CELLULAR AND CHEMICAL DYNAMICS WITHIN THE NUCLEUS ACCUMBENS DURING REWARD-RELATED LEARNING AND DECISION MAKING

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ABSTRACT

JEREMY DAY: Cellular and Chemical Dynamics within the Nucleus Accumbens during Reward-related Learning and Decision Making
(Under the direction of Regina M. Carelli)

The ability to form and maintain associations between environmental cues, actions, and rewarding stimuli is an elementary yet fundamental aspect of learned behavior. Moreover, in order for organisms to optimize behavioral allocation after learning has occurred, such associations must be able to guide decision making processes as animals weigh the benefits and costs of potential actions. Multiple lines of research have identified that reward-related learning and decision making are mediated by a distributed network of brain nuclei that includes the nucleus accumbens (NAc) and its innervation from dopamine neurons located in the midbrain. However, the precise neural processing that underlies this function is unclear. The first set of experiments detailed in this dissertation took advantage of technological advances to characterize patterns of NAc dopamine release in real time, during behavioral performance. The results of the first experiment demonstrate for the first time that rapid dopamine release in the NAc is dramatically altered during stimulus-reward learning. Before learning, reward delivery produced robust increases in NAc dopamine concentration. After learning, these increases had completely transferred to the predictive cue and were no longer present when rewards were delivered. Further experiments revealed that cue-evoked increases in NAc dopamine concentration did not signal reward prediction alone, but reflected the

work required to obtain rewards. Together, these results suggest that NAc dopamine encodes both the benefits and costs of predicted rewards. A second set of experiments used electrophysiological techniques to measure neural activity within the nucleus accumbens during decision making tasks. These experiments show that when rats were choosing between rewards with different effort requirements, a subset of NAc neurons tracked the degree of effort predicted by cues, while other neurons exhibited prolonged activation or inhibition as animals overcame large effort requirements to obtain rewards. Finally, when rats were choosing between rewards that came at different temporal delays, many NAc neurons exhibited changes in activity that correlated with reward delay. Such activity represents a candidate mechanism for linking actions with outcomes, and may also provide insight into the role of the NAc in psychiatric disorders characterized by maladaptive goal-directed behavior and decision making processes.

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PREFACE

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

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ABBREVIATIONS

ACC	Anterior cingulate cortex
ANOVA	Analysis of variance
BLA	Basolateral amygdala
CeA	Central nucleus of the amygdala
CoV	Coefficient of variation
CS	Conditioned stimulus
FR	Fixed ratio
FSCV	Fast-scan cyclic voltammetry
NAc	Nucleus accumbens
OFC	Orbitofrontal cortex
PCA	Principal components analysis
PEH	Peri-event histogram
PFC	Prefrontal cortex
S:B	Signal-to-baseline
SEM	Standard error of the mean
US	Unconditioned stimulus
VP	Ventral pallidum
VTA	Ventral tegmental area

CHAPTER 1

INTRODUCTION

Diverse lines of research have implicated the nucleus accumbens (NAc) and its dopaminergic innervation from the ventral tegmental area (VTA) in multiple facets of reward-related behavior, including reinforcement, learning, and decision making (Di Chiara and Imperato, 1988; Schultz et al., 1997; Berridge and Robinson, 1998; Salamone and Correa, 2002; Wise, 2004; Frank and Claus, 2006; Nicola, 2007; Phillips et al., 2007). However, the precise means by which NAc activity or dopamine release within the NAc contributes to these processes is a topic of current debate. The experiments described in this dissertation seek to investigate several aspects of NAc and dopamine function during learning and decision making tasks. Therefore, this chapter will focus on reviewing the previous literature on the role of the NAc and the mesocorticolimbic dopamine system in reward learning and decision making. This chapter will first review the overall relevance of, and processes that govern, learning and choice behavior with respect to rewards. Secondly, this chapter will discuss the cellular and systems-level mechanisms underlying neural communication within the mesolimbic dopamine system and the NAc. Finally, these ideas will be integrated in order to examine theoretical and empirical links between dopamine release in the NAc, NAc neural activity, and reward-directed behavior.

Reward-related learning and decision making

Organisms forage and survive in demanding environments by learning about the

events surrounding them and adapting behavioral strategies accordingly. Such learning is present in two well-studied forms. In *stimulus-outcome* (classical or Pavlovian) conditioning, organisms learn to associate a previously neutral stimulus (the conditioned stimulus, or CS) with a biologically salient event such as the delivery of food (the unconditioned stimulus, or US). As a result, the CS gains salience and can influence ongoing behavior by generating both prepatory and consummatory conditioned responses (Pavlov, 1927; Konorski, 1967; Brown and Jenkins, 1968; Jenkins and Moore, 1973). This type of learning is sensitive to a number of factors, including the temporal delay between the CS and US, the frequency of CS-US pairings, and the intensity of stimuli employed. However, another critical variable in Pavlovian conditioning involves the contingency between the CS and the US, or the degree to which the CS predicts the US (Rescorla, 1968, 1969, 1988). This relationship forms the basis of numerous efforts to model Pavlovian learning (Sutton and Barto, 1981; Rescorla, 1988; Sutton and Barto, 1998).

In *action-outcome* (operant or instrumental) conditioning, animals learn to associate actions or responses with biologically salient outcomes, and thus those actions increase or decrease in frequency (Thorndike, 1933; Skinner, 1938, 1981). Similar to Pavlovian conditioning, the frequency of responding observed following operant conditioning is subject to a number of variables, including the rate of reinforcement, the number of responses required for reinforcement, and the concurrent presence of other reinforcers. Over time, such responses can become habitual, and are more dependent upon the stimuli that precede them than the outcome that follows them (Watson, 1913; Dickinson, 1994). In contrast, goal-directed instrumental responses are characterized and identified by their relationship with the outcome (Balleine and Dickinson, 1992; Dickinson et al., 1996; Balleine and Dickinson,

1998). Even under instrumental contexts, environmental cues (here called discriminative stimuli) still play an important role in signaling when and whether actions will be reinforced. Once established, Pavlovian and instrumental processes interact in interesting ways. For example, it has long been realized that strong CSs can be used to reinforce instrumental actions (Zimmerman, 1957), indicating that they maintain their own reinforcing properties. Moreover, the presentation of Pavlovian cues can exert robust motivational effects on instrumental behavior, even when there is no specific connection between the cue and the response. In this phenomenon, known as Pavlovian-to-instrumental transfer (PIT), animals that were separately trained to associate a CS with delivery of a US and to press a lever for delivery of the same US are then presented with the CS in the instrumental context under extinction. Under this condition, presentation of the Pavlovian CS increases response rates, demonstrating its ability to drive goal-directed behavior (Estes, 1948; Holland, 2004).

As they relate to rewarding or reinforcing stimuli such as food, water, and copulation, these learning mechanisms are fundamental and clearly adaptive in that animals are better able to predict, prepare for, and obtain future rewards. However, natural environments present organisms with a complex array of response options that compete for behavioral resources (Stevens and Krebs, 1986). Therefore, once organisms have learned the predictive relationship between stimuli and rewards or actions and rewards, they must use this information to guide and optimize future behavior. This is critical in that available rewards may vary along multiple dimensions including their magnitude and preferability (Doya, 2008). Moreover, available responses can be burdened by different costs, such as the time required to wait for a reward and the amount of effort or work associated with obtaining a reward (Weiner, 1994; Green and Myerson, 2004; Rudebeck et al., 2006; Walton et al.,

2006). Each of these parameters can be altered separately through a number of environmental or economic constraints. In order to be efficient, decision making processes must weigh the costs and benefits of available options, consider the deprivation state of the animal, and engage motor systems to select the optimal action. It follows that in times of scarcity (when available options are few or poor), organisms must be able to overcome high costs to obtain rewards. Likewise, when options with different costs are available, behavioral allocation should shift to the lower-cost option. Decades of behavioral research indicates that this is the case. Thus, organisms routinely exhibit a preference for low-effort rewards unless the magnitude of higher-effort rewards is increased (Bautista et al., 2001; Salamone et al., 2003; Stevens et al., 2005; Walton et al., 2006; Phillips et al., 2007). Similarly, organisms (including humans) discount the value of delayed rewards in comparison to immediate rewards (a phenomenon termed delay discounting) and match response allocation to reward rate on schedules of reinforcement that involve temporal components (Herrnstein, 1970, 1974; Ainslie, 1975; Herrnstein and Loveland, 1975; Davison, 1988; Cardinal et al., 2002a; Green and Myerson, 2004). These results demonstrate that organisms use cost-related information to guide selection between actions, even when both actions will be rewarded.

The mesolimbic dopamine system

Anatomy of the VTA: Afferent and efferent projections. The mesolimbic dopamine projection originates from dopamine neurons in the VTA, which lies ventrally to the red nucleus in the midbrain. Although dopamine neurons are also present in the more lateral substantia nigra, there is a dissociation between the projection targets of these neurons. Thus, whereas dopamine neurons in the substantia nigra comprise the striatonigral dopamine system and project most prominently to the dorsal striatum (caudate and putamen), axons

emanating from dopaminergic neurons in the VTA project to diverse brain targets, including the NAc, prefrontal cortex (PFC), amygdala, hippocampus, ventral pallidum, and olfactory tubercle (Anden et al., 1964; Ungerstedt, 1971; Swanson, 1982; Haber and Fudge, 1997; Fields et al., 2007; Ikemoto, 2007). However, the projection to the NAc represents the densest pathway of dopaminergic axons leaving the VTA (Fields et al., 2007). Inputs onto dopamine neurons in the VTA also arise from diverse brain nuclei, including the PFC, lateral hypothalamus, superior colliculus, pedunculopontine tegmental nucleus, central nucleus of the amygdala, and NAc (Phillipson, 1979; Geisler and Zahm, 2005; Geisler et al., 2007). However, the precise density and origin of inputs is segregated based on the projection target of the neuron (Carr and Sesack, 2000b; Omelchenko and Sesack, 2005; Margolis et al., 2006b; Balcita-Pedicino and Sesack, 2007).

Dopamine neurophysiology and release. In vivo, dopamine neurons typically fire at a "tonic" pace (2-5 Hz), but can also exhibit glutamate-dependent "phasic" bursts of activity at greater than 20 Hz (Grace and Bunney, 1984a, b; Chergui et al., 1993; Hyland et al., 2002; Schultz, 2007). While tonic firing patterns are thought to contribute to a low-level basal concentration of dopamine at the synapse, phasic activity can produce robust yet transient increases dopamine concentration (Garris et al., 1994; Garris et al., 1999). Current estimates suggest that the basal concentration of dopamine is within the 5-20 nM range (Watson et al., 2006), whereas stimulation of dopamine neurons at frequencies that mimic phasic bursting produces concentrations in the range of 100-2000 nM (Garris et al., 1999; Phillips et al., 2003a). Such phasic or transient dopamine release events are dependent upon cell firing within the VTA (Sombers et al., 2009), yet are highly variable across different microenvironments of the ventral striatum (Wightman et al., 2007).

The precise amount of dopamine released within the NAc due to an action potential undergoes rich modulation that is based largely on the recent history of dopamine release events (Montague et al., 2004b). A host of factors converge to alter dopamine release in response to dopamine neuron activity. Thus, enhanced glutamate transmission in the NAc serves to increase dopamine release in response to the same neuronal stimulation, presumably by activation of NMDA receptors on presynaptic dopaminergic terminals (Imperato et al., 1990; Youngren et al., 1993; Howland et al., 2002). Likewise, dynorphin-induced activation of kappa opioid receptors on dopamine terminals inhibit release (Di Chiara and Imperato, 1988; Spanagel et al., 1992), and the ongoing activity of striatal cholinergic interneurons exhibits complex frequency-dependent effects on dopamine release (Rice and Cragg, 2004; Zhang and Sulzer, 2004; Cragg, 2006). Finally, dopamine release itself can inhibit future dopamine release by activating D_2 autoreceptors located on dopamine terminals (Kennedy et al., 1992; Phillips et al., 2002; Schmitz et al., 2003).

Once released, dopamine readily diffuses from the synaptic cleft (Garris et al., 1994), thereby operating as a volume neurotransmitter at target sites (including presynaptic and postsynaptic receptors). At the level of the striatum, the duration and sphere of dopamine action is regulated primarily by the presence of dopamine transporters (Gainetdinov et al., 1998; Cragg and Rice, 2004), which terminate dopamine signaling via reuptake into the presynaptic terminal where it can be repackaged into vesicles. Dopamine transporters are expressed at high levels in the dorsal and ventral striatum (Ciliax et al., 1995), and represent a major site of action for a number of drugs of abuse, including cocaine and amphetamine (Kilty et al., 1991; Giros et al., 1996; Jones et al., 1998). These drugs disrupt normal dopamine reuptake and therefore greatly increase the extracellular dopamine concentration

within the NAc (Di Chiara and Imperato, 1988; Jones et al., 1995; Jones et al., 1998; Aragona et al., 2008).

Dopamine receptors. Dopamine exerts its action at two subclasses of G-protein coupled receptors (Kebabian and Calne, 1979), most of which are located extrasynaptically (Sesack et al., 1994; Yung et al., 1995). One subclass, the "D_1-like" family of receptors (D_1 & D_5), are coupled to $G_{s/olf}$ proteins that activate adenylyl cyclase, increase levels of intracellular cyclic adenosine monophosphate (cAMP), and activate a host of ion channels and intracellular signaling pathways (such as protein kinase A) which alter the physiological and nuclear activity of the cell (Greengard et al., 1999; Greengard, 2001; Stipanovich et al., 2008). Conversely, another subclass, the "D_2-like" family of receptors (D_2, D_3, & D_4) is coupled to $G_{i/o}$ proteins which inhibit cAMP production. Although the existence of opposing receptor systems for the same neurotransmitter within the same brain region at first appears to be paradoxical, two observations suggest that this dichotomy lends itself to unique functional properties of the mesolimbic dopamine system. First, these receptors do not bind dopamine with the same affinity. Thus, whereas most D_1 receptors in the striatum exist in a low affinity state (and therefore require high concentrations of dopamine to elicit meaningful levels of receptor activation), D_2 receptors typically exhibit a high affinity for dopamine, and are therefore likely to be activated by very low levels of dopamine concentrations (Richfield et al., 1989). Secondly, neurons within the NAc exhibit mostly non-overlapping expression of D_1 and D_2 receptors (Bertran-Gonzalez et al., 2008), although not to the same degree observed among neurons in the dorsal striatum (Surmeier et al., 2007; Shen et al., 2008). Thus, phasic high concentration surges in dopamine release may specifically activate striatal D_1 dopamine receptors and therefore produce altered activity in only a subset of neurons.

Likewise, tonic changes in dopamine firing may generate differential activation at D_2 dopamine receptors to alter the activity of a different class of neurons.

In vivo dopamine measurement techniques. The evidence reviewed above suggests that dopamine release in a terminal area can vary based on a number of factors. Therefore, thorough examination of the functional role of dopamine requires measurement techniques that can directly assess dopamine concentration within terminal regions. There are presently two commonly employed methods to do so: microdialysis and electrochemical methods (Watson et al., 2006; Wightman, 2006). In microdialysis, a probe with a thin, semi-permeable membrane is placed in the brain region of interest, and a dialysate solution is perfused within the probe. As this occurs, small molecules present in the extracellular fluid will diffuse across the membrane into the dialysate, which can be collected and analyzed offline using high pressure liquid chromatography or capillary electrophoresis (Westerink, 1995). This approach has been used with success to measure dopamine concentration in the NAc during reward-related behavior and drug administration (Di Chiara and Imperato, 1988; Bassareo and Di Chiara, 1997, 1999b). Although microdialysis possesses excellent chemical selectivity and sensitivity (in the femtomolar-picomolar range) and is therefore excellent for determining the basal concentration of a molecule, the temporal resolution of measurements is typically poor (1 collection per 2-10 minutes). Thus, microdialysis is not ideally suited to measure the phasic changes in dopamine produced by bursting of dopamine neurons.

In comparison, electrochemical methods detect neurotransmitter content *in situ*, usually at a carbon-fiber microelectrode (Phillips et al., 2003b; Robinson et al., 2003). These techniques take advantage of the electroactive nature of specific analytes such as dopamine, which can undergo oxidation and reduction in response to changes in voltage. Although other

electrochemical methods have previously been used to assess changes in dopamine concentration (Doherty and Gratton, 1992), the most common electrochemical technique is fast-scan cyclic voltammetry (FSCV; **Fig. 1.1**) . Here, a carbon fiber electrode is encased in a glass pipette and pulled to a sharp tip, such that only 75-100μm of the carbon fiber is exposed. Measurements are made by ramping the voltage of the electrode to a level that oxidizes dopamine (to dopamine-ortho quinone) and then back to its original potential, which reduces dopamine-ortho quinone back to dopamine. This change in applied voltage typically

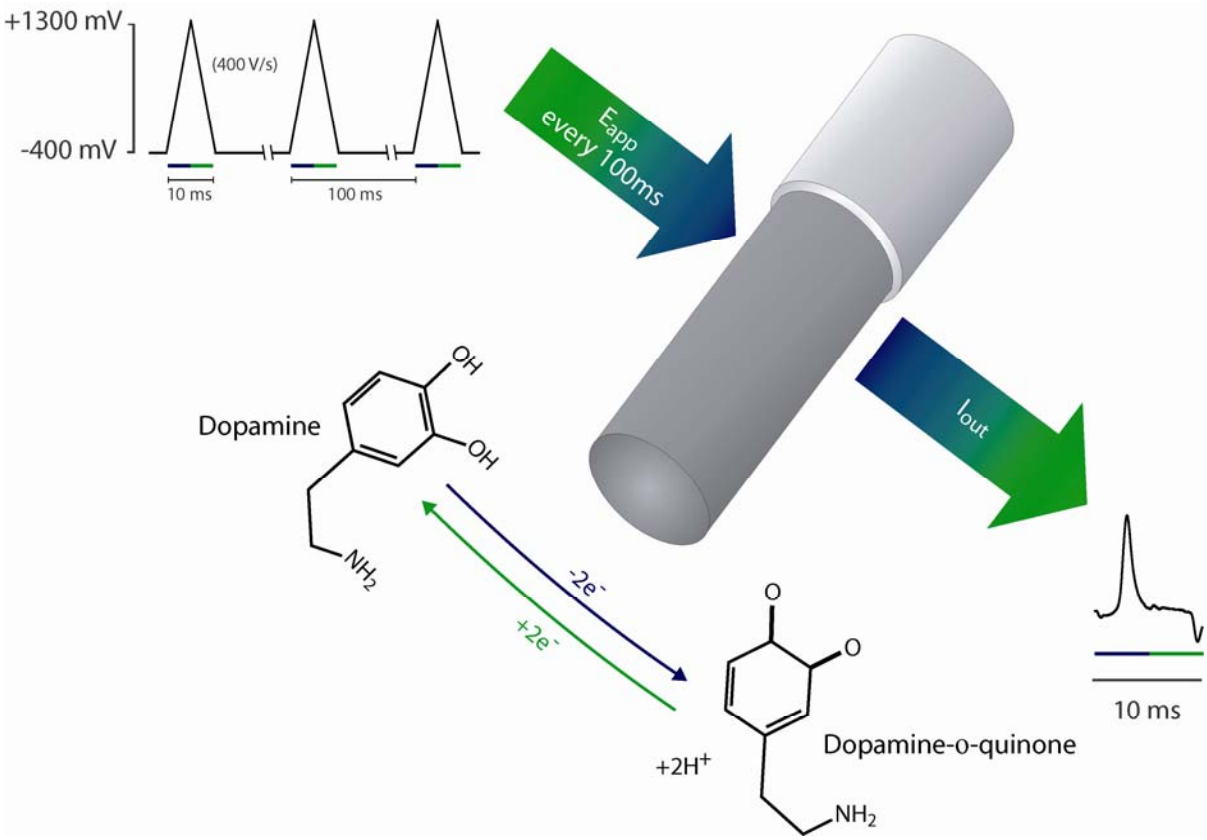

Figure 1.1. Fast-scan cyclic voltammetry. A glass-encased carbon fiber microelectrode is inserted into the target brain region. Dopamine molecules present at the carbon fiber electrode are oxidized (to dopamine-ortho-quinone) in a two-electron transfer by ramping the voltage of the electrode from its resting potential of -0.4 volts to +1.3 volts. Dopamine-o-quinone is reduced back to dopamine when the voltage is returned to its resting potential. Each reaction produces a change in current at the carbon fiber electrode which is used as a chemical signature for dopamine. The change in voltage (E_{app}) takes only 10ms is repeated every 100ms to produce a new measurement (I_{out}).

takes 10ms, and is repeated every 100ms. The result of each scan is a large faradaic current that results from oxidation and reduction of electroactive chemical species near the electrode as well as changes on the surface of the carbon fiber electrode (Kawagoe et al., 1993). This current can be detected at the exposed carbon fiber and plotted against the applied potential to produce a cyclic voltammogram, which can be subtracted from other cyclic voltammograms to provide information on how the current changed over time. As electroactive species oxidize and reduce at different voltages, background-subtracted cyclic voltammograms also provide information on the specific analyte in question (Heien et al., 2004; Heien et al., 2005), allowing dissociable measurement of ascorbate, serotonin, DOPAC, and pH (Cahill et al., 1996; Bunin and Wightman, 1998; Heien et al., 2004). Thus, FSCV provides subsecond (100ms) temporal resolution in detecting changes in dopamine at terminal regions, and has recently been applied successfully to real-time measurement of dopamine release in behaving animals (Robinson et al., 2002; Phillips et al., 2003b; Phillips et al., 2003a; Roitman et al., 2004). Aims 1 & 2 of this dissertation will therefore employ FSCV to determine changes in dopamine concentration during reward-related learning and decision making.

The nucleus accumbens

NAc cellular composition and neurophysiology. The NAc has received intense electrophysiological investigation as a part of the brain's reward pathway. The majority (>90%) of neurons in the NAc are GABAergic medium spiny projection neurons (MSNs) (Groves, 1983; Gerfen and Wilson, 1996). These neurons possess a closed-field morphology with a thin but lengthy unmyelinated axon and dendrites that radiate outwards in all directions from the soma (cell soma ~15μm in diameter) (Groves, 1983; Kawaguchi, 1993).

MSNs stain positively for a number of immunohistochemical markers, including enkephalin, dynorphin, substance P, and neurotensin, and these markers often predict the output target of the neuron (Meredith, 1999). Moreover, enkephalin-containing MSNs exhibit higher levels of D_2 receptor expression, whereas dynorphin positive neurons exhibit greater D_1 receptor expression (Le Moine and Bloch, 1995). In brain slices, medium spiny neurons exhibit a bistable membrane potential characterized by hyperpolarized "down states" at ~ -85mV, and depolarized "up states" close to the threshold for spike generation (~ -60mV) (Wilson and Kawaguchi, 1996). The transition between these states is triggered by synaptic input, and MSNs are only able to generate action potentials from the up state (Nicola et al., 2000; O'Donnell, 2003).

Less than 5% of cells in the nucleus accumbens are cholinergic interneurons (Groves, 1983; Aosaki et al., 1994; Aosaki et al., 1995; Berlanga et al., 2003). These neurons are characterized by short myelinated axons, radial yet irregular dendrites, and relatively large cell bodies (20-50μm in diameter) (Kawaguchi, 1993; Kawaguchi et al., 1995). A third type of neuron found in the NAc is the medium sized GABAergic interneuron, which also accounts for less than 5% of all striatal cells (Kawaguchi et al., 1995) yet is divisible into parvalbumin, calretinin, and somatostatin/neuropeptide Y containing populations that are believed to have unique functional roles (Kawaguchi et al., 1995; Meredith, 1999; Berke et al., 2004; Berke, 2008). In addition to differences in morphological characteristics mentioned above, NAc neurons also exhibit different firing rates when measured in vivo or in vitro. MSNs typically fire irregularly at a low rate (1-3 Hz), whereas cholinergic interneurons have firing rates often ranging from 8-15 Hz and GABAergic interneurons typically fire at >20 Hz (Yim and Mogenson, 1982; Aosaki et al., 1994; Koos and Tepper, 1999; Berke et al., 2004).

NAc anatomy: Afferent and efferent projections. The rodent NAc receives afferent projections from a variety of cortical and subcortical structures, including the basolateral amygdala (Zahm and Brog, 1992; Brog et al., 1993; Wright et al., 1996), the prefrontal cortex (McGeorge and Faull, 1989; Zahm and Brog, 1992; Brog et al., 1993), the subiculum of the hippocampus (Groenewegen et al., 1987; Groenewegen et al., 1991; Zahm and Brog, 1992; Brog et al., 1993), and a dense dopaminergic projection from the ventral tegmental area (Zahm and Brog, 1992). NAc neurons in turn impact behavior through their projections to the substantia nigra, ventral pallidum, and lateral hypothalamus (Zahm, 1999).

Given the anatomic arrangement of the NAc (**Fig. 1.2**), it was proposed by Mogenson (Mogenson, 1987) and elaborated upon by others (Everitt and Robbins, 1992; Pennartz et al., 1994; Ikemoto and Panksepp, 1999) that the NAc functions as a site for the integration of limbic information related to memory, drive and motivation, and the generation of goal-directed motor behaviors (termed 'limbic-motor integration'). Consistent with this view is the observation that NAc afferents make convergent synaptic contacts onto MSNs. Studies using immunocytochemistry in conjunction with electron microscopy showed that hippocampal and dopaminergic inputs make synaptic connections with the same NAc neuron (Totterdell and Smith, 1989; Sesack and Pickel, 1990). Likewise, Van Bockstaele and Pickel (Van Bockstaele and Pickel, 1993) reported that 5-HT terminals were in direct contact with dopaminergic axons. In addition, a convergence of inputs from the medial prefrontal cortex and the ventral subiculum on NAc neurons has recently been identified (French and Totterdell, 2002) as well as the BLA and ventral subiculum (French and Totterdell, 2003). These findings indicate that NAc afferents are capable of influencing NAc cell firing in

behaving animals (Pennartz et al., 1994; O'Donnell and Grace, 1995; Carr and Sesack, 2000a; Pinto and Sesack, 2000).

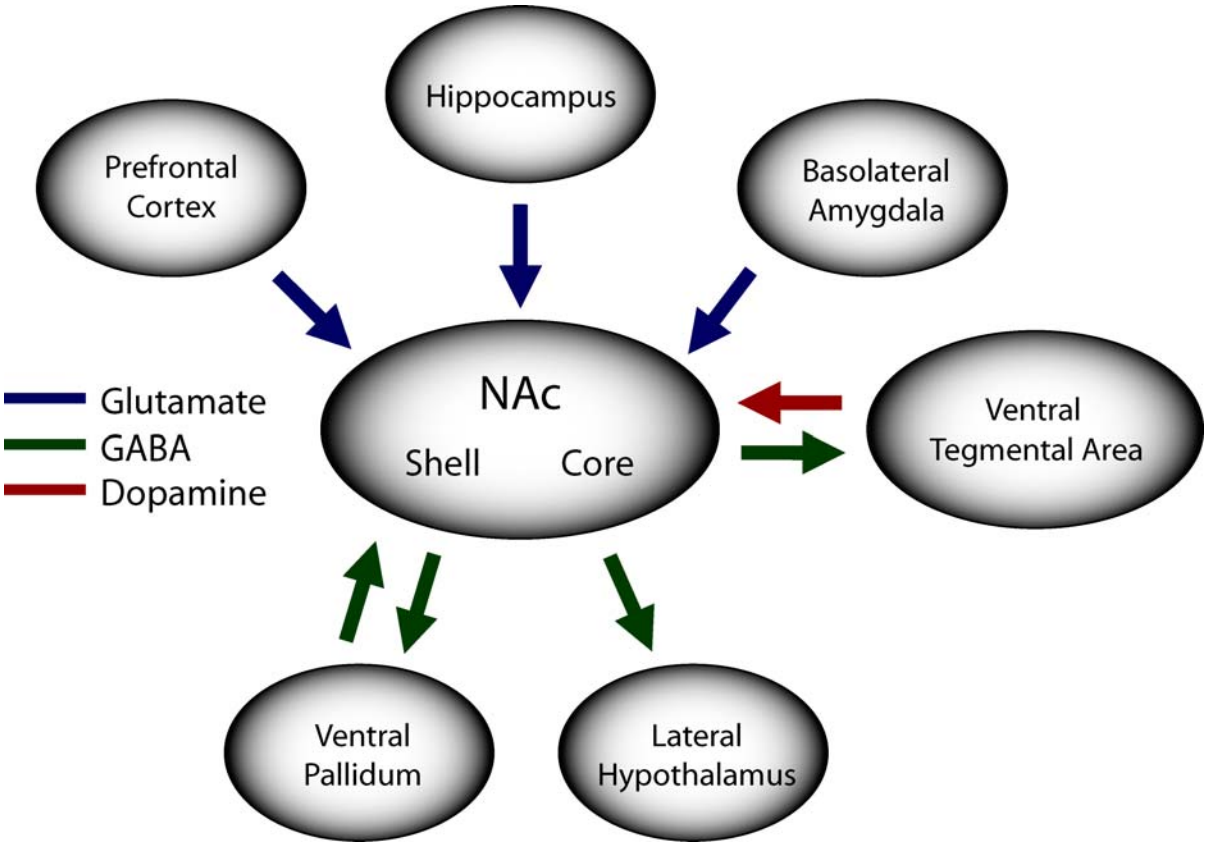

Figure 1.2. Simplified circuit diagram of afferent and efferent connections of the NAc. Locations of arrows do not necessarily indicate precise location or extent of projections. Figure has been modified from Day, J.J. & Carelli, R.M. (2007). The nucleus accumbens and pavlovian reward learning. *The Neuroscientist, 13*(2).

NAc anatomy: Subdivisions. The NAc possesses two subterritories that can be delineated both physically and functionally. Evidence suggests that the shell subregion plays a larger role in integrating emotional limbic information, while the core is necessary for the generation and direction of reward-related movements (Stratford and Kelley, 1997; Kalivas and Nakamura, 1999; Parkinson et al., 1999). Importantly, afferent projections to the NAc are not homogeneously distributed across the core and shell (Groenewegen et al., 1987;

McGeorge and Faull, 1989; Groenewegen et al., 1991; Zahm and Brog, 1992; Brog et al., 1993; Heimer et al., 1995; Heimer et al., 1997). For example, Brog and co-workers (Brog et al., 1993) showed that a number of cortical afferents of the shell and core originate in separate areas (e.g., the orbitofrontal, infralimbic, and posterior piriform cortices to the medial shell versus the dorsal prelimbic and anterior cingulate to the core). VTA input to the NAc also differs by subregion, with more medially located VTA neurons projecting to the medial shell, and more lateral VTA neurons projecting mostly to the NAc core and lateral shell (Ikemoto, 2007). Likewise, the efferent projections from the NAc differ between the core and shell subregions in the rat (Heimer et al., 1991; Zahm and Brog, 1992; Zahm and Heimer, 1993; Zahm, 1999). The NAc core parallels basal ganglia circuitry, sending outputs through the ventral pallidum (dorsolateral district), subthalamic nucleus, and substantia nigra, and these outputs in turn project via the motor thalamus to premotor cortical areas. In contrast, the shell projects preferentially to subcortical limbic regions including the lateral hypothalamus, ventral pallidum (ventromedial district) and VTA (Zahm, 1999). Interestingly, recent findings show direct interconnections between core and shell neurons, providing anatomic evidence that these NAc subregions do not function completely independently, but instead comprise interacting neuronal networks (van Dongen et al., 2005).

Synaptic actions of dopamine within the NAc. Dopamine axons terminate onto the necks of synapses in MSNs, mostly at locations where the head of the striatal synapse also receives an excitatory input (Groves et al., 1994; Moss and Bolam, 2008). This anatomical arrangement, together with a wealth of *in vitro* studies, suggests that instead of having direct excitatory or inhibitory actions, dopamine serves to modulate ongoing activity at glutamatergic synapses (O'Donnell et al., 1999; Nicola et al., 2000; Brady and O'Donnell,

2004; Goto and Grace, 2005). As mentioned above, MSNs exhibit bistable membrane potentials that are driven by convergent synaptic input (Nicola et al., 2000). In vivo, one effect of dopamine may be to "gate" glutamatergic inputs in the NAc, such that only the strongest inputs can control NAc output (Nicola et al., 2000; Floresco et al., 2001b; Floresco et al., 2001a). Glutamatergic synapses at MSNs can undergo bidirectional synaptic plasticity (both long term potentiation, or LTP, and long-term depression, or LTD) as a result of stimulated activity or drug administration (Kombian and Malenka, 1994; Nicola et al., 2000; Thomas et al., 2001). Recent studies suggest that dopamine in the NAc may direct this synaptic plasticity at glutamatergic synapses, in effect determining which ones become strengthened or weakened by activity (Thomas et al., 2000; Boudreau et al., 2007; Kourrich et al., 2007; Conrad et al., 2008; Shen et al., 2008). Dopamine receptor activation is required for the induction of synaptic plasticity at MSNs, and the overall effect of dopamine is dependent upon the type of dopamine receptor expressed within the MSN (Pawlak and Kerr, 2008; Shen et al., 2008). Thus, spike-timing dependent plasticity protocols in which presynaptic stimulation precedes postsynaptic cell firing induce LTP at cortical inputs onto D_1 containing MSNs, and this can be prevented by blocking D_1 receptors. Likewise, in D_2 containing neurons, both LTP and long-term depression LTD can be induced by different spike-timing dependent protocols, and each can be reversed by D_2 receptor antagonism (Shen et al., 2008). Importantly, this arrangement appears to offer a mechanism by which temporally coincident stimulation of D_1 and NMDA receptors can initiate complex intracellular cascades that drive changes in gene expression in a specific set of neurons (Kelley, 2004; Valjent et al., 2005; Day, 2008; Stipanovich et al., 2008). As D_1 receptors require higher concentrations of dopamine to become activated, this suggests that one role of

phasic dopamine may be to engage these molecular mechanisms and generate long-term synaptic plasticity.

Neural substrates of reward

The initial evidence that specific brain regions controlled reward processing came more than fifty years ago from studies in which rats were implanted with stimulating electrodes in multiple brain nuclei and given the opportunity to press a lever to deliver stimulation (Olds and Milner, 1954; Olds, 1958, 1962). These pioneering studies revealed that although animals would not press for brain stimulation at all brain locations, robust stimulation-directed behavior was observed when electrodes were placed near a group of projection fibres known as the medial forebrain bundle. Although it was not known at the time, it was later discovered that a portion of these axons projected from dopamine-containing neurons in the midbrain to forebrain structures, including the NAc (Anden et al., 1964; Anden et al., 1965; Ungerstedt, 1971; Nauta et al., 1978). Subsequent experiments demonstrated that the midbrain dopaminergic projection to the NAc is critical for a number of reward-related behaviors, and several decades of experimental research have attempted to elucidate the precise functional role of this connection. Early support for the involvement of the mesocorticolimbic dopamine pathway in reward processing came from several studies demonstrating that the blockade of dopamine receptors produced a decrease in goal-directed behavior for food and other rewards (Wise et al., 1978b; Wise et al., 1978a; Gallistel et al., 1982; Wise et al., 1992). Interestingly, although animals that received dopamine antagonists still worked to obtain rewards, responding decreased as a function of time, similar to what would be expected if rewards were removed altogether (Fouriezos and Wise, 1976; Wise et al., 1978b). These findings initially led to the suggestion that dopamine release in the NAc

mediates the hedonic or "pleasure" aspects of rewarding stimuli, and, in turn, that both natural and drug rewards could be defined by this common path of activation (Wise and Bozarth, 1985). However, this hypothesis has been questioned on a number of grounds. For example, dopamine antagonism in the NAc does not impair orofacial movements characteristic of reward "liking" (Pecina et al., 1997), indicating that the hedonic value of a stimulus is not based on NAc dopamine transmission. Moreover, proper NAc dopamine function is also required for tasks that are motivated by aversion rather than by rewards (Blackburn et al., 1992; Salamone, 1994). Finally, NAc dopamine depletion disrupts behavioral performance when large amounts of effort are required to obtain rewards, but has little effect on easy tasks (Aberman and Salamone, 1999). Taken together, these and other findings support a larger role for NAc dopamine beyond simple hedonic pleasure (Blackburn et al., 1987; Salamone et al., 1991; Schultz et al., 1993; Hollerman and Schultz, 1998; Waelti et al., 2001; Salamone et al., 2002; Pecina et al., 2003).

Although the precise role of dopamine in reward processing is presently under much debate, new findings and technological advances have contributed greatly to our understanding of this issue. While microdialysis investigations have long reported increases in NAc dopamine levels during goal-directed behaviors and/or receipt of rewards (Di Chiara, 2002), these investigations lack the temporal resolution necessary to associate dopamine with precise (real-time) behavioral observations. Recently, the ability to measure dopamine release on a physiologically and behaviorally relevant timescale has led to a focus on rapid NAc dopamine release events (Garris et al., 1999; Phillips et al., 2003a; Robinson et al., 2003). Using an electrochemical technique that permitted sub-second detection of dopamine, research from the present laboratory has demonstrated that operant responses for a sucrose

reward were associated with brief but robust increases in NAc dopamine concentration (Roitman et al., 2004; Stuber et al., 2004; Stuber et al., 2005). Similar dopamine signals have also been observed in male rats during exposure to and approach towards receptive females (Robinson et al., 2001), suggesting that phasic changes in dopamine release in the NAc may dynamically modulate a variety of reward-directed behaviors. Furthermore, recent results indicate that subsecond increases in NAc dopamine concentration are promoted by primary rewards but not aversive stimuli, and that this response is innate (Roitman et al., 2008).

In addition to manipulation of NAc dopamine function, numerous studies have implicated NAc processing itself in reward-related behavior. These studies have discovered that the NAc plays a direct and critical role in both the appetitive and consummatory phases of goal-directed responses (Stratford and Kelley, 1997; Swanson et al., 1997; Berridge and Robinson, 1998; Kelley, 2004). Both GABA agonism and glutamate antagonism in the NAc produce increases in food consumption, further indicating that neuronal inhibition in this structure may play an important role in the initiation or maintenance of feeding behavior (Kelley, 2004). Intra-NAc μ-opioid agonists have also been shown to boost food intake, while animals receiving μ-opioid antagonists exhibit attenuated consumption (Kelley et al., 1996; Pecina and Berridge, 2000). Interestingly, manipulations that increase food intake are most effective in the shell of the NAc, indicative of a functional division between NAc subregions. In addition, a spatially restricted area within the medial NAc shell has been specifically implicated in the ability of opioid agonists to alter hedonic reactions to both rewarding and aversive stimuli (Pecina and Berridge, 2005). Thus, some categories of reward-related information may be processed by distinct neurotransmitter systems in functionally isolated regions of the NAc.

Successful reward-related behaviors require the ability of brain systems to process information about the identity and value of unconditioned stimuli that act as rewards and, once a reward is obtained, to engage motor systems to redirect behavior and gain maximal utility from the reward. More recently, in vivo electrophysiological methods have been applied to investigate the role of the NAc in food and drug seeking behaviors. These approaches provide a unique perspective of NAc function because they elucidate the precise correlation between neural activity and behavioral events. Using these techniques, researchers have demonstrated that NAc neurons exhibit patterned changes in activity (increases and decreases in firing rate) before, during, and after the completion of operant responses for food and drug rewards as well as during the presentation of cues that signal the availability of rewards (Carelli and Deadwyler, 1994; Peoples et al., 1997; Carelli et al., 2000; Nicola and Deadwyler, 2000; Carelli, 2002a, 2004; Nicola et al., 2004b; Peoples et al., 2004; Day et al., 2006). However, these patterns of cellular activity are not homogenous. In fact, some NAc cells display enhanced activation before a lever press, while the activity of other neurons may increase or decrease immediately after the lever press (Carelli and Deadwyler, 1994, 1997; Carelli, 2002a).

Electrophysiological studies typically investigate NAc reward processing using tasks in which reward acquisition and goal-directed behaviors occur concurrently or in close apposition, making it difficult to distinguish NAc activity specific to rewards from activity related to reward seeking behaviors. However, a few recent studies have controlled for or circumvented this complication to assess reward-specific NAc activity. In one study, NAc cellular activity was monitored while naive rats received experimenter-controlled intra-oral infusions of rewarding sucrose (Roitman et al., 2005). Consistent with other reports (Nicola

et al., 2004a; Taha and Fields, 2006), the predominant response of NAc neurons to sucrose infusions was a decrease in activity. However, the same neurons exhibited opposite responses when an aversive quinine solution was delivered intra-orally. One hypothesis suggests that inhibitions observed during reward delivery occur among GABA-containing NAc neurons that project to important motor areas such as the ventral pallidum (VP). Through the dis-inhibition of target neurons, such a change in activity could provide a gating signal for reward-related behaviors such as consumption (Nicola et al., 2004a; Roitman et al., 2005; Taha and Fields, 2006). In support of this hypothesis, a recent study found that individual VP neurons exhibit increases in firing rate during consumption of a rewarding sucrose solution (Tindell et al., 2006). Notably, a separate but small subset of NAc neurons exhibit increases in activity when sucrose rewards are delivered (Taha and Fields, 2005). However, the magnitude of activation varies based on the concentration of sucrose, indicating that these neurons encode the palatability of a food reward instead of reward delivery or consumption. Interestingly, not all inhibitory and excitatory NAc responses observed during the delivery of primary rewards are fixed or unconditional. Rather, a subgroup of NAc neurons exhibit differential responses based on the relative context of reward delivery, including the availability of more and/or less preferred rewards (Wheeler et al., 2005). Thus, NAc neurons seemingly process remarkably different types of reward-related information, which could reflect the dual role of this structure in both reward seeking and reward consumption (Nicola et al., 2004a).

