An Examination of Phasic Dopamine Release in
Distinct Striatal Subregions during Reward-Directed Behavior

BY

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B.S., University of Michigan, 2005

THESIS

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<tr>
<td>6-OHDA</td>
<td>6-hydroxydopamine</td>
</tr>
<tr>
<td>Ag/AgCl</td>
<td>Silver/Silver Chloride</td>
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<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
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<tr>
<td>AP</td>
<td>anterior-posterior</td>
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<tr>
<td>Core</td>
<td>nucleus accumbens core</td>
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<tr>
<td>cm</td>
<td>centimeter</td>
</tr>
<tr>
<td>[DA]</td>
<td>dopamine concentration</td>
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<td>DAT</td>
<td>dopamine transporter</td>
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<td>DLS</td>
<td>dorsolateral striatum</td>
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<td>DMS</td>
<td>dorsomedial striatum</td>
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<tr>
<td>DS+</td>
<td>rewarded discriminative stimulus</td>
</tr>
<tr>
<td>DS-</td>
<td>non-rewarded discriminative stimulus</td>
</tr>
<tr>
<td>DV</td>
<td>dorsal-ventral</td>
</tr>
<tr>
<td>FSCV</td>
<td>fast-scan cyclic voltammetry</td>
</tr>
<tr>
<td>GABA</td>
<td>ɣ-aminobutyric acid</td>
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<tr>
<td>HCl</td>
<td>hydrochloric acid</td>
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<tr>
<td>Hz</td>
<td>hertz</td>
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<td>im</td>
<td>intramuscular</td>
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<td>ip</td>
<td>intraperitoneal</td>
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<td>kg</td>
<td>killograms</td>
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<td>mg</td>
<td>milligrams</td>
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<td>mL</td>
<td>milliliters</td>
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<th>Abbreviation</th>
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<tbody>
<tr>
<td>ML</td>
<td>medial-lateral</td>
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<tr>
<td>mV</td>
<td>millivolts</td>
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<tr>
<td>mm</td>
<td>millimeter</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>PCA</td>
<td>principal component analysis</td>
</tr>
<tr>
<td>μA</td>
<td>microamps</td>
</tr>
<tr>
<td>s</td>
<td>second</td>
</tr>
<tr>
<td>Shell</td>
<td>nucleus accumbens shell</td>
</tr>
<tr>
<td>SNpc</td>
<td>substantia nigra pars compacta</td>
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<tr>
<td>VTA</td>
<td>ventral tegmental area</td>
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SUMMARY

Learning about stimuli that signal the attainment of food and other rewards is critical for survival. Evidence suggests that the midbrain dopamine system plays a critical role in reward learning and goal-directed behavior. Recordings of midbrain dopamine neurons in awake and behaving subjects suggest that phasic dopamine signals are uniformly broadcast throughout terminal regions in response to unpredicted reward or environmental stimuli that predict reward. However, studies sampling dopamine release from the nucleus accumbens during unpredicted reward or predictive stimuli show that phasic dopamine signaling may occur in a more regionally-selective manner. However, there has not been a systematic examination of whether phasic dopamine release from dorsal and ventral striatal subregions occurs uniformly throughout the striatum or in a regional-selective manner. To address this, I measured phasic dopamine release, using fast-scan cyclic voltammetry, in four striatal regions (nucleus accumbens shell and core, dorsomedial and dorsolateral striatum) during electrical stimulation of the ventral midbrain, unpredicted food reward or during a discriminative stimulus paradigm.

The results from all experiments indicate that dopamine signaling occurs in a regional-specific manner. Electrical stimulation of the SNpc/VTA evoked dopamine release in all striatal regions but the rate of reuptake was fastest in the dorsolateral striatum and slowest in the nucleus accumbens shell. Unpredicted food reward only evoked phasic dopamine release in the nucleus accumbens core. In the discriminative stimulus task, a cue predictive of reward evoked a phasic dopamine signal in the nucleus accumbens core and dorsomedial striatum. Following performance of the discriminative stimulus task, unpredicted food reward increased phasic dopamine release in both the nucleus accumbens core and the dorsomedial striatum. No condition
evoked phasic dopamine release in the nucleus accumbens shell or dorsolateral striatum. These findings provide the first demonstration that phasic stimulation, reward stimuli and reward predictive cues evoke highly compartmentalized changes in phasic dopamine release across multiple striatal regions. This incongruence with electrophysiological recordings may be due to several factors including prior task experience, selection criterion for dopamine neuronal recording, or presynaptic modification of dopamine release. Together, these results suggest distinct roles of phasic dopamine release across striatal subregions.
Chapter I
Introduction

A. **Historical background of the relationship between reward and dopamine**

The ability to acquire knowledge in a complex environment is critical for daily living and survival. Learning about the associations between environmental stimuli and rewards (e.g. food or social interaction) can powerfully reinforce behavior to attain those rewards. Understanding the neural mechanisms that underlie reward, associative learning and reinforcement has been the subject of intense study for several decades. One of the landmark studies in understanding these neural mechanisms was by James Olds and Peter Milner in 1954. In their study, stimulating electrodes were implanted into various brain regions and fiber tracts. Rats were able to press a lever that delivered electrical stimulation to a specific brain region. From this, the ability for stimulation to elicit lever pressing behavior was evaluated. Olds and Milner (1954) observed that electrical stimulation of distinct forebrain and midbrain regions produced significant lever pressing behavior, while other brain areas produce avoidance of lever pressing or had no effect. This finding was interpreted that electrical stimulation in distinct regions was positively reinforcing (or rewarding) and produced similar behavioral effects to that observed with natural rewards (i.e. food and water, reviewed in Trowill et al., 1969). These results were significant because it generated focus on these brain systems to understand the neurobiological mechanisms underlying reward.

Today, the phenomena in which animals administer brief bursts of weak electrical stimulation to specific brain sites is referred to as intracranial self-stimulation. Since
intracranial self-stimulation was supported with electrode placements only in certain brain regions, this led to the prediction that there were specialized reward systems in the brain. As physiological psychologists were using intracranial self-stimulation paradigms to study brain circuitry involved in reward, there were concomitant advances in neuroanatomy that led to the identification of catecholamines as neurotransmitters in the brain and neural pathways in which particular catecholamines, such as dopamine, were located. For example, dopamine was found in to be present in high concentrations in the striatum (Carlsson & Waldeck, 1958 and Bertler & Rosengren, 1959 as cited in Carlsson, 1987) and the dopaminergic pathways were mapped from the ventral midbrain to terminal forebrain regions (Dahlstrom and Fuxe, 1964; Andént al., 1964; 1965 as cited in Carlsson, 1987).

Several experiments indicated that intracranial self-stimulation could be easily elicited from stimulation of dopamine regions such as dopamine cell bodies in the ventral midbrain (Olds and Olds, 1963; Mogenson et al., 1979; Robertson et al., 1981; Hand and Franklin, 1983), fiber bundles containing dopamine axons (Olds, 1956; Corbett, 1990) or several dopamine terminal regions in the forebrain (Routtenberg and Sloan, 1972; Mogenson et al., 1979; Robertson et al., 1981; Hand and Franklin, 1983; Corbett, 1990). Furthermore, not only was intracranial self-stimulation elicited by stimulation of brain dopamine systems, but it could be pharmacologically manipulated by drugs acting on the dopamine system. Systemic administration of drugs that increase dopamine, including amphetamine and cocaine, increased lever pressing for intracranial self-stimulation (Crow, 1970; Phillips and Fibiger, 1973) while dopamine antagonists, such as pimozide or chlorpromazine, decreased lever pressing (Olds and
Travis, 1960; Fibiger et al., 1976; Fouriezos and Wise, 1976; Fouriezos et al., 1978).

Interestingly, the decrease in lever pressing behavior followed a pattern similar to an extinction paradigm, where a lever press no longer results in electrical stimulation. This suggests that the stimulation is no longer “pleasurable” or rewarding after dopamine antagonist administration. From these experiments, the midbrain dopamine system emerged as a critical player in the rewarding aspects of intracranial self-stimulation (Wise, 1978a). Not only were the reinforcing behaviors elicited by stimulation of dopamine pathways, but the pleasurable or rewarding effects of the stimulation were altered by dopamine manipulations. It remained unclear, however, if these dopamine mediated behaviors were specific to the rewarding effects of intracranial self-stimulation, or if dopamine was also important for natural rewards, such as food or sex.

To examine this, a seminal experiment by Wise et al. (1978a) evaluated the effects of a systemic injection of the dopamine antagonist, pimozide, on lever pressing behavior for food reward over multiple days. In a methodology similar to intracranial self-stimulation paradigms, animals were able to press a lever to receive a food reward. When administered and tested over multiple days, pimozide had no effect initially, but lever pressing behavior gradually declined. The decrease in responding after pimozide seemed to have been acquired or learned over several days of testing. No effect was observed in rats administered the dopamine antagonist in their home cages for several days, but later evaluated for lever pressing behavior. From this, it was suggested that pimozide resulted in a loss of the pleasurable effects of food reward. Based upon this relationship of dopamine and reward, Roy Wise (1978b) proposed that dopamine signaled the hedonic or pleasurable value of rewards and that dopamine antagonism
resulted in a lack of pleasure, or anhedonia from those rewards. Known as the anhedonia hypothesis, this idea served as a basis for our understanding of dopamine function as the "pleasure" neurotransmitter for more than a decade.

Technical advances in the ability to record dopamine concentration in terminal areas further supported the anhedonia hypothesis. In vivo microdialysis, a technique which became popular in behavioral experiments in the 1990s, measures changes in dopamine concentration in specific brain regions. In behaving animals, increases in dopamine concentration at terminal sites, such as the striatum and nucleus accumbens, occur in response to rewarding stimuli such as food reward (Bassareo et al., 1995; Bassareo and Di Chiara, 1999; Bassareo et al., 2011; Ostlund et al., 2011), a sexually receptive mate (Pfaus et al., 1990; Damsma et al., 1992), drugs of abuse (Robinson et al., 1988; Zetterstrom et al., 1988; Kalivas and Duffy, 1993) and intracranial self-stimulation (Fibiger et al., 1987; Berridge et al., 1989; Phillips et al., 1989; Hernandez et al., 2006; Cheer et al., 2007; Owesson-White et al., 2008; Beyene et al., 2010). Taken together, dopamine release is not only increased by a variety of rewarding stimuli but dopamine receptor blockade decreases their reinforcing effects.

Work in the mid-1990’s produced a series of results that were inconsistent with what had become the widely accepted view that dopamine signaling was directly responsible for the pleasurable aspects of reward (the anhedonia hypothesis). In particular, studies demonstrated a dissociation between dopamine activity and its pleasurable effects. Work by Berridge and colleagues (Berridge et al., 1989; Berridge and Robinson, 1998) examined orofacial responses of rats to pleasurable rewarding stimuli. Using a toxin selective for dopamine neurons (6-hydroxydopamine; 6-OHDA), they showed that
orofacial response were unaffected by severe dopamine depletion, i.e. rats still showed pleasurable responses to reward after dopamine depletion. Similarly, the dopamine antagonist pimozide did not alter pleasurable orofacial responses to reward (Pecina et al., 1997). This provided strong evidence that dopamine may not be directly responsible for the hedonic or pleasurable qualities of a reward. Furthermore, Salamone and colleagues examined the role of dopamine in behaviors that required varying levels of effort to obtain rewards. Specifically, dopamine depletion with 6-OHDA lesions and dopamine antagonists reduced operant responding in an effort-dependent manner. Low effort tasks, such as fixed ratio 1 where rats only had to press a lever once to receive a reward, were relatively unaffected by dopamine depletion or antagonism (Aberman et al., 1998; Aberman and Salamone, 1999; Salamone et al., 2001). However, on tasks requiring increasing motivational demands, such as progressive ratio where the number of lever presses increased exponentially to obtain each reward (Ex. 1, 2, 4, 16, 64, etc), the level of responding was profoundly decreased following dopamine depletion or antagonism (Aberman et al., 1998; Aberman and Salamone, 1999; Salamone et al., 2001). These results suggested that dopamine played a role in mediating the level of effort exerted in motivationally challenging tasks.

These studies and others drew support away from the anhedonia hypothesis and resulted in the development of many new proposals for dopamine’s function that are still debated today. Currently, there are numerous hypotheses regarding the relationship between dopamine and reward including dopamine’s involvement in reward seeking (Ikemoto and Panksepp, 1999), reward craving (Berridge and Robinson, 1998), action reinforcement (Redgrave and Gurney, 2006), motivated reinforcement (Salamone and
Correa, 2002) and reward prediction error (Schultz, 1997, 1998). While no single psychological role of dopamine has emerged and remains a topic for debate, there is a clear relationship between dopamine, reward and behaviors directed at achieving rewards.

