entry than Go+ trials. Additionally, reward seeking varied as a function of drug treatment, $F(3, 21) = 9.43$, $p < 0.001$. Follow-up analysis using Tukey's HSD revealed that animals treated with 125ng demonstrated impaired reward seeking as compared to vehicle, 15.63, and 62.5ng treated animals ($p < 0.05$). There was not a significant interaction of trial type and drug treatment on reward seeking behavior, $F(3, 21) = 0.52$, *ns* (see Figure 3.7c).

5. Blockade of Glutamate NMDA Receptors Impairs Go+ Responding

D-AP5 Microinjections Impair Go+ Responding. In order to assess the impact of glutamate NMDA receptor blockade in the NAc core on Go+/NoGo+ behavioral performance, D-AP5 was infused immediately prior to behavioral testing and behavioral performance on Go+ and NoGo+ trials was monitored. Data was expressed as the percentage of trials that were performed correctly (responding following Go+ cues and withholding responding following NoGo+ cues). To determine the effects of NMDA receptor blockade on Go+/NoGo+ behavioral performance, a 2 (trial type: Go+, NoGo+) X 4 (drug treatment: 0, 0.5, 1.0, 2.0μg D-AP5) repeated-measures analysis of variance (ANOVA) was calculated. Behavioral performance (as measured by percentage of trials completed correctly) varied as a function of trial type, $F(1, 6) = 6.59$, $p < 0.05$. NoGo+ trials were completed with greater accuracy than Go+ trials. Additionally, behavioral performance varied as a function of drug treatment, $F(3, 18) = 5.37$, $p < 0.01$. Follow-up analysis using Tukey's HSD revealed that animals treated with 2.0μg of D-AP5

performed less accurately on the Go+/NoGo+ paradigm than vehicle, 0.5, and 1.0μg treated animals. There was a trend towards a significant interaction between trial type and drug treatment moderating these results, $F(3, 18) = 3.05$, $p = 0.05$. Follow-up analysis with Tukey's post-hoc testing revealed that while treatment with D-AP5 had no impact on NoGo+ trial performance, 2.0μg of D-AP5 significantly impaired Go+ trial responding (see Figure 3.8a; $p < 0.05$).

D-AP5 Trends Towards Increasing Go+ Trial Reaction Time. To assess the impact of glutamate NMDA receptor blockade in the NAc core on reaction time, the amount of time between cue presentation and behavioral response was monitored for both correctly performed Go+ trials and incorrectly performed NoGo+ trials. Reaction time data for NoGo+ trials were not analyzed as very few NoGo+ trials were associated with responses (errors). As a result, only reaction time data for Go+ trials were further analyzed. To determine the impact of glutamate NMDA receptor blockade on Go+ reaction time, a one way (drug treatment: 0, 0.5, 1.0, 2.0μg D-AP5) repeated-measures analysis of variance (ANOVA) was calculated. Reaction time (s) trended towards varying as a function of drug treatment, $F(3, 18) = 3.07$, $p = 0.05$, however follow-up analysis using Tukey's HSD did not reveal any change in reaction time across doses of D-AP5 (see Figure 3.8b; *ns*).

D-AP5Impairs Reward-Seeking Behavior on Go+ Trials. To assess the impact of glutamate NMDA receptor blockade in the NAc core on reward seeking, the amount of time between cue presentation and a head entry into the reward port was monitored for Go+ and NoGo+ trials. A 2 (trial type: Go+, NoGo+) X 4 (drug treatment: 0, 0.5, 1.0, 2.0μg D-AP5) repeated-measures ANOVA was calculated. Reward seeking behavior varied as a function of trial type, $F(1, 6) = 8.73$, $p < 0.05$. NoGo+ trials were more likely to be followed by a head entry

than Go+ trials. Reward seeking did not vary as a function of drug treatment, $F(3, 18) = 2.88$, *ns*. A significant interaction between trial type and drug treatment moderated these results, $F(3, 18) = 3.45$, $p < 0.05$. Follow up analysis with Tukey's post hoc test revealed that animals entered the reward port following cue presentation on a similar number of Go+ and NoGo+ trials following treatment with vehicle, 0.5, and 1.0μg D-AP5. However, following treatment with 2.0 μg D-AP5, animals entered the reward port following the NoGo+ cue more often than the Go+ cue (see Figure 3.8c; $p < 0.05$).

6. Blockade of Glutamate AMPA Receptors Impairs Go+ Responding

CNQX Microinjections Selectively Impair NoGo+ Responding. In order to assess the impact of glutamate AMPA receptor blockade in the NAc core on Go+/NoGo+ behavioral performance, CNQX was infused immediately prior to behavioral testing and behavioral performance on Go+ and NoGo+ trials was monitored. Data was expressed as the percentage of trials that were performed correctly (responding following Go+ cues and withholding responding following NoGo+ cues). To determine the effects of CNQX receptor blockade on Go+/NoGo+ behavioral performance, a 2 (trial type: Go+, NoGo+) X 4 (drug treatment: 0, 0.25, 0.50, 1.00μg CNQX) repeated-measures analysis of variance (ANOVA) was calculated. Behavioral performance (as measured by percentage of trials completed correctly) varied as a function of trial type, $F(1, 17) = 23.08$, $p < 0.001$. Go+ trials were completed with greater accuracy than NoGo+ trials. Additionally, behavioral performance varied as a function of drug treatment, $F(3, 51) = 18.23$, $p < 0.001$. Follow-up analysis using Tukey's HSD revealed that animals treated with 1.00μg of CNQX performed less accurately on the Go+/NoGo+ paradigm than vehicle, 0.25, and 0.50μg treated animals ($p < 0.001$). These results were moderated by a significant interaction between trial type and drug treatment, $F(3, 51) = 11.62$, $p < 0.001$. Follow-up

analysis with Tukey's post-hoc testing revealed that while treatment with CNQX had no impact on Go+ trial performance, 0.50 and 1.00μg of CNQX significantly impaired NoGo+ trial responding (see Figure 3.9a; $p < 0.05$).

CNQX Does Not Impact Go+ or NoGo+ Trial Reaction Time. To assess the impact of glutamate AMPA receptor blockade in the NAc core on reaction time, the amount of time between cue presentation and behavioral response was monitored for both correctly performed Go+ trials and incorrectly performed NoGo+ trials. A 2 (trial type: Go+, NoGo+) X 4 (drug treatment: 0, 0.25, 0.50, 1.00μg CNQX) repeated-measures ANOVA was calculated. Reaction time did not vary as a function of trial type, $F(1, 12) = 0.75$, *ns*, drug treatment, $F(3, 36) = 2.08$, *ns*, or an interaction between trial type and drug treatment, $F(3,36) = 0.22$, *ns* (see Figure 3.9b).

CNQX Does Not Impact Reward-Seeking Behavior. To assess the impact of glutamate AMPA receptor blockade in the NAc core on reward seeking, the amount of time between cue presentation and a head entry into the reward port was monitored for Go+ and NoGo+ trials. A 2 (trial type: Go+, NoGo+) X 4 (drug treatment: 0, 0.25, 0.50, 1.00μg CNQX) repeated-measures ANOVA was calculated. Reward seeking behavior did not vary as a function of trial type, $F(1, 17) = 3.05$, *ns*, drug treatment, $F(3, 51) = 0.83$, *ns*, or an interaction between trial type and drug treatment, $F(3, 51) = 1.81$, *ns*.

D. Discussion

The NAc is well-established as a structure integral for numerous aspects of goal-directed behavior. Pharmacological manipulations of the NAc potentiate hedonic behavioral responses to taste stimuli (Baldo & Kelley, 2007; Kelley et al., 2005; Peciña & Berridge, 2000; Will, Pratt, & Kelley, 2006). NAc neurons encode the rewarding affective stimuli with reductions in firing rate and aversive affective stimuli with increases in firing rate (Nicola et al., 2004a; Roitman et al.,

2005; Taha & Fields, 2006; Wheeler et al., 2008; Wilson & Bowman, 2005). While the NAc encodes the rewarding properties of taste stimuli, many studies suggest the NAc also plays an important role in the execution of goal-directed behaviors. Neurons of the NAc modulate their firing rate during reward-predictive cues (Ambroggi et al., 2011; Day et al., 2006; Day et al., 2011; Roitman et al., 2005), anticipation of operant responding (Ambroggi et al., 2011; Carelli & Deadwyler, 1994; Carelli, 2002), and immediately following the response during reward delivery (Carelli & Deadwyler, 1994; Carelli, 2002; Day et al., 2006). Additionally, the NAc may mediate the pattern of actions selected as inactivation of the NAc increases premature responding (Cardinal, Pennicott, Sugathapala, Robbins, & Everitt, 2001), impulsive responding (Cardinal et al., 2001; Christakou, Robbins, & Everitt, 2004), and perseveration on incorrect responding (Christakou et al., 2004). At times, it is to our advantage to resist approaching and engaging with behavioral stimuli, that is to exert behavioral inhibition, in order to maximize rewards. Therefore, we given that the NAc has been implicated in numerous aspects of goal-directed behavior, we undertook a series of pharmacological manipulations in an effort to determine whether the NAc is essential for approach behavior, or rather more globally involved goal-directed behavior including the inhibition of approach to obtain reward.

1. Blockade of Dopamine D_1 and D_2 Receptors Suppress Operant Responding

To determine the importance of NAc DA receptor activation during Go+/NoGo+ performance, we systematically blocked D_1 and D_2 receptors. Blockade of both D_1 activity with SCH23390 and D_2 activity with raclopride resulted in a reduction in Go+ trial accuracy while leaving NoGo+ trial accuracy intact. All infused doses of both SCH23390 and raclopride resulted in a selective decrease in the number of correctly performed Go+ trials, indicating that the animals no longer responded following either cue. These results extend previous work

suggesting DA within the NAc plays a strong role in motivated behavior (Salamone, 1996; Kiyatkin, 2002; Kelley et al., 2005; Ikemoto, 2007; Carlezon & Thomas, 2008).

Historically, generalized destruction of the NAc afferent dopaminergic pathway via 6-hydroxydopamine (6-OHDA) lesions has long been known to impair behavior directed at obtaining rewards (Aberman et al., 1998; Hamill et al., 1999; Ikemoto & Panksepp, 1999). 6-OHDA lesions of the NAc greatly reduce operant responding in animals in an effort-dependent manner. Low effort paradigms, such as fixed ratio (FR) 1 or 5 responding, are relatively unaffected by DA-depleting lesions (Aberman & Salamone, 1999; Salamone et al., 2001). However, following exposure to paradigms requiring increasing motivational demands, such as the progressive ratio schedule, 6-OHDA lesions produce significant and profound decreases in the levels of responding (Aberman & Salamone, 1999; Salamone et al., 2001). Thus, the dopaminergic projections from the VTA to the NAc appear critical in mediating effort exertion in motivationally challenging tasks.