Role of mesolimbic system in reward-related learning

Since the original "anhedonia" hypothesis, a number of new and/or revised theories have been developed to explain the function of NAc dopamine in reward processing

(Blackburn et al., 1992; Ikemoto and Panksepp, 1999; Schultz, 2001; Di Chiara, 2002; Ungless, 2004; Wise, 2004; Salamone et al., 2005). One of the most influential has come from electrophysiological recordings of midbrain dopamine neurons in both rats and primates. A majority of these neurons exhibit brief increases in activity when rewards are delivered unexpectedly (Mirenowicz and Schultz, 1994; Hollerman and Schultz, 1998; Pan et al., 2005). However, if rewards are fully predicted by a CS, they no longer evoke activation among dopamine neurons. Instead, conditioned stimuli alone elicit increases in dopamine burst firing, and this signal varies in magnitude based on the probability of reward delivery, the delay between the CS and reward, and the value of the expected reward (Schultz et al., 1997; Fiorillo et al., 2003; Tobler et al., 2005; Roesch et al., 2007; Fiorillo et al., 2008; Kobayashi and Schultz, 2008). A current hypothesis based on these observations proposes that dopamine neurons may provide a "prediction error" signal consistent with contemporary reward learning theories (Montague et al., 1996; Schultz et al., 1997; Sutton and Barto, 1998). According to this hypothesis, phasic activation of dopamine neurons signal unexpected reward delivery because this produces an error in ongoing predictions about reward availability. Likewise, as conditioned stimuli become valid reward predictors, reward delivery does not constitute a violation of expectancy and therefore does not produce phasic dopamine cell firing. By computing the difference between expected and actual outcomes, dopamine neurons are thought to play a key role in reward-related learning.

At the cellular level, phasic dopamine signals in the NAc may facilitate synaptic modification (Calabresi et al., 2000a; Arbuthnott and Wickens, 2007), enabling NAc neurons to incorporate new information. With respect to Pavlovian learning, such plasticity could help organisms identify cues that predict rewards and update evaluation of those cues based

on actual outcomes. However, actual dopamine release during the presentation of conditioned stimuli may not be identical across all terminal regions. Using microdialysis to determine extracellular dopamine levels in the NAc core and shell, Bassareo and Di Chiara (Bassareo and Di Chiara, 1997, 1999a) observed that while food rewards preferentially evoked increases in dopamine concentration in the NAc shell, conditioned stimuli paired with those rewards only elicited dopamine release in the NAc core. Furthermore, dopamine release in response to conditioned stimuli paired with cocaine rewards occurs selectively in the NAc core as well (Ito et al., 2000). Based on these findings, it has been tentatively suggested that dopamine transmission in the NAc core specifically mediates associative learning processes, whereas dopamine increases in the NAc shell reflect primary reinforcement (Di Chiara, 2002). However, to date no experiments have specifically addressed whether sub-second dopamine release in the NAc reflects the content of Pavlovian learning. Therefore, Aim 1 of this dissertation will examine dopamine release in the NAc using fast-scan cyclic voltammetry during several stages of appetitive conditioning.

The evidence mentioned above, together with the anatomic arrangement of the NAc, suggests that it is an ideal location for the encoding, storage, and/or application of associative information. However, because NAc neurons are not believed to process primary sensory information, this function would likely require that individual NAc circuits undergo dynamic modification during stimulus-reward learning. Two intriguing studies have recently indicated that this may be the case. Setlow and colleagues (Setlow et al., 2003) paired olfactory cues with rewarding sucrose in a go-no go task while monitoring the activity of neurons in the ventral striatum (including the NAc). Initially, delivery of olfactory cues produced a change in activity among very few neurons. However, as animals learned to associate olfactory

stimuli with sucrose delivery, those cues began to evoke time-locked phasic responses in a number of neurons. In another study that employed a strictly Pavlovian design (Roitman et al., 2005), NAc neurons developed responses to reward-predictive audiovisual cues on the first day that these stimuli were paired. Thus, although the majority of individual NAc neurons do not exhibit innate phasic responses to environmental stimuli, such responses quickly emerge as animals come to associate those stimuli with impending outcomes.

The ability of conditioned stimuli to elicit changes in NAc cell activity may only increase as stimulus-reward associations become stronger. In one experiment, rats were repeatedly exposed to a CS that was always followed by a sucrose reward as well as a control stimulus that was not paired with a reward (Day et al., 2006). Across several conditioning sessions, rats gradually developed selective conditioned approach responses towards the reward predictive cue, but not towards the unpaired cue. Consistent with another recent study that employed a similar paradigm (Wan and Peoples, 2006), a majority of NAc neurons exhibited marked changes (increases and decreases) in firing rate during presentation of the reward-paired CS in well-conditioned rats. Of these cells, roughly half responded with a prolonged inhibition, while the other half were activated by the presence of the cue, again suggesting that individual neurons within the NAc may operate as a part of microcircuits with distinct functional responsibilities (Carelli and Wightman, 2004). It has been suggested that such excitations among NAc neurons may originate from glutamatergic inputs from cortical and limbic structures that compete for access to motor resources through striatal circuits (Pennartz et al., 1994). Through this mechanism, higher-order processing centers could gain direct influence over motor areas and promote behavioral responses to conditioned stimuli and other important cues. Importantly, recent evidence indicates that

similar cue-evoked excitations among NAc neurons are dependent upon the activity of dopamine neurons (Yun et al., 2004b).

The functional role of the NAc and its dopaminergic innervation during Pavlovian conditioning has been explored extensively using site-specific lesions and pharmacological manipulations. These studies have also identified distinctions between NAc core and shell subregions. Parkinson and colleagues (Parkinson et al., 1999) used an autoshaping paradigm to train rats to associate the presence of a previously neutral stimulus with the delivery of a food reward. Selective lesions were then made to either the core or shell of the NAc, and rats underwent additional pairing sessions in which conditioned approach responses towards the reward-paired cue were monitored. Lesions to the NAc core (but not shell) significantly impaired the expression of these approach responses, indicating that CS-US associations were disrupted. Similarly, dopamine antagonism or depletion in the NAc core also produces a profound impairment in the ability of animals to learn and express conditioned approach responses (Di Ciano et al., 2001; Parkinson et al., 2002). By comparison, NMDA antagonism in the NAc disrupts conditioned responses only during acquisition, whereas AMPA antagonism preferentially impairs the expression of Pavlovian approaches (Di Ciano et al., 2001). Taken together, these findings suggest that the reliance of conditioned approach responses on an intact NAc core reflects the contributions of dopamine and glutamate transmission within this structure.

The precise role of dopamine in associative reward learning may be selectively mediated by specific receptor subtypes within the NAc. Dopamine D1 and D2 receptors oppositely modulate the same intracellular cascade, and D1 receptor antagonism inhibits long term potentiation of striatal synapses (Calabresi et al., 2000b; Kerr and Wickens, 2001).

Consistent with the distinct cellular effects attributed to these receptors, Eyny and Horvitz (Eyny and Horvitz, 2003) reported that selective D1 and D2 antagonists also differentially affect stimulus-reward learning. In this study, the systemic blockade of D1 receptors produced a reduction in conditioned approaches towards reward-paired stimuli, while D2 antagonists actually promoted the expression of learned associations (Eyny and Horvitz, 2003). Intra-NAc D1 receptor antagonism immediately after Pavlovian conditioning also blocks the performance of subsequent conditioned approach responses in an autoshaping task, indicating that D1 receptors in the NAc may play a vital role in the consolidation of learned stimulus-reward associations (Dalley et al., 2005).

In addition to the dopaminergic projection from the VTA, a number of other structures may contribute specific information to the NAc during associative learning (Robbins and Everitt, 2002). For example, excitotoxic lesions to the anterior cingulate cortex (ACC) impair the acquisition and performance of Pavlovian approach responses towards conditioned stimuli (Cardinal et al., 2002b). However, in contrast to NAc core lesions, ACC lesions do not abolish approach responses, but rather increase that likelihood that animals will approach non-predictive cues (Bussey et al., 1997). One potential explanation for this effect is that the ACC rapidly acquires the ability to discriminate between stimuli and then "teaches" this discrimination to other regions, such as the NAc (Cardinal et al., 2002b; Robbins and Everitt, 2002). In agreement with this view, disconnection lesions between the NAc core and ACC also impair the expression of learned associations (Parkinson et al., 2000). Importantly, other brain structures may also contribute to stimulus-reward learning in a NAc-independent manner. Indeed, a number of studies have indicated that a separate neural circuit consisting of the central nucleus of the amygdala, substantia nigra pars compacta, and

dorsolateral striatum mediates the learning and expression of conditioned orienting responses elicited by cues that predict favorable outcomes as well as the potentiation of feeding by conditioned stimuli (Han et al., 1997; Lee et al., 2005; El-Amamy and Holland, 2006).

Although dopamine and NAc activity appear to have a clear role in Pavlovian learning, their relation to acquisition of an instrumental response remains somewhat controversial (Kelley, 2004; Fields et al., 2007; Belin et al., 2008; Yin et al., 2008). Thus, a series of studies have found that NMDA and D_1 receptor antagonism in the NAc disrupt learning in instrumental tasks (Maldonado-Irizarry and Kelley, 1995; Kelley et al., 1997; Smith-Roe and Kelley, 2000). Moreover, this effect appears to be dependent upon downstream signaling cascades and alterations in protein expression, as inhibition of protein kinase A (a signaling molecule downstream of D_1 receptor activation) or protein synthesis in the NAc produce similar effects on instrumental learning (Baldwin et al., 2002; Hernandez et al., 2002). Conversely, other studies have found that neither dopamine nor an intact NAc are required for instrumental learning (Corbit et al., 2001; de Borchgrave et al., 2002; Cardinal and Cheung, 2005; Robinson et al., 2005) or instrumental responding *per se* (McCullough et al., 1993a; Balleine and Killcross, 1994; Aberman and Salamone, 1999). However, lesions to the dorsomedial striatum have profound impact on learned action-outcome associations (Yin and Knowlton, 2004; Yin et al., 2005a; Yin et al., 2005b), leading to the hypothesis that that while the NAc mediates Pavlovian learning with respect to rewards, the dorsomedial striatum regulates reward-related instrumental learning (Yin et al., 2008).

Role of mesolimbic system in instrumental performance and decision making

Despite the controversy over the role of the NAc in instrumental learning, there is widespread agreement that NAc manipulations can have profound effects on behavioral

performance in instrumental tasks, particularly when behavior is elicited by environmental cues (Ikemoto and Panksepp, 1999; Balleine, 2005; Fields et al., 2007; Nicola, 2007; Yin et al., 2008). Thus, dopamine antagonism in the NAc or VTA inactivation reduces lever presses evoked by reward-paired discriminative stimuli (Yun et al., 2004a; Yun et al., 2004b), and manipulations that increase NAc dopamine also increases the number of cues to which animals respond (Nicola et al., 2005). The NAc is also necessary for the ability of Pavlovian cues to enhance instrumental responding (Pavlovian to instrumental transfer). Lever pressing in the presence of Pavlovian cues is robustly enhanced by intra-NAc administration of amphetamine, which increases dopamine transmission (Wyvell and Berridge, 2000). Likewise, lesions of the NAc or interference of dopamine neurotransmission in the NAc markedly disrupt Pavlovian to instrumental transfer (Corbit et al., 2001; Hall et al., 2001; Murschall and Hauber, 2006; Lex and Hauber, 2008) In combination, this evidence suggests a global role for NAc activity in the ability of animals to respond appropriately to reward-paired cues during instrumental performance.

NAc disruptions also have profound effects on instrumental responses that require the exertion of significant effort to produce reinforcement. Indeed, a series of investigations have discovered that NAc dopamine is required for animals to overcome high effort requirements to obtain food (Correa et al., 2002; Salamone and Correa, 2002; Salamone et al., 2005). Specifically, DA depletions in the NAc significantly decrease response rates on FR16 and FR64 schedules of reinforcement, but have little or no effect on FR1 and FR4 schedules (Aberman and Salamone, 1999). Doses of D_1 and D_2 receptor antagonists that impair lever pressing on an FR5 schedule of reinforcement actually increase food intake (Salamone et al., 2002). Finally, DA release in the NAc (measured over minutes) is correlated with operant

response rates but not with the quantity of food an animal receives (McCullough et al., 1993a). These results suggest that NAc DA may act as a cost-benefit calculator, regulating the amount of effort that animals will expend to obtain food rewards (Salamone et al., 2003; Walton et al., 2006; Phillips et al., 2007). However, it is less clear exactly why interrupting NAc dopamine transmission has such profound effects on high effort schedules but not low effort schedules (Niv et al., 2007; Phillips et al., 2007). The design of Aim 2 of this dissertation will enable us to determine whether phasic NAc dopamine release differs when animals are required to exert low and high amounts of effort to obtain rewards.

Under the majority of real-world circumstances, organisms are not simply learning and responding to individual stimuli, but are engaged in making decisions between multiple competing response options. In addition to their effects on performance in static instrumental tasks, NAc manipulations also alter behavior in more dynamic decision making tasks, in which animals are allowed to choose between two or more outcomes. In the initial demonstration of this phenomenon, hungry rats were given the opportunity to respond for a preferred food reward or consume freely available (but less preferred) rat chow (Salamone et al., 1991). Under normal circumstances, rats willingly pressed the lever for the preferred food while largely ignoring the concurrently available chow. However, following either systemic or intra-NAc injections of the dopamine antagonist haloperidol and intra-NAc dopamine depletion, rats no longer pressed the lever to obtain the previously preferred food, and instead consumed more chow. This basic effect has been confirmed by numerous subsequent observations, in which NAc lesions, dopamine antagonism, or dopamine depletion all produced a similar switch from preference for larger rewards that were available at higher costs to smaller rewards that were available at lower costs (Cousins and Salamone, 1994;

Salamone et al., 1994; Cousins et al., 1996; Floresco et al., 2007; Hauber and Sommer, 2009). Furthermore, many recent studies have expanded this line of research to include decisions that are based on reward delay (so-called inter-temporal choice or delay discounting) rather than response cost. These reports have noted similar deficits of NAc lesions. That is, although animals will normally choose to wait for larger rewards, animals with lesions to the NAc core robustly prefer immediate rewards to delayed rewards, even when the delayed rewards are larger (Cardinal et al., 2001; Acheson et al., 2006; Bezzina et al., 2007). Although this effect does not occur with NAc dopamine depletion (Winstanley et al., 2005b), systemic dopamine manipulations do bias inter-temporal choice (Wade et al., 2000), indicating a separate locus of action. Critically, although the NAc appears to be involved in both effort and delay based decisions, cortical involvement in these types of tasks is heterogeneous, with lesions of the anterior cingulate cortex disrupting effort-related choices (Walton et al., 2003; Rudebeck et al., 2006) and lesions to the orbito-frontal cortex disrupting delay-related choices (Kheramin et al., 2002; Mobini et al., 2002; Rudebeck et al., 2006). Thus, delay and effort based decision making are both behaviorally and neurally dissociable.

The evidence reviewed above suggests that in addition to playing a direct role in reward learning, NAc dopamine release may also contribute to reward-related decision making (McClure et al., 2003a; Doya, 2008; Floresco et al., 2008). Recordings from dopamine neurons are consistent with this idea, and suggest that dopamine responses to predictive cues provide a wealth of information about the magnitude, delay, and probability of future rewards, all of which are critical to decisions. This information is generally reflected in the magnitude of cue-evoked dopamine activity, with sooner, larger, and more

probable rewards eliciting larger phasic responses (Fiorillo et al., 2003; Tobler et al., 2005; Roesch et al., 2007; Fiorillo et al., 2008; Kobayashi and Schultz, 2008). However, no studies to date have evaluated whether dopamine signals in the NAc reflect the variables critical to decision making, or whether NAc neurons encode such information.

Goals of this dissertation

The NAc and dopamine release in the NAc have long been implicated in a number of behavioral phenomena, including Pavlovian reward learning and reward-related decision making. Previous investigations from this laboratory have found that NAc neurons exhibit time-locked phasic changes in activity during the presentation of reward-paired cues (Nicola et al., 2004b; Roitman et al., 2005; Day et al., 2006) and operant responses to obtain rewards (Carelli et al., 2000; Carelli and Wightman, 2004). Likewise, dopamine release within the NAc is elevated in relation to both conditioned (Phillips et al., 2003a) and discriminative stimuli (Roitman et al., 2004) as well as operant responses (Phillips et al., 2003a; Roitman et al., 2004; Stuber et al., 2004; Stuber et al., 2005). However, little is known about how these phasic NAc dopamine signals emerge and change during conditioning and whether they modulate cost-related decision making in well-trained animals. Similarly, the ability of NAc neurons to encode cost-related information is unclear. The proposed studies seek to elucidate the behavioral role of neurochemical and neurophysiological signals in the NAc by assessing subsecond NAc dopamine release and NAc cellular activity during a variety of behavioral tasks.

Specific Aims:

1. Characterize phasic dopamine release in the NAc during different stages of reward learning.

Environmental stimuli that consistently predict rewards can develop biological salience and promote reward-seeking behaviors in a manner that is both NAc and dopamine dependent (Dickinson et al., 2000; Everitt et al., 2001; Robbins and Everitt, 2002; See, 2002; Kalivas and McFarland, 2003; Yun et al., 2004a; Yun et al., 2004b). This aim will employ fast-scan cyclic voltammetry to detect dopamine release at different stages of conditioning in the NAc core, a brain region in which dopamine release has been directly implicated in appetitive conditioning (Di Ciano et al., 2001; Dalley et al., 2002; Dalley et al., 2005). By providing insight into how rewards and reward-paired cues evoke NAc dopamine release during both the acquisition and expression of a learned association, the results will improve our understanding of how rapid NAc dopamine signaling contributes to reward learning. This aim has been published (Day, J.J., Roitman, M.F., Wightman, R.M., & Carelli, R.M. (2007). Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. *Nature Neuroscience, 10*(8) 1020-1028).

2. To examine rapid DA release in the NAc during effort-based decision making.

In addition to its proposed role in reward learning, dopamine transmission in the NAc has also been heavily implicated in goal-directed behavior and decision making. Phasic dopamine signals in the NAc modulate food and drug seeking (Phillips et al., 2003a; Roitman et al., 2004), and dopamine (DA) depletion or antagonism in the NAc produces profound effects on operant responding for food, but primarily when reinforcement is contingent upon high work-related response costs (Aberman et al., 1998; Aberman and Salamone, 1999; Correa et al., 2002; Salamone et al., 2002; Salamone et al., 2003). These observations have led to the related hypotheses that NAc dopamine functions to promote behavioral output when increased effort is required (Salamone et al., 2003) or to compute the maximal effort

that the organism should expend to obtain a predicted reward (Phillips et al., 2007). However, the precise way in which phasic NAc dopamine signals contribute to effort-related decision making remains unknown. In this study, NAc dopamine concentration will be measured in real time while rats engage in an effort-based decision making task. Rats will be trained to associate visual cues with the availability of rewards at either low (FR1), high (FR16), or choice (FR1 or FR16) effort requirements. As cues will be presented well before the opportunity to respond, this design will enable us to dissect whether NAc dopamine signaling encodes differences in predicted response costs, the actual exertion of effort, and/or reward delivery produced by different amounts of effort. The results will help clarify the role of phasic NAc dopamine in effort-based choice behavior. Data from this aim are currently being prepared as a brief report for submission to *The Journal of Neuroscience*.

3. To examine neurophysiological output of NAc neurons during an effort-based decision task.

The dramatic effects of pharmacological manipulations in the NAc on effort-based tasks strongly suggest that it plays a critical role in the ability to overcome high-effort requirements to obtain rewards and in effort-related decision making in general (Salamone et al., 1991; Salamone, 2002; Ishiwari et al., 2004; Ishiwari et al., 2007; Font et al., 2008; Hauber and Sommer, 2009). The NAc sends outputs directly to motor areas involved in goal-directed behavior, and proper NAc function is also required for feeding behavior and instrumental actions that produce food (Kelley, 2004). Likewise, NAc neurons selectively encode information related to food and drug-seeking behaviors as well as cues that signal reward delivery and availability (Carelli and Deadwyler, 1994; Nicola et al., 2004a, b; Roitman et al., 2005; Day et al., 2006). However, little is known about whether effort-related

information is encoded in the activity of NAc neurons. This aim will advance the existing literature by employing the same decision-making task described in Aim 2. Individual NAc neurons will be monitored using *in vivo* electrophysiological techniques during the performance of this task to assess whether NAc neurons encode the amount of effort required to obtain a reinforcer and/or whether cues that signal low and high response costs differently modulate the output of NAc neurons. Results from this aim are currently being prepared as a manuscript for submission to *Neuron*.

4. To examine neurophysiological output of NAc neurons during a delay-based decision task.

Animals routinely prefer immediate rewards to delayed rewards of the same value, a phenomenon termed delay discounting (Green and Myerson, 2004). Lesions to the NAc core produce alterations in delay-based decision making in which animals exhibit impulsive preference for immediate but small rewards even when much larger reward amounts are available at slight time delays (Cardinal et al., 2001). Although several recent investigations have examined dopamine cell firing during decision or conditioning tasks that involve different reward delays (Roesch et al., 2007; Fiorillo et al., 2008; Kobayashi and Schultz, 2008), no studies to date have assessed whether NAc neurons encode information about reward delay. In this study, individual NAc neurons will be monitored electrophysiologically in behaving animals during a task similar to that employed in Aims 2 and 3. However, instead of altering the amount of effort associated with rewards, this study will change the temporal delay between operant responses and rewards. Thus, separate visual cues will signal the availability of either immediate (0s delay), delayed (4-8s delay), or choice (0s or 4-8s) rewards. Importantly, as the design of this study is otherwise identical to that used in Aim 3,

the results will provide a direct comparison between NAc signaling relative to delay and effort-based choice behavior.

CHAPTER 2

ASSOCIATIVE LEARNING MEDIATES DYNAMIC SHIFTS IN DOPAMINE SIGNALING WITHIN THE NUCLEUS ACCUMBENS

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ABSTRACT

The ability to predict favorable outcomes using environmental cues is an essential aspect of learned behavior. Dopamine neurons in the midbrain encode such stimulus-reward relationships in a manner consistent with contemporary learning models, but it is unclear how this translates into actual dopamine release in target regions. Here, we sampled dopamine levels in the rat nucleus accumbens on a rapid (100 ms) timescale using electrochemical technology during a classical conditioning procedure. Early in conditioning, transient dopamine release events signaled a primary reward but not predictive cues. After repeated cue-reward pairings, dopamine signals shifted in time to predictive cue onset and were no longer observed at reward delivery. In the absence of stimulus-reward conditioning, there was no shift in the dopamine signal. Consistent with proposed roles in reward prediction and incentive salience, these results indicate that rapid dopamine release provides a reward signal that is dynamically modified by associative learning.

INTRODUCTION

Organisms forage and survive in demanding environments by learning about the events surrounding them and adapting behavioral strategies accordingly. One simple yet biologically critical form of learning involves the ability to link environmental stimuli with favorable outcomes that they predict. Recent investigations indicate that midbrain dopamine neurons encode such stimulus-reward associations (Schultz et al., 1997), and current hypotheses suggest that dopamine may act as a teaching signal consistent with contemporary models of animal learning (Sutton and Barto, 1998; Montague et al., 2004a; Bayer and Glimcher, 2005; Pan et al., 2005). Within these models, phasic changes in the firing rate of dopamine neurons are thought to provide a "prediction error" signal that compares expected outcomes with actual outcomes (Mirenowicz and Schultz, 1994; Schultz et al., 1997; Bayer and Glimcher, 2005). Unexpected rewards produce brief synchronous bursts among dopamine neurons (Schultz et al., 1997), whereas fully predicted rewards typically evoke little or no phasic activity. Moreover, the events that serve as predictors come to elicit brief dopamine bursts even though they often possess no inherent biological value, and the magnitude of these conditioned neuronal responses is correlated with the certainty of the reward being predicted (Fiorillo et al., 2003). This and other information provided by dopamine neurons may not only influence reward learning, but also impact decision-making strategies (Morris et al., 2006).

Dopamine neurons are not alone in processing reward-related information. In fact, expanding research has identified a distributed network of brain nuclei involved in this process. At the center of this network is the nucleus accumbens (NAc), which receives convergent glutamatergic input from the prefrontal cortex, hippocampus, and basolateral

amygdala, as well as a dopaminergic projection from the ventral tegmental area (VTA). The NAc projects to motor areas such as the ventral pallidum, making it an ideal location for detailed reward information to be turned into motivated action (Mogenson et al., 1980). NAc neurons strongly encode stimuli that predict rewarding events (Roitman et al., 2005; Day et al., 2006), and dopaminergic input is required for NAc neurons to exhibit such responses (Yun et al., 2004b). Further, pharmacological manipulations in this region markedly affect the acquisition and expression of Pavlovian conditioned responses (Di Ciano et al., 2001; Di Chiara, 2002; Dalley et al., 2005), indicating that dopamine signaling within the NAc likely plays a critical role in stimulus-reward learning.

Although dopamine neurons clearly provide a reward prediction signal, it is less apparent how this translates into dopamine release in target regions such as the NAc. This is a key concern for several reasons. First, there is not always a one-to-one correspondence between dopamine cell firing and dopamine release, which is subject to active facilitation and depression by a number of terminal factors (Cragg, 2003; Zhang and Sulzer, 2004; Cragg, 2006). Indeed, direct stimulation of dopamine cell bodies can produce remarkably different release profiles based on the recent history of release events (Montague et al., 2004b). Moreover, there are times at which such stimulation produces no detectable change in dopamine concentration at target areas (Garris et al., 1999). Secondly, midbrain dopamine neurons project to multiple targets with different functional roles, including the prefrontal cortex, amygdala, dorsal striatum (caudate and putamen), and NAc. It is uncertain if these heterogeneous regions receive the same or even overlapping information from dopamine neurons, although several microdialysis studies suggest otherwise (Bassareo and Di Chiara, 1997; Di Chiara, 2002). Finally, while the majority of electrophysiological examinations

distinguish dopamine neurons based on waveform properties (Waelti et al., 2001; Fiorillo et al., 2003), it is unclear if the neuronal population isolated using this method is limited to dopamine neurons (Ungless et al., 2004; Margolis et al., 2006a). Measuring dopamine release directly avoids all of these concerns, and the ability to do so on a sub-second timescale ensures that measurements are both physiologically and behaviorally relevant.

The present study investigated how stimulus-reward learning impacts sub-second dopamine release within the NAc. *In vivo* detection of dopamine was accomplished using fast-scan cyclic voltammetry (FSCV), an electrochemical technique that permits rapid sampling on a timescale analogous to extracellular activity (Phillips et al., 2003a; Heien et al., 2004; Heien et al., 2005). Dopamine release characteristics were first examined in naïve rats during a single conditioning block that paired an experimental stimulus with natural rewards. Next, we characterized dopamine signals in rats that received either many stimulus-reward pairings or unpaired stimuli and rewards. Consistent with its role as a reward prediction error signal, our observations demonstrate that phasic dopamine release events in the NAc initially marked primary rewards, but shifted to a predictive cue following Pavlovian conditioning. However, when the same stimulus was presented in an explicitly unpaired manner, primary rewards still evoked rapid dopamine release. Thus, midbrain dopamine reward signals are transmitted to the NAc and are dynamically modified as a result of Pavlovian learning.

METHODS

Animals. Male Sprague Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) aged 90−120 d and weighing 260−330 gm were used as subjects and individually housed with a 12:12 light: dark cycle. All experiments were conducted between 9:00 am and 5:00 pm. Bodyweights were maintained at no less than 85% of pre-experimental levels by food restriction (10−15 gm of Purina laboratory chow each day, in addition to approximately 1 gm of sucrose consumed during daily sessions). This regimen was in place for the duration of behavioral testing, except during the post-operative recovery period, when food was given *ad libitum*. All procedures were approved by the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee.

Conditioning procedures.

Experiment 1: Early conditioning Naïve rats (n = 6) were surgically fitted for voltammetric recordings using methods described below. After full recovery, rats were placed in a standard experimental chamber (Med Associates, St. Albans, VT) and received a magazine training session in which single sucrose pellets (45 mg) were delivered at random intervals to a food dish. This served to acquaint the animal with the location and taste of sucrose before conditioning began. On the next day, electrochemical data were collected in the NAc core during a conditioning session that consisted of 50 individual trials. On 25 trials, a compound stimulus (extension of a retractable lever and illumination of cue light above the lever) was presented to the animal for 10 s. At the end of the stimulus presentation, a sucrose pellet was immediately delivered to a food dish. On the other 25 trials, another compound stimulus (extension of a separate retractable lever and illumination of associated cue light) was presented for 10 s, but was not followed by sucrose delivery (**Fig. 2.1a**). Thus, the first

stimulus (termed the CS+) provided a positive predictor of sucrose delivery, while the second stimulus (the CS−) was a negative predictor of sucrose delivery (i.e., the cue signaled the absence of sucrose). The order of CS+ and CS− trials was semi-random, with no more than two of either trial type occurring in sequence. Individual trials were initiated on a variable schedule every 45−75 s; the average inter-trial interval was 60 s. Additionally, the lever and cue light that served as the CS+ were counterbalanced across animals. The CS+ and CS− stimuli were symmetrically located on the same wall as the food receptacle, with a horizontal separation of 17 cm. Contact with conditioned stimuli (registered as a lever presses) was recorded during each trial. However, sucrose delivery was independent of contact with the CS+. Manual frame-by-frame videotape analysis of the entire conditioning session was used to pinpoint the timing of each sucrose retrieval for each animal. For this analysis, a Sony video cassette recorder received video input from a camera fastened to the ceiling of the experimental chamber, allowing a complete view of the subject and experimental setup. This input was recorded on VHS tapes along with time-stamped session information from a Video Character Generator (University of North Carolina Electronics Facility), which enabled the electrochemical data to be re-aligned with respect to the actual recovery of the sucrose pellet. Sucrose retrieval was operationally defined as the first 100 ms bin after sucrose delivery in which the rat's nose and mouth were lowered into the food receptacle.

Experiment 2: Extended conditioning A second group of naïve rats (n = 6) received ten conditioning sessions nearly identical to that described above (50 total trials, 25 CS+ and 25 CS− presentations; one session per day). After the fifth conditioning session, cue lights were no longer illuminated during the CS+ and CS−, making the retractable levers the only stimuli with predictive value. After the tenth conditioning session, animals were fed ad

libitum and surgically prepared for voltammetric recordings. Following a one-week recovery period, rats received another 50-trial conditioning session. On the test day, electrochemical data were collected in the NAc core during the twelfth and final conditioning session. Thus, at the start of the recording session, rats had received 275 pairings between the CS+ and sucrose delivery, as well as 275 CS− presentations that were not followed by sucrose delivery.

Experiment 3: Unpredicted reward Another group of rats (n = 6) underwent 10 sessions in which sucrose delivery and stimulus presentations occurred in an explicitly unpaired manner. Each session consisted of 50 unpaired stimulus presentations (extension of right or left retractable lever and associated cue light for 10 s) and the unpredicted delivery of 25 sucrose pellets to the food dish. Here, unpaired stimulus trials were initiated on a variable time interval every 60−90 s (mean 75 s). Sucrose deliveries were timed to occur sporadically between lever presentations, but never occurred within 15 s of stimulus onset or offset. Again, after five sessions the cue lights were no longer illuminated with lever extension. After the tenth session, animals were fed ad libitum and surgically fitted for electrochemical recordings. Following full recovery, rats received another session of unpredicted reward delivery. The result was that, prior to the test session, rats had experienced 550 cue presentations that did not predict sucrose and 275 unsignaled sucrose deliveries. On the experimental test day, [DA] was measured in the NAc core during the twelfth and final session.

Surgery Rats were surgically prepared for voltammetric recordings as described previously (Phillips et al., 2003b). After establishing an anesthetic plane with ketamine hydrochloride (100 mg/kg, intramuscular) and xylazine hydrochloride (20 mg/kg,

intramuscular), rats were placed in a stereotaxic frame. A guide cannula (Bioanalytical Systems, West Lafayette, IL) was positioned dorsally to the core subregion of the NAc (1.3 mm anterior, 1.3 mm lateral from bregma). An Ag/AgCl reference electrode was placed contralateral to the stimulating electrode in the left forebrain. Stainless steel skull screws and dental cement were used to secure all items. A bipolar stimulating electrode was placed dorsally to the ventral tegmental area (5.2 mm posterior, 1.0 mm lateral from bregma and 7 mm ventral from the dural surface). A detachable micromanipulator containing a glass-sealed carbon-fiber electrode (75−100 μm exposed tip length, 7 μm diameter, T-650; Amoco, Greenville, SC) was inserted into the guide cannula, and the electrode was lowered into the NAc core. The bipolar stimulating electrode was then lowered in 0.2 mm increments until electrically evoked dopamine release was detected at the carbon-fiber electrode in response to a stimulation train (60 biphasic pulses, 60 Hz, 120 μA, 2 ms per phase). The stimulating electrode was then fixed with dental cement and the carbon-fiber electrode was removed.

Fast-scan cyclic voltammetry Following surgery, animals were allowed one week to recover pre-surgery body weight. Food intake was then reduced to ensure motivation during conditioning. To collect electrochemical data on the test day, a new carbon-fiber electrode was placed in the micromanipulator and attached to the guide cannula. The carbon-fiber electrode was then lowered into the NAc core. The carbon-fiber and Ag/AgCl electrodes were connected to a head-mounted voltammetric amplifier attached to a commutator (Crist Instrument Company, Hagerstown, MD) at the top of the experimental chamber. All electrochemical data were digitized and stored using computer software written in LabVIEW (National Instruments, Austin, TX). To minimize current drift, the carbon-fiber electrode was allowed to equilibrate for 30−45 min prior to the start of the experiment.

The potential of the carbon-fiber electrode was held at −0.4 V versus the Ag/AgCl reference electrode. Voltammetric recordings were made every 100 ms by applying a triangular waveform that drove the potential to +1.3 V and back at a rate of 400 V/s. The application of this waveform causes oxidation and reduction of chemical species that are electroactive within this potential range, producing a change in current at the carbon-fiber. Specific analytes (including dopamine) are identified by plotting these changes in current against the applied potential to produce a cyclic voltammogram(Heien et al., 2004). The stable contribution of current produced by oxidation and reduction of surface molecules on the carbon-fiber was removed by using a differential measurement (i.e., background-subtraction) between a time when such signals were present but dopamine was not. For data collected during the behavioral session, this background period (500 ms) was obtained during the baseline window (10 s prior to cue onset). This practice does not subtract the presence of phasic dopamine events during the baseline, because the background was explicitly selected for the absence of fast dopamine signals. Following equilibration, dopamine release was electrically evoked by stimulating the VTA (24 biphasic pulses, 60 Hz, 120 μA, 2 ms per phase) to ensure that carbon-fiber electrodes were placed close to release sites. The position of the carbon-fiber was secured at the site of maximal dopamine release. Experiments began when the signal-to-noise ratio of electrically evoked dopamine release exceeded 30. During conditioning sessions, experimental and behavioral data were recorded with a second computer, which translated event markers to be time-stamped with electrochemical data. VTA stimulation was repeated following the experiment to verify electrode stability and ensure that the location of the electrode could still support dopamine release.

Signal identification and separation After in vivo recordings, dopamine release evoked by VTA stimulation was used to identify naturally occurring dopamine transients using methods described previously (Heien et al., 2004; Heien et al., 2005). Stimulation of the VTA leads to two well-characterized electrochemical events: an immediate but transient increase in [DA] and a delayed but longer-lasting basic pH shift. To separate these signals, a training set was constructed from representative, background-subtracted cyclic voltammograms for dopamine and pH. This training set was used to perform principal component regression on data collected during the behavioral session. Principal components were selected such that at least 99.5% of the variance in the training set was accounted for by the model. All data presented here fit the resulting model at the 95% confidence level. After use, carbon-fiber electrodes were calibrated in a solution of known [DA] to convert observed changes in current to differential concentration.

Data Analysis Significant changes in NAc [DA] were evaluated using a one-way repeated measures ANOVA with Tukey *post hoc* tests for multiple comparisons of 100 ms time bins and a baseline window (mean [DA] during 10 s preceding cue onset or reward delivery (unpaired group only)). To determine whether cue-related dopamine responses emerged for each animal in the early conditioning group, data were divided into blocks of 5 trials and a one-way repeated measures ANOVA was performed for the first and final blocks. Differences between CS+ and CS− cues were evaluated using paired t-tests on peak [DA]. In a separate analysis, the signal-to-baseline ratio (S:B) was computed by dividing the maximal differential [DA] observed during an event (signal) by the average differential [DA] observed during the 10s baseline window preceding cue onset (or preceding reward delivery in cases where sucrose was not signaled by a cue). Differences in S:B relative to CS+, CS−, reward,

and control cue presentations within groups were assessed by conducting one-way repeated measures ANOVAs (Early and Extended conditioning groups) or one-tailed paired Student's T tests (Unpaired group). Tukey *post hoc* tests for multiple comparisons were employed following ANOVAs to determine S:B differences between individual events.

Pavlovian approach responses directed at conditioned stimuli were recorded as lever presses. For each behavioral session, the probability of approach was calculated for the CS+ and CS− by dividing the total number of approaches (lever presentations in which at least one lever press occurred) by the number of opportunities for approach. For the initial conditioning group, approach probabilities for the CS+ and CS− were compared using a paired Student's T-test. For the extended conditioning group, differential acquisition of stimulus-selective approach behavior was evaluated using a within-subjects cue (two levels) x session (12 levels) repeated measures ANOVA. Bonferroni *post hoc* tests were employed to identify sessions in which approaches directed at the CS+ and CS− differed. The relationship between the latency or vigor of approach responses and dopamine release was evaluated using linear regression analysis. Statistical significance was designated at $\alpha = 0.05$. All statistical analyses were carried out using InStat version 3.0 for Windows (Graphpad Software, San Diego, CA) and SPSS version 12.0 for Windows (SPSS Inc., Chicago, IL). Three-dimensional graphical analyses were performed using Matlab software (MathWorks, Natick, MA).

Histological verification of electrode placement. Upon completion of each experiment, rats were deeply anesthetized with a ketamine/xylazine mixture (100 mg/kg and 20 mg/kg, respectively). In order to mark the placement of electrode tips, a 50−500 μA current was passed through a stainless steel electrode for 5 seconds. Transcardial perfusions

were then performed using physiological saline and 10% formalin, and brains were removed. After post-fixing and freezing, 50 μm coronal brain sections were mounted on microscope slides. The specific position of individual electrodes was assessed by visual examination of successive coronal sections. Placement of an electrode tip within the NAc was determined by examining the relative position of observable reaction product to visual landmarks (including the anterior commissure and the lateral ventricles) and anatomical organization of the NAc represented in a stereotaxic atlas (Paxinos and Watson, 2005).

RESULTS

Phasic dopamine release during initial conditioning

Primary rewards produce bursts in the firing rate of dopamine neurons unless animals have learned to predict rewards using experimental cues (Mirenowicz and Schultz, 1994). However, important questions about this signal remain unanswered. For example, the majority of existing studies have only assessed dopamine signaling in well-trained or experienced animals, making it difficult to resolve dopamine's function when an organism is foraging and learning associations in novel environments. To address this issue, we performed FSCV in experimentally naive rats (n = 6) during a single conditioning block that consisted of 50 discrete trials. On 25 trials, one conditioned stimulus (the CS+, a retractable lever and cue light) was presented for 10 s and then retracted. Upon retraction, a reward (45 mg sucrose pellet) was immediately delivered to a food receptacle (**Fig. 2.1a**). Thus, the CS+ predicted reward delivery on each trial, which was independent of any behavioral response. On the other 25 trials, another conditioned stimulus (the CS−, a spatially separate retractable lever and cue light) was presented for 10 s, but was not followed by a reward. Trial type was selected semi-randomly, with a variable inter-trial interval (45−75 s; see Conditioning Procedures for details). Using a similar conditioning design, previous reports demonstrate that approach responses towards reward-predictive cues develop as a function of conditioning (Di Ciano et al., 2001; Day et al., 2006). Termed "sign-tracking" or "autoshaping", these responses are believed to reflect Pavlovian learning and the incentive salience of predictive cues (Robbins and Everitt, 2002; Everitt and Robbins, 2005; Uslaner et al., 2006). These responses were therefore recorded and interpreted as a behavioral measure of the strength of stimulus-reward associations. We chose the NAc core as a dopamine

detection site for FSCV in all experiments because this sub-region receives input from dopamine axons and plays a critical role in this form of associative reward learning (Parkinson et al., 1999; Cardinal et al., 2002b; Robbins and Everitt, 2002).