One of the more prominent hypotheses of dopamine’s function was proposed by Wolfram Schultz (1997), suggesting a role of dopamine in signaling information about the prediction of reward. Schultz and colleagues recorded the activity of single dopamine neurons in the ventral midbrain in awake and behaving non-human primates. They demonstrated that a majority of dopamine neurons briefly increase their activity to unpredicted rewards – perhaps on the surface a result consistent with the ‘anhedonia hypothesis.’ However, when the reward is fully expected, little change in dopamine neuronal activity was observed. Furthermore, when environmental cues come to reliably predict the delivery of a reward, the increase in dopamine activity shifts to the onset of the predictive cue (Schultz et al., 1993; Mirenowicz and Schultz, 1994, 1996). Surprisingly, the response of dopamine neurons to the reward itself was no longer present. That is, the increase in dopamine activity appeared to shift from the primary reward to the earliest reliable predictor of reward delivery. This shift demonstrated that dopamine activity was not linked directly to primary reward but indicated that dopamine may play a role in reward prediction (Schultz, 1997). Additional work has further developed this hypothesis, demonstrating a majority of recorded dopamine neurons respond to unexpected reward (75%) and reward-predictive cues (55-70%) in well-trained non-human primates (Schultz, 2002). Furthermore, changes in dopamine neuron activity also encode the expected value of the reward and the probability of
receiving the reward (Hollerman and Schultz, 1998; Tobler et al., 2005). Taken together, Schultz (2002) suggested that dopamine neurons uniformly increase their activity to encode information about reward expectancies and cues that predict the rewards, producing a global dopamine signal across all terminal regions.

However, at present there is conflicting evidence of how dopamine transmission may occur related to reward and reward predictive cues. While the work by Schultz and others suggest that dopamine neurons uniformly respond to reward and reward-associated stimuli, measurements of dopamine concentration using in vivo microdialysis suggest otherwise (Bassareo and Di Chiara, 1999; Bassareo et al., 2011; Ostlund et al., 2011). The central aim of this dissertation project was to determine whether dopamine signaling is uniformly broadcast or regionally selective in subregions of the striatum to reward and stimuli predictive of reward. To provide a context for this central aim, below is a description of the anatomy of striatal subregions and the midbrain dopamine system, as well as additional background on how reward-associated activity is encoded by dopamine neuron activity and release.

**B. Anatomical organization of the striatum**

As mentioned previously, the relationship between dopamine signaling and reward is, at present, a topic of considerable debate. One possible reason for the inability to fit a single function to dopamine activity is that dopamine neurons project widely across the forebrain, possibly playing a unique function at each terminal region. One major target of midbrain dopamine neurons is the striatum, the main input structure of the basal ganglia. The striatum serves as an integration center for topographic projections
from cortical, limbic, thalamic and midbrain dopamine regions. These anatomical connections result in functional distinctions in which striatal subregions differentially contribute to aspects of goal-directed behavior, learning and motor behavior. While there is not universal agreement on the functional roles of each of the striatal subregions, there is little doubt for regional specificity.

All striatal regions receive input from thalamic and cortical regions onto medium spiny neurons, GABA-ergic projection neurons that compose 90-95% of the neurons in the striatum (Meredith et al., 2008). Striatal medium spiny neurons project to basal ganglia output circuitry which ultimately drives motor output (Bateup et al., 2010). Cortical and thalamic inputs largely connect to the head of dendritic spines on medium spiny neurons. Midbrain dopamine neurons, however, primarily make symmetrical synapses onto the necks of dendritic spines, sometimes shared with another bouton forming an asymmetrical synapse from the cortex or thalamus (Moss and Bolam, 2008). Given this anatomical arrangement, dopamine is in a prime position to modulate striatal output (Nicola and Deadwyler, 2000; Bamford et al., 2004a; Bamford et al., 2004b; Surmeier et al., 2009; Gerfen and Surmeier, 2010). Therefore, dopamine modulation of medium spiny neuron activity is perfectly poised to alter basal ganglia output circuitry which ultimately drives motor responses.

The striatum can be divided into four subregions primarily based on input-output relationships and cytoarchitecture: the nucleus accumbens shell (Shell), the nucleus accumbens core (Core), the dorsomedial striatum (DMS) and the dorsolateral striatum (DLS). The Shell receives afferents from the infralimbic and piriform cortex, amygdala, hippocampus, lateral hypothalamus, paraventricular thalamic nucleus, and
dopaminergic information from the medial VTA (Kelley et al., 1982; Berendse and Groenewegen, 1990; Berendse et al., 1992; Zahm and Brog, 1992; Groenewegen et al., 1999). Functionally, the Shell is thought to process novel stimuli (Bassareo and Di Chiara, 1999). Medium spiny output neurons in the Shell are differentially modulated by rewarding and aversive stimuli and are thought to encode the hedonic value of stimuli with dopamine playing a key role, especially when the valence of the stimulus is altered (Roitman et al., 2008; 2010). A distinct “hedonic hot spot” in the rostromedial Shell is thought to mediate the hedonic impact of rewarding stimuli but primarily through an opioid mechanism (Pecina and Berridge, 2000, 2005). Furthermore, its role in encoding hedonic valence along with its anatomical connections from lateral hypothalamic, amygdala, and gustatory thalamic regions have suggested a role of the Shell in feeding behavior (Stratford and Kelley, 1997; Kelley, 2004; Stratford, 2005; Stratford and Wirtshafter, 2011).

Another ventral striatal subdivision, the Core, receives excitatory input from limbic regions including the medial prefrontal cortex, amygdala, hippocampus, ventral medial and parafascicular thalamic nuclei and dopaminergic innervation primarily from the lateral VTA and medial SNpc (Kelley et al., 1982; Berendse and Groenewegen, 1990; Berendse et al., 1992; Sadikot et al., 1992; Zahm and Brog, 1992; Groenewegen et al., 1999). The Core, and specifically its dopamine innervation, is involved in mediating goal-directed behaviors, encoding information about rewards and reward-associated cues (Bassareo and Di Chiara, 1999; Kelley, 1999; Carelli et al., 2000; Day et al., 2006). Proposed as a limbic-motor interface (Mogenson et al., 1980), the limbic regions
projecting to the Core are integrated by the medium spiny neurons, modified by
dopamine release and then project to downstream motor circuitry that drives behavior.

The DMS receives excitatory projections from the medial prefrontal cortex,
parafascicular thalamic nucleus as well as dopaminergic projections from the SNpc and
VTA (McGeorge and Faull, 1989; Berendse et al., 1992; Sadikot et al., 1992) The DMS
has been shown to be critical for reward learning, specifically the ability to flexibly shift
behavior from one choice pattern to another in order to obtain a reward (Featherstone
and McDonald, 2004; Kimchi and Laubach, 2009a; Ragozzino et al., 2009). While
dopamine in the DMS increases during operant responding for rewards (Stefani and
Moghaddam, 2006; Ostlund et al., 2011), dopamine’s role in the DMS remains unclear.
Interestingly, while the DMS and Core receive many overlapping inputs form cortical
and thalamic nuclei, each striatal region seems to play different roles in goal-directed
behavior

The DLS receives inputs from sensorimotor and motor cortex, centromedian
thalamic nucleus, and dopamine inputs primarily from the lateral portion of the SNpc
(McGeorge and Faull, 1989; Sadikot et al., 1992; Cheatwood et al., 2003; Reep et al.,
2003; Cheatwood et al., 2005). The DLS is a region critical for the development and
expression of a habit, or a well-learned stimulus-response association (Yin et al., 2004).
Consistent with this idea, neurons in the DLS do not respond to reward (Root et al.,
2010). Dopamine depletion in the DLS leads to impairment in motor behaviors, including
forepaw use to retrieve reward (Evenden and Robbins, 1984; Sabol et al., 1985). Thus,
while the DLS is thought to play a role in habitual responding, dopamine activity in the
DLS seems to underlie motor behaviors necessary for reward retrieval.
Taken together, striatal subregions receive a diversity of inputs that uniquely integrate distinct sets of information, resulting in functional differences across the striatum. While behavioral studies have suggested that dopamine has different functional roles across striatal subregions, single dopamine neurons have vast striatal projection fields with dense axonal arborizations. A single nigrostriatal dopamine neuron can cover more than 6% of the striatal volume on one side of the striatum, relaying dopamine signals to large terminal areas (Matsuda et al., 2009). The authors used these results to suggest that a single dopamine neuron broadcasts a uniform signal across large striatal areas. While dopamine activity in each subregion seems to have a distinct function, the expansive arborization of single dopamine neurons may suggest that dopaminergic information is uniformly transmitted across the striatum.

C. Midbrain dopamine neurons receive unique afferent information and project topographically to the striatum.

As described above, the midbrain dopamine system is part of a neural network proposed to be critical for signaling aspects of reward and stimuli predictive of reward. The ventral midbrain contains the largest group of dopamine neurons, comprised of the VTA, SNpc, and retrorubral nucleus (RRN; Dahlstrom and Fuxe, 1964). While staining for the dopamine precursor tyroxine hydroxlase reveals a continuous band of dopamine neurons, this region is actually made up of pools of dopamine neurons that receive different sets of inputs which differentially modulate select pools of neurons. The VTA contains the most medial group of dopamine neurons, receiving information primarily from the pendunculopontine tegmental nucleus, rostral medial tegmental nucleus,
lateral hypothalamus, superior colliculus, prefrontal cortex, amygdala, ventral pallidum and reciprocal connections from the nucleus accumbens (Conrad and Pfaff, 1976; Domesick, 1988; Semba and Fibiger, 1992; Sesack and Pickel, 1992; Wallace et al., 1992; Fudge and Haber, 2000; Geisler and Zahm, 2005). Continuous with the VTA, the SNpc extends laterally, with major afferents from the subthalamic nucleus, globus pallidus, pendunculopontine tegmental nucleus, bed nucleus of the stria terminalis, amygdala, substantia nigra pars reticulata and considerable reciprocal connections with the dorsal striatum (Bunney and Aghajanian, 1977; Grace and Bunney, 1985; Fudge and Haber, 2000; Haber et al., 2000). The RRN lies dorsal and lateral to the SNpc. While it is suggested that it receives input from similar areas as the SNpc, the specific afferent projections to the RRN remain largely unknown (Joel and Weiner, 2000). Thus, although the midbrain dopamine nuclei all relay dopamine information, each region receives afferents from somewhat distinct brain regions.

Not only do these dopamine nuclei receive unique inputs, but they also topographically project to different terminal destinations, including the striatum. Distinct populations of dopamine neurons terminate in the striatum in a ventromedial to dorsolateral gradient (Ikemoto, 2007). Medial dopamine neurons in the VTA primarily project to the medial Shell while lateral dopamine neurons in the SNpc primarily project to the DLS (Haber et al., 2000; Voorn et al., 2004). Reciprocal connections directly link the midbrain dopamine neurons and striatum, creating a spiral of connections between the medial VTA and medial Shell and terminating with the lateral SNpc and DLS (Haber et al., 2000; Voorn et al., 2004; Ikemoto, 2007). The RRN is an exception to this, as it projects widely throughout the striatum and also to dopaminergic neurons in the VTA.
and SNpc, but receives little reciprocal projections back from the striatum (Arts et al., 1996). Thus, midbrain dopamine nuclei not only receive unique afferent projections but send topographic projections to the striatum, supporting that pools of dopamine neurons may transmit regionally selective information.

D. **Midbrain dopamine neurons transmit reward-associated signals**

While anatomical findings characterize the afferents and efferents of midbrain dopamine neurons, it is critical to understand how these connections translate into functional roles. One approach to understand the function of midbrain dopamine neurons has been to record the electrophysiological activity of single neurons during behavior. This next section provides a basic electrophysiological characterization of midbrain dopamine neurons, as well as how dopamine neuronal activity responds to reward and reward predictive cues across the extent of the VTA/SNpc.

When recorded *in vivo*, midbrain dopamine neurons can exhibit regular, tonic activity and high bursting, phasic activity (Hyland et al., 2002). While burst activity is thought to be responsible for the high frequency, short lasting phasic (100s of ms) alterations in dopamine neuronal activity (Hyland et al., 2002; Schultz, 2007), specific burst activity is not examined in many studies (Mirenowicz and Schultz, 1996; Matsumoto and Hikosaka, 2009). These brief, phasic changes in the activity of dopamine neurons are both important (Zweifel et al., 2009) and sufficient for (Tsai et al., 2009) for reinforcement. Building upon the electrical stimulation evoked by intracranial self-stimulation that reinforced behavior (Olds and Milner, 1954), selective phasic activation of dopamine neurons in the ventral midbrain also reinforces behavioral patterns (Tsai et
Thus, it is the phasic activations of dopamine neurons that are thought to be important for reinforcement and associative learning.

Electrophysiological recordings demonstrate that dopamine neurons consistently respond with robust phasic increases in activity to unpredictable rewards and to cues that reliably predict reward across many studies (Schultz, 1986; Ljungberg et al., 1991; Schultz et al., 1993; Mirenowicz and Schultz, 1994, 1996; Hollerman and Schultz, 1998; Hyland et al., 2002; Matsumoto and Hikosaka, 2009). Electrophysiological responses of dopamine neurons were recorded in the VTA and SNpc of awake, behaving non-human primates during presentation of an unpredictable juice reward or a cue conditioned to reliably predict juice delivery. Delivery of the unpredicted juice reward evoked a phasic increase in dopamine neuron activity. When the cue became conditioned to predict juice delivery, the phasic response shifts from the primary reward to the earliest reliable predictor of reward and dopamine neurons no longer increased their activity to the reward itself. Work by Schultz and colleagues (2002) showed that these response are evoked in dopamine neurons across the medial-lateral extent of the midbrain, with approximately 75% of all dopamine neurons responding to unexpected reward and 55-70% responding to reward-predictive cues in well-trained non-human primates. Coupled with the fact that dopamine neurons form extensive arborizations in the striatum (Matsuda et al., 2009), primate electrophysiological studies strongly suggest that primary reward and predictive cues evoke an elevation in extracellular dopamine that is homogenously broadcast throughout the striatum (Schultz, 1997). Together with dopamine’s role as a neuromodulator, this would therefore support that dopamine uniformly modulates activity of striatal output neurons throughout all subregions.
E. Dopamine release in striatal terminal regions

Dopamine neurons project from the ventral midbrain to all areas of the striatum where they modulate ongoing activity (Nicola and Deadwyler, 2000; Bamford et al., 2004b; Surmeier et al., 2009; Gerfen and Surmeier, 2010). Specifically, phasic changes in dopamine release are correlated with several critical functions of the striatum including modulation of striatal neuron excitability (Bamford et al., 2004b; Goto and Grace, 2005; Tseng et al., 2007), reinforcement learning (Waelti et al., 2001; Bayer and Glimcher, 2005; Tsai et al., 2009) and goal-directed behavior (Zweifel et al., 2009; Flagel et al., 2010).

As described in the previous section, electrophysiological responses of dopamine neurons to rewarding stimuli have been studied extensively in vivo. However, based upon electrophysiological characteristics that limit the classification of midbrain neurons as dopaminergic, recent in vitro evidence suggests that these recordings may be biased to a subset of dopamine neurons (Margolis et al., 2006; Lammel et al., 2008; 2011). Additionally, fluctuations in extracellular dopamine in terminal regions may not be faithful to dopamine neuronal activity (Trulson, 1985; Montague et al., 2004). Dopamine neuronal activity is also significantly modulated at the presynaptic terminal which cannot be detected by recording action potentials at dopamine cell bodies (Zhou et al., 2001; Rice and Cragg, 2004; Zhang and Sulzer, 2004; Britt and McGehee, 2008; Zhang et al., 2009). Thus, recording of dopamine release at terminal regions can measure the actual dopamine release events as they occur in the striatum during behavioral manipulations.

Recording of dopamine release from terminal areas has primarily utilized in vivo microdialysis, measuring extracellular dopamine changes over a timescale of several
minutes. In contrast to the global signal supported by electrophysiological studies, microdialysis experiments have revealed regional differences in dopamine concentration across the striatum (Bassareo and Di Chiara, 1999; Stefani and Moghaddam, 2006; Bassareo et al., 2011; Ostlund et al., 2011). For example, dopamine levels increase to novel food reward presentation in the Shell, but rapidly habituate with repeated exposure (Bassareo and Di Chiara, 1999; Bassareo et al., 2011). In contrast, after associative learning, increases in dopamine to both predictive stimuli and food reward develop in the Core (Bassareo and Di Chiara, 1999; Bassareo et al., 2011). However, microdialysis recordings cannot capture potential changes in dopamine as a result of phasic activations due to its limited temporal resolution (minutes). Given that phasic dopamine activity is critical for reinforcement and associative learning, measurement of these phasic changes in dopamine release is necessary for our understanding of dopamine’s role in these behaviors.

Recently, fast-scan cyclic voltammetry (FSCV) has been used to detect fluctuations in extracellular dopamine on a timescale akin to that achieved with electrophysiological recordings in awake and behaving animals. Using this technique, several experiments have examined phasic changes in dopamine release during reward-associated tasks, but have selectively focused on recording in the nucleus accumbens – and most often just the Core. Consistent with previous electrophysiological findings, unpredicted reward evokes an increase in phasic dopamine time-locked to the reward (Day et al., 2007; Stuber et al., 2008). This response shifts to predictors of reward following extended training (Roitman et al., 2004; Day et al., 2007; Jones et al., 2010) but has primarily been examined in a single subregion, usually the Core. Taken together, most
studies examining regional specificity in dopamine terminal release have either used techniques that lack the temporal resolution to detect phasic changes (Bassareo et al., 2011; Ostlund et al., 2011), focused on a subset of striatal regions (Aragona et al., 2009; Wanat et al., 2010) or both. Therefore, it remains unclear if dopamine is globally broadcast across the striatum or is evoked in a regionally selective manner.

F. Uniformly broadcast versus regionally-selective phasic dopamine signaling during reward-directed behavior

The striatum is divided into four subregions (Shell, Core, DMS and DLS) that all receive input from midbrain dopamine neurons. Electrophysiological recordings of these dopamine neurons show uniform neuronal firing patterns in response to primary reward and predictive cues, suggesting that dopamine neurons broadcast a global signal throughout the striatum. However, pools of dopamine neurons receive different inputs and project topographically to striatal subregions, suggesting the possibility of regional specificity of dopamine activity. Furthermore, studies examining striatal dopamine concentration with in vivo microdialysis have empirically shown regional specificity. Therefore, at present, there is evidence that supports dopamine being either uniformly broadcast throughout striatal terminal regions or occurring in a regionally selective manner. To date, there has not been a systematic examination of striatal phasic dopamine release to behaviorally relevant stimuli. The goal of the current set of experiments was to determine whether phasic dopamine signaling is uniformly broadcast or regionally selective using stimuli known to reliably evoke phasic dopamine activity.
To accomplish this, I recorded phasic dopamine release, using FSCV, during conditions that reliably evoke phasic changes in dopamine neuronal activity in one of four striatal subregions (Shell, Core, DMS and DLS). In Chapter II, findings are described from an experiment that investigated regional differences in striatal phasic dopamine release by applying current directly to the ventral midbrain, which contains dopamine cell bodies, to phasically drive electrophysiological activity. I found that dopamine was released in each of the four subregions assayed. However, stimulation-evoked phasic dopamine release also revealed regional differences with respect to the magnitude of release and the duration of the dopamine release event. This is the first systematic investigation of regional differences in awake and behaving subjects.

In Chapters III and IV, experiments are described in which rats were presented with reward stimuli that are widely believed to activate an overwhelming majority of dopamine neurons. Chapter III describes a study that examined phasic dopamine release in response to unpredicted food reward (sugar pellet). Chapter IV describes the results examining phasic dopamine release in response to reward predictive cues during a discriminative stimulus task used in Jones et al. (2010) and the subsequent delivery of unpredicted food reward. If these reward stimuli activate a majority of dopamine neurons, then phasic dopamine release should be observed throughout all striatal subregions. However, if dopamine release is regionally evoked, then select striatal regions will show changes in dopamine release to reward-associated stimuli in a regionally specific manner.

These experiments are the first to systematically examine phasic dopamine release in multiple striatal areas in response to stimulation-driven phasic dopamine release,
unpredicted food reward and reward predictive cues. Analysis of phasic dopamine release events in each of these different conditions allows for a methodical examination of the ability for behaviorally relevant stimuli to evoke phasic dopamine release in striatal subregions. Thus, the experiments determined whether phasic dopamine under these conditions is uniformly broadcast or regionally selective in subregions of the striatum.
Chapter II

Electrical stimulation of midbrain dopamine neurons evokes phasic dopamine release throughout the striatum

A. Introduction

Animals will work for electrical stimulation to the ventral midbrain, the location of dopamine cell bodies (Olds and Milner, 1954). Not only is ventral midbrain stimulation reinforcing, but it also evokes dopamine release in the striatum as measured by in vivo microdialysis (Fibiger et al., 1987; Phillips et al., 1989; Hernandez et al., 2006). The temporal resolution afforded by FSCV has revealed that stimulation trains mimicking phasic activation of dopamine neurons evoke a sharp, phasic increase in extracellular dopamine concentration with two main components (Jones et al., 1995b). First, current injection causes a rising phase in extracellular dopamine. Underlying this rise is vesicular dopamine release. The magnitude of release is limited by a number of factors including ongoing dopamine reuptake by the dopamine transporter (DAT). After the current pulses cease, dopamine levels fall, roughly following a single exponential decay rate. This falling phase is primarily regulated by the rate of dopamine reuptake by the DAT. Indeed, DAT blockers significantly increase the magnitude of the rising phase and decrease the rate of the falling phase of stimulation-evoked extracellular dopamine release (Jones et al., 1995a; Cragg et al., 2000). Taken together, the dynamics of electrically-evoked dopamine release are significantly influenced by the DAT.

After dopamine is released, it diffuses through the extracellular space where it can act on dopamine receptors (primarily low affinity D1 and high affinity D2 receptors).
Importantly, and especially in the striatum, the DAT is thought to limit the effective range of evoked dopamine concentration changes via removal from the extracellular space (Cragg and Rice, 2004). The DAT is expressed selectively on dopamine neurons in the perisynaptic regions, the area just outside the synapse. Thus, DATs are in an excellent position to strongly regulate phasic dopamine signaling in the striatum.

Phasic dopamine release evoked by the delivery of stimulation trains is qualitatively similar to phasic dopamine release evoked in behavioral contexts. Thus, electrical stimulation allows for the ability to carefully evaluate regional differences throughout the striatum. Indeed, there is some evidence that phasic dopamine signals may be differentially regulated in a subregion specific manner. For example, regional differences in the DAT density have been demonstrated within the striatum (Marshall et al., 1990; Richfield, 1991; Ciliax et al., 1995; Nirenberg et al., 1997). Previous studies however, have primarily focused on comparing functional differences in the DAT across broad areas such as comparing the nucleus accumbens to the dorsal striatum (Jones et al., 1995b; Jones et al., 1995a). Moreover, most of the work establishing gross regional differences in the regulation of phasic dopamine signals has employed in vitro or anesthetized preparations (Jones et al., 1995b; Cragg et al., 2000; Wu et al., 2001). Thus, it remains unclear whether phasic dopamine changes are differentially regulated in awake, behaving animals.

Here I applied stimulation trains to the ventral midbrain neurons to explore whether phasic signals are differentially regulated within the striatum based on subregion. Electrical stimulation allows for an identical stimulus to be examined across all subjects. I systematically examined electrically-evoked phasic dopamine release and the rate of
reuptake in four striatal regions (Shell, Core, DMS and DLS) in awake, behaving animals. Results demonstrate that dopamine release can be phasically evoked in all striatal regions – although the magnitude of dopamine release significantly differed. In addition, the rate of reuptake was also regionally distinct.

B. **Experimental Methods**

1. **Subjects**

   Male, Sprague-Dawley rats (n = 46; Charles River Laboratories) weighting 325-425 g were individually housed in plastic cages (26.5 x 50 x 20 cm) and maintained on a 12/12 hour light/dark cycle in a temperature (22°C) and humidity (30%) controlled environment. Food and water were available *ad libitum* during the post-operative recovery period. Animal care and use was in accordance with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals and approved by the University of Illinois Institutional Laboratory Animal Care and Use Committee.

2. **Apparatus**

   Rats were tested in a standard operant chamber (Med Associates, St. Albans, VT, USA). A hole in the top of the chamber allowed for the attachment of the headstage for voltammetric measurements. The headstage, in turn, was attached to an electric swivel (Crist Instrument Company, MD, USA) mounted above the chamber and permitted free movement throughout the chamber during recording.
3. **Electrodes**

Carbon fiber microelectrodes were constructed as previously described (Heien et al., 2005). Individual 5-μm diameter carbon fibers were aspirated into glass capillaries and pulled in a vertical micropipette puller. Each electrode was examined under an optical microscope to determine if there was a good seal between the carbon fiber and the glass. If a good seal was observed, the carbon fiber was cut to a length of 50-100 μm using a scalpel and all others were discarded. Electrodes were then loaded into custom-designed micromanipulators (University of Illinois at Chicago Engineering Design Shop) which allowed them to be raised and lowered in micrometer increments and soaked in isopropyl alcohol until use (~2-12 hours).

4. **Surgery**

Rats were prepared for voltammetric recording as previously described (Day et al., 2007; Ebner et al., 2010; Jones et al., 2010) Rats were anesthetized with a cocktail of ketamine hydrochloride (100 mg/kg, intraperitoneal (IP)) and xylazine hydrochloride (10 mg/kg, IP). The fur was then removed from the top of the scalp between the eyes and ears. Rats were placed in the stereotaxic frame and the scalp was wiped first with betadine iodine solution and then with isopropyl alcohol. A midsagittal incision was made to retract the scalp and expose the skull. The skull was made horizontal by ensuring that the dorsal-ventral (DV) coordinates of lambda and bregma were within 0.2 mm of each other. After the coordinates of bregma were determined, coordinates for the working electrode cannula and stimulating electrode were calculated. A guide cannula (Bioanalytical Systems) for the carbon fiber working electrode was positioned dorsal
(approximately 2.5 mm below the skull) to one of four striatal subregions on the right side of the brain according to the following coordinates: Shell +1.7 anterior/posterior (AP), -0.9 medial/lateral (ML); Core +1.3 AP, -1.5 ML; DMS +0.6 AP, -2.1 ML; DLS +0.6 AP, -4.0 ML. The guide cannula was pseudo-randomly placed in one of the striatal subregions. The plastic sheathing of the guide cannula was trimmed to approximately 1.5 mm and the metal obdurator was cut to extend approximately 1 mm past the plastic sheathing. A chlorinated sliver wire (Ag/AgCl) reference electrode was implanted in the left forebrain, contralateral to guide cannula and stimulating electrode. Reference electrodes were made with 1.5 cm silver wire inserted into a plated pin that was attached to the negative side of a 9 V battery and insulated copper wire was attached to the positive side of the battery. Both the silver wire and copper wire end were placed into 1N HCl to develop a AgCl coating on the silver wire. Three stainless steel jeweler screws and dental cement secured the guide cannula and reference electrode to the skull.

Before the stimulating electrode was cemented into place, a removable custom micromanipulator (UIC engineering shop) loaded with a carbon fiber microelectrode was lowered just dorsal to the region of interest. A twisted bipolar stimulating electrode (Plastics One) with ~1 mm tip separating the tips was initially lowered in the VTA/SNpc region (-5.2 AP, -1.0 ML, -7.0 DV). The stimulating electrode was lowered from -7.0 mm (relative to surface of the brain) at 0.2 mm increments. At each increment a train of current pulses was delivered (60 pulses delivered at 60 Hz, 120 µA). After stimulation evoked a phasic increase in dopamine, the position of the stimulating electrode was optimized (maximal evoked dopamine) and cemented in place. The carbon fiber
electrode was then removed. Rats were given ~3 mL saline subcutaneously in the hind regions. Rats recovered under a heat lamp until they were awake and then placed into a clean cage with rat chow made into a mash. Rats were given free access to rat chow and water until they had reached pre-operative weight (3-5 days).

5. **Fast-Scan Cyclic Voltammetry Recordings**

FSCV allows for the real-time identification and monitoring of extracellular concentrations of electroactive compounds such as dopamine with high temporal and spatial resolution. Its application to awake and behaving rats has been described previously (Day et al., 2007; Roitman et al., 2008; Ebner et al., 2010). During FSCV, a voltage waveform is applied to a carbon fiber electrode lowered into a striatal subregion. The potential of the carbon fiber electrode is held at -0.4 V relative to the Ag/AgCl reaction on the reference electrode. A triangular waveform is applied to drive the potential to +1.3 V and back to -0.4 V at a rate of 400 V/s. Voltammetric measurements were made once every 100 ms. Chemical species that are electroactive within this voltage range will oxidize and reduce at different potentials along the waveform. Current due to oxidation and reduction are measured at the surface of the carbon fiber electrode (see Figure 2.1A). Dopamine is electroactive within this applied voltage range and is identified by its oxidation and reduction potentials. Dopamine oxidizes at ~0.6 V, undergoing a conformational change into dopamine-α-quinone shedding two electrons, which is detected as oxidative current at the carbon fiber electrode. Dopamine-α-quinone slowly reduces back to dopamine at about -0.2 V, which is detected as a reductive current at the surface of the electrode.
The change in current at the oxidation potential was used to quantify phasic dopamine release. The stable contribution of current produced by oxidation and reduction of surface molecules on the carbon fiber is removed by subtracting a background obtained when dopamine was not present. The background period (1 s) was obtained within the 10 s before electrical stimulation. These REDOX reactions are visualized by plotting the changes in current against the triangular voltage waveform, known as a cyclic voltammograms (see Figure 2.1B). This cyclic voltammogram serves as an identification signature of dopamine based on the oxidation and reduction potentials. Further, changes in current from dopamine oxidation are directly proportional to dopamine concentration changes at the electrode surface (Heien et al., 2004). All electrochemical data were then relayed through the headstage, digitized and recorded on a computer using programs written with LabView software (National Instruments; (Robinson et al., 2003; Heien et al., 2004; Hermans et al., 2008). Thus, FSCV can resolve changes in dopamine concentration from background changes to reveal subsecond fluctuations in dopamine concentration.

6. Experimental Procedure

On the day of testing, rats were placed into the operant chamber and a new carbon fiber recording microelectrode was lowered into the selected striatal subregion using a custom-made micromanipulator and locked into place. Of the 46 rats used in this study, 11 rats had recordings in the Shell, 11 rats had recordings in the Core, 12 rats had recordings in the DMS and 12 rats had recordings in the DLS. The Ag/AgCl reference electrode, stimulating electrode, and carbon fiber recording electrode were connected to
a headstage containing a voltammetric amplifier attached via a tether to the electric swivel (Crist Instrument Company) at the top of the operant chamber. This allowed a rat to move freely in the chamber during voltammetric recording. Once the carbon fiber was lowered into position, the waveform was turned on and allowed to equilibrate for 40 minutes (30 minutes at 60 Hz and 10 minutes at 10 Hz) to minimize current drift. Equilibration is critical because recordings taken immediately after lowering the electrode into place show significant drift in current (Phillips et al., 2003). Once the carbon fiber electrode had equilibrated, the VTA/SNpc (24 pulses, 60Hz, 120 μA, 4 ms/pulse) was stimulated while recordings were made. If no dopamine was recorded, the electrode was lowered in 0.15 mm increments. Once electrically evoked dopamine was located, another stimulation of 24 pulses at 60 Hz was taken and used for further analysis. These stimulation parameters (24 pulses at 60 Hz, 120 μA) were selected as they have been previously shown to elicit responding for intracranial self-stimulation as well as evoke a significant increase in phasic dopamine release in both the nucleus accumbens and dorsal striatum (Ewing et al., 1983; Kuhr et al., 1984; Cheer et al., 2005; Cheer et al., 2007; Owesson-White et al., 2008).

7. Data Analysis

First, changes in phasic dopamine release in response to electrical stimulation were determined within each striatal region. Two distinct epochs within the evoked dopamine concentration traces were utilized for further analysis: a Baseline epoch (5 s prior to stimulation) and a Stimulation (1 s after stimulation). Paired t-tests then compared epochs (e.g. Baseline versus Stimulation) within each striatal region. Next, the peak
dopamine concentration for each stimulation was measured and a one-way analysis of variance (ANOVA) and post hoc Tukey's test examined dopamine release across striatal subregions. Differences in peak dopamine release could reflect several factors so I focused on analyzing the rate of dopamine reuptake by the DAT across regions. To account for differences in the magnitude of dopamine release, the peak dopamine concentration evoked by stimulation was set to 100% and I measured the latency for dopamine concentration to decay to 50% of the maximum (half-life). Differences in half-life between regions were examined with a one-way ANOVA and post hoc Tukey's test.

8. **Histology**

Once all voltammetric recordings were completed, rats were injected with a lethal dose of sodium pentobarbital (~100mg/kg). To determine recording location, a stainless steel electrode (A-M Systems #571500, Sequim, WA, USA) was lowered to the same depth as recording and an electrolytic lesion was made. Rats were then transcardially perfused with 0.9% phosphate buffered saline followed by a 10% formalin solution. Brains were removed and stored in 10% formalin solution until being frozen. Using a -20°C cryostat (Leica CM1850), coronal sections were sliced at 50 μm and mounted on gelatin coated slides. Slides were stained with cresyl violet and coverslipped using Permount (Fisher Scientific). After the slides had dried, the location of the recording electrode was identified using a light microscope with the aid of the stereotaxic atlas by Paxinos and Watson (1998).
C. Results

1. Electrode Placement Verification in Striatal Subregions

Electrode locations for all recordings are shown in Figure 2.2. Recordings in the dorsal striatum were between 0.48 and 1.7 mm anterior to bregma. DLS placements were located 3.6 to 4.5 mm lateral to the midline and 3.8 to 5.5 mm ventral to the surface of the brain. DMS placements were found 1.0 to 1.8 mm lateral to the midline and 3.8 to 5.5 mm ventral to brain surface. For recordings in the nucleus accumbens core and shell electrode placements were located between 0.7 and 1.7 mm anterior to bregma. Electrode placements in the Core were located 1.0 to 2.2 mm from the midline and 6.6 to 7.2 mm ventral to the brain surface. Electrode placements in the Shell were located 0.6 to 1.6 mm from the midline and 6.5 to 8.0 mm ventral to the brain surface.

2. Peak dopamine release evoked by electrical stimulation

Midbrain neurons in the VTA/SNpc were electrically stimulated and the resultant phasic dopamine release was recorded in the Shell, Core, DMS and DLS. A single stimulation of 24 pulses at 60 Hz was selected for each animal. Electrical stimulation evoked a significant increase in dopamine concentration across all striatal subregions (Baseline vs. Stimulation epochs, P’s < 0.01, paired t-tests). However, there was a difference in the peak dopamine concentration across regions. Specifically, stimulation evoked similar dopamine levels in the Shell (Peak dopamine: 363.6 ± 97.2 nM), Core (Peak dopamine: 383.6 ± 71.3 nM) and DMS (475.5 ± 115.9 nM), but lower dopamine was evoked in the DLS (173.2 ± 35.3 nM).
3. **Reuptake dynamics for electrically-evoked phasic dopamine release in striatal subregions**

In order to compare differences in dopamine reuptake across regions, peak dopamine concentration was set at 100 and subsequent values were normalized as a percentage (Figure 2.3B). The time to decay to 50% of the maximum (halflife), an index of dopamine reuptake rate used previously (Dugast et al., 1994), was determined and statistically compared across regions (Figure 2.3C). There was a clear ventromedial to dorsolateral striatal gradient where halflife in the Shell (1.04 ± 0.05 s) was nearly double that observed in the DLS (0.55 ± 0.05 s). A one-way ANOVA revealed a main effect of subregion ($F_{3,45} = 12.40$, $p < 0.0001$). As can be seen in Figure 2.3C, and confirmed by a post hoc Tukey's test, halflife in the DLS was significantly shorter relative to that in the Core and Shell ($P's < 0.01$). Further, DMS halflife was significantly shorter than the Shell ($p < 0.01$).

**D. Discussion**

Electrical stimulation of the VTA/SNpc evoked phasic dopamine release in all striatal regions sampled (Shell, Core, DMS and DLS). However, this response was not uniform as there were significant differences in the magnitude of dopamine release and the rate of dopamine reuptake. The magnitude of electrically-evoked dopamine was significantly smaller in the DLS relative to all other regions. Further, the rate of reuptake, as measured by the halflife of dopamine in the falling phase, varied across striatal regions. Reuptake rate increased in a ventromedial to dorsolateral gradient, with the fastest reuptake rate in the DLS and slowest in the Shell. Thus, while electrically-evoked
dopamine release was evoked in all striatal regions, both the magnitude and rate of reuptake varied across subregions.

The concentration of electrically-evoked dopamine was greater in the Shell, Core and DMS as compared to the DLS. There are several possibilities for this difference. First, the bipolar stimulating electrode was primarily aimed towards the VTA and medial SNpc and thus it is possible that more lateral portions of the SNpc, which primarily project to the DLS, were less stimulated (Bjorklund and Lindvall, 1984; Haber et al., 2000; Voorn et al., 2004). However, it is unknown how far the stimulation current can spread and thus how many dopamine neurons were excited. Another possibility is that dopamine release is more tightly regulated in the DLS resulting in an attenuated response. This regulation could be due to several factors including presynaptic regulation of dopamine release (Threlfell and Cragg, 2011) as well as a higher density of DATs and rate of reuptake in the DLS than other striatal regions, which will be discussed later in greater detail (Richfield, 1991; Cass et al., 1993; Ciliax et al., 1995; Nirenberg et al., 1997; Cragg et al., 2000, 2002). Interestingly, in slice preparations, direct electrical stimulation of the DLS evokes a greater dopamine concentration as compared to other striatal areas (Cragg, 2003), which is thought to be due to a greater density of dopamine release sites in the DLS (Beal and Martin, 1985; Doucet et al., 1986; Widmann and Sperk, 1986). However, as I demonstrated in a previous study (Daberkow et al., submitted), dopamine dynamics are significantly different in a brain slice preparation as compared to an awake, behaving animal. Therefore, while differences in peak dopamine release could be due to a variety of factors, electrical
stimulation of the VTA/SNpc was able to evoke a significant increase in dopamine concentration in all striatal areas recorded.

The rate of reuptake varied across striatal regions. Reuptake was slowest in the ventromedial Shell region and the fastest in the DLS (Figure 2.3B/C). One explanation for these differences is that the density of striatal DATs follows a similar gradient: the lowest DAT density in the Shell which increase in a ventromedial to dorsolateral gradient with the highest density of DATs in the DLS (Marshall et al., 1990; Richfield, 1991; Ciliax et al., 1995; Nirenberg et al., 1997). Importantly, the affinity of the DAT for dopamine is similar across the striatum (Marshall et al., 1990). Therefore, a greater density of DATs results in quicker reuptake into the dopamine neurons, limiting the duration of phasic dopamine release events across the striatum.

Here, using in vivo voltammetry in awake, behaving animals, I have demonstrated, with greater regional specificity, differences in the rate of reuptake of electrically-evoked phasic dopamine release. The current results support previous examinations using immunohistochemical methods to examine DAT densities directly or in vitro or anesthetized voltammetric recordings to record functional differences in striatal DATs. However, voltammetric recordings have focused on examining general functional differences between the nucleus accumbens and dorsal striatum (Cass et al., 1993; Jones et al., 1995b; Wu et al., 2001). Variation in reuptake rates across striatal regions likely alters the time and distance that dopamine can diffuse after release. A smaller sphere of influence due to a high density of DATs, such as in the DLS, results in dopamine only binding to nearby dopamine receptors, as opposed to a larger sphere of influence due to a lower density of DATs, such as in the Shell, where dopamine can act
on more distant dopamine receptors (Cragg and Rice, 2004). Thus, a greater number of DATs as in the DLS, for example, would suggest tighter control of phasic dopamine release events as compared to areas with fewer DAT, such as the Shell. Taken together, experimenter-delivered electrical stimulation of the VTA/SNpc evoked phasic dopamine release throughout the striatum. There were, however, regional differences in both peak evoked dopamine concentration as well as in the rate of reuptake. These regional differences have significant implications in how dopamine release is regulated as well as in its involvement in reward and goal-directed behaviors.
Figure 2.1: Increases in phasic dopamine concentration in response to electrical stimulation of the VTA/SNpc. A. Voltammetric response to VTA/SNpc stimulation. The color plot indicates changes in current as a function of electrode potential and time. Time is on the abscissa, the applied electrode potential is on the ordinate, and the current changes are encoded in false color. Stimulation of midbrain neurons (t = 0) evoked current at several applied potentials along the triangular waveform. B. Cyclic voltammograms at time = 0.7 s after stimulation. Voltage is on the abscissa (negative and positive going scans), and change in current is on the ordinate. Current changes at the time of stimulation are due to the presence of dopamine at the recording electrode, identified by its oxidation (~0.6 V) and reduction (~-0.2 V; on the negative going scan) potentials. The identification of dopamine on this cyclic voltammograms matched identically with previously work using FSCV to measure exogenous dopamine in a flow cell system (Heien et al., 2004). C. Dopamine concentration increase in response to electrical stimulation of the VTA/SNpc. Dopamine concentration is directly proportional to the oxidative current at 0.6 V (1 nA = ~66 nM dopamine). Time is the abscissa and dopamine concentration is the ordinate.
Figure 2.2: Location of recording electrodes for electrically evoked phasic dopamine release. Carbon fiber recording electrodes were located in discrete striatal regions. Placements are color-coded: Shell, blue; Core, green; DMS, orange; DLS, red. Numbers are distances in mm anterior from bregma. Brain histological images were adapted from the stereotaxic atlas of Paxinos & Watson (1998).
Figure 2.3: The rate of dopamine reuptake varies as a function of striatal subregion. A. Average change in dopamine concentration evoked by electrical stimulation (24 pulses, 60 Hz) of the VTA/SNpc. The black box along the x-axis indicates stimulation onset and offset (400ms in length) B. Reuptake of dopamine across striatal subregions. For each stimulation, data were normalized to percentage of peak dopamine concentration. C. Half-life (latency to decay to 50% of peak dopamine concentration) significantly differed across striatal subregions. Reuptake was faster in the DLS than in the Core and Shell. In addition, reuptake was faster in the DMS relative to the Shell. ** p < 0.01 versus the Core and Shell; * p < 0.05 versus the Shell.
Chapter III

Regionally-distinct phasic dopamine release to unpredicted food reward

A. Introduction

Proposed by Wise (1978), the anhedonia hypothesis proposed a function for dopamine in signaling pleasure and that a lack of dopamine resulted in anhedonia from rewarding stimuli. While his hypothesis no longer holds much credence, the association Wise made between dopamine and rewarding stimuli remains extremely strong today. Currently, numerous proposals support a functional relationship between dopamine and reward, but no single psychological role of dopamine has emerged (Schultz, 1997; Berridge and Robinson, 1998; Schultz, 1998; Ikemoto and Panksepp, 1999; Salamone and Correa, 2002; Redgrave and Gurney, 2006). This lack of a single underlying function for dopamine may be a result of dopamine’s extensive projections throughout the forebrain. It is possible that dopamine may play different roles across its terminal regions.

Electrophysiological recordings from the VTA and SNpc demonstrate that unpredicted food reward evokes an increase in the firing rate of a majority of dopamine neurons. The increase occurs with short latency and lasts just a few hundred ms (Mirenowicz and Schultz, 1994, 1996; Hyland et al., 2002; Bayer and Glimcher, 2005; Matsumoto and Hikosaka, 2009). That upwards of 80% of dopamine neurons measured in the VTA and SNpc are synchronously activated by the same stimulus and with the same latency led to the proposal that all dopamine terminal regions receive a brief increase in dopamine concentration (Schultz, 1997). One way to empirically
evaluate this hypothesis is to measure dopamine concentration changes in different striatal subregions during reward. Indeed, extracellular dopamine concentration has been assayed during reward directed behaviors including in response to food reward (Wilson et al., 1995; Ahn and Phillips, 1999; Bassareo and Di Chiara, 1999; Day et al., 2007; Roitman et al., 2008; Zhang et al., 2009; Bassareo et al., 2011). Here, the data have been inconclusive. Using microdialysis, a technique that samples extracellular dopamine, studies have shown large increases in the nucleus accumbens during the consumption of food (Wilson et al., 1995; Ahn and Phillips, 1999; Bassareo and Di Chiara, 1999; Bassareo et al., 2011). When subregions have been directly compared, differences in dopamine fluctuations between regions have emerged. Di Chiara and colleagues have dissociated Shell and Core dopamine concentration changes in response to food reward using microdialysis (Bassareo and Di Chiara, 1999; Bassareo et al., 2011). However, microdialysis lacks the temporal resolution to capture fluctuations in extracellular dopamine due to brief changes in dopamine neural activity – especially the brief, phasic activations described by Schultz and colleagues. FSCV has this capability (Sombers et al., 2009). Similar to electrophysiological studies, unpredicted food reward clearly evokes a brief phasic increase in extracellular dopamine in the Core (Day et al., 2007; Roitman et al., 2008; Zhang et al., 2009). However, virtually no data exists that has examined potential subregional differences.

The goal of the current study was to examine phasic dopamine release to unpredicted food reward (sugar pellet) in four striatal subregions (Shell, Core, DMS and DLS). As described above, this stimulus has been shown to evoke a phasic increase in a very large percentage of midbrain dopamine neurons by a number of different
laboratories (Mirenowicz and Schultz, 1996; Schultz, 1997; Bayer and Glimcher, 2005; Matsumoto and Hikosaka, 2009). Thus, it represents the very best stimulus to use to evaluate whether reward related signals are uniformly broadcast throughout the striatum or in a more regionally specific manner. Results demonstrate that unpredicted sugar pellet reward selectively evoked phasic dopamine release in striatal subregions, supporting that dopamine signals are not uniformly broadcast throughout the striatum.

B. Experimental Methods

1. Subjects:

Male, Sprague Dawley rats (n= 23; Charles River Laboratories) weighing 325-400g at the time of testing were used. Animals were individually housed in plastic cages (26.5 x 50 x 20 cm) in a temperature (22°C) and humidity (30%) controlled environment on a 12/12 h light/dark cycle. Prior to training and during recovery from surgery rats had *ad libitum* access to both standard lab chow and water. During training and testing, rats were food restricted to ~95% of their *ad libitum* body weight with free access to water. Animal care and use was in accordance with the National Institutes for Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at the University of Illinois at Chicago.

2. Apparatus

Rats were trained and tested in a standard operant chamber (Med Associates, St. Albans, VT, USA). A houselight and two different sound generators were located on one wall of the chamber. A custom designed acrylic pellet receptacle was located in the
center of the opposite wall. A retractable lever with a circular white cue light above it was positioned on either side of and equidistant to the pellet receptacle. A hole in the top of the chamber allowed for the attachment of the headstage for voltammetric measurements. The headstage, in turn, was attached to an electric swivel (Crist Instrument Company, MD, USA) mounted above the chamber and permitted free movement throughout the chamber during recording.

3. **Pellet Retrieval Training:**

Prior to surgery, rats were food-restricted and trained on two separate days to retrieve 45 mg sugar pellets (BioServe, Sugar Dustless Precision Pellets, #F0042) delivered with pseudorandom inter-trial intervals (range 30-90s; 30 trials). Rats then underwent surgery and, after recovery, were again food restricted and given at least one session to retrieve pellets while connected to a headstage to acclimate for voltammetric recording.

G.

4. **Electrodes:**

Carbon fiber microelectrodes were constructed as previously described in Chapter II (page 23).

5. **Surgery:**

Rats were prepared for voltammetric recording as previously described in Chapter II (page 23).
6. **Fast-Scan Cyclic Voltammetry Recordings:**

Voltammetric data was recorded as described in Chapter II (page 25).

7. **Experimental Procedure:**

On the day of testing, rats were placed into the operant chamber and a carbon fiber electrode was lowered into a striatal region (Shell, n=6; Core, n=5; DMS, n=6; DLS, n=6). Rats were connected and prepared for voltammetric recording as described in Chapter II (page 26). When the carbon fiber electrode had equilibrated, dopamine was electrically evoked by stimulating the VTA/SNpc (24 pulses, 60Hz, 120 μA, 4 ms/pulse) to initially determine whether the dopamine was detected by the carbon fiber electrode as shown. Once phasic dopamine release was located, the VTA/SNpc was stimulated several times at various parameters (10-24 pulses, 30-60Hz, 120μA, 4 ms/pulse) to generate release events with different magnitudes. Stimulation reliably evokes two responses: an increase in dopamine followed by a basic pH change (Roitman et al., 2004). Representative current by voltage plots (cyclic voltammograms) are obtained for each of these responses. Training sets were constructed from cyclic voltammograms for dopamine and pH to allow for principal component regression on data collected during the behavioral session as previously described (Heien et al., 2004; Day et al., 2007). In all experiments, principal component analysis was used to extract the dopamine component from the voltammetric recordings.

8. **Data Analysis**

Principal Component Analysis (PCA): Following behavioral sessions, a training set was generated from 5 to 10 background-subtracted cyclic voltammograms from
stimulations by varying the number of pulses and frequency of the electrical stimulation at the same location, evoking different concentrations of dopamine release. An example electrically evoked dopamine color plot with cyclic voltammograms is shown in Figure 3.1A. Similarly, a training set was created for extracellular pH using background subtracted cyclic voltammograms 5-10 s after each stimulation. Five to 10 cyclic voltammograms of both dopamine and pH were extracted from the stimulations and the current amplitude was converted to concentration based upon calibration factors (1 nA = 66.6 nM for dopamine, and 1 nA = 0.0958 nM for pH). The background-subtracted cyclic voltammograms used in the training set were reduced by principal component analysis to approximately 3-9 factors, which captured 99.5% of the variance in the training set. These results were used with regression analysis to evaluate the behavioral evoked responses for dopamine and pH. An example of the resulting extraction from the color plot by PCA is shown in Figure 3.1B. In response to unpredicted food reward, the phasic dopamine response is shown below the color plot.

For each behavioral session, data files were cut to 20 s files, with 10 s before and after the onset of the pellet delivery. Backgrounds were selected for each individual trial at a location where dopamine was not present. PCA analysis was used to extract dopamine concentration changes for each trial and a snapshot of the background subtracted color plot was recorded. These color plots were then averaged together for each rat and PCA was performed on this average color plot. Further analyses utilized this averaged data as well as the concentration traces from individual trials. Two distinct epochs within the average dopamine concentration traces were utilized for further analysis: a Baseline epoch (5 s prior to the pellet delivery) and a Pellet (1 s after pellet
delivery). Paired t-tests then compared epochs (e.g. Baseline versus Pellet) within each striatal region. Statistical analyses were carried out using GraphPad Prism and Statistica software and an alpha level of 0.05 was set for significance.

9. Histological Verification of Electrode Placement

As described in Chapter II (page 28), rats were injected with a lethal dose of sodium pentobarbital, lesioned at the location of recording, and transcardially perfused. Brains were removed and stored in 10% formalin solution until being frozen and mounted in a -20°C cryostat (Leica CM1850). Coronal sections were sliced at 50 μm and mounted on gelatin coated slides. Slides were stained with cresyl violet and coverslipped using Permount (Fisher Scientific). After the slides had dried, the location of the recording electrode was identified using a light microscope with the aid of the stereotaxic atlas by Paxinos and Watson (1998).

C. Results

1. Electrode Placements Resulted In Selective Sampling Within Distinct Striatal Subregions

Electrode locations for all recordings are shown in Figure 3.2. For recordings in the Shell and Core, electrode placements were located between 0.7 and 1.7 mm anterior to bregma. Electrode placements in the Shell were located between 0.6 to 1.6 mm lateral to the midline and 6.5 to 8.0 mm ventral to brain surface. Electrode placements in the Core were located 1.0 to 2.2 mm lateral to the midline and were dorsal to the anterior commissure from 6.6 to 7.2 mm ventral to brain surface. Recordings in the dorsal striatum were between 0.48 and 1.7 mm anterior to bregma. DMS placements were
found 1.0 to 1.8 mm lateral to the midline and 3.8 to 5.5 mm from brain surface. DLS placements were located 3.6 to 4.5 mm lateral to the midline and ventral 3.8 to 5.5 mm from the surface of the brain.

2. **Unpredicted reward selectively evokes phasic dopamine in the Core**

Here, phasic changes in striatal dopamine release were recorded in response to unpredicted reward and extracted from voltammetric data utilizing PCA as described above (see Figure 3.1 for example). While all rats exhibited significant electrically evoked dopamine release, dopamine evoked by sugar pellet delivery varied as a function of subregion (Figure 3.3). In the Core, peak dopamine (53.4 ± 11.1 nM) was significantly elevated during the pellet epoch – a greater than 5 fold increase relative to the baseline epoch ($t(4) = 3.50, p < 0.05$; Figure 3.3B). Unpredicted pellet delivery failed to evoke a change in dopamine in the Shell ($t(5) = 0.67, p > 0.05$.; Figure 3.3A), DMS ($t(5) = 1.61, p > 0.05$.; Figure 3.3C) or DLS ($t(5) = 2.41, p > 0.05$.; Figure 3.3D). In all panels, insets show the average baseline and pellet dopamine for individual rats. These data not only confirm previous evidence that unpredicted reward evokes a phasic dopamine increase in the Core but also indicate that phasic dopamine is not similarly evoked across striatal subregions by unpredicted reward.

**D. Discussion**

Unpredicted food reward evokes phasic increases in activity from a majority of dopamine neurons across the medial-lateral extent of the VTA/SNpc (Mirenowicz and Schultz, 1994, 1996; Schultz, 1998; Hyland et al., 2002; Bayer and Glimcher, 2005;
Matsumoto and Hikosaka, 2009). However, it remains unclear how dopamine is transmitted to terminal regions in response to unpredicted food reward. To resolve this, phasic changes in dopamine concentration were recorded in the Shell, Core, DMS and DLS in response to unpredicted sugar pellet reward. While electrical stimulation of the VTA/SNpc evoked a robust phasic signal in all striatal regions (as described in Chapter II), unpredicted food reward selectively evoked phasic dopamine release in the Core with no change observed in the Shell, DMS or DLS. These results clearly demonstrate that in response to unpredicted food reward, phasic dopamine is not uniformly transmitted to striatal terminal regions.

Based upon electrophysiological and anatomical evidence, it has been suggested that dopamine neurons broadcast a uniform signal across the striatum (Schultz, 1998). The current results, however, support previous neurochemical findings revealing regional differences in dopamine concentration to reward stimuli (Bassareo and Di Chiara, 1999; Aragona et al., 2008; Aragona et al., 2009; Bassareo et al., 2011). In the Shell, dopamine concentration, as measured by microdialysis, increases in response to novel reward stimuli, but is rapidly attenuated with repeated exposure (Bassareo and Di Chiara, 1999). Results from the current study further support these findings, as the sugar pellets were not novel during testing and no dopamine response was observed in the Shell. However, reward evoked phasic dopamine release has been previously observed in the Shell (Roitman et al., 2008). Rats had catheters inserted into their oral cavities that allowed for infusion of solutions into their mouth during behavioral testing. This technique ensures the animals taste the solution but do not have to consume it. Naïve rats were randomly given intra-oral infusions of rewarding sucrose while changes
in phasic dopamine release were recorded in the Shell. In response to an unexpected infusion of sucrose, phasic dopamine release was increased in the Shell following the infusion. Unique from the current study, these intra-oral infusions of a rewarding solution were novel and unexpected, possibly eliciting the phasic dopamine activity observed in the Shell. Furthermore, the mode of delivery, an intra-oral infusion, is very distinct from pellet delivery as animals are passively presented with the solutions and did not have to pay attention to reward delivery or locomote to retrieve a reward. Given that the paradigms used in these studies were significantly different, it still remains unclear if phasic dopamine is transmitted uniformly throughout the striatum or in a regionally selectively manner in response to unpredicted food reward.

In the current study, unpredicted food reward selectively evoked phasic dopamine release in the Core in rats with limited experience. This is consistent with previous studies recording changes in dopamine concentration in the Core to reward stimuli (Bassareo and Di Chiara, 1999; Day et al., 2007; Zhang et al., 2009). For example, microdialysis recordings by Bassareo & Di Chiara, (1999) showed that increases in dopamine release develop in the Core to both predictive stimuli and food reward after associative learning. In the current experiment, rats had several sessions to retrieve and consume pellets prior to voltammetric recording. It is possible that, given this experience, rats developed expectancies based on contextual cues and the cues associated with sugar pellet delivery.

No change in phasic dopamine release was observed in the dorsal striatum (DMS or DLS) to unpredicted food reward, consistent with neural activity in these regions. While the response in the DLS exhibits a trend toward significance, relative to baseline the
increase in phasic dopamine release is negligible. Medium spiny neurons (the striatal output neurons) in the DMS are activated when a response, such as a lever press, must be made to obtain a reward or when choice pattern has to be selected in a flexible manner (Kawagoe et al., 1998; Ragozzino et al., 2001; Pasupathy and Miller, 2005; Kimchi and Laubach, 2009b). Neurons in the DLS, however, do not respond to reward stimuli (Root et al., 2010). Thus, the absence of phasic dopamine to unpredicted reward in the DMS and DLS is in concert with neural activity in these regions.

The current findings demonstrate that phasic dopamine release is differentially evoked in the striatum to unpredicted food reward. There remains, however, a disconnection between the uniform response of dopamine neurons and the regional selectively of phasic dopamine release to unpredicted food reward. Several factors may underlie this divergence including the level of previous experience, modification of dopamine activity at the terminal region, and the identification of dopamine neurons used in electrophysiological recordings. These hypotheses will be further discussed in Chapter V. Taken together, the selective increase in phasic dopamine release in the Core by unpredicted food reward suggests that regional changes in phasic dopamine release may play unique functional roles across terminal regions.
Figure 3.1: Individual trial examples of phasic dopamine evoked by electrical stimulation of the VTA/SNpc and by unpredicted food reward. (A) Electrical stimulation evokes a phasic change in dopamine concentration. Top: Color plot shows current changes (in color) across the applied voltages (E\text{app}; ordinate) over time (abscissa). Dopamine is identified by its oxidation (green feature, \sim 0.6 V) and reduction (dark blue/yellow feature, \sim -0.2 V) peaks that arise just after stimulation onset. Inset: Cyclic voltammogram plotted at the time of peak dopamine release. Cyclic voltammograms for dopamine and pH obtained after stimulation are used to build a training set for PCA. (B) In the same rat, unpredicted food reward (sugar pellet) evokes a phasic increase in dopamine concentration. Top: Color plot shows current changes as a function of applied voltage over time. Dopamine is identified by its oxidation and reduction features occurring just after pellet delivery. Bottom: Changes in dopamine concentration extracted from the color plot above using PCA.
Figure 3.2: Location of carbon fiber recording electrodes examining phasic dopamine release evoked by unpredicted reward. Carbon fiber recording electrodes were located in distinct striatal regions. Placements are color-coded: Shell, blue; Core, green; DMS, orange; DLS, red. Numbers are distances in mm anterior from bregma. Brain histological images were adapted from the stereotaxic atlas of Paxinos & Watson (1998).
**Figure 3.3**: Unpredicted food reward selectively evoked an increase in phasic dopamine release in the Core, but not the Shell, DMS or DLS. Top: Color plots show current changes (in color) over time (abscissa) across the different voltages (ordinate) of the recording electrode. Bottom: Average dopamine concentration traces extracted from the voltammetric data using PCA, aligned to the pellet delivery. Mean dopamine concentration is represented by the points and error bars represent ± SEM. Inset: Average dopamine concentration during each epoch (Baseline and Pellet) for individual rats. A. Unpredicted reward did not alter phasic dopamine signaling in the Shell. B. Unpredicted reward evokes phasic dopamine release in the Core. * p < 0.05 Baseline versus Pellet epochs. C. No change in phasic dopamine release to unpredicted reward in the DMS. D. Phasic dopamine release is unaltered during unpredicted reward in the DLS.
Chapter IV
Regionally-distinct phasic dopamine response to reward predictive cues and subsequent unpredicted delivery of a food reward

A. Introduction

For decades, dopamine projections to the striatum, specifically from the SNpc, were thought to play a critical role in voluntary movement. This idea was primarily based on the selective degeneration of the nigrostriatal dopamine pathway in Parkinson’s disease, a neurodegenerative disorder in which aberrant motor behavior is a primary symptom (Ehringer & Horneykinewcz, 1960 as cited in Carlsson, 1987). In animal models, selective destruction of dopamine neurons also results in disordered motor behavior (Schultz, 1982; Perese et al., 1989; Kirik et al., 1998). To better understand this link between dopamine and movement, Schultz (1986) measured the electrophysiological activity of dopamine neurons in non-human primates during arm movements to retrieve rewards. The rewards were used to motivate the monkeys to perform the task. The results demonstrated, however, that a trigger stimulus used to initiate the arm reaching movement phasically increased dopamine neural activity whereas the actual limb movements were associated with far less neural activity. In a series of additional experiments (Romo and Schultz, 1990; Ljungberg et al., 1992; Schultz et al., 1993; Mirenowicz and Schultz, 1994, 1996), it was established that dopamine neuronal activity increases to unpredicted rewards but this increase shifts to the onset of reliable reward predictors with training. Activity of dopamine neurons also encodes the expected value of the reward and the probability of receiving the reward.
Collectively, these experiments suggested that dopamine neurons signal information about reward expectancies and cues that predict the rewards. Evidence from electrophysiological recordings suggests that a majority of the dopamine neurons recorded across the medial-lateral extent of the VTA/SNpc respond with increases in firing rate to unpredicted reward (~75% of neurons) and reward predictive cues (~55-70% of neurons; Schultz 2002). Further, dopamine neurons respond to reward stimuli with similar latencies, magnitude and duration (Mirenowicz and Schultz, 1996; Hyland et al., 2002; Matsumoto and Hikosaka, 2009; Kim et al., 2010). Given that a majority of dopamine neurons recorded are synchronously activated by reward predictive stimuli, it has been proposed (Schultz, 1997) that dopamine neurons produce a global dopamine signal throughout all striatal regions.

The broadcasting of a uniform dopamine signal would suggest a global increase in extracellular dopamine concentration across the striatum to reward predictive stimuli. However, very few studies have examined changes in extracellular dopamine concentration across striatal regions. Using in vivo microdialysis, experiments have demonstrated regionally selective changes in striatal dopamine release to various rewards and cues predictive of reward (Barrot et al., 1999; Bassareo and Di Chiara, 1999; Stefani and Moghaddam, 2006; Bassareo et al., 2011; Ostlund et al., 2011). In several of the studies (Stefani and Moghaddam, 2006; Ostlund et al., 2011) however, changes in dopamine concentration to the cues and rewards were unable to be separated due to the lack of temporal resolution with in vivo microdialysis. The brief, phasic changes in dopamine concentration to rewards and their associated cues that
have been recorded with electrophysiology cannot be captured with *in vivo* microdialysis. This leaves unanswered whether phasic dopamine in distinct striatal regions is evoked by cues predictive of reward.

Fast-scan cyclic voltammetry operates on a subsecond time-scale that is more appropriate for measuring phasic dopamine release events. Previous studies have focused almost exclusively on recording in the nucleus accumbens, primarily in the Core. Using many different behavioral paradigms, phasic dopamine release is reliably evoked by reward predictive cues in the Core (Roitman et al., 2004; Day et al., 2007; Stuber et al., 2008; Aragona et al., 2009; Jones et al., 2010; Wanat et al., 2010). However, examination of phasic dopamine release in distinct subregions of the nucleus accumbens (Core and Shell) have led to conflicting results (Aragona et al., 2009; Wanat et al., 2010). One possibility for these disparate results could be the significant differences in the methodologies used including behavioral paradigms, the level of prior experience of the task, as well as the recording location in the Shell. To address this, it is critical that to record phasic dopamine release to reward predictive cues in multiple striatal regions using the same parameters and task.

In the current experiment, rats were trained and tested on a discriminative stimulus task that has been previously shown to reliably evoke phasic dopamine release in the Core (Jones et al., 2010). Rats were trained to associate an audiovisual cue (tone, left cue light; DS+) with the ability to press the left lever for food reward. Another audiovisual cue (white noise, right cue light; DS-) was associated with the ability to press the right lever with no consequence (no food reward). Rats learned to press the DS+ lever and not press the DS- lever with training. Jones and colleagues (2010) found
that in the Core, the DS+ evoked a significant increase in phasic dopamine release, while the DS- cue evoked a much attenuated phasic dopamine response.

Using FSCV, I recorded changes in phasic dopamine release in the Shell, Core, DMS or DLS during performance of a discriminative stimulus task similar to Jones et al., (2010). Reward predictive cues (DS+) evoked phasic dopamine release in distinct striatal subregions and no response was observed to the DS- cue. Following the discriminative stimulus task, rats were presented with unpredicted food reward (sugar pellets) as described in Chapter III. Unpredicted food reward evoked regionally specific phasic dopamine release, but the response was different from that demonstrated in Chapter III. These results further argue against the uniform broadcast of dopamine to striatal areas and instead support a more complex, regionally selective response that may be influenced by training or task demands.

**B. Experimental Methods**

1. **Subjects:**

Male, Sprague-Dawley rats (n=23; Charles River Laboratories) weighing 325-425 g were individually housed and maintained on a 12/12 hour light/dark cycle in a temperature and humidity controlled environment. Rats were maintained at approximately 90% *ad libitum* body weight during behavioral training and testing portions of the experiments with free access to water. Food was available *ad libitum* during the post-operative recovery period. All procedures were in accordance with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals and
approved by the University of Illinois Institutional Laboratory Animal Care and Use Committee.

2. **Apparatus:**

Rats were trained and tested in the same standard operant chambers as described in Chapter II (page 22).

3. **Discriminative Stimulus Task:**

Food deprived rats were first exposed to 45 mg sugar pellets (BioServe, Sugar Dustless Precision Pellets, #F0042) in their home cage. Rats were next trained in the operant chamber to press either the right or left lever for a sugar pellet. The house light was illuminated and both levers extended in the chamber. Depression of either lever resulted in both levers immediately retracting and a sugar pellet delivered into the food cup located between the levers. After 5 s, the levers re-extended into the chamber and this process continued for 30 minutes. Once rats acquired the lever press-reward association by pressing at least 50 times on either of the levers for 2 consecutive days, they began the discriminative stimulus paradigm adapted from Jones et al., 2010 on the following day. In this task (Figure 4.1), two sets of compound stimuli were associated with spatially distinct levers, one of which was predictive of reward. A tone was played for 1 s accompanied by illumination of the left cue light (rewarded discriminative stimulus; DS+). Three seconds after cue onset the left lever was extended. A lever press resulted in lever retraction, cue light termination and delivery of a sugar pellet. A second set of distinct audiovisual cues (white noise and right cue light) were associated
with the right lever, however depression of this lever did not result in sugar pellet delivery (non-rewarded discriminative stimulus; DS-). Levers were retracted and cue lights were turned off after 5 s if no press was made and the trial was concluded. DS+ and DS- levers were counterbalanced such that stimuli and levers associated with the DS+ and DS- cues were counterbalanced. Each training session consisted of 60 trials (30 DS+, 30 DS-) that were presented pseudorandomly with an inter-trial interval of 15 ± 4 s. Criterion was reached once rats correctly pressed 90% DS+ trials and correctly abstained from pressing on 70% of DS- trials for 2 consecutive days. Rats were allowed free access to food for 48 hours and then underwent surgery for voltammetric recording as described above. Following recovery from surgery, rats were again food restricted and trained to criterion performance. During post-operative training, rats were connected to a dummy headstage to familiarize rats with the voltammetric recording apparatus. Once rats reached criterion, testing began the following day.

4. **Electrodes:**

Carbon fiber microelectrodes were constructed as previously described in Chapter II (page 23).

5. **Surgery:**

Rats were prepared for voltammetric recording as previously described in Chapter II (page 23).
6. **Fast-Scan Cyclic Voltammetry Recordings:**

Voltammetric data was recorded as described in Chapter II (page 25).

7. **Experimental Procedure:**

On the day of testing, a carbon fiber electrode was prepared, lowered into the select striatal subregion (Shell, n=5; Core, n=6; DMS, n=6; DLS, n=6), and allowed to equilibrate as previously described in Chapter II (page 26). Once equilibrated, dopamine was evoked by electrically stimulating the VTA/SNpc (24 pulses, 60Hz, 120 μA, 4 ms/pulse) until lowered into a region that supported phasic dopamine release. Approximately 20 trials (10 DS+, 10 DS-) were then presented to determine any changes in phasic dopamine in response to the task stimuli. If no phasic events were observed, the carbon fiber electrode was lowered 0.3 mm and the electrical stimulation was repeated. In sessions where phasic dopamine release was observed in response to task stimuli, rats were presented with 60 trials (30 DS+, 30 DS-). However, in sessions where phasic dopamine was unaltered in response to task stimuli, rats were presented with approximately 20 trials at a minimum of 4 locations (each 0.3 mm apart). This was done in order to ensure that no dopamine response was present throughout the selected region.

Immediately following recording of the discriminative stimulus paradigm, rats were presented with unpredicted sugar pellets in a manner identical to Chapter III (page 40) while recording continued. During the session, sugar pellets were delivered after a pseudorandomly selected inter-trial interval (range 30-90s; 30 trials). Once the behavioral task was complete, a series of electrical stimulations (10-24 pulses, 30-60Hz, 120μA, 4 ms/pulse) were taken to use for additional analysis. Representative
current by voltage plots (cyclic voltammograms) are obtained for each of these responses. Training sets were constructed from cyclic voltammograms for dopamine and pH to allow for principal component regression on data collected during the behavioral session as previously described (Heien et al., 2004; Day et al., 2007). In all experiments, PCA was used to extract the dopamine component from the voltammetric recordings.

8. Data Analysis

PCA was performed as described in Chapter III (page 41). Data files were cut to 20 s files, with 10 s before and after the onset of the discriminative stimulus or pellet delivery. Backgrounds (for background subtraction) were selected for each individual trial at a location where dopamine was not present. Chemometric analysis was performed on each of these files to extract concentration traces and a snapshot of the background subtracted color plot was recorded. These color plots were then averaged together for each rat and chemometrics were performed on this average color plot. Further analyses utilized this averaged data as well as the concentration traces from individual trials. Two distinct epochs within the average dopamine concentration traces were utilized for further analysis: a Baseline epoch (5 s prior to the onset of the DS cue) and a DS+ or DS- (1 s after DS cue onset) for the discriminative task. For the unpredicted reward task, epochs used for analysis were the Baseline epoch and Pellet epoch (1 s after pellet delivery). Paired t-tests then compared epochs (Baseline versus DS cue, DS+ versus DS- and Baseline versus Pellet) within each striatal region. Statistical analysis was carried out using GraphPad Prism software.
9. **Histology**

As described in Chapter II (page 28), rats were injected with a lethal dose of sodium pentobarbital, lesioned at the location of recording, and transcardially perfused. Brains were removed and stored in 10% formalin solution until being frozen and mounted in a -20°C cryostat (Leica CM1850). Coronal sections were sliced at 50 μm and mounted on gelatin coated slides. Slides were stained with cresyl violet and coverslipped using Permount (Fisher Scientific). After the slides had dried, the location of the recording electrode was identified using a light microscope with the aid of the stereotaxic atlas by Paxinos and Watson (1998).

C. **Results**

1. **Electrode Placement Verification in Striatal Subregions**

Electrode locations for all recordings are shown in Figure 4.2. For recordings in the nucleus accumbens Shell and Core electrode placements were located between 0.7 and 1.7 mm anterior to bregma. Shell placements were located between 0.6 to 1.6 mm lateral to the midline and 6.5 to 8.0 mm ventral to brain surface. Core placements were located 1.0 to 2.2 mm lateral to the midline and were dorsal to the anterior commissure from 6.6 to 7.2 mm ventral to brain surface. Recordings in the DMS were located between 0.48 and 1.7 mm anterior to bregma, 1.0 to 1.8 mm lateral to the midline and from 3.8 to 5.5 mm ventral to brain surface. DLS placements were located 3.6 to 4.5 mm lateral to the midline and ventral 3.8 to 5.5 mm from the surface of the brain.
2. **Phasic dopamine release in striatal subregions during a discriminative stimulus paradigm.**

Rats were trained on a discriminative stimulus paradigm where distinct cues signaled the ability to obtain or not obtain a reward. When presented with the reward predictive cue (DS+), rats pressed a lever to receive a sugar pellet. When the cue that did not predict reward (DS-) was presented, rats abstained from pressing the other lever which did not have any consequence. On the day of testing, rats performed this task with near perfect performance, pressing 97.26 ± 0.97 % on the DS+ lever and 0.23 ± 0.002 % on the DS- lever.

Dopamine concentration traces were aligned to the onset of the DS+ or DS- cue (t = 0) and averaged for each rat, separated by trial type (Figure 4.3). Data from individual rats, comparing the Baseline epoch to the Cue epoch (1 s after cue onset), are shown in the insets for each region. Similar to unpredicted reward (Chapter III), the DS+ evoked a greater than 4 fold increase in Core dopamine relative to baseline. The DS+ also elicited a greater than 3 fold increase in the DMS relative to baseline. Paired t-tests (Figure 4.3, insets) comparing Baseline epoch (5 s prior to cue presentation) to the DS+ epoch (1 s post cue presentation) revealed that the DS+ cue selectively evoked phasic dopamine in the Core (84.2 ± 21.7 nM peak dopamine for DS+, t(5) = 3.19, p < 0.05; Figure 4.3B) and DMS (49.2 ± 8.6 nM peak dopamine for DS+, t(5) = 3.45, p < 0.05; Figure 4.3C). The DS+ cue failed to evoke changes in phasic dopamine in the Shell (t(4) = 0.94, p > 0.05; Figure 4.3A) or DLS (t(5) = 1.65, p > 0.05; Figure 4.3D) Interestingly, the nature of the response in the Core and DMS is different. In the Core (Figure 4.3B), there are two distinct peaks in dopamine concentration corresponding to the onset of the DS+ cue
and extension of the associated lever, whereas in the DMS (Figure 4.3C) there is a single peak in dopamine concentration occurring at the onset of the DS+ cue.

As shown in Figure 4.3, the DS- failed to evoke a change in dopamine in all striatal subregions (P’s > 0.05). To determine if phasic dopamine responses were selectively evoked by a reward predictive cue, I compared, within each subregion, the difference between dopamine evoked by the DS+ versus DS- (Figure 4.4). Paired t-tests revealed that the DS+ evoked a greater increase in phasic dopamine than the DS- in the Core (t(5) = 3.23, p < 0.05; Figure 4.4B), and DMS (t(5) = 3.09, p < 0.05; Figure 4.4C). In contrast, there was no significant difference in dopamine concentration following a DS+ cue compared to that following a DS- cue in the Shell (t(4) = 0.71, p > 0.05; Figure 4.4A), and DLS (t(5) = 0.66, p > 0.05; Figure 4.4D). These effects are consistent among individual rats within each striatal region. Thus, reward predictive (DS+) cues elicited an increase in phasic dopamine only in the Core and DMS as compared to both the baseline epoch and the DS- cue.

3. Phasic dopamine release to unpredicted reward following discriminative stimulus training.

Immediately following administration of the discriminative stimulus recording session, rats were presented with unpredicted sugar pellets as described in Chapter III. Average dopamine concentration traces, aligned to pellet delivery are shown in Figure 4.5. Baseline and Pellet epochs were compared using paired t-tests for each striatal subregion (Figure 4.5 insets). Similar to results obtained from rats without discriminative stimulus training, unpredicted food reward evoked a greater than 5 fold phasic increase
in dopamine concentration in the Core ($t(4) = 3.38, p < 0.05$; Figure 4.5B) and failed to evoke a change in the Shell ($t(4) = 0.76, p > 0.05$; Figure 4.5A) and DLS ($t(5) = 0.54, p > 0.05$; Figure 4.5D). In contrast to results described in Chapter III, after discriminative stimulus training, unpredicted food reward evoked a greater than 3 fold and significant increase in phasic dopamine in the DMS ($t(5) = 3.29, p < 0.05$; Figure 4.5C).

### D. Discussion

Both unpredicted food reward and cues that predict reward elicit synchronous activity in a majority of dopamine neurons across the medial-lateral extent of the ventral midbrain (Mirenowicz and Schultz, 1996; Hyland et al., 2002; Matsumoto and Hikosaka, 2009). This global increase in activity is thought to transmit a uniform increase in phasic dopamine release across the striatum in response to these reward-associated stimuli. However as described in Chapter III, phasic dopamine release was not globally increased, but was selectively evoked in the Core to unpredicted food reward in rats with limited experience. In the current experiment, I further demonstrate that phasic dopamine release is also selectively evoked by reward predictive cues and the subsequent presentation of unpredicted food rewards. Both reward predictive cues and unpredicted food reward following training on the discriminative stimulus task evoked phasic dopamine release in the Core and DMS. In the Shell and the DLS, no change in phasic dopamine signaling was observed during either of the tasks. These findings demonstrate that phasic dopamine release is not uniformly broadcast across the striatum, but is selectively evoked in distinct striatal regions. Furthermore, selectivity is
dependent on the specific conditions in which a reward or associated cues are delivered.

In the current study, reward predictive cues evoked phasic dopamine in distinct striatal subregions. Similar to results from previous experiments (Roitman et al., 2004; Day et al., 2007; Stuber et al., 2008; Jones et al., 2010), phasic dopamine release was evoked in the Core in response to reward predictive cues. However, no response was observed to reward predictive cues in the Shell, concordant with a study by Aragona et al. (2009). In a Pavlovian conditioning paradigm where no response was required to obtain the reward, a cue light and tone were associated with intra-oral sucrose while phasic dopamine was recorded in the Core or medial Shell. Sucrose predictive cues evoked phasic dopamine in the Core, but not in the Shell, demonstrating regionally distinct phasic dopamine release. Rats were naïve until the recording session and only experienced the Pavlovian task on the day of testing. However, in an experiment by Wanat et al. (2010) a different result was observed. Using an instrumental paradigm (progressive ratio) where rats had to lever press exponentially more to obtain each reward, cues predictive of reward evoked phasic dopamine release in both the Core and Shell. Importantly, the locations of the Shell recordings in Wanat et al. (2010) were ventral to the anterior commissure, not in the medial Shell as in Aragona et al. (2009) and the current study. This point is critical as the Shell is a very heterogeneous area (Park et al., 2010) and the region recorded from in Wanat et al. (2010) receives dopaminergic input from a similar population of neurons as the Core (Ikemoto, 2007). Thus, phasic dopamine release evoked in these different Shell locations further support
a regional specificity in evoked phasic dopamine release greater than the four striatal subregions examined here.

Furthermore, the paradigms used in these two experiments were substantially different. During Pavlovian conditioning, as used in Aragona et al (2009), rats learned to associate cues with reward delivery. However in Wanat et al. (2010), rats associated a cue with the ability to lever press for reward in a progressive ratio paradigm where the number of lever presses required increased exponentially with each reward. Interestingly, previous studies have suggested that striatal regions may play distinct roles in Pavlovian and instrumental conditioning (Floresco et al., 2008; Corbit and Janak, 2010; Lex and Hauber, 2010), which may therefore influence regional differences in evoked phasic dopamine release.

Here, I observed a robust increase in phasic dopamine release in the DMS to both reward predictive cues as well as to unpredicted food rewards following discriminative stimulus task performance. The DMS been proposed to facilitate a response selection process (Balleine et al., 2007) such as during instrumental task performance or when the ability to select a choice pattern requires flexibility (Kawagoe et al., 1998; Ragozzino et al., 2001; Pasupathy and Miller, 2005; Kimchi and Laubach, 2009b). During the discriminative task performance, animals have to pay attention to the predictive cues to guide their behaviors. Following performance of the discriminative stimulus task, animals must flexibly shift their behavioral pattern from paying attention to the cues and levers to focusing on the reward receptacle for retrieve the unpredicted food reward. This change in behavioral responding may recruit dopamine signaling to the DMS to facilitate the flexible shift in behavioral patterns.
The current results suggest a disconnection between the uniform response of dopamine neurons and the regional selectivity of phasic dopamine release to reward-associated stimuli. Mechanisms involved in these underlying differences could be presynaptic modification at dopamine terminals, the selection criterion for dopamine neurons in previous studies, as well as the level of prior experience and type of tasks used to examine dopamine activity. These possibilities will be further discussed in Chapter V. Taken together with the results from Chapter III, these findings demonstrate that different patterns of phasic dopamine release are evoked to reward-associated stimuli depending on the specific conditions in which the reward or associated cues are delivered.
Figure 4.1

Figure 4.1: A schematic diagram of the behavioral task. Animals were pseudorandomly presented with one of two trial types (DS+ or DS-). Each trial type was associated with a different auditory cue (white nose or tone), cue light (left or right), and subsequent extension of the respective lever (left or right) below the illuminated cue light. A response on the DS+ lever resulted in the delivery of a sugar pellet. Responses on the DS- lever had no consequence.
Figure 4.2: Location of carbon fiber recording electrodes examining phasic dopamine release during the discriminative stimulus task and subsequent unpredicted food reward presentation. Placements are color-coded: Shell, blue; Core, green; DMS, orange; DLS, red. Numbers are distances in mm anterior from bregma. Brain histological images were adapted from the stereotaxic atlas of Paxinos & Watson (1998).
Figure 4.3: Discriminative stimuli differentially evoke phasic dopamine signaling across striatal subregions. Average dopamine (black line) ± SEM (gray vertical bars) to predictive cues in striatal subregions during the discriminative stimulus test. Top: A cue predictive of reward (DS+) selectively evokes phasic dopamine release in the Core (B) and DMS (C) but not the Shell (A) or DLS (D). Insets: Average dopamine concentration for each rat during both Baseline and Cue epochs. Note that in the Core (B), the scale for the ordinate, dopamine concentration ([DA]) in nM, is 100 nM, twice that of the other striatal regions. * P < 0.05 for Baseline versus DS+ epoch. Bottom: A cue predictive of no reward (DS-) fails to alter phasic dopamine signaling in all striatal subregions. Insets: Average dopamine concentration for each rat during both Baseline and Cue epochs.
Figure 4.4: Cue-evoked dopamine is dependent on a cue-reward association. Average dopamine concentration for each rat during both Cue (DS+ versus DS-) epochs. The DS+ evoked significantly greater dopamine relative to the DS- in the Core (B) and DMS (C). Note that in the Core (B), the scale for the ordinate, [DA] in nM, is 100 nM, twice that of the other striatal regions. * $P < 0.05$ for DS+ versus DS- epochs. No differences were observed in the Shell (A) or DLS (D).
**Figure 4.5**: In rats trained in the discriminative stimulus paradigm, unpredicted food reward evokes a different pattern of phasic dopamine release across striatal subregions. Average dopamine (black line) ± SEM (gray vertical bars) in different striatal regions in response to unpredicted food reward (sugar pellet; time = 0). Insets: Average dopamine concentration for each rat during both Baseline and Pellet epochs. Unpredicted food reward evokes phasic dopamine release in the Core (B) and DMS (C) but not the Shell (A) or DLS (D). Note that in the Core (B), the scale for the ordinate, [DA] in nM, is 100 nM, twice that of the other striatal regions. * $P < 0.05$ for Baseline versus Pellet epochs.
Chapter V
General Discussion

Phasic changes in dopamine are critical for signaling reward (Mirenowicz and Schultz, 1996; Roitman et al., 2008; Matsumoto and Hikosaka, 2009) and play a role in learning about cues that predict reward (Waelti et al., 2001; Day et al., 2007; Tsai et al., 2009; Zweifel et al., 2009; Jones et al., 2010). Electrophysiological studies have demonstrated that a majority of dopamine neurons in the VTA/SNpc uniformly increase their activity in response to reward and reward-predictive cues (Mirenowicz and Schultz, 1996; Schultz, 2002; Matsumoto and Hikosaka, 2009). It has been proposed (Schultz, 1997) that this increase in dopamine neuronal activity relays a synchronous dopamine signal throughout all terminal areas. The goal of the current studies was to systematically examine phasic dopamine signaling in four striatal regions (Shell, Core, DMS and DLS) in response to stimuli known to evoke phasic dopamine activity.

In the current experiments, electrical stimulation of the VTA/SNpc evoked phasic dopamine release across all four striatal regions. However, unpredicted reward delivery and reward predictive cues selectively evoked dopamine in the Core and DMS. Further, the phasic dopamine response in the DMS to unpredicted food reward only occurred in rats trained on a discriminative stimulus paradigm and when pellets were delivered immediately following discriminative stimulus performance. The reward-associated stimuli used here failed to evoke phasic dopamine release in the Shell or DLS. Results therefore demonstrate that phasic dopamine release is not uniformly transmitted to striatal regions under these behavioral conditions, but is selectively evoked in distinct subregions.
A. The selection of striatal subregions for examination of phasic dopamine release

The four striatal subregions used here were selected based upon anatomical and functional divisions used in previous experiments (Roitman et al., 2004; Palencia and Ragozzino, 2005; Roitman et al., 2008; Brown et al., 2010; Ebner et al., 2010). However, the rodent striatum is composed of a gradient of inputs without having distinct regional borders in certain cases (McGeorge and Faull, 1989; Cheatwood et al., 2003; Cheatwood et al., 2005). Thus, there are other ways to divide up the striatum and investigate whether phasic dopamine responses occur under specific behavioral conditions in different areas of the striatum. It is important to note that the selection of regions in the current study was specific to the dorsal-ventral and medial-lateral planes, but did not focus on the anterior-posterior plane. Several studies have proposed differences between anterior and posterior striatal regions such as the DMS (Yin et al., 2005; Corbit and Janak, 2010; Pielock et al., 2011) and Shell regions (Pecina and Berridge, 2005; Pecina et al., 2006; Park et al., 2010). Again for the current project, the striatal subregions selected were based on common subdivisions used to describe anatomically and functionally distinct regions of the striatum. Specifically, the dorsal and ventral divisions were further divided into the Core and Shell in the ventral division and the DMS and DLS in the dorsal division. Importantly, the selection of the four subregions used in the current study was sufficient to identify differences in phasic dopamine release in different areas of the striatum. Based on results from the current experiments, future studies can examine regional differences in more specific striatal regions, such along the anterior-posterior axis.
B. **Midbrain stimulation evoked regional differences in phasic dopamine release**

I demonstrated that phasic dopamine release was supported at all recording sites. Application of current directly to the dopamine cell bodies in the VTA/SNpc evoked phasic dopamine release in all four striatal regions (Shell, Core, DMS and DLS). This demonstration was critical, specifically in regions that did not show any phasic dopamine response to the reward-associated stimuli. The ability to evoke dopamine in all regions indicates that all areas were capable of producing phasic dopamine signals. Thus, if no change in phasic dopamine release was observed to any of the stimuli in the behavioral paradigms tested in Chapters III and IV, it would be due to the failure of particular stimuli to evoke phasic dopamine release and not due to the inability to detect phasic dