The Nucleus Accumbens Core Dopamine D1 and Glutamate AMPA/NMDA Receptors Play a Transient Role in the Performance of Pavlovian Approach Behavior

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THE NUCLEUS ACCUMBENS CORE DOPAMINE D1 AND GLUTAMATE AMPA/NMDA RECEPTORS PLAY A TRANSIENT ROLE IN THE PERFORMANCE OF PAVLOVIAN APPROACH BEHAVIOR

by

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A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

2017
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This manuscript has been read and accepted for the Graduate Faculty in Psychology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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ABSTRACT

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Veronica Dobrovitsky

Advisor: Jon C. Horvitz

The role of the nucleus accumbens core (NAc core) continues to be redefined with newly acquired data on neurochemical mechanisms mediating the learning and performance of behavior. Previous empirical data showed that dopamine transmission at the D1 receptor (D1R) plays a transient role in the expression of learned Pavlovian approach behavior. Here we show that, prior to overtraining, dopamine activity at D1Rs specifically within the NAc core is critical for the performance of approach behavior elicited by the recently-acquired reward-paired cue. Blockade of D1Rs in the NAc core, but not the dorsomedial striatum or NAc shell, disrupted approach responses during early training; however, the dependence of Pavlovian approach on D1R transmission declined throughout training. Upon blockade of NAc core D1Rs during extended training, the expression of Pavlovian approach responses remained intact. Given these findings we next explored whether a) neuronal activity within the core of accumbens still mediates cued approach during the late training stages in the absence of D1R transmission by relying on glutamatergic transmission or b) whether mediation of the cued approach becomes independent of the NAc core itself, i.e., shifts to another substrate. We blocked AMPA/NMDA receptors in the NAc core during early versus extended training and showed that loss of neuronal activation in the NAc core only disrupted expression of conditioned stimulus-elicited responses during early training. Our results indicate that NAc core activity is not necessary for the expression of well-acquired approach.
ACKNOWLEDGMENTS

I wholeheartedly thank my mother for her support, patience, and kindness during my time as a graduate student. Thanks also to the rest of my family for being supportive and understanding. I express my appreciation and gratitude to my academic advisor, Dr. Jon C. Horvitz, for his guidance and support during the writing of this dissertation and in the years that proceeded its writing. Extending the gratitude to the members of my committee, Dr. Jennifer Mangels, Dr. Ratna Sircar, and Dr. James Stellar, I especially would like to express gratitude for the invaluable learning experience they provided me with in the preparation of the dissertation and during its defense. Many thanks to the Horvitz laboratory undergraduate research assistants who helped with the running of the experiments presented herein. Special thanks to Dr. Rosa Caamaño-Tubio for her technical expertise on various research matters.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AMPA</td>
<td>α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid</td>
</tr>
<tr>
<td>BLA</td>
<td>basolateral amygdala</td>
</tr>
<tr>
<td>cAMP</td>
<td>cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>CS</td>
<td>conditioned stimulus</td>
</tr>
<tr>
<td>D1Rs</td>
<td>dopamine D1 receptors</td>
</tr>
<tr>
<td>D2Rs</td>
<td>dopamine D2 receptors</td>
</tr>
<tr>
<td>DA</td>
<td>dopamine</td>
</tr>
<tr>
<td>DLS</td>
<td>dorsolateral striatum</td>
</tr>
<tr>
<td>DMS</td>
<td>dorsomedial striatum</td>
</tr>
<tr>
<td>DS</td>
<td>discriminative stimulus</td>
</tr>
<tr>
<td>FR</td>
<td>fixed ratio</td>
</tr>
<tr>
<td>GABA</td>
<td>γ-aminobutyric acid</td>
</tr>
<tr>
<td>GLU</td>
<td>glutamate</td>
</tr>
<tr>
<td>GPe</td>
<td>external segment of globus pallidus</td>
</tr>
<tr>
<td>GPI</td>
<td>internal segment of globus pallidus</td>
</tr>
<tr>
<td>ITI</td>
<td>inter-trial interval</td>
</tr>
<tr>
<td>MSNs</td>
<td>medium spiny neurons</td>
</tr>
<tr>
<td>NAc</td>
<td>nucleus accumbens</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-Methyl-D-aspartate</td>
</tr>
<tr>
<td>OFC</td>
<td>orbitofrontal cortex</td>
</tr>
<tr>
<td>PD</td>
<td>Parkinson’s Disease</td>
</tr>
<tr>
<td>PFC</td>
<td>prefrontal cortex</td>
</tr>
<tr>
<td>SN</td>
<td>substantia nigra</td>
</tr>
<tr>
<td>SNC</td>
<td>substantia nigra pars compacta</td>
</tr>
<tr>
<td>SNr</td>
<td>substantia nigra pars reticulata</td>
</tr>
<tr>
<td>S-R</td>
<td>stimulus-response</td>
</tr>
<tr>
<td>VP</td>
<td>ventral pallidum</td>
</tr>
<tr>
<td>VTA</td>
<td>ventral tegmental area</td>
</tr>
<tr>
<td>US</td>
<td>unconditioned stimulus</td>
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AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; BLA: basolateral amygdala; cAMP: cyclic adenosine monophosphate; CS: conditioned stimulus; D1R: dopamine D1 receptors; D2R: dopamine D2 receptors; DA: dopamine; DLS: dorsolateral striatum; DMS: dorsomedial striatum; DS: discriminative stimulus; FR: fixed ratio; GABA: γ-aminobutyric acid; GLU: glutamate; GPe: external segment of globus pallidus; GPI: internal segment of globus pallidus; ITI: inter-trial interval; MSNs: medium spiny neurons; NAc: nucleus accumbens; NMDA: N-Methyl-D-aspartate; OFC: orbitofrontal cortex; PD: Parkinson’s Disease; PFC: prefrontal cortex; SN: substantia nigra; SNC: substantia nigra pars compacta; SNr: substantia nigra pars reticulata; S-R: stimulus-response; VP: ventral pallidum; VTA: ventral tegmental area; US: unconditioned stimulus
1. INTRODUCTION TO DISSERTATION
1.1 GENERAL INTRODUCTION

Parkinson’s Disease (PD) patients are known to suffer cognitive and behavioral deficits that are believed to result from impaired neurochemical signaling in the striatum due to the degeneration of midbrain dopamine (DA) neurons. PD patients face difficulties with internally generated movements towards simple goals. However, under certain conditions, they show surprisingly normal motor function. One anecdotal example of the surprising movement ability, called paradoxical kinesia, is that of a skilled tennis player afflicted with PD. During the course of PD progression, his daily movements become progressively impaired, but his ability to hit the tennis ball remains intact. This example is one of several suggesting that overtrained behaviors may be performed normally even under conditions of severe DA loss. The experimental work in this dissertation employs a rat model to ask whether the role of DA transmission in a behavioral response diminishes when the response becomes overtrained. The work begins by asking whether a well-acquired behavior can be carried out even when DA transmission is strongly compromised. If so, does the behavior shift to non-DA innervated anatomical sites? Does the behavior continue to depend upon the same brain regions as it did early in training, with DA’s role within the region(s) diminishing?
1.1.1 Functional anatomy of the striatum and its connections

The striatum, its subregions, and behavior

The basal ganglia are believed to enable movements by setting the premotor frontal lobe into a mode ready for movement execution (Alexander and Crutcher 1990; Chevalier and Deniau 1990). The striatum contributes to this function by gating proper signals to relevant motor circuits (Chevalier and Deniau 1990). Although the specific nature of the computations carried out by the striatum is not known, an early formulation proposed that the striatum (particularly the ventral striatum) is an “anatomical link between motivation and action” (Mogenson et al. 1980). Contemporary theories highlight the importance of the striatum in carrying out motivated action selection (Nicola 2007) appropriate to existing environmental conditions (Horvitz 2002).

The dorsal striatum is comprised of a) ‘associative striatum’, principally the caudate (along with anterior parts of putamen), and the ‘sensorimotor striatum’, mostly made up of putamen (except the most anterior region). In rodents, caudate and putamen are not morphologically distinguishable and are referred to by their respective homologues: the dorsomedial striatum (DMS) and the dorsolateral striatum (DLS). For simplicity (and given that the dissertation experiments are carried out in rats), the rodent terminology will be used in this review of DA and the striatum. Studies on operant behavior in rodents (described in more detail in a later section) suggest that facilitation of outcome-dependent behaviors (e.g., reaching a goal) involves DMS processing while stimulus-response (S-R) behaviors (e.g., habits) rely on DLS activity (Balleine et al. 2009). The DLS is heavily innervated by sensory and motor cortices; in contrast, the DMS receives most cortical input from association areas, including areas coding behavioral goals (Selemon and Goldman-Rakic 1985; Flaherty and Graybiel 1993; Tremblay and Schultz 1999; Schilman et al. 2008).

In contrast to the dorsal striatum (at least the DLS) which has been associated with sensorimotor aspects of behavior, the ventral striatum is strongly linked to motivational aspects of behavior. In 1980, Mogenson theorized that the ventral striatum translates motivational signals, of limbic origin, into behavioral actions (Mogenson et al. 1980); however, even today, knowledge about the ventral striatum’s exact behavioral function and what its neurons encode remains controversial (see Nicola et al. 2007 for
review). The major anatomical constituent of the ventral striatum is the nucleus accumbens (NAc). (Also, included in the definition of ventral striatum are the olfactory tubercle and the ventromedial portion of the caudate-putamen.) The NAc receives preferential amygdala inputs (Groenewegen et al. 1999; Fudge et al. 2002) which have been hypothesized to encode environmental stimuli that predict rewards (Ambroggi et al. 2008). Placing a heavier emphasis on the affective elements of behavior, partly due to the origin of its projections, the ventral portion of the striatum is sometimes referred to as the limbic striatum (Voorn et al. 2004).

The NAc is a compartmentalized unit with core and shell subregions (Heimer et al. 1997; Groenewegen et al. 1999; van Dongen et al. 2008) that may mediate different aspects of behavior. The dorsal part of the NAc core is morphologically continuous with the dorsal striatum; the shell surrounds the core’s medial, ventral, and lateral sides (Heimer et al. 1997). Both core and shell neurons exhibit reward-related neuronal excitations and inhibitions; however, shell neurons are particularly inhibited during behavioral responses for reward, and core neurons become far more inhibited than shell neurons during reward consumption (Ambroggi et al. 2011). When excitatory transmission in NAc core is abolished, behavioral responding for a reward-predictive cue decreases; however, in the NAc shell, inactivation produces significant increases in behavioral responding for a neutral cue. Nicola (2007) has proposed that under normal conditions, NAc neurons exert an inhibitory modulation on behavior; the experimental inactivation of NAc neurons produces an unnatural disinhibition of non-specific behavioral actions (Nicola 2007). Natural activation of NAc neurons by environmental events allows the organism to perform desired actions in order to obtain wanted goals such as food rewards.

**The striatum receives DA signals from the midbrain**

DA neurons located in the substantia nigra (SN) and ventral tegmental area (VTA) of the midbrain (Lindvall et al. 1984) send projections to various brain sites including primary motor cortex, prefrontal cortex (PFC), orbitofrontal cortex (OFC), DMS, DLS, and the NAc (Fallon 1981; Björklund and Lindvall 1984; Haber 2003; Hosp et al. 2011; Chandler et al. 2013). Diverse terminations of DA terminals in the brain appear to arise from distinct populations of SN-VTA neurons (Fallon 1981). The location of DA cell bodies is divided into the dorsal tier containing VTA and the dorsal SN compacta (SNc) and a ventral tier
containing the rest of SNc (Heimer et al. 1997; Haber 2003). The DMS and DLS mainly receive projections from the ventral tier SNc (Heimer et al. 1997; Haber 2003). The NAc receives major input from the dorsal tier (mostly the VTA) (Heimer et al. 1997; Groenewegen et al. 1999; Haber 2003).

Numerous factors determine the effects of VTA neuronal firing upon neurochemical activity within NAc. With phasic firing being strictly dependent upon external environmental events, VTA neurons fluctuate their electrophysiological state between tonic and phasic firing patterns (Bayer et al. 2007). The phasic NAc DA release evoked by reward-predictive cues occurs with subsecond latency (Roitman et al. 2005). Firing of VTA DA neurons produces robust DA transients at various NAc sites; however, the precise locations of DA transients within NAc depend upon which subpopulations of VTA neurons are activated (Wightman et al. 2007). DA neurons are heterogeneously distributed throughout the VTA and have diverse neurophysiological properties (Lammel et al. 2014; Barker et al. 2016). Some DA neurons projecting to the NAc are capable of co-releasing of glutamate (GLU) (Hnasko et al. 2010; Stuber et al. 2010; Zhang et al. 2015) and γ-aminobutyric acid (GABA) (Tritsch et al. 2012; Tritsch et al. 2014). In some conditions, DA released from midbrain terminals in the NAc may also transmit contemporaneously with endocannabinoids released from NAc neurons themselves (Freund et al. 2003; Seif et al. 2011; Fitzgerald et al. 2012; Wang et al. 2012). Such diverse neuropharmacological actions may be important factors in real-time regulation of synapses and transmission inside the NAc.

Horvitz (2002) has hypothesized that phasic DA activity in the ventral striatum gates GLU input signals to striatal output neurons (Horvitz 2002). DA produces a variety of neuropharmacological effects in the striatum (Nicola et al. 2000) by acting on its own heterogeneous receptor systems. Direct actions of DA in the striatum are through the activation of postsynaptic DA receptors (White and Wang 1986; Levey et al. 1993; Nicola et al. 2000). Indirect effects of DA on striatal neurons are mediated through DA receptors located presynaptically (Levey et al. 1993; David et al. 2005). The gating of GLU inputs to select subsets of striatal neurons for downstream processing is believed to reflect DA’s role as a neuromodulator that preferentially amplifies strong compared to weak GLU inputs to the striatum.

Striatal DA enhances intra-striatal neuronal excitation by sustaining the membrane potential ‘up states’ which increase the probability of action potentials (O’Donnell 2003; Plotkin et al. 2011). GLU released from striatal afferents (Groenewegen et al. 1999; David et al. 2005) elicits excitatory activity in
striatal cell bodies by activating several types of ionotropic GLU receptors (Hu and White 1996; Plotkin et al. 2011) located postsynaptically (David et al. 2005; Hallett et al. 2006; Capper-Loup et al. 2009; Wolf 2010; Ferrario et al. 2011). The neuronal 'up states' depend upon activation of N-Methyl-D-aspartate (NMDA) receptors (Plotkin et al. 2011) which bind GLU and depolarize the neuron (Flores-Hernandez et al. 2002); sustainability of 'up states' is mediated through the DA D1 receptors (D1Rs) (O'Donnell 2003) also located postsynaptically (White and Wang 1986; Levey et al. 1993; David et al. 2005). The activation of striatal D1Rs enhances currents mediated by neighboring NMDA receptors (Flores-Hernandez et al. 2002). Corticostriatal afferents are not known to co-release DA (Benoit-Marand and O'Donnell 2008); instead, concurrent DA transmission originating from the midbrain selectively sustains the neuronal excitation evoked by GLU (O'Donnell 2003; Plotkin et al. 2011). The effects of D1R activation on neuronal activity depend upon the strength of the current excitatory synaptic input (Nicola et al. 2000) and the state of the postsynaptic NMDA receptors (Cepeda et al. 1993).

DA also activates D2 receptors (D2Rs) located pre- and post-synaptically (Levey et al. 1993; David et al. 2005). When presynaptic D2Rs located on some corticostriatal afferents are stimulated by DA, GLU release from their terminals becomes inhibited (Bamford et al. 2004). This effect is specific for the corticostriatal afferents that have weak electrophysiological activity at the time of D2R activation; therefore, activation of presynaptic D2Rs further diminishes weak inputs to the striatum (Bamford et al. 2004).

**Striatal medium spiny neurons receive converging inputs**

Over 95% of striatal neurons are classified as GABAergic medium spiny neurons (MSNs) (Matamales et al. 2009) which remain silent most of the time (Wilson 1992). Experimental results from intracellular recordings indicate that a hyperpolarized 'down state' predominates in MSNs of the dorsal striatum (Wilson and Kawaguchi 1996). MSN action potentials are believed to reflect membrane potential shifts resulting from a barrage of excitatory afferent signals received via a rich number of synapses (Wilson 1992; Wilson and Kawaguchi 1996). Electrophysiological recordings in freely behaving animals suggest that striatal activity emulates the pattern of activations from its various inputs (Teagarden and Rebec 2007).
Intracellular staining has revealed that the dendrites of striatal MSNs have the highest density of spines (Wilson and Groves 1980) out of all central nervous system neurons (Heimer et al. 1997). Midbrain and limbic inputs commonly synapse on spines, and cortical inputs synapse with MSN spines exclusively (Wilson and Groves 1980). Given this dendrite morphology, each MSN is able to receive multiple inputs.

Striatal MSNs, similarly present in all striatal subregions (Voorn et al. 2004; Matamales et al. 2009), receive converging inputs (Selemon and Goldman-Rakic 1985; Flaherty and Graybiel 1993; Groenewegen et al. 1999). Cell bodies of MSNs give off branching dendrites that form large receptive fields (Wilson and Groves 1980; Kawaguchi et al. 1990; Humphries et al. 2010). The extensive dendrites allow MSNs to receive wide synaptic convergence. A cortical pyramidal neuron need not make many contacts in one confined striatal area or on the same neuron; it instead innervates multiple MSNs (Bolam et al. 2000). Optogenetic work suggests that each GLU input from hippocampus, basolateral amygdala (BLA), and PFC innervates each NAc shell MSN (Britt et al. 2012). Indicating presence of limbic divergence, stimulation of any one afferent pathway elicits excitatory potentials in 95% of all NAc shell MSNs. In the NAc core, thalamic inputs converge with those from amygdala and PFC (Heimer et al. 1997; Groenewegen et al. 1999). Figure 1 illustrates some examples of converging inputs to the NAc where VTA axon terminals innervate MSNs that receive cortical and allocortical projections.

![Figure 1. Simplified diagram of inputs converging in the rat ventral striatum.](image_url)

The nucleus accumbens (NAc) receives dopamine (DA) modulation from the midbrain (e.g., ventral tegmental area = VTA) and integrates information from various nonstriatal sources signalling with glutamate (GLU) (e.g., basolateral amygdala = BLA, prefrontal cortex = PFC, orbitofrontal cortex = OFC, hippocampus = HIPP). Sagittal rat brain image adapted from a BrainMaps.org virtual slide dataset by Karten (2007) was retrieved from http://brainmaps.org/index.php?action=viewslides&datid=107.
Cortical and thalamic inputs to the dorsal striatum

Although striatal MSNs receive converging anatomical inputs, some degree of separation between inputs across the striatum is present as well. For instance, anatomical mapping has revealed that motor inputs from neighboring cortical areas preserve segregation of their terminal fields in the dorsal striatum (Nambu et al. 2002). The supplementary motor area, a cortical region with activity related to speed and direction of movements (Tankus et al. 2009), predominantly projects to the medial DLS whereas the primary motor cortex maps on laterally (Nambu et al. 2002). While conserving their cortical topography, supplementary motor area and primary motor cortex inputs overlap in only 20% of the DLS (Nambu et al. 2002).

Forming striatal sensorimotor maps in DLS, somatosensory afferents converge onto the topographically organized motor input zones (Haber 2003) thereby conserving organization of cortical limb- and body-part representations (Kunzle 1977; Flaherty and Graybiel 1993; Flaherty and Graybiel 1995). By tracking primary motor and somatosensory projections Flaherty and Graybiel (1993) showed that inputs from ipsilateral somatosensory and motor cortices representing same body-parts overlap their terminations in the striatum (Flaherty and Graybiel 1993). The sensorimotor representations of proximal to distal body-parts are arranged in a dorsal-to-ventral fashion throughout the DLS (Flaherty and Graybiel 1993; Flaherty and Graybiel 1995). Although a given region of the DLS can receive converging inputs from somatosensory and motor regions of the cortex, the DLS nevertheless retains some topography or 'separation' of inputs, i.e., different body parts are represented in different DLS zones.

Topographically organized projections from intralaminar and midline thalamic nuclei are the major source of thalamic input to the striatum (Haber 2003). Thalamic terminals map onto the same striatal areas receiving cortical input (Cheatwood et al. 2005). The converged cortical and thalamic inputs are contained on spines of individual MSNs; however, cortical synapses are more prominent (Huerta-Ocampo et al. 2014).