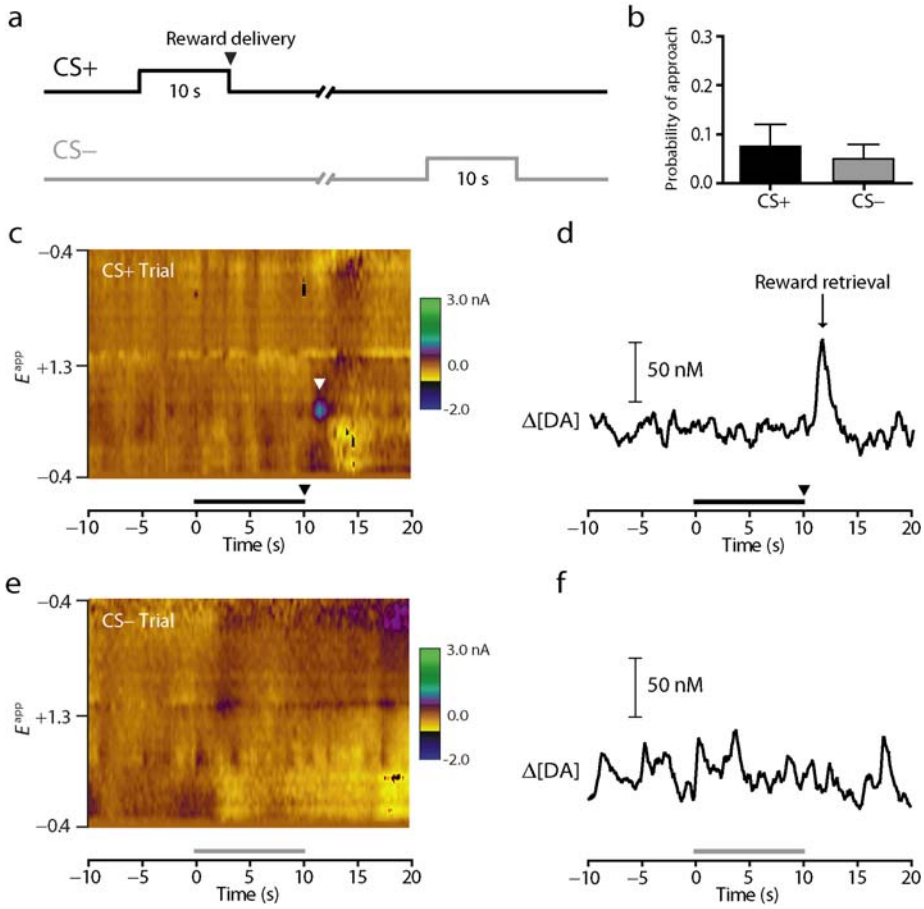

Figure 2.1. Early in associative learning, rapid elevations in NAc dopamine concentration were time-locked to receipt of reward but not conditioned stimuli. (**a**) Conditioning procedure. Conditioned stimuli were semi-randomly presented to naïve rats in a single conditioning block of 50 trials. The appearance of one stimulus (the CS+) predicted reward delivery (45 mg sucrose pellet), whereas the other stimulus (the CS−) did not. Each 10 s CS was presented 25 times. (**b**) Mean (+SEM) approach probability. There was no cue difference in approach probability, indicating that rats made no behavioral distinction between stimuli. (**c**) Two-dimensional representation of electrochemical data collected during a single CS+ trial. The applied voltage (ordinate) is plotted during a 30 s window surrounding CS+ presentation (horizontal black bar beginning at time-point zero, abscissa). Changes in current at a carbon-fiber electrode located in the NAc are encoded in color. The inverted black triangle denotes reward delivery, whereas the inverted white triangle marks reward retrieval. Dopamine is visible as a green-encoded spike in current at reward retrieval. (**d**) Differential dopamine concentration obtained from representative example in panel C. Data are plotted relative to CS+ presentation (horizontal black bar) and reward delivery (inverted black triangle). On this trial, a robust increase in dopamine concentration corresponded to reward retrieval. (**e**) Two-dimensional representation of electrochemical data during a CS− trial. The horizontal gray bar denotes cue presentation. (**f**) Differential dopamine concentration obtained from representative example in panel E. No robust changes in dopamine concentration were observed at any time-point.

Approach behaviors directed at the CS+ and CS− during the initial conditioning block were not statistically distinguishable from zero (both 95% confidence intervals contained 0) and were not significantly different from each other ($t = 0.933$, $df = 5$, $p = 0.39$; **Fig. 2.1b**), indicating that the animals did not behaviorally discriminate between the cues. To determine how conditioning and rewarding stimuli altered subsecond dopamine concentration ([DA]) in the NAc core, electrochemical data were evaluated as single-trial traces (see **Figure 2.1c−f** for representative CS+ and CS− traces from a single animal). Interestingly, a brief yet robust elevation in NAc [DA] occurred when this animal retrieved a sucrose reward from the food dish (**Fig. 2.1c**,**d**; timing of retrieval determined using detailed videotape analysis). In contrast, there were no phasic changes in NAc [DA] when the CS+ (**Fig. 2.1c**,**d**) and CS− (**Fig. 2.1e**,**f**) were presented. Re-alignment of averaged electrochemical data with respect to reward retrieval for all animals (**Fig. 2.2a**,**b**) revealed a significant increase in extracellular [DA] at the precise time of retrieval ($F_{40,200} = 5.272$, $p < 0.001$; Tukey *post hoc* comparisons vs. baseline $p < 0.05$ at −0.1 to 0.4 s surrounding sucrose retrieval). Thus, the phasic increase in NAc [DA] began before rewards were actually procured or consumed (**Fig. 2.2a**), indicating that visual, auditory, or even olfactory information may contribute to the initiation of this signal. Pooled across trials and animals, peak [DA] during sucrose retrieval was 42.9 ± 6 nM. Additionally, this reward-related increase in dopamine was not altered by conditioning, but was steady throughout the experimental session ($F_{1,111} = 0.08$, $p = 0.77$; test for linear trend between trial number and [DA] at sucrose retrieval; see **Fig. 2.2b**).

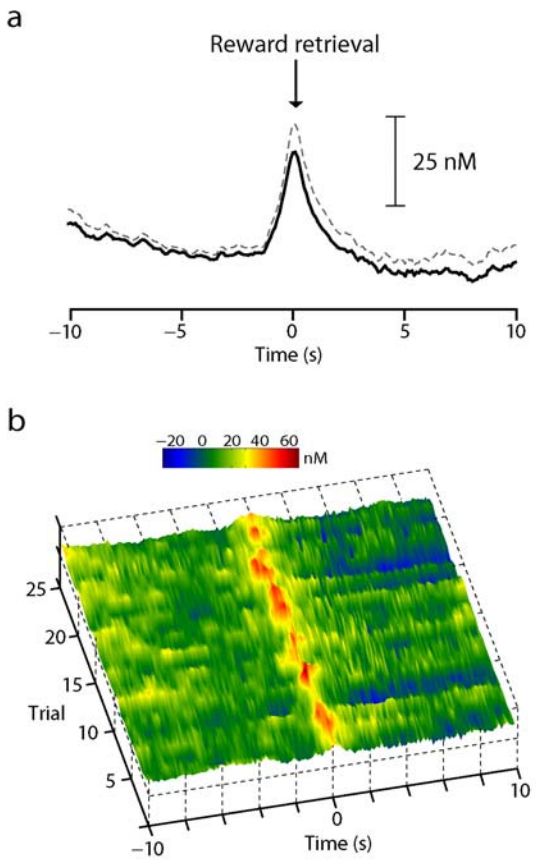

Figure 2.2. Rapid increase in NAc dopamine relative to reward retrieval during initial conditioning block. (**a**) Mean dopamine concentration (solid line) ± SEM (dashed line) relative to reward retrieval (time zero). At retrieval, dopamine concentration was significantly higher than baseline levels. (**b**) Trial-by-trial mean [DA] relative to reward retrieval (at time zero). A reward-related increase in dopamine signal was observed early and did not change throughout the conditioning session. Negative concentrations are considered because measurements are differential rather than absolute (see Methods for details).

To determine whether dopamine signals gradually became time-locked to experimental cues as conditioning progressed (as electrophysiological findings would suggest (Pan et al., 2005)), we divided the initial conditioning session into blocks of five trials for both the CS+ and CS−. Neither cue produced an increase in NAc [DA] in the first block of trials ($p > 0.05$ for all comparisons; **Fig. 2.3a**, top traces), suggesting that cues did not initially evoke an increase in NAc dopamine. Visual inspection of mean [DA] from the final 5 trials revealed an apparent (but statistically insignificant) increase in [DA] within seconds of both CS+ and CS− onset (**Fig. 2.3a**, bottom traces). As the CS+ and CS− did not evoke significantly different changes in [DA] ($p > 0.05$) or approach probability (**Fig. 2.1b**), dopamine recordings were collapsed across cue type and examined in chronological order.

Although cues did not produce a significant increase in [DA] on average, there was remarkable between-animal variability. NAc [DA] was significantly increased following cue presentation in four out of six animals during the last ten trials ($p < 0.05$ in at least one time bin within 2 s of cue onset), while two animals exhibited no cue-evoked increase. Interestingly, the time interval between cue offset and reward retrieval during the entire session predicted the existence of a cue-related dopamine signal by the end of the session (**Fig. 2.3b**). Animals that retrieved the reward quickly after the CS+ elapsed exhibited a phasic dopamine response to cue (CS+ and CS−) onset by the end of the session, whereas those with a more delayed retrieval response did not exhibit a significant cue-evoked response ($r^2 = 0.72$, $p < 0.03$; **Fig. 2.3b**). For animals that exhibited relatively rapid (< 5 s) retrieval responses during the session, cue-related dopamine signals increased in strength as a function of conditioning (positive linear relationship between the maximal change in [DA] produced by cues and trial number ($r^2 = 0.27$, $p < 0.001$; **Fig. 2.3c**,**d**). Even when cue responses developed, there was no significant difference in the magnitude of dopamine signals following the CS+ and CS− (comparison between CS+ and CS− Δ[DA]max on last 5 trials, $p > 0.05$). Moreover, the development of a cue-evoked dopamine signal was not linked to a difference in general cue approach behavior or CS+/CS− discrimination ($p > 0.3$ for both t-tests). Thus, we observed no behavioral or electrochemical differences between the CS+ and CS− for this group during the first conditioning session.

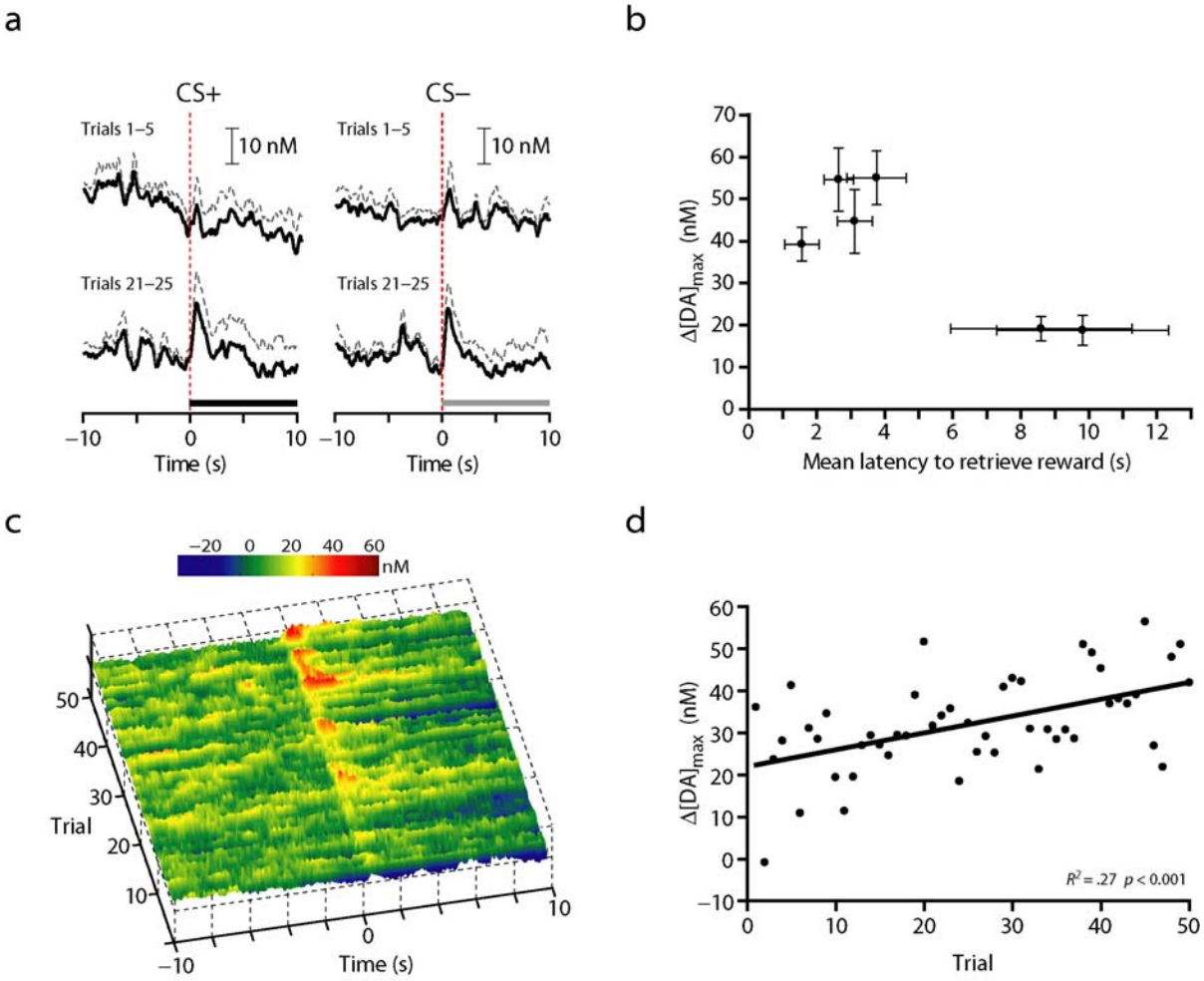

Figure 2.3. Dopamine signaling in response to conditioned stimuli during the initial conditioning block. (**a**) On average, neither the CS+ (horizontal black bar, left traces) nor the CS− (horizontal gray bar, right traces) elicited a significant increase in NAc dopamine concentration during the first five or last five conditioning trials (mean ± SEM). (**b**) Cue-evoked peak Δ[DA] (± SEM) during the last 10 trials (collapsed across cues) as a function of mean (± SEM) latency to retrieve sucrose reward after CS+ offset for individual animals. Animals that retrieved the reward at shorter latency after CS+ offset exhibited a greater cue-evoked dopamine signal. (**c**) Trial-by-trial mean [DA] in response to cue onset (time zero) for the 4 animals with relatively short (< 5 s) retrieval latencies. Again, negative concentrations are considered because of differential measurements. For these animals, cue-evoked dopamine signals emerged as conditioning progressed. (**d**) Cue-related dopamine signals (peak Δ[DA]) taken from the mean traces in panel c. Peak [DA] evoked by cue onset became significantly stronger during the course of the experimental session.

Transition in dopamine release after associative learning

To further determine how Pavlovian learning modified NAc dopamine signaling, another group of rats (n = 6) received a total of 12 conditioning sessions on 12 separate days. As above, each conditioning session consisted of 50 trials (25 CS+/reward and 25 CS−), and FSCV was performed during the final conditioning session. A repeated measures ANOVA revealed a significant cue-session interaction in approach responding ($F_{11,110} = 21.57$, $p < 0.001$). Consistent with previous reports on autoshaping (Di Ciano et al., 2001; Day et al., 2006), approach responses directed at the CS+ increased as a function of conditioning, whereas CS− approaches did not (**Fig. 2.4a**). CS+ approach probability was greater than that for the CS− for conditioning sessions 6−12 (Bonferroni *post hoc* tests, all p-values < 0.05), which indicated that animals could discriminate behaviorally between the conditioned stimuli, and that the CS+ possessed enhanced incentive-motivational salience as a cue that signaled reward.

After extended Pavlovian conditioning, both conditioned stimuli evoked changes in NAc [DA] within seconds of cue onset (CS+, $F_{40,200} = 10.12$, $p < 0.001$; CS−, $F_{40,200} = 4.635$, $p < 0.001$). Consistent with previous reports that visual and auditory cues can excite dopamine neurons at very brief latency(Dommett et al., 2005; Pan and Hyland, 2005), we observed that conditioned increases in NAc [DA] were typically of short onset and short duration (see **Fig. 2.4b** for examples). The CS+ (**Fig. 2.4c**,**d**) produced robust increases in NAc [DA] from 0.3−1.4 s following cue onset ($p < 0.05$). Peak [DA] (53.9 ± 15.0 nM) occurred at 550 ± 56 ms after CS+ onset. Despite their close temporal proximity, there was no indication that the rapid rise in [DA] preceded or caused the Pavlovian approach response.

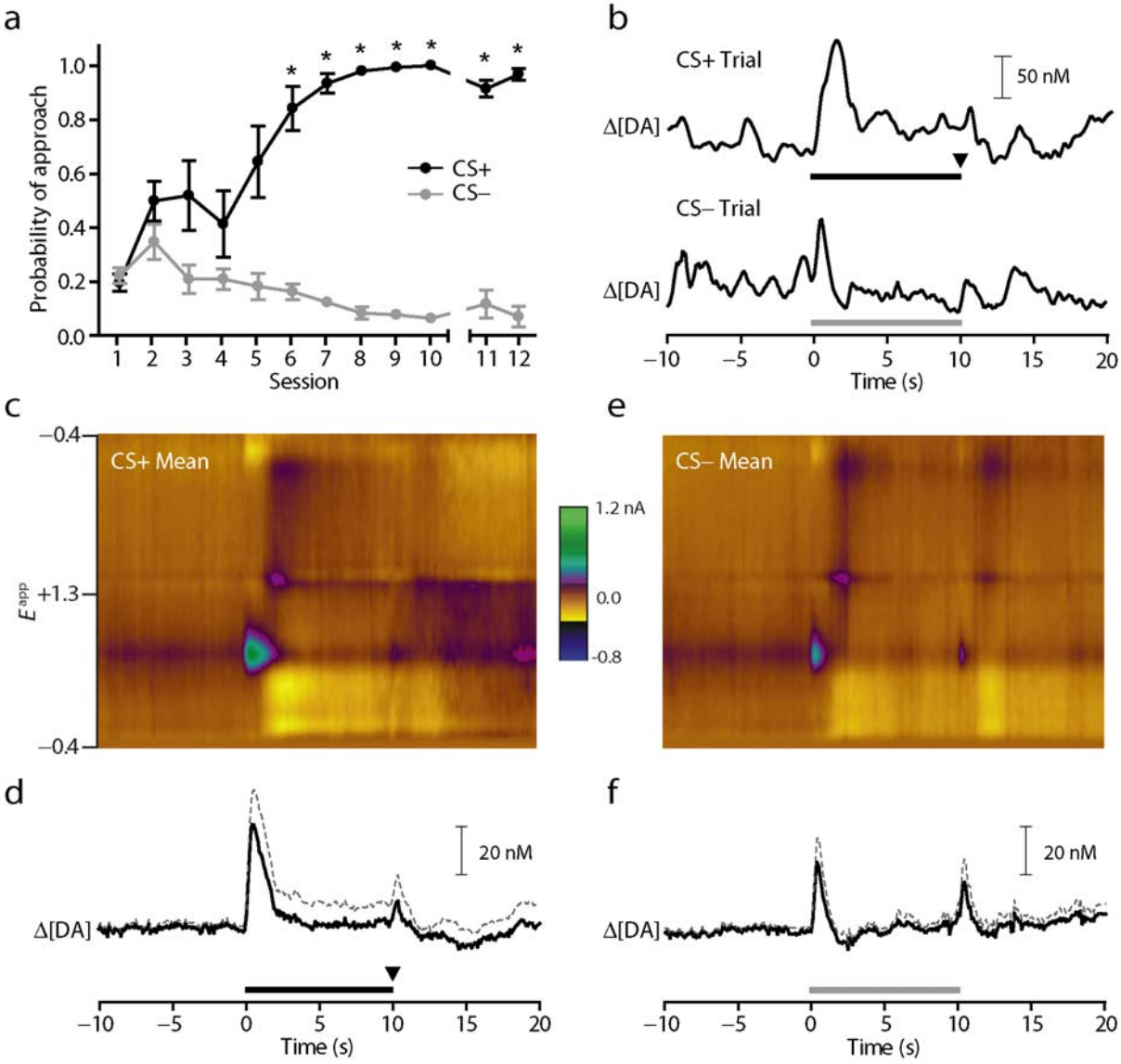

Figure 2.4. After extended conditioning, rapid dopamine release events in the NAc shift to conditioned stimuli and no longer signal primary rewards. (**a**) Behavioral discrimination (mean ± SEM approach probability) between conditioned stimuli based on predictive value. Rats approached the predictive CS+ significantly more than the non-predictive CS− in sessions 6−12. After 10 conditioning sessions animals underwent surgery for implantation of voltammetric recording apparatus (indicated by break in graph). (**b**) Representative changes in dopamine signaling during individual CS+ (top) and CS− (bottom) trials. (**c**) Three-dimensional representation of mean electrochemical data collected during reward-predictive CS+ trials. CS+ presentations evoked an immediate rise in signal that returned to baseline levels within seconds. Conventions are the same as Fig. 1c. (**d**) Mean (± SEM) increase in dopamine concentration evoked by CS+ onset was significantly greater than baseline dopamine concentration at 0.3−1.4 s after CS+ onset. No increase in signal was observed relative to reward delivery. (**e**) Three-dimensional representation of mean electrochemical data collected during CS− trials. CS− presentations evoked relatively smaller increases in signal. (**f**) Mean (± SEM) dopamine concentration also changed after CS− onset. *Post hoc* comparisons revealed a rapid increase in dopamine at 0.4−0.5 s after CS− onset. The CS− also produced a significant increase in NAc dopamine concentration at 0.4 s following cue offset.

Indeed, although approach responses were generally completed during the seconds surrounding the peak [DA] response, the timing of these variables was not significantly correlated ($r^2 < 0.01$, $p = 0.76$). Additionally, there was no relationship between the magnitude of the dopamine signal observed on a given CS+ trial and the vigor (number of lever presses) after the approach response on that trial ($r^2 = 0.014$, $p = 0.21$). Unlike early in learning, reward delivery did not evoke a significant increase in NAc [DA] ($p > 0.05$ for all comparisons; **Fig. 2.4d**).

CS− presentation evoked an increase in [DA] at 0.4−0.5 s after cue onset ($p < 0.05$; **Fig. 2.4e**,**f**). Peak [DA] occurred at 383 ± 31 ms after CS− onset and reached 37.3 ± 11.2 nM. Peak dopamine responses to the CS− were significantly smaller than those produced by the CS+ ($t = 2.917$, $df = 5$, $p = 0.033$). Additionally, the dopamine response evoked by the CS− was significantly lower than that evoked by the CS+ at 0.5−0.8 s following cue onset. In addition to the phasic response at cue onset, a significant increase in [DA] occurred at 0.4 s following CS− offset ($p < 0.05$; **Fig. 2.4f**).

Nucleus accumbens dopamine and unpredicted reward

Previous investigations in nonhuman primates indicate that phasic activation of dopamine neurons signals reward when there is no predictor available, even after repeated exposure (Schultz et al., 1997). To determine how unpredicted reward delivery affected NAc [DA], we exposed another group of rats ($n = 6$) to 12 non-conditioning sessions. During each session, 25 sucrose rewards were delivered at random to a food dish. Additionally, 10 s cues (identical to those used above) were presented 50 times in an explicitly unpaired design. FSCV was performed during the final (12^{th}) session. In this group, reward delivery produced a significant increase in NAc [DA] (**Fig. 2.5a**; $F_{40,200} = 7.27$, $p < 0.001$; $p < 0.05$ for specific

comparisons at 1.0−1.3 s after reward delivery). Peak reward-related [DA] across animals was 54.3 ± 13.7 nM. The explicitly unpaired stimulus (**Fig. 2.5b**) also produced a change in [DA] ($F_{40,200}$ = 3.073, $p < 0.001$). However, the onset and offset of this cue produced decreases in [DA] ($p < 0.05$ at 1.0−1.2 s and 10.5−13.0 s time bins).

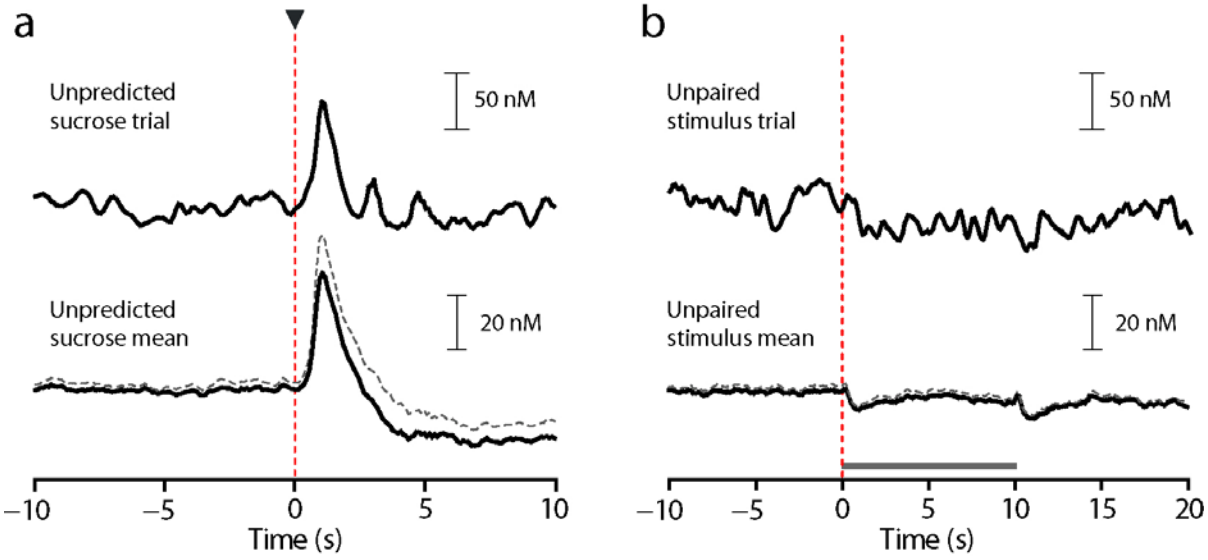

Figure 2.5. Phasic dopamine signals remained timelocked to reward delivery in the absence of a predictor. (**a**) Single-trial and mean (± SEM) dopamine signals during the final session. Unpredicted reward delivery (vertical dashed line) evoked significant increases in NAc dopamine levels at 1.0−1.3 s after delivery. (**b**) Single-trial and mean (± SEM) dopamine concentration relative to presentation of an explicitly unpaired stimulus (horizontal gray line at time-point zero). This cue produced decreases in NAc dopamine concentration at 1.0−1.2 and 10.5−13.0 s time bins relative to cue onset.

Differential dopamine signals and conditioning history

To compare the relative magnitude of dopamine signals in response to cue and reward stimuli within each experimental group, electrochemical data was converted to signal-to-baseline (S:B) ratios (defined as peak differential [DA] during event/average baseline differential [DA]). In the early conditioning group, the CS+ and CS− evoked relatively small S:B ratios (2.18 ± .42 and 2.52 ± .38, respectively), indicating that phasic dopamine signals were only weakly modified by the presentation of these cues (**Fig. 2.6a**). Conversely, the maximal

dopamine signal during reward retrieval was nearly a five-fold increase over baseline (actual S:B = 4.65 ± .99), significantly more than produced by either CS ($F_{2,17} = 8.089$, $p = 0.008$; Tukey multiple comparisons test, $p < 0.05$ for both reward vs. cue comparisons).

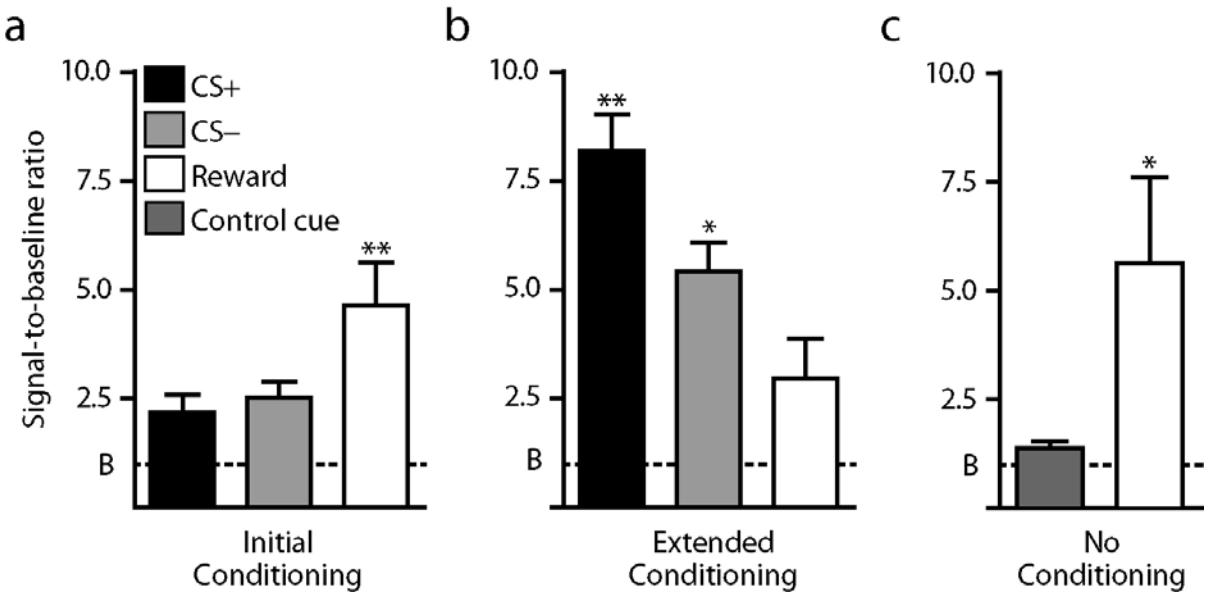

Figure 2.6. Comparison of dopamine changes relative to cue and reward stimuli using signal-to-baseline transformation. (**a**) For the initial conditioning group, the reward signal (mean ± SEM) was significantly greater than signals for either conditioned stimulus (**Tukey multiple comparisons test, $p < 0.05$ for both reward vs. cue comparisons). (**b**) After extended conditioning, dopamine signals were significantly greater for both conditioned stimuli than for reward delivery. Additionally, the S:B ratio for the CS+ was greater than that for the CS− (*Tukey multiple comparisons test, $p < 0.05$ for CS− vs. reward; **Tukey multiple comparisons test, $p < 0.05$ for CS+ vs. CS− and CS+ vs. reward). (**c**) In the absence of a predictive cue, the reward signal was significantly greater than the unpaired cue signal.

After extended conditioning (12 experimental sessions) in a second group of animals, peak dopamine signals were greatest in response to conditioned stimuli and smallest when rewards were delivered ($F_{2,17} = 28.538$, $p < 0.0001$; **Fig. 2.6b**). Specifically, mean peak [DA] increased over eight-fold from baseline levels during CS+ presentation. Peak dopamine signals relative to CS− presentation and reward delivery were significantly smaller (Tukey multiple comparisons test, $p < 0.05$ for each comparison; CS+ > CS− > reward). This result suggests that NAc dopamine signals were no longer time-locked to reward delivery or

retrieval, but instead corresponded to the presentation of a reward predictive cue and (to a lesser extent) a separate but similar cue that did not predict rewards.

In the group that received no conditioning (i.e., stimuli and rewards were explicitly unpaired), the maximal S:B ratio during reward delivery was significantly greater than that for the cue period ($t = 2.618$, $df = 5$, $p = 0.047$; **Fig. 2.6c**). Thus, non-conditioning sessions did not produce a shift in the phasic dopamine signal. Moreover, unlike the CS− from the previous experiment, the unpaired cue in this condition did not produce increases in [DA].

DISCUSSION

The use of environmental cues to predict impending outcomes is a fundamental aspect of learned behavior. By sampling at different stages of conditioning, our design enabled us to determine how such associative learning alters real-time NAc dopamine signaling in response to predictive cues and rewarding stimuli. Here, we demonstrated that sub-second dopamine release within the NAc core signals reward in naïve rats. However, when animals were trained to associate an experimental cue with the delivery of a reward, the dopamine signal shifted to this predictor and was no longer present when the reward was made available. In the absence of a predictor, phasic elevations in NAc [DA] remained time-locked to reward delivery. Taken together, these findings reveal that associative learning dynamically alters NAc dopamine responses to both predictive cues and primary rewards.

The present results are highly consistent with "prediction error" models of dopamine function (Bayer and Glimcher, 2005; Pan et al., 2005). Early in learning, reward delivery was not yet associated with the CS+ and therefore occurred unpredictably. In this condition, phasic dopamine release events were time-locked to the receipt of a reward but not the CS+. As conditioning progressed, both the CS+ and CS− came to evoke increases in NAc [DA] in some animals but not others. Individually, this development was predicted by the duration between the CS+ and reward; animals that obtained the reward sooner after cue offset exhibited a phasic cue-evoked dopamine signal by the end of the behavioral session. Thus, the acquisition of dopamine signals during conditioning corresponded to the temporal proximity of the cue and reward, providing an early link between associative strength (Sutton and Barto, 1998) and NAc dopamine signaling. Furthermore, the emergence of an acquired dopamine response at cue onset was not selective for the reward-predictive CS+, but also

occurred when the CS− was presented. This finding may underscore the limits of the dopamine system. Faced with the task of successfully predicting reward delivery in a novel environment, rapid increases in dopamine may signal not only predictive cues, but also similar cues which may turn out to provide valuable information. Such a function could prove beneficial in natural environments where food could be predicted by spatially separate but physically similar cues.

After many conditioning sessions, animals developed a behavioral discrimination between the CS+ and CS−, indicating that they had learned the existing predictive relationships. Consistent with dopamine cell recordings in primates (Mirenowicz and Schultz, 1994; Waelti et al., 2001), rapid dopamine release events shifted to the cue that predicted future rewards. In contrast, predicted reward delivery lost the ability to elicit increases in NAc [DA]. This change in dopamine signaling was only present in animals that underwent stimulus-reward pairings; dopamine release events still signaled reward delivery in animals that received equal exposure to rewards without a predictor. Although stimulus-reward learning clearly altered dopamine signaling in the NAc, it should be noted that not all cues paired with rewards produce phasic dopamine responses. In a previous report that employed a blocking paradigm, reward-predictive cues did not produce an increase in dopamine cell firing when it was blocked by a previously predictive cue during conditioning (Waelti et al., 2001). Thus, prediction errors (and not stimulus-reward associations alone) are the determining factor in the generation of phasic cue-related dopamine responses.

Even after extended conditioning, a CS− which predicted the absence of rewards evoked a brief increase in NAc [DA] (Fig. 2.4f). While this response may seem paradoxical, it should be noted that electrophysiological studies have reported similar patterns in burst

firing among a subset of dopamine neurons when CS− cues are presented (Waelti et al., 2001), and that these responses have also been modeled using temporal difference algorithms (Kakade and Dayan, 2002). One interpretation suggests that this response reflects a form of stimulus generalization (Waelti et al., 2001; Kakade and Dayan, 2002). The initiation of both CS+ and CS− dopamine signals likely begins with the audio component of cue onset, as reward-predictive audio stimuli evoke increases in dopamine cell firing at shorter latency than do visual cues (Pan and Hyland, 2005). However, since the cues used here generated highly similar sounds (and were only spatially distinct), audio information alone may not enable adequate discrimination. Accordingly, cue onset may produce a rapid increase in dopamine cell firing that corresponds to the expected value predicted by *both* cues, which is ½ of a reward (average of 0 for CS− and 1 for CS+). When the identity of the cue is fully ascertained through visual input, the dopamine response may adjust to reflect the updated prediction. Thus, the CS+ signals a better-than-expected outcome and the increase in dopamine continues, while the CS− signals a worse-than-expected outcome and [DA] rapidly decreases in a manner consistent with electrophysiological results from dopamine neurons (Waelti et al., 2001). A similar phenomenon may occur at CS− offset, when the existing prediction is the absence of a reward. Here, the sound of cue offset is associated with reward on 50% of trials, and so a small positive prediction error may be generated on CS− but not CS+ trials. This position is further strengthened by the observation that no phasic increases in dopamine were produced by an unpaired cue when animals did not have concurrent exposure to a predictive cue (Fig. 2.5b). Here, cue onset and offset produced decreases in NAc [DA] even though this cue and the CS− carry highly similar information with respect to reward

delivery. This result highlights the potential impact of learning environment, and especially the presence of other cues, in the promiscuity of the dopamine signal.

Behavioral discrimination between reward-predictive cues and other stimuli likely requires concerted activity in a distributed network of brain structures that includes the NAc and its dopaminergic innervation, the anterior cingulate cortex (ACC) and the central nucleus of the amygdala (CeA) (Robbins and Everitt, 2002). Conditioned approaches towards a predictive CS+ are impaired by D1/D2 dopamine receptor antagonism and dopamine depletion within the NAc core (Di Ciano et al., 2001; Parkinson et al., 2002). Moreover, excitotoxic lesions to the ACC or CeA also significantly alter the allocation of conditioned approach responses (Cardinal et al., 2002b). Within this circuit, it has been proposed that excitatory ACC input into the NAc facilitates discrimination between sensory cues, while the CeA augments the firing of dopamine cells that project to the NAc (Robbins and Everitt, 2002). However, the precise behavioral role of phasic dopamine release within the NAc remains unclear. One possibility is that these signals are responsible for the generation of approach responses towards predictive stimuli (Ikemoto and Panksepp, 1999). Although recent reports suggest that dopamine can actively produce or modulate operant reward-seeking behaviors (Phillips et al., 2003a; Roitman et al., 2004), several results argue against this interpretation with respect to the Pavlovian approach responses observed in the present context. First, the CS+ and CS− both evoked brief increases in NAc [DA] in animals that received extended conditioning, but the same animals approached the CS− on only 6% of trials while approaching the CS+ on over 95% of trials (Fig. 2.4a). It is uncertain how this clear behavioral discrimination could be made based on a phasic dopamine signal that is highly similar for the CS+ and CS− immediately after cue onset. Second, the timing and

magnitude of the dopamine signal on CS+ trials was unrelated the timing or degree of behavioral activation. We therefore hypothesize that dopamine-related reward prediction information may be processed by the NAc and utilized to instruct or strengthen (Wise, 2004) (but not generate) certain motor responses as they occur or after they occur. A related and intriguing explanation posits that rapid dopamine release may reflect the incentive value of the CS+ and reward (Berridge and Robinson, 1998; Berridge, 2006). Early in conditioning, the sight or sound of a reward may signal an "incentive" to retrieve the reward and produce a phasic increase in NAc [DA]. During learning, the CS+ comes to predict the reward in the same manner, thereby acquiring its own incentive value and evoking a similar dopamine response.

The ability of the NAc and other striatal regions to influence behavioral output based on Pavlovian associations almost certainly involves the modification of individual synaptic inputs during learning. Indeed, recent studies have demonstrated that although the majority of NAc neurons do not innately respond to neutral environmental cues, responses quickly emerge when cues begin to predict rewarding events (Setlow et al., 2003; Roitman et al., 2005). Moreover, the majority of NAc neurons display robust changes in activity when reward predictive cues are presented after an extended conditioning design similar to the one used here (Day et al., 2006). It has been suggested that dopamine-glutamate interactions within the NAc may play a key role in this cellular plasticity, with dopamine gating the efficacy of NAc glutamatergic inputs from limbic and cortical structures (Cepeda and Levine, 1998). Consistent with this hypothesis, blockade of dopamine D1 receptors inhibits long-term potentiation in corticostriatal slices (Kerr and Wickens, 2001) and prevents the proper expression and consolidation of learned stimulus-reward relationships (Eyny and

Horvitz, 2003; Dalley et al., 2005). We propose that the phasic dopamine signals observed here possess a special role with respect to D1 receptor activation during stimulus-reward learning. Recent no-net-flux microdialysis studies have placed the basal concentration of dopamine at levels far below those needed to activate low-affinity D1 receptors (Richfield et al., 1989; Watson et al., 2006). However, by rapidly increasing the local concentration of dopamine, phasic release events are capable of providing a signal that can stimulate D1 receptors on a timescale commensurate with behavioral events and environmental stimuli. In turn, D1 receptors could act through well-described signaling cascades (Greengard, 2001) to prolong recent memory traces and allow fast synaptic communications to interact with those traces. Understanding the complexities of this interplay within brain regions such as the NAc may provide critical insight into the neurobiology of both natural and aberrant stimulus-reward learning.