However, while DA in general has been demonstrated to mediate motivated behaviors, DA activity within the NAc is not homogenous. As discussed previously (see Chapter I, page 11) DA released in the NAc can activate any of five different receptor subtypes. These receptors are traditionally grouped into two categories: D_1-like receptors (D_1 and D_5) and D_2-like receptors (D_2, D_3, and D_4). The majority (83-94%) of direct and indirect pathways of NAc neurons are thought to uniquely express D_1 and D_2 receptors, respectively, with approximately 6-17% co-expressing both receptors at detectable levels (Bertran-Gonzalez et al., 2008; Lu, Ghasemzadeh, & Kalivas, 1998; Matamales et al., 2009). Activation of D_1-expressing neurons in the NAc has been associated with in an increase in motor output and a reduction in freezing behavior. In contrast, activation of the D_2-expressing neurons in the NAc is associated with a reduction in

motor behavior and an increase in freezing behavior (Kravitz et al., 2010). As D_1 receptors enhance the excitability of direct pathway MSNs and D_2 receptors reduce the excitability of indirect pathway MSNs, selective blockade of D_1 and D_2 receptors might produce similar results on operant behavior. Indeed, our results indicate that blockade of both the direct or indirect pathways (via D_1 and D_2 blockade respectively) resulted in reductions in operant performance.

Our results support a role for both D_1 and D_2 receptors in mediating Go+ responding in our Go+/NoGo+ paradigm. Interference with DA activity via the direct pathway has a rich history of interfering with goal-directed behavior. Multiple studies have found that D_1 receptor blockade in the NAc reduces operant responding (Aberman et al., 1998; Koch, Schmid, & Schnitzler, 2000; McGregor & Roberts, 1993; Nowend et al., 2001; Salamone, Correa, Farrar, & Mingote, 2007; Yun, Nicola, et al., 2004; Yun, Wakabayashi, Fields, & Nicola, 2004). Similar to the results of our study, intra-accumbens infusions (0.3-4.0ug/side) or systemic injection (0.038-0.3mg/kg) of SCH23390 consistently suppress operant responding for food reward in demanding fixed ratio (Koch et al., 2000; Nowend et al., 2001), progressive ratio (Aberman et al., 1998), and discriminative stimulus paradigms (Yun, Nicola, et al., 2004; Yun, Wakabayashi, et al., 2004) while also preventing cue-induced reinstatement of food-seeking behavior (Guy, Choi, & Pratt, 2011). Blockade of D_1 receptors has also been shown to reduce overall operant responding for cocaine reward (McGregor & Roberts, 1993) in addition to attenuating cocaine seeking following a priming dose of drug (Anderson, Bari, & Pierce, 2003). Blockade of D_1 receptors appears to efficiently suppress behavior during operant paradigms.

The mediation of Go+ responding by D_1 receptors and the direct pathway support the theory that while activation of the direct pathway encourages movement (Kravitz et al., 2010), blockade of D_1 receptors on the direct pathway neurons may suppress movement and operant

responding. However, the reduction in goal-directed behavior may not be a direct effect of D_1 receptor blockade. Activation of D_1 receptors within the NAc has been demonstrated to increase the excitability of direct pathway neurons via an increase in the surface expression of NMDA and AMPA receptors (Hallett, Spoelgen, Hyman, Standaert, & Dunah, 2006; Snyder et al., 2000). Therefore, these cells are more excitable to glutamatergic inputs. D_1 receptor activation also functions to enhance excitatory currents in NAc neurons produced by glutamatergic afferents (Cepeda, Buchwald, & Levine, 1993). As a result, increases in motor activity following D_1 receptor activation may result indirectly from increased excitability of NAc MSNs to glutamatergic afferents. Therefore, blockade of D_1 receptors, such as the SCH23390 manipulations performed here, may not directly block activation of the direct pathway, but rather reduce the excitability of the NAc MSNs leading to less activation overall.

Traditionally, the indirect pathway is thought to be the counterpart, or functional opposite, of the direct pathway. As mentioned previously, activation of the indirect pathway results in increased freezing and a reduction in motor behavior (Kravitz et al., 2010). As the majority of indirect pathway MSNs colocalize D_2 receptors, and D_2 receptor activation reduces the excitability of MSNs (Surmeier et al., 2007), D_2 receptor activation should inhibit the function of the indirect pathway and result in an increase in goal-directed behavior. Therefore, blockade of D_2 receptors may result in enhanced excitability of indirect pathway MSNs which would result in an increase in freezing behavior. This is supported by numerous studies suggesting that intra-accumbens infusions or systemic administration of the D_2 receptor antagonists raclopride or sulpiride reduce operant responding in challenging fixed ratio (Koch et al., 2000; Nowend et al., 2001), progressive ratio (Aberman et al., 1998), or discriminative stimulus paradigms (Yun, Wakabayashi, et al., 2004) in addition to blocking cue-induced

reinstatement to food-seeking behavior (Guy et al., 2011). These results suggest that blockade of D_2 receptors within the NAc suppresses operant behavior. A recent study by Cui and colleagues (2013) found that selecting an action to execute evoked increases in activity of both direct and indirect pathways of striatal MSNs. These authors maintain that activation of the direct pathway promotes the appropriate or "wanted" motor program while activation of the indirect pathway functions to suppress competing motor programs (Cui et al., 2013). If this is the case, while reducing the excitability of the direct pathway via D_1 receptor blockade would interfere with performance of the appropriate motor program, increasing the excitability of the indirect pathway via D_2 receptor blockade may enhance the suppression of competing motor programs.

While the tendency to explain the reduction in Go+ responding following D_1 and D_2 receptor blockade in terms of reduced motor capabilities is tempting, our work does not support a reduction in operant responding due to an inability to move. DA has been shown critical for motor behavior in general, however, the motivation to act is impaired long before the ability to move is compromised (Wise, 2004). Studies by Salamone and colleagues (1994; 1996) reveal that reduced responding following DA depletion does not arise from a general motor impairment as depleted animals are capable of exerting effort to obtain food rewards, but are unwilling to do so if a lower effort option is available (Cousins, Atherton, Turner, & Salamone, 1996; Salamone et al., 1994). Animals maintain the ability to respond in less motivationally demanding operant paradigms following blockade of DA receptors (Yun, Nicola, et al., 2004). Though large depletions of DA with 6-OHDA can indeed produce locomotor deficits, once again, the motivation to act is impaired long before the ability to move is compromised. Reductions in locomotor behavior or attenuations in the locomotor stimulatory effects of DA agonists such as cocaine following blockade of D_1 and D_2 receptors (Baldo, Sadeghian, Basso, & Kelley, 2002;

Dreher & Jackson, 1989; Kaddis, Wallace, & Uretsky, 1993; McGregor & Roberts, 1993; Neisewander, O'Dell, & Redmond, 1995) may instead reflect a failure of the animals to engage with salient behavioral stimuli (Ikemoto & Panksepp, 1999). Animals given the option between responding in an operant paradigm for a preferred food source and eating a freely available less preferred food source will usually perform the operant task for the highly palatable food. However, following blockade of both D_1 and D_2 receptors the reduction in operant performance is matched with an increase in less palatable chow intake (Baldo, Sadeghian, Basso, & Kelley, 2002; Koch et al., 2000; Nowend et al., 2001; Salamone et al., 2007) supporting the idea that DA receptor blockade within the NAc does not impair all movement, but rather reduces the willingness of animals to exert effort to obtain food rewards.

A shift in the willingness to exert effort for food rewards following blockade of DA receptors is reflected in our reaction time and reward-seeking data. Intra-accumbens infusions of D_1 receptor antagonist SCH23390 increased reaction time following Go+ cues. In addition, even though D_1 receptor antagonism caused a dose-dependent reduction in responding following the Go+ cue, animals continued to actively seek reward as evidenced by continued head entries into the reward port. Intra-accumbens infusions of the D_2 receptor antagonist raclopride was highly effective in reducing responding following the Go+ cue to the extent that too few Go+ responses were available for detailed analysis of reaction time. Remarkably, animals still continued to actively seek reward as evidenced by head entries in to the reward port. While increases in reaction time alone may suggest a motor impairment, animals continued checking the reward port for sucrose pellet rewards even after they stopped making operant responses. Following blockade of both D_1 and D_2 receptors, animals continued checking the reward port following NoGo+ cues and to a lesser extent following Go+ cues. This supports not only that animals still

possessed the capability to move freely following blockade of D_1 and D_2 receptors , but also that they remained sensitive to reward-predictive cues. Head entries into the reward port were more frequent following NoGo+ cues, where rewards required no additional effort or operant response to obtain. Therefore, animals were still sensitive to cues which provided reward without exerting additional effort, and as a result checked the reward port more frequently following the NoGo+ cues. While DA receptor blockade reduced willingness to perform the motivationally challenging Go+/NoGo+ paradigm, animals still remained sensitive to reward-predictive cues and demonstrated their ability to move around and check the reward port for sucrose pellets.

2. Activation of $GABA_A$ and $GABA_B$ Receptors Suppress Operant Responding

Termed a bridge between motivation and action, the NAc is optimally positioned to integrate cortical and limbic afferents and translate them into motivated behavior (Mogenson et al., 1980). As NAc activity has been demonstrated to modulate responses to rewarding stimuli, impact goal-directed behavior, and influence the selection of certain actions at the expense of others, the NAc as a whole is a structure of much interest. However, the role of the NAc in mediating goal-directed behavior is still controversial and largely unknown. While the NAc is itself composed of GABA-producing MSNs, the NAc also contains receptors for $GABA_A$ and $GABA_B$ (Bowery, Hudson, & Price, 1987). Therefore to examine the role of the NAc as a whole in Go+/NoGo+ responding, we infused the $GABA_A$ agonist muscimol and $GABA_B$ agonist baclofen immediately prior to task performance. Muscimol/baclofen infusions dose-dependently decreased Go+ trial responding in addition to increasing response latency and reducing reward-seeking as measured by the number of trials on which the animal checked the reward port following the cue.

A large divide exists in the literature on NAc inactivation studies and their effects on motivated behavior. Electrolytic lesions and inactivation of the NAc with either $GABA_A$ or $GABA_B$ agonists traditionally result in significant increases in consummatory behavior especially when infused into the medial NAc shell (Baldo & Kelley, 2007; Kelley et al., 2005; Lorens et al., 1970; Pulman, Somerville, & Clifton, 2010; Stratford & Kelley, 1997; Stratford & Wirtshafter, 2011, 2012b; Wirtshafter et al., 2012). Lesions and inactivation of the NAc have been demonstrated to both increase (Kubos et al., 1987; Lorens et al., 1970; Starkstein et al., 1988; Wong, Eshel, Dreher, Ong, & Jackson, 1991) and conversely also reduce motor output (Fuchs et al., 2004; Płaznik, Stefański, & Kostowski, 1990). Similar discrepancies surround the effects of NAc inactivation on goal-directed behavior. Several studies demonstrate that lesions and inactivation of the NAc using GABA agonists not only increase responding on reinforced and non-reinforced levers, but also increase responding during cue-induced reinstatement to drug seeking (Blaiss & Janak, 2009; Bowman & Brown, 1998; Floresco, McLaughlin, & Haluk, 2008; Pulman et al., 2012; Wirtshafter & Stratford, 2010).

As demonstrated in the current study, global interference with NAc functioning may also reduce operant responding for sucrose pellets (Balleine & Killcross, 1994; Floresco et al., 2008; Gill, Castaneda, & Janak, 2010; Trojniar et al., 2007). Similar inactivations also diminish responding for self-stimulation (Hayes, Hoang, & Greenshaw, 2011), drugs of abuse such as morphine (Yoon et al., 2009) and heroin (Alderson, Parkinson, Robbins, & Everitt, 2001; Hutcheson, Parkinson, Robbins, & Everitt, 2001), and interfere with both drug- and cue-induced reinstatement to drug seeking behavior (Chaudhri, Sahuque, Schairer, & Janak, 2010; Fuchs et al., 2004; McFarland & Kalivas, 2001). Given the increase in response latency and reduction in overall reward-seeking behavior on all trial types, our results support that global inactivation of

the NAc with muscimol and baclofen reduces goal-directed behavior. However, there are more than a few studies supporting the converse conclusion. This suggests that perhaps there are multiple populations of neurons in the NAc that may be performing different functions.

As mentioned previously, the NAc is composed of both a direct and indirect pathway each of which receive unique inputs and have differing efferent paths (Carlezon & Thomas, 2009; Gerfen et al., 1990; Wilson, 2007). The unique efferent projections may underlie some of the behavioral differences observed following global inactivation of the NAc. Kravitz and colleagues (2010) found that optogenetic activation of the direct pathway resulted in an increase in ambulation and fine motor movement concurrent with a reduction in freezing behavior. However, activation of the indirect pathway resulted in an increase in freezing behavior and a reduction in ambulation (Kravitz et al., 2010). Therefore, direct and indirect pathways may play different roles in the generation of goal-directed behavior. It's possible that previous studies examining the effects of global inactivation of the NAc have inadvertently targeted one of these pathways over the other. The studies finding increases in locomotor activity and operant behavior may have damaged or inactivated more of the indirect than the direct pathway. Conversely, studies (including our own) which have found reductions in locomotor activity and operant behavior following infusions of GABA agonists or electrolytic lesions may have accidentally caused more damage to the direct than indirect pathway. Regardless of the underlying reason for the discrepancies in the effects of NAc inactivation on behavior, global inactivation of the NAc is too blunt of a tool to effectively examine the functioning of this region. Instead, it would be more valuable to selectively target afferents to the NAc or activity of specific neurotransmitters within the NAc to tease apart the role of the NAc in goal-directed behavior.

3. Blockade of Glutamate NMDA and AMPA Receptors Have Opposing Effects on Behavioral Performance

Global inactivation of the NAc using GABA receptor agonists, in our hands, produced a reduction in operant behavior as evidenced by impairment in task performance, slower reaction times, and a reduction in the amount of reward seeking behavior as animals checked the reward port less frequently on all trials. Another means of NAc inactivation would be to block excitatory inputs with glutamate receptor antagonists. Similar to the literature surrounding inactivation of the NAc with GABA agonists, the effects of NMDA blockade in the NAc are also unclear. Blockade of NMDA receptors with the highly selective antagonist D-AP5 resulted in a reduction in Go+ trial performance at the highest dose (2ug). However, we found no changes in overall reaction time on Go+ trials, and animals still displayed sensitivity to reward-predictive cues as they sought checked for rewards more frequently following NoGo+ cues than Go+ cues. Therefore, while NMDA receptor blockade impaired task performance, animals maintained the ability to move about the chamber and check the reward port.

Our results differ with respect to the majority of the published literature to date. Though several studies implicate a role for NAc NMDA receptor activation in reducing general locomotor activity or activity stimulated by a variety of DA agonists (David, Sissaoui, & Abraini, 2004; Maldonado-Irizarry & Kelley, 1994; Pulvirenti, Berrier, Kreifeldt, & Koob, 1994), the role of NMDA receptors in goal-directed behavior suggests a very different story. NMDA receptors have been demonstrated to play a very important role in long term potentiation and learning. Blockade of NMDA receptors during task learning impairs the acquisition of Pavlovian (Dalley et al., 2005; Di Ciano et al., 2001) and operant paradigms (Hernandez, Andrzejewski, Sadeghian, Panksepp, & Kelley, 2005; Kelley et al., 1997). Similarly, blockade

of NMDA receptors immediately before altering a task, such as reinstatement following extinction, also has been demonstrated to impair task performance (Bäckström & Hyytiä, 2007; Bespalov, Dravolina, Zvartau, Beardsley, & Balster, 2000) However, typically inactivation of NMDA receptors after a task has been learned has no effect on task performance (Burns, Everitt, Kelley, & Robbins, 1994; Dalley et al., 2005; Hernandez et al., 2005; Kelley et al., 1997).

As previous work strongly supports a role for NMDA receptors during acquisition of a task and not during later expression, our findings that D-AP5 reduced performance in a Go+ paradigm may seem out of place. However, similar to our findings, Ambroggi and colleagues (2011) found that AP-5 (2ug) reduced responding during a discriminative stimulus and increased response latency when infused into the NAc in conjunction with the AMPA antagonist CNQX. The greater impairment in operant responding observed in our study may due in part to the chosen drug. Due to solubility issues, our study utilized only the active enantiomer of AP-5, D-AP5. As a result, a more potent antagonist of NMDA receptors was infused into the NAc as compared to most of the previously reported experiments. Our experiments revealed a deficit in Go+ responding only on the highest dose (2ug) of D-AP5 which is at least twice as potent as the same dose of DL-AP-5.

In addition to potential dosing issues, tyrosine hydroxylase-expressing neurons within the NAc have also been demonstrated to colocalize NMDA receptors (Gracy & Pickel, 1996; Krebs et al., 1991). The localization of NMDA receptors on neurons releasing DA provides a potent mechanism by which blockade of glutamate activity can modulate DA signaling within the NAc. Indeed, perfusion of NMDA in the NAc has been demonstrated to enhance DA release (Ohno, Arai, & Watanabe, 1995). Recent work by Parker and colleagues (2010) examined phasic DA signaling during a Pavlovian approach paradigm in animals lacking NMDA receptors on DA

neurons. They found that these knockout animals had significantly attenuated phasic DA release within the NAc core during goal-directed behavior (Parker et al., 2010). Given NMDA activation enhances DA release within the NAc and deletion of NMDA receptors on DA neurons attenuates phasic DA release, blockade of NMDA receptors may in fact inhibit DA release. As previously discussed, blockade of DA receptors in our studies resulted in a reduction in Go+ trial accuracy similar to what was seen with D-AP5 infusion. In addition, similar to our results with D_1 and D_2 receptor blockade, following inactivation of NMDA receptors within the NAc the animals maintained their sensitivity to rewarding cues as evidenced by their greater tendency to check the reward port following NoGo+ cues as opposed to Go+ cues. Therefore, it is possible that blockade of NAc NMDA receptors in our hands resulted in a reduction in DA signaling, and as a result animals displayed impaired motivation to exert effort in our Go+/NoGo+ paradigm.

In contrast to our results following DA D_1, D_2, and NMDA receptor antagonism and GABA receptor agonism, blockade of glutamate AMPA receptors resulted in a selective impairment in NoGo+ trial performance. Reduced accuracy on NoGo+ trials indicates that animals were inappropriately making operant responses following NoGo+ cues instead of exercising behavioral inhibition. However, intra-NAc inactivation of AMPA receptors with CNQX resulted in no changes in Go+ trial response latency or reward-seeking behavior. Blockade of glutamate AMPA receptors within the NAc has distinct effects depending on the location of infusion. Infusions of AMPA antagonists into the NAc core have been demonstrated to reduce the locomotor stimulatory effects of cocaine (Kaddis et al., 1993) in addition to impairing operant responding for food and drug rewards (Ambroggi et al., 2011; LaLumiere & Kalivas, 2008; Yun, Nicola, et al., 2004). AMPA receptors appear to play a very different role in the shell. Drugs impairing the action of AMPA within the NAc shell increase feeding behavior

(Maldonado-Irizarry, Swanson, & Kelley, 1995; Stratford, Swanson, & Kelley, 1998) and locomotor activity (Burns et al., 1994). Similar to the results of the current study, intra-NAc shell infusion of CNQX results in an increase in responding following cues and during times that are never rewarded (Ambroggi et al., 2011; Yun, Nicola, et al., 2004). While our infusions were aimed at the NAc core, assessment of our histology reveals that a number of the placements were located in close proximity to the ventral NAc core/shell border (see Figure 3.8). Additionally, our infusion volume of 0.5μL is estimated to spread approximately $1mm^3$ within neural tissue (Routtenberg, 1972) which is more than enough distance for our infusions to diffuse from the core to the shell. Collectively, this suggests that the selective impairment in NoGo+ performance may be driven by the blockade of AMPA receptors within the NAc shell, rather than the core.

While previous work suggests that glutamate activity within the NAc shell is important for exercising behavioral inhibition, the NAc shell receives glutamatergic inputs from a number of structures, including the infralimbic prefrontal cortex, basolateral amygdala, and ventral subiculum of the hippocampus, all of which are believed to provide the NAc with different pieces of information (Britt et al., 2012; Voorn et al., 2004; Zahm & Brog, 1992; Zahm, 2000). These cortical afferents have been demonstrated to greatly influence goal-directed behavior with the ventral subiculum hypothesized to provided spatial and contextual information, the basolateral amygdala provides both affective information and information about conditioned associations, and the prefrontal cortex allows for executive control and behavioral inhibition (Sesack & Grace, 2010). Given the roles that each NAc glutamatergic afferent is believed to play, our results may be mediated by the projection from the infralimbic prefrontal cortex to the NAc shell. Multiple studies from Fields and colleagues have systematically inactivated the dorsal (prelimbic) and ventral (infralimbic) prefrontal cortex in order to examine the effects on a DS

task for food reward. These results suggest that while inactivation of the prelimbic prefrontal cortex (which projects to the NAc core) reduces overall operant responding (Ishikawa, Ambroggi, Nicola, & Fields, 2008a; Ishikawa, Ambroggi, Nicola, & Fields, 2008b), inactivation of the infralimbic prefrontal cortex increases unrewarded responding (Ghazizadeh, Ambroggi, Odean, & Fields, 2012; Ishikawa et al., 2008a). Similar to the results found with operant responding for food reward, inactivation of the prelimbic prefrontal cortex is interferes with drug-induced reinstatement to cocaine seeking (Stefanik et al., 2013) while inactivation of the infralimbic prefrontal cortex enhances cocaine-seeking and reinstatement behavior (LaLumiere, Smith, & Kalivas, 2012; Peters, LaLumiere, & Kalivas, 2008). Therefore, our data would suggest an inactivation of the infralimbic to NAc shell glutamatergic projection resulting in a reduction in behavioral inhibition during NoGo+ trials and therefore an increase in operant behavior.

4. Conclusions

Given the demonstrated role of the NAc in signaling reward and influencing reward-seeking behavior, we sought to tease apart the role of various neurochemical signals within the NAc in mediating goal-directed behavior during our Go+/NoGo+ paradigm. Our results support the NAc as a structure that integrates information from numerous limbic afferents and translates that information into goal-directed behavior. Glutamatergic pathways from the prefrontal cortex to the NAc may be involved in implementing behavioral inhibition as inactivation of glutamatergic AMPA receptors within the NAc resulted in an increase in non-rewarded responding. Blockade of both types of DA receptors resulted in a reduction in responding, possibly reflecting the role of DA receptor activation in motivation to engage in operant tasks. While global inactivation of the NAc with muscimol/baclofen resulted in a reduction in operant responding, this may reflect the heterogeneity of the cell populations in the NAc. As the NAc is

composed of both a direct and indirect pathway that are hypothesized to play potentially opposing roles in the generation of goal-directed behavior, global inactivation of the NAc may affect one or both of these pathways leading to unpredictable results on operant responding. Taken together, these results suggest that the vast array of inputs to the NAc may indeed encode different components of goal-directed behaviors which are integrated in the NAc before being translated into action.

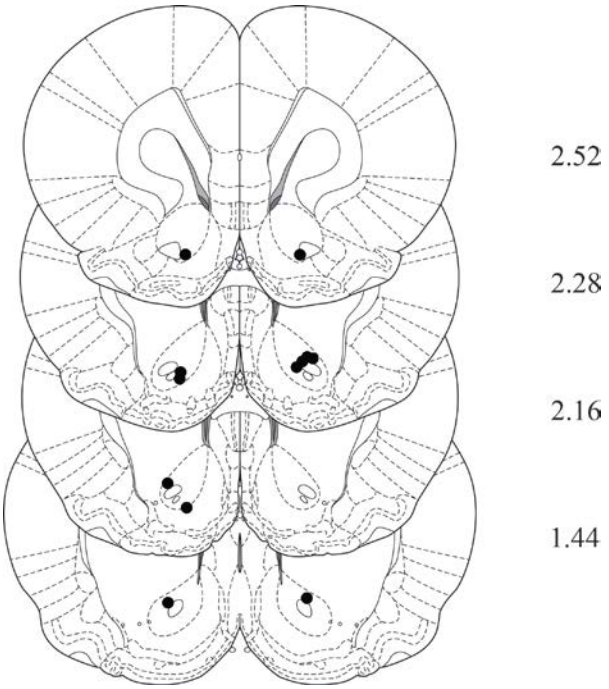

Figure 3.1: Histology of microinjection cannulae in the NAc core in animals infused with the dopamine D_1 receptor antagonist SCH23390 and D_2 receptor antagonist raclopride. Each histological image is paired with a number which represents the distance in mm anterior from bregma. Brain histological images were adapted from the stereotaxic atlas of Paxinos & Watson (1998).

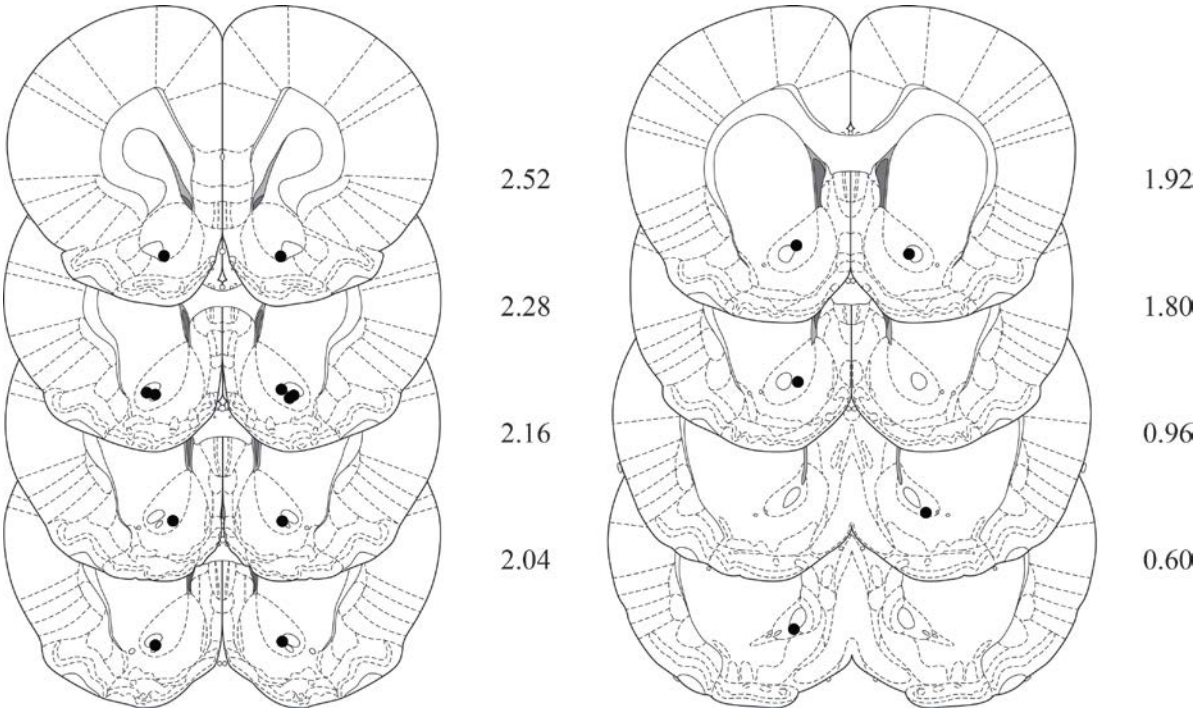

Figure 3.2: Histology of microinjection cannulae in the NAc core in animals infused with the $GABA_A$ receptor agonist muscimol and the $GABA_B$ receptor antagonist baclofen. Each histological image is paired with a number which represents the distance in mm anterior from bregma. Brain histological images were adapted from the stereotaxic atlas of Paxinos & Watson (1998).

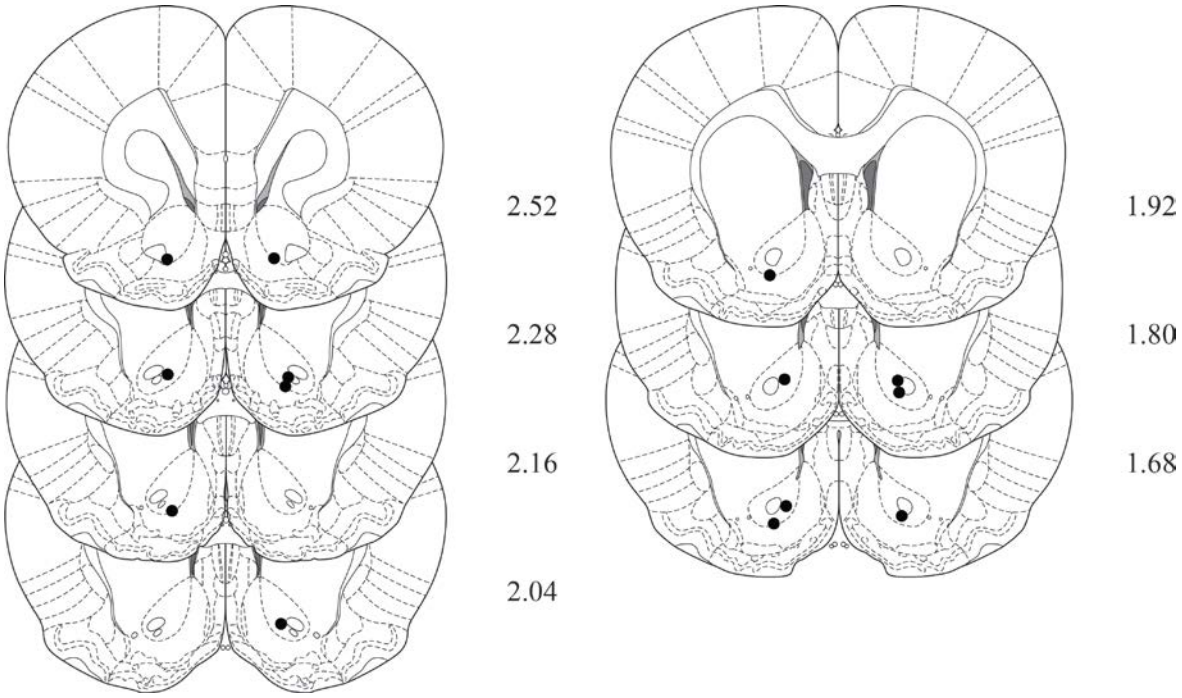

Figure 3.3: Histology of microinjection cannulae in the NAc core in animals infused with the glutamate NMDA receptor antagonist D-AP5. Each histological image is paired with a number which represents the distance in mm anterior from bregma. Brain histological images were adapted from the stereotaxic atlas of Paxinos & Watson (1998).