Striatal MSNs are classified based on the expression of DA receptors

Striatal MSNs are classified into two main populations based on the expression of two main categories of surface DA receptors. The D1-like receptors which through a G-coupled complex increase...
intracellular second messenger cyclic adenosine monophosphate (cAMP) are one category; in the second category are the D2-like receptors which inhibit cAMP (Kebabian and Calne 1979; Andersen et al. 1990; Civelli et al. 1993). Enough evidence has accumulated to suggest that central nervous system cAMP is involved in the control of behavior by conditioned rewards (Sutton and Beninger 1999). In an immunoreactivity study of DA receptor expression across the brain, D1Rs and D2Rs were found to be densely concentrated in the striatum (Levey et al. 1993). Upon close inspection of the expression pattern, it was observed that MSN dendrites express either D1Rs or D2Rs (Levey et al. 1993). Given this neuronal differentiation, the pattern of cAMP formation likely varies across the striatum.

Striatal MSNs predominantly express either D1Rs or D2Rs (David et al. 2005). In a study using a fluorescent reporter protein controlled by specific D1R or D2R gene promoters, MSNs in dorsal and ventral striatum expressed either D1Rs or D2Rs in about a 1:1 ratio with only a small percentage expressing both receptor types (Matamales et al. 2009). This estimate seems at odds with reports indicating that virtually all MSNs show electrophysiological responses to D1R agonist application during whole-cell recordings (Flores-Hernandez et al. 2002). Earlier studies hinted at existence of other D1-like receptor types (Andersen et al. 1990). It is now known that the D1R superfamily includes D5 receptors which bind D1R ligands (Civelli et al. 1993). Low expression of D5 receptors has been observed in similar densities on both the D1R- and D2R-expressing MSNs (Rivera et al. 2002) which may explain responses of D2R-expressing MSNs to D1R agonists in whole-cell preparations. The role of D5 receptors however is presently not well-known (Beaulieu and Gainetdinov 2011).

In a study using specific antibodies for D1R and D2R and for a presynaptic DA marker, it was found that MSNs which express D1R exclusively are packed together in columnar arrangements in the NAc shell (Jansson et al. 1999). Surrounding these D1R-expressing regions are the DA terminals (Jansson et al. 1999) which provide a source of DA transmission. Interestingly, the D1R-rich columns constituted only a small fraction of the total DA receptor expression in the NAc. In NAc core, D1R-rich areas were found as well; however, these tissue patches also expressed D2Rs indicating that D1R- and D2R-expressing MSNs are intermixed there (Jansson et al. 1999).

MSNs types differ in terms of dendrite morphology. Immunostains for D1Rs and D2Rs are primarily observed on spines of dendrites that are at synapses with excitatory terminals (Levey et al.
Although spine density in D1R- and D2R-expressing MSNs is similar, MSNs exclusively expressing D1Rs have more primary dendrites (Gertler et al. 2008). Consequently, the dendritic span may be larger in D1R-expressing MSNs allowing them to receive 50% more GLU input (Gerfen and Surmeier 2011) than their D2R-expressing counterparts. Individual GLU afferents, however, have been traced to innervate both MSN types, and a close microscopic analysis revealed that cortical and thalamic innervation of D1R- and D2R-expressing MSNs is proportionally similar between the two MSN types (Doig et al. 2010).

**Local striatal neurons and their interactions**

Within the dorsal striatum, MSNs participate in lateral inhibition and influence activity of neighboring MSNs (Bolam et al. 2000) through local GABAergic axon collaterals (Kawaguchi et al. 1990). Interestingly, some dorsal MSNs send axonal projections to the contralateral dorsal striatum (Mensah and Deadwyler 1974; Kawaguchi et al. 1990). Intrastratal projections have also been observed between NAc core/shell compartments (van Dongen et al. 2005). Although dendrites of MSNs do not cross the core/shell boundary, axon collaterals extend into neighboring compartments (van Dongen et al. 2008). Thus, NAc core and shell have reciprocal axonal connections that target other MSNs and interneurons (van Dongen et al. 2005).

The stratum contains a small percentage of cholinergic and GABAergic interneurons (Andersen et al. 1990; Matamales et al. 2009) which are capable of lateral interactions. The interneurons, which make up approximately 5% of all striatal neurons (Matamales et al. 2009), have been described as aspiny and medium or large in size (Heimer et al. 1997; Tepper and Bolam 2004; Tepper et al. 2010). Striatal cholinergic interneurons are innervated by thalamic GLU fibers and participate in feedforward microcircuits with GABA interneurons and MSNs (Tepper and Bolam 2004; Gerfen and Surmeier 2011). These tonically active cholinergic interneurons pause their firing during presentation of reward-paired cues and may modulate MSN activity in a behaviorally-relevant manner (Tepper and Bolam 2004).

The striatum is modeled as an inhibitory network system (Yim et al. 2011). The fastspiking GABA interneurons, which account for about 1% of striatum's neurons, also inhibit MSN activity (Tepper and Bolam 2004; Humphries et al. 2010). An interneuron may influence MSN activity in striatal areas where
numerous MSN dendritic fields overlap with the axonal field of the interneuron (Humphries et al. 2010). GABA interneurons are innervated by the cortical pyramidal neurons (Bolam et al. 2000). Cortical stimulation produces spikes in GABA interneurons quicker than spikes produced in MSNs and evokes these spikes at lower intensities; therefore, when/where cortical input to the striatum is weak, the fastspiking GABA interneurons provide a source of inhibition to MSNs (Mallet et al. 2005). This feedforward inhibition mechanism has been proposed to suppress MSN activity associated with unwanted behavioral actions at a given moment (Gerfen and Surmeier 2011). Prefrontal NAc afferents interact with NAc's fastspiking GABA interneurons to produce differential NAc MSN responses observed under some conditions (Gruber et al. 2009; Asher and Lodge 2012).

**Direct and indirect striatal output pathways**

Striatum's only output is via its two distinct populations of MSNs (Levey et al. 1993; Beaulieu and Gainetdinov 2011) which terminate principally within the pallidum and the SN pars reticulata (SNr) (Mensah and Deadwyler 1974; Kawaguchi et al. 1990). (As described earlier and noted below, DA cell bodies are located within the SNc). The pallidum is composed of three main regions: the internal (GPI) and external segments (GPe) of globus pallidus and the ventral pallidum (VP) (Haber and Knutson 2010). The GPI and GPe receive GABA inputs from the dorsal striatum while the VP receives its GABA terminals from the ventral striatum (including the NAc). The SNc, which contains DA cell bodies, as well as the SNr, which does not contain DA cell bodies, both have reciprocal connections with the striatum (Haber and Knutson 2010). Basal ganglia's main output nuclei (i.e., GPI and SNr) appear to be one continuous topographical region with representations of axial and limb movements in GPI and head and eye movements in SNr (Gerfen and Surmeier 2011).

Striatal MSNs that extend to GPI/SNr comprise the direct output pathway of the basal ganglia (Gerfen and Surmeier 2011). These direct pathway MSNs express D1Rs almost exclusively (Matamales et al. 2009). Striatal MSNs expressing D2Rs comprise the indirect output pathway and project to GPe without branching off to other regions (Kawaguchi et al. 1990). The GPe, in turn, projects to GPI/SNr (Bevan et al. 1998; Haber 2003). Therefore, the indirect pathway involves multisynaptic projections from...
the striatum to the GPi/SNr, and the direct pathway involves monosynaptic projections from the striatum to GPi/SNr.

The striatum is considered the ‘input’ stage of basal ganglia circuits (Alexander and Crutcher 1990). All striatal output is GABAergic, i.e., inhibitory (Gerfen and Surmeier 2011). Disinhibition is believed to be the basic mechanism that translates striatal activity (and perhaps its cognitive/perceptual correlates) into action (Chevalier and Deniau 1990). The GPi and SNr exert a tonic inhibitory influence over motor output regions (including thalamic areas that project to the motor cortex). When the organism is at rest, the striatum is mostly quiet, and GPi and SNr (i.e., the striatal target regions) have sustained impulses. As described below, this GPi/SNr output produces a sustained inhibitory influence on motor systems (Chevalier and Deniau 1990). Activation of striatal GABA neurons silences the GPi/SNr impulses (Chevalier and Deniau 1990). Actual movements are believed to occur during pauses in the tonic firing of the GPi/SNr GABA neurons (Gerfen and Surmeier 2011). When a relevant environmental stimulus is encountered or a goal-directed action is initiated, GPi and SNr cells pause or decrease their firing (Chevalier and Deniau 1990).

As noted above, the GPi/SNr send inhibitory signals to the thalamus thereby inhibiting the thalamic GLU (i.e., excitatory) projections to motor regions of the cortex (Haber 2003). Because the GPi and SNr receive inhibitory projections from the striatum and send inhibitory projections to the thalamus, in order for the cortex to receive excitatory signals from the thalamus, inhibition placed on thalamus by GPi/SNr must be removed. The GPi/SNr can be disinhibited by ‘activation’ of the direct pathway. The inhibitory output signal to GPi/SNr produces inhibition of GPi/SNr GABA neurons thereby reducing their inhibitory control of thalamus and thus allowing the thalamic GLU neurons to signal areas like the premotor cortex.

In contrast to the direct pathway, whereby increased corticostriatal input leads to increased behavioral output, activation of the indirect pathway has been hypothesized to decrease behavioral output (Freeze et al. 2013). Striatal projections to the GPe and from the GPe to GPi/SNr are inhibitory. When the GPe is inhibited, GPi/SNr GABA signals to thalamus increase. Additionally, the GPe connects to the subthalamic nucleus which sends GLU to the GPi (Haber 2003). Striatal signals to the GPe reduce the inhibitory influence of GPe on the subthalamic nucleus: this permits the subthalamic nucleus to send
excitatory signals to GPi/SNr thereby increasing inhibition placed on the thalamus (Alexander and Crutcher 1990). The striatal indirect pathway signals to the GPe thus contribute to the net inhibitory influence placed on the thalamus and on motor behavior.

The direct and indirect output pathways appear to have opposing effects on behavior (Alexander and Crutcher 1990). In an optogenetic study where stimulation of direct and indirect MSNs was paired with distinct reward presentations, mice avoided reward location paired with stimulation of the indirect pathway MSNs and preferred the reward location paired with stimulation of direct pathway D1R-expressing MSNs (Morita et al. 2013). This suggests that opposing effects of direct versus indirect pathway activities may not only pertain to motor behavior but also to the processing of reward information.

Although the NAc contains D1R- and D2R-expressing MSNs in proportions similar to the dorsal striatum (Matamales et al. 2009) and extends its main output terminals to the GPe and VP (Groenewegen et al. 1999; Haber 2003; Shiflett and Balleine 2011), it is not clear whether NAc outputs segregate into direct and indirect pathways in a manner analogous to that described above for the dorsal striatal outputs. About 40-50% of D1R-expressing NAc MSNs project to the VP (David et al. 2005). The GABAergic projection from NAc to VP has been hypothesized to underlie "integration of motivation into behavior" (Mogenson et al. 1980) as well as hedonic responding for rewards (Smith and Berridge 2005; Smith et al. 2009). Nicola (2007) has characterized the VP as being similar to GPi and suggests that projections from the NAc may have separate direct- and indirect-like paths through basal ganglia circuits (Nicola 2007). Future work will need to reveal whether the direct/indirect pathways scheme holds for these ventral striatal outputs.

Loop arrangement of basal ganglia circuits gate information to the cortex

Basal ganglia networks are organized in arrangements described as segregated loops (Alexander et al. 1986). For instance, the DLS participates in a segregated ‘motor’ loop which includes corticostriatal, striatothalamic, and thalamocortical connections (Alexander et al. 1986). Functional topography from cortex to striatum is maintained in the striatal connection to the GPi/SNr, from the GPi/SNr to thalamus, and from thalamus to cortex (Haber 2003).
The circuit loops permit information flow to the cortex. No matter the type of circuit, thalamic nuclei form the “last links” in the loops (Haber 2003) and influence specific cortical regions. The NAc, for example, is in a communication circuit with the PFC and sends output signals to the VP (Mogenson et al. 1980; Heimer et al. 1997; Haber 2003). VP projects to the thalamus from where subsequent thalamic signals are sent back to the PFC (Heimer et al. 1997). Projections from thalamic nuclei are directed at some of the same cortical areas which input the striatum (Alexander et al. 1986; Alexander and Crutcher 1990). Therefore, the ‘motor’ circuit and multiple other basal ganglia circuits dedicated to various areas of the cortex (Alexander and Crutcher 1990) can all be conceptualized as “partially-closed circuit loops” (Alexander et al. 1986) that transmit information regarding behavior back to the cortex.
1.1.2 Striatum and behavior

Striatal neurons respond phasically during reward-related behavior

Body movements

Physiological responses of striatal MSNs correlate strongly with behavioral measures. Numerous studies have associated changes in striatal activity with body movements, limb actions (Crutcher and DeLong 1984; West et al. 1990; Carelli and West 1991), and reward-related behavioral performance (Schultz et al. 1992; Hollerman et al. 1998; Nicola et al. 2004; Samejima et al. 2005). Individual MSN activity linked to behavioral events is phasic; that is, movement-related neuronal responses occur on the order of seconds or less. Although striatal interneurons can significantly influence striatal MSNs (Mallet et al. 2005), for simplicity, MSNs, which are the striatum’s most frequently encountered neurons (Matamales et al. 2009) and its only output mechanism, are the primary focus of the following sections.

Striatal MSNs become activated during specific limb and facial movements, and cells dedicated to movements of particular body parts are clustered together (DeLong et al. 1984). The arrangement of neurons that respond in a time-locked manner to movement of any one body part (e.g., face, head, neck, shoulder, trunk, or limbs) extends topographically throughout the lateral striatum (Carelli and West 1991; Cho and West 1997). Compared to limb-related movements, clusters of cells relating to face and head movements are located ventrolaterally (Crutcher and DeLong 1984; Cho and West 1997). Concentrated in the DLS are the limb-related neurons while the more medial portions of the striatum are largely devoid of these cells (West et al. 1987) and of the dense motor cortex innervation (that the DLS receives) (Kunzle 1975).

A differential pattern of motor cortical input to regions of the striatum corresponds to regional differences in directional movement coding within the striatum. The dorsal striatum, particularly the DLS, receives ipsilateral inputs from motor cortices and gets additional contralateral motor cortex input (Flaherty and Graybiel 1993). Most limb-related neurons in the DLS encode contralateral movements (West et al. 1990). In contrast to the contralateral movement coding of these dorsal cells, NAc neurons sensitive to directional movements in an appetitive operant task do not display such lateralization bias.
(Taha et al. 2007). This lack of lateralized neuronal coding likely relates to the fact that the NAc receives no direct motor cortex innervation (Kunzle 1975).

**Movement planning**

Striatal neurons exhibiting changes in activity during a delay prior to movement initiation are implicated in movement planning. In many cases, this neural activity is sensitive to the direction of movement and is therefore believed to underlie the planning of directional movements. For instance, some NAc neurons become activated during the delay prior to movement initiation in a directional operant task (Taha et al. 2007). The selective activation during the task delay suggests that these NAc neurons ‘anticipate’ the upcoming movement direction (Taha et al. 2007). In the DMS, pre-movement activations of neurons tuned to saccade direction are tied to planning of directional eye movements (Lau and Glimcher 2007). Interestingly, direction-sensitive saccade neurons are less likely to be reward-responsive which suggests that eye movement planning may be governed by a select population of DMS neurons that do not process rewards (Lau and Glimcher 2007). The DLS also contains a groups of neurons that are only sensitive to upcoming movement direction; the rest of DLS is either sensitive to expected reward probability or has a combined sensitivity to both parameters (Pasquereau et al. 2007).

Because some activations occur irrespective of whether the movement is rewarded, they appear to encode movements or their planning rather than reward expectation. Many neurons in the anterior portions of DMS, DLS, and ventral striatum of primates performing a delayed go/no-go task discharge in response to task cues and prior to reward delivery (Hollerman et al. 1998). In this task, the instructional stimulus informs subjects a) whether a behavior should be performed or withheld and b) whether a reward (or merely an auditory cue) will be delivered as a result of the behavior. A triggering cue notifies when to perform the behavioral reaction and is an important factor in movement initiation. Data gathered by Hollerman et al. (1998) indicate that over one third of all task activations occur around the time of the triggering cue, and about half of such activations occur in movement trials only (Hollerman et al. 1998).

**Reward expectation**

Some striatal neurons encode behavioral reactions only when a reward is expected. During go/no-go task performance, these neurons exhibit activations that precede the instructed behavior but
only on trials when subjects expect to receive a liquid reward (rather than a sound) after the movement (Hollerman et al. 1998). The sustained pre-movement activations in response to the informative instructional stimulus are proposed to reflect reward expectation but were also dependent upon the planned movement (Hollerman et al. 1998). So, for instance, some neurons were activated prior to the instructed performance but not the instructed withholding of movement and only on trials when the behavior was expected to be followed by a reward. Other neurons were activated prior to the instructed withholding and not the performance of the movement and only on the to-be-rewarded trials (Hollerman et al. 1998).

Striatal activations are modulated by reward information. Some activations are selective for the reward probability expected prior to movement initiation (Samejima et al. 2005; Pasquereau et al. 2007). The probabilistic reward context signaled by target cues indicating the reward probability associated with a particular movement direction appears to modulate the pre-movement activation of DLS neurons (Pasquereau et al. 2007). In addition to the DLS, a significant portion of DMS neurons also encodes motor actions based on reward probabilities (Samejima et al. 2005). Numerous other studies hint at the possibility that striatal MSNs integrate expected value of the outcome into their activity (Hollerman et al. 1998; Hassani et al. 2001; Cromwell and Schultz 2003). For example, in a version of go/no-go task where the instructional stimulus indicated which reward amount was to be expected in a trial, about half of all activations exhibited differential pre-movement responses that depended upon the expected reward magnitude (Cromwell and Schultz 2003). The possibility that the expected reward value and movement encoding can become integrated by individual striatal MSNs is in agreement with the idea that striatal MSNs integrate information from multiple sources.

Nicola (2004) has hypothesized that striatal neurons perform integrations that allow for the encoding of motor responses driven by stimuli predicting that a motor response can result in reward (Nicola et al. 2004). For example, the major role of NAc neurons appears to fall on the encoding of movements made in order to obtain predictable rewards. No changes in NAc neuronal activity are detected when animals make operant responses in the absence of the reward-predictive stimulus (Nicola et al. 2004; Ambroggi et al. 2011). Evidently, NAc neuronal responses linked to rewarding behaviors depend upon the reward-predicting cue.
Most NAc activity patterns are governed by sensory information predicting rewards or signaling their availability (Nicola et al. 2004). In a modified version of the delayed go/no-go task where different rewards were signaled by different instructional stimuli, specific reward expectation produced differential effects on pre-movement activations (Hassani et al. 2001). The task-related activations were reliably of larger or smaller magnitude for preferred compared to non-preferred rewards and occurred not only in response to instructional stimuli but also in response to the rewards themselves (Hassani et al. 2001). Thus, reward preference is reflected in the activations that encode reward expectation or reward detection differentially.

Striatal MSNs may process rewards differentially based on the input information they receive. The OFC, which sends terminals to the striatum (Selemon and Goldman-Rakic 1985; Groenewegen et al. 1999), also processes reward expectation and preference (Tremblay and Schultz 1999; Tremblay and Schultz 2000). The idea that striatal neurons can be tuned to specific rewards is supported by findings showing that NAc neurons activated by natural reinforcement are not readily activated when animals are switched to reinforcement with a drug (Carelli 2002). Carelli (2002) has proposed that reinforcement with different classes of rewards is encoded by distinct neuronal population within NAc (Carelli 2002).

*Expected outcome value is conveyed to the NAc by limbic afferents*

 Preferential input from OFC to the NAc (Selemon and Goldman-Rakic 1985; Haber and Knutson 2010) is hypothesized to signal reward events (Ostlund and Balleine 2007). The OFC is activated upon the rewarding outcome regardless of whether the outcome was predicted and regardless of movement contingencies (Tremblay and Schultz 2000). However, sustained activation of OFC during reward delays suggests that OFC also conveys reward expectation to its targets (Furuyashiki and Gallagher 2007).