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CHAPTER 3

ROLE OF PHASIC NUCLEUS ACCUMBENS DOPAMINE IN EFFORT-RELATED DECISION MAKING

ABSTRACT

Optimal reward seeking and decision making requires that organisms correctly evaluate both the costs and benefits of multiple potential choices. One such cost is the amount of effort required to obtain rewards, which can be increased through a number of environmental and economic constraints. Dopamine transmission within the nucleus accumbens (NAc) has been heavily implicated in theories of reward learning and cost-based decision making, and is required for organisms to overcome high response costs to obtain rewards. Here, we monitored dopamine concentration within the NAc core on a rapid timescale using fast-scan cyclic voltammetry during an effort-related decision task. Rats were trained to associate different visual cues with rewards that were available at low cost (FR1), high cost (FR16), or choice (FR1 or FR16) effort levels. Behavioral data indicate that animals successfully discriminated between visual cues to guide behavior during the task, that behavioral output increased when required to obtain reinforcement on high cost trials, and that choice allocation was sensitive to cost requirements. Electrochemical data indicate that cues predicting low-cost effort requirements evoked significantly greater increases in dopamine concentration than cues which predicted high-cost effort requirements. On choice trials, cue-evoked dopamine concentration was similar to low-cost cues presented alone. There were no differences in dopamine concentration during the response period or upon reward delivery.

These findings are consistent with previous reports that implicate NAc dopamine function in reward prediction and the allocation of response effort during reward-seeking behavior, and indicate that dopamine may influence decision making by reflecting the effort requirements associated with available rewards.

INTRODUCTION

An organism's ability to obtain food in natural environments often requires considerable expenditures of time and energy that must be correctly evaluated to optimize decision making strategies. A fundamental cost in all goal-directed behaviors is the amount of effort required, which can be increased through a number of environmental and economic constraints. Overcoming high work-related response costs associated with reward seeking allows animals to capitalize on feeding opportunities, providing maximal caloric intake in situations of inelastic demand. Effort-related decision making likely involves the concerted activation of a specific network of brain nuclei including the nucleus accumbens (NAc) and its dopaminergic input. Subsecond dopamine release within the NAc is believed to modulate food and cocaine seeking behaviors (Phillips et al., 2003a; Roitman et al., 2004), and drugs that alter dopamine transmission bias effort-related decision making (Floresco et al., 2007). Dopamine depletion or antagonism in the NAc produces profound effects on operant responding for food, but primarily when reinforcement is contingent upon high work-related response costs (Cousins and Salamone, 1994; Aberman et al., 1998; Aberman and Salamone, 1999; Correa et al., 2002; Salamone et al., 2002; Salamone et al., 2003; Mingote et al., 2005). Moreover, dopamine concentration (as measured via microdialysis) is more closely correlated to response output than overall reinforcement rate (McCullough et al., 1993a; Sokolowski et al., 1998).

These and other observations have led to the hypothesis that one function of NAc dopamine is to promote behavioral output when reward acquisition demands increased effort (Salamone et al., 2003; Niv et al., 2007; Phillips et al., 2007). However, NAc dopamine is also heavily implicated in behavioral responses to reward-paired cues and the ability of such

cues to influence decision making (Di Ciano et al., 2001; Dayan and Balleine, 2002; Nicola et al., 2005; Berridge, 2006; Morris et al., 2006; Pessiglione et al., 2006). Discriminative and conditioned stimuli evoke robust dopamine release in the NAc (Roitman et al., 2004; Day et al., 2007), and recent evidence suggests that dopamine neurons relay complex reward-related information concerning the probability, value, and temporal delay of predicted rewards (Fiorillo et al., 2003; Tobler et al., 2005; Roesch et al., 2007). Thus, dopamine release in the NAc may not only be necessary to overcome large effort requirements, but may also facilitate choice behavior when available options have different effort-related costs. However, it is presently unclear whether effort-related information is encoded by phasic dopamine release in the NAc.

This experiment will extend previous findings by monitoring NAc dopamine concentration on a rapid timescale using fast-scan cyclic voltammetry during an effort-related decision task. In this design, sucrose rewards will be made available under both low-cost (fixed ratio 1; FR1) and high-cost (FR16) schedules of reinforcement in discrete trials, each of which will be predicted by separate 5s discriminative stimuli. As each cue predicts different effort requirements and precedes the opportunity to respond, this design enables separate yet direct comparison of both cue-related and response-related NAc dopamine signals. Moreover, during a third trial type, animals will be presented with both discriminative stimuli and allowed to choose either the low- or high-cost response option. The aims of this experiment are thus three-fold: 1) to determine whether cue-evoked increases in NAc dopamine concentration encode information about the effort requirements associated with future rewards, 2) to reveal potential differences in phasic NAc dopamine signaling during the completion of different effort requirements, and 3) to examine

differences in NAc dopamine signaling under choice situations wherein available options present different response costs. As such, this experiment will provide novel insight into how dopamine could promote behavioral activation when required by environmental constraints and/or bias decision making when multiple choices with different costs are available.

METHODS

Animals Male, Sprague Dawley rats (n=8, Harlan Sprague Dawley, Indianapolis, IN) aged 90-120 d and weighing 260-350 gm were used as subjects and individually housed with a 12:12 light: dark cycle. All experiments were conducted between 9:00 am and 5:00 pm. Bodyweights were maintained at no less than 85% of pre-experimental levels by food restriction (10-15 gm of Purina laboratory chow each day, in addition to approximately 1 gm of sucrose consumed during daily sessions). This regimen was in place for the duration of behavioral testing, except during the post-operative recovery period when food was given *ad libitum*. All procedures were approved by the Institutional Animal Care and Use Committee.

Behavioral training Lever pressing behavior in all rats was initially reinforced on a continuous schedule of reinforcement on two levers, such that every response on either lever resulted in the delivery of a 45mg sucrose pellet to a centrally located food receptacle. A maximum of 100 reinforcers (50 per lever) were available per session (with 1 session per day). After stable responding developed (5 sessions), rats were transferred to a multiple schedule task in which reinforcement was contingent on operant responses in 90 discrete trials. Each trial was initiated randomly after a variable time interval, with an average of 20s between trials. In this task, distinct cue lights (located above two response levers) were illuminated for 5s before lever extension to signal which lever was active (i.e., which lever produced reinforcement). Response levers were available for 15s unless response requirements were completed, in which case the levers were retracted and the reward was delivered. On 60 forced-choice trials, one cue was presented alone and only a response on the corresponding lever was reinforced. On these trials, responses made on the uncued lever (termed “errors”) resulted in the termination of the houselight for the remainder of the trial

period and the absence of sucrose delivery for that trial. The number of errors served as a behavioral measure of discrimination between low and high cost cues. On another 30 free-choice trials, both cues were presented simultaneously, allowing a choice between both options. For the first 11 days of training, the response cost of each option was identical (an FR1 schedule of reinforcement). In order to produce an effort disparity between response options, the required fixed ratio on one lever (termed the "high cost" option) was gradually increased from 1 to 16 according to the following schedule: Sessions 1-11, FR1; Session 12, FR2; Session 13, FR4; Sessions 14-16, FR8; Sessions 17-20, FR12; Sessions 21-25, FR16. The fixed ratio on the other lever (termed the "low cost" option) remained the same throughout training (see **Fig. 3.1**). Choice behavior on free-choice trials served as a measure of an animal's overall sensitivity to changes in the work-related response costs of available options. In this task, work-related response costs are minimized by selecting the low-cost option on the 30 choice trials. Similarly, reinforcement is maximized by overcoming high costs when required on forced-choice trials. Following 25 training sessions, all rats were prepared for electrochemical recording in the NAc as described below. After recovery, rats underwent additional training sessions until behavior was stable (at least 5 sessions).

Surgery Surgical techniques were identical to those described in chapter two (see chapter two, pages 41-42 for details).

Fast-scan cyclic voltammetry Electrochemical procedures were identical to those described in chapter two (see chapter two, pages 42-43 for details).

Signal identification and separation Dopamine was identified and separated from electrochemical data using methods identical to those described in chapter two (see chapter two, page 44 for details).

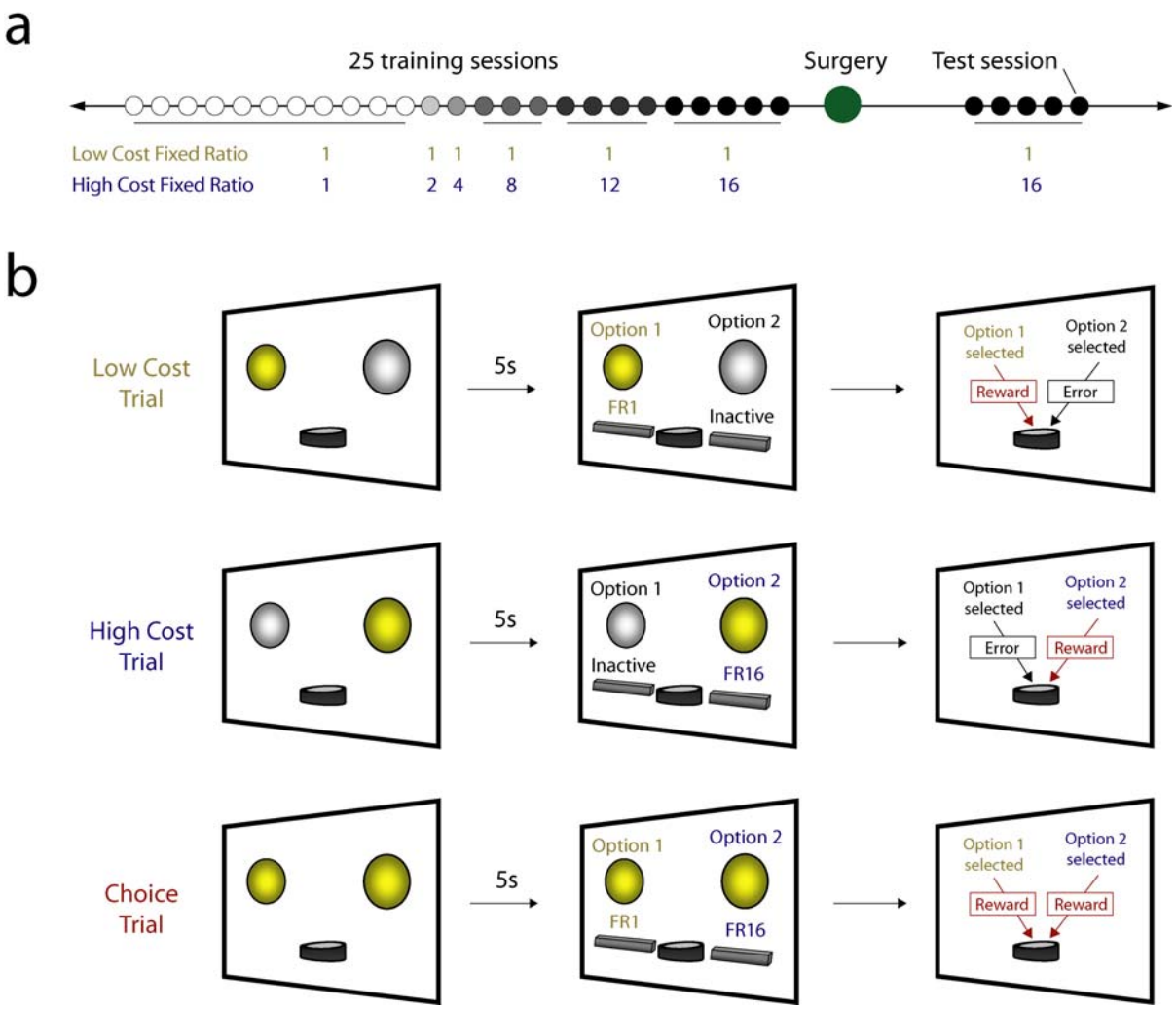

Figure 3.1. Experimental timeline and design of effort-based choice task. (**a**) Experimental timeline. Animals received 25 total training sessions before surgical implantation of guide cannula above the NAc (each circle = 1 session). Additional training sessions occurred after surgery, and dopamine concentration was recorded during the task. Numbers below circles indicate number of responses required to produce reinforcement on low and high cost trials. Costs were gradually increased on high cost trials across training. (**b**) Behavioral task during the recording session. On low cost trials (top panels), a cue light was presented for 5s and was followed by lever extension into the chamber. A single lever press on the corresponding lever led to reward delivery in a centrally located receptacle. Responding on the other lever did not produce reward delivery and terminated the trial. On high cost trials, the other cue light was presented for 5s before lever extension. Here, sixteen responses on the corresponding lever were required to produce reward delivery. Responses on the low-cost lever terminated the trial and no reward was delivered. On choice trials (lower panels), both cues were presented, and animals could select either low or high cost options.

Data Analysis All behavioral events (cue onset and offset, lever presses, lever extension/retraction, and reward delivery) occurring during training and electrochemical recording were recorded and available for analysis. Analysis of behavioral data collected

during training sessions included examination of overall response rates and allocation, latency to initiate and complete response requirements, number of reinforcers obtained, number of errors committed, and preference between the low and high costs options on choice trials. Effects of training on total reinforcement and number of errors committed were assessed using a repeated measures ANOVA that tested for a linear trend between session number and the dependent variable. Effects of response cost on choice allocation were evaluated using a two-way repeated measures ANOVA of average choice probability as a function of cost, with Bonferroni post-hoc tests used to correct for multiple comparisons between low and high cost choice probability. Response times on high and low trials during the recording session were compared using t-tests.

Phasic changes in extracellular DA concentration during the task were assessed by aligning DA concentration traces to relevant behavioral events (specifically, cue presentations, lever extension, and reward delivery). Individual data were smoothed using a Gaussian filter (kernel width = 3 bins). Group increases or decreases in NAc dopamine concentration were evaluated separately for each trial type and for each event using a one-way repeated measures ANOVA with Tukey's correction for multiple comparisons. This analysis compared the baseline average dopamine concentration to each data point obtained within 2.5s following an event. The effects of predicted and experienced response costs on group DA levels were assessed using a one-way repeated measures ANOVA that compared peak changes in DA levels following each event (within 2.5s of the event), with Tukey's correction for multiple post-hoc comparisons. This comparison was performed separately for data collected in the core and shell of the NAc. All analyses were considered significant at $\alpha=$

0.05. Statistical and graphical analyses were performed using Graphpad Prism and Instat (Graphpad Software, Inc) and Neuroexplorer for Windows version 4.034 (Plexon, Inc).

Histological verification of electrode placement. Histological techniques and identification of electrode locations were identical to methods described in chapter two (see chapter two, pages 45-46 for details).

RESULTS

Behavior during the effort-based decision task

Dopamine recordings were obtained from seven male rats that were trained on the effort-based decision task. Results demonstrate that animals could discriminate between cues preceding lever presentation, could overcome large (FR16) response costs when necessary, and were sensitive to changes in cost (**Fig. 3.2**). On forced-choice trials, animals initially responded at equal levels on each trial type (**Fig. 3.2a**). However, as response costs were increased (beginning in session 12), animals increased response output on the high cost trials in order to meet the requirements. In the recording session, 97.6 ± 0.02% (mean ± SEM) of all trials and 90.8 ± 0.04% of the forced choice high cost trials resulted in reward delivery, indicating that when no alternatives were available, animals were capable of overcoming required costs. Across sessions, the number of rewards obtained increased (test for linear trend, $F_{1,187} = 89.82$, $p < 0.001$; **Fig. 3.2b**), whereas the number of errors decreased ($F_{1,187} = 115.1$, $p < 0.001$; **Fig. 3.2c**), indicating that performance improved with training and animals used cues to guide responding on forced choice trials. On free choice trials, preference changed as a function of imposed response cost (repeated measures ANOVA; interaction between option and cost; $F_{6,42} = 5.187$, $p < 0.001$; **Fig. 3.2d,e**). Specifically, animals preferred the low cost option over the high cost option at all cost ratios after 2:1, including the 16:1 ratio on the recording day (Bonferroni *post hoc* tests, all p's < 0.05). Response latencies on high and low cost trials differed on the recording day, with animals exhibiting faster responses on the low cost option (paired *t*-test, $t = 2.592$, $df = 7$, $p = 0.036$; low cost latency = 0.39 ± 0.07s, high cost latency = 0.78 ± 0.1s).

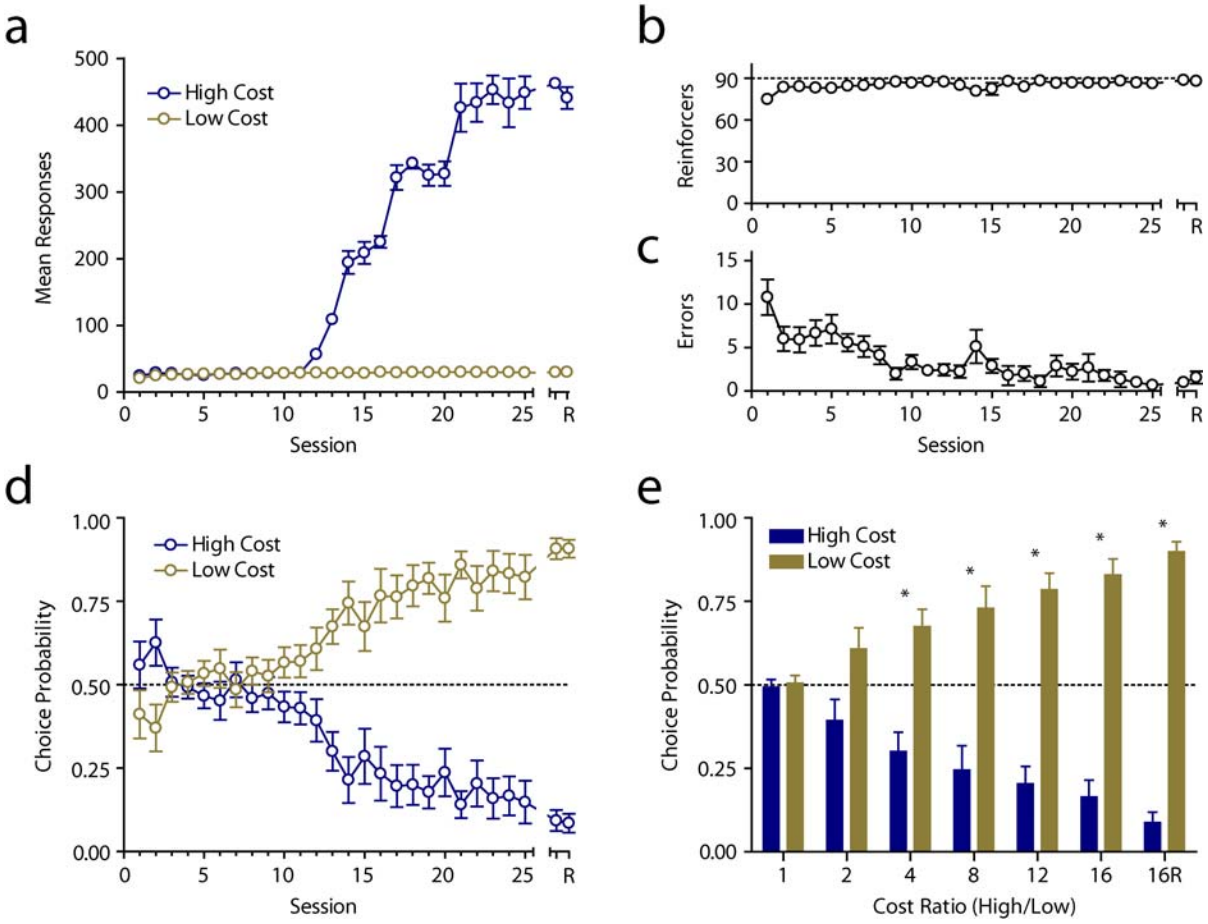

Figure 3.2. Behavior during the effort-based choice task. (**a**) Mean responses on forced choice trials. Response output (mean ± SEM) increased as response requirements were raised on high choice trials, beginning with session 12. Fixed ratio requirements on high cost trials were increased to FR2 (session 12), FR4 (session 13), FR8 (sessions 14-16), FR12 (sessions 17-20), and FR16 (remaining sessions, including recording session, R). Response requirements on low cost trials were not altered. (**b**) Total reinforcers across training sessions (mean ± SEM). Reinforcers obtained increased with training ($p < 0.01$), and was near maximal levels during the recording session. Dashed line indicates maximal number of reinforcers available. (**c**) Total errors across sessions (mean ± SEM). Errors decreased as training progressed ($p < 0.001$), indicating animals could discriminate between cues. (**d**) Choice probability as a function of session (choice trials only). Dashed line indicates behavioral indifference point (chance selection). When given a choice, animals initially exhibited little preference. As response requirements were increased for the high cost option, animals began to select the low cost option. (**e**) Choice probability as a function of the ratio between lever presses required on high cost and low cost trials. Dashed line indicates indifference point. Choice allocation shifted as a function of response cost (two-way repeated measures ANOVA, $p < 0.05$). Asterisks indicate ratios at which preference for the low-cost option was significant (Bonferroni *post hoc* tests, $p < 0.05$). 16R denotes choice preference during the recording session.

Reward-associated discriminative stimuli evoke phasic dopamine signals in the NAc

On the recording day, electrochemical data were collected while animals performed the effort-based choice task. Characteristic phasic dopamine signals occurring during this

session are shown in **Fig. 3.3** (single trial color plots and dopamine traces) and **Fig. 3.4** (dopamine traces and average for an entire session). Consistent with previous results (see Chapter 2), we found that reward-associated cues evoked the strongest increase in phasic dopamine release. Thus, cue onset produced a robust increase in dopamine concentration that was visible both on single trials, and in averages across the session. These increases were present across low cost, high cost, and choice trials types (**Figs. 3.3, 3.4**). In contrast, neither lever extension, lever presses, or reward delivery appeared to evoke any robust change in dopamine concentration.

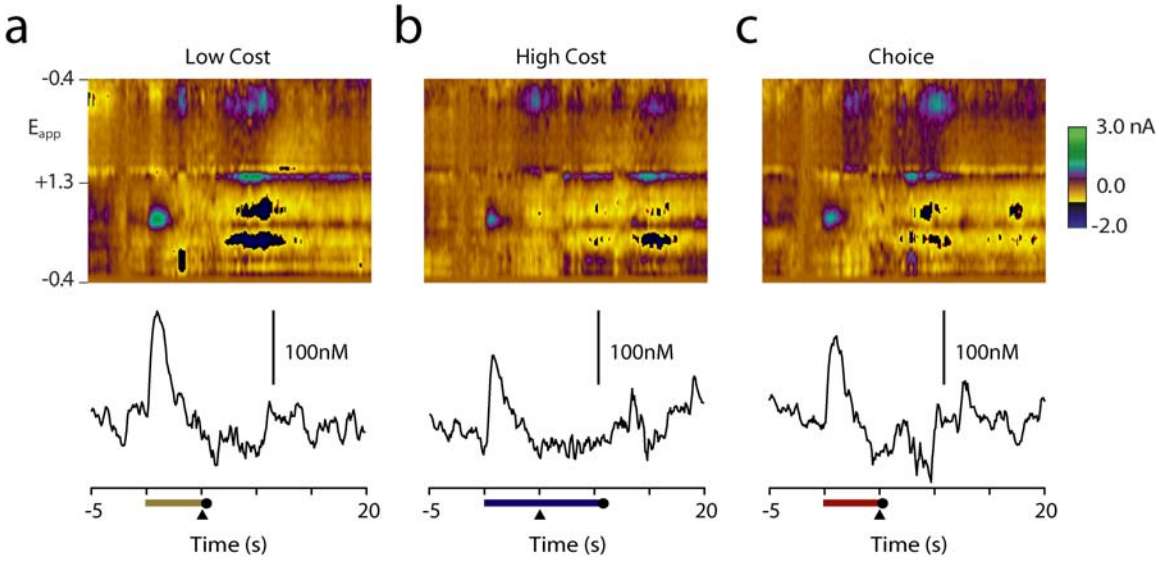

Figure 3.3. Representative electrochemical data collected during individual behavioral trials. (**a**) Two-dimensional representation (color plot) of electrochemical data collected during a single low cost trial (top) and corresponding dopamine concentration trace (bottom). The applied voltage (ordinate) is plotted during a 25 s window aligned to cue onset (horizontal gold bar beginning at time-point zero, abscissa). Changes in current at a carbon-fiber electrode located in the NAc are encoded in color. The black triangle denotes lever extension, whereas the black circle marks reward retrieval. Dopamine is visible as a green-encoded spike in current at cue onset in the color plot. (**b**) Color plot and dopamine trace from a high cost trial. Blue bar denotes cue presentation. All other conventions follow panel a. (**c**) Color plot and dopamine trace on choice trial, when both cues are presented. Here, the animal selected the low cost option. Red bar denotes cue presentation; all other conventions follow panel a. All cues evoked dopamine release in the NAc.

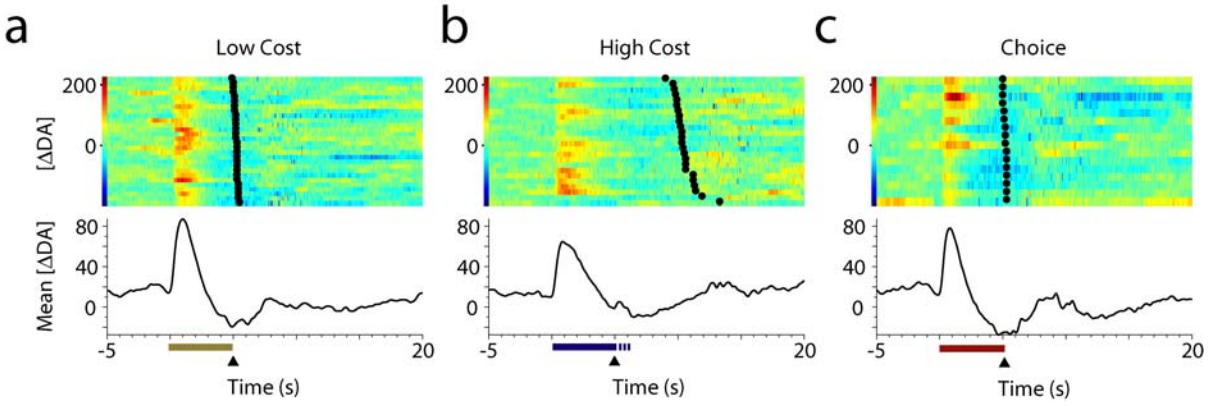

Figure 3.4. Changes in dopamine across multiple trials for a representative animal. (**a**) Changes in dopamine concentration on low cost trials, aligned to cue onset (gold bar, time zero). Top panel (heat plot) represents individual trial data (rank ordered by distance from lever extension to reward delivery), whereas bottom trace represents average from all trials. Black triangle indicates lever extension, black circles on heat plot indicate reward delivery. Dopamine release peaks shortly after cue onset and returns to baseline levels. (**b**) Relative dopamine concentration on high cost trials, aligned to cue onset (blue bar; other conventions same as panel a. Again, cue presentation evoked robust increases in NAc dopamine concentration that shortly returned to baseline levels. (**c**) Change in dopamine concentration on choice trials aligned to cue presentation (red bar). Dopamine peaks after cue onset.

Cue-evoked dopamine signals within the NAc core reflect predicted response cost

Electrode locations from four separate animals were histologically verified to be located in the core subregion of the NAc (**Fig. 3.5a**). Group changes in dopamine concentration recorded at these sites are shown in **Fig. 3.5b**, timelocked to cue onset. Repeated measures ANOVA revealed that cue presentation in each trial type significantly increased dopamine concentration ($p < 0.05$ for each type). However, a one-way repeated measures ANOVA comparing the mean peak dopamine concentration evoked on each trial type indicated that the amount of dopamine release varied based on cost ($F_{2,6} = 9.98$, $p = 0.012$; **Fig. 3.5c**). Specifically, cues that predicted higher response costs (FR16) generated less dopamine release than cues which predicted low costs (FR1) or the presentation of both cues on choice trials (Tukey's *post hoc* test, $p < 0.05$ for both comparisons). However, there was no difference between dopamine release evoked by low cost cues and cue presentation on choice trials, when the animals overwhelmingly chose the low cost option ($p > 0.05$).

Confirming observations from single trials and single animals, neither lever extension nor reward delivery evoked significant levels of dopamine release on any trial type (repeated measures ANOVAs, $p > 0.05$ for each trial type). Thus, only presentation of reward-paired discriminative stimuli evoked changes in dopamine concentration. Furthermore, there was no difference in peak dopamine concentration observed at lever extension or reward delivery across trials types (repeated measures one-way ANOVA, $p > 0.05$).

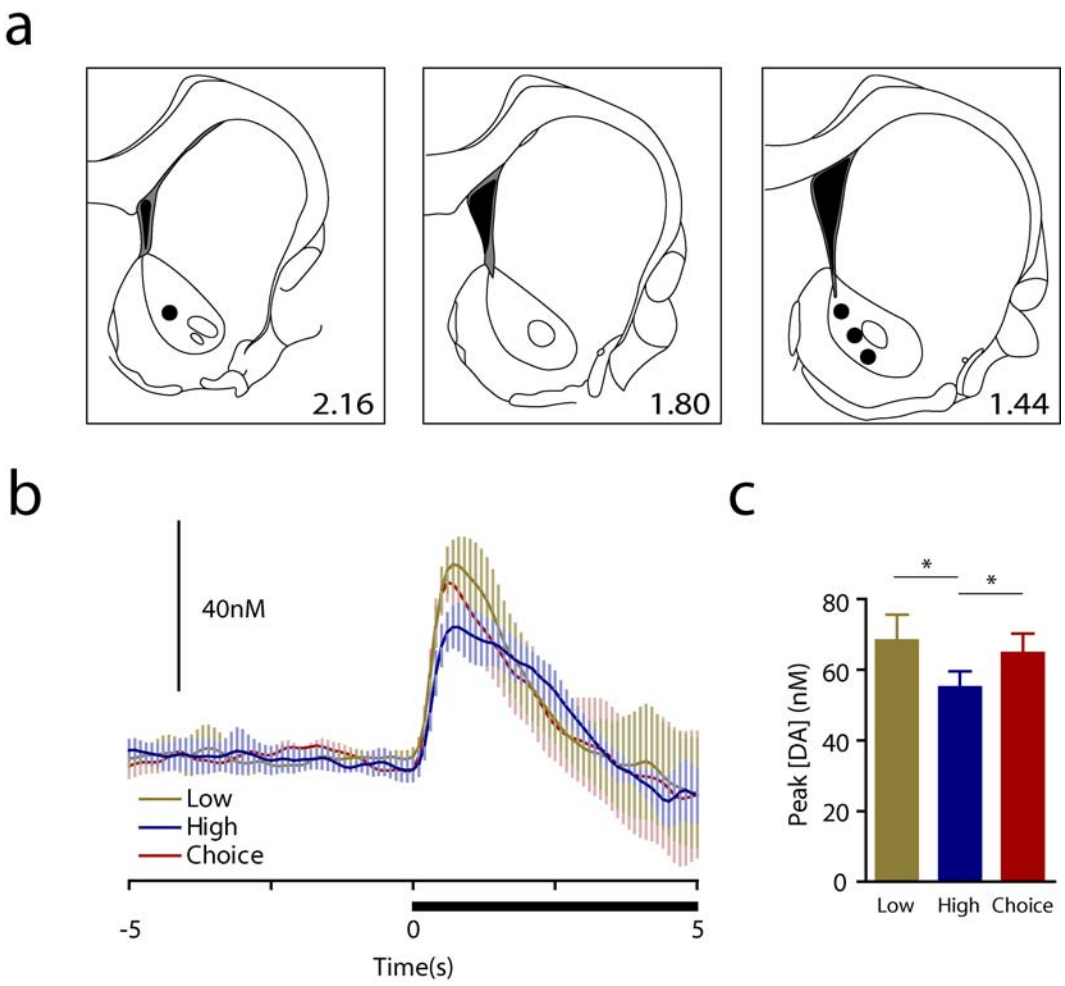

Figure 3.5. Cue-evoked dopamine release in the NAc core. (**a**) Coronal diagrams illustrating confirmed location of carbon-fiber electrodes within the NAc core. Black circles indicate recording sites. Numbers in lower right corner of each panel indicate location anterior to bregma, in mm. (**b**) Mean (solid lines) ± SEM (shaded lines) change in dopamine concentration for each trial type, relative to cue onset (black bar, time zero). All cues evoked significant increases in dopamine concentration (repeated measures ANOVAs, $p < 0.05$). (**c**) Average peak cue-evoked dopamine signal (± SEM) across trial type. Cue presentation on low cost and choice trials led to significantly larger increases in dopamine concentration than high cost cue presentation (repeated measures ANOVA, $p < 0.05$; Tukey *post hoc* test, $p < 0.05$ for both comparisons).

Cue-evoked dopamine release in the NAc shell does not encode future costs

Histological examination revealed that four additional electrode placements were located in the shell subregion of the NAc (**Fig. 3.6a**). These locations covered a similar rostro-caudal extent as NAc core locations, but were located more ventrally and more medially. At these sites, cue presentation also evoked significant increases in dopamine concentration over baseline levels (repeated measures ANOVAs, $p < 0.05$ for each trial type; **Fig. 3.6b**). However, unlike data recorded in the NAc core, there was no cost-related difference in peak cue-evoked dopamine responses in the NAc shell ($F_{2,6} = 0.04$, $p = 0.95$; **Fig. 3.6c**). Thus, low and high cost cues (as well as the presentation of both cues on choice trials) evoked the same increase in NAc shell dopamine concentration. Similar to data obtained from the NAc core, there were also no significant increases in NAc shell dopamine concentration upon lever extension or reward delivery (repeated measures ANOVAs, $p > 0.05$ for each trial type). There were also no differences in peak dopamine concentration following either event across trial types (repeated measures ANOVA, $p > 0.05$ for each comparison). Finally, there was no difference in the behavioral performance between animals during core and shell recording sessions (*t*-test comparisons for choice allocation, number of errors, number of rewards, all p's > .10), indicating that differences in dopamine release patterns between these regions could not be explained by altered patterns of behavior.

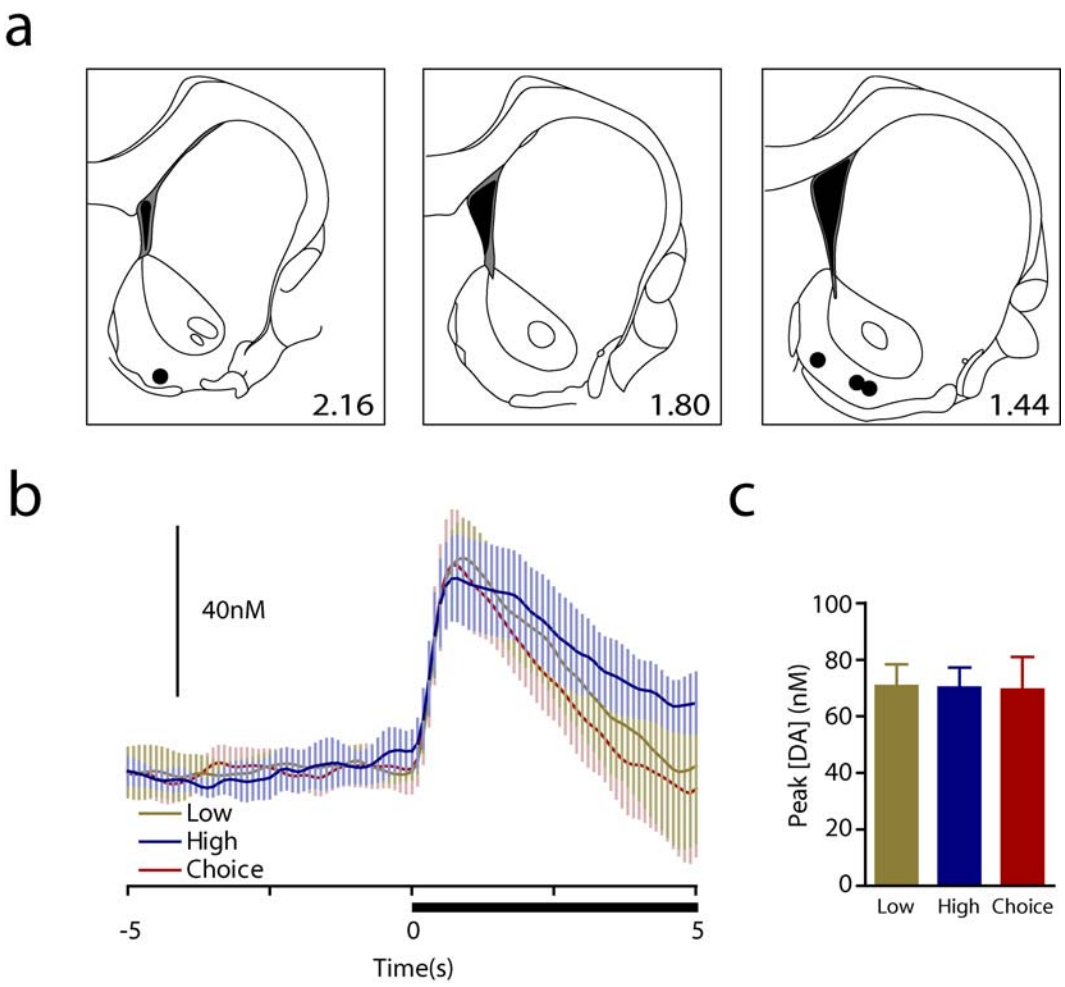

Figure 3.6. Cue-evoked dopamine release in the NAc shell. (**a**) Coronal diagrams illustrating confirmed location of recording sites. Conventions follow Figure 3-5a. (**b**) Mean (solid lines) ± SEM (shaded lines) changes in dopamine concentration for each trial type, relative to cue onset (black bar, time zero). All cues evoked significant increases in dopamine concentration (repeated measures ANOVAs, $p < 0.05$). (**c**) Average peak cue-evoked dopamine signal (± SEM) across trial type. There were no differences in the magnitude of dopamine released in response to low cost, high cost, and choice cues (repeated measures ANOVA, $p > 0.05$).

DISCUSSION

Dopamine neurons in the VTA and substantia nigra encode a reward prediction error signal in which cues that predict rewards evoke phasic increases in firing rate, whereas fully expected rewards do not alter dopamine activity (Schultz et al., 1997). This cue-evoked signal is also sensitive to a number of features of the upcoming reward. Thus, cues which predict rewards that are larger, immediate, or more probable elicit larger spikes in dopamine neuron activity than cues which predict rewards that are smaller, delayed, or less probable (Fiorillo et al., 2003; Tobler et al., 2005; Roesch et al., 2007; Fiorillo et al., 2008). This signal has been hypothesized to contribute to reward-based decision making in a number of ways (Morris et al., 2006; Roesch et al., 2007). However, no studies have investigated whether effort-based information is encoded by this signal, even though multiple studies have implicated dopamine in cost-related decision making (Salamone et al., 2007). Further, none of these studies examined dopamine release directly in target regions, where dopamine is known to have different roles in behavior (Di Chiara, 2002).

In the present study, dopamine release was recorded directly at terminal regions while animals performed an effort-based choice task. Importantly, this task allowed us to assess whether independent cues that predicted rewards with different costs affected patterns of dopamine release. Furthermore, free choice trials allowed direct examination of how NAc dopamine may contribute to decision making. The results suggest that cue-evoked dopamine signals in the NAc core (but not shell) are sensitive to the future costs of rewards. Increases in dopamine concentration within the NAc core were observed upon the presentation of discriminative stimuli that signaled the opportunity to respond for a reward that came at low costs (FR1 schedule of reinforcement) or high costs (FR16 schedule of reinforcement).

However, there were significant differences in the magnitude of dopamine release evoked by these cues. Specifically, cues that signaled low cost rewards evoked greater increases in dopamine concentration than cues that signaled high cost rewards. These results are consistent with evidence that NAc manipulations alter effort-based decision making (Salamone et al., 1991; Salamone et al., 1994; Salamone et al., 2007), and suggest that information about the costs of impending rewards is integrated with reward-prediction signals in the NAc core.

Phasic dopamine release in the NAc core has been proposed to play the crucial role of acting as a cost-benefit calculator to determine the overall utility of available behavioral options (Phillips et al., 2007). Conceptually, such measures of utility would include the costs that animals must pay to obtain available rewards, whether those costs come in the form of increased energy expenditure or longer wait times (opportunity costs). Rewards that come at high costs would therefore come with a lower perceived utility, leading animals away from them. However, in order to be advantageous, information about reward utility must be prospective (i.e., it must be available before a choice is made). In the present task, cues presented to animals signaled not only which option would be rewarded, but also how much rewards would cost. This information was available before response options were presented, allowing us to dissociate dopamine release produced by instructive cues from dopamine release produced by responses or rewards. Animals revealed behavioral preferences for low cost rewards on free choice trials, confirming that the information provided by cues was useful in guiding behavior towards rewards with higher utility. We found that on these choice trials, cue-evoked dopamine release was highly similar to cue-evoked dopamine release on forced low cost trials, suggesting that although the actual choice had not yet been made,

dopamine release was either 1) signaling the better of the two options, or 2) reflecting the intention of the animal to choose the low cost option. Therefore, these results suggest that phasic cue-evoked dopamine release within the NAc core may indeed play an important role in signaling the utility of available options, and that such information may be used to either facilitate or strengthen choices that involve the same reward but lower costs.

Importantly, this effect was not observed in the NAc shell, demonstrating that dopamine transmission of cost-related information is site-specific. Although all cues evoked dopamine release in the shell, there were no differences in dopamine concentration on low cost, high cost, or choice trials. These results indicate that reward prediction is signaled in the NAc shell independently of reward cost. Furthermore, the difference between the core and shell suggests that these regions may have different roles in weighing effort-related decisions. Consistent with this idea, dopamine depletions that do not include part of the NAc core are ineffective at altering choice allocation on an effort-based task (Sokolowski and Salamone, 1998). Moreover, although the effect of shell-specific NAc lesions have not been investigated, recent evidence have revealed that lesions of the NAc core alone reduce high-cost choices on a two-arm maze (Hauber and Sommer, 2009). It is not presently clear whether the core-shell differences in dopamine release observed here are purely attributable to differences in the population of dopamine neurons that project to these structures (Ikemoto, 2007) or differences in terminal regulation of release patterns (Cragg and Rice, 2004; Cragg, 2006).

Although reward-paired discriminative cues evoked increases in NAc dopamine in both the core and shell subregions in the present study, we saw no changes in dopamine release when animals initiated responses to obtain rewards or when rewards were delivered.

This result was somewhat striking and unexpected given that previous studies have found robust increases in subsecond NAc dopamine concentration relative to individual operant responses for both food, cocaine, and intracranial stimulation rewards (Phillips et al., 2003a; Roitman et al., 2004; Stuber et al., 2004; Stuber et al., 2005; Cheer et al., 2007a). However, it is important to note that in most of these studies, cues that may have been used to guide behavior (such as cue light presentation or lever extension) also produced their own robust increases in dopamine concentration. Furthermore, in these studies animals were typically trained for a very short time (generally ~300 total trials) before recordings were made, whereas in the present study animals received ~2800 trials before recordings were made. Therefore, one intriguing possibility is that as operant responses become automated with extended practice, response-related changes in phasic NAc dopamine are no longer observed, while cue-evoked increases are left intact. Future studies will be required to determine exactly how prolonged training affects multiple parameters of dopamine release across brain regions.

The role of NAc dopamine in effort-based decision making has received much attention, with a number of studies revealing two related yet dissociable deficits following dopamine depletion or antagonism in the NAc. First, in fixed choice tasks in which animals can only gain reinforcement on one response lever, dopamine blockade produces robust decreases in response rates, even when reinforcement rates are held constant (Aberman et al., 1998; Aberman and Salamone, 1999; Salamone et al., 2003). Interestingly, the decrease in response rate is linearly related to the baseline response rate, with schedules that support higher response rates being the most sensitive to dopamine depletion (Salamone et al., 2003). Microdialysis investigations have also found that dopamine levels in both the core and shell

are positively correlated with operant response rates but not with reward rates (McCullough et al., 1993b; Sokolowski et al., 1998; Cousins et al., 1999). Taken together, these findings suggest that increases in dopamine may be acting to as an "activator" to help animals overcome particularly high costs to obtain rewards (Salamone and Correa, 2002; Salamone et al., 2003). However, given that operant responses in the present task were not associated with phasic increases in dopamine levels, it is possible that the rate-decreasing effects of NAc dopamine depletions operate through another aspect of dopaminergic transmission. A candidate mechanism is tonic release of dopamine , which has been used successfully in free-operant models of behavior to explain how NAc dopamine depletions could impact response vigor and response rate (Niv et al., 2007). One possibility is that tonic dopamine levels increase before or during the behavioral session, and that these changes serve to prime or enable reward seeking, especially when it is attended by high costs. Indirect support for this idea comes from the finding that response rates can be decreased by antagonism of D_2 receptors (Salamone et al., 1991; Denk et al., 2005), which are typically high affinity and therefore should be susceptible to small changes in tonic extracellular dopamine concentration (Richfield et al., 1986; Richfield et al., 1989). Here, dopamine changes were recorded with a differential, background subtracted technique, making it hard to determine whether tonic concentration changed over the behavioral session.

A second observation from studies of dopamine depletion or antagonism is that in tasks that allow animals to choose between multiple sources of reinforcement that come with different costs, dopamine manipulation alters the relative allocation of responses. In one study, rats were trained to perform on a T-maze task in which one arm of the maze contained a large food reward that was blocked by a barrier, and the other arm contained a lesser

reward but no barrier (Cousins et al., 1996). Under normal circumstances, rats chose to climb the barrier to obtain the larger reward. However, following dopamine depletion in the NAc, animals changed their preference to the lesser reward that was easier to obtain. Although these results at first indicate that dopamine is necessary for animals to overcome high costs, further tests indicated that this was not the case. Thus, when the no-barrier arm did not contain food, even dopamine depleted rats were able to climb the barrier to obtain food. These and other results indicate that NAc dopamine depletion specifically reduces the relative allocation of behavior towards response options that require high costs (Cousins and Salamone, 1994; Salamone et al., 1994; Cousins et al., 1996; Salamone et al., 2003). Importantly, such effects do not seem to be due to impaired reward processing or decreased reward sensitivity, as NAc dopamine depletion does not change positive hedonic reactions to rewarding stimuli, and mice that completely lack dopamine still exhibit normal reward preferences (Cannon and Palmiter, 2003; Berridge, 2006). The results are consistent with the idea that NAc dopamine is involved in choices between two rewarding alternatives that differ in their degree of effort.

Emerging evidence suggests that effort-based decision making is regulated by a complex brain circuit, which includes the anterior cingulate cortex (ACC), basolateral amygdala (BLA), NAc core, and dopamine release within the NAc core (Floresco and Ghods-Sharifi, 2007; Floresco et al., 2007; Phillips et al., 2007; Salamone et al., 2007; Bezzina et al., 2008b; Hauber and Sommer, 2009). Lesions of the ACC or disconnection of the ACC and NAc core disrupt effort-based choice behavior, leading animals to choose lesser rewards that cost less (Rudebeck et al., 2006; Hauber and Sommer, 2009). Likewise, BLA inactivation or disconnection of the BLA and ACC induces similar behavioral deficits,

biasing animals away from high cost options (Floresco and Ghods-Sharifi, 2007). These findings suggest that the serial transfer of information between these structures is critical for normal effort-based decision making.

Precisely why dopamine disruption in the NAc alters choice behavior on effort-related tasks remains a question for open investigation. However, the difference in cue-evoked NAc core dopamine signals on high and low cost trials may indicate one substrate for dopamine's role in effort-based decision making. Dopamine release is thought to modulate synaptic plasticity through a number of mechanisms within the NAc (Nicola et al., 2000; Kauer and Malenka, 2007), determining which glutamatergic inputs drive NAc output. Thus, cue-evoked release of dopamine would presumably engage synaptic plasticity mechanisms to strengthen coincidently active glutamatergic inputs onto NAc neurons, which provide sensory, context, and outcome specific information related to those cues (Shidara and Richmond, 2002; Saddoris et al., 2005; Schoenbaum and Roesch, 2005; Ambroggi et al., 2008; Lapish et al., 2008). Likewise, cues that evoke greater release of dopamine, such as those that predict lower-cost rewards, would facilitate certain inputs, allowing them to exhibit enhanced control over NAc output and motivated behavior, biasing animals towards the options they represent. This idea is supported by evidence that interrupting NAc dopamine transmission alters neuronal responses and disrupts behavioral responses to reward-paired cues (Di Ciano et al., 2001; Yun et al., 2004a; Yun et al., 2004b; Cheer et al., 2005), and that striatal neurons encode the action value of future choices (Samejima et al., 2005).

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CHAPTER 4

NUCLEUS ACCUMBENS NEURONS ENCODE BOTH PREDICTED AND EXPENDED RESPONSE COSTS DURING EFFORT-BASED DECISION MAKING

ABSTRACT

Efficient decision making requires that animals consider both the benefits and costs of potential actions. The nucleus accumbens (NAc) has been implicated in the ability to choose between options with different costs and overcome high costs when necessary, but it is not clear how NAc processing contributes to this role. Here, NAc neuronal activity was monitored using multi-neuron electrophysiology during an effort-based choice task. After initial training on a continuous schedule of reinforcement, rats were placed on a multiple schedule task in which distinct 5s visual cues predicted low cost (FR1) or high cost (FR16) lever press requirements for a sucrose rewards in separate trials. Additionally, in other trials, both cues were presented simultaneously, allowing a choice between low and high cost options. On choice trials the low cost option was selected on over 85% of trials by the end of training, demonstrating that animals could discriminate between cues to produce nearly optimal choice behavior. Electrophysiological analysis indicated that a subgroup of NAc neurons (41 of 110 cells; 37%) exhibited phasic increases in firing rate during cue presentations. For nearly one-third of these cells, the degree of phasic activity was sensitive to the amount of effort predicted, with significantly greater cue-evoked increases in firing rate occurring on low cost trials than on high cost trials. In contrast, other subgroups exhibited either increases (15 of 110 cells; 13.6%) or decreases (24 of 110 cells; 21.8%) in

firing rate preceding the onset of behavioral responses. Remarkably, these changes in firing rate were sustained until response requirements were met, thereby encoding differences in the amount of effort expended. Finally, neurons that were excited during reward delivery exhibited larger activations when high response costs preceded the reward. These findings are consistent with previous reports that implicate NAc function in reward prediction and the allocation of response effort during reward-seeking behavior, and suggest a mechanism by which NAc activity contributes to decision making and overcoming high response costs.

INTRODUCTION

Obtaining food and other rewards often requires organisms to invest considerable resources such as time and the expenditure of energy. Recent evidence suggests that the NAc is part of a brain circuit that mediates the ability of organisms to overcome very large costs to obtain rewards, and to choose between rewards that come at different costs. Although animals typically prefer larger rewards, and will work harder for them, NAc lesions produce an abrupt shift in behavior, as animals reallocate behavior towards easier response options that pay smaller rewards (Hauber and Sommer, 2009). Likewise, animals with NAc core lesions reach lower break points on progressive ratio schedules of reinforcement, but exhibit no change in sensitivity to reinforcement (Bezzina et al., 2008b). Dopamine antagonism or depletion in the NAc produces a similar deficit, as animals continue to respond on an FR1 schedule of reinforcement, but will no longer complete larger effort requirements (e.g., an FR16) for the same reward (Aberman et al., 1998; Aberman and Salamone, 1999). These observations suggest that normal processing within the NAc is necessary for animals to overcome large response costs to obtain rewards.

Previous studies from this and other laboratories demonstrate that NAc neurons encode operant responding for food and other reinforcers as well as cues that predict rewards (Carelli, 2002b; Nicola et al., 2004a, b; Taha and Fields, 2005, 2006; Taha et al., 2007). The NAc receives and integrates information from other brain nuclei (such as the basolateral amygdala and anterior cingulate cortex) that have been implicated in effort-based decision making (Walton et al., 2002; Rudebeck et al., 2006; Floresco and Ghods-Sharifi, 2007; Hauber and Sommer, 2009) and projects directly to motor output structures (Zahm, 2000). Therefore, the NAc (and the activity of NAc neurons) represents a candidate site for the

storage or application of cost-related information. The previous study demonstrated that phasic dopamine release in the NAc core (but not shell) encoded the difference in response costs predicted by reward-paired cues. However, no studies to date have investigated the response of NAc neurons when increased constraints are imposed on food-seeking behaviors. Furthermore, it is unknown how NAc cell firing is altered by cues that predict such constraints. In this study, rats were trained to lever-press for sucrose rewards presented in the same effort-related decision making task described in chapter 3. Electrophysiological data were collected during the performance of this task to assess whether NAc neurons encode the amount of effort required to obtain a reinforcer and exhibit different responses to discriminative cues that specifically predict reward cost.

METHODS

Animals Male, Sprague Dawley rats (n=12, Harlan Sprague Dawley, Indianapolis, IN) aged 90-120 d and weighing 260-350 gm were used as subjects and individually housed with a 12:12 light: dark cycle. All experiments were conducted between 9:00 am and 5:00 pm. Bodyweights were maintained at no less than 85% of pre-experimental levels by food restriction (10-15 gm of Purina laboratory chow each day, in addition to approximately 1 gm of sucrose consumed during daily sessions). This regimen was in place for the duration of behavioral testing, except during the post-operative recovery period when food was given *ad libitum*. All procedures were approved by the Institutional Animal Care and Use Committee.

Behavioral training Lever pressing behavior in all rats was initially reinforced on a continuous schedule of reinforcement on two levers, such that every response on either lever will result in the delivery of a 45mg sucrose pellet to a centrally located food receptacle. A maximum of 100 reinforcers (50 per lever) were available per session (with 1 session per day). After stable responding developed (5 sessions), rats were transferred to the same multiple schedule task described in chapter three (see Fig. 3.1). Again, reinforcement was contingent on operant responses in 90 discrete trials. Each trial was initiated randomly after a variable time interval, with an average of 20s between trials. In this task, distinct cue lights (located above two response levers) were illuminated for 5s before lever extension to signal which lever was active (i.e., which lever produced reinforcement). Response levers were available for 15s unless response requirements were completed, in which case the levers were retracted and the reward was delivered. On 60 forced-choice trials, one cue was presented alone and only a response on the corresponding lever was reinforced. On these trials, responses made on the uncued lever (termed “errors”) resulted in the termination of the

houselight for the remainder of the trial period and the absence of sucrose delivery for that trial. The number of errors served as a behavioral measure of discrimination between low and high cost cues. On another 30 free-choice trials, both cues were presented simultaneously, allowing a choice between both options. For the first 11 days of training, the response cost of each option was identical (an FR1 schedule of reinforcement). In order to produce an effort disparity between response options, the required fixed ratio on one lever (termed the "high cost" option) was gradually increased from 1 to 16 according to the following schedule: Sessions 1-11, FR1; Session 12, FR2; Session 13, FR4; Sessions 14-16, FR8; Sessions 17-20, FR12; Sessions 21-25, FR16. The fixed ratio on the other lever (termed the "low cost" option) remained the same throughout training. Choice behavior on free-choice trials served as a measure of an animal's overall sensitivity to changes in the work-related response costs of available options. In this task, work-related response costs are minimized by selecting the low-cost option on the 30 choice trials. Similarly, reinforcement is maximized by overcoming high costs when required on forced-choice trials. Following 25 training sessions, all rats were prepared for electrophysiological recording in the NAc as described below. After recovery, rats underwent additional training sessions until behavior was stable (usually 3-5 sessions).

Surgery Animals were anesthetized with ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (20 mg/kg) and microelectrode arrays were implanted with the NAc, using established procedures (Carelli et al., 2000). Electrodes were custom-designed and purchased from a commercial source (NB Labs, Dennison, TX). Each array consisted of eight microwires (50 μm diameter) arranged in a 2x4 bundle that measured ~1.5 mm anteroposterior and ~.75 mm mediolateral. Arrays were targeted for permanent, bilateral

placement in the core and shell subregions of the NAc (AP, +1.3-1.8 mm; ML, ±0.8 or 1.3 mm; DV, -6.2 mm; all relative to bregma on a level skull, (Paxinos and Watson, 2005)). Ground wires for each array were coiled around skull screws and placed 3-4mm into the ipsilateral side of the brain, ~5mm caudal to bregma. After implantation, both arrays were secured on the skull using surgical screws and dental cement. All animals were allowed at least 5 post-operative recovery days before being reintroduced to the behavioral task.

Electrophysiological Recordings Electrophysiological procedures have been described in detail previously (Carelli et al., 2000; Carelli, 2002a; Hollander and Carelli, 2005). Briefly, before the start of the recording session, the subject was connected to a flexible recording cable attached to a commutator (Crist Instruments) that allowed virtually unrestrained movement within the chamber. The headstage of each recording cable contained 16 miniature unity-gain field effect transistors. NAc activity was recorded differentially between each active and the inactive (reference) electrode from the permanently implanted microwires. The inactive electrode was examined before the start of the session to verify the absence of neuronal spike activity and served as the differential electrode for other electrodes with cell activity. Online isolation and discrimination of neuronal activity was accomplished using a neurophysiological system commercially available (multichannel acquisition processor, MAP System, SIG board filtering, 250 Hz to 8 kHz; sampling rate, 40 kHz, Plexon, Inc., Dallas, TX). Another computer controlled behavioral events of the experiment (Med Associates Inc., St. Albans,VT) and sent digital outputs corresponding to each event to the MAP box to be time stamped along with the neural data. Principle component analysis (PCA) of continuously recorded waveforms was performed prior to each session and aided in the separation of multiple neuronal signals from the same electrode. This analysis generates a

projection of waveform clusters in a three-dimensional space, enabling manual selection of individual waveforms. Before the session, an individual template made up of many "sampled" waveforms was created for each cell isolated using PCA. During the behavioral session, waveforms that "matched" this template were collected as the same neuron. Cell recognition and sorting was finalized after the experiment using the Offline Sorter program (Plexon, Inc., Dallas, TX), when neuronal data were further assessed based on PCA of the waveforms, cell firing characteristics, autocorrelograms, cross-correlograms, and interspike interval distributions. Units with excessively low or sporadic firing rates over the course of the behavioral session were identified by computing the coefficient of variation (CoV = σ/μ). If the variance in a given cell's firing rate was more than three times the mean firing rate, (i.e., the CoV was greater than 3), the cell was excluded from further analysis. The CoV was used as it is highly susceptible to instability in firing rate across time, which makes accurate assessment and discrimination of phasic activity across trials nearly impossible. Additionally, units that exhibited pre-event mean firing rates exceeding 10 Hz were considered unlikely to be medium spiny neurons and were excluded from analysis (Berke, 2008).

Determining phasic response patterns of NAc neurons Statistical analysis of spike-train data collected during behavioral sessions had two main goals. First, we sought to identify neurons that exhibited increased or decreased activity in response to three relevant behavioral events: cue presentation, lever press responses, and reward delivery. Secondly, we sought to determine whether such response patterns were sensitive to differences in cost. Each analysis is described in detail below.

Changes in neuronal firing patterns relative to behavioral events were analyzed by constructing peri-event histograms and raster displays (bin width, 250ms) surrounding each event using commercially available software (Neuroexplorer, Plexon, Inc). For this analysis, a cell could exhibit a change in activity relative to cue onset (0 to 2.5s following cue presentation), prior to the initial lever press on a given trial (-2.5 to 0s before the response), or following reward delivery (0 to 2.5s after response completion/reward delivery). Individual units were categorized as either excitatory or inhibitory during one of these epochs if the firing rate was greater than or less than the 99.9% confidence interval (CI) projected from the baseline period (10s before cue onset) for at least one 250ms time bin. This stringent CI was selected such that only robust responses were categorized as excitatory or inhibitory. Some neurons in this analysis exhibited low baseline firing rates, and the 99.9% CI included zero. Where this was the case, inhibitions were assigned if $e_0 > 2b_0$ (where e_0 = the number of consecutive 0 spikes/s time bins during the event epoch and b_0 = the maximal number of consecutive 0 spikes/s time bins during the baseline period). Units that exhibited both excitations and inhibitions within the same epoch were classified by the response that was most proximal to the event in question, unless the most proximal response was ongoing when the event occurred (e.g., during reward delivery). Importantly, the above analysis was completed separately for both low and high cost trial types to determine how many neurons responded to each cue, lever press initiation, and reward. However, the resultant categories of neuronal response profiles were not mutually exclusive. Thus, a neuron could potentially exhibit an excitation to the low-cost cue and an inhibition to the low cost reward, or an inhibition to both the low cost cue and the high cost cue. Neuronal responses were classified as "specific" if they exhibited a given response on one trial type but not another. The

duration of a neuronal response to a specific event was determined by computing the onset of the response (first time bin in which firing rate crossed the 99.9% CI) and the offset of the response (first time bin in which cell firing returned to non-significant levels). For responses that persisted across time yet were sporadic (i.e., non-consecutive), the offset was considered to be the first time bin where the response returned to non-significant levels for at least 1s.

Cost-sensitive neurons were identified by comparing the firing rate of event-responsive neurons on low cost and high cost trials. Neurons were categorized as cost-sensitive when the firing rate during a given epoch of the low-cost trial differed significantly from the firing rate during the same epoch of a high-cost trial (differences assessed using Wilcoxon rank-sum test on data 2.5s following the event (cues and rewards) or before the event (initial lever press)). Comparisons of response durations and peaks across trial type within subpopulations of neurons were performed using paired *t*-tests (for comparisons between two trial types) or repeated measures ANOVA with Tukey *post-hoc* tests (for comparisons between three trial types). Differences in the frequency or proportion of neuronal responses across different trial types or subregions were examined using Fisher's exact test. All analyses were considered significant at $\alpha = 0.05$. For population activity graphs, the firing rate of each cell was normalized by a Z-score transformation (using baseline mean and standard deviation) to reduce the potential influence of baseline differences in this analysis.

Behavioral Data Analysis All behavioral events (cue onset and offset, lever presses, lever extension/retraction, and reward delivery) occurring during training and electrophysiological recording were recorded and available for analysis. Analysis of behavioral data collected during training sessions included examination of overall response

rates and allocation, latency to initiate and complete response requirements, number of reinforcers obtained, number of errors committed, and preference between the low and high costs options on choice trials. Effects of training on total reinforcement and number of errors committed were assessed using a repeated measures ANOVA that tested for a linear trend between session number and the dependent variable. Effects of response cost on choice allocation were evaluated using a two-way repeated measures ANOVA of average choice probability as a function of cost, with Bonferroni post-hoc tests used to correct for multiple comparisons between low and high cost choice probability. Response times on high and low trials during the recording session were compared using paired *t*-tests. All analyses were considered significant at $\alpha = 0.05$. Statistical and graphical analyses were performed using Graphpad Prism and Instat (Graphpad Software, Inc).

Histology Upon completion of the experiment, rats were deeply anesthetized with a ketamine and xylazine mixture (100 mg/kg and 20 mg/kg, respectively). In order to mark the placement of electrode tips, a 13.5μA current was passed through each microwire electrode for 5 seconds. Transcardial perfusions were then performed using physiological saline and a 10% formalin mixture containing potassium ferricyanide, which reveals a blue dot reaction product corresponding to the location of each electrode tip. Brains are then removed, post-fixed using a 10% formalin solution, and frozen. Successive 50 μm coronal brain sections extending from the rostral to caudal extent of the NAc are then mounted on microscope slides. The specific position of individual electrodes was assessed by visual examination of successive coronal sections. Placement of an electrode tip within the NAc core or shell was determined by examining the relative position of observable reaction product to visual landmarks (including the anterior commissure and the lateral ventricles) and anatomical

organization of the NAc represented in a stereotaxic atlas (Paxinos and Watson, 2005). Differences in the prevalence of neuronal responses across the core and shell of the NAc were examined using Fisher's exact test. All analyses were considered significant at $\alpha = 0.05$.

RESULTS

Behavior during the effort-based decision task

Animals (n=12) received 25 training sessions on the effort-based choice task before being bilaterally implanted with a chronic microelectrode bundle in the NAc. Similar to results obtained in animals performing the same task in the chapter 3 (see Fig. 3.2), multiple behavioral measures indicated that animals successfully acquired the task and could discriminate between cues to guide behavior, overcome large response costs when necessary, and allocate behavior appropriately on choice trials to avoid high costs (**Fig. 4.1**). During initial training, animals distributed responses evenly across levers on forced-choice trials (**Fig. 4.1a**). However, as the fixed-ratio was increased on the high-cost option (beginning with session 12), rats exhibited increased response output on high cost trials to match the requirements. By the end of training (final pre-surgery session), animals emitted 436 ± 20 (mean ± SEM) responses on high cost trials while responding only 29.8 ± 0.1 times on forced low cost trials. Despite this difference, animals still completed 89% of forced high cost trials, demonstrating the ability to overcome high costs to maximize reinforcement. The total number of reinforcers obtained in each session remained near the maximal possible level across training and did not change (test for linear trend, $p > 0.05$; **Fig. 4.1b**). Conversely, the number of errors committed decreased with training (test for linear trend, $F_{1,322} = 26.42$, $p < 0.001$; **Fig. 4.1c**), demonstrating that the animals used the cues to guide ongoing behavior and select the response option that would be rewarded. However, on choice trials, when both cues were presented and animals were free to respond on either option, behavioral allocation changed as a function of imposed cost ($F_{6,77} = 14.19$, $p < 0.001$; **Fig. 4.1d,e**). Thus, early in training when the options presented no difference in cost (sessions 1-

11), animals chose each option equally (Bonferroni *post hoc* test, $p > .05$). However, as the response cost was gradually increased for the high-cost option, animals demonstrated a significant behavioral preference for the low-cost option, choosing it more frequently. This preference was present at all comparisons after the 4:1 high:low cost ratio, including the recording day ($p < .05$ for all comparisons). Thus, animals avoided paying high costs when possible by selecting low-cost options. There was no significant difference on any behavioral metric (total reinforcers, total errors, choice probability) between performance levels attained by the end of training and performance during electrophysiological recording session (all p's > 0.05). Analysis of responding during the electrophysiological recording session revealed a significant main effect of trial type on response latency, or the time between lever presentation and initial lever press (paired *t*-test, $t = 3.964$, $df = 11$, $p = 0.002$). This effect was attributable to shorter response latencies on low-cost trials as compared to high cost trials (low cost, 0.40 ± 0.05s; high cost, 1.18 ± 0.18s). There was no difference in response latency for low cost trials and choice trials in which the low cost option was selected ($p > 0.05$). After the initial response on high cost trials, animals required an additional 5.05 ± 0.48s to complete the FR16 requirement.

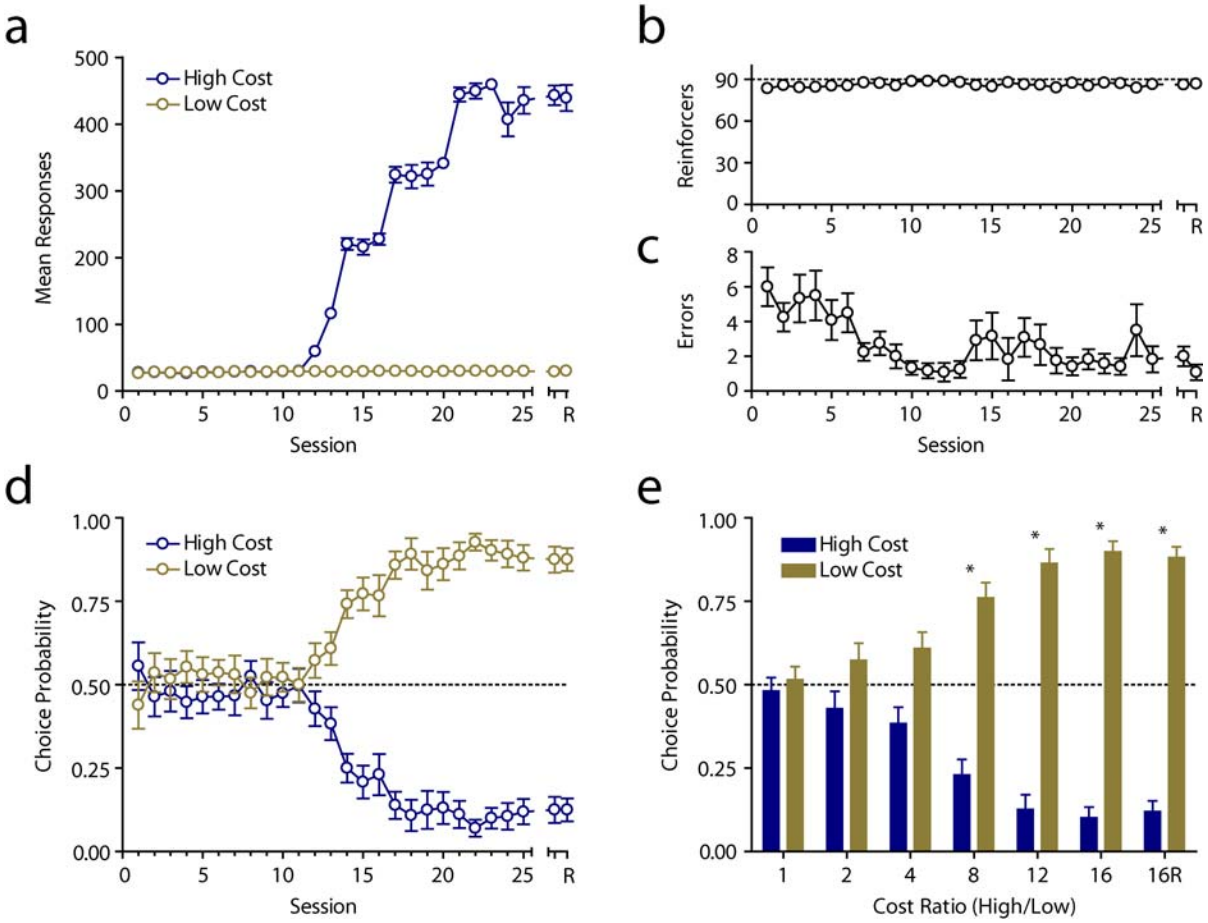

Figure 4.1. Behavior during the effort-based choice task. (**a**) Mean responses on forced choice trials. Response output (mean ± SEM) increased as response requirements were raised on high choice trials, beginning with session 12. Fixed ratio requirements on high cost trials were increased to FR2 (session 12), FR4 (session 13), FR8 (sessions 14-16), FR12 (sessions 17-20), and FR16 (remaining sessions, including recording session, R). Response requirements on low cost trials were not altered. (**b**) Total reinforcers across training sessions (mean ± SEM). Reinforcers obtained were near maximal levels across training, including the recording session. Dashed line indicates maximal number of reinforcers available. (**c**) Total errors across sessions (mean ± SEM). Errors decreased as training progressed ($p < 0.001$), indicating animals could discriminate between cues. (**d**) Choice probability as a function of session (choice trials only). Dashed line indicates behavioral indifference point (chance selection). When given a choice, animals initially exhibited little preference. As response requirements were increased for the high cost option, animals began to select the low cost option. (**e**) Choice probability as a function of the ratio between lever presses required on high cost and low cost trials. Dashed line indicates indifference point. Choice allocation shifted as a function of response cost (two-way repeated measures ANOVA, $p < 0.05$). Asterisks indicate ratios at which preference for the low-cost option was significant (Bonferroni *post hoc* tests, $p < 0.05$). 16R denotes choice preference during the recording session.

Overview of NAc firing patterns during behavioral task

A total of 110 individual NAc neurons were recorded from 12 rats during performance of the effort-based choice task. Of these, 98 (89.1%) exhibited significant

modulation in firing rate during at least one task event. Seventy-nine neurons (71.8%) exhibited changes in firing rate during cue presentation, 77 (70%) exhibited changes preceding the initial lever press on low or high cost trials, and 92 (83.6%) exhibited changes during response requirement completion/reward delivery. A more detailed description of each response type is presented below.

Cue-evoked activity in a subset of NAc neurons is modulated by predicted cost

Previous studies indicate that a substantial number of NAc neurons exhibit phasic changes in activity during presentation of reward-paired cues, whether those cues signal reward itself or the opportunity to respond for a reward (Nicola et al., 2004b; Roitman et al., 2005; Day et al., 2006; Ambroggi et al., 2008). Consistent with these results, we observed that presentation of reward-paired discriminative stimuli evoked changes in firing rate in the majority of NAc neurons recorded (79 of 110, 71.8%). Of these, 41 (51.9%) were marked by significant increases in firing rate on at least one trial type (see **Fig. 4.2a** for a characteristic example). The majority of these neurons exhibited significant increases in activity during the presentation of both low and high cost cues (**Fig. 4.2b**). As a population, these activations were not significantly different on low cost, high cost, and choice trials in either peak or average cue-related activity (repeated measures ANOVA; $p > .05$ for both comparisons; **Fig. 4.2c,d**).

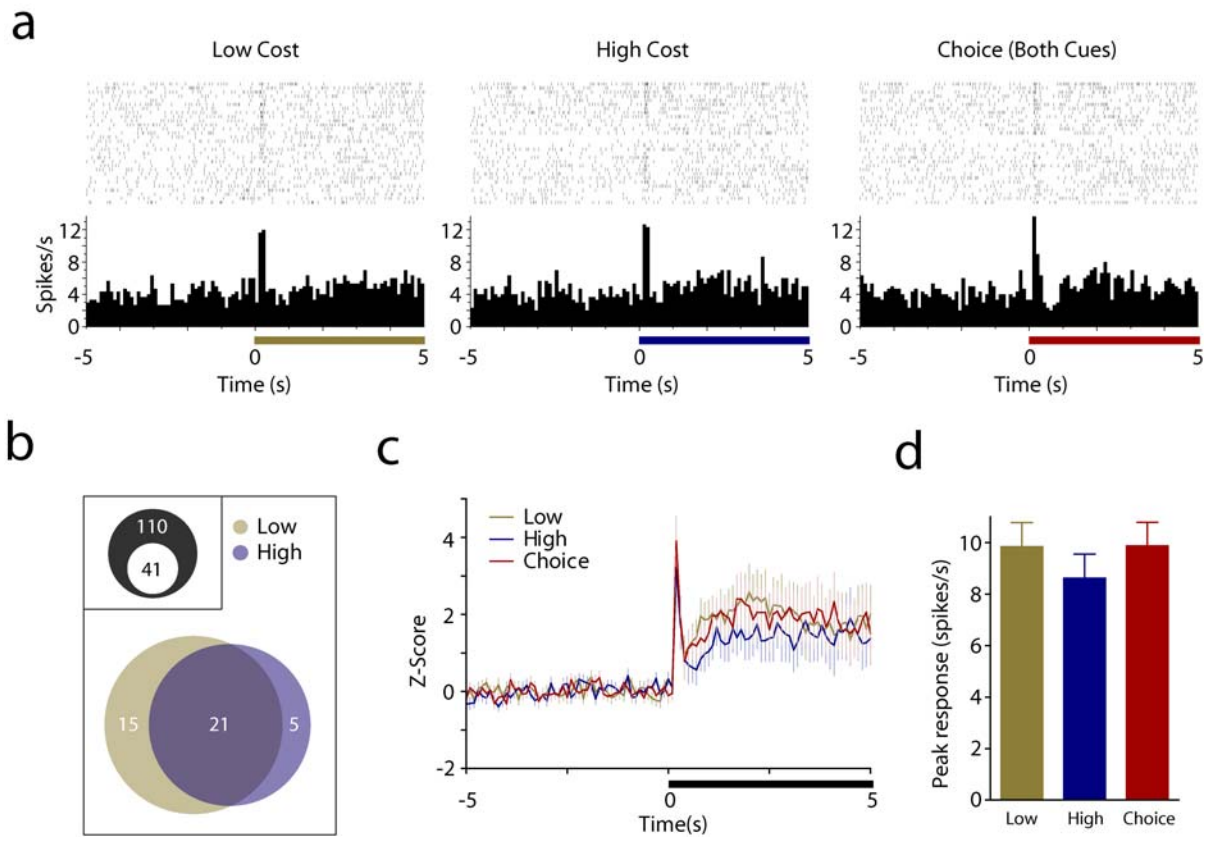

Figure 4.2. Discriminative stimuli activate a subset of NAc neurons. (**a**) Representative NAc neuron exhibiting a cue-evoked increase in firing rate. Left panel, raster plot (top) and peri-event histogram (PEH; bottom) aligned to onset of low cost cue (gold bar). Center panel, raster plot and PEH aligned to high cost cue (blue bar). Right panel, raster plot and PEH aligned to onset of choice trials (presentation of both cues). This neuron was equally excited by all cues. (**b**) Venn diagrams illustrating the distribution of cue-evoked excitations. Inset, 41 of 110 cells were excited by low cost or high cost cues. Of these, 36 were excited by the low cost cue, and 26 were excited by the high cost cue. Twenty-one neurons were excited by both cues. (**c**) Mean Z-score (± SEM) of neural activity for neurons excited by cues on either trial type. (**d**) Peak cue-evoked activity (± SEM) for all neurons across trial type. There was no significant difference in cue-evoked excitation (repeated measures ANOVA, $p > 0.05$).

Although the amplitude of excitations on low and high cost trials did not differ across the population, further examination revealed that a substantial portion of these neurons (20 of 41, 48.8%) exhibited cue-specific responses (i.e., changes that were present on only one trial type). Critically, a significantly higher proportion of these cue-specific neurons were responsive to the low-cost cue but not the high cost cue (**Fig. 4.2b**; Fisher's exact test, $p = .019$). Moreover, comparison of peri-event histograms aligned to cue onset across trial types

indicated that many cue-evoked excitations were modulated by predicted cost, with greater activation to low cost cues than high cost cues (see **Fig. 4.3a,b** for specific examples). A more detailed analysis of all cue excitatory cells revealed that a number of neurons (17 of 41; 41.5%) exhibited significant differences in firing rate following the presentation of low and high cost cues. Of these, the significant majority were found to be selective for the low cost cue (**Fig. 4.3c**; low cost selective, 13 of 41, 31.7%; high cost selective, 4 of 41, 9.8%; Fisher's exact test, $p = .027$). As a class, these low-cost selective neurons exhibited a significantly greater peak response and greater overall activation to cue presentation on low cost and choice trials as compared to high cost trials (repeated measures ANOVA; peak activity comparison: $F_{2,38} = 10.81$, $p < 0.001$; mean activity comparison: $F_{2,38} = 26.28$, $p < 0.001$ **Fig. 4.3d,e**). Importantly, there were no differences in the peak or average activity evoked by low cost cues and dual cue presentation on choice trials ($p > 0.05$ for both post-hoc comparisons), suggesting that these excitations encode information related to the relative costs of each option irrespective of choice situation. Thus, while the population of cue-evoked excitations in NAc neurons seemingly signal reward prediction alone (and provide no information on the costs of future rewards), a unique subset of neurons appear to exhibit activity that is preferential for low-cost options.

Cue-evoked inhibitions do not reflect predicted response cost

A total of 38 neurons (34.5%) exhibited significant decreases in firing rate upon cue presentation (data not shown). Overall, this population exhibited no difference in degree of inhibition across low cost, high cost, and choice trials (repeated measures ANOVA for mean inhibition; $F_{2,113} = 0.10$, $p = 0.9$). Interestingly, the majority of cue inhibitions (24 of 38, 63.2%) were trial-type specific. However, unlike specific cue-evoked excitations, that

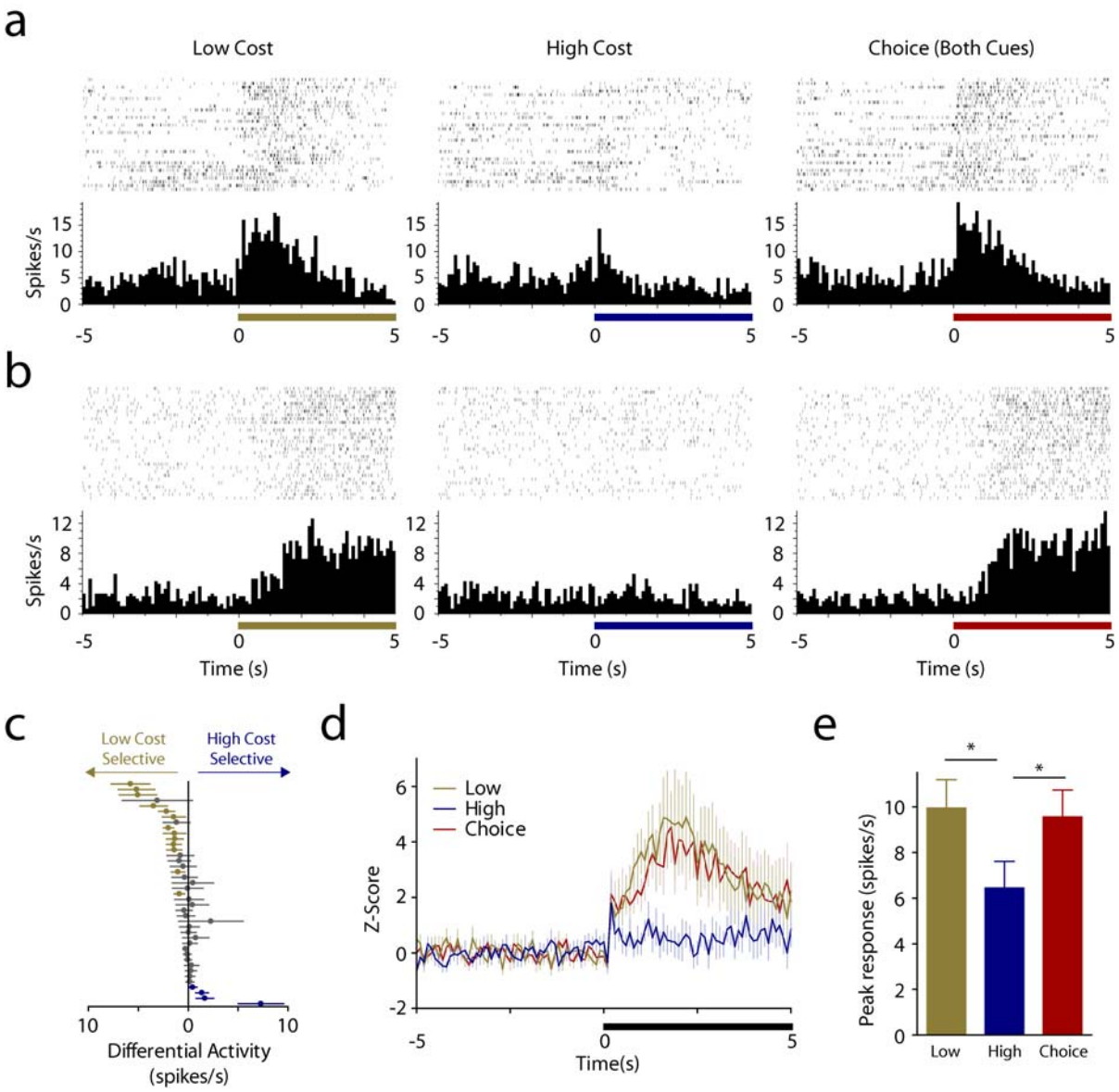

Figure 4.3. A subset of cue-evoked excitations reflect predicted response cost. (**a,b**) Raster plots and PEHs from representative NAc neurons that exhibited greater activity on low cost and choice trials than high cost trials. (**c**) Differential activity across population of cue-excited cells. Points represents difference in activity (± 95% confidence interval) between high and low cost trials for each neuron. Leftward placement indicates greater activity on low cost trials, rightward placement indicates greater activity on high cost trials. Confidence intervals that do not cross zero (gold or blue data points) indicate significant cue-selective activity. A significantly greater number of neurons were selective for the low cost cue (Fisher's exact test, $p < 0.05$). (**d**) Mean (SEM) Z-score for low-cost selective neurons, aligned to cue onset (black bar, time zero). (**e**) Peak cue-evoked activity of low-cost selective neurons was significantly greater on low cost and choice trials than on high cost trials (Tukey *post-hoc* comparisons, $p < 0.05$).

favored low-cost cues, specific cue-related inhibitions were equally distributed across low and high cost trials (n=12 for each; Fisher's exact test, p = 1.0). Likewise, although 12

neurons were found to be selective for a certain trial type (Wilcoxon test, all p's < 0.05), selectivity was equally distributed across trial types (Fisher's exact test, p = 0.75). Such selectivity has been reported previously for a discriminative stimulus task in which animals must make right or left movements to obtain rewards (Taha et al., 2007). However, as these responses were not meaningfully modulated by response cost, they are not considered further.

Response-related changes in NAc activity are maintained until reward delivery

Previous examinations of NAc function during goal-directed behavior have reported both phasic excitations and inhibitions immediately preceding operant responses for rewards (Carelli and Deadwyler, 1994; Carelli et al., 2000; Ghitza et al., 2004; Nicola et al., 2004a; Taha and Fields, 2006). Consistent with these results, we found that 77 of 110 (70%) neurons recorded during the effort based choice task exhibited significant alterations in firing rate within the seconds preceding the lever press (on low cost trials) or the onset of lever pressing (high cost trials). Of these, 31 of 77 (40.3%) were characterized by increases in firing rate (**Fig.4.4a,b**), whereas the majority (46 of 77, or 59.7%) displayed decreases in firing rate (**Fig 4.5a,b**). Previous studies have suggested that a significant portion of neurons that exhibit responses during reward-directed behavior are selective for the direction of movement (Taha et al., 2007). In the present study, we found that a large percentage of response-related changes in activity were specific for one trial type (16 of 31 or 51.6% of response-related excitations, **Fig. 4.4b**; 22 of 46 or 47.8% of response-related inhibitions, **Fig. 4.5b**). However, the distribution of these response-selective cells did not differ based on response cost (Fisher's exact test, p > 0.8 for both comparisons). Therefore, neuronal activations or depressions which were response-specific were excluded from group analyses.

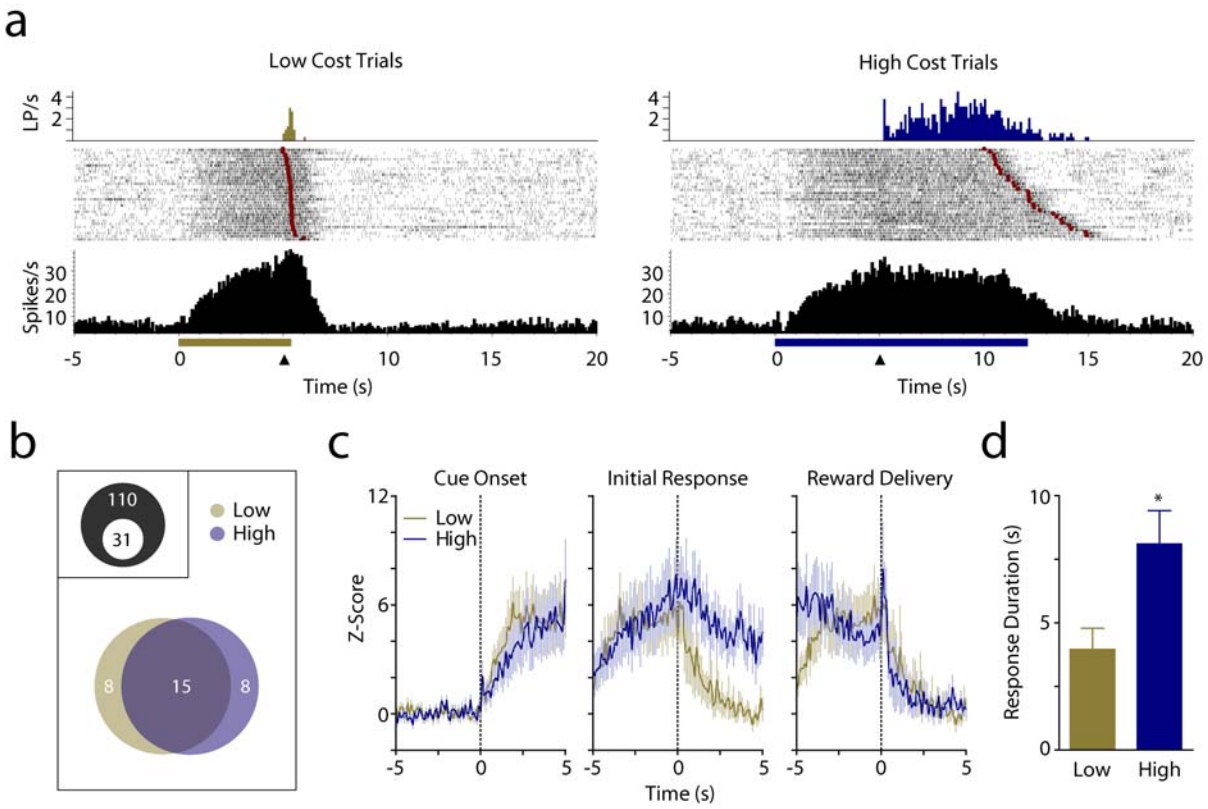

Figure 4.4. Response-activated NAc neurons. (**a**) Top panel, PEH of lever presses on low and high cost trials. Middle and bottom panels, raster plots and PEHs from representative NAc neuron exhibiting a pre-response excitation on both low and high cost trials. For both, data are aligned to cue onset, and the black triangle denotes lever extension (at 5s). Trials in raster plots are sorted based on the latency between lever extension and reward delivery (red circles). (**b**) Venn diagrams illustrating distribution of response activated NAc neurons for low and high cost trials. Inset, 31 of 110 neurons exhibited increased activity preceding the initial level press on low or high cost trials. Of these, 15 were excited before both responses, whereas 16 were specific to trial type. (**c**) Mean (± SEM) Z-score of 15 neurons that were excited before the initial response on both trials. Data are aligned to cue onset (left panel), the initial response (center panel), and reward delivery (right panel). (**d**) Duration of excitation for response-activated neurons from (c). Excitations were longer on high cost trials than on low cost trials ($p < 0.05$).

Of the remaining cells, we found that changes in activity which began during the pre-response period exhibited no differences in mean or peak activity on low cost and high cost trials (repeated measures ANOVA, $p > 0.05$ for response excitatory and response inhibitory cells on both comparisons). However, these cells typically exhibited lasting changes in altered firing rate, even after the initial response was made. Of 15 neurons that were excited preceding the initial response on both trials, 14 (93%) were characterized by long-duration

activations (defined as significant increase in firing rate for 1s or more) for at least one trial type. Likewise, all 24 neurons that were inhibited preceding operant responses were characterized by long durations on at least one trial type. Interestingly, changes in activity that occurred leading up to the initial response persisted while animals completed the response requirements on high cost trials. Thus, neurons that became activated preceding the initial response on both options exhibited an increased firing rate until the response requirement was completed and the reward was delivered (**Fig. 4.4c**). This maintained firing rate was evident in two ways. First, even though animals took an average 5.05 ± 0.48s (mean ± SEM) to complete response requirements on high cost trials after the initial lever press, these cells still exhibited increased activity over baseline in the time epoch (2.5s) immediately preceding reward delivery (*t*-test, t=3.089, df = 14, p = 0.008). Secondly, these neurons exhibited significantly longer duration responses than those observed on low-cost trials (*t*-test, t=3.77, df = 14, p = 0.002; **Fig. 4.4d**). Likewise, cells that became inhibited during the pre-response period continued this inhibition until reward delivery on high cost trials (**Fig. 4.5c**). Similar to response-related excitations, this was evident in both a decreased firing rate (as compared to baseline) for these cells during the time epoch immediately preceding high-cost reward delivery (*t*-test, t=6.919, df = 23, p < 0.0001), and also in a prolonged response duration on high cost trials as compared to low cost trials (*t*-test, t=2.549, df = 23, p = 0.018; **Fig. 4.5d**).

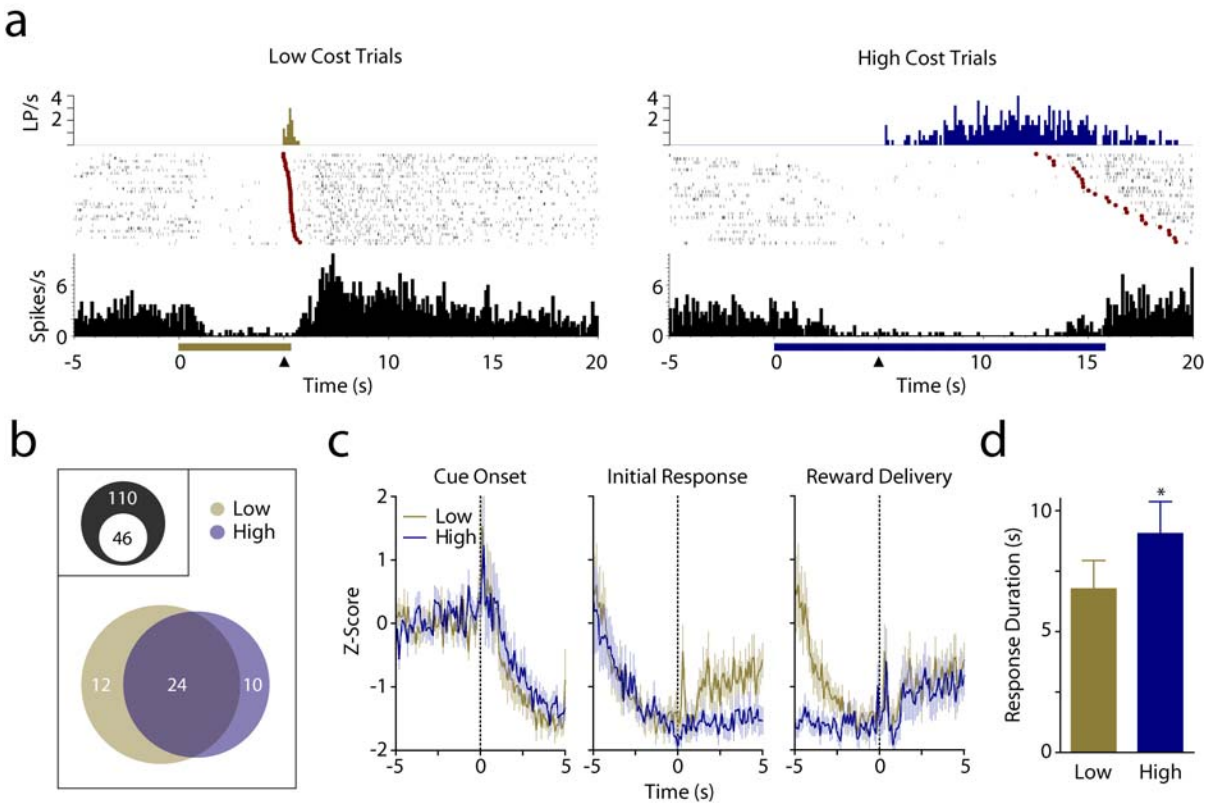

Figure 4.5. Response-inhibited NAc neurons. (**a**) Top panel, PEH of lever presses on low and high cost trials. Middle and bottom panels, raster plots and PEHs from representative response-inhibited NAc neuron on low and high cost trials. For both, data are aligned to cue onset, and the black triangle denotes lever extension (at 5s). Trials in raster plots are sorted based on the latency between lever extension and reward delivery (red circles). (**b**) Venn diagrams illustrating distribution of response inhibited NAc neurons for low and high cost trials. Inset, 46 of 110 neurons exhibited decreased activity preceding the initial level press on low or high cost trials. Of these, 24 exhibited inhibitions preceding both responses, whereas 22 were specific to trial type. (**c**) Mean (± SEM) Z-score of 24 neurons that were inhibited before the initial response on both trials. Data are aligned to cue onset (left panel), the initial response (center panel), and reward delivery (right panel). (**d**) Duration of inhibition for response-inhibited neurons from (c). Inhibitions were longer on high cost trials than on low cost trials ($p < 0.05$).

Reward-related changes in NAc neuronal activity

The vast majority of NAc neurons recorded here (92 of 110, 83.6%) exhibited a phasic change in activity during the time epoch following response completion/ reward delivery. Of these, excitations (45 of 92, 48.9%; **Fig. 4.6**) and inhibitions (47 of 92, 51.1%; data not shown) were equally prevalent. Previous reports indicate that reward-evoked increases in NAc cell firing occur independently of previous behavioral actions (Schultz et

al., 2000), yet are related to the palatability of the reinforcer, with greater activations observed when rewards are more palatable (Taha and Fields, 2005). Here, reward-related excitations were often specific to trial type, with 15 of 45 (33%) neurons specifically responding to high cost rewards and 9 of 45 (20%) neurons specifically responding to low-cost rewards. There was no significant difference in the distribution of specific responses according to preceding cost (Fisher's exact test, $p = 0.23$). In the overall population of reward excited cells, there was a small yet significant difference in the response magnitude (peak) between trial types, with greater activation occurring in response to rewards that were preceded by higher costs (*t*-test, $t = 3.4$, $df = 44$, $p = 0.001$; **Fig. 4.6c,d**). The majority (28 of

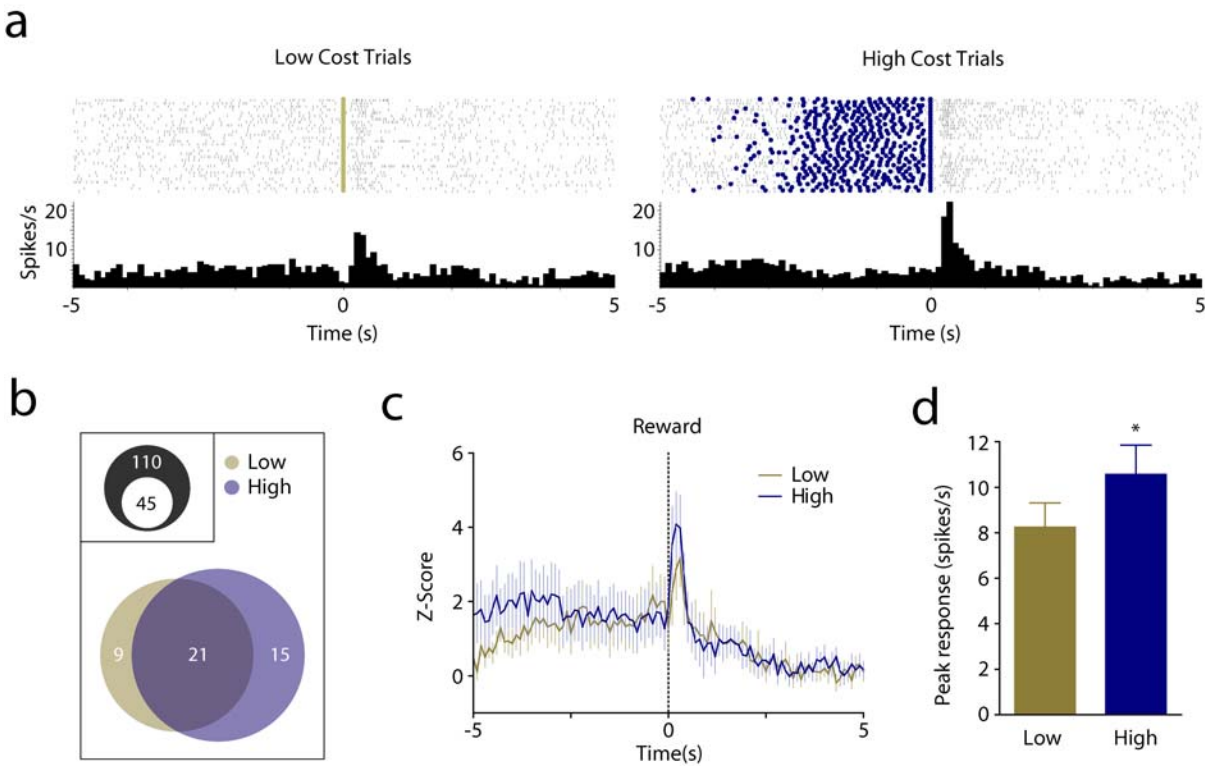

Figure 4.6. Reward-related activation of NAc neurons. (**a**) Raster plots and PEHs from representative NAc neuron on high and low cost trials. Data are aligned to response completion/reward delivery. Gold and blue circles in raster plot indicate low cost and high cost lever presses, respectively. (**b**) Venn diagrams illustrating distribution of reward activated NAc neurons for low and high cost trials. Inset, 45 of 110 neurons exhibited increased activity following reward delivery on low or high cost trials. Of these, 21 exhibited excitations for rewards on either trial type, whereas 24 were specific to trial type. (**c**) Mean (± SEM) Z-score of neural activity for all neurons that were activated by either reward. Data are aligned to reward delivery. (**d**) Peak (± SEM) activity for all reward-excited neurons. Rewards that were preceded by higher costs evoked greater increases in activity than rewards that were preceded by low costs ($p < 0.05$).

46, 61%) of reward-evoked inhibitions in neuronal activity occurred regardless of trial type. In the overall population, there were no significant difference in degree of inhibition between high and low cost trials (comparison of mean response, $t = 0.4$, $df = 46$, $p = 0.68$). Likewise, there was no difference in the proportion of neurons that responded specifically or selectively to the low cost or high cost reward (Fisher's exact test, $p > 0.05$).

Electrode placement

A total of 192 microelectrodes (16 per animal) were implanted bilaterally and aimed at the nucleus accumbens. Histological verification of electrode placements confirmed that 55 neurons were recorded from 41 electrodes located in the NAc core, whereas 55 neurons were recorded from 42 electrodes located in the NAc shell. Across animals, electrode placements ranged from 0.84 - 2.96mm anterior to bregma, 0.6 - 2.05mm lateral to the midline, and 6.8 - 8.3mm ventral from the brain surface. The precise placement of marked electrode tips in the NAc are shown in **Figure 4.7**. Data from electrodes located outside the NAc were excluded from analysis. There was no difference in the distribution of any response type between the core and shell of the NAc (Fisher's exact test on response frequencies across region, $p > 0.05$ for all comparisons).

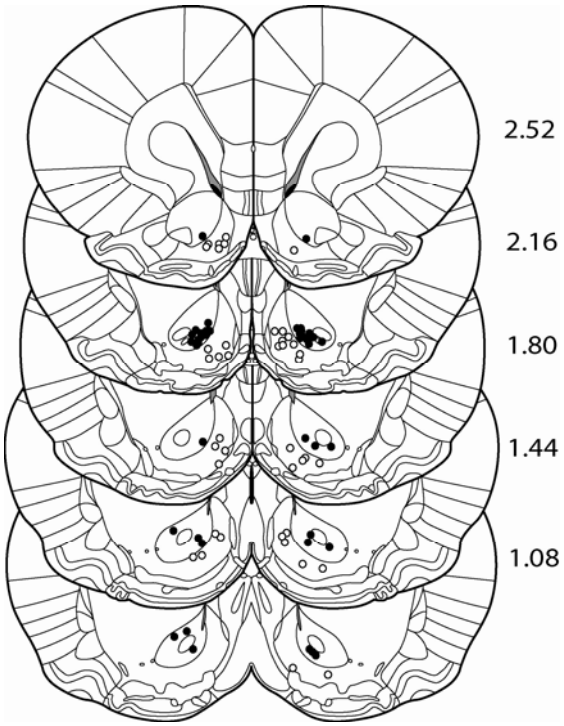

Figure 4.7. Successive coronal diagrams illustrating anatomical distribution of electrode locations across core and shell of the NAc. Marked locations are limited to electrodes that contributed to data presented here. Filled circles indicate electrode location in the NAc core, open circles indicate electrode locations in the NAc shell. Numbers to the right of each diagram indicate anteroposterior coordinates rostral to bregma (in mm).

DISCUSSION

The NAc has been implicated in a wide range of reward-related functions, including responding to reward-paired incentive cues and decision making. The present experiment used electrophysiological techniques to record the activity of NAc neurons during the effort-based choice task used in chapter 3. Consistent with those results, animals exhibited behavioral preferences for response options with lower costs on choice trials, demonstrating sensitivity to effort differences. Neurophysiological results reveal that NAc neurons exhibit phasic patterns of activity (both excitations and inhibitions) relative to all aspects of the task, including cue presentations, operant responses, and reward delivery. However, specific components of these responses were sensitive to effort requirements. First, a portion of cue-evoked excitations exhibited greater activation on low cost trials than high cost trials, even before responses were performed. Second, two classes of response-related phasic responses were also modulated by cost. In these cells, changes in activity began prior to the response, but when higher costs were required to obtain rewards, these responses were sustained until response completion. Finally, neurons that exhibited excitations upon reward delivery responded with larger excitations when greater levels of effort preceded the reward. These response patterns reveal that the NAc encodes information about costs in three unique ways, and are consistent with the hypothesis that the NAc is involved in effort-based decision making or selection of appropriate actions after decision making processes have been engaged (Nowend et al., 2001; Salamone and Correa, 2002; Salamone et al., 2007; Hauber and Sommer, 2009).

Cues that predict rewards (conditioned stimuli) or precede the opportunity to respond for rewards (discriminative stimuli) have the ability to redirect ongoing behavior and

facilitate reward acquisition by speeding reaction times (Konorski, 1967; Brown and Bowman, 1995; Ikemoto and Panksepp, 1999). Numerous electrophysiological investigations of NAc function indicate that NAc neurons are responsive to both conditioned and discriminative stimuli (Ghitza et al., 2003; Ghitza et al., 2004; Nicola et al., 2004b; Roitman et al., 2005; Wilson and Bowman, 2005; Day et al., 2006; Wan and Peoples, 2006; Wheeler et al., 2008). This responsivity appears to be determined by the relationship between such cues and the future reward, as they are usually either specific (i.e., responsive only to reward paired cues) or selective (i.e., responsive to both reward paired and unpaired cues, but exhibit larger responses to cues that predict rewards) (Nicola et al., 2004b; Day et al., 2006). Moreover, cue responses in many striatal neurons are sensitive to the magnitude and identity of the predicted reward, with greater activations occurring for cues that predict larger or more preferred rewards (Hassani et al., 2001; Cromwell and Schultz, 2003; Cromwell et al., 2005).

In the present task, both low and high cost discriminative stimuli signaled the opportunity to respond for an identical reward volume, although one signaled that more effort was required. On choice trials, animals revealed a preference for the option that signaled less effort, demonstrating that the cues were being used to guide behavior. Not surprisingly, a large subset of NAc neurons was activated by the presentation of discriminative cues. As a population these responses were not different on low effort, high effort, and choice trials. However, a subpopulation of cue-responsive cells appeared to encode the difference between cost requirements by exhibiting greater activity on low cost trials than on high cost trials. Moreover, although both cues were presented on choice trials, the response of these cells reflected the preferred low cost option. Thus, the magnitude of cue responses in these neurons was not determined solely by the final outcome. Rather, the activity of these neurons

appears to signal that the less costly option is available, even before the animal selects that option. Such activity is consistent with the idea that this class of NAc cue responses encode the relative identity and value of future rewards (Hassani et al., 2001; Cromwell and Schultz, 2003; Cromwell et al., 2005; Samejima et al., 2005; Wilson and Bowman, 2005).

Previous investigations using electrophysiological recordings and/or pharmacological inactivation have revealed that NAc dopamine is required for both neuronal and behavioral responses to reward paired cues (Yun et al., 2004b; Nicola et al., 2005). These studies suggest a potential link between the cue-evoked excitations reported here and the phasic dopamine responses reported in the previous chapter (Aim 2). Phasic dopamine release likely activates D_1 dopamine receptors on medium spiny neurons in the NAc, which can potentiate synaptic strength in an NMDA dependent manner (Pennartz et al., 1993; Pawlak and Kerr, 2008; Shen et al., 2008). As discussed in chapter 3, different levels of D_1 receptor activation (arising from different concentrations of dopamine release) could lead to the relative strengthening of glutamatergic inputs that carry information about one cue or response option, allowing those inputs to selectively drive NAc output. In the present case, cues that signal low cost options also produce greater dopamine release in the NAc core and greater activity in a subgroup of NAc neurons. Although such activity may not be required for appropriate responses when only one option is available, it is possible that this coincident pattern of neuronal activity and dopamine release is integral to choice situations, such as those presented in the current task. Consistent with this idea, previous studies have also reported that striatal neurons encode information about reward value, which is also encoded by dopamine neurons by way of larger magnitude responses (Cromwell and Schultz, 2003; Samejima et al., 2005; Tobler et al., 2005).

In addition to its role in responding to reward paired cues, the NAc has been implicated in goal-directed behavior in general (Pennartz et al., 1994; Ikemoto and Panksepp, 1999; Wise, 2004). Particularly relevant to the present design, a host of studies suggest that the NAc plays a key role in permitting and/or instructing behavioral responses when large amounts of effort are required. Thus, NAc lesions, dopamine depletion in the NAc, and adenosine agonism in the NAc have all been found to decrease choices that involve high response costs but superior rewards in a two-choice task (Cousins et al., 1996; Font et al., 2008; Hauber and Sommer, 2009). The present study found that two different response patterns reflected the level of effort exerted on each trial type. The first consisted of neurons that became excited during the period prior to responding and remained activated until requirements were complete. On low cost trials, this resulted in a relatively short duration of activity. However, on high cost trials, the same neurons remained active over a longer period of time, as animals were required to perform 16 responses to obtain rewards. Such responses may have multiple behavioral functions. One interpretation of this activity is that it reflects response anticipation and contributes to the performance of specific responses over others (Pennartz et al., 1994; Chang et al., 1996; Taha et al., 2007). Indeed, one theory of NAc function suggests that competing responses are encoded by groups of NAc neurons, and that one action is ultimately performed when one group 'wins out' over another (Pennartz et al., 1994; Nicola, 2007). The result is not only influence over downstream motor structures, but mutual inhibition of competing neuronal networks within the NAc. The observation that activations in the present study typically began before responses were made is consistent with this view. Moreover, a number of neurons were responsive specifically before the execution of responses on low or high cost trials, suggesting that they encoded unique

actions. However, such explanations would not explain why activations were often present during both trial types, or why many activations persisted after responding was initiated on one option. Another possibility is that this activity reflects the expectation that action sequences will be reinforced (Cromwell and Schultz, 2003). This type of activity could act as a memory trace that works to keep motivational goals in a state where they can influence behavior. Consistent with this view, such responses are rarely observed when animals must make movements that do not lead to rewards (Hollerman et al., 1998). Deficits in such processing, induced by manipulations in the NAc, would therefore lead to an impaired ability to maintain a representation of action values over time and across large workloads, making animals less likely to overcome high effort requirements to obtain rewards and more likely to choose smaller rewards that come at lesser costs.

A second group of NAc neurons reflected patterns of motivated behavior by exhibiting inhibitions preceding responses and maintaining those inhibitions until reward delivery. Again, this led to relatively shorter duration inhibitions on low cost trials than on high cost trials. Previous studies have also reported inhibitions among a subset of NAc neurons during goal-directed behavior (Taha and Fields, 2006). Similar to the present results, that study found that such inhibitions typically preceded the onset of reward-seeking behavior and continued through reward consumption. Considering the cellular composition and circuitry of the NAc, these types of responses are proposed to have a role in permissively 'gating' actions that lead to rewards, irrespective of the specific action (Roitman et al., 2005; Taha and Fields, 2006; Taha et al., 2007). The majority of NAc neurons are GABAergic projection neurons that should inhibit target neurons under baseline conditions. However, when NAc neurons undergo decreases in firing rate, such activity would be associated with

disinhibition of target structures. Since two major output nuclei of the NAc are the ventral pallidum and lateral hypothalamus (both of which play a role in food consumption), such disinhibition could produce or help to maintain appetitive behavior. This hypothesis is consistent with pharmacological studies demonstrating that inhibition of the NAc produces neuronal excitation in the ventral pallidum and lateral hypothalamus and induces feeding behavior (Stratford and Kelley, 1997, 1999). It has also been speculated that the ability of intra-NAc dopamine agonism to increase response rates and break point on progressive ratio schedules is produced by inhibition of this class of neurons (Wyvell and Berridge, 2000; Zhang et al., 2003; Taha and Fields, 2006).

In addition to activations and depressions following cue onset and preceding responses, we found that a class of NAc neurons were activated upon reward delivery. Similar excitations have previously been reported in primates performing a go/no go task in which reward delivery (a squirt of juice to the monkey's mouth) were contingent upon either making the correct movement (go trials) or withholding a movement (no go trials) (Apicella et al., 1991). Importantly, these excitations were observed following both go and no go trials, indicating that they are not solely the result of movements that accompany or precede reward acquisition. Other studies have found that these activations are sensitive to the palatability of rewards, with more palatable rewards evoking greater increases in firing rate (Taha and Fields, 2005). In the present study, reward-related excitations were larger on high cost trials than on low cost trials, indicating that the cost required to obtain the reward may be encoded in the reward response. Thus, one interpretation of this result is that animals find rewards that come at higher costs more palatable. Unfortunately, we have no behavioral evidence of palatability, and therefore cannot confirm or refute this idea. However, another more likely

scenario is that the exact timing of reward delivery on high cost trials was less predictable than on low cost trials, and that the unexpected nature of reward delivery evoked greater activity in these neurons. Consistent with this idea, fMRI BOLD signals in the human NAc are higher when rewards are delivered unpredictably than when they occur in an expected fashion (Berns et al., 2001).

The core and shell of the NAc are marked by dramatically different behavioral functions (Zahm, 1999; Di Chiara, 2002; Everitt and Robbins, 2005), and previous investigations have uncovered differences between these subregions in neural response profiles during reward-related tasks. Specifically, cue-responsive neurons are more prevalent in the NAc core than the shell, and more core neurons have been found to exhibit increases in activity prior to operant responses for cocaine reinforcement (Ghitza et al., 2003; Ghitza et al., 2004; Day et al., 2006; Ghitza et al., 2006). Differences in neuronal activity between NAc subregions are consistent with the differential roles of these structures in behavior (Parkinson et al., 1999; Di Chiara, 2002). However, in the current investigation, we found no differences in the distribution of any response type between the core and shell of the NAc. Although this is particularly puzzling given the core and shell differences in dopamine release reported in chapter 3, it is important to note that the bulk of neurophysiological investigations in the NAc have reported no core/shell differences (Carelli et al., 1993; Nicola et al., 2004a, b; Taha and Fields, 2005; Carelli and Wondolowski, 2006; Taha and Fields, 2006; Taha et al., 2007). Moreover, although these subregions receive different afferents (Zahm and Brog, 1992), the presence of direct connections between the core and shell indicate that they share information (van Dongen et al., 2005). Additionally, because the core and shell differ in efferent output, it is likely that the same types of activity have very different effects on downstream activity

(Zahm and Brog, 1992; Zahm and Heimer, 1993; Zahm, 1999). Therefore, unique activity within the NAc core and shell may not be necessary for these regions to contribute to different aspects of behavior.

Individual NAc neurons receive diverse cortical and subcortical inputs, and can carry a heavy information processing load (Kincaid et al., 1998; Zahm, 1999). A number structures that project to the NAc, including the anterior cingulate and orbitofrontal cortices and basolateral amygdala (BLA), are known to process reward-related information (Critchley and Rolls, 1996; Watanabe, 1996; Behrens et al., 2007; Belova et al., 2007; Doya, 2008; Tye et al., 2008). These inputs may be the basis for the ability of NAc neurons to distinguish cues that predict rewards from cues that don't, distinguish cues that signal different outcomes, and to become activated during periods of reward expectancy. Consistent with this idea, recent studies demonstrate that inactivation of the BLA or dorsomedial prefrontal cortex abolish excitatory NAc responses to reward paired cues (Ambroggi et al., 2008; Ishikawa et al., 2008a, b). Given that these areas already process information about rewards, it is unclear why proper NAc function is required for proper responding in decision making tasks. However, one possibility is that these inputs converge at NAc neurons, where the information they provide is integrated in order to promote selection of a single behavioral response over competing actions (Nicola, 2007). Alternatively, such information could serve to set a motivational threshold, and that NAc neurons operate to drive acquisition of rewards up to this threshold. Such processing would be consistent with the effects of NAc lesions (which presumably disrupt this signaling) in effort-based tasks (Bezzina et al., 2008b; Hauber and Sommer, 2009).

CHAPTER 5

NUCLEUS ACCUMBENS NEURONS ENCODE REWARD DELAYS DURING DELAY-BASED DECISION MAKING

ABSTRACT

Choosing between rewards that come at different delays is a fundamental component of decision making that is disrupted in multiple psychiatric disorders. The NAc is part of a distributed neural circuit that regulates such choice behavior and helps animals overcome long delays to obtain reinforcement. However, how neuronal processing within the NAc may contribute to delay-based decisions in poorly understood. Here, rats were trained to respond for both immediate and delayed rewards that were predicted by separate discriminative stimuli. Additionally, the task included choice trials, in which rats could choose between immediate and delayed rewards. After training, rats exhibited the ability to discriminate between cues to guide behavior and demonstrated a preference for immediate rewards on choice trials. NAc unit activity was measured using multi-neuron electrophysiological techniques during the performance of this task. Analysis revealed that NAc neurons exhibited phasic changes in firing rate during multiple components of the task, including cue presentation, response initiation, and reward delivery. However, the delay between responses and reward delivery was encoded specifically by two populations. A subpopulation of neurons (12 of 67; 17.9%) became inhibited preceding the operant response on both immediate and delayed reward trials, and this inhibition was prolonged on delayed reward trials, lasting until rewards were delivered. Another class of neurons (25 of 67, 37.3%)

exhibited progressively higher firing rates during the delay period s which peaked at reward delivery on delayed reward trials. These patterns of activity may reflect dissociable processes linked to accurately reflecting and overcoming reward delays, and are consistent with a role for the NAc in guiding delay-based decision making.

INTRODUCTION

Animals in natural environments often face decisions between rewards that are available at different temporal delays. When the rewards are identical, these decisions are simple: the animal chooses the one that is delivered sooner. This phenomenon, termed delay discounting, summarizes the observation that the subjective value of delayed rewards is discounted as compared to the same immediate reward (Rachlin, 1992; Green and Myerson, 2004; Rachlin, 2006). However, when available rewards differ in both delay and magnitude, animals must make trade-offs between two preferences – one for larger magnitude rewards and another for rewards at shorter delays. Such tradeoffs are at the center of decision making models, as they show to considerable individual variability, with some individuals greatly discounting delayed rewards, and others showing very little discounting (Green et al., 1996; Cardinal, 2006; Kable and Glimcher, 2007). Furthermore, studies of delay discounting may possess particular relevance for a number of disorders such as drug addiction and attention deficit disorder, which are often characterized in part by impulsivity, or a preference for small immediate rewards over delayed larger rewards (American Psychological Association, 2000; Green and Myerson, 2004; Cardinal, 2006). Therefore, understanding how neural systems encode and process information related to reward delays may provide insight into both normal and aberrant forms of decision making.

The NAc of both humans and other animals is responsive to rewards and cues that predict rewards (Breiter et al., 2001; Knutson et al., 2001a; Knutson et al., 2001b; Cromwell and Schultz, 2003; Cromwell et al., 2005; Knutson and Cooper, 2005; Day et al., 2006; Strohle et al., 2008) and has been heavily implicated in decision making processes for rewards that involve different temporal delays (Cardinal, 2006; Kable and Glimcher, 2007).

Thus, lesions to the NAc core impair instrumental learning when rewards are delayed (Cardinal and Cheung, 2005) and produce profound effects on delay-related decision making by biasing animals away from larger delayed rewards when smaller, immediate rewards are also available (Cardinal et al., 2001; Bezzina et al., 2007; Bezzina et al., 2008a). Previous studies indicate that neurons in the primate ventral striatum (including the NAc) become active during periods of reward anticipation, and that this activity increases as animals wait for rewards (Hollerman et al., 1998; Schultz et al., 2000). However, it is presently unclear whether delay-related information is encoded by NAc neurons during choice tasks.

Data presented in the previous section (chapter 4) suggests that NAc neurons encode different aspects of reward cost, including the amount of effort predicted by discriminative cues. However, because that task combined delay with effort (i.e., animals took longer to complete 16 responses versus 1), it is possible that the results were influenced by the delay between the onset of lever pressing and the reward. This experiment proposes to investigate NAc signaling using multi-unit electrophysiology during a delay-based decision task similar to the one used in chapter four. Here, rats will be trained to associate different discriminative stimuli with the availability of response options that produce either immediate or delayed rewards. Importantly, as these options differ only in reward delay (and not effort or reward magnitude), the results will also provide insight into how NAc signaling may contribute differently to decisions based on effort and reward delay.

METHODS

Animals Male, Sprague Dawley rats (n=9, Harlan Sprague Dawley, Indianapolis, IN) aged 90-120 d and weighing 260-350 gm were used as subjects and individually housed with a 12:12 light: dark cycle. All experiments were conducted between 9:00 am and 5:00 pm. Bodyweights were maintained at no less than 85% of pre-experimental levels by food restriction (10-15 gm of Purina laboratory chow each day, in addition to approximately 1 gm of sucrose consumed during daily sessions). This regimen was in place for the duration of behavioral testing, except during the post-operative recovery period when food was given *ad libitum*. All procedures were approved by the Institutional Animal Care and Use Committee.

Behavioral Procedures The behavioral design employed to investigate the effects of reward delay on NAc response patterns was similar to that used to investigate the effects of effort requirements in chapter four. Animals were first trained to press for sucrose rewards on a continuous schedule of reinforcement on two levers, such that every response on either lever resulted in the delivery of a 45mg sucrose pellet to a centrally located food receptacle (with no delay between responses and reinforcement). A maximum of 100 reinforcers (50 per lever) were available per session (with 1 session per day). After stable responding developed (5 sessions), rats were transferred to a multiple schedule task in which reinforcement was contingent on operant responses in 90 discrete trials. Importantly, each trial was initiated randomly after a variable time interval, with an average of 20s between trials. In this task, distinct cue lights (located above two response levers) were illuminated for 5s before lever extension to signal which lever was active (i.e., which lever produced reinforcement on an FR1 schedule). Response levers were available for 15s unless response requirements were completed, in which case the levers retracted and the reward was delivered. On 60 forced-

choice trials, one cue was presented alone and only a response on the corresponding lever was reinforced. On these trials, responses made on the uncued lever (termed "errors") resulted in the termination of the houselight for the remainder of the trial period and the absence of sucrose delivery for that trial. The number of errors served as a behavioral measure of discrimination between cues. On another 30 free-choice trials, both cues were presented simultaneously, allowing a choice between both options. For the first 11 days of training, the reward delay of each option was identical (0s; the reward was delivered immediately upon response completion). Thereafter, the reward delay for one option (termed the "delayed" option) was gradually increased from 0s to 4s according to the following schedule: Sessions 1-11, 0s; Session 12, 0.25s; Session 13, 0.5s; Sessions 14-16, 1s; Sessions 17-20, 2s; Sessions 21-25, 4s. The reward delay for the other option (termed the "immediate" option) remained at 0s throughout training (**Fig. 5.1**). Choice behavior on free-choice trials served as a measure of an animal's overall sensitivity to changes in reward delay associated with available options. Following 25 training sessions, all rats were prepared for electrophysiological recording in the NAc as described below. After recovery, rats underwent additional training sessions until behavior was stable. For 5 animals, reward delay on the delay option was extended to 8s during an additional five post-surgery training sessions. On the test day, the electrophysiological activity of NAc neurons was recorded in a single session during the delay-based decision making task.

Surgery Surgical procedures are identical to those described in chapter four (see chapter four, pages 95-96 for details). All animals were allowed at least 5 post-operative recovery days before being reintroduced to the behavioral task.

Electrophysiological Recordings Electrophysiological procedures were identical to those described in chapter four (see chapter four, pages 96-97 for details).

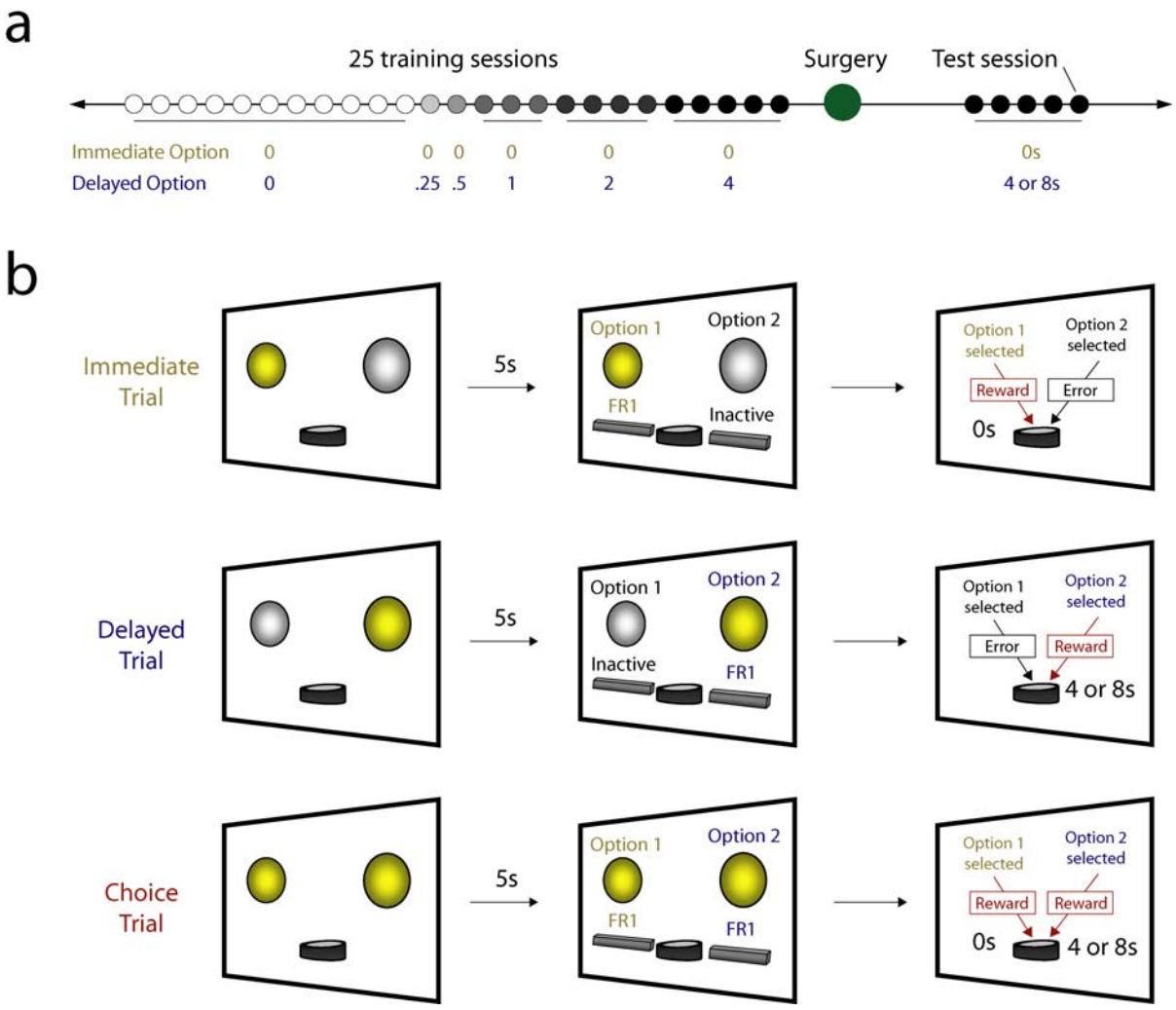

Figure 5.1. Experimental timeline and behavioral task. (**a**) Experimental timeline. Animals received 25 total training sessions before surgical implantation of guide cannula above the NAc (each circle = 1 session). Additional training sessions occurred after surgery until behavior was stable, and neuronal activity in the NAc was recorded during the task. Numbers below circles indicate the delay between the lever press (FR1 schedule for both levers) and reward on immediate reward and delayed reward trials. The delay was gradually increased on delayed reward trials across training. For 5 animals, the delay was increased to 8s after surgery. (**b**) Behavioral task during the recording session. On immediate reward trials (top panels), a cue light was presented for 5s and was followed by lever extension into the chamber. A single lever press on the corresponding lever led to reward delivery in a centrally located receptacle. Responding on the other lever did not produce reward delivery and terminated the trial. On delayed reward trials, the other cue light was presented for 5s before lever extension. Here, a lever press on the corresponding lever led to reward delivery 4 or 8s later. Responses on the immediate reward lever terminated the trial and no reward was delivered. On choice trials (lower panels), both cues were presented, and animals could select between immediate and delayed rewards.

Determining phasic response patterns of NAc neurons Analysis of neuronal responses was similar to that performed in chapter four. Here, we first sought to identify neurons that exhibited increased or decreased activity in response to three relevant behavioral events: cue presentation, lever press responses, and reward delivery. Secondly, we sought to determine whether such response patterns were sensitive to differences in reward delay. Each analysis is described in detail below.

Changes in neuronal firing patterns relative to behavioral events were analyzed by constructing peri-event histograms and raster displays (bin width, 250ms) surrounding each event using commercially available software (Neuroexplorer, Plexon, Inc). For this analysis, a cell could exhibit a change in activity relative to cue onset (0 to 2.5s following cue presentation), prior to the initial lever press on a given trial (-2.5 to 0s before the response), or following reward delivery (0 to 2.5s after reward delivery). Individual units were categorized as either excitatory or inhibitory during one of these epochs if the firing rate was greater than or less than the 99.9% confidence interval (CI) projected from the baseline period (10s before cue onset) for at least one 250ms time bin. This stringent CI was selected such that only robust responses were categorized as excitatory or inhibitory. Some neurons in this analysis exhibited low baseline firing rates, and the 99.9% CI included zero. Where this was the case, inhibitions were assigned if $e_0 > 2b_0$ (where e_0 = the number of consecutive 0 spikes/s time bins during the event epoch and b_0 = the maximal number of consecutive 0 spikes/s time bins during the baseline period). Units that exhibited both excitations and inhibitions within the same epoch were classified by the response that was most proximal to the event in question, unless the most proximal response was ongoing when the event occurred (e.g., during reward delivery). Importantly, the above analysis was completed

separately for both immediate and delayed reward trial types to determine how many neurons responded to each cue, lever press initiation, and reward. However, the resultant categories of neuronal response profiles were not mutually exclusive. Thus, a neuron could potentially exhibit an excitation to the no delay cue and an inhibition to the delay reward, or an inhibition to both the no delay cue and the delay cue. Neuronal responses were characterized as "specific" when the neuron responded with a change in firing rate during an event on one trial type but not the other trial type. The duration of a neuronal response to a specific event was determined by computing the onset of the response (first time bin in which cell firing crossed the 99.9% CI) and the offset of the response (first time bin in which cell firing returned to non-significant levels). For responses that persisted across time yet were sporadic (i.e., non-consecutive), the offset was considered to be the first time bin where the response returned to non-significant levels for at least 1s.

Delay-sensitive neurons were identified by comparing the firing rate of event-responsive neurons on immediate and delay trials. Neurons were categorized as delay-sensitive when the firing rate during a given epoch of the immediate reward trial differed significantly from the firing rate during the same epoch of a delayed reward trial (differences assessed using Wilcoxon rank-sum test on data 2.5s following the event (cues and rewards) or before the event (lever press)). Comparisons of response durations and peaks across trial type within subpopulations of neurons were performed using paired *t*-tests (for comparisons between two trial types) or repeated measures ANOVA with Tukey *post-hoc* tests (for comparisons between three trial types). Differences in the frequency or proportion of neuronal responses across different trial types or subregions were examined using Fisher's exact test. All analyses were considered significant at $\alpha = 0.05$. For population activity

graphs, the firing rate of each cell was normalized by a Z-score transformation (using baseline mean and standard deviation) to reduce the potential influence of baseline differences in this analysis.

Behavioral Data Analysis All behavioral events (cue onset and offset, lever presses, lever extension/retraction, and reward delivery) occurring during training and electrophysiological recording were recorded and available for analysis. Analysis of behavioral data collected during training sessions included examination of overall response rates and allocation, latency to initiate and complete response requirements, number of reinforcers obtained, number of errors committed, and preference between the delay and no delay options on choice trials. Effects of training on total reinforcement and number of errors committed were assessed using a repeated measures ANOVA that tested for a linear trend between session number and the dependent variable. Effects of reward delay on choice allocation were evaluated using a two-way repeated measures ANOVA of average choice probability as a function of delay, with Bonferroni post-hoc tests used to correct for multiple comparisons between delay and immediate choice probability. Response times on delay and immediate trials during the recording session were compared using paired two-tailed *t*-tests. All analyses were considered significant at $\alpha = 0.05$. Statistical and graphical analyses were performed using Graphpad Prism and Instat (Graphpad Software, Inc).

Histology Histological procedures were identical to those described in chapter four (see chapter four, pages 100-101 for details). Differences in the prevalence of neuronal responses across the core and shell of the NAc were examined using Fisher's exact test. All analyses were considered significant at $\alpha = 0.05$.

RESULTS

Behavior during the delay-based decision task

Animals (n=9) received 25 training sessions on the delay-based choice task before being bilaterally implanted with a chronic microelectrode bundle in the NAc. Multiple behavioral measures indicated that animals successfully acquired the task and could discriminate between cues to guide behavior, wait for rewards on delay trials, and allocate behavior appropriately on choice trials to avoid delays (**Fig. 5.2**). The total number of reinforcers obtained in each session increased significantly with training (test for linear trend, $F_{1,241} = 70.73$, $p < 0.001$; **Fig. 5.2a**), whereas the number of errors committed decreased with training (test for linear trend, $F_{1,241} = 65.92$, $p < 0.001$; **Fig. 5.2b**). Thus, animals used the cues to guide ongoing behavior and select the response option that would be rewarded on forced choice trials. However, on choice trials, when both cues were presented and animals were free to respond on either option, behavioral allocation changed as a function of imposed reward delay for the delayed option ($F_{7,60} = 5.32$, $p < 0.001$; **Fig. 5.2c**). Thus, early in training when reward delays were not different (sessions 1-11), animals chose each option equally (Bonferroni *post hoc* test, $p > .05$). However, as the delay was gradually increased for the delayed option, animals demonstrated a significant behavioral preference for the immediate reward option, choosing it more frequently. This preference was present at delays of 4s, 8s, and on the recording day ($p < .05$ for all comparisons). Thus, animals avoided long delays when possible by selecting options that produced immediate rewards. There was no significant difference on any behavioral metric (total reinforcers, total errors, choice probability) between performance levels attained by the end of training and performance during electrophysiological recording session (all p's > 0.05). There was no difference in

response latency on immediate and delayed reward trials during the recording session (paired *t*-test, p = 0.21).

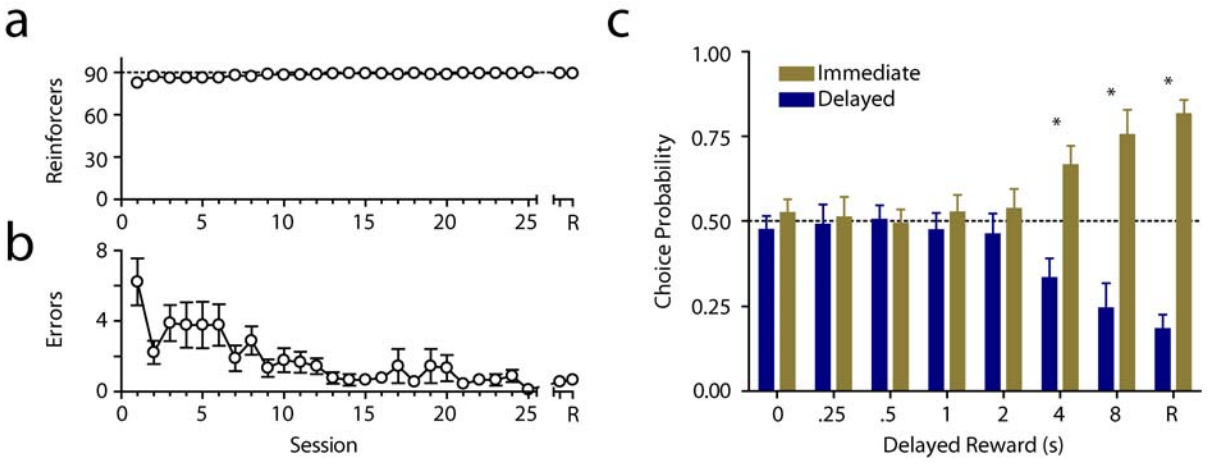

Figure 5.2. Behavior during the delay-based decision task. (a) Total reinforcers across training sessions (mean ± SEM). Reinforcers obtained were near maximal levels across training, including the recording session (R). Dashed line indicates maximal number of reinforcers available. (b) Total errors across sessions (mean ± SEM). Errors decreased as training progressed ($p < 0.001$), indicating animals could discriminate between cues. (**c**) Choice probability for immediate and delayed reward options as a function of the interval between responses and rewards on delayed reward trials. Dashed line indicates indifference point. Choice allocation shifted as a function of reward delay (two-way repeated measures ANOVA, $p < 0.05$). Asterisks indicate ratios at which preference for the immediate reward option was significantly greater (Bonferroni *post hoc* tests, $p < 0.05$). "R" denotes choice preference during the recording session.

Overview of NAc firing patterns during behavioral task

A total of 67 individual NAc neurons were recorded from 9 rats during performance of the delay-based choice task. Of these, 56 (83.6%) exhibited significant modulation in firing rate during at least one task event. Thirty-six neurons (53.7%) exhibited changes in firing rate during cue presentation, 46 (68.7%) exhibited changes preceding the operant responses, and 48 (71.6%) exhibited changes during reward delivery. In addition, 25 of 67 neurons exhibited increased activity following the lever press or preceding reward delivery on delayed reward trials, when animals were waiting for rewards. A more detailed description of each response type is presented below.

Cue-evoked activity in NAc neurons is not sensitive to predicted reward delay

The presentation of reward-paired discriminative stimuli evoked changes in firing rate in the majority of NAc neurons recorded (36 of 67, 53.7%). Of these, 14 (38.9%) were marked by significant increases in firing rate on at least one trial type (see **Fig. 5.3a** for a representative example). Less than half (6 of 14 neurons, 42.9%) of these neurons exhibited significant increases in activity during the presentation of both immediate reward and delayed reward cues (**Fig. 5.3b**). As a population, these activations were not significantly different on immediate reward, delayed reward, and choice trials in either peak or average cue-related activity (repeated measures ANOVA; $p > .05$ for both comparisons; **Fig. 5.3c,d**). Unlike cue-evoked excitations reported in chapter four, there were no significant differences in the distribution of cue specific or cue selective responses in the present population (Fisher's exact test, $p > 0.05$ for both comparisons). The majority of cue-responsive neurons (23 of 36, 63.9%) exhibited decreased firing rate during cue presentation on at least one trial type (data not shown). Overall, this population exhibited no difference in degree of inhibition across low cost, high cost, and choice trials (repeated measures ANOVA for mean inhibition; $F_{2,26} = 1.23$, $p = 0.31$). Moreover, there were no significant differences in the distribution of cue specific or cue selective responses (Fisher's exact test, $p > 0.05$ for both comparisons). Thus, overall there were no differences in cue-evoked response patterns on delayed and immediate reward trials, indicating that reward delay was not encoded by this population.

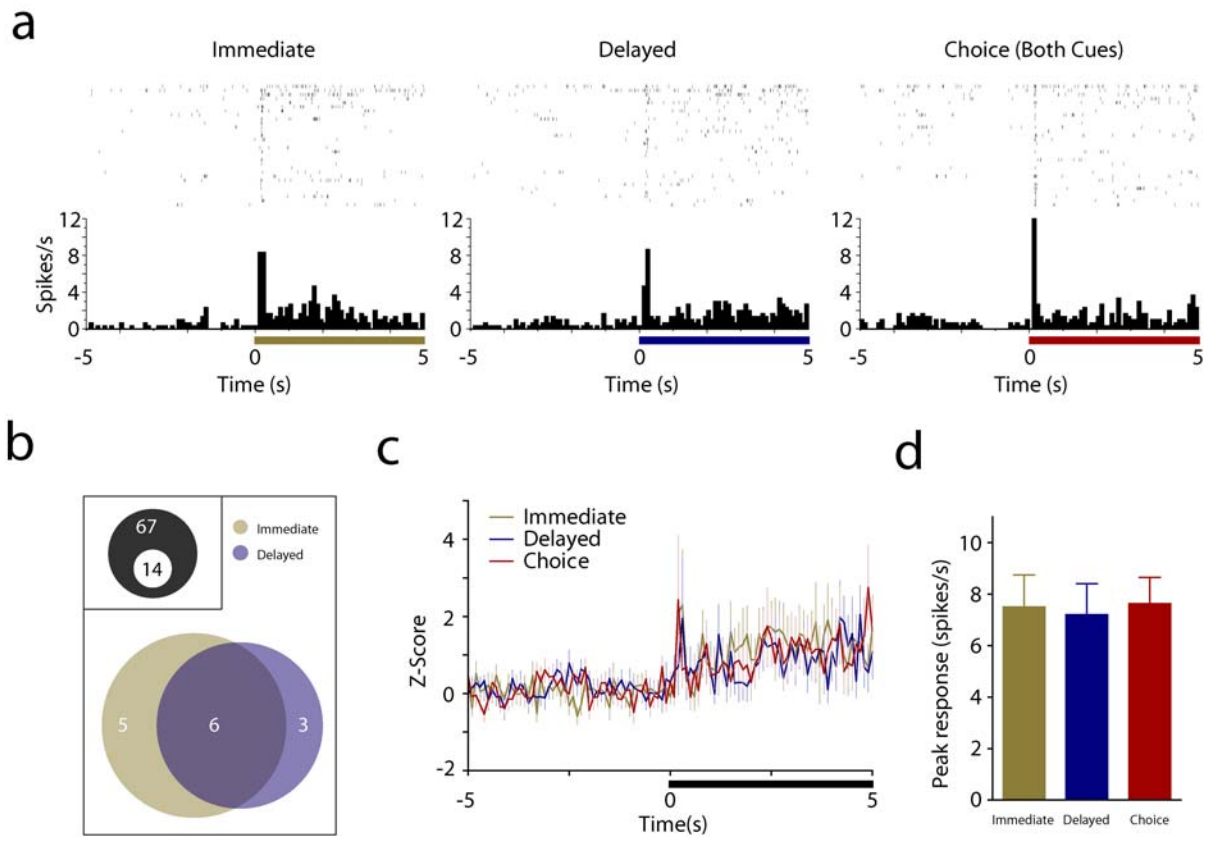

Figure 5.3. Cue-evoked excitations in NAc neurons. (**a**) Representative NAc neuron exhibiting a cue-evoked increase in firing rate. Left panel, raster plot (top) and peri-event histogram (PEH; bottom) aligned to onset of cue that predicts immediate reward (gold bar). Center panel, raster plot and PEH aligned to cue that predicts delayed reward (blue bar). Right panel, raster plot and PEH aligned to onset of choice trials (presentation of both cues). This neuron exhibited an excitation at cue onset regardless of trial type. (**b**) Venn diagram illustrating the distribution of responses across immediate and delayed reward trial types. Inset, 14 (white circle) of 67 total neurons (black circle) responded to cues with an excitation. Of these, 5 to the immediate reward cue alone (gold circle), 3 to the delayed reward cue alone (blue circle), and 6 responded to both cues (overlap). (**c**) Mean Z-score (± SEM) of neural activity for all cue-excitatory neurons (n=14). (**d**) Peak cue-evoked activity (± SEM) for all cue-excitatory neurons across trial type. There was no significant difference in cue-evoked excitation (repeated measures ANOVA, $p > 0.05$).

Response-evoked firing patterns

Forty-six of 67 (68.7%) neurons recorded during the delay based choice task exhibited significant alterations in firing rate within the seconds preceding operant responses. Of these, 25 of 46 (54.3%) were characterized by increases in firing rate on at least one trial type (see **Fig. 5.4a** for example neuron), whereas 24 of 46 (52.2%) displayed decreases in firing rate on at least one trial type (see **Fig. 5.5a** for example neuron). For each of these

groups, a large proportion of phasic responses were specific for trial type (14 of 25, or 56% of response-related excitations, **Fig. 5.5b**; 12 of 24, or 50% of response-related inhibitions, **Fig. 5.5b**). However, the distribution of response-specific or response selective cells did not differ based on reward delay (Fisher's exact test, $p > 0.05$ for both comparisons). Therefore, neuronal activations or depressions which were response-specific were excluded from group analyses.

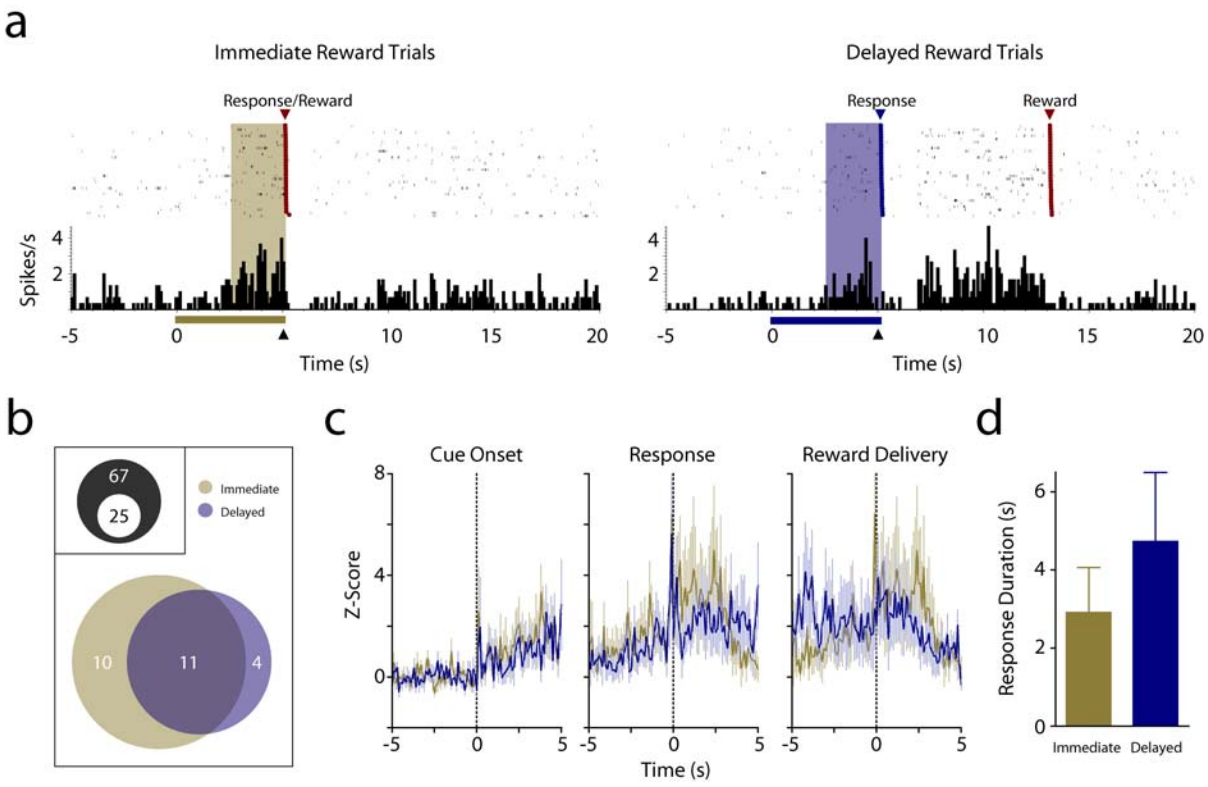

Figure 5.4. Response-activated NAc neurons. (**a**) Raster plots and PEHs from representative NAc neuron that exhibited an excitation preceding the operant response on immediate and delayed reward trials. Data are aligned to cue onset, and rasters are sorted based on the latency between lever extension (at 5s, black triangle) and reward delivery (red triangle, red circles in raster plot). Shaded areas indicate the classification window for pre-response activity. Blue circles in right raster plot denote timing of lever press on delayed reward trials (lever presses and reward delivery occurred simultaneously on immediate reward trials). Other conventions follow Fig. 5.3a. (**b**) Venn diagrams illustrating frequency of response activated NAc neurons for immediate and delayed reward trials. Inset, 25 of 67 neurons exhibited increased activity preceding the operant response on immediate or delayed reward trials. Of these, 10 were excited before the immediate reward lever press alone (gold circle), 4 were excited before the delayed reward lever press alone, and 11 were excited prior to both responses (overlap). (**c**) Mean (± SEM) Z-score of 11 neurons that were excited before the initial response on both trials. Data are aligned to cue onset (left panel), the operant response (center panel), and reward delivery (right panel). (**d**) Duration of excitation for response-activated neurons from (c). There was no difference in the length of excitations on immediate and delayed reward trials ($p > 0.05$).

The population activity of neurons that exhibited increased activity before responses on both immediate and delayed reward trial types is shown in **Fig. 5.4c**. There was no difference in the mean or peak activity of these neurons during the pre-response period on immediate and delayed reward trials (repeated measures ANOVA, $p > 0.05$ on both comparisons). Unlike the pre-response excitations reported in chapter four (see Fig. 4.4), neurons that exhibited increases in firing rate before responses in this task did not maintain this excitation until the reward was delivered. Thus, these cells did not exhibit increased activity over baseline in the time period (2.5s) immediately preceding reward delivery (*t*-test, $t = 1.745$, $df = 10$, $p = 0.11$), and there was no difference in the duration of these excitations across immediate and delayed reward trials (*t*-test, $t = 1.3$, $df = 10$, $p = 0.22$; **Fig. 5.4d**). However, many of these same neurons (such as the example neuron in Fig. 5.4a) were also activated at some point during the delay period on delayed reward trials, as animals were waiting for rewards. These neurons are analyzed separately below (see Fig. 5.6).

Cells that became inhibited during the seconds preceding the operant response on both immediate and delayed reward trial types are shown in **Fig. 5.5c**. There was no difference in the magnitude of inhibitions preceding responses between trial types (repeated measures ANOVA, $p > 0.05$). However, in contrast to pre-response excitations, units that became inhibited during the pre-response period continued this inhibition until reward delivery on delayed reward trials (**Fig. 5.5c**). This was evident in both a decreased firing rate (as compared to baseline) for these cells during the time epoch immediately preceding delayed reward delivery (*t*-test, $t = 3.16$, $df = 11$, $p = 0.009$), and also in a prolonged response duration on delayed reward trials as compared to immediate reward trials (*t*-test, $t = 3.701$, df

= 11, p = 0.004; **Fig. 5.5d**). These cells resemble the pre-response inhibitions from chapter four (see Fig. 4.5), which were also inhibited until rewards were delivered. Thus, although pre-response excitations were not maintained while animals were waiting for rewards to be delivered in delayed reward trials, pre-response inhibitions were.

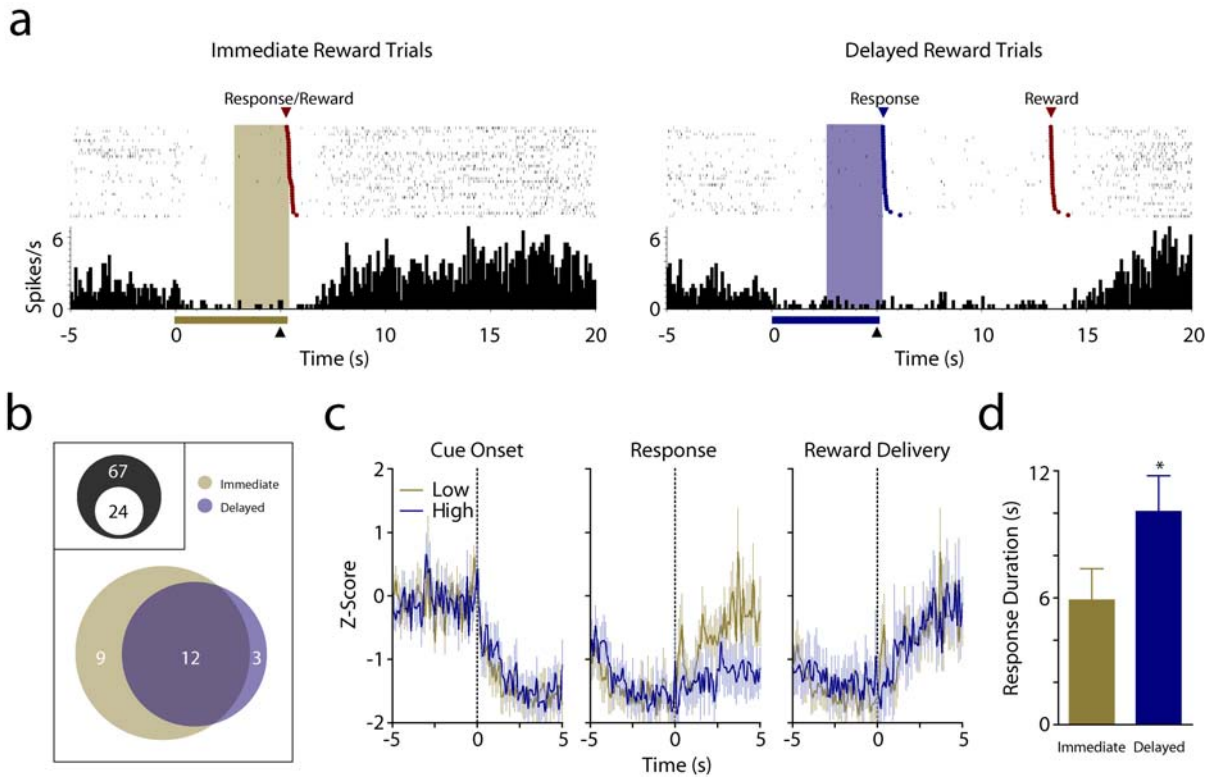

Figure 5.5. Response-inhibited NAc neurons. (**a**) Raster plots and PEHs from representative response-inhibited NAc neuron on immediate and delayed reward trials. Conventions follow Fig. 5.4a. (**b**) Venn diagrams illustrating frequency of response inhibited NAc neurons for immediate and delayed reward trials. Inset, 24 of 67 neurons exhibited decreased activity preceding the operant response on immediate or delayed reward trials. Of these, 9 were inhibited before the immediate reward lever press alone (gold circle), 3 were inhibited before the delayed reward lever press alone, and 12 were inhibited prior to both responses (overlap). (**c**) Mean (± SEM) Z-score of 12 neurons that were inhibited before the operant response on both trials. Data are aligned to cue onset (left panel), the operant response (center panel), and reward delivery (right panel). (**d**) Duration of inhibition for response-activated neurons from (c). Response-inhibited neurons exhibited longer duration inhibitions on delayed reward trials than on immediate reward trials ($p < 0.05$).

NAc excitations during reward delay

Previous studies indicate that neurons in the ventral striatum (including the NAc) exhibit increases in activity in anticipation of reward delivery, even after responses that

produce the reward have been made (Hollerman et al., 1998). Therefore, we examined neuronal activity during the time window between the response and reward delivery on delayed reward trials. Consistent with previous results, we found that a sizeable subgroup of NAc neurons (25 of 67, 37.3%) exhibited increases in firing rate during this period (**Fig. 5.6**). Of these, 16 of 25 were also activated during the pre-response period (presented in Fig. 5.4). Since there is no directly comparable period for immediate reward trials, between-trials contrasts were not performed for these neurons. However, comparisons to baseline activity revealed that the same neurons were activated following the operant response on immediate reward trials (repeated measures ANOVA for mean firing rate with Dunnett's *post hoc* comparisons to baseline; $F_{2,48} = 5.241$, $p = 0.009$; **Fig. 5.6b,c**). On delayed reward trials, these cells were excited during the post-response period and remained significantly activated through reward delivery ($F_{4,96} = 4.718$, $p = 0.002$; Dunnett's *post hoc* comparisons to baseline, $p < 0.05$; **Fig. 5.6b,c**). Interestingly, firing rate on each trial type exhibited a linear increase across time (test for linear trend, $p < 0.05$ for each trial type), with the greatest activity coming during reward delivery.

Reward-related changes in NAc neuronal activity

A majority of NAc neurons recorded here (46 of 76, 71.6%) exhibited increased or decreased activity during reward delivery. Of these, excitations (23 of 46, 50%; **Fig. 5.7**) and inhibitions (29 of 46, 63%; data not shown) were both common (note: these percentages sum to over 100 because neurons could be both inhibitory on one trial type and excitatory on another). A characteristic reward-evoked excitation is shown in **Figure 5.7a**. Here, only 9 of 23 reward-related excitations were specific trial type, with 7 of 23 (30%) neurons specifically responding to immediate rewards and 2 of 23 (8.7%) neurons specifically responding to

delayed rewards. There was no significant difference in the distribution of specific responses according to preceding delay (Fisher's exact test, p = 0.13). In the overall population of reward excited cells, there was no difference in response magnitude (peak) between trial types (*t*-test, t = 0.1, df = 22, p = 0.91; **Fig. 5.7c,d**). Nearly half of reward-related inhibitions were specific to trial type, with 6 of 29 (21%) exhibiting an inhibition following immediate reward delivery and 8 of 29 (28%) exhibiting an inhibition specifically following delayed reward delivery. However, most of these neurons (15 of 29, 52%) exhibited inhibitions during reward delivery regardless of trial type. There was no significant difference in the

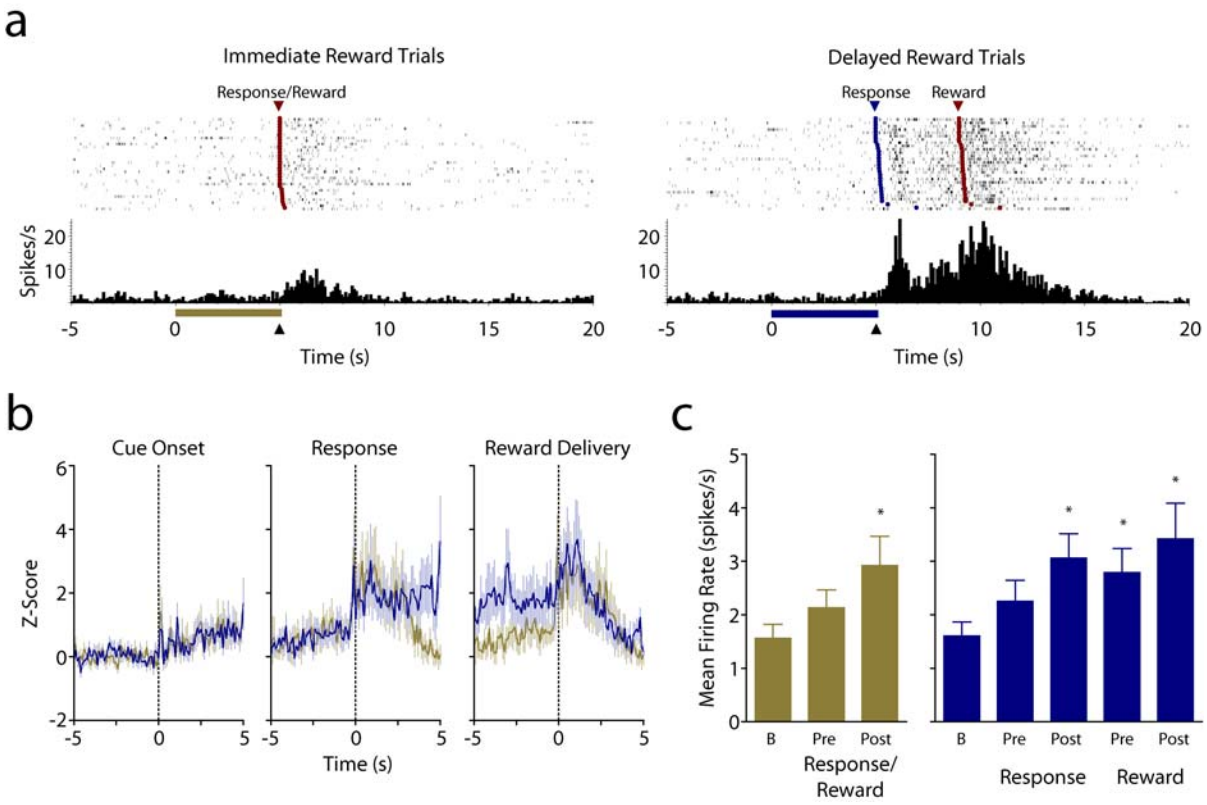

Figure 5.6. A subset of NAc neurons are activated during reward delay. (**a**) Raster plots and PEHs from representative NAc neuron on immediate and delayed reward trials. Data are aligned to cue onset, but sorted based on the latency between lever extension and reward delivery (red circles in raster plot). (**b**) Mean (± SEM) Z-score of 25 neurons that were excited during the delay period on delayed reward trials. Data are aligned to cue onset (left panel), response (center panel), and reward delivery (right panel). (**c**) Comparison of mean firing rate vs. baseline during 2.5s time epochs before and after relevant events from (b). These neurons were activated during reward delivery on both trial types, but exhibited significantly increased activity for the duration of the delay period on delayed reward trials (Dunnett's *post hoc* comparisons with baseline, p < 0.05).

degree of inhibition between immediate and delayed reward trials (comparison of average response, t = 0.5, df = 28, p = 0.61). Likewise, there was no difference in the proportion of neurons that responded specifically or selectively to the immediate or delayed reward (Fisher's exact test, p > 0.05).

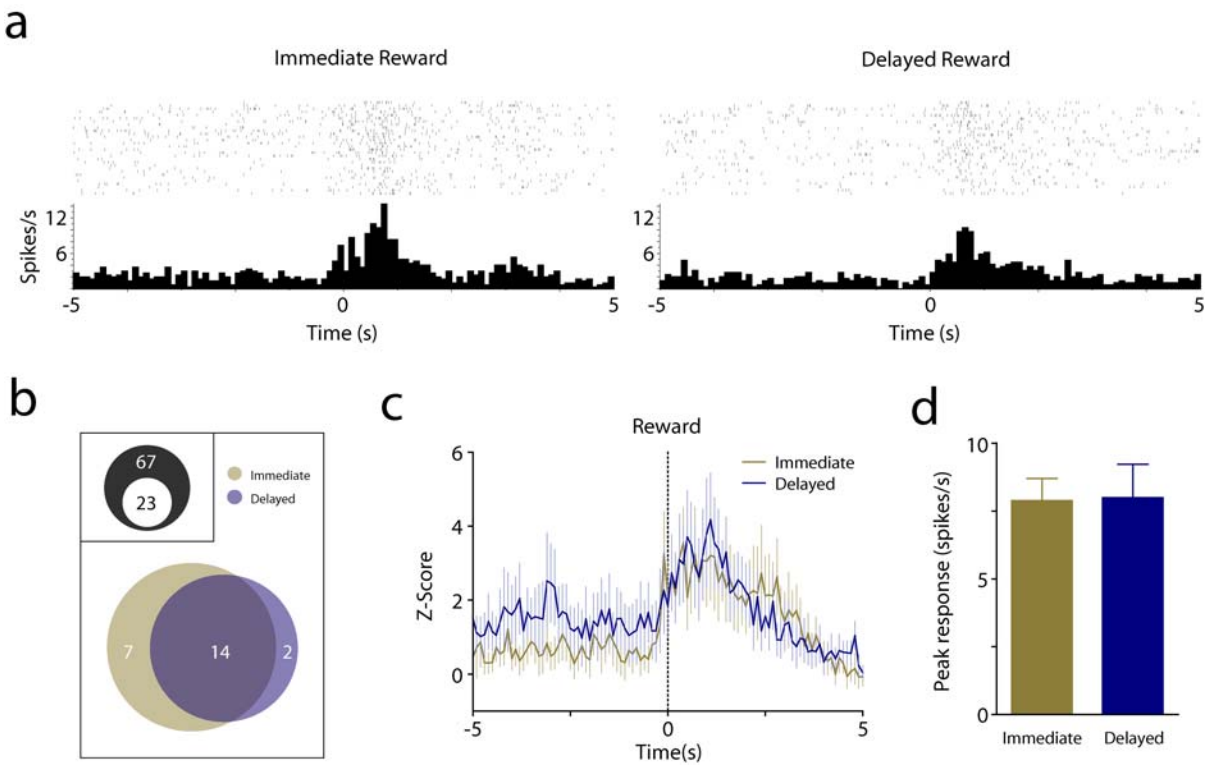

Figure 5.7. Reward-excited NAc neurons. (**a**) Raster plots and PEHs from representative NAc neuron exhibiting an increase in firing rate upon reward presentation on both immediate and delayed reward trials. Data are aligned to reward delivery. (**b**) Venn diagrams illustrating distribution of reward-excited NAc neurons for immediate and delayed reward trials. Inset, 23 of 67 neurons exhibited decreased activity preceding the operant response on immediate or delayed reward trials. Of these, 7 were excited by immediate rewards alone (gold circle), 2 were excited by delayed rewards alone, and 14 were excited by both rewards (overlap). (**c**) Mean (± SEM) Z-score of 14 neurons that exhibited excitations upon reward delivery on both trial types. Data are aligned to reward delivery. (**d**) Mean magnitude (peak spikes/s) of reward-evoked increase in neurons from (c). There was no difference in response magnitude (p > 0.05).

Electrode placement

A total of 144 microwires (16 per rat; 9 rats) were implanted bilaterally and aimed at the nucleus accumbens. Histological verification of electrode placements confirmed that 34 neurons were recorded from 26 electrodes located in the NAc core, whereas 33 neurons were

recorded from 28 electrodes located in the NAc shell. Across animals, electrode placements ranged from 1.08 – 3.0mm anterior to bregma, 0.6 – 1.8mm lateral to the midline, and 6.2 – 8.0mm ventral from the brain surface. The precise placement of marked electrode tips in the NAc are shown in **Figure 5.8**. Data from electrodes located outside the NAc were excluded from analysis. There was no difference in the distribution of any response type between the core and shell of the NAc (Fisher's exact test on response frequencies across region, $p > 0.05$ for all comparisons).

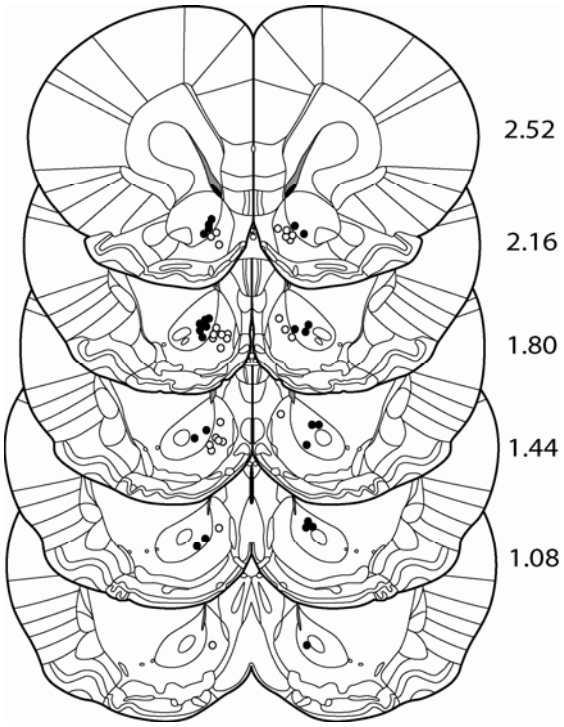

Figure 5.8. Successive coronal diagrams illustrating anatomical distribution of electrode locations across core and shell of the NAc. Marked locations are limited to electrodes that contributed to data presented here. Filled circles indicate electrode location in the NAc core, open circles indicate electrode locations in the NAc shell. Numbers to the right of each diagram indicate anteroposterior coordinates rostral to bregma (in mm).

DISCUSSION

The present study investigated neuronal activity in the NAc during a delay-based choice task, in which rats were presented with cues that signaled the opportunity to respond for rewards at different temporal delays. Behavioral results suggest that animals learned the task and could distinguish between the discriminative cues. Further, rats exhibited a behavioral preference for immediate rewards on choice trials, when they were free to choose between immediate and delayed reward options. Neurophysiological data revealed that subsets of NAc neurons exhibited phasic responses during each portion of the task. Specifically, one population exhibited changes in activity relative to the presentation of cues that signal reward opportunities, but did not encode the temporal delay predicted by cues. Distinct subsets of NAc neurons also responded with either excitations or inhibitions before animals responded on immediate and delayed reward trials, but only inhibitions were sustained as animals waited for delayed rewards. A class of NAc cells also showed excitations as animals were waiting for reward delivery, and for these cells the magnitude of excitation increased linearly with wait time. Finally, subgroups of NAc cells were responsive during reward delivery, although there were no differences between rewards delivered on immediate and delayed trials. Consistent with results reported in chapter 4, there were no differences in the distribution of these response types across the core and shell of the NAc. These results demonstrate that the NAc encodes delay-related information that may be useful to action selection during intertemporal choice tasks.

Similar to previous reports and data presented in chapter 4 (Cromwell and Schultz, 2003; Nicola et al., 2004b; Day et al., 2006), a subset of NAc neurons recorded during the delay-based decision task exhibited excitatory responses during the presentation of reward-

paired discriminative stimuli. As mentioned previously (see Chapter 4 discussion section), such cue responses have been found to encode unique information about upcoming rewards, including their motivational valence (Setlow et al., 2003; Roitman et al., 2005), identity (Hassani et al., 2001), magnitude (Cromwell and Schultz, 2003), location (Taha et al., 2007), and cost (Chapter 4). In contrast to these studies, the present report found no difference in the overall activity of cue responsive neurons on immediate and delayed reward trials, indicating that this population of neurons does not encode future reward delay. Moreover, although some neurons exhibited larger responses to cues that predicted immediate rewards, the frequency of these neurons did not differ from the frequency of neurons that responded preferentially to cues that signaled delayed rewards.

Given that cue-evoked excitations are thought to reflect the motivational value of cues, and that this information may be relevant to action selection (Nicola, 2007), it is somewhat surprising that we found no delay-related differences in cue excitations. Importantly, animals exhibited clear preferences for immediate rewards over delayed rewards on choice trials. Therefore, the lack of delay-sensitive cells does not indicate that animals could not discriminate between the cues or that animals were insensitive to reward delay in general. Moreover, since cost-sensitive neurons were common in animals responding on a very similar task in Chapter 4, it is not likely that a lack of cue selectivity was due to the level of training or the specific design of the task. One potential explanation for this difference is that the discriminative stimuli used here signaled different reward delays *from the time of the lever press* rather than from the time of the cue. Thus, whereas the immediate reward cue was at least 5s removed from the reward, the delayed reward cue preceded reward delivery by at least 9-13s (for 4 and 8s delays, respectively, assuming animals responded

immediately upon lever extension). Therefore, both cues signaled delayed rewards, although one was more delayed than another. Although this was also the case in the previous experiment (chapter 4), the cues presented in that study also signaled differences in effort in addition to differences in delay. While this indicates that reward delay alone was not encoded in cue-evoked NAc excitations, future parametric studies will be required to parse the precise effects of reward delay and response cost on NAc cue responses. Indeed, it is possible that larger differences in reward delay are required NAc neurons to prospectively encode delay-related information.

Changes in neuronal firing before the operant response may reflect both instructive signals, which contribute to the performance of a specific response over another, and permissive signals, which contribute to goal-directed responding in general (Carelli, 2002b, 2004; Roitman et al., 2005; Taha and Fields, 2006; Taha et al., 2007). Conversely, such activity could reflect the anticipation of rewards associated with specific actions (Hollerman et al., 1998). The previous chapter reported that NAc excitations which began prior to the response were maintained until reward delivery, even on high cost trials. Here, we found that as a population, neurons that were excited prior to the response failed to maintain this activity through reward delivery on delayed reward trials, and were not significantly longer in duration than excitations observed on immediate reward trials (Fig. 5.4). In contrast, another subset of neurons became activated during the delay period, and exhibited the greatest increase in activity following reward delivery (Fig. 5.6). These activations may therefore reflect dissociable levels of reward processing, with the first type encoding planning or execution of movements required to obtain rewards, and the second type encoding reward expectation or anticipation, which should be low prior to the response and grow as the time

of reward delivery approaches. Importantly, there is much overlap between these neuronal populations, indicating that some neurons may exhibit both types of activity.

In contrast, neurons which exhibited inhibitions that began before operant responses on immediate and delayed reward trials tended to maintain this activity until reward delivery, leading to longer periods of inhibition on delayed reward trials (Fig. 5.5). These types of responses have previously been interpreted as permissive signals that gate the onset of motivated behavior (Taha and Fields, 2006; Taha et al., 2007). In the task used in the present study, the delay period may be considered as part of the general sequence of events that leads to reward delivery, as the animal must move from the response lever to the reward receptacle and await reward delivery. Thus, it is not surprising that inhibitory responses were extended through this period. In fact, this type of activity may play an integral role in keeping motor systems engaged and ready for reward delivery across delays, instead of allowing the animal to become disengaged. As such, these prolonged inhibitions may contribute to animals' ability to wait long periods of time for large rewards, and may help explain the deficits in delay-based decision making induced by NAc lesions (Cardinal et al., 2001; Bezzina et al., 2007).

Reward-related activations in the present task were observed on both immediate reward and delayed reward trials, indicating that these responses are not simply due to lever retraction or cue offset, but signal reward delivery. In contrast to the results from the effort-based decision making task (where reward-evoked excitations were found to be greater following higher costs), we observed no differences in the magnitude of reward responses on trials in which reward delivery was immediate or delayed. This suggests that the differences in reward-evoked excitatory responses in the effort-based task were not simply a function of

reward delay. However, we should again note that in the effort-based task reward delivery was controlled by the animal (in terms of response rate), and was therefore inherently variable. In the present task, the reward was always delivered at a set interval following the response, and was therefore independent of the animal's response rate. Thus, reward delivery on delayed trials in the present task may have been more predictable or expected than reward delivery on high cost trials in the effort-based task, which may explain the lack of differences in the magnitude of excitations.

Conceptually, the impairments in delay-based decision making produced by NAc lesions may arise from disruption of several different processes (Cardinal et al., 2001; Cardinal, 2006). First, NAc lesions may alter reward sensitivity, or the ability to discriminate between different volumes of reward. An impairment in this ability may lead animals to select more immediate rewards, because the difference between large and small rewards would be less discernable. Secondly, NAc lesions may impair the ability to discriminate actual changes in reward delay during a session or between sessions. Finally, NAc lesions may increase the actual rate of reward discounting that occurs with time, such that future rewards are devalued at a faster pace. Current evidence suggests that deficits in delay-based decision making are not due to decreased reward sensitivity. NAc lesioned animals behaving in operant tasks are still sensitive to outcome devaluations such as prefeeding (Balleine and Killcross, 1994), and generally prefer larger rewards when there are no delays between responses and rewards (Bezzina et al., 2007). However, other evidence demonstrates that NAc lesions may both impair the ability to discriminate different delays and increase the rate at which future rewards lose value (Pothuizen et al., 2005; Acheson et al., 2006; Bezzina et al., 2007), although mathematical models indicate that discounting rate is the parameter most

affected (Bezzina et al., 2007). Importantly, these disruptions may be associated with distinct impairments in types of NAc activity reported here. Responses that are maintained across a delay, such as the excitations and inhibitions reported here, may operate as a memory trace that bridges responses with delayed rewards and makes those responses more probable (Cardinal, 2006). Therefore, future rewards in NAc lesioned animals may lose the ability to flexibly guide behavior, leading animals to select immediate rewards regardless of size.

The NAc is part of a distributed neural network that regulates decisions regarding reward delays, which includes the orbitofrontal cortex (OFC), subthalamic nucleus, and basolateral amygdala (Winstanley et al., 2004; Winstanley et al., 2005a; Rushworth and Behrens, 2008). OFC neurons, which send glutamatergic efferents to many places (including the NAc), encode a number of highly specific features about predicted rewards, including their taste, smell, texture, identity, and delay (Rolls and Baylis, 1994; Rolls et al., 1996; Rolls et al., 1999; Padoa-Schioppa and Assad, 2006; Roesch et al., 2006). The OFC appears to play an especially critical role in guiding delay-related decisions (Cardinal, 2006; Rudebeck et al., 2006; Rushworth and Behrens, 2008), as lesions to the OFC or disconnection of the OFC and NAc induce preference for small, immediate rewards over large delayed rewards (Kheramin et al., 2002; Rudebeck et al., 2006). Further, OFC lesions impair the ability to learn about rewards that are available at long delays (Mobini et al., 2002). However, the OFC is not required for animals to choose between rewarding options on the basis of response effort, indicating that its role in decision making may be selective (Rudebeck et al., 2006). In addition to the contribution of distinct nuclei, delay discounting also involves a complex interplay between neurotransmitter systems within the NAc. Thus, NAc dopamine depletion has no effect on delay discounting, but serotonin antagonism increases impulsivity in a

dopamine-dependent manner (Winstanley et al., 2005b). Understanding how this neural circuit interacts with the response types observed in the present study may enhance our knowledge of the neural basis of delay discounting and lead to better explanations and treatments for disorders characterized by impulsivity.

CHAPTER 6

GENERAL DISCUSSION

Summary of experiments

The studies described in the previous chapters were designed to extend our understanding of the role of dopamine and NAc signaling in both reward-related learning and decision making. The results demonstrate that the NAc dopamine can dynamically encode new reward associations, that both the value and cost of such associations are reflected in NAc dopamine signaling, and that NAc neurons reflect information about the cost and delay of rewards. A brief summary of each experiment is presented below.

Phasic dopamine signaling in the NAc during Pavlovian reward learning

The experiments described in chapter two represent the first observation that phasic NAc dopamine release is dramatically altered as a result of reward learning. This study employed an appetitive conditioning task in which one cue (the CS+) predicted a sucrose reward and another (the CS–) predicted the absence of reward. We observed that during the initial stages of conditioning, when animals had not yet learned to associate the CS+ and the reward, rewards alone evoked subsecond increases in NAc dopamine concentration. However, during the initial session, as animals were exposed to stimulus-reward pairings, dopamine release produced by cue presentation increased for several animals. After extended conditioning, when animals demonstrated behavioral evidence of a learned stimulus-reward association, phasic elevations in NAc dopamine concentration were observed at cue onset,

but were no longer observed at reward delivery. This was due to the formation of a learned association, as phasic NAc dopamine release in animals that received unpaired stimuli and rewards was still timelocked to reward delivery.

Rapid NAc dopamine signaling during effort-based decision making

The experiments reported in chapter three demonstrate for the first time that in addition to signaling reward prediction, rapid NAc dopamine release may also signal the costs of future rewards. Animals were trained on a decision making task in which distinct cues predicted the availability of sucrose rewards at either low effort requirements (FR1) or high effort requirements (FR16). Furthermore, animals were given choice trials in which they revealed a preference for low cost rewards. Interestingly, cue-evoked dopamine release in the NAc core was smaller when cues predicted high cost rewards than when cues predicted low cost rewards. Additionally, on choice trials, cue-evoked dopamine release appeared to signal the better of two options. In contrast, cue-evoked dopamine release in the NAc shell signaled reward prediction alone and was not sensitive to reward cost. These results establish that NAc dopamine may encode information that is relevant to decision making, although in a region-specific manner.

NAc neurophysiology during effort-based decision making

The study described in chapter four employed the effort-based choice task used in chapter three to investigate the activity of individual NAc neurons during the same behavior. The results provide the first demonstration that individual NAc neurons encode information about the costs associated with rewards. A subset of NAc neurons exhibited increased activity when cues signaled low effort rewards as compared to high effort rewards. On choice trials, when either reward was available, the activity of these neurons was consistent with the

behavioral preference for low cost rewards. Likewise, two different classes of neurons exhibited increases and decreases in activity preceding response initiation that were maintained during the exertion of effort. These responses are consistent with the idea that the NAc contributes to effort-based decision making and may help to explain the role of the NAc in overcoming large response costs to obtain rewards.

NAc neurophysiology during delay-based decision making

The study described in chapter five examined whether NAc neurons encode information about reward delays. This experiment employed a decision task in which animals responded for both immediate and delayed rewards on two different levers with the same effort requirements (FR1 on both levers). Animals were also given choice trials in which they demonstrated a preference for immediate rewards over delayed rewards. Importantly, NAc neurons were not sensitive to the differences in reward delay predicted by discriminative stimuli. However, two groups of neurons showed changes in activity as animals were actually experiencing delays. One class of cells exhibited decreased firing rate leading up to the operant responses and maintained this activity until reward delivery, even after animals had performed the response on delayed reward trials. Conversely, another class was activated during the delay, and exhibited gradually heightened activity that peaked at reward delivery. These signals are similar to those observed in the effort-based choice task, indicating that they are present when animals overcome either large response costs or long wait times to obtain rewards.

General discussion and relevance of findings

Although the unique implications of each study are discussed individually following each original data chapter, these findings also have further implications for how the

mesolimbic dopamine system functions in vivo, and how this function relates to its role in learning, decision making, and psychiatric disorders such as drug addiction. Therefore, these topics are addressed below.

Effects of dopamine signaling on NAc activity

The first two studies reported here discuss changes in phasic NAc dopamine concentration during behavior. However, this phasic release does not occur in a vacuum, but exerts its effect via postsynaptic changes in cellular activity at MSNs. Therefore, one of the key issues that arise from such studies is how dopamine signals may contribute to MSN output. This has traditionally been a contentious question, with some studies indicating that dopamine directly inhibits MSNs, and others reporting that dopamine excites MSNs (White and Wang, 1986; Yim and Mogenson, 1988; Yim and Mogenson, 1991; Gonon, 1997; Nicola et al., 2000). In reality, the precise function of dopamine on the postsynaptic neuron likely depends on a range of factors, including the coincidence of afferent input, the present firing rate of the cell, the tonic extracellular concentration of dopamine, and the type of dopamine receptor expressed in the cell (Surmeier and Kitai, 1993; Nicola et al., 2000; Surmeier et al., 2007).

In addition to these direct actions, dopamine also has effects on long-term synaptic plasticity that outlasts the activation of dopamine receptors. MSNs receive glutamatergic input from diverse brain regions and exhibit an NMDA dependent form of LTP (Pennartz et al., 1993; Pawlak and Kerr, 2008; Shen et al., 2008). Dopamine, particularly at D_1 receptors, is required for this plasticity (Pawlak and Kerr, 2008; Shen et al., 2008). Due to the differential affinity states of dopamine receptors (discussed in chapter 1), the phasic dopamine signals reported here are likely necessary to elevate the extracellular concentration

of dopamine in ways that can activate D_1 receptors. It has been proposed that coincident glutamatergic activation of NMDA receptors and stimulation of D_1 receptors initiates a host of intracellular signaling cascades that are relevant for the generation of long term potentiation (Cepeda and Levine, 1998; Fienberg et al., 1998; Valjent et al., 2005; Girault et al., 2007). Thus, behaviorally meaningful changes in synaptic strength produced by learning may require both convergent glutamatergic input into the NAc and the rapid dopamine signals observed here. This idea is consistent with the observation that both dopamine and NMDA antagonism in the NAc impairs the formation of stimulus-reward associations (Di Ciano et al., 2001).

Recently, technological advances have allowed simultaneous recording of both subsecond dopamine release and postsynaptic cell firing at the same carbon fiber electrode (Cheer et al., 2005). These studies have shown that although patterns of neuronal activity are diverse, "phasic" neurons that exhibit increases or decreases in activity timelocked to behavioral events are only found in locations where rapid dopamine release is also evident (Cheer et al., 2005; Cheer et al., 2007a). Such reports confirm that dopamine likely plays a key role in driving MSN activity, whether this effect is due to immediate changes in neuronal excitability or prolonged changes in the ability of afferents to influence firing rate. However, because the activity of NAc neurons does not always reflect the pattern of dopamine signaling, it appears likely that additional circuit-level mechanisms interact with dopamine to determine the precise pattern of NAc activity.

Role of dopamine and NAc signaling in reward learning

As discussed in the introduction, a number of theories have tied the activity of dopamine neurons to computational models of reward learning (Montague et al., 1996;

Schultz et al., 1997; McClure et al., 2003a; Montague et al., 2004a; Redish, 2004; Pan et al., 2005; Roesch et al., 2007). The majority of these models, such as temporal difference learning algorithms, seek to explain how an agent learns to predict rewards in the environment (Sutton and Barto, 1981; Sutton and Barto, 1998). At the core of these models is the idea that stimuli or contexts (known in these algorithms as "states") are not randomly associated with future rewards, and therefore can be employed to predict rewards. In temporal difference learning, the agent seeks to estimate the value of these states as predictors. In order for this to occur, the learning agent must compute the difference between the value of a reward it expects in a state from the value of a reward it receives in the same state. Within these algorithms, this difference is modeled in an error term, known as δ. During a learning situation, δ can be used to push the estimated predictive value associated with a certain stimulus towards more accurate estimations. Thus, when an animal receives a reward that is unexpected, δ is high and drives up the value of stimuli that preceded the reward. Conversely, when stimuli have high values due to positive associations with rewards, these stimuli themselves will generate high error terms, as they predict states that are better than expected. However, when rewards are predicted but do not occur, δ will be negative, and therefore push the predictor to a lower value (McClure et al., 2003a).

A multitude of neurophysiological investigations indicate that the firing rate of dopamine neurons encodes a signal similar to that of the error term (δ) in temporal difference learning models (Schultz et al., 1997; Bayer and Glimcher, 2005; Pan et al., 2005). Thus, when rewards are unexpected (and therefore generate high δ values), they elicit increases in dopamine neuron firing rate. Following learning, stimuli that predict rewards (and high δ) produce increases in dopamine neuron firing, whereas predicted rewards do not. Finally,

predicted rewards that are omitted evoke negative prediction errors and decreases in dopamine neuron activity (Schultz, 2004). The data presented in chapter one are clearly applicable to these models, and indicate that this signal is faithfully transferred to terminal regions. Thus, early in learning, rewards generated high prediction errors and also evoked dopamine release. However, after learning, conditioned stimuli alone produced increases in phasic dopamine release. In contrast, when rewards were not predicted (and therefore generated positive prediction errors), they still evoked phasic surges in dopamine concentration. Such activity is a candidate mechanism for reward learning, and is consistent with the deficits induced by NAc dopamine depletion or antagonism (Di Ciano et al., 2001; Parkinson et al., 2002). Furthermore, the observation that NAc neurons become excited by reward predictive cues also indicates that this information can be incorporated into NAc output (Setlow et al., 2003; Nicola et al., 2004b; Roitman et al., 2005; Day et al., 2006).

Human brain imaging studies during reward learning tasks have confirmed and extended experimental links between dopamine signals and NAc activity during associative learning (Knutson and Cooper, 2005). A number of investigations using fMRI techniques to assess blood oxygenation have reported increased activity in the ventral striatum (including the NAc) during exposure to rewards ranging from water to money to sexual stimuli (McClure et al., 2004). Consistent with the animal literature, reward prediction is a key feature in this pattern of activation (Pagnoni et al., 2002). Berns and others (Berns et al., 2001) found that unpredicted delivery of a rewarding juice substance to a volunteer's mouth evoked a significantly greater change in activity of the ventral striatum than when rewards were delivered in a predictable fashion. Moreover, when rewards are predicted by a discrete conditioned stimulus, this CS itself can evoke a change in activity in the ventral striatum

(McClure et al., 2003b; Ramnani et al., 2004). Notably, the ventral striatum seems to encode such deviations from reward prediction in both passive (Pavlovian) and active (operant) tasks, whereas the dorsal striatum is only activated by prediction errors that occur in an operant situation (O'Doherty et al., 2004). Thus, the ventral striatum and the NAc may have a wider role in linking stimuli with outcomes in both stimulus-outcome and action-outcome learning situations.

Role of dopamine and NAc signaling in decision making

Organisms commonly face situations in which they must choose between multiple options in order to maximize the value of rewarding outcomes. Although such decisions are often relatively simple, others require trade-offs between different variables, such as reward magnitude and reward cost. Interestingly, prediction error signaling by dopamine neurons has also been applied to computational models of decision making situations (Egelman et al., 1998; McClure et al., 2003a; McClure et al., 2004; Daw and Doya, 2006). In this context, dopamine could conceivably alter action selection in two ways. First, dopamine's role in learning may lead to different learning rates for different rewards, leading animals to select one action over another because the predictive value of one stimulus or action is less than another. Secondly, even when their predictive value has been fully established, dopamine may attribute higher values to stimuli or actions that lead to better rewards. In this case, the positive prediction errors associated with specific actions or stimuli may make them more likely to be chosen in the future (McClure et al., 2003a). The results presented in chapter three are consistent with this hypothesis. Here, animals were trained with equivalent reward costs in order to remove potential differences in learning rate for each option. During the recording session, we found that cues that predicted rewards at lower costs evoked larger

increases in NAc core dopamine than cues that predicted rewards at higher costs. These findings offer a potential substrate by which dopamine may contribute to decisions between two rewarding options. Higher-value cues that signal better rewards (and therefore more dopamine release) may work to increase the likelihood that those options are selected in the future. Electrophysiological evidence is consistent with this idea, as dopamine neurons have been found to exhibit larger responses for cues that signal immediate rewards, larger rewards, and more probable rewards (Fiorillo et al., 2003; Tobler et al., 2005; Roesch et al., 2007; Fiorillo et al., 2008). Moreover, the relevance of dopamine to NAc output is again supported by the observation that NAc neurons also code for action values (Samejima et al., 2005), predicted reward magnitude (Hassani et al., 2001), and predicted reward cost (chapter 4).

Although phasic dopamine signals may clarify dopamine's role in reward learning and decision making, they do not explain all of the deficits that arise following NAc dopamine depletion. For example, dopamine depletions in the NAc clearly have an adverse effect on animal's ability to overcome large response requirements to obtain rewards (Ishiwari et al., 2004; Mingote et al., 2005; Salamone et al., 2007). However, we found that rapid dopamine signals are not observed during actual responses in a decision making task (chapter 3), suggesting that phasic increases in dopamine concentration do push responding to overcome large costs. One explanation for these diverse results is that different aspects of dopamine transmission contribute to different facets of reward-directed behavior. Thus, while phasic dopamine may directly contribute to learning and action selection, tonic dopamine may contribute to incentive motivation to bias reward "wanting" and help animals surmount large costs when required to obtain rewards (Berridge and Robinson, 1998; Berridge, 2006; Niv et al., 2007). Such a role would explain the beneficial effect of dopamine transmission on

large fixed ratio schedules of reinforcement. However, this account is entirely speculative at the present time, and future studies are required to determine the differential contribution of tonic and phasic dopamine.

Neuronal activity in the NAc is also critical for overcoming large costs or long delays to obtain rewards (Cardinal et al., 2001; Bezzina et al., 2008b; Hauber and Sommer, 2009). The cost- and delay-modulated changes in NAc activity reported in chapters 4 & 5 may represent neural substrates that are important for this capacity. In both cases, we observed changes in neural activity that were maintained until rewards were obtained, regardless of whether animals were actively engaged in responding for rewards or simply waiting for reward delivery. This type of activity may represent multiple levels of reward processing, including reward expectation, a 'gate' for motivated behavior, and the representation of the goals of particular actions (Hollerman et al., 1998; Taha and Fields, 2006; Samejima and Doya, 2007). In any case, such activity would seem to be especially necessary when rewards are not immediately available and easy to procure. However, future studies will be required to elucidate whether such response profiles are necessary or sufficient for animals to overcome large costs.

Implications for drug addiction

In the studies presented here, NAc dopamine release or neural activity was monitored as animals were learning about or responding for natural rewards. However, the same behavioral processes are relevant to many other rewards, including drugs of abuse. Learned associations between cues and drug rewards are extremely important in addiction, as they evoke drug craving in human subjects (Gawin, 1991; O'Brien et al., 1992; O'Brien et al., 1998; Volkow et al., 2006), and leads to relapse in both human and animals (O'Brien et al.,

1998; Shaham et al., 2003; Fuchs et al., 2004). Importantly, drug addiction also involves the same brain circuits discussed here (Kalivas and McFarland, 2003; Kalivas and O'Brien, 2008). Addictive substances such as cocaine, alcohol, heroin, nicotine, and amphetamine all increase dopamine levels in the NAc (Di Chiara and Imperato, 1988; Cheer et al., 2007b). Additionally, cues associated with drug taking gain the ability to evoke increases in dopamine release and NAc cell firing as a result of learning (Carelli, 2000; Phillips et al., 2003a; Stuber et al., 2004; Stuber et al., 2005). This feature may prove especially important to the ability of cues to drive drug seeking. As discussed above, when natural rewards are fully predicted, they lose their ability to evoke increases in NAc dopamine concentration. However, due to the pharmacological properties of addictive drugs, they should continue to elicit dopamine release regardless of predictions, in effect signaling that the drug was better than predicted. This property of addictive compounds would lead to a situation in which drugs continuously elevate the estimated value of stimuli that predict them, and therefore bias decision making in favor of actions or stimuli associated with drug delivery (Montague et al., 2004a; Redish, 2004; Hyman, 2005). In this way, mechanisms that evolved to support natural reward-related learning and decision making could be maladaptive in the context of drug addiction (Hyman, 2005; Hyman et al., 2006). However, although this hypothesis may help to explain drug taking behavior, it does not explain why most animals and humans that take drugs do not become addicted (Deroche-Gamonet et al., 2004), or why dopamine release appears to become less important to drug taking in addicted individuals (Everitt and Robbins, 2005; Kalivas and O'Brien, 2008). Therefore, future studies are required to examine the relationship between dopamine, learning, and other risk factors for addiction (Nestler, 2000; Kreek et al., 2005).

Future directions

The experiments described in the preceding chapters comprise initial and basic experiments designed to begin investigations of the role of the NAc and NAc dopamine release in reward learning and reward-based decision making. However, the results left many questions unanswered and also generated new questions that will provide the basis for future research. Below are suggestions for additional experiments that will help to clarify the role of NAc and dopamine systems in behavior, specifically in reward learning and decision making.

The role of phasic dopamine signaling in NAc synaptic plasticity during learning

The observation that reward-paired cues acquire the ability to evoke phasic release of dopamine during learning suggests that excitatory inputs onto VTA dopamine neurons undergo plastic modification during conditioning. A recent study used in vitro electrophysiological techniques in combination with fast-scan cyclic voltammetry to elegantly demonstrate that this is the case (Stuber et al., 2008). Rats were trained to associate a predictive cue with reward delivery to a food cup, and electrochemical data indicated that cues gained the ability to elicit phasic dopamine release in the NAc. In vitro analyses revealed that the ratio between AMPA and NMDA receptor mediated excitatory currents in dopamine neurons (a measure of LTP) transiently increased in the same conditioning session that learning was first expressed. Moreover, NMDA receptor antagonism in the VTA blocked both this increase in synaptic strength and learning, but had no effect on the expression of a previously learned association (Stuber et al., 2008).

Previous studies indicate that striatal neurons undergo dopamine and NMDA dependent forms of synaptic plasticity (Pennartz et al., 1993; Shen et al., 2008), and that both dopamine and NMDA receptor activation in the NAc are required for pavlovian reward

learning (Di Ciano et al., 2001). However, although synaptic plasticity in the VTA is evidently required for learning, it remains unclear whether a similar form of plasticity occurs in the NAc during conditioning and whether such plasticity is required for learning. Therefore, future studies will be required to examine how excitatory synapses onto NAc neurons are modified as a result of learning. These studies will also have the benefit of being able to determine the synaptic strength of different excitatory inputs into the NAc, thereby elucidating which NAc afferents undergo synaptic plasticity.

Intracellular pathways mediating reward learning

Cue-evoked dopamine signals in the NAc are hypothesized to facilitate stimulus-outcome learning by regulating mechanisms of synaptic plasticity at MSNs (Kheirbek et al., 2008). Such plasticity may involve a number of intracellular effectors downstream of dopamine receptor activation, including dopamine and adenosine regulated phosphoprotein (32 kilodaltons), or DARPP-32 (Fienberg et al., 1998; Stipanovich et al., 2008), extracellular signal-related kinase (ERK) (Girault et al., 2007; Day, 2008; Shiflett et al., 2008), cAMP response element binding protein (CREB) (Self et al., 1998; Shiflett et al., 2009), and epigenetic modifications (Levenson and Sweatt, 2005). Each of these may produce a host of short and long term changes within the cell. However, it is presently unclear which pathways are involved in reward learning, and in what ways. Therefore, future studies will be required to probe these pathways using site-specific treatments that prevent or facilitate the action of these pathways during learning.

Phasic dopamine release in other terminal regions during learning

The experiments described in chapter 2 demonstrated that stimulus-reward conditioning altered the temporal pattern of dopamine release in the NAc core. However,

dopamine neurons project to other targets in the striatum, including the NAc shell and dorsal striatum. Previous studies have used microdialysis to investigate tonic changes in dopamine levels in the NAc core and shell during conditioning (Bassareo and Di Chiara, 1997, 1999a; Cheng et al., 2003). Although one of these studies reported that reward-paired cues evoke increases in dopamine only within the NAc core (Bassareo and Di Chiara, 1999a), the other study reported that conditioned stimuli elicited dopamine release equally in both the core and shell (Cheng et al., 2003). However, both of these studies lack the temporal resolution to distinguish specific behavioral events. To clarify this controversy, future studies should employ the same behavioral design used here to examine phasic release of dopamine within the NAc shell. Such experiments may reveal why dopamine antagonism in the core and shell produce very different behavioral impairments (Everitt et al., 1999; Parkinson et al., 1999; Di Chiara, 2002).

Examination of individual differences in reward learning

In chapter two, stimulus-reward pairings induced a pavlovian approach response directed at the stimulus that predicted reward (the CS+) but not the stimulus that predicted the absence of reward (the CS–). This 'sign-tracking' response demonstrated that animals learned the association between the CS+ and reward delivery. However, recent studies have revealed that animals can demonstrate the content of learning in another way, by approaching the food cup during the same type of conditioning (Flagel et al., 2007; Robinson and Flagel, 2008). This 'goal-tracking' response occurs in roughly one-third of rodents, and suggests that there are tremendous individual differences in behavioral responses elicited by reward-paired cues. Moreover, goal tracking is associated with differential expression of tyrosine hydroxylase, dopamine transporters, and dopamine receptors (Flagel et al., 2007). Thus,

future studies should address whether reward learning differently alters phasic dopamine release in this population. The results would provide insight into dopamine's role in this manifestation of learning. Additionally, since sign-trackers and goal-trackers exhibit different responses to both acute and repeated administration of psychostimulant drugs, the results may also have potential implications for drug addiction (Flagel et al., 2008a; Flagel et al., 2008b).

The role of rapid dopamine signaling in coding for other parameters during decision making

The results from chapter three argue that the cost of future rewards is encoded in phasic dopamine signals in the NAc core. However, a number of variables other than cost enter into decision making processes, including reward magnitude, delay, probability, and uncertainty (Doya, 2008). As discussed above, all of this information appears to be encoded at the level of dopamine neurons (Fiorillo et al., 2003; Tobler et al., 2005; Roesch et al., 2007; Kobayashi and Schultz, 2008). However, it is unclear how this information may be translated into dopamine release in terminal areas. Therefore, future studies are required to address whether cue-evoked dopamine release in specific terminal regions (including the dorsal striatum and NAc core and shell) reflects these variables. Additionally, as real-life decisions also entail the possibility of loss or aversive stimuli (Tversky and Kahneman, 1974, 1981), it is important to study how these variables alter decisions about rewards and whether they are reflected in NAc dopamine release (Roitman et al., 2008).

Afferent modulation of NAc activity during effort and delay based decision making

The NAc receives afferent input from a number of brain nuclei that have been implicated in different forms of decision making (Rudebeck et al., 2006; Floresco and Ghods-Sharifi, 2007; Floresco et al., 2008; Rushworth and Behrens, 2008). However, it is

unclear how these afferents differentially contribute to NAc output during behavior. The results described in chapters 4 & 5 demonstrate that the NAc exhibits different patterns of behavior-related activity, each of which may contribute in unique ways during decision making. Although recent studies have found that inactivation of the basolateral amygdala or dorsomedial prefrontal cortex attenuates cue-evoked responses in the NAc (Ambroggi et al., 2008; Ishikawa et al., 2008a), it is unclear which inputs drive prolonged increases and decreases in activity during high effort requirements or long delays. To test this, NAc neurons could be recorded during tasks similar to the ones reported here. In combination, specific regions could be inactivated via microinjection of GABA agonists while neuronal recordings are performed in the NAc. As bilateral inactivation would likely have dramatic effects on behavior (and therefore make it difficult to examine NAc output during behavior), specific nuclei should be inactivated unilaterally while both ipsilateral and contralateral NAc recordings are performed. Such studies would permit investigation of which NAc afferents contribute to the ability to overcome delays or costs to obtain rewards.

The role of rapid dopamine release and NAc activity in decisions that involve drugs of abuse

Experimental evidence suggests that drug addiction is associated with altered decision making processes. For example, human addicts typically discount future rewards at a much faster rate than ex users or normal controls, with the fastest rates of discounting occurring for future drug rewards (Madden et al., 1997; Bickel et al., 1999; Kirby et al., 1999; Bickel and Marsch, 2001; Green and Myerson, 2004). This pattern of discounting suggests that drug addiction is associated with a heightened value for immediate rewards (regardless of identity), and is consistent with links between impulsivity and addiction (Bickel et al., 1999; Bickel and Marsch, 2001; Kreek et al., 2005). However, it is unclear if this different

valuation system is associated with altered patterns of phasic dopamine release and NAc activity. To test whether this is the case, future studies should be designed to examine dopamine release in the NAc and NAc neurophysiology during decision making tasks that involve drug rewards. For example, rats could be trained to associate one cue with the availability of an immediate (but small) drug reward and a second cue with the availability of a large yet delayed or high cost drug infusion. After learning, dopamine release and neural activity in the NAc could be examined to investigate whether these cues lead to different patterns of activity. Importantly, this design may eventually enable comparisons between cues that signal natural rewards and cues that signal drug rewards within the same animal, in the same task. Moreover, in order to determine how repeated drug experience leads to alterations within this neural circuit, these types of studies could also be performed separately in animals with limited drug experience and animals that exhibit signs of addiction (Deroche-Gamonet et al., 2004). The results of such experiments would elucidate the role of NAc activity and dopamine release within the NAc during drug-related decision making.

Concluding remarks

Learning to obtain, predict, and choose between rewarding stimuli such as food, water, sex, social attachment, and drugs of abuse lies at the foundation of human behavior. These abilities are mediated by a highly conserved network of brain nuclei, including the NAc and mesolimbic dopamine system. The experiments described in this dissertation reveal how patterns of activity and neurotransmitter release within this system are linked to ongoing behavior in real time. As such, these studies provide critical insight into how this circuit processes information during the formation and maintenance of reward-related memories and during key aspects of decision making. However, the importance of this network is also

highlighted by decades of research demonstrating that the NAc-dopamine system is altered in numerous human disease states, including depression, schizophrenia, addiction, obesity, attention deficit/hyperactivity disorder (ADHD), and Parkinson's disease (Cotzias et al., 1969; Carlsson, 1972, 1978; Spiegel et al., 2005; Volkow and Li, 2005; Cardinal, 2006; Nestler and Carlezon, 2006; Waltz et al., 2007), which are often marked by problematic deficits in reward-related processing and decision making. Therefore, understanding how this neural circuit operates provides key insight not only for normal goal-directed behaviors, but also serves as a window through which disorders in this system can be observed and interpreted. Indeed, studies similar to those presented here have already provided the basis for new explanations of behavioral deficits that occur in Parkinson's disease, ADHD, and schizophrenia (Frank et al., 2004; Frank and Claus, 2006; Frank et al., 2007a; Waltz et al., 2007; Moustafa et al., 2008), and have helped to explicate how human genetic differences confer unique behavioral traits (Frank et al., 2007b). Future applications of such basic research will hopefully result in a better understanding of complex interactions between the environment, genes, and behavior, leading to the production of more sophisticated and effective courses of treatment for disorders such as addiction.

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