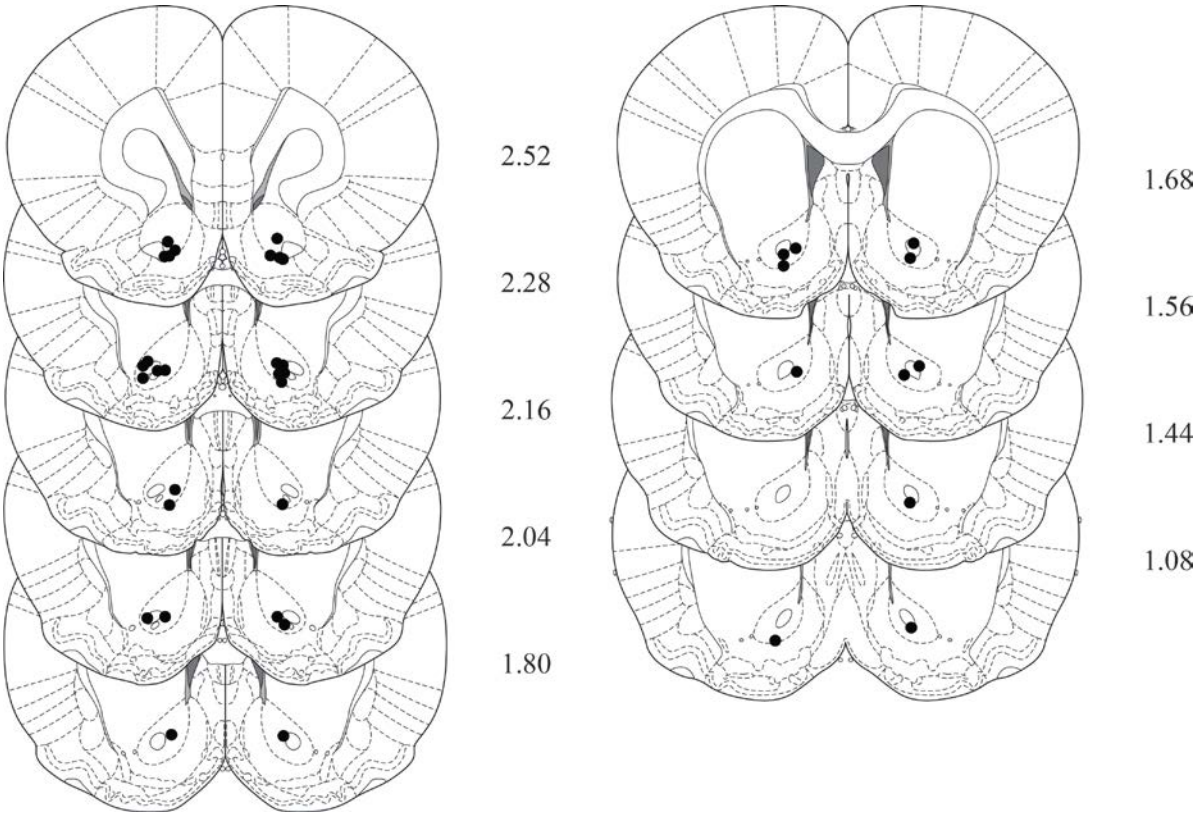

Figure 3.4: Histology of microinjection cannulae in the NAc core in animals infused with the glutamate AMPA receptor antagonist CNQX. Each histological image is paired with a number which represents the distance in mm anterior from bregma. Brain histological images were adapted from the stereotaxic atlas of Paxinos & Watson (1998).

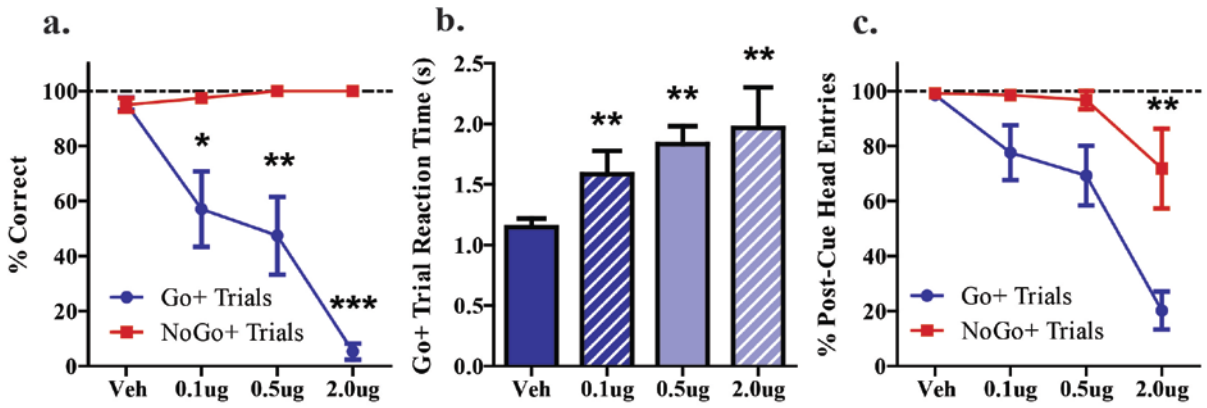

Figure 3.5: Behavioral performance following microinjection of the dopamine D_1 antagonist SCH23390 into the NAc core (a; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to vehicle). SCH23390 selectively impaired Go+ trial performance at all doses. Reaction time to Go+ trials was increased for all doses of SCH23390 (b; ** $p < 0.01$ compared to vehicle). 2.0µg SCH23390 impaired reward seeking behavior on Go+ trials as measured by the percentage of trials in which the animal made a head entry following the cue (c; ** $p < 0.01$ compared to NoGo+).

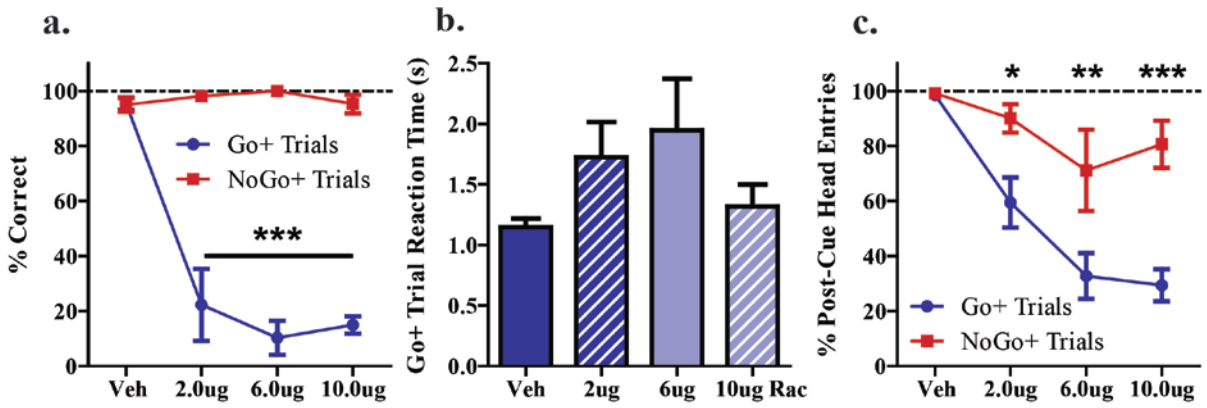

Figure 3.6: Behavioral performance following microinjection of the dopamine D_2 antagonist raclopride into the NAc core (a; *** $p < 0.001$ compared to vehicle). Raclopride selectively impaired Go+ trial performance at all doses. Reaction time to Go+ trials was not impacted by any dose of raclopride (b). Raclopride impaired reward seeking behavior on Go+ trials at all doses as measured by the percentage of trials in which the animal made a head entry following the cue (c; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to NoGo+).

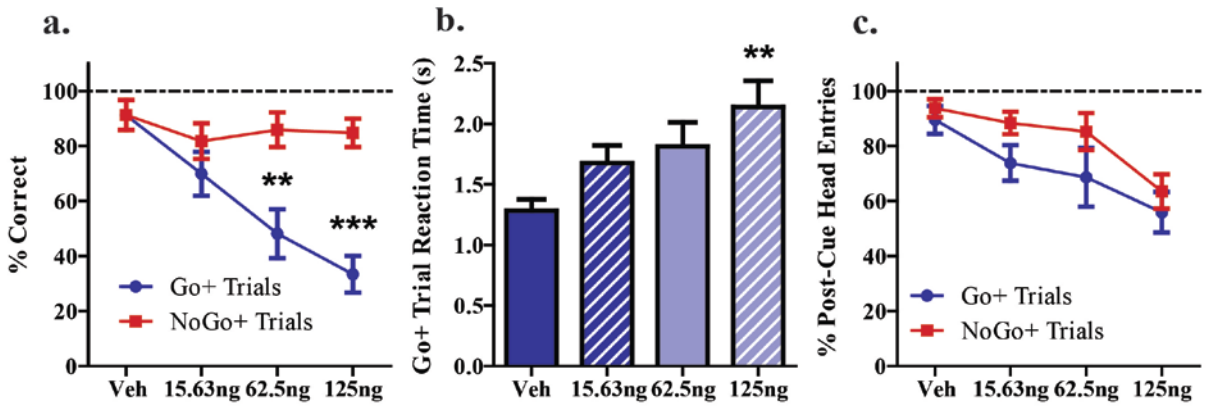

Figure 3.7: Behavioral performance following microinjection of the $GABA_A$ agonist muscimol and $GABA_B$ agonist baclofen into the NAc core (a). Muscimol/baclofen selectively and dose-dependently impaired Go+ trial performance at 62.5ng and 125ng (** $p < 0.01$, *** $p < 0.001$ compared to vehicle). Muscimol/baclofen (125ng) increased Go+ trial reaction times (b; ** $p < 0.01$ compared to vehicle).Muscimol/baclofen treatment decreased reward seeking similarly on both Go+ and NoGo+ trials (c; $p < 0.05$).

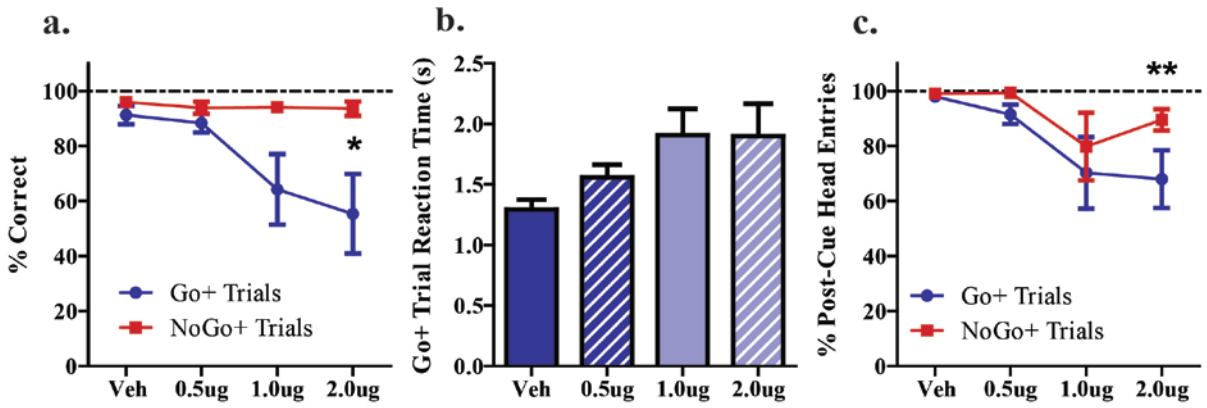

Figure 3.8: Behavioral performance following microinjection of the glutamate NMDA antagonist D-AP5 into the NAc core (a; * $p < 0.05$ compared to vehicle). D-AP5 selectively impaired Go+ trial performance at 2.0µg. Go+ trial reaction times were not affected by any dose of D-AP5 (b). D-AP5 impaired reward seeking behavior on Go+ trials at 2.0µg as measured by the percentage of trials in which the animal made a head entry following the cue (c; ** $p < 0.01$ compared to NoGo+).

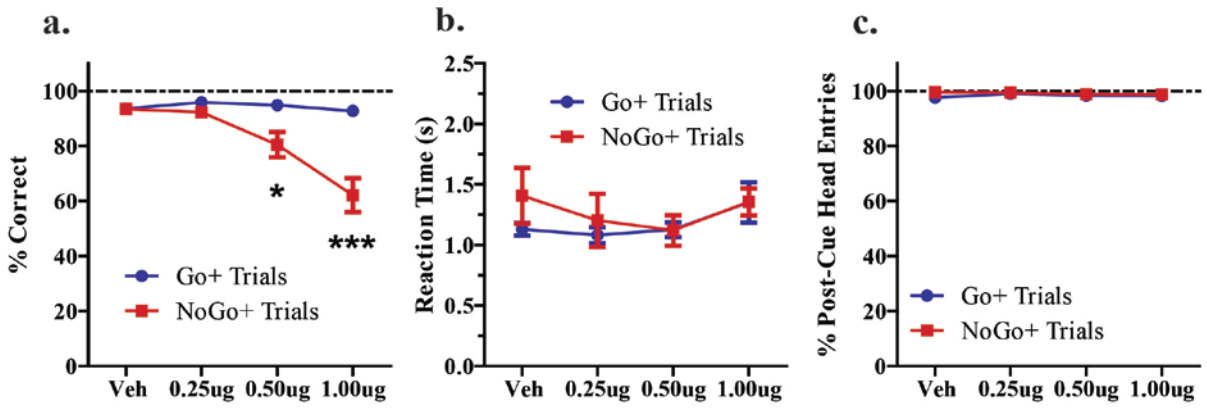

Figure 3.9: Behavioral performance following microinjection of the glutamate NMDA antagonist CNQX into the NAc core (a; * $p < 0.05$, *** $p < 0.001$ compared to vehicle). CNQX selectively impaired NoGo+ trial performance at 0.5 and 1.00µg. Reaction times were not affected by any dose of CNQX (b). CNQX did not impact reward seeking behavior following Go+ or NoGo+ trials as measured by the percentage of trials in which the animal made a head entry following the cue (c).

Chapter IV

General Discussion

A. Afferents to the NAc Encode Reward and Goal-Directed Behavior

The manner in which we behave is motivated and energized by the desire to seek and enjoy rewarding stimuli. In order to maximize our encounters with rewarding stimuli, we remain vigilant for cues and predictors that signal the delivery or presence of a reward. As individuals motivated to pursue and engage rewarding stimuli, we easily learn what situations lend themselves to reward. Utilizing what we have learned, we modify our behavior and direct our actions, or exercise behavioral inhibition, to maximize reward. A distributed network of structures contributes to various aspects of reward and goal-directed behavior. However, at the center of this network is the NAc. As described in Chapter I, the NAc is well situated to process affective stimuli in the service of motivated behavior. Indeed, the NAc is vital in the signaling of rewarding stimuli as demonstrated by pharmacological manipulations (Baldo & Kelley, 2007; Kelley et al., 2005; Peciña & Berridge, 2000; Will, Pratt, & Kelley, 2006), and self-administration of drugs of abuse (Carlezon et al., 1995; Hoebel et al., 1983; Kelsey et al., 1989; Phillips, Howes, et al., 1994; Phillips, Robbins, et al., 1994; Roberts et al., 1980). Electrophysiological recordings of NAc neurons confirm NAc MSNs are modulated by the delivery of rewarding taste stimuli (Nicola et al., 2004a; Roitman et al., 2005; Taha & Fields, 2006; Wheeler et al., 2008; Wilson & Bowman, 2005). Truly, the NAc is well ensconced as a structure critical in the signaling of primary rewards.

In addition to the confirmed role of the NAc in encoding rewarding stimuli, the NAc critically contributes to the execution of goal-directed behavior. Neurons in the NAc encode all aspects of operant responding, with modulations in firing rate during reward-predictive cues

(Ambroggi et al., 2011; Day et al., 2006; Day et al., 2011; Roitman et al., 2005), anticipation of operant responding (Ambroggi et al., 2011; Carelli & Deadwyler, 1994; Carelli, 2002), and immediately following the response during reward delivery (Carelli & Deadwyler, 1994; Carelli, 2002; Day et al., 2006). These same neurons exhibit pre-movement (Bowman, Aigner, & Richmond, 1996; Schultz, Apicella, Scarnati, & Ljungberg, 1992) and pre-operant response (Ambroggi et al., 2011; Carelli & Deadwyler, 1994; Carelli, 2002) changes in firing rate subsequent to cue presentation. Modulations in firing rate are tightly correlated with the direction of future movement suggesting that at least a subpopulation of NAc neurons encode information about the selection of actions during goal-directed operant tasks (Taha et al., 2007). The high degree of responsiveness of the NAc to all aspects of goal-directed behavior suggests that the NAc may be a key structure in synthesizing limbic information from a number of afferent projections in order to translate that information into action.

Indeed, Mogenson and colleagues (1980) long ago postulated that the NAc represents a bridge between motivation and action, or a “limbic-motor interface” as it receives numerous inputs from limbic structures involved in the regulation of affect and motivation such as the amygdala, hippocampus, thalamus, prefrontal cortex, and VTA (Salamone, 1996; Groenewegen, Wright, Beijer, & Voorn, 1999; Kiyatkin, 2002; Voorn, Vanderschuren, Groenewegen, Robbins, & Pennartz, 2004; Kelley et al., 2005; Ikemoto, 2007; Carlezon & Thomas, 2008) and sends efferent projections to areas involved in motor control such as the globus pallidus and subthalamic nucleus (Mogenson et al., 1980; Kalivas & Volkow, 2005; Carlezon & Thomas, 2008). Thus, the NAc is ideally positioned to receive afferent information about rewards and the cues that predict them, in addition to the pattern of actions designed to obtain the rewards.

The current experiment sought to clarify the role of the NAc afferents in different aspects of goal-directed behavior. We assessed the role of phasic DA release within the NAc core in signaling cues predictive of reward delivery versus the pattern of actions selected to execute. In concordance with past work, NAc core phasic DA increased in response to cues that were predictive of reward availability (Brown et al., 2011; Day et al., 2007; Jones et al., 2010; McCutcheon et al., 2012; Roitman et al., 2004; Stuber et al., 2008) while cues not predictive of reward availability failed to drive phasic DA signaling to the same degree (Brown et al., 2011; Day et al., 2007; Jones et al., 2010). The current work also demonstrates for the first time that phasic DA within the NAc core does not encode the behavioral pattern of actions that will be executed. Irrespective of whether the animal made an operant response, cues signaling the availability of reward elicited increases in phasic DA release while cues unrelated to reward delivery failed to stimulate changes in DA signaling.

Pharmacological manipulations of DA, GABA, and glutamate activity within the NAc advocate differential roles of afferents in goal-directed behavior. In the vein of other research, blockade of DA activity, both at D_1 and D_2 receptors, resulted in cessation of operant responding (Aberman et al., 1998; Koch et al., 2000; McGregor & Roberts, 1993; Nowend et al., 2001; Salamone et al., 2007; Yun, Nicola, et al., 2004; Yun, Wakabayashi, et al., 2004). Despite the fact that traditionally the direct pathway of NAc MSNs (which colocalize D_1 receptors) are thought to encourage movement while the indirect pathways of MSNs (colocalizing D_2 receptors) promotes freezing behavior and the cessation of movement, recent work has found that both pathways are active during goal-directed behavior (Cui et al., 2013). Rather than one pathway promoting behavior and the other inhibiting movement, the direct pathway may stimulate appropriate behavioral responding while the indirect pathway suppresses inappropriate

responding. T`herefore, as both pathways are important for generating the correct pattern of actions during behavioral responding, blockade of either pathway interferes with operant performance. Activation of NAc $GABA_A$ and $GABA_B$ receptors resulted in similar reductions in Go+ trial performance. Once again, simultaneous inhibition of both direct and indirect pathways in the NAc may result in both an inability to perform the correct response, and a failure to inhibit incorrect competing responses. Blockade of glutamate NMDA receptors dose-dependently reduced responding in a Go+/NoGo+ paradigm. While most work suggests that NMDA receptor blockade within the NAc impairs only the acquisition of a behavioral tasks (Dalley et al., 2005; Di Ciano et al., 2001; Hernandez et al., 2005; Kelley et al., 1997), there are NMDA receptors located within the NAc on neurons containing tyrosine hydroxylase (Gracy & Pickel, 1996). Therefore, the reduction in operant responding following NMDA receptor blockade may be the result of a reduction in DA signaling.

While NMDA receptor blockade reduced operant responding, infusion of a glutamate AMPA receptor antagonist into the NAc resulted in an increase in inappropriate operant responding during NoGo+ trials. This increase in operant responding may reflect impairment in behavioral inhibition caused by interference with a NAc shell glutamatergic afferent from the infralimbic (ventromedial) prefrontal cortex. Selective blockade of this pathway has previously been demonstrated to increase inappropriate and non-rewarded operant responding (Ghazizadeh et al., 2012; Ishikawa et al., 2008; LaLumiere et al., 2012; Peters et al., 2008). Therefore, while activation of GABA and blockade of DA and NMDA activity within the NAc suppress operant behavior, glutamate AMPA receptors appear to critically mediate the ability to behaviorally inhibit in order to obtain a reward.

B. A Potential Role of Dopamine in Behavioral Selection

As demonstrated by the current experiments, phasic DA within the NAc core does not encode the selected pattern of actions to be performed. However, this does not preclude DA from exercising any control over the selection of actions and behavioral switching within the NAc. Manipulations of DA within the NAc enhance goal-directed behavior and the ability of animals to switch between strategies for performing operant tasks (Cools, 1980; van den Bos & Cools, 1989). It has been theorized that while mild to moderate increases in the activity of DA within the NAc may facilitate behavioral switching, similar reductions in signaling may hinder switching (Redgrave et al., 1999). Therefore, while phasic DA does not directly signal the pattern of action that will be executed, DA activity within the NAc does appear to exert control over task performance.

1. DA as a Modulator of NAc MSN Excitability

As previously described, glutamate AMPA activity within the NAc may represent a critical element of behavioral selection and inhibition during goal-directed operant behavior. Inactivation of the afferent glutamatergic projections from the prelimbic prefrontal cortex to the NAc core reduces operant responding aimed at food (Ishikawa et al., 2008a,b) and drug (Stefanik et al., 2013) reward. In contrast, inactivation of afferent glutamatergic projections from the infralimbic prefrontal cortex to the NAc shell increases unrewarded responding (Ghazizadeh et al., 2012; Ishikawa et al., 2008a) and enhances cocaine-seeking and reinstatement behavior (LaLumiere et al., 2012; Peters et al., 2008). Manipulations of the glutamatergic connections between the prefrontal cortex and the NAc have a powerful impact on the execution and inhibition of goal-directed responses.

As changes in NAc DA may facilitate or prevent behavioral switching, and glutamate signaling from the prefrontal cortex may mediate behavioral inhibition, DA may influence behavioral selection through interactions with glutamate signaling within the NAc. Indeed, striatal MSNs have been demonstrated to receive inputs from multiple afferent structures simultaneously (French & Totterdell, 2003; Stuber et al., 2011). Specifically, dopaminergic and cortical afferents from the hippocampus, amygdala, and prefrontal cortex to the NAc come into close apposition, even converging on the same MSNs (Bouyer, Park, Joh, & Pickel, 1984; Sesack & Pickel, 1990, 1992; Smith & Bolam, 1990; Totterdell & Smith, 1989), suggesting that DA and glutamate potentially have the capacity to reciprocally modulate the excitability of MSNs. As activation of D_2 receptors within the striatum has been demonstrated to reduce the opening of voltage-dependent Na^+ channels (Surmeier et al., 1992) and promote the opening of K^+ channels (Greif, Lin, Liu, & Freedman, 1995), DA is ideally positioned to modulate the excitability of striatal MSNs by producing long-lasting changes in membrane conductance that can enhance or inhibit the ability of glutamate to depolarize the neurons (Lavin et al., 2005; Mercuri et al., 1985). Indeed, multiple studies have examined the excitability of NAc MSNs to glutamatergic afferents following changes in DA signaling.

Stimulation of the dopaminergic neurons of the VTA attenuates NAc neuronal excitability to hippocampal (Yang & Mogenson, 1984), amygdala (Yim & Mogenson, 1982), and prefrontal cortex stimulation (Brady & O'Donnell, 2004; O'Donnell, Greene, Pabello, Lewis, & Grace, 1999). Activation of DA receptors within the NAc also suppresses the amplitude of glutamatergic excitatory post-synaptic currents (Harvey & Lacey, 1996), and reduced currents in response to prelimbic cortical stimulation (Nicola, Kombian, & Malenka,

1996; O'Donnell & Grace, 1994). Therefore, stimulation of afferent dopaminergic projections to the NAc reduces the likelihood that glutamatergic afferents will drive NAc cells to fire.

While DA may mediate the excitability of NAc MSNs through changes in membrane conductance, DA has been demonstrated to have direct effects on the numbers of surface glutamate receptors on striatal MSNs. Activation of DA D_1 receptors triggers a second messenger cascade that can directly impact the function and trafficking of AMPA and NMDA receptors. DA D_1 receptors result in the activation of protein kinase A (PKA) which has been directly linked to increases in the surface expression of AMPA and NMDA receptors on striatal MSNs (Hallett et al., 2006; Lee et al., 2002; Scott et al., 2006; Snyder et al., 2000). Conversely, DA D_2 receptor activation promotes the trafficking of AMPA receptors away from the synaptic membrane (Håkansson et al., 2006). Collectively, this work advocates that DA may gate the excitability of NAc MSNs to glutamatergic afferents by directly modulating the number of NMDA and AMPA receptors trafficked to the synaptic membrane.

2. Direct Modulation of Pre-Synaptic Glutamate Signaling By DA

As described above, one potential mechanism for dopaminergic modulation of glutamatergic activity within the striatum is through gating the excitability of MSNs to afferents either via changes in membrane conductance or mediating surface levels of glutamate receptors. However, a separate mechanism exists for further interaction between DA and glutamate signaling within the striatum. DA receptors have been identified on the terminals of glutamatergic projections from cortical afferents (Filloux, Liu, Hsu, Hunt, & Wamsley, 1988; Godukhin, Zharikova, & Budantsev, 1984; Schwarcz, Creese, Coyle, & Snyder, 1978). Thus, DA receptors are ideally positioned to modulate pre-synaptic glutamate release.

At first glance, the effects of DA receptor activation on glutamate signaling appear contradictory. Activation of DA receptors within the striatum via DA agonists or evoking DA release via electrical stimulation of DA cell bodies has been demonstrated to enhance glutamate release within the striatum (Cepeda et al., 1993; Godukhin et al., 1984). Paradoxically, DA agonists have also been demonstrated to attenuate the release of glutamate within the striatum (Bamford et al., 2004; Crowder & Bradford, 1987; Donzanti, Hite, & Yamamoto, 1993; Godukhin et al., 1984; Rowlands & Roberts, 1980; Yamamoto & Davy, 1992; Yin & Lovinger, 2006). A recent synthesis of the literature by Wang and colleagues (2012) postulates that modulation of glutamate release within the NAc depends on the frequency of both cortical and dopaminergic activity. At lower frequencies of cortical activity (<10Hz), tonic levels of DA function to inhibit glutamate release via activation of D_2 receptors. However, at higher levels of DA activity, DA D_1 receptors are activated and enhance glutamate release via pre-synaptic mechanisms. When cortical glutamatergic activity increases, DA has an inhibitory effect on cortical activity mediated primarily through adenosine and endocannabinoid activity (Wang et al., 2012). Thus, DA and glutamate have a complex interaction within the NAc in which DA can selectively enhance or inhibit pre-synaptic glutamate release depending on the level of cortical activity.

C. Resolving Dopamine Function

At first glance, our results in chapters II and III may seem contradictory. We established that phasic DA release within the NAc core encodes cues that are predictive of reward availability, and not approach behavior. However, pharmacological manipulations suggest that blockade of DA D_1 and D_2 receptors may impair the motivation of animals to engage in operant paradigms as demonstrated by reduced responding. Thought it seems counterintuitive that phasic

DA release encodes one aspect of goal-directed behavior while DA receptor activation or blockade could serve a different function, this is not necessarily the case. First, DA receptor blockade is not simply preventing DA released during phasic activity from activating DA receptors. Rather, DA receptor blockade prevents the activation of these receptors by DA released at any time. As mentioned in Chapter I, DA neurons typically fire action potentials at tonic lower frequencies (Grace & Bunney, 1984). However, periodically DA neurons exhibit brief (<1s) high frequency (20-60Hz) phasic increases in activity (Grace & Bunney, 1984b; Hyland, Reynolds, Hay, Perk, & Miller, 2002; Schultz, 1998). Thus, there are two separate mechanisms to increase DA release within DA terminal regions such as the NAc. Phasic increases in the firing rate of DA neurons, or burst firing, evoke greater DA release compared with single-spike firing activity (Floresco et al., 2003; Gonon, 1988; Grace, 1991). However DA is also released separately from burst firing of DA neurons during single-spike activity. Therefore, DA receptor blockade has much larger implications than preventing phasic DA activity within the NAc.

In addition, phasic DA release encodes cues predictive of reward, and reward-predictive cues have been demonstrated to energize behavior. Thus, blockade of DA receptors prevents the NAc from receiving information about these cues and therefore may result in a lack of energizing behavior. As discussed earlier in this chapter, there are numerous opportunities for DA activity to mediate the excitability of NAc MSNs to glutamatergic afferents. Direct manipulations of prefrontal glutamatergic afferents to NAc core MSNs modulate goal-directed behavior (Ishikawa et al., 2008a; Stefanik et al., 2013). If phasic DA release traditionally functions to mediate the excitability to glutamatergic afferents which modulate behavior, blockade of DA receptors would impact the excitability of NAc neurons. If transmitting

information about cues that are predictive of reward availability to the NAc is an important component of energizing and motivating behavior, potentially through interactions with glutamate signaling, blockade of DA receptors would impact behavior by failing to alter the excitability of NAc neurons to glutamatergic inputs. Therefore, DA receptor blockade may result in reduced motivation to engage in operant paradigms because cues that predict reward availability fail to energize behavior through interactions with glutamate signaling within the NAc core.

D. Conclusions

Though the current experiment does not support a role for phasic DA in encoding the pattern of actions to execute, it does suggest that glutamatergic afferents to the NAc, may encode information about behavioral selection and inhibition. Phasic DA may still execute some control over behavioral selection via interactions with glutamate signaling within the NAc. DA alters the membrane conductance of NAc MSNs which in turn modulates neuronal excitability to glutamatergic afferents from regions such as the hippocampus, amygdala, and prefrontal cortex. Activation of DA receptors triggers intracellular signaling cascades that mediate the trafficking of glutamate AMPA and NMDA receptors to and from the synaptic membrane, once again impacting the excitability of the NAc. Additionally, DA modulates glutamate via receptors located on presynaptic terminals. Phasic DA release within the NAc may encode the availability of rewards in the environment, and modulate the ability of glutamate to signal behavioral inhibition. Interference with the intricate balance between DA and glutamate signaling in the NAc may be required in some cases for adaptation to relevant behavioral stimuli (Pennartz et al., 1994), but also may underlie maladaptive behaviors such as the inability to control excessive food intake or drug-seeking behavior (Kalivas, 2009).

E. Future Directions

The current set of experiments suggests several intriguing directions in which future research should proceed. Voltammetric recordings during Go+/NoGo+ and Go+/NoGo- task performance were performed in the NAc core due to the role of the NAc core in reward and goal-directed behavior. However, the dorsomedial region of the striatum is also responsive to reward-predictive cues (Brown et al., 2011) and is thought to mediate behavioral flexibility, the ability to shift from one pattern of behavior to another during reward-related learning (Kimchi & Laubach, 2009; Ragozzino, Jih, et al., 2002; Ragozzino et al., 2009; Ragozzino, Ragozzino, et al., 2002), and strategy shifting (Ragozzino & Choi, 2004; Ragozzino, Ragozzino, et al., 2002). Neuronal activity within the dorsal striatum increases prior to self-initiated movements and in response to trigger stimuli provided that a movement follows (Romo et al., 1992; Schultz & Romo, 1988). Therefore, while the current work supports phasic DA within the NAc in encoding cues that signal reward availability, phasic DA activity within the dorsal striatum may be potentially important for facilitating behavioral switching and flexibility. Recording from the dorsomedial striatum during Go+/NoGo+ and Go+/NoGo- responding would reveal whether phasic DA activity in this region participates in a similar function as the NAc core, or possibly encodes information about future patterns of action to execute.

While pharmacological manipulation of the NAc was aimed at the NAc core, the volume of infusion (0.5μL) was large enough to result in diffusion of the drugs over a distance of approximately $1mm^3$ (Routtenberg, 1972) resulting in receptor activation and blockade in both the NAc core and shell.The NAc core and shell are traditionally believed to have disparate effects on behavior. The NAc core performs an important role in goal-directed behavior, encoding information about rewards and reward-predictive cues (Brown et al., 2011; Day et al.,

2007; Di Ciano & Everitt, 2001; Fuchs et al., 2004; Jones et al., 2010; McCutcheon et al., 2012; Parkinson et al., 1999; Parkinson et al., 2002), and may mediate the level of effort exerted in motivationally challenging tasks (Sokolowski & Salamone, 1998). In contrast, the NAc shell is believed to mediate the hedonic value of rewarding taste stimuli (Peciña & Berridge, 2000; Peciña & Berridge, 2005) and feeding behavior (Basso & Kelley, 1999; Stratford & Kelley, 1997, 1999). Given differences in function, future studies should employ a smaller infusion volume in order to selectively confine pharmacological manipulations to the NAc core or shell.

The current experiments revealed that blockade of NAc glutamate AMPA receptors dose-dependently increased inappropriate operant responding during NoGo+ cues. The results are in accord with studies blocking AMPA receptors within the NAc shell that find an increase in responding following cues and during times that are never rewarded (Ambroggi et al., 2011; Yun, Nicola, et al., 2004). As the infusion volume in this study was large enough to diffuse to the shell subregion of the NAc and some of the infusion cannulae were located in close proximity to the ventral core/shell border, the effects in the current experiment may be driven by the blockade of NAc shell AMPA receptors. The NAc shell receives glutamatergic input from the infralimbic prefrontal cortex that, when inactivated, also increases unrewarded responding for food (Ghazizadeh, Ambroggi, Odean, & Fields, 2012; Ishikawa et al., 2008a) and drug reward (LaLumiere et al., 2012; Peters et al., 2008). As the effects of the current study appear to be driven by the glutamatergic connection from the infralimbic prefrontal cortex to the NAc shell, selective inactivation of both pathways in a double dissociation should be performed in order to validate that the connections between the two structures are mediating behavioral inhibition.

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Animal Care Committee Protocol Approval

This research was approved by the University of Illinois Animal Care Committee under protocol 12-110.

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EDUCATION

Post-Doctoral Research Associate University of Minnesota Minneapolis, MN

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Ph.D., 2013 University of Illinois at Chicago Chicago, IL

- Dissertation: *Dissociating Reward Prediction from Action Selection: Distinct Roles for Nucleus Accumbens Inputs*
  - Proposal: April 24, 2012
  - Defense: May 10, 2013
- Major: Behavioral Neuroscience
- Minor: Psychopharmacology
- Cumulative Grade Point Average: 4.0
- Committee: Mitchell Roitman (Advisor/Chair), Jamie Roitman, Michael Ragozzino, David Wirtshafter, Daniel Corcos

M.A., 2009 University of Illinois at Chicago Chicago, IL

- Thesis: *The Effects of Recreational Hallucinogen Salvia Divinorum on Phasic Dopamine Signaling and Motivation*
  - Proposal: March 17, 2009
  - Defense: May 20, 2009
- Committee: Mitchell Roitman (Advisor/Chair), Jamie Roitman, Michael Ragozzino, David Wirtshafter

Honors B.A., 2005 College of St. Benedict/St. John's University St. Joseph, MN

- Senor Honors Thesis: *Antidepressant Efficacy and Behavioral Comparisons of Two Animal Models of Depression*
  - Defense: April 18, 2005
- Major: Psychology
- Minors: Mathematics
- Cumulative Grade Point Average: 3.97
- Graduated Summa Cum Laude
- Committee: Linda Tennison (Chair), Rodger Narloch, Jan Holtz

PROFESSIONAL MEMBERSHIPS

The Society for the Study of Ingestive Behavior (2010-present)
The Society for Neuroscience (2008-present)
The Chicago Chapter for the Society for Neuroscience (2008-present)

RESEARCH GRANTS

University of Illinois at Chicago ***Chicago, IL***

- Chancellor's Graduate Research Fellowship, 2011-2013
  - Project Title: *Nucleus Accumbens Signals Behavioral Inhibition*

ACADEMIC AWARDS AND HONORS

University of Illinois at Chicago ***Chicago, IL***

- Chicago Chapter of Society for Neuroscience Poster Competition Winner, 2013
- Brain Research Foundation Neuroscience Day Poster Competition Winner, 2013
- Society for the Study of Ingestive Behavior New Investigator Travel Award, 2012
- UIC LAS PhD Student Travel Award, 2011-2012
- UIC Graduate College Student Presenter Award, 2008-2013
- UIC Graduate Student Council Travel Award, 2008-2012
- UIC Department of Psychology Travel Award, 2008-2012

College of St. Benedict/St. John's University ***St. Joseph, MN***

- Graduated Summa Cum Laude, 2005
- Regents'/Trustees' Scholar, 2001-2005
- Dean's List, 2001-2005
- Graduated with Distinction in Psychology

MANUSCRIPTS IN PRESS

Cone, J.J., Chartoff, E.H., Potter, D.N., Ebner, S.R., & Roitman, M.F. (2013). Prolonged exposure to a high fat diet induces a deficit in dopamine reuptake by decreasing membrane associated transporters, *PLoS One, 8*(3), 1-10.

McCutcheon, J.E., Ebner, S.R., Loriaux, A.L., & Roitman, M.F. (2012). Encoding of aversion by dopamine and the nucleus accumbens, *Frontiers in Neuroscience*, *6*(137), 1-10.

Ebner, S.R., Roitman, M.F., Potter, D.N., Rachlin, A.B., & Chartoff, E.H. (2010). Depressive like effects of the kappa opioid receptor agonists salvinorin A are associated with decreased phasic dopamine release in the nucleus accumbens. *Psychopharmacology*, *210*(2), 241-252.

MANUSCRIPTS IN PREPARATION

Chartoff, E.H., Ebner, S.R., Potter, D.N., Roitman, M.F. Temporal interaction between the kappa-opioid receptor agonist salvinorin A and cocaine on reward and dopamine signaling (*Manuscript in preparation*).

Ebner, S.R., Roitman, J.D., & Roitman, M.F. Dissociating reward prediction from action selection: Distinct roles for nucleus accumbens inputs (*Manuscript in preparation*).

Ebner, S.R., Roitman, J.D., & Roitman, M.F. Nucleus accumbens phasic dopamine signals reward prediction rather than action selection. (*Manuscript in preparation*).

ORAL PRESENTATIONS

Ebner, S. R., Roitman, J. D., Amaya, A. A., & Roitman, M. F. (2012). Nucleus accumbens phasic dopamine signals reward prediction rather than action selection, The Society for the Study of Ingestive Behavior, Zurich, Switzerland.

Ebner, S. R., Chartoff, E. H., & Roitman, M. F. (2010). The kappa receptor agonist salvinorin A suppresses phasic dopamine signaling and motivated for food reward, The Society for the Study of Ingestive Behavior, Pittsburgh, PA.

POSTER PRESENTATIONS

Ebner, S.R., Roitman, J.D., Amaya, A.A., & Roitman, M.F. (2013). Phasic dopamine recordings and behavioral pharmacology reveal differential roles for nucleus accumbens inputs in reward prediction and action selection, Brain Research Foundation Annual Neuroscience Day, Chicago, IL.

Ebner, S.R., Roitman, J.D., Amaya, A.A., & Roitman, M.F. (2012). Phasic dopamine recordings and behavioral pharmacology reveal differential roles for nucleus accumbens inputs in reward prediction and action selection, Annual Meeting for the Society for Neuroscience, New Orleans.

Amaya, A.A, Ebner, S.R., McMurray, M.S., & Roitman, J.D. (2012). Neural activity in the medial prefrontal cortex during performance of a go-nogo task, Annual Meeting for the Society for Neuroscience, New Orleans.

McCutcheon, J.E., Ebner, S.R., Loriaux, A.L., & Roitman, M.F. (2012). Intraoral infusions of sucrose suppress dopamine release in nucleus accumbens shell after induction of a conditioned taste aversion, Annual Meeting for the Society for Neuroscience, New Orleans.

Ebner, S.R., Roitman, J.D., Amaya, A.A., & Roitman, M.F. (2012). Nucleus accumbens phasic dopamine signals reward prediction rather than action selection, Chicago Chapter of the Society for Neuroscience, Chicago, IL.

Chartoff, E.H., Ebner, S.R., Potter, D.N., & Roitman, M.F. (2011). Temporal interaction between the kappa-opioid receptor agonist salvinorin A and cocaine on reward and dopamine signaling, Annual Meeting of the Society for Neuroscience, Washington D.C.

Ebner, S.R., Roitman, M.F. (2011). The rostromedial tegmental nucleus does not provide inhibitory tone to dopamine neurons, Annual Meeting of the Society for Neuroscience, Washington D.C.

Ebner, S.R., Roitman, M.F. (2011). The rostromedial tegmental nucleus does not provide inhibitory tone to dopamine neurons, The Society for the Study of Ingestive Behavior, Clearwater, FL.

Ebner, S. R., Roitman, M. F., Potter, D., Saxena, R., & Chartoff, E. H. (2010). The kappa receptor agonist salvinorin A exerts acute and delayed effects on reward function and phasic dopamine release, Brain Research Foundation Annual Neuroscience Day, Chicago, IL.

Ebner, S. R., Roitman, M. F., Potter, D., Saxena, R., & Chartoff, E. H. (2010). The kappa receptor agonist salvinorin A exerts acute and delayed effects on reward function and phasic dopamine release, Annual Meeting of the Society for Neuroscience, San Diego, CA.

Ebner, S. R., Chartoff, E. H., & Roitman, M. F. (2010). The kappa receptor agonist salvinorin A suppresses phasic dopamine signaling and motivated behavior, Chicago Chapter of the Society for Neuroscience, Chicago, IL.

Ebner, S. R., Chartoff, E. H., Potter, D., & Roitman, M. F. (2009). The kappa opioid receptor agonist salvinorin A suppresses phasic dopamine signaling and motivation, Annual Meeting of the Society for Neuroscience, Chicago, IL.

Ebner, S.R., Chartoff, E.H., & Roitman, M.F. (2009). The recreational hallucinogen *salvia divinorum* depresses phasic dopamine signaling and motivation, Chicago Chapter of the Society for Neuroscience, Chicago, IL.

Roitman, M.F., Dikopf, M., Thompson, J., Ebner, S., Ragozzino, M.E., & Fall, C.P. (2008). Neuropeptide Y increases electrically-evoked dopamine release in the nucleus accumbens, Brain Research Foundation Annual Neuroscience Day, Chicago, IL.

Roitman, M.F., Dikopf, M., Thompson, J., Ebner, S., Ragozzino, M.E., & Fall, C.P. (2008). Neuropeptide Y increases electrically-evoked dopamine release in the nucleus accumbens, Annual Meeting of the Society for Neuroscience, Washington D.C.

Ebner, S.R. (2005). Antidepressant efficacy and behavioral comparisons of two animal models of depression, National Conference for Undergraduate Research, Lexington, VA.

Ebner, S.R. (2005). Antidepressant efficacy and behavioral comparisons of two animal models of depression, St. John's University Board of Academic Affairs Meeting, Collegeville, MN.

Ebner, S.R. (2004). Blunted diurnal variation in startle during withdrawal from chronic opiate exposure in rats, Summer Research Experience for Undergraduates, Minneapolis, MN.

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TEACHING/MENTORING EXPERIENCE

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