The NAc also receives major amygdaloid projections (Wright et al. 1996; Fudge et al. 2002; Fudge et al. 2004) which contribute to signaling the expected reward value. Electrophysiological recordings suggest that BLA neurons preferentially encode stimuli with reward values, and inactivation of the BLA disrupts behavioral responding driven by reward-predictive cues (Ambroggi et al. 2008). Consolidation of stimulus-reward associations relies on the intact function of both BLA and OFC (Delamater 2007). Lesions of either region render rats insensitive to outcome devaluation (Delamater 2007; Ostlund and Balleine 2007). This insensitivity suggests that the neural network has become
incapable of updating the outcome; therefore, both the BLA and OFC contribute to signaling expected reward outcomes. Based on a neural model, it was inferred that OFC assigns values to particular stimuli by accessing values of specific rewards and categories of reward types encoded in the amygdala (Grossberg et al. 2008).

Prefrontal, amygdaloid, and hippocampal projections to the NAc (Groenewegen et al. 1999) participate in signaling reward magnitude information. By eliciting excitatory activity along the BLA-to-NAc projection during an operant task, Stuber et al. (2011) provided evidence that activation of this pathway is sufficient for positive reinforcement (i.e., animals reliably performed operant responses in order to obtain experimentally delivered stimulation of this projection) (Stuber et al. 2011). This result is consistent with reports implicating the BLA in processing reward value; however, stimulation of the dense ventral hippocampal projection to NAc shell likewise supports operant responding and may thus convey additional incentive information (Britt et al. 2012). Animals also perform operant responses to receive optogenetic stimulation of the PFC-to-NAc projection (Britt et al. 2012). Lesion studies suggest that BLA (Ostrander et al. 2011) and medial PFC (Gill et al. 2010) are required for normal levels of effortful responding for rewards of large magnitude. Rats with lesions of these limbic centers instead choose low-effort options that yield smaller rewards (Gill et al. 2010; Ostrander et al. 2011). This behavioral disruption results from abolished incentive value processing produced by the limbic lesions.

Although GLU signaling to the striatum supports operant responding on its own (i.e., animals perform nose-pokes to receive stimulation of the hippocampal, BLA, or PFC-to-NAc shell projections which all release GLU in NAc) (Stuber et al. 2011; Britt et al. 2012), elicitation of excitatory postsynaptic currents along the DA projection from the VTA facilitates behavioral responding only when natural reward is also available (Adamantidis et al. 2011). Such findings place the limbic GLU projections at the forefront of reward-affect processing; however, animals also perform behavioral responses to receive excitatory stimulation of striatal neurons directly (Britt et al. 2012). Thus, it cannot be ruled out that activities at both DA and GLU receptors located on striatal neurons (David et al. 2005) contribute to the motivational effects. Excitatory synaptic input (i.e., GLU) and neuromodulation (i.e., DA) both factor into the depolarization of striatal neurons (Wilson 1992; Plotkin et al. 2011). It is possible that motivational effects of primary and conditioned rewards depend upon the overall amount of striatal depolarization (i.e., the
combined influence of both GLU and DA on striatal neurons contributes to the motivation to perform behavioral responses for rewarding outcomes).

**Differential NAc responses to rewarding and aversive US**

The NAc receives (Zahm 2000) and differentially processes pleasurable and aversive valence associated with affective (Carlezon and Thomas 2009) and behavioral states (Roitman et al. 2005; Roitman et al. 2010). NAc neurons are primarily inhibited during reward and excited during aversive events (Roitman et al. 2005). Because learning systems permit environmental stimuli to acquire conditioned properties (Sutton and Beninger 1999), NAc neurons develop inhibitions to the conditioned stimulus (CS) associated with a rewarding unconditioned stimulus (US). As a result, a CS-US bond forms. Accordingly, the time-locked responses to an aversive CS are excitatory (Roitman et al. 2005). The hedonic values of the outcome and the environmental stimulus are encoded and tracked by strikingly differential NAc responses.

The mechanism behind NAc's differential neuronal responses to rewarding versus aversive events is not fully understood. NAc neurons are known to receive both segregated and convergent inputs from the PFC and OFC (Groenewegen et al. 1999). The NAc neurons with evoked excitation responses to stimulation of either pathway tend to respond synergistically when the PFC and OFC are simultaneously activated (Asher and Lodge 2012). Interestingly, the NAc neurons with evoked excitations only to PFC stimulation become inhibited with pre-stimulation of the OFC (Asher and Lodge 2012). Conversely, pre-stimulation of the PFC produces inhibitory neuronal responses in the NAc neurons that are otherwise activated by OFC stimulation only (Asher and Lodge 2012). Although this may be a potential mechanism for differential NAc neuronal responses, this finding, alone, does not explain the plasticity behind NAc's processing of hedonic valence or how the direction (excitation versus inhibition) of the NAc neuronal response can be modulated by learning. Roitman et al. (2010), for instance, showed that NAc neurons with previous time-locked inhibitions to a sucrose reward switch their responses to excitation when conditioned taste aversion is experimentally induced (Roitman et al. 2010).

Striatal neurons appear to be importantly involved in the coding for reward events. For instance, in operant tasks, striatal MSNs respond phasically at reward delivery time (Schultz et al. 1992; Hollerman et al. 1998; Nicola et al. 2004), and these responses are differential for specific rewards (Hassani et al. 20
and reward amounts (Cromwell and Schultz 2003). Additionally, phasic NAc responses occur at the time of a rewarding Pavlovian US (Wan and Peoples 2006) and even during non-contingently delivered reward (Roitman et al. 2005). Because no behavior was required for reward procurement in the latter example, these activations seem to link to reward exclusively. Alternatively or additionally, such phasic responses encode the oral muscle activity associated with passive liquid sucrose ingestion (e.g., swallowing). As noted above, the majority of the phasic responses associated with delivery of a rewarding US are inhibitory (Roitman et al. 2005; Wan and Peoples 2006).

**Phasic NAc responses relating to food reward consumption**

Also consistent with an inhibitory NAc response to food reward, NAc neurons are more frequently inhibited than excited at the time that animals consume rewards after an operant lever-press (Ambroggi et al. 2011) or nose-poke (Nicola et al. 2004). At the time of unrewarded food receptacle entry, neurons that exhibited sustained responses during rewarded trials do not exhibit such responses (Nicola et al. 2004). The sustained inhibitions in NAc MSNs at the time of reward procurement have been postulated to disinhibit consummatory motor actions in order to allow animals to engage in ingestion (Nicola et al. 2004; Nicola 2007).

If the consumption-related NAc inhibitions are occurring in the direct pathway NAc output MSNs, then Nicola’s proposal on disinhibition of consumption (Nicola 2007) seems to contradict the classic stance on behavioral disinhibition which assumes that movements occur during pauses in the firing of basal ganglia GABA neurons located in the main output nuclei (Chevalier and Deniau 1990). If the direct pathway GABAergic output MSNs are inhibited, basal ganglia neurons are less likely to pause their firing which would instead result in behavioral inhibition. To get around this contradiction, one must either assume that the consumption-related NAc inhibitions occur 1) in the D1R output pathway to suppress behaviors that oppose consumption, 2) that they occur only in the indirect pathway MSNs to disinhibit consummatory actions, or 3) that they communicate out through an alternative output mechanism. Kelley (2004) hypothesized that some NAc neurons, particularly those located in the medial NAc shell, output to the lateral hypothalamus which has connections to the motor pattern generator units located in brainstem systems that control motor actions involved in feeding (e.g., chewing) (Kelley 2004).
Alternating patterns of NAc activations are believed to govern action selection (Nicola 2007). It is proposed that in order to promote proper actions associated with either the appetitive or the consummatory behavioral state, NAc neurons alternate their phasic excitatory and inhibitory activity patterns (Nicola 2007). Many of the neurons that become excited during reward-predictive cues, sustain their excitations during operant responses and then become inhibited during consumption of the food reward (Nicola et al. 2004). These neurons are believed to facilitate the "appetitive to consummatory switch" (Nicola et al. 2004; Nicola 2007) utilized by behavior. This proposition is supported by the ethological assumption that animals have no need to engage in consummatory actions when no rewards are available and are less likely to seek rewards at the time of ingestion. When neurons with consumption-related inhibitions switch their activity to excitation, ingestion is inhibited and reward seeking resumes. The idea that NAc excitations relate to reward-seeking behavioral states falls in line with the behavioral disinhibition theory of basal ganglia circuits.

**NAc MSNs guide appetitive behavior driven by reward-paired cues**

*Behavioral responses driven by reward-paired cues*

The NAc plays a key role in expression of behavior driven by the rewarding stimuli which activate NAc neurons (Nicola et al. 2004; Ambroggi et al. 2011). Under normal conditions, many NAc neurons become inhibited at the time of a rewarding operant response, but many of these neurons are first excited by discriminative stimulus (DS) presentation (Nicola et al. 2004). Also, the magnitude of the operant-related neuronal response is enhanced if the operant response is performed during the DS duration (Nicola et al. 2004). Reward-predictive cues, thus, exert a powerful influence on the same neurons that exhibit firing related to operant actions. Loss of this neuronal communication due to NAc inactivation appears to disrupt the ‘translation’ of motivational signals into behavior.

Loss of NAc activity can resemble the loss of previously stored reward-predictive information (accessible to motor functions). Lesions of NAc reduce behavioral expression of CS-US associations in rats that were trained prior to receiving the lesion (Parkinson et al. 1999; Cardinal et al. 2002), and inhibition of NAc with a GABA agonist in rats that have been trained drug-free produces similar disruptions in expression of conditioned approach (Blaiss and Janak 2009). These findings suggest the
NAc is a reward-predictive memory locus or that NAc retrieves this information when animals perform learned motor actions for rewards.

Appetitive learning depends upon excitatory neurotransmission within NAc. By pretreating animals with ionotropic GLU antagonists prior to training sessions during the early learning phase of a lever-press task, Hernandez et al. (2005) showed that activation of NAc α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and NMDA receptors while animals are engaged in the learning context is required for the encoding of instrumental memory (Hernandez et al. 2005). Animals with intra-NAc AMPA or NMDA receptor blockade required more training sessions to reach optimal performance levels (Hernandez et al. 2005). Permanent lesions of NAc core similarly impede acquisition of lever pressing (Corbit et al. 2001). Perhaps the abnormal learning results from a reduced ability to encode associations involving the reinforcing outcome. The lever itself can serve as an optimizing stimulus as its features and location can become associated with the reward and the behavioral response. In a Pavlovian discriminated approach task where the CS+ was a 10 sec insertion of a lever into the testing chamber, animals typically learn to approach the CS+ lever in order to obtain the noncontingently delivered food reward (Cardinal et al. 2002). Inactivation of NAc with an NMDA antagonist impairs acquisition of this discriminated approach behavior: animals do not acquire discrimination of CS+ from CS- (which in this case was another lever but with no reward association) (Di Ciano et al. 2001). Proper formation of the CS-US association is required for normal levels of discriminative approach which NAc inactivation apparently disrupts.

Subregions of the dorsal striatum play differential roles in the shift from goal-directed to S-R instrumental performance

The DLS is particularly important for acquiring S-R associations (Devan et al. 2011). In a task where rats normally learn to discriminative lever-pressing for a CS+, subjects that received DLS lesions prior to training were severely impaired in S-R acquisition; these DLS-lesioned rats exhibited lower levels of CS+ responding and thus nonoptimal instrumental behavior (Featherstone and McDonald 2004). Unlike DLS-lesioned subjects, rats with DMS or sham lesions learned to increase their responding during CS+ presentation (Featherstone and McDonald 2004). This suggests that an intact DLS allows for the formation a normal S-R association. To make sure that the DLS impairment was in fact S-R-related and
not resulting from loss of association with the rewarding outcome, authors also tested DLS-lesioned subjects for their ability to learn conditioned place preference and found that DLS lesions do not impair the learning of the association between the environmental context and the reward (Featherstone and McDonald 2004).

Two sources of motivational control give rise to 1) acquisition of goal-directed behavior governed by the representation of the outcome and behavioral response and 2) habit acquisition where behavioral actions become controlled by the association between the stimulus and behavioral response (Balleine et al. 2009; Balleine and O'Doherty 2010). Habit learning is composed of a shift in behavior from outcome mediation to direct elicitation by the reward-predictive stimuli (Ashby et al. 2010). Yin et al. (2004) used a (variable interval) schedule of reinforcement which renders the rats’ behavior habitual then devalued the rewarding outcome and measured persistence of responding during an extinction test. The investigators hypothesized that formation of an S-R association relies on DLS processing and that lesions of DLS should increase sensitivity to outcome devaluation because animals would not be able to rely on the S-R processing mechanism destroyed by the lesion (Yin et al. 2004). DLS-lesioned rats did in fact show sensitivity to goal devaluation at a stage of training when control animals had become habit-like in their behavior, i.e., insensitive to outcome devaluation; in contrast, lesions of the DMS resulted in enhanced S-R habit formation (Yin et al. 2004). These findings suggest that DLS is concerned with formation of habits defined by the S-R bond while the DMS processes outcome-response associations.

The role of DLS in S-R learning relates to the nature of its inputs. The type of information the DLS receives is more stimulus-related than outcome-related (Horvitz 2009). For instance, the DLS primarily receives projections from sensorimotor regions of the cerebral cortex rather than the more anterior frontal cortical inputs sent to the DMS (Selemon and Goldman-Rakic 1985).

Behavior becomes habitual as a function of training length, and the DMS and DLS appear to mediate this progression differentially (Ashby et al. 2010). Although it has been proposed that real-time skill-acquisition processing by the DMS and DLS proceeds in parallel (Yin et al. 2009), simultaneous recordings of DMS and DLS MSNs during t-maze learning indicate that activity in these two regions diverges into opposite patterns during early training (Thorn et al. 2010). The DMS processes the outcome-response association (Balleine and O'Doherty 2010) and is therefore critically engaged early in
learning. Recordings from primates learning a procedural button-press task suggest that the DMS becomes activated during performance of new sequences of button-presses and that this neuronal activity is mostly absent during performance of already learned button-press responses (Miyachi et al. 2002). More abundantly found in the DLS are the neurons that are preferentially responsive during already learned button-press sequences (Miyachi et al. 2002) and neurons that exhibit greater activity during the later training periods when the skill behavior is already habitual (Yin et al. 2009). Interestingly, recordings made from DLS neurons of rodents learning to perform a t-maze task show that activity of DLS neurons correlated with improved performance becomes more refined throughout training (Barnes et al. 2005; Thorn et al. 2010).

Activity in the DLS is not only necessary for the transition from goal to S-R mediation but also for the expression of already learned S-R behavior (Ashby et al. 2010). Inactivation of the DLS has been shown to disrupt the performance of previously learned button-press sequences (Miyachi et al. 1997). Thus, DLS is not just critical at encoding S-R memories but may also store or retrieve them. Featherstone and McDonald (2005) evaluated the effects of DLS lesions on the expression of the S-R bond in previously learned instrumental CS+/CS- discrimination task. DLS-lesioned rats, unlike DMS- and sham-lesioned subjects, omitted the greater portion of trials after receiving the lesion which suggested that they had difficulty responding to the previously learned CS+ (Featherstone and McDonald 2005).
1.1.3 Dopamine and behavior

**DA neurons report salient environmental occurrences**

Over the span of multiple decades, DA has been implicated in an array of functions ranging from its role as a subjective mediator of the hedonic impact of rewards (Wise 1982) to its more objectively-defined function of serving learning and performance systems as a synapse-strengthening (Wickens et al. 2003) or time-stamping mechanism (Coull et al. 2011).

Early work on the role of DA in reward evaluated effects of DA antagonists on reward function and suggested that DA primarily mediates hedonia (Wise 1982). DA antagonists produced within-session performance decrements resembling extinction of behavior for primary rewards (Wise 1982; Wise 2004) and blocked conditioned place preference to the environment with which their administration was paired (Berridge and Robinson 1998). Although many findings supported the idea that DA antagonists blunt the rewarding impact of reinforcement (Wise 1982; Berridge and Robinson 1998; Wise 2004), the effects DA lesions had on taste reactivity responses for food reward (presumably reflecting an affective state) were in discord with this hypothesis. Casting doubt on many of the original stipulations about DA’s role as the key mediator of pleasure in the brain, DA lesions surprisingly failed to affect and even enhanced rewarding-taste reactivity (Berridge and Robinson 1998).

Single neuron data also support the notion that DA neurons are unlikely to transmit ‘reward value’ to their targets. In 1998, Schultz reported that DA neurons discharge in response to unpredictable rewards (Schultz 1998). However, upon repeated exposure to the reward and the experimental context, DA neurons with instant neuronal discharges to the primary reward developed time-locked responses to its earliest predictor (i.e., a CS) and failed to discharge at the time of the reward (Schultz 1998; Fiorillo et al. 2003). Unless one assumes that predicted rewards lose all hedonic value, the fact that predicted rewards do not activate DA neurons (Schultz 1998; Fiorillo et al. 2003) argues against the view that DA neurons code reward values. The shift of the time-locked DA responses to CSs signaling the impending reward delivery suggests that DA neurons are capable of calculating or accessing predictions about appetitive events.
Interestingly, when a predicted reward is withheld, inhibitory activity occurs in DA neurons at the time that the reward was expected (Schultz 1998; Schultz 2013). On the basis of this electrophysiological result and those described above, Schultz (1998) yielded the “reward prediction error hypothesis” by suggesting that DA system can process differences between received and predicted rewards (Schultz 1998). Optogenetic work has demonstrated that about half of all VTA neurons behave in a manner consistent with reward prediction error processing (Cohen et al. 2012) described by Schultz (1998). The finding that DA neurons are inhibited at the time of unexpected reward omission demonstrates that their reward-related predictions are in fact time-sensitive (Bermudez and Schultz 2014).

Several pieces of empirical evidence suggest that the DA system may signal important environmental events which lack obvious subjective reward information. For example, DA neurons respond to unexpected events with a latency that precedes any possible eye gaze to a reward location in the environment (Redgrave and Gurney 2006). Latency to perform a shift in eye gaze is about 150-200 milliseconds, and DA neurons respond with a latency of <100 msec. Horvitz et al. (2007) has proposed that this is not enough time for the nervous system to deduce whether an event is rewarding (Horvitz et al. 2007). DA responses consistent with post-saccadic latencies of >200 msec have not been reported (Redgrave and Gurney 2006). Additionally, some DA neurons discharge in response to nonrewarding stimuli such as loud sounds and light flashes (Horvitz et al. 1997; Horvitz et al. 2007; Lammel et al. 2014) which suggests that DA neurons are tuned to environmental saliency. Phasic responses to unconditioned auditory stimuli have also been detected in the DLS (Zhong et al. 2014) which gets midbrain DA input and sends reciprocal output back to the midbrain (Haber 2003). The precise role of reciprocal connections between the midbrain and the striatum in saliency processing, however, remains elusive.

Although the DA system is preferentially activated by appetitive events (Mirenowicz and Schultz 1996; Schultz 2013), under some conditions aversive stimuli do elicit DA release within its target region NAc (Wilkinson et al. 1998; Young 2004). Others have reported that primary and conditioned aversive stimuli either fail to activate DA neurons or produce neuronal responses that are weaker than the reward-related firing (Mirenowicz and Schultz 1996). Nonetheless, large increases in NAc DA concentration during footshock presentation have been reported; however, these DA responses declined with subsequent shocks while the DA response to the CS emerged over the course of aversive conditioning.
(Young 2004). Interestingly, another study detected no increases in extracellular NAc DA the first time a CS was presented with footshock, but the CS paired with footshock did later elicit increased DA within NAc (Wilkinson et al. 1998). According to Roitman et al. (2008), the motivational valence of primary reward and aversive events produces differential subsecond NAc DA responses. When rats ingest a liquid reward, NAc DA concentrations increase phasically; however, when given an unpleasant oral liquid, DA release within the NAc seemingly decreases (Roitman et al. 2008). The exact mechanism of how midbrain firing or lack of firing during aversive events contribute to neurochemical responses within NAc has not been elucidated.

The role of the DA system in aversive processing remains unclear. Mirenowicz and Schultz (1996) found that only a few DA neurons were phasically activated by aversive events (Mirenowicz and Schultz 1996). However, given that a single DA neuron can influence 2.7-5.7% of striatal neurons via its extensive axonal arborizations (Matsuda et al. 2009), the firing of even a few DA neurons may significantly influence DA concentrations in the striatum. Nevertheless, claims that excitation of DA neurons is actually caused by the termination rather than onset of an aversive stimulus (Lammel et al. 2014) have complicated the identification of a unitary DA mechanism in aversive processing. Additionally, many studies report that DA neurons are inhibited by aversive cues and during primary aversive events (Schultz 2013). Schultz (1998) has proposed that the DA system processes aversive stimuli on a slower time scale compared to rewarding ones (Schultz 1998); others have suggested that VTA neurons with aversive responses may not always be of DA origin (Lammel et al. 2014). About half of all VTA neurons are activated during the delay between the predictive cue and the outcome (Cohen et al. 2012). This special subset of VTA neurons is made up of DAergic neurons that increase their firing rate for rewarding events only and of GABAergic neurons that increase their activity in both rewarding and aversive instances (Cohen et al. 2012).

**DA in the NAc promotes expression of appetitive behavior**

Studies employing systemic blockade of DA receptors have suggested that DA transmission in the brain is critical for performance of behaviors driven by reward-paired stimuli (Sutton and Beninger 1999). Loss of D1R transmission increases latency for both Pavlovian approach and operant lever-
presses to reward-paired cues (Choi et al. 2009). In contrast, D2R blockade spares responses to reward-paired cues but increases duration of individual response elements (e.g., the amount of time between a head entry and exit from the food compartment or the duration of individual lever-presses) (Choi et al. 2009). It is possible that D2R blockade impairs the termination of the behavioral response. This may be relevant to the role of the indirect D2R-expressing basal ganglia output stream in inhibition of motor output (Alexander and Crutcher 1990).

Under some conditions, DA transmission within the NAc is required for the expression of reward-directed behavior. In these cases, the VTA-to-NAc DA projection appears to critically mediate behavioral performance. For instance, inhibition of VTA neuronal firing or blockade of DA transmission in the NAc reduce the performance of previously learned lever-pressing for food (Yun et al. 2004). Conversely, optogenetically delivered stimulation of VTA neurons reliably elicits NAc DA release similarly to that elicited by food reward (Adamantidis et al. 2011) and establishes conditioned place preference (Tsai et al. 2009) traditionally produced by primary rewards.

NAc DA promotes the performance of reward-directed behavior guided by reward-paired stimuli (Parkinson et al. 2002; Lex and Hauber 2008). Reward-predictive cues (e.g., DS) evoke NAc excitatory neuronal responses, and these NAc response precede reward-seeking behavior (Nicola et al. 2004). Approximately 10 minutes after delivery of a D1R antagonist into the NAc, rats a) reduce their lever-pressing for food reward signaled by a DS (du Hoffmann and Nicola 2014), b) show increased latencies to respond to an appetitive DS, or c) fail to respond to the DS altogether (Yun et al. 2004; Yun et al. 2004; Nicola et al. 2005; du Hoffmann and Nicola 2014). NAc DA therefore appears to be necessary for eliciting the NAc neuronal activity that drives cued behavior (i.e., DA is causal to the reward-seeking response) (Nicola et al. 2005).

Interestingly, NAc D1R blockade leaves the performance of a tightly-linked chain of appetitive behavior unaffected but at the same time reduces the tendency of animals to return to the reward-related operandum to initiate another chain of behavioral responses (Nicola 2010). Thus, DA within NAc has been described as mediating “flexible approach” behavior when there are pauses in rewarding actions in-between DS presentations (Nicola 2010). Loss of DA transmission at NAc D1Rs reduces reward-related approach thereby lowering chances of rewarding actions subsequent to the approach behavior.
Both loss of NAc DA transmission (Di Ciano et al. 2001; Parkinson et al. 2002) and lesions of NAc (Parkinson et al. 1999; Cardinal et al. 2002) reduce the ability of a previously food-paired CS to elicit Pavlovian approach; however, DA transmission also enables CSs to selectively enhance instrumental performance (Dickinson et al. 2000). This effect appears to depend upon activation of NAc DA. Unlike animals with intact DA transmission, animals with blockade of either D1Rs or D2Rs in the NAc fail to elevate their instrumental responding when presented with a CS previously paired with food reward during Pavlovian training (Lex and Hauber 2008).

DA activity in the NAc may help optimize rewarding behavior by signifying appetitive value to cues. In 1997, Swanson et al. infused DA directly into the NAc shell and found that feeding behavior increased (Swanson et al. 1997). Specifically, rats with enhanced NAc shell DA transmission spent more time feeding and had a greater number of feeding bouts during the session (Swanson et al. 1997). Over a decade later, Faure et al. (2008) found that abolishing excitatory transmission within the NAc shell by blocking AMPA receptors also increases feeding (Faure et al. 2008). More importantly, when D1R and D2R antagonists were combined with an AMPA antagonist and were infused into the NAc shell, generation of feeding behavior, typically observed after local GLU disruption, became suppressed (Faure et al. 2008). Faure et al. (2008) interpret these findings to suggest that under natural conditions, in order to correctly tag environmental stimuli as appetitive, DA indirectly inhibits AMPA-evoked GLU transmission in select regions of NAc (Faure et al. 2008). When NAc DA transmission is intact but ionotropic GLU transmission is blocked, animals make incorrect instrumental responses and abnormally elevate behavioral responding to nonrewarding cues (Di Ciano et al. 2001; Yun et al. 2004; Ambroggi et al. 2011). Therefore, when NAc GLU transmission is inadequate, DA abnormally tags neutral in-session cues as appetitive thus increasing reward-related actions even if those actions are unrewarded.

Convergence between DA and GLU in the striatum provides a framework for integrating DA signals with sensory and motivational signals and for generating motor output (Horvitz 2002; Wickens et al. 2003; Horvitz 2009). For instance, NAc DA may gate BLA-signaled emotive sensory information to proper output MSNs. The BLA is believed to underlie associations between sensory features and emotional feedback (Balleine and O'Doherty 2010). BLA and midbrain DA projections interact critically within NAc in order to promote reward-seeking behavior (Ambroggi et al. 2008). Similar to the effects of
intra-NAc D1R antagonists, inhibition of BLA reduces conditioned approach and increases latency to respond to the DS (Jones et al. 2010). Given that DS-related neuronal responses in the BLA precede those of NAc, the BLA-to-NAc projection appears to drive the NAc neuronal responses to reward-predictive cues (Ambroggi et al. 2008). Disconnecting the NAc from the BLA disrupts both the NAc neuronal responses and behavioral responding driven by the DS (Ambroggi et al. 2008; Jones et al. 2010). Thus, the NAc DS-excited neurons appear to be activated by this specific afferent connection. DA may facilitate the selective activation of such MSNs via its neuromodulatory effects in the NAc.

DA in the NAc appears to mediate differential behavioral responses driven by the discrimination of differential incentive predictions. Although administration of the indirect DA agonist amphetamine into the NAc core decreases latency to respond to cues predictive of the upcoming reward magnitude, the accuracy of cue discrimination becomes severely impaired (Giertler et al. 2003). Normally, relative to cues signaling smaller rewards, larger magnitude reward cues are more rapid at eliciting behavioral responses. After receiving the intra-NAc DA agonist, animals are quicker to respond to the magnitude-signaling cues, but their responses are no longer driven by the size of the predicted reward magnitude (Giertler et al. 2003). Impairment of the selective invigoration of behavior by differential cues could be a result of the altered phasic DA signals within NAc due to intra-NAc amphetamine which elevates synaptic DA levels via blockade of DA reuptake. Normal NAc DA transmission is thus important for production of behavioral responses driven by cues signaling different reward expectations. Lesions of NAc are known to disrupt the ability of animals to preferentially choose delayed or uncertain rewards that are of larger magnitude (Cardinal et al. 2001; Cardinal and Howes 2005) which may relate to impaired processing of probabilistic cues signaling these types of rewards. After NAc lesions, rats behave as if the predicted outcome signaled by the probabilistic cue is less certain than it really is (Cardinal and Howes 2005) and are more likely to choose the poor immediate rewards instead of the usually preferred delayed rewards of larger magnitude (Cardinal et al. 2001). It could be argued that because of the potentially costly temporal expenditures, making behavioral responses for uncertain or delayed reinforcers is by nature more effortful. Therefore, a reduction in high effort expenditures may explain these lesion effects. Although NAc DA has been implicated in physical effort during behavioral performance (Nowend et al. 2001; Correa et
al. 2002; Salamone and Correa 2002; Salamone et al. 2007; Salamone et al. 2015), its role in the cognitive aspects of effort is less established.

Tasks that require demanding behavioral output are susceptible to loss of DA transmission. After depleting DA within NAc, Correa et al. (2002) found that rats were less willing to make responses on high requirement fixed ratio (FR) schedules of reinforcement (Correa et al. 2002). While responding for food pellets on the FR1 schedule of reinforcement was not affected by NAc DA depletion, the rate of responding on FR5 decreased substantially (Correa et al. 2002). Berridge and Robinson (1998) have hypothesized that during performance of rewarding actions, DA-release signals incentive salience. That is, DA communicates how much something is wanted (Berridge and Robinson 1998). It is possible that the signal incorporates ‘cost’ of the action that reward delivery is contingent upon into its function. The DS task lacks a ratio component but does require a response within the 10 sec DS; for the subjects, this could mean giving up another behavioral or cognitive activity in order to engage in an instrumental response. Therefore, responding on the DS task may be more taxing on subjects, as they are alerted of the imposed time to make reinforced responses: this constitutes a demand component absent in FR1 and raises susceptibility to the disruptive effects of D1R blockade. Additionally, frequent motor responses on the FR schedule of reinforcement may serve as reference stimuli that guide performance of subsequent actions even under NAc DA blockade. Interestingly, although intra-NAc D1R blockade increases reaction time and reduces instrumental responding for DSs (Wakabayashi et al. 2004; Yun et al. 2004; du Hoffmann and Nicola 2014), willingness to wait for reward delivery after a response to the DS has already been made remains intact (Wakabayashi et al. 2004).

In part supported by results suggesting that loss of DA transmission leads to an impairment in instrumental responding resembling a general state of satiety (Dickinson et al. 2000), earlier theories argued that DA disruptions affect “directional aspects of primary motivation” (Salamone and Correa 2002; Salamone and Correa 2012). However, rather than mediating the rewarding effects of primary reinforcement, DA appears to activate the production of effortful behavioral responses for primary rewards (Correa et al. 2002; Salamone et al. 2007; Bardgett et al. 2009; Ostlund et al. 2012; Salamone and Correa 2012; Salamone et al. 2015). Although the impairment after NAc DA depletion seems similar
to the effect prefeeding has on instrumental responding, the DA-related impairment does not result from a reduced rewarding value of the outcome which is what prefeeding accomplishes.

Several pieces of experimental evidence support this view. As mentioned above, NAc DA-depleted rats respond for primary rewards on FR1 without any disruptions (Correa et al. 2002). Additionally, systemic and intra-NAc D1R antagonists impair instrumental responding for food on FR5 and at the same time shift behavioral output to a concurrently available low-effort source of less preferred food (Nowend et al. 2001; Salamone et al. 2002). When the free source of food is concurrently available on FR5 or higher, the total amount of food consumed by rats with DA disruptions is comparable to the total amount of food consumed on FR5 under normal conditions (Salamone and Correa 2002). Thus, when DA is disrupted, behavioral output is still directed by primary motivation (Salamone and Correa 2002).

Unlike DA disruption, prefeeding or administration of an appetite suppressant reduces both consumption from freely available food source and from instrumental responding on high-effort reinforcement schedules (Salamone et al. 2002; Salamone and Correa 2002). In contrast, reduced DA function reliably shifts behavioral allocation from ‘costly’ or demanding options to low-effort alternatives without affecting the primary motivation (Salamone et al. 2015). Therefore, as a result of lost DA transmission, rats are simply less likely to engage in effortful or ‘costly’ instrumental behaviors.

The role of NAc DA in effortful performance may be extended to Pavlovian-like behavior. Rats with intra-NAc D1R blockade are less likely to initiate conditioned approach to a food source, but the total amount of food they consume is similar to pre-treatment levels (Baldo et al. 2002). NAc D1R blockade prolongs the feeding bout duration (Baldo et al. 2002) which may constitute a lower effort option inadvertently available during testing. The NAc along with its PFC and BLA afferents make up a regulatory system of effort-related behavioral operations (Salamone et al. 2007). In this system, DA may act as a real-time reinforcer of effortful behavioral allocations through its neuromodulatory actions on NAc MSNs and the motivation-related afferents that synapse with them.

**Activation of NAc D1Rs is required during appetitive learning**

A trace of cue-related DA release within NAc core may compose a neurochemical signature of appetitive learning. Food reward and food-predictive cues elicit DA release within the NAc core (Roitman...
et al. 2004; Brown et al. 2011) where the DA release in response to the cue signaling food availability becomes reliable across behavioral training as the cue gains its reward-predictive strength (Sunsay and Rebec 2008). Thus, as animals become familiar with associations between food reward and the stimuli that precede the food reward, phasic DA release shifts forward in time to correlate tightly with the occurrence of CSs (Berridge and Robinson 1998). This ‘evolution’ of NAc DA release in response to reward-predictive cues is consistent with the shifts and persistence of midbrain DA responses to the earliest environmental predictors of reward reported by Schultz (1998).

DA in the NAc plays an important role during acquisition of appetitive behaviors (Horvitz et al. 2007; Meredith et al. 2008). The region receives reward-related subsecond DA signals (Roitman et al. 2005), has a high D1R expression density (Bardo and Hammer 1991; Levey et al. 1993; Duffy et al. 2000), and is believed to be critically involved in acquisition and maintenance of associations between environmental stimuli and rewards (Di Ciano and Everitt 2003; Wise 2004). Both NAc DA depletion and selective blockade of NAc D1Rs impair the learning of discriminated approach behavior for a CS+ in a Pavlovian autoshaping task (Di Ciano et al. 2001; Parkinson et al. 2002). NAc DA lesions given prior to acquisition have a greater disruption on the output of Pavlovian approach than lesions given post acquisition (Parkinson et al. 2002). Therefore, NAc DA appears to have a more critical role in establishing rather than expressing learned appetitive behavior. It is then not surprising that intra-NAc D1R antagonist pretreatments disrupt acquisition of lever-pressing behavior for food reward (Hernandez et al. 2005).

Excitatory transmission along the D1R NAc output pathway allows for the formation of learned associations between temporally-related events like stimuli and rewards or actions (Kelley 2004). GLU and DA signaling on NAc output neurons together play a cooperative role in appetitive learning. Both NAc NMDA and D1R transmission are required for learning Pavlovian discriminated approach (Di Ciano et al. 2001), and co-activation of NAc D1Rs and NMDA receptors is necessary for instrumental appetitive learning (Smith-Roe and Kelley 2000). It is believed that the co-activation of D1Rs and NMDA receptors initiates cellular plasticity that underlies appetitive learning (Kelley 2004). Normal transmission at NAc D1Rs and NMDA receptors immediately after training is required for the early consolidation of appetitive instrumental memories (Dalley et al. 2005) formation of which is accompanied by and critically dependent upon protein synthesis within NAc (Hernandez et al. 2002).
Striatal GLU and DA interact through a functional, physiological, and molecular relationship (Morari et al. 1998; Kelley 2004; David et al. 2005). D1R activation enhances NMDA-mediated cellular events and AMPA-mediated excitatory postsynaptic currents (Nicola et al. 2000), and ionophoretic delivery of DA or a D1R agonist onto D1R MSNs potentiates the NMDA-mediated ‘up states’ (Cepeda et al. 1993; Plotkin et al. 2011). Stimulation of D1Rs also facilitates increased expression of AMPA receptors on NAc MSN synapses (Sun et al. 2008; Wolf 2010) and promotes increased expression of NMDA receptors on MSN dendrites (Hallett et al. 2006; Missale et al. 2006). Concurrent activation of striatal D1Rs and NMDA receptors leads to the formation of a D1/NMDA heterodimerized receptor complex at postsynaptic sites (Cahill et al. 2014). To form the heterodimer, which boosts D1R-enhanced NMDA currents (Cahill et al. 2014) and prevents internalization and desensitization of D1Rs (Missale et al. 2006), D1 and NMDA receptors fuse their structures through an assemblage of some of their subunits. All of these rapid neuroplastic effects selectively enhance excitatory transmission in MSNs and promote subsequent plasticity.

Connections between the currently active striatal output neurons and the concurrently active corticostriatal GLU inputs representing environmental stimuli are believed to be selectively strengthened by D1R transmission (Horvitz 2002; Horvitz 2009). DA released into the striatum results in synaptic potentiation of cortical inputs, and the magnitude of this potentiation correlates with successful acquisition of rewarding instrumental actions (Reynolds et al. 2001). Blockade of D1Rs prevents the potentiation of synapses between afferents and MSNs (Reynolds et al. 2001). The effects of phasic NAc DA signals on reward-associated learning involve subsets of NAc MSNs and selective afferents that target specific neurons (Grace et al. 2007). The excitatory signals sent via GLU inputs (Capper-Loup et al. 2009; Doig et al. 2010) conveying sensory (Pidoux et al. 2011) and motivational information (Ambroggi et al. 2008) are capable of inducing a long-term potentiation of motor output neurons (Fino et al. 2005; Fino et al. 2010) that is enhanced by D1R activation (Beninger and Miller 1998; O'Donnell 2003).

Therefore, during striatal DA transmission, the relationship between a GLU input and MSN output may be modified (Wickens et al. 2007). DA pulses acting on corticostriatal and limbicostriatal synapses within the striatum lead to a strengthening of some synapses and weakening of others (Horvitz 2002; Wickens et al. 2003). Thus, by fine-tuning the responses of output MSNs to behaviorally relevant
environmental stimuli (Horvitz 2002; Horvitz 2009), the relationship between striatal GLU and DA inputs shapes plasticity relevant to learned appetitive behavior (Shiflett and Balleine 2011).

Most long-term synaptic memories depend upon the second messenger cAMP (Kandel 2014). D1R activation initiates an intracellular cascade that involves adenylyl cyclase activation (Andersen et al. 1990) which in turn increases cAMP, protein kinase A, and other downstream messengers that contribute to the modification of corticostriatal synaptic transmission associated with the processing of reward-paired stimuli (Beninger and Miller 1998). Mice genetically lacking a striatum specific isoform of adenylyl cyclase are severely disrupted at learning Pavlovian approach behavior and are unable to use appetitive Pavlovian cues to guide their operant responding (Kheirbek et al. 2008). Under normal conditions, previous food-paired CSs elicit increased levels of behavioral motivation which correlate with increased transcription of immediate early genes within NAc (Schiltz et al. 2007).

**Role of DA in neuronal responses relating to the expression of overtrained behavior**

Over the course of habit learning, overtrained S-R representations may shift away from being mediated by corticostriatal–basal ganglia circuits to control by other (e.g., corticocortical) brain circuits (Ashby et al. 2007). Consistent with this possibility, the proportion of DLS neurons that respond to an operant lever-press (Carelli et al. 1997) or an operant head movement (Tang et al. 2007) becomes strongly reduced after extended training. That is, as operant performance becomes habitual, some DLS neurons abolish their firing rate altogether (Carelli et al. 1997; Tang et al. 2007). Similarly, overtraining produces a reduction in the proportion of DMS neurons that respond to a discriminative cue in an operant t-maze task (Thorn et al. 2010).

It is not clear whether neurons in the NAc show a similar reduction in training-related neuronal activity. In several experiments examining NAc neuronal responses in appetitive tasks, NAc neuronal responses to reward-predictive stimuli and during operant behaviors were observed even after trials exceeded the number employed in the DLS studies above (Nicola et al. 2004; Ghitza et al. 2006). However, these findings do not come from experiments specifically employing the length of training as a design variable.
Training of new behaviors relies on the continuous communication between the striatum and cortex (Suvorov and Shuvaev 2004) and can result in increased synaptogenesis between cortical neurons (Kleim et al. 2004). Upon transfer of control to the cortex, loss of striatal activity should no longer have an effect on behavioral production. Work on birds provides support for a temporary striatal role in learned behavior (i.e., a role which diminishes with extended training). For instance, area X, an avian homolog of the basal ganglia containing a striatal-like region (Doupe et al. 2005; Kojima and Doupe 2009) appears to be important during early and not later stages of learned behavioral performance. Disconnecting the communication pathway from Area X to the cortex (Kojima and Doupe 2009) disrupts the initial learning of a birdsong, but the same lesion given after the behavior has been well-acquired fails to disrupt expression of well-learned birdsong behavior (Doupe et al. 2005). The proposition that the mediation of overtrained behaviors may shift to corticocortical circuits is supported by empirical research suggesting that activity in subregions and even in single neurons of the motor cortex may underlie whole behavioral repertoires rather than abstract components of movement (Graziano and Gross 1993).

It has not been determined whether mediation of Pavlovian behavior shifts to a nonstriatal substrate after overtraining. Bespalov et al. (2007) showed that overtraining Pavlovian approach produces resistance of cued-approach behavior to reward devaluation and slows extinction (Bespalov et al. 2007). Prior to overtraining, acquisition (Di Ciano et al. 2001) and expression (Parkinson et al. 1999; Cardinal et al. 2002) of CS-US associations critically depend upon NAc. However, after extended training, approach behavior becomes insensitive to systemic D1R blockade (Choi et al. 2005; Bespalov et al. 2007). It has been suggested that a shift to S-R mediated responding coincides with a shift to DA-independent control of behavior (Horvitz et al. 2007). Interestingly, Bespalov et al. (2007) also showed that, even during extended training, behavioral vulnerability to D1R blockade may be restored by co-administration of a systemic AMPA antagonist which suggests the possibility that, as a result of overtraining, the NAc adapts in a way that leaves the expression of Pavlovian approach dependent upon excitatory GLU activity within the NAc or another substrate. The current set of experiments aims to explore whether habitual responding shifts D1R-mediated Pavlovian approach expression out of the NAc core and whether NAc core’s excitatory neurotransmission is still necessary to drive appetitive behavior during the habitual stages of learning.
1.2 BRIEF DISSERTATION PROPOSAL

1.2.1 Significance and specific aims

To understand the changes in brain reward systems associated with expression of drug-seeking behaviors and habits, it is important to acquire a more precise knowledge of the neural processes underlying, presumably evolved to mediate, natural (e.g., food) reward-seeking actions. Cues (e.g., CSs) previously paired with primary rewards, such as drugs of abuse or food, are powerful elicitors of reward-directed responses (Davis and Smith 1976; See et al. 1999; Di Ciano and Everitt 2003; Di Ciano and Everitt 2004) and serve as ‘relapse cues’ in addicted individuals (O’Brien et al. 1981; Childress et al. 1988; O’Brien et al. 1991; O’Brien et al. 1992; Childress et al. 1993). DA action in the NAc critically underlies the associations between rewards and CSs (Di Ciano et al. 2001; Parkinson et al. 2002; Wise 2004). Given the discoveries on behavioral CS effects (e.g., enhanced drug-seeking) and the mechanisms mediating CSs (e.g., DA activity), it is of interest to explore DA’s role in habits formed for natural rewards.

Often leading to abnormal processing of reward-related CSs, addictive substances are known to produce long-lasting neuroplastic changes within the NAc and other reward-processing regions. Reward-related striatal neuroplasticity depends upon activation of D1Rs (O’Donnell 2003; Wickens et al. 2003; Wickens et al. 2007; Horvitz 2009; Gerfen and Surmeier 2011) which are expressed in high density in the NAc core (Bardo and Hammer 1991; Levey et al. 1993). In order to better conceptualize the role of NAc core in reward processing, it is essential to examine if NAc core or its D1Rs become differentially engaged in the expression of reward-directed responses throughout habit training.

DA activity in the striatum has been implicated in both learning and performance, but the importance of empirically distinguishing these two aspects of behavior should not be overlooked (Balleine et al. 2009). For example, activation of D1Rs is critical for learning CS-signaled approach to a reward location (Eyny and Horvitz 2003) as well as for the learning of motor skills (Willuhn and Steiner 2008); however, under some conditions, blockade of DA transmission at D1Rs can disrupt the performance of a previously learned response (Choi et al. 2005; Bespalov et al. 2007; Nicola 2010). Examining effects of pharmacological DA loss on learning typically involves experimental designs in which DA activity is
altered during each training session, and the extent of learning is then tested by assessing performance in an uncompromised state. Conversely, experiments on performance usually involve a design in which effects of DA blockade are assessed after the subjects have undergone drug-free behavioral training. As can be seen in the method below, the present work adopts the latter strategy because the focus here is on the changing role of NAc core neurochemical activity in the expression rather than acquisition of reward-directed responding.

Experimental studies have shown that DA transmission at D1Rs plays an important role in performance of behaviors during the early learning phases. Interestingly, previous results from our laboratory, which suggested that activation of D1Rs is not necessary for conditioned response expression late in training, showed that behavioral vulnerability to systemic D1R blockade diminishes as a function of the exposure to the CS/reward pairings during training of approach (Choi et al. 2005). Although these data suggested that central D1Rs play a decreasing role in the performance of CS-elicited approach over the course of training, it remained unclear whether D1Rs located specifically in the NAc core mediate this type of behavior. Moreover, it became of interest to test whether the involvement of NAc core D1Rs continues beyond the early training stages or declines with training.

Interestingly, there is evidence to suggest that brain mechanisms involved in performance of learned behavior may shift over the course of training (Carelli et al. 1997; Miyachi et al. 1997; Jog et al. 1999; Miyachi et al. 2002; Barnes et al. 2005; Doupe et al. 2005; Tang et al. 2007; Tang et al. 2009; Yin et al. 2009; Ashby et al. 2010; Restivo and Frankland 2010; Thorn et al. 2010). These data along with accumulating evidence of DA-independence post acquisition and overtraining of approach behavior (Choi et al. 2005; Bespalov et al. 2007) have raised the question of whether extended training leads to a diminishing role of NAc core DA specifically or whether it more generally reduces the involvement of the NAc core output neurons. Using a simple head entry paradigm in which rats learn to enter a food compartment to collect a CS-signaled food reward, we planned to investigate the roles of NAc core DA and ionotropic GLU transmission in the performance of recently-acquired versus extensively-trained Pavlovian approach. Specifically, we aimed to examine the effects of pharmacological blockade within NAc core, of first the D1 and then the AMPA and NMDA receptors, all with regard to reward-directed approach expression during early versus extended training stages. The work also asks whether the role
of D1Rs in the performance of early-stage approach includes the DMS or NAc shell, regions in close anatomical proximity to NAc core, also receiving heavy DA innervation (Fallon 1981; Heimer et al. 1997; Haber 2003).

**Specific Aim 1: Examining the effects of intra-NAc core SCH23390 on Pavlovian approach expression during early and extended training stages (Study 1)**

Study 1 will investigate the function of NAc core D1Rs in the performance of approach behavior during the early versus extended stages of training. D1R are targeted because previous work has shown that CS-elicited approach depends upon D1R but not D2R transmission (Choi et al. 2009). The effects of intra-NAc core D1R antagonist microinjection on the latency and frequency of approach behavior will be measured and compared to that of a vehicle microinjection administered during each training stage. If behavioral dependence on D1R activity in the NAc core decreases as a function of training, blockade of NAc core D1Rs during extended training should produce no disruptive effects on the expression of Pavlovian responses. However, during early learning, expression of CS-elicited responses should rely upon NAc core D1R transmission.

**Specific Aim 2: Examining the effects of intra-DMS and intra-NAc shell SCH23390 on early-stage Pavlovian approach expression (Study 2)**

To determine whether the behaviorally disruptive effects of D1R blockade on early-stage approach behavior are specific to the loss of D1R activity in the NAc core and not a neighboring region that also receives DA innervation, Study 2 will examine the expression of recently-acquired CS-elicited approach under the blockade of D1Rs in the DMS and the NAc shell. The effect of these control D1R antagonist microinjections on latency and frequency of recently-acquired approach behavior will be measured and compared to that of vehicle microinjections administered to each respective control site.

**Specific Aim 3: Examining the effects of intra-NAc core CNQX/AP-5 on Pavlovian approach expression during early and extended training stages (Study 3)**

Study 3 will examine the role of excitatory NAc core transmission in the performance of approach behavior during early versus extended stages of training. Activation of NAc core output neurons can be
blocked by co-antagonism of the local ionotropic GLU receptors—namely, the AMPA and the NMDA receptors (Hu and White 1996); thus, the effects of the combined blockade of AMPA and NMDA receptors will be examined. The effects of NAc core combined AMPA and NMDA antagonists microinjection on the latency and frequency of approach behavior will be compared to that of a vehicle microinjection administered during each training stage. If the well-acquired behavior has become independent of NAc core neuronal activity, then normal levels of responding should be observed even under AMPA/NMDA receptor blockade during extended training. Consequently, if extended-stage performance no longer depends upon NAc core AMPA/NMDA transmission, our results may suggest that mediation of approach expression has shifted to another anatomical substrate.
1.3 GENERAL MATERIALS AND METHODS

1.3.1 Subjects

Sprague Dawley rats weighing 270-310 g were obtained from Charles River Laboratories (Wilmington, MA) and housed in pairs within clear Plexiglas cages (20 cm high × 26 cm wide × 46 cm deep) in an animal colony. The colony was maintained at approximately 23°C with 12 hr light/dark cycle. During acclimatization to the colony, food (Purina Lab Chow) and water was available *ad libitum*. After acclimatization, rats were handled daily and were subsequently placed on a 22 hr food-restriction schedule in order to accustom them to the food regimen that would be in place during the behavioral experiments.

1.3.2 Surgery

In all three studies, rats underwent cannulae implantation surgery prior to receiving any behavioral training. At the time of surgery, rats (300-340 g at the time of surgery) were anesthetized with Nembutal (50 mg/kg; Henry Schein, Melville, NY) intraperitoneally and administered Atropine Sulfate (0.25 mg/kg; Henry Schein, Melville, NY) intramuscularly to reduce bronchial secretions. After being secured in a stereotaxic instrument (Stoelting Co., Wood Dale, IL), animals were implanted with 23 gauge bilateral stainless steel cannulae (Plastics One, Roanoke, VA) according to standard flat-skull stereotaxic procedures. Using a 6° lateral to medial angle in order to avoid cannula passage through lateral ventricles, guide cannulae were implanted 2 mm above the NAc core (anterior-posterior [AP] +1.7; medial-lateral [ML] ±2.0 mm; dorsal-ventral [DV] -5.5 mm relative to the skull surface) or DMS (AP +1.7, ML ±2.0 mm, DV -3.5 mm). A 12° angle was used for implanting cannulae 2 mm above the NAc shell (AP +1.7; ML ±2.6 mm; DV -5.9 mm). Stainless steel stylets (Plastics One, Roanoke, VA) were placed inside guide cannulae to prevent blockage. After surgery, animals received 5 ml of bacteriostatic saline (Henry Schein, Melville, NY) subcutaneously for hydration and spent the recovery period on a warm surface. Upon gaining consciousness, a single subcutaneous injection of Rimadyl (5 mg/kg; Henry Schein, Melville, NY) was administered for pain relief. Rats were given a minimum of five days for recovery in their
home colony before starting behavioral training and were singly housed for the remainder of the experiment. During the first four to five days of recovery, animals were given *ad libitum* food and water and were subsequently returned to the 22 hr food-restriction schedule for the remainder of the experiment.

1.3.3 Apparatus

Behavioral training sessions were carried out in test chambers (29 cm high × 29 cm wide × 25 cm deep; Coulbourn Instruments, Allentown, PA) that were individually contained within sound- and light-attenuated isolation cubicles equipped with fans (120VAC 3"; Radioshack, Fort Worth, TX) positioned at the top right corner of one wall. A house light was centered at the top of one test chamber wall (26 cm above the chamber floor), and a liquid delivery trough (4.2 cm high × 3.3 cm wide × 3.5 cm deep) was centered at the bottom 2 cm above the floor. Prior to liquid sucrose delivery, a 500 msec 70 dB (2500 Hz) tone that served as the CS was generated by a speaker. The liquid sucrose (0.04 ml of 20% sucrose) was delivered into the reward compartment by a dipper arm (Coulbourn Instruments, Allentown, PA) which lifted upward to permit access to the liquid reward. The liquid sucrose was pumped into the reward arm during each trial by a single speed syringe pump (Razel Scientific, Saint Albans, VT) which drove a syringe containing the liquid sucrose connected to tubing which extended into the reward cup located on the dipper arm. Interruption of a photocell emitter and sensor (H20-94; Coulbourn Instruments, Allentown, PA) located on the sides of the reward compartment detected head entries into the compartment via a computer (Cobalt 4114 LabMax Series, Cobalt, Allentown, PA) and Habitest LabLink interface (Coulbourn Instruments, Allentown, PA). Time of food compartment head entries and withdrawals, sucrose delivery, and tone presentations were time-stamped by the computer running the Coulbourn Instruments Graphic State Notation software with a 50 msec sampling rate.

1.3.4 Data analysis

The presence of the rat’s head in the food compartment was recorded during the time period from 10 sec before to 10 sec after each CS presentation. This period was divided into 100-msec bins, and the
frequency with which the rat’s head was in the compartment during each time bin was used to generate individual and group plots of head entries time-locked to the CS.

Dependent measures included *baseline head entries*, i.e., the number of head entries emitted during the 10 sec period before the CS, *latency*, i.e., the time to enter the food compartment upon CS onset, with the 10 sec maximum scores assigned to trials with latencies of >10 sec excluded, and the *proportion of trials missed*, i.e., the number of trials with >10 sec latency.

### 1.3.5 Sucrose acclimation procedure

Rats were habituated to 20% liquid sucrose in their homecage during their 2 hr feeding period one day prior to the start of any behavioral training. Because some rats in Study 1 had to be excluded from analyses after failing to retrieve rewards on the first day of CS-US training, an additional acclimation procedure was added in which rats entered the test chamber with the dipper arm in the raised position, a single sucrose bolus in the reward cup, and the reward compartment light illuminated. Rats typically found and consumed the sucrose drop while exploring the chamber. Once it was observed that the rat consumed the sucrose, it was taken out of the chamber and the procedure was repeated to allow the rat to consume the liquid sucrose bolus one more time. If the rat did not consume the sucrose within 45 min, the entire procedure was repeated the next day.

### 1.3.6 Behavioral procedure

Rats received daily drug-free CS-US training sessions for three or nine consecutive days. At the start of each session, the house light was illuminated and remained illuminated until the end of the session. Each session consisted of 28 trials. During each trial, the liquid reward was presented along with the illumination of the food compartment light 250 msec after the onset of a 500 msec tone (CS). Each trial consistent of a variable-time 70 sec inter-trial interval (ITI) with a minimum inter-trial interval of 30 sec. Upon entry into the food compartment within 10 sec of liquid sucrose presentation, the reward arm remained raised for 4 sec allowing the animal to consume the liquid sucrose. Failure to enter the food compartment within 10 sec of liquid sucrose presentation was counted as a *missed trial*. At the cessation
of each conditioning session, on average lasting 32 min, animals were returned to the home colony where they received food *ad libitum* for two hours.

During the two ‘Test’ days following the training period (i.e., on days 4/5 for the *Early* training groups or on days 9/10 for the *Extended* training groups), each animal received a single bilateral drug microinfusion and a single vehicle infusion delivered in a volume of 0.5 μl per side of the striatal target site. The days of drug and vehicle infusion were counterbalanced across rats so that for half of the rats, the vehicle day preceded the drug infusion day; for the other half, the order was the reverse. Microinfusions were also randomized in a way which ensured that rats getting microinjection treatment on a particular day did not receive the same condition only. ‘Test’ day sessions were otherwise identical to the drug-free training sessions described above.

1.3.7 Microinfusion procedure

At the time of the microinfusion, stylets were removed, and internal cannulae (30 gauge; Plastics One, Roanoke, VA) extending 2 mm beyond guide cannulae were inserted. The internal cannulae were connected to a 10 μl microsyringe (Hamilton Co., Reno, NV) with polyethylene tubing (PE-50, Plastics One, Roanoke, VA). All microinfusions were bilateral with a volume of 0.5 μl/side. A microdrive pump (Razel Scientific, Saint Albans, VT) drove the microsyringe and delivered fluid at a rate of 0.5 μl/min. Internal cannulae were left in place for an additional 60 sec to allow for diffusion. After the microinfusion was complete, internal cannulae were removed and stylets were re-inserted. Animals were tested within 5 min of receiving the microinfusion.

1.3.8 Drugs

D1R antagonist SCH23390 (Sigma-Aldrich, St. Louis, MO; R(+)-7-chloro-b-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride) was dissolved in sterile distilled water (1 μg/side or 2 μg/side) in a volume of 0.5 μl/side. Doses of the D1R antagonist were chosen based on previous work examining the effects of central SCH23390 injections on approach behavior (Choi et al.)
2005; Choi et al. 2009). Vehicle microinfusions in SCH23390 experiments consisted of 0.5 μl/side infusion of sterile distilled water.

The AMPA and NMDA receptor antagonists were infused as a mixture. We dissolved 1 μg of the AMPA antagonist CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) and 2 μg of the NMDA antagonist AP-5 (2-amino-5-phosphonopentanoic acid) in phosphate buffered saline to prepare a CNQX/AP-5 combination infusion (1 μg & 2 μg/side, respectively) with a volume of 0.5 μl/side. Doses of CNQX and AP-5 were chosen based on a previous study examining the effects of intra-NAc CNQX/AP-5 microinjections on reward-directed responding (Yun et al. 2004). Vehicle microinfusions in Study 3 consisted of 0.5 μl/side infusion of phosphate buffered saline.

1.3.9 Histological analysis

Rats were deeply anesthetized with Nembutal and perfused transcardially with 0.9% isotonic saline followed by 10% formalin. Brains were harvested and stored in a post-fixative solution of formalin for 24 hours before being transferred to 30% sucrose-formalin mixture until sunken and thereafter were sliced on a microtome in 40 μm sections. Sections were mounted on glass slides, stained with cresyl violet, and viewed with light microscopy to verify correct placement of cannulae.

Only rats with infusion sites within the NAc core, DMS, or NAc shell were included in data analyses. If the implantation site was estimated to be outside the particular target region on either one or both hemispheres, the subject was excluded from the analysis. Figure 2 shows the estimated microinjection sites of the all subjects included in present results.
Figure 2. **Microinjection sites of all rats included in statistical analyses.** Locations of the NAc core, NAc shell, and dorsomedial striatum (DMS) microinjection sites are shown with regard to distance from bregma. The bilateral microinjection sites from the Early Training intra-NAc core SCH23390 2 μg (●), Early Training intra-NAc core SCH23390 1 μg (■), Extended Training intra-NAc core SCH23390 2 μg (○), Extended Training intra-NAc core SCH23390 1 μg (□), Early Training intra-NAc core CNQX/AP-5 (◆), Extended Training intra-NAc core CNQX/AP-5 (◇), intra-DMS SCH23390 2 μg (★), and intra-NAc shell SCH23390 2 μg (◆) groups were determined according to the coronal bregma-based atlas diagrams adapted with permission from Paxinos and Watson (1998).
2. DISSERTATION EXPERIMENTS AND RESULTS
2.1 Effects of intra-NAc Core SCH23390 on Pavlovian approach expression during early and extended training stages (Study 1)

2.1.1 Introduction

Experimental data have suggested that DA neurotransmission mediates the performance of reward-directed behaviors during early learning phases. A key study from our laboratory (Choi et al. 2005) showed that transmission at D1Rs is vital for the intact performance of recently-acquired Pavlovian approach. To be more specific, during early training, a systemically injected D1R antagonist reduced the probability of performing a simple approach response signaled by a reward-predictive CS. It is therefore believed that the performance of CS-signaled approach is dependent upon D1R transmission during early training. In contrast, D2R blockade failed to affect Pavlovian approach probability.

However, the behavioral dependence on central D1R transmission declines throughout training. Unlike subjects from the early training group, animals treated with the D1R antagonist late in training (after receiving extensive CS/reward pairings) do not exhibit a disruption of CS-driven behavioral responding. Thus, approach responses elicited by extensively-trained CSs appear to be less vulnerable to disruption by central D1R blockade.

We hypothesized that the critical role of DA in the performance of Pavlovian approach during early learning may be attributed to DA activity at D1Rs within the NAc core. This site receives major DA projections from the midbrain (Fallon 1981) and expresses D1Rs in high density (Bardo and Hammer 1991; Levey et al. 1993). DA activity within the NAc core has been implicated in the initiation of approach behavior (du Hoffmann and Nicola 2014), vigor of appetitive responses (Nowend et al. 2001; Salamone and Correa 2002), and the expression of associations between stimuli and rewards (Di Ciano et al. 2001).

It was therefore of interest to ask: 1) Do NAc core D1Rs critically mediate the performance of recently-acquired Pavlovian approach, and 2) does dependence on NAc core D1R transmission for the performance of Pavlovian approach decline throughout training? Using a simple Pavlovian approach paradigm in which rats learn to make head entries into a food compartment to collect CS-signaled reward, susceptibility of approach responses to NAc core D1R blockade was examined after three and nine drug-free training days in separate groups of animals. In order to block D1R transmission, we microinfused
SCH23390, a selective D1R antagonist with high affinity for D1Rs, into the NAc core. At each training stage, dependent measures of approach behavior when under the effect of the intra-NAc core D1R antagonist were compared to measures taken after vehicle microinjections. Because systemic D2R blockade failed to disrupt Pavlovian approach behavior previously (Choi et al. 2005; Choi et al. 2009), striatal D2Rs are not experimentally evaluated presently.

It is presently hypothesized that blockade of NAc core D1Rs will disrupt the expression of recently-acquired Pavlovian approach. Given the systemic results with the D1R antagonist, the role of NAc core D1R transmission in the performance of Pavlovian approach is hypothesized to be transient. If we find that blockade of NAc core D1Rs fails to produce disruptive effects on the performance of CS-signaled approach during extended training, our data would suggest that over time behavioral performance becomes less dependent upon NAc core DA transmission.

2.1.2 Materials and methods

Experimental design
Rats were divided into Early and Extended training groups. Prior to getting any of the bilateral microinjections, rats in the Early training group received three consecutive drug-free training days while the Extended training group received nine drug-free training days. Each of the two training groups were subdivided to receive either the 2 μg/side dose of the D1R antagonist SCH23390 and vehicle (0 μg/side) or the 1 μg/side dose of SCH23390 and vehicle (0 μg/side). Rats received the microinfusions prior to start of sessions taking place on the two ‘Test’ days that followed the initial training period (i.e., on days 4/5 for the Early training group and days 9/10 for the Extended training group). Microinjections were counterbalanced so that half of the rats in each group received the drug first and the other half received vehicle first.

Statistical analysis
As noted above, each rat received only two microinfusion ‘Test’ days during which vehicle or one SCH23390 dose were administered. Therefore the Dose of SCH23390 (1 μg or 2 μg) was a between-
subject factor, i.e., while half the rats received 1 μg SCH23390 and vehicle infusion, the other half received 2 μg of SCH23390 and vehicle infusion. Because separate groups of rats received two different amounts of training (i.e., three or nine drug-free days) prior to the microinfusion test sessions, Stage of Training was another between-subject factor. Assignment of SCH23390 Dose and Stage of Training to between-subjects factors had the advantage of avoiding the repeated-dosing effects on behavior that are often observed with DA antagonists (Fowler and Liou 1994).

Each rat received a vehicle and a drug microinfusion and therefore served as its own control. Treatment (vehicle vs SCH23390 infusion) was therefore a within-subject factor. We ensured that all microinjections were counterbalanced so that half of the rats in each group received drug first while the other half received the vehicle microinjection first. Treatment order effects therefore were not expected; however, we used Treatment Order (SCH23390 or vehicle first) as an additional between-subject factor to verify that no order effect was seen.

2.1.3 Results

Over nine drug-free training days, proportions of ‘trials missed’ decreased from ~0.26 ± 0.07 on day 1 to ~0.01 ± 0.01 on day 9 (Figure 3). ANOVAs were conducted on SCH23390 data to determine whether the NAc core D1R blockade produced effects on approach responses during the Early (days 4 and 5) or Extended (days 9 and 10) training stages. A 3-way Treatment (vehicle vs SCH23390 dose) × Dose (1 μg vs 2 μg of SCH23390) × Stage of Training (3 drug-free days vs 9 drug-free days) ANOVA conducted on ‘missed trials’ (in which rats failed to enter the food compartment within 10 sec of CS onset) revealed a main effect of Treatment, $F_{(1, 33)} = 15.97, p < .001$, showing that the D1R antagonist significantly reduced the number of trials for which rats emitted a cued head entry response into the food compartment. A Treatment × Dose interaction, $F_{(1, 33)} = 11.27, p = .002$, revealed that the intra-NAc core 2 μg dose of SCH23390 led to a greater proportion of ‘missed trials’ than the 1 μg dose. A Treatment × Dose × Stage of Training interaction, $F_{(1, 33)} = 4.37, p < .05$, indicated that the effect of intra-NAc core SCH23390 dose on ‘missed trials’ depended upon the stage of training.

Figure 4 depicts the mean probability that a rat’s head was inside the food compartment from 10 sec before to 10 sec after the CS for rats treated with intra-NAc core vehicle or SCH23390 during the
Early (top panel) or Extended (bottom panel) training stages. Vehicle scores for the two SCH23390 dose groups (1 μg group vs 2 μg group) did not differ significantly and were therefore pooled in Figure 4. As can be seen in the top panel, in comparison to vehicle and the 1 μg dose of SCH23390, the 2 μg dose administered during Early training dramatically lowered the probability of (the proportion of trials for which) the rats’ head was inside the food compartment following the CS. Conversely, SCH23390 failed to produce a substantial reduction in the proportion of trials for which rats entered the food compartment in response to the CS when the drug was administered during Extended training (bottom panel of Figure 4).

Two representative rats that received vehicle and the high dose of SCH23390 during either the Early (left panel) or the Extended (right panel) training stage are shown in Figure 5. It can be seen that intra-NAc core SCH23390 reduced noncued head entries (i.e., the entries occurring 10 sec prior to CS) during both Early and Extended training. However, the CS-driven entries were only suppressed by the intra-NAc core D1R blockade during Early training (bottom left of Figure 5). During this stage, SCH23390 led to a substantial number of ‘missed trials’ within the session. For example, the representative rat from the Early training group missed trials around the middle of the session. (However, other rats from this group missed trials in other parts of the session.) In contrast, cued head entries of the rat that received the D1R antagonist during Extended training were not suppressed (bottom right of Figure 5).

Shown in Figure 6 are the mean proportions of trials missed by the Early and Extended training groups when under the influence of vehicle or the high dose of the D1R antagonist. Paired samples t-tests comparing vehicle and SCH23390 ‘missed trial’ scores showed that when administered during Early training, the high dose of intra-NAc core SCH23390 significantly increased the proportion of trials that animals missed ($t(9) = 2.82, p < .05$). In contrast, the high dose of the D1R antagonist failed to produce a significant elevation of ‘missed trials’ when administered during Extended training ($t(10) = 1.89, p = n.s.$).

Latencies to enter the food compartment following the CS were calculated for each session by excluding the ‘missed trials’ which were assigned a maximum score of 10 sec. A 3-way Treatment (vehicle vs SCH23390 dose) × Dose (1 μg vs 2 μg of SCH23390) × Stage of Training (3 drug-free days vs 9 drug-free days) ANOVA conducted on latency scores revealed a main effect of Treatment, $F(1, 33) = 19.01, p < .001$, indicating that SCH23390 increased the latency to enter the food compartment and a marginal Treatment (SCH23390 vs vehicle) × Dose (1 μg vs 2 μg of SCH23390) interaction, $F(1, 33) = $
3.68, \( p = .06 \), showing that the 2 μg dose of SCH23390 had a greater impact on latencies than the 1 μg dose. A marginal \( Treatment \times Dose \times Stage \ of \ Training \) interaction, \( F_{(1, 33)} = 3.63, p = .07 \), indicated that the effects of intra-NAc core SCH23390 dose on latency to make a cued entry into the food compartment depended upon the stage of training. As depicted in Figure 7, paired samples \( t \)-tests showed that 2 μg of SCH23390 significantly increased latency during \( Early \) \( (t_{(9)} = 3.64, p < .05) \) but not during \( Extended \) training \( (t_{(10)} = 1.60, p = \text{n.s.}) \).

A 3-way \( Treatment \) (vehicle vs SCH23390 dose) \( \times \) \( Dose \) (1 μg vs 2 μg of SCH23390) \( \times \) \( Stage \ of \ Training \) (3 drug-free days vs 9 drug-free days) ANOVA on ‘baseline head entries’ revealed a main effect of \( Treatment, F_{(1, 33)} = 22.83, p < .001 \). No 3-way interaction was observed \( (F_{(1, 33)} = 1.19, p = \text{n.s.}) \). Unlike the measures of cued approach behavior (i.e., ‘missed trials’ and latency), ‘baseline head entries’ were significantly suppressed during both \( Early \) \( (t_{(9)} = 3.10, p < .05) \) and \( Extended \) \( (t_{(10)} = 2.59, p < .05) \) training stages (Figure 8). Therefore, extended training did not reduce the NAc core D1R-dependence of approach behavior per se but specifically reduced dependence of the approach behavior on NAc core D1R transmission when the approach behavior was elicited by the well-acquired CS.

As noted above, all animals received vehicle and SCH23390 microinfusions on the last two days of training. The order of microinfusions was counterbalanced in both \( Early \) and \( Extended \) training groups so that half of the animals in each group received vehicle first and the other half received the D1R antagonist first. \( Treatment \) \( Order \) (SCH23390 or vehicle first) produced neither a main effect nor an interaction with any other factor(s) on any of the dependent measures \( (p = \text{n.s.} \) for ‘missed trials’, latency, and ‘baseline head entries’).
Figure 3. Drug-free Pavlovian performance across training. The figure shows mean proportion of 'trials missed', i.e., trials during which rats took longer than 10 sec to enter the food compartment in response to the conditioned stimulus (CS), across the 9 drug-free training days.
Figure 4. Effects of intra-NAc core D1R antagonist on the mean probability of rats’ head being inside the food compartment 10 sec before and 10 sec after CS presentation during Early (top panel) and Extended (bottom panel) training stages. The mean proportion of trials for which the rats’ head was inside the food compartment during consecutive 0.1 sec bins is plotted on the y-axis. The x-axis represents trial time relative to the 500 msec CS (vertical line at 0 sec). Lower peaks after CS presentation in rats receiving intra-NAc SCH23390 during Early training reflect a reduced proportion of trials in which rats responded to the CS within 10 sec.
Figure 5. Effects of intra-NAc core D1R antagonist on approach responses in individual rats from *Early* (left panel) and *Extended* (right panel) training groups. The 28 consecutive trials of each session are plotted on the y-axis (bottom to top). Time relative to CS presentation (0 sec) is shown on the x-axis. Each horizontal line represents the occurrence and duration of the rat's food compartment entries. Both rats received high dose of SCH23390 and vehicle on the last two days of training. In comparison to each respective vehicle session, after being treated with the intra-NAc core D1R antagonist, both rats exhibited a suppression of the food compartment entries that occur during the 10 sec prior to CS. Food compartment entries occurring within 10 sec after CS-onset became disrupted only in the rat that received the D1R antagonist during the *Early* training stage.
Figure 6. Proportions of trials missed by Early and Extended training groups following intra-NAc core vehicle or D1R antagonist treatment. The figure shows mean proportion of trials missed. Blockade of NAc core D1Rs produced a significant elevation of ‘missed trials’ during Early training only. Asterisk denotes a significant difference from the within-subjects vehicle control, *p < .05.
Figure 7. Latencies of *Early* and *Extended* training groups to enter the food compartment in response to the CS following intra-NAc core vehicle or D1R antagonist treatment. A significant increase in mean latency with ‘missed trials’ excluded was observed during *Early* training only. Asterisk denotes a significant difference from the within-subjects vehicle control, \( * p < .05 \).
Figure 8. Noncued approach responses of *Early* and *Extended* training groups following intra-NAc core vehicle or D1R antagonist treatment. The figure shows mean frequency of ‘baseline head entries’ (during the 10 sec prior to CS presentation). Blockade of NAc core D1Rs produced significant suppression of noncued approach during both *Early* and *Extended* training stages. Asterisks denote significant differences from the within-subjects vehicle control, *p < .05.*
2.2 Effects of intra-DMS and intra-NAc shell SCH23390 on early-stage Pavlovian approach expression (Study 2)

2.2.1 Introduction

Preliminary results from Study 1 indicated that performance of a recently-acquired Pavlovian approach requires transmission at D1Rs in the NAc core; however, it is also known that the NAc core neighbors other striatal areas which likewise receive strong DA innervation (Fallon 1981; Heimer et al. 1997; Haber 2003) and similarly express D1Rs on their own output MSNs (Levey et al. 1993; Matamales et al. 2009). The anatomical similarity of neuronal networks in the NAc core and its surround raised questions about the anatomical specificity of the behavior-disruptive effects observed in Study 1. Thus, it became of interest to determine whether the effects of D1R blockade observed during early training result from loss of transmission at D1Rs in the NAc core exclusive of neighboring striatal areas. Due to the possibility of drug-spread and an inability to visualize the diffusion of the microinfusion solution, we microinfused the D1R antagonist into neighboring striatal regions to detect possible disruptions in Pavlovian approach during early training.

Because of potential drug-spread up the cannula shaft from the NAc core into the dorsal direction, we chose the DMS, located 2 mm above the NAc core, as our first anatomical control site. A strong disruption of cued approach following infusions to this DMS region would raise the possibility that behavioral disruptions observed in Study 1 following intra-NAc core infusions were in fact caused by dorsal spread of the drug to the overlying DMS. Around the NAc core’s medial, ventral, and lateral sides, the NAc shell extends asymmetrically and lies within a 2 mm radius from the central NAc core. We therefore chose the NAc shell as our second anatomical control. This left us with a third potential site from the striatal complex, i.e., the DLS. However, given that the most of the DLS is more than 2 mm away from the central part of the NAc core, it is highly unlikely that the microinfusion solution would have spread there in Study 1. We therefore chose not to examine the effects of D1R blockade in the DLS.

With the exception of the new microinfusion target sites, experimental design and procedures of Study 2 were identical to those used in the early training conditions of Study 1. Because preliminary infusions of the high 2 μg SCH23390 dose into the DMS (Dobrovitsky et al. 2011) or the NAc shell
(Dobrovitsky et al. 2013) produced no disruption of the cued approach, we chose not to test the low dose of the D1R antagonist in these regions. Separate groups of rats were used to examine the effects of DMS or NAc shell D1R antagonist and vehicle on the expression of approach behavior.

2.2.2 Materials and methods

Experimental design

Rats were trained for three consecutive drug-free training days prior to being tested under the influence of either DMS or NAc shell D1R blockade. Following the initial training period, rats received bilateral microinfusions prior to start of sessions on days 4 and 5. During these two 'Test' days, each animal received microinfusions of the 2 μg/side dose of the D1R antagonist SCH23390 and vehicle (0 μg/side). The order of drug versus vehicle microinjections was counterbalanced across the two 'Test' days (i.e., days 4/5). Half of all rats in each group received 2 μg of the D1R antagonist first and vehicle second, and the other half received vehicle first and the 2 μg dose of the D1R antagonist second.

2.2.3 Results

Microinfusions of the D1R antagonist into the DMS and NAc shell produced a set of effects clearly distinguishable from the impairments observed after NAc core D1R blockade during the Early training conditions of Study 1. Figures 9 and 10 depict the mean probability that a rat’s head was inside the food compartment from 10 sec before to 10 sec after CS-onset for rats treated with intra-DMS and NAc shell vehicle or SCH23390 during Early training. As can be seen, the probability of the head being inside the food compartment following CS presentation was not lowered by either DMS (Figure 9) or NAc shell (Figure 10) D1R blockade. This contrasts with the effect of intra-NAc core SCH23390 administered during Early training (top of Figure 4).

Intra-DMS D1R blockade failed to significantly affect the production of cued head entry responses. Figure 11 depicts the mean proportions of trials missed by the Early training group under the influence of vehicle or the high dose of the D1R antagonist infused into the DMS. As can be seen in the figure, elevation of ‘trials missed’ was not observed with blockade of DMS D1Rs. Paired samples t-tests
comparing vehicle and SCH23390 'missed trial’ scores showed that the high dose of intra-DMS
SCH23390 produced no significant increase in the proportion of trials that animals missed \( t(9) = 1.66, p = \) n.s.). Similarly, as shown in Figure 12, the latency to make cued head entry responses was not increased by the intra-DMS SCH23390 treatment. Upon examination of latency scores (with ‘missed trials’
excluded), no significant difference between vehicle and the 2 μg SCH23390 latency scores of rats
infused into the DMS was found \( t(9) = 1.33, p = \) n.s.).

In addition to leaving measures of cued approach unaffected, the intra-DMS SCH23390 treatment
also failed to disrupt the production of noncued approach responses. As seen in Figure 13, no
suppression of ‘baseline head entries’ (i.e., the noncued food compartment entries occurring during the
10 sec of ITI immediately preceding CS onset) was observed in rats with D1R blockade in the DMS. No
significant difference between the intra-DMS SCH23390 and vehicle ‘baseline head entry’ scores was
revealed by the paired samples \( t \)-test \( t(9) = 1.88, p = \) n.s.). These findings suggest that, during early
training, blockade of D1Rs in the DMS leaves both the ability to make spontaneous (noncued) as well as
CS-elicited approach responses into the food-compartment intact.

Our results from NAc shell microinjections show that cued approach is not vulnerable to blockade
of this region’s D1Rs. Blockade of NAc shell D1Rs with 2 μg of SCH23390 failed to elevate the number of
trials animals missed (Figure 11) as well as the latency to make head entry responses within 10 sec of CS
onset (Figure 12). Paired samples \( t \)-tests on the measures of cued approach from the intra-NAc shell
infused rats showed no significant effect of SCH23390 on ‘missed trails’ \( t(8) = 0.35, p = \) n.s.) or latency
\( t(8) = 0.05, p = \) n.s.). However, as shown in Figure 13, the intra-NAc shell SCH23390 treatment did impair
the animals’ ability to make noncued head entries. The paired samples \( t \)-test revealed a significant
difference between the NAc shell SCH23390 and vehicle ‘baseline head entry’ scores \( t(8) = 2.65, p < .05.
Although it appears that spontaneous approach into the food compartment was in fact reduced by the
intra-NAc shell D1R antagonist, because NAc shell D1R blockade failed to affect measures of cued
approach, testing the effects of the intra-NAc shell SCH23390 during \textit{Extended} training fell outside the
scope of present work.

Consistent with Study 1, all animals in the control groups (i.e., the intra-DMS or NAc shell
microinfused rats) received vehicle and SCH23390 microinfusions on the last two days of training. The
order of microinfusions (vehicle first vs SCH23390 first) was counterbalanced in both the DMS and NAc shell groups so that half of the animals in each group received vehicle first and the other half received the D1R antagonist first. Treatment Order (SCH23390 or vehicle first) for the intra-DMS and NAc shell microinfused rats produced neither a main effect nor an interaction with other factor(s) on any of the dependent measures ($p = \text{n.s.}$ for ‘missed trials’, latency, and ‘baseline head entries’.)

Results of Study 2 allowed us to conclude that the NAc core microinjections (from Study 1) were anatomically specific. However, similarly to the result obtained with intra-NAc core SCH23390, a reduction in noncued approach was observed in rats with NAc shell D1R blockade. Although the effect of intra-NAc shell SCH23390 on noncued approach is significant, it is not likely that the intra-NAc core SCH23390 treatment produced its effects on simple approach by spreading to the shell. If drug-spread from core to shell is to account for the effects of intra-NAc core SCH23390 on approach behavior, a disruption of the cued approach would have also been observed with the intra-NAc shell SCH23390 treatment. The lack of effect of DMS or NAc shell D1R blockade on the measures of cued approach taken during Early training persuaded us not to examine the effects of D1R blockade in these regions duringExtended training.
Figure 9. Effects of intra-DMS D1R antagonist on the mean probability of rats’ head being inside the food compartment 10 sec before and 10 sec after CS presentation during the Early training stage. The mean proportion of trials for which the rats’ head was inside the food compartment during consecutive 0.1 sec bins is plotted on the y-axis. The x-axis represents trial time relative to the 500 msec CS (vertical line at 0 sec). Intra-DMS SCH23390 given during Early training produced no effect on cued head entry performance. Neither the proportion of trials for which rats approached the food compartment in response to the CS nor their latency to do so were affected.
Figure 10. Effects of intra-NAc shell D1R antagonist on the mean probability of rats' head being inside the food compartment 10 sec before and 10 sec after CS presentation during the Early training stage. The mean proportion of trials for which the rats' head was inside the food compartment during consecutive 0.1 sec bins is plotted on the y-axis. The x-axis represents trial time relative to the 500 msec CS (vertical line at 0 sec). Intra-NAc shell SCH23390 given during Early training produced no effect on cued head entry performance. Neither the proportion of trials for which rats approached the food compartment in response to the CS nor their latency to do so were affected.
Figure 11. Proportions of trials missed by Early training groups following intra-DMS and NAc shell vehicle or D1R antagonist treatment. The figure shows mean proportion of trials missed by the intra-DMS and intra-NAc shell microinfused groups. Neither DMS or NAc shell D1R blockade resulted in any significant increases of 'missed trials'.
Figure 12. Latencies of rats to enter the food compartment in response to the CS following intra-DMS and NAc shell vehicle or D1R antagonist treatment during *Early training*. No significant increase in mean latency was observed with either DMS or NAc shell D1R blockade.
Figure 13. Noncued approach responses following intra-DMS and NAc shell vehicle or D1R antagonist treatment during Early training. The figure shows mean frequency of ‘baseline head entries’ by the intra-DMS and intra-NAc shell microinfused groups. A significant suppression of noncued approach during Early training was produced by blockade of D1Rs in the NAc shell. Asterisk denotes significant differences from the respective within-subjects vehicle control, *$p < .05$. 

Baseline head entries

Vehicle
SCH23390 2 μg

DMS
NAc shell

$*_{p < .05}$
2.3 Effects of intra-NAc core CNQX/AP-5 on Pavlovian approach expression during early and extended training stages (Study 3)

2.3.1 Introduction

The NAc is believed to critically underlie the expression of associations between CSs and rewards. Rats with post-training lesions of the NAc core show disruption in the performance of previously learned approach driven by a reward-paired CS (Cardinal et al. 2002). Activity within the NAc core also appears to be required for the expression of operant responses under some experimental conditions. For example, inactivation of NAc core reduces instrumental responding driven by a reward-paired cue (Ambroggi et al. 2011). Excitatory activity within the NAc core thus appears to be required for at least some forms of reward-directed responding.

Action potentials in striatal MSNs result from a barrage of excitatory signals sent by striatal afferents (Wilson 1992; Wilson and Kawaguchi 1996). For instance, BLA neuronal responses elicited by a reward-paired cue precede those of the NAc core (Ambroggi et al. 2008). Furthermore, inactivation of the BLA prevents the cue-elicited NAc core neuronal activations (Jones et al. 2010). The excitatory striatal inputs (Capper-Loup et al. 2009; Doig et al. 2010) conveying motivational information from the amygdala (Ambroggi et al. 2008; Jones et al. 2010) or sensory information from the cortex (Pidoux et al. 2011) activate the output MSNs via released GLU (Hu and White 1996) which binds the ionotropic AMPA and NMDA receptors located postsynaptically (David et al. 2005). Concurrent D1R activation potentiates the activated states of NAc MSNs (Cepeda et al. 1993; Nicola et al. 2000; Flores-Hernandez et al. 2002; Plotkin et al. 2011).

The results of Study 1 suggest that NAc core D1Rs play only a temporary role in the performance of CS-elicited approach behavior. Whereas NAc core D1R blockade disrupted Pavlovian approach performance during early training, the same manipulation failed to significantly affect the behavior after extended training. It remained unknown, however, whether or not NAc core output neurons continue to mediate Pavlovian approach during extended training (i.e., even when the behavior is no longer dependent upon D1R activity) or whether extended training of Pavlovian approach reduces the overall
involvement of NAc core neurons (and not just NAc core DA transmission) in mediating the cued approach behavior (Figure 14).

**Early Training**

![Early Training Diagram](image)

**Extended Training**

![Extended Training Diagram](image)

1. NAc core is still necessary for cued approach expression but DA is not

2. Neither NAc core or DA are necessary for cued approach expression

**Figure 14. Hypothetical mechanisms mediating well-acquired Pavlovian approach.** Figure depicts two possible mechanisms underlying expression of cued approach during *Extended* training. In the absence of D1R activation, GLU transmission may facilitate CS-elicited approach either via GLU input to 1) NAc core output neurons or 2) to another brain region. Study 3 examines the likelihood of each scenario in order to determine whether the mechanisms underlying expression of well-acquired Pavlovian responses 1) simply rely on NAc core GLU signalling without NAc core D1R transmission or 2) whether neuronal activation of NAc core is altogether not necessary for the expression of cued approach during *Extended* training.

Evidence suggests that brain mechanisms involved in the performance of learned behavior may indeed shift over the course of training (Miyachi et al. 2002; Yin et al. 2004; Yin et al. 2008; Yin et al. 2009; Ashby et al. 2010; Restivo and Frankland 2010). In the dorsal striatum, activity that is typically time-locked to a recently-acquired operant response loses this relation in rats trained extensively (Carelli et al. 1997; Barnes et al. 2005; Tang et al. 2007). Rats receiving lesions of NAc core after undergoing extensive training on a simple instrumental task do not exhibit any disruptions in the performance on the task (Murphy et al. 2008). These results suggest that other brain sites can take over the mediation of well-acquired instrumental actions; however, it is not clear whether substrate shifts also occur as Pavlovian behaviors become well-acquired. To our best knowledge, a comparison of the effects of NAc core
inactivation on the performance of Pavlovian approach during early versus extended training stages has not been carried out. Thus, the purpose of Study 3 is to evaluate whether NAc core activity plays a differential role in the performance of Pavlovian approach during early versus extended stages of training.

To determine whether NAc core activity is still required for the performance of Pavlovian approach late in learning, we abolished excitatory activity in the NAc core during early and extended learning stages and observed the effects of the treatment on the expression of approach behavior. Excitatory transmission in striatal MSNs is primarily mediated by GLU with AMPA receptors facilitating the initial depolarization and NMDA receptor transmission mediating the secondary source of excitatory transmission. Concurrent blockade of AMPA and NMDA receptors is known to reliably prevent the activation of NAc output neurons (Hu and White 1996). Therefore, in order to completely abolish excitatory NAc core activity, we blocked both the AMPA and NMDA receptors in our experimental manipulation. To block NAc core GLU activity at these receptors concurrently, we combined CNQX, a competitive AMPA receptor antagonist, with AP-5, a selective NMDA receptor antagonist, and microinjected the mixture into the NAc core. Rats were trained drug-free for three or nine days prior to receiving a single intra-NAc core cocktail microinjection of the AMPA and NMDA receptor antagonists and a single vehicle microinjection randomized and counterbalanced on the last two training days.

2.3.2 Materials and methods

Experimental design

We previously found that the role of NAc core D1Rs in the expression of CS-elicited approach diminishes as a function of training. The purpose of Study 3 was thus to determine if NAc core activation, even independent of D1R activation, is still necessary for performance of Pavlovian approach late in learning. In order to determine whether neuronal activation of NAc core retains its role in mediating CS-elicited approach, we examined the effects of blocking excitatory transmission within NAc core during the same stages of training evaluated in Study 1.

Rats were divided into two groups that received a single bilateral infusion of 1 μg of AMPA receptor antagonist CNQX combined with 2 μg of NMDA receptor antagonist AP-5 (1 μg & 2 μg/side,
respectively and a vehicle (0 μg/side) microinjection delivered into the NAc core during either the Early or Extended stages of training. Prior to receiving the microinjections, rats in the Early training group received three consecutive drug-free training days while the rats in the Extended training group received nine drug-free training days. Rats were administered the microinfusions prior to start of sessions taking place on the two ‘Test’ days that followed the initial training period (i.e., on days 4/5 for the Early training group and days 9/10 for the Extended training group). Microinjections were counterbalanced in each group so the half of the rats in each group received the drug combination infusion first and the other half received vehicle first.

2.3.3 Results

ANOVA conducted on intra-NAc core CNQX/AP-5 data allowed us to determine whether blockade of GLU transmission in the NAc core has differential effects on simple approach behavior during Early and Extended training. A 2-way Treatment (vehicle vs CNQX/AP-5) × Stage of Training (3 drug-free days vs 9 drug-free days) ANOVA conducted on ‘missed trials’ (in which rats failed to enter the food compartment within 10 sec of CS onset) revealed a main effect of Treatment, \( F_{(1,14)} = 13.43, p = .003, \) showing that concurrent blockade of NAc core AMPA and NMDA receptors significantly reduced the number of trials for which rats emitted a cued head entry response into the food compartment. A Treatment × Stage of Training interaction, \( F_{(1, 14)} = 5.53, p < .05, \) revealed that the effect of intra-NAc core AMPA/NMDA blockade on ‘missed trials’ depended upon the stage of training.

Shown in Figure 15 is the mean probability of a rat’s head being inside the food compartment from 10 sec before to 10 sec after CS presentation when under the intra-NAc core vehicle or CNQX/AP-5 treatment during the Early (top panel) or Extended (bottom panel) training stages. As can be seen in the top panel, in comparison to vehicle, the CNQX/AP-5 microinjections administered during Early training dramatically lowered the probability of (the proportion of trials for which) the rats’ head was inside the food compartment following the CS. Conversely, the CNQX/AP-5 treatment failed to produce a reduction in the proportion of trials for which rats entered the food compartment in response to the CS when the antagonist combination was administered during Extended training (bottom panel of Figure 15).
The approach behavior exhibited by two representative rats that received vehicle and the AMPA/NMDA receptor antagonist combination during either Early (left panel of Figure 16) or Extended (right panel of Figure 16) training stages illustrates the way in which blockade of excitatory activity within the NAc core suppressed CS-driven food compartment entries during Early training only. By increasing the number of trails missed within the session (bottom left of Figure 16), the intra-NAc core CNQX/AP-5 treatment lead to a suppression in CS-driven responding in the rat that received the combination infusion during Early training. In contrast, cued head entries of the rat that received the CNQX and AP-5 combined antagonists during Extended training were not suppressed (bottom right of Figure 16).

The pattern of disruption that the NAc core AMPA/NMDA receptor blockade produced on cued approach resembles that of Study 1 where the D1R antagonist infused into NAc core disrupted production of cued head entry responses during Early training only (Figure 6). The mean proportions of trials missed by the Early versus Extended training groups when under the influence of vehicle or the GLU antagonists delivered into the NAc core is shown in Figure 17. Paired samples t-tests comparing vehicle and CNQX/AP-5 ‘missed trials’ scores indicated that during Early training, the intra-NAc core CNQX/AP-5 treatment significantly increased the proportion of trials that animals missed ($t_{(8)} = 3.54, p < .05$). In contrast, the combined AMPA and NMDA antagonists failed to produce a significant elevation of ‘missed trials’ when administered during Extended training ($t_{(8)} = 1.65, p = \text{n.s.}$). These results suggest that during Extended training, performance of cued approach is not only less dependent on DA transmission within the NAc core but appears to not require NAc core activation altogether.

It can be seen in the top panel of Figure 15 that not only did AMPA/NMDA receptor blockade lead to a reduced probability of being inside the food compartment post CS-onset during Early training, the treatment also shifted the probability of being inside the food compartment to a later time point relative to CS occurrence. This suggested that during Early training, NAc core AMPA/NMDA receptor blockade also increased latency to perform the cued head entry responses. A 2-way Treatment (vehicle vs CNQX/AP-5) $\times$ Stage of Training (3 drug-free days vs 9 drug-free days) ANOVA on latencies (with ‘missed trials’ excluded) revealed a main effect of Treatment, $F_{(1, 14)} = 14.63, p = .002$. A significant Treatment $\times$ Stage of Training interaction, $F_{(1, 14)} = 7.36, p < .05$, suggested that the effect of NAc core AMPA/NMDA receptor blockade on the ability of rats to emit a timely CS-driven head entry depended upon the stage of training.
As shown in Figure 18, paired samples $t$-tests revealed a significant difference between the vehicle and CNQX/AP-5 latency scores of rats microinjected during *Early* ($t_{(8)} = 4.60, p < .05$) but not during *Extended* ($t_{(8)} = 0.98, p = \text{n.s.}$) training. Reduced dependence of the cued approach behavior on NAc core excitatory transmission appears to result from extended training on the Pavlovian approach task.

Main effects of combined CNQX and AP-5 antagonists infused into NAc core suggest that blockade of excitatory transmission within the NAc core disrupts recently-acquired cued (Figures 17 and 18) but not noncued approach. In fact, blockade of NAc core AMPA/NMDA receptors failed to reduce food compartment entries that occur during the 10 sec of the ITI preceding CS-onset (i.e., ‘baseline head entries’) regardless of training stage. A 2-way *Treatment* (vehicle vs CNQX/AP-5) $\times$ *Stage of Training* (3 drug-free days vs 9 drug-free days) ANOVA on ‘baseline head entries’ failed to reveal a main effect of *Treatment* ($F_{(1, 14)} = 0.47, p = \text{n.s.}$). A *Treatment $\times$ Stage of Training* interaction was not found ($F_{(1, 14)} = 0.34, p = \text{n.s.}$). Unlike the measures of cued approach behavior (i.e., ‘missed trials’ and latency) which were disrupted when rats were under the effects of GLU receptor blockade during *Early* training, noncued food compartment entries occurring in the 10 sec prior to CS presentation (i.e., ‘baseline head entries’) were not significantly suppressed in either the *Early* ($t_{(8)} = 1.29, p = \text{n.s.}$) or *Extended* ($t_{(8)} = 0.01, p = \text{n.s.}$) training stage (Figure 19). Thus, NAc core excitatory transmission appears necessary specifically for normal performance of cued approach rather than approach generally and only during *Early* training.

All animals received vehicle and GLU antagonists on the last two days of training. The order of microinfusions (vehicle first vs CNQX/AP-5 first) was counterbalanced in both *Early* and *Extended* training groups so that half of the animals in each group received vehicle first and the other half received the AMPA/NMDA antagonists first. *Treatment Order* (CNQX/AP-5 or vehicle first) produced neither a main effect nor an interaction with any other factor(s) on any of the dependent measures ($p = \text{n.s.}$ for ‘missed trials’, latency, and ‘baseline head entries’).
Figure 15. Effects of intra-NAc core combined AMPA and NMDA antagonists on the mean probability of rats' head being inside the food compartment 10 sec before and 10 sec after CS presentation during Early (top panel) and Extended (bottom panel) training stages. The mean proportion of trials for which the rats' head was inside the food compartment during consecutive 0.1 sec bins is plotted on the y-axis. The x-axis represents trial time relative to the 500 msec CS (vertical line at 0 sec). Lower peaks after CS presentation in rats with AMPA/NMDA blockade during Early training reflect a reduced proportion of trials in which rats responded to the CS within 10 sec.
Figure 16. Effects of intra-NAc core combined AMPA and NMDA antagonists on approach responses in individual rats from Early (left panel) and Extended (right panel) training groups. The 28 consecutive trials of each session are plotted on the y-axis (bottom to top). Time relative to CS presentation (0 sec) is shown on the x-axis. Each horizontal line represents the occurrence and duration of the rat’s food compartment entries. Both rats received the AMPA/NMDA antagonist combination and vehicle on the last two days of training. In comparison to each respective vehicle session, after being treated with the intra-NAc core AMPA/NMDA antagonist combination, food compartment entries occurring within 10 sec after CS-onset became disrupted only in the rat that received the CNQX/AP-5 treatment during Early training.
Figure 17. Proportions of trials missed by Early and Extended training groups following intra-NAc core vehicle treatment or treatment with combined AMPA and NMDA antagonists. Blockade of NAc core AMPA/NMDA receptors produced a significant elevation of ‘missed trials’ during Early training only. Asterisk denotes a significant difference from the within-subjects vehicle control, *p < .05.
Figure 18. Latencies of *Early* and *Extended* training groups to enter the food compartment in response to the CS following intra-NAc core vehicle treatment or treatment with combined AMPA and NMDA antagonists. A significant increase in mean latency was observed during *Early* training only. Asterisk denotes a significant difference from the within-subjects vehicle control, *p* < .05.
Figure 19. Noncued approach responses of Early and Extended training groups following intra-NAc core vehicle treatment or treatment with combined AMPA and NMDA antagonists. Blockade of NAc core AMPA/NMDA receptors produced no significant suppression of noncued approach during either Early or Extended training stages.
3. DISSERTATION CONCLUSIONS
3.1 SUMMARY

The key findings of these studies were that behavioral expression of recently-acquired Pavlovian approach depends 1) upon the ability of DA to activate D1Rs in the NAc core and 2) upon NAc core AMPA/NMDA receptor transmission. This was clear from the fact that, in separate groups of rats, NAc core D1 or AMPA/NMDA receptor blockade disrupted the recently-acquired Pavlovian approach responses. However, 3) neither intra-NAc core D1 nor AMPA/NMDA receptor blockade (with CNQX & AP-5) disrupted the performance of cued approach after extended training.

An additional surprising finding was that NAc core ionotropic GLU transmission appears to mediate early-stage approach when the approach is cued by an external stimulus but not when this behavior occurs spontaneously. The CNQX/AP-5 treatment produced no disruption of spontaneous (noncued) approach within the baseline ITI during either early or extended training. This contrasts with the effects of blocking NAc core D1Rs during the early training stage wherein the microinfusion treatment disrupted both cued and noncued entries into the food compartment. With extended training, noncued responses remained dependent upon NAc core D1Rs, and (as noted above) cued approach became invulnerable to NAc core D1R blockade. In summary, the results are consistent with the hypothesis that expression of an overtrained Pavlovian approach response becomes DA-independent. The DA-independence appears to occur within the NAc core alongside an altogether reduced requirement of NAc core excitatory transmission for the performance of overtrained Pavlovian approach.

Choi et al. (2005) showed that when D1Rs were blocked during early training with systemic administration of a D1R antagonist, cued approach became disrupted (Choi et al. 2005). This finding raised the question as to where in the brain D1Rs mediate the performance of recently-acquired Pavlovian approach. Presently, we answered this question by showing that D1R blockade localized to the NAc core disrupts the recently-acquired cued behavioral responding. When D1Rs were blocked during the early training stage with microinfusions of intra-NAc core D1R antagonist, rats emitted the CS-signaled approach responses with longer latency and made fewer cued head entries into the reward compartment (i.e., the proportion of trials missed became elevated). These results suggest that the performance of recently-acquired Pavlovian approach depends upon DA transmission at NAc core D1Rs.
Given that previous findings showed no disruption of Pavlovian approach with systemic D2R blockade (Choi et al. 2005), effects of NAc core D2R blockade were not examined here.

Other striatal regions surrounding the NAc core (Heimer et al. 1997; Voorn et al. 2004), which are implicated in reward processing (i.e., DMS and NAc shell) (Balleine et al. 2007; Nicola 2007; Yin et al. 2008), also receive DA innervation (Fallon 1981; Heimer et al. 1997; Haber 2003) and express D1Rs (Levey et al. 1993; Matamales et al. 2009) similarly to the NAc core. If the disruptive effects seen during early training in Study 1 resulted from an uncontrolled spread of the D1R antagonist into a neighboring region, we could not conclude that NAc core D1R transmission is necessary for the performance of recently-acquired approach. In order to determine if the effect of the intra-NAc core D1R antagonist on cued approach is anatomically specific, we examined the effects of D1R blockade on approach by microinjecting the D1R antagonist into the DMS or NAc shell of separate rat groups. D1R blockade in these anatomical control sites failed to disrupt cued approach suggesting that the disruptions in cued approach seen following D1R antagonist infusions to the NAc core were indeed acting within the target site. Although the three key findings in this work (as noted above) pertain to the reduced involvement of NAc core D1Rs (and NAc core generally) in cued approach over the course of training, it is of interest to consider the implications of the negative effects of D1R blockade during early training in the two anatomical control sites.
3.2 GENERAL DISCUSSION

3.2.1 Contrasting effects of D1R blockade in the NAc core, DMS, and NAc shell on recently-acquired cued approach

Compared to the set of disruptions observed with NAc core D1R blockade, a different profile of effects emerged when D1Rs were blocked in the NAc shell. Unlike the disruption produced by NAc core D1R blockade on the recently-acquired cued approach, blockade of NAc shell D1Rs during early training failed to elevate 'missed trials' or to increase latency to make cued head entry responses. At least with respect to these behavioral measures, this finding suggests that NAc shell DA activity is not necessary for the performance of recently-acquired Pavlovian approach behavior. Consistent with the current findings, Gore and Zweifel (2013) found that NAc core, and not NAc shell, D1Rs facilitated Pavlovian approach (Gore and Zweifel 2013). In their study involving D1R knockout mice, conditional D1R expression restricted to the NAc core produced what appeared to be an enhanced form of Pavlovian responding not exhibited by mice with the NAc shell D1R expression.

The NAc shell has been construed as a site critical for the cue-specific effects of reward-predictive CSs on instrumental actions and may thus guide instrumental responses falling under the influence of specific Pavlovian cues (Corbit et al. 2001). In addition to this general role of NAc shell in Pavlovian-to-instrumental transfer, DA activity within the shell has been proposed to mediate the influence of CSs on operant behavior. Consistent with this hypothesis, blockade of NAc shell D1Rs abolishes the enhancement of response rate that a previous reward-paired cue usually produces on instrumental responding (Lex and Hauber 2008). Given the more prominent role of NAc shell DA in instrumental conditioning, the lack of effect of NAc shell D1R blockade on expression of conditioned approach observed in the present study is not surprising.

Similarly, we found that blockade of DMS D1Rs during early training produced no significant increase in latency to emit CS-signaled approach responses or elevation of 'missed trials'. These infusions also failed to suppress even the noncued approach responses occurring during the ITI. Consistent with these data, Yun et al. (2004) have shown that neither performance of lever-press...
responses elicited by a reward-paired DS or unrewarding lever-presses during presentation of a nonreward-paired stimulus are affected by DMS D1R blockade (Yun et al. 2004).

One explanation for the lack of effect of intra-DMS D1R blockade on cued responding may be related to the well-documented role of the DMS in processing outcome information during initial learning stages. Neurons in the DMS are believed to encode outcome information (Hollerman et al. 1998; Lau and Glimcher 2007) deemed to be critical during goal-oriented acquisition and performance of behavior (Yin et al. 2004; Yin et al. 2009; Balleine and O'Doherty 2010; Thorn et al. 2010) when the learning and performance systems are more likely to utilize outcome-related information (Ashby et al. 2010). Experimental data have shown that goal-related neuronal activity within the DMS diminishes throughout training (Thorn et al. 2010). It is possible that, by day three of training, activity at D1Rs in the DMS plays little to no role in the performance of already acquired Pavlovian actions because the outcome representation has already become less important in the production of well-cued behavior. Of course, this is speculative (in the absence of an outcome-devaluation test). These results, however, make it clear that the early-stage cued approach is disrupted by D1R blockade in the NAc core and that this disruption is anatomically specific.

It is interesting that intra-DMS D1R blockade left noncued responding during the ITI intact while blockade of D1Rs in either the NAc core or shell produced a suppression in noncued responses. Thus, the ability to generate spontaneous approach responses in the absence of the CS appears to depend upon intact D1R transmission within either NAc region. Because approach responses not elicited by the CS (i.e., noncued approach responses) are sensitive to D1R blockade in either region of the NAc, neither core or shell DA is unique in facilitating noncued approach during early training. These results suggest the possibility that activation of D1Rs in either NAc site invigorates spontaneous approach not associated with an explicit reward-paired cue. In accordance with this hypothesis, activation of either the NAc core or NAc shell D1Rs by DA or by a selective D1R agonist produces psychostimulant-like effects on locomotor behavior (Swanson et al. 1997).

In summary, blockade of NAc core but not NAc shell or DMS D1Rs disrupted performance of cued approach during early training. In addition to demonstrating anatomical specificity of the intra-NAc core D1R antagonist, the ineffectiveness of DMS and NAc shell D1R blockade at disrupting CS-elicited
approach suggests that D1Rs in the NAc core are somehow specialized to mediate the performance of (at least recently-acquired) conditioned approach responses. Although, in comparison to its neighboring regions, the NAc core appears to be a special structure mediating recently-acquired CS-elicited approach, D1Rs in the entire NAc including the core play a role in approach responses not elicited by a discrete reward-paired cue.

The unique role of NAc core in mediating recently-acquired approach behavior may relate to the specialized afferents received by this structure. The striatum receives segregated projections which target the DMS, DLS, and NAc differentially (Kunzle 1975; Selemon and Goldman-Rakic 1985; Flaherty and Graybiel 1993; Fudge et al. 2002). Specifically, the NAc is known to receive preferential input from the amygdala (Fudge et al. 2002). The cue-evoked excitations in the NAc core appear to depend upon BLA input, and intact BLA activity is required for the behavioral responses elicited by the reward-predictive stimuli (Ambroggi et al. 2008; Jones et al. 2010). Given that D1Rs located postsynaptically are capable of enhancing excitatory activity in NAc core neurons (Nicola et al. 2000; O'Donnell 2003; Plotkin et al. 2011), it is possible that NAc core D1Rs participate in processing cue-evoked signals sent from the BLA during performance of the recently-acquired approach responses that are being driven by the reward-paired cue.

3.2.2 NAc core D1R blockade produces only a transient disruption of cued approach

In light of the preceding discussion, the current finding for a unique NAc core role in cued approach is consistent with a number of earlier findings. More surprising, however, is that the type of neural processing carried out by NAc core D1Rs during performance of recently-acquired CS-elicited approach appears to be unnecessary for the expression of CS responses during the extended training stage. As noted earlier, the intra-NAc core D1R antagonist disrupted CS-elicited approach only during early training; NAc core D1R blockade failed to significantly affect the well-acquired CS-elicited approach. No significant increase in latency to perform CS-elicited responses or elevation of ‘missed trials’ were observed when we blocked NAc core D1Rs on days 10 or 11 of training. This result is consistent with the previously proposed hypothesis (Choi et al. 2005; Horvitz et al. 2007) on the diminishing role of DA in the performance of well-acquired CS-elicited approach and extends the hypothesis by pointing specifically to the NAc core as the site where DA plays this transitory role. The finding also extends the earlier
hypotheses by showing that both NAc core DA and GLU transmission play a transitory role in CS-elicited approach, rather than DA alone.

In the work by Choi et al. (2005), blockade of D1Rs led to a disruption of Pavlovian performance during early but not late stages of training. When given systemic injections of the D1R antagonist on day 17 as opposed to day 3 of training, rats were no longer disrupted in performing cued approach responses for food reward (Choi et al. 2005). The currently observed failure to disrupt Pavlovian approach with NAc core D1R blockade during extended training suggests that DA transmission at NAc core D1Rs is not necessary for the expression of approach responses elicited by an overtrained CS.

It is important to note that while extended training reduced vulnerability of the CS-elicited approach behavior to NAc core D1R blockade, noncued ITI head entries remained vulnerable to NAc core D1R blockade during both early and extended training. The reduced ability of NAc core D1R blockade to disrupt the cued approach response therefore cannot be attributed to a reduction in the effectiveness of the D1R antagonist or to an overall reduced sensitivity of approach behavior to the D1R antagonist. Thus, the behavioral transition to DA-independence appears to involve conditioned rather than unconditioned behavioral responses.

The fact that noncued ITI approach remained dependent upon D1Rs, even after the cued response had become DA-independent, may be related to the observation that the most severe behavioral disruptions in PD patients involve internally generated movements (Jahanshahi 1998) rather than movements driven by salient environmental cues (Suteerawattananon et al. 2004; Stern et al. 2005). The approach responses into the reward compartment in the absence of the CS (i.e., during the ITI) may be described as internally generated: they are not under the influence of explicit environmental cues (i.e., at least not cues under experimental control). Presently, blockade of NAc core D1Rs suppressed noncued approach regardless of training stage suggesting that DA actions in the NAc are necessary for behavioral responses that are not elicited by discrete environmental stimuli. (Given that we did not examine the effects of the intra-NAc shell microinjections during extended training, it is not clear whether the noncued approach response remains dependent upon NAc shell D1R transmission.) The heightened vulnerability of noncued behavior to DA receptor blockade may relate to the difficulties PD patients face in making self-generated rather than routine (i.e., overtrained) movements (Jahanshahi 1998) and supports
the idea that loss of DA activity in the NAc may contribute to the specific symptomology of PD. Although this suggestion is speculative, it is in accordance with recent evidence that PD patients show anatomical abnormalities within the NAc (Mavridis et al. 2011; Mavridis 2014) in addition to the well-known nigrostriatal DA loss.

3.2.3 Why is the NAc core D1R role in cued approach transient?

According to one theoretical model (Horvitz 2002; Horvitz et al. 2007), D1Rs are involved in setting the threshold for a CS to elicit a conditioned behavioral response for a goal. The present results could be explained by this model, as it could be viewed that the blockade of NAc core D1Rs increases the threshold for environmental stimuli to elicit approach responses to the reward compartment. Non-drugged animals express the cued approach on the early training ‘test’ day: the response eliciting strength of the CS under these conditions is, by definition, above threshold. According to the model, administration of the D1R antagonist raises the threshold for cues to drive approach behavior; consequently, the previously-acquired response-eliciting strength of the CS now falls below this raised threshold. In contrast, the extensively-trained CS in the extended training group is presumed to have gained enough response-eliciting strength to remain above even the raised threshold produced by NAc core D1R blockade. Interestingly, from this theoretical perspective, noncued approach responses during the ITI, presumably elicited by (contextual) environmental stimuli present in the chamber only weakly paired with reward, remain ‘below threshold’ for eliciting approach responses—even after extended training—because the contextual cues were never explicitly paired with reward. Therefore, a model in which D1R blockade raises the threshold for environmental cues to generate an approach response accounts both for the disruptive effect of D1R blockade on cued approach during early training only and also for the fact that noncued approach during the ITI is disrupted by D1R blockade during both early and extended training.

The exact mechanism of DA in setting the threshold for motivational cues to elicit behavior remains to be fully elucidated. DA release in target regions, such as the NAc, is believed to gate incoming stimuli to proper motor outputs (Horvitz 2002). In addition to this proposed effect on the throughput of current input signals to the striatum, DA activity has been hypothesized to mediate early-stage learning and performance (Horvitz et al. 2007) by strengthening the connections between the currently active NAc
motor output neurons and the concurrently active cortical/allocortical neurons that carry sensory information (about environmental stimuli) to the NAc (Horvitz 2002; Horvitz 2009). After extensive occurrence of CS-reward pairings over time and therefore a stronger CS-response connection, expression of behavioral responses elicited by the well-acquired CS may become less dependent on DA modulation because the approach response now becomes elicited by strong excitatory input that drives Pavlovian approach expression.

3.2.4 The role of NAc core excitatory (GLU) transmission in cued approach is also transient

As noted above, excitatory input/output activity in the NAc core is driven by GLU transmission at AMPA and NMDA receptors, and the efficacy of this excitatory activity is modulated by DA. Based on the premise that the strong excitatory input, to the NAc, encoding information about a well-acquired CS is strong enough to elicit a Pavlovian response into the food compartment even in the absence of DA activity, we hypothesized that while DA transmission within the NAc core is only necessary during early-stage performance, excitatory (GLU-driven) neuronal activity within the NAc core would be required for the performance of CS-elicited approach regardless of training stage. In other words, we postulated a model in which the NAc core remained critical to response expression even as DA’s role within the core diminished. We therefore predicted that co-blockade of NAc core AMPA and NMDA receptors would produce disruptive effects on cued responding during both early and extended training stages. In light of our results showing decreasing NAc core D1R involvement in cued approach performance across training, these results would suggest that the role of D1R transmission in Pavlovian approach is temporary while the role for NAc core output activity is maintained. Such results would imply that during extended training, via activation of NAc core output neurons, GLU signaling conveying sensory and/or motivational information can elicit approach responses even in the absence of DA transmission.

Conversely, a failure of the NAc core AMPA/NMDA receptor blockade to disrupt Pavlovian approach during extended training would suggest that, once overtrained, CS-elicited approach responses no longer depend upon NAc core excitatory transmission. Thus, the alternative outcome would suggest that another substrate takes over the mediation of Pavlovian approach late in learning. The
representation of the well-acquired behavior might have moved to another anatomical circuit, bypassing the NAc core. (This pattern of results would also be consistent with the possibility that, with extended training, another anatomical substrate mediates the behavior in addition to the NAc core.)

The results show that the NAc core AMPA/NMDA receptor blockade significantly increased latency to enter the food compartment upon CS-onset and produced a significant elevation of ‘missed trials’ during early training. Consistent with these observations, previous data have suggested that NAc core activity is required for the expression of recently-acquired CS-US associations. For example, rats trained on four days of discriminated approach reduced their responding to the CS+ once the NAc core was inactivated (Blaiss and Janak 2009). The present work, however, additionally shows that this NAc core role in conditioned approach is transient, i.e., that the well-acquired Pavlovian approach becomes independent of NAc core excitatory (GLU) transmission.

The fact that the well-acquired conditioned approach becomes independent of NAc core GLU transmission would explain the fact that it becomes independent of NAc core DA. After all, if the behavior no longer depends upon NAc core activation, it is not surprising that it no longer depends upon DA (D1R) modulation of this activation.

It is alternatively possible that the diminished role of NAc core DA is of primary importance and that the diminishing role of NAc core GLU is a secondary observation. For instance, activation of AMPA and NMDA receptors may enhance striatal DA release (Morari et al. 1998; David et al. 2005). This enhanced DA release could, under normal conditions, facilitate Pavlovian performance during the early learning phases. Based on this idea, the ionotropic GLU antagonist combination may have inadvertently reduced DA release in the NAc core thereby disrupting Pavlovian performance during early training (a behavioral effect we also observed with direct intra-NAc core D1R blockade). In this case, blockade of AMPA/NMDA receptors during extended training may have failed to alter CS-elicited approach because DA transmission at D1Rs within NAc core was no longer required for behavioral expression. Assuming this scenario, DA would appear to retain the primary role in the progression to a NAc core independent state in the performance of overtrained Pavlovian approach.

However, given that the AMPA/NMDA receptor antagonist combination likely fully abolished NAc core excitatory signaling (Hu and White 1996), the failure of NAc core inactivation to disrupt overtrained
Pavlovian approach seems highly likely to reflect the fact that the signaling of CS information to the NAc core via GLU transmission was no longer necessary once the CS-elicited response became overtrained.

3.2.5 Excitatory transmission in the NAc core is not needed for noncued approach responses

Although the loss of NAc core excitatory transmission during early training produced a suppression of cued responding, noncued approach into the food compartment remained unaffected. This contrasted with NAc core D1R blockade which disrupted both cued and noncued approach during early training. Our data suggest that during early learning, transmission at NAc core’s ionotropic GLU receptors is necessary for reward-directed approach responses elicited by an explicit CS but not for the spontaneous approach behavior. The findings support the notion that NAc core ionotropic GLU transmission mediates specifically the behavioral responses elicited by a recently-acquired CS.

However, the fact that intra-NAc core GLU receptor blockade failed to affect noncued approach is at odds with some previous work showing that blockade of NAc core GLU receptors increases ‘irrelevant’, or nonrewarded, behavioral responses (Yun et al. 2004; Ambroggi et al. 2011). Specifically, upon blockade of AMPA/NMDA receptors in the NAc core, rats significantly increased lever-presses directed toward an inactive lever, lever-presses directed toward an active lever in the absence of a DS signaling reward availability, and lever-press responses for a neutral cue not paired with reward (Yun et al. 2004; Ambroggi et al. 2011). In contrast to these increases in unnecessary operant responses, we failed to observe increased noncued (and thus nonrewarded) approach into the reward compartment following intra-NAc core AMPA/NMDA receptor blockade administered during either training stage.

Besides paradigm differences and the inclusion of NAc shell inactivations (which appear more prominent in disinhibition of ‘irrelevant’ operant actions) in the aforementioned operant results, the seeming discrepancy may stem from the fact that the previously observed increases in nonrewarded behavior occurred during the 10 sec following a rewarded response but not during the ITI period immediately preceding stimulus presentation within trials (Ambroggi et al. 2011). Given that our noncued behavioral measure consisted of the 10 sec preceding the CS, previous data examining effects of ionotropic GLU receptor blockade on operant responses are mostly consistent with the present findings.
showing a disruption of early-trained CS-elicited approach and neither a suppression nor a disinhibition of noncued approach in the ITI baseline period during NAc core inactivation.

3.2.6 If the NAc core plays a diminishing role in well-acquired behavior, does another brain region take over?

We presently show that blockade of NAc core D1Rs disrupted early-stage Pavlovian approach but failed to disrupt Pavlovian approach during the extended training stage. The fact that even the GLU (AMPA/NMDA) receptor blockade failed to block the well-acquired behavior suggests that excitatory signals sent to the NAc core were no longer necessary for the expression of overtrained cued approach.

If so, one might predict that the NAc core output neurons fire less during the expression of overtrained Pavlovian responses. While this hypothesis has not been tested, output neurons in a dorsal striatal region, the DLS, showed reduced neuronal firing as lever-pressing behavior became overtrained (Carelli et al. 1997). Perhaps the NAc core output neurons similarly become less engaged during extended training of the Pavlovian approach response.

If one assumes that there is a fine-tuning of, or even a diminished, neuronal firing of NAc core MSNs (i.e., the output neurons) during overtraining of Pavlovian approach (similar to that seen by Carelli et al. 1997 in the DLS), what kind of mechanism might lead to the reduced NAc activity? It is possible that it is a function of the interaction between DA and GLU transmission resulting in neuroplastic effects.

Usually, D1/NMDA receptor interactions are seen as potentiating neurotransmission. For instance, DA activity at currently active corticostriatal synapses within the NAc core has been hypothesized to strengthen those synapses (Horvitz 2002; Horvitz 2009). NAc core neuroplasticity underlying behavioral learning is initiated by the co-activation of D1 and NMDA receptors (Kelley 2004). Additionally, it has been proposed that the induced neuroplastic changes strengthening excitatory synapses on NAc core MSNs result in enhanced output in response to subsequent excitatory input (Wolf 2010). Such neuroplastic effects may help confine NAc core neuronal responses to specific MSNs or MSN populations throughout behavioral training.

But can DA/NMDA receptor interactions also lead to reduced excitatory transmission? Interactions between GLU and DA at the “synaptic triad” in the NAc (i.e., the circuit between GLU inputs,
MSN output neurons, and DA modulation) that are mediated by D1Rs can either potentiate or inhibit NMDA transmission (Missale et al. 2006). For example, co-activation of D1 and NMDA receptors located on MSNs induces increased expression of hybrid D1/NMDA receptor complexes (Missale et al. 2006; Cahill et al. 2014). Depending on which subunit of the NMDA receptor is incorporated into the hybrid D1/NMDA receptor, subsequent activation of the hybrid receptor types can result in either enhanced or inhibited subsequent NMDA currents (Missale et al. 2006). By such a mechanism, diminished NAc core involvement in the overtrained behavior might result from reduced NAc core responses to excitatory inputs. Thus, the progression to a NAc core independent state might involve mechanisms relying on neurochemical transmission within the structure. However, it is also possible that reduced excitatory input to the NAc core (rather than reduced NAc core responsiveness to the input) accounts for the region's diminished role in overtrained CS-elicited approach.

Our data showing a diminished role of NAc core excitatory transmission in the performance of overtrained Pavlovian approach suggest that the mediation of this behavior has transferred to a non-NAc core substrate. This raises the question as to which brain areas are needed for the expression of cued approach following extended training. The Choi et al. (2005) results showed that overtrained cued approach is invulnerable to systemically administered DA receptor blockade (Choi et al. 2005). This suggests that mediation of overtrained cued approach may shift to a brain region that is not subject to the modulatory effects of DA transmission. There is evidence, for instance, to suggest that automatized responses may engage the cerebellum (Lu et al. 1998; Lang and Bastian 2002) or occur via corticocortical connections (Ashby et al. 2010) that do not require basal ganglia involvement. On the other hand, at least one study suggests that overtrained Pavlovian approach may depend upon interactions between D1 and AMPA receptors (Bespalov et al. 2007). It may therefore be premature to rule out a shift to a non-NAc core but still DA modulated brain area, such as the DLS, as a mediator of the overtrained Pavlovian approach response. Having established that the NAc core is not needed for expression of the well-acquired reward-directed behavior, the question remains, what is (are) the anatomical and neurochemical mediator(s) of the overtrained behavioral response?
REFERENCES


Dobrovitsky, V., G. Canales, M. Briones, S. Ma, M. O. West and J. C. Horvitz (2011). Dopamine D1 receptor blockade within the nucleus accumbens, but not the dorsomedial striatum, disrupts the expression of a reward-directed approach response. *Society for Neuroscience Abstracts*. 41st Annual Society for Neuroscience Conference, Washington, DC.


Nowend, K. L., M. Arizzi, B. B. Carlson and J. D. Salamone (2001). D1 or D2 antagonism in nucleus accumbens core or dorsomedial shell suppresses lever pressing for food but leads to compensatory increases in chow consumption. *Pharmacology, Biochemistry, and Behavior* 69(3-4): 373-82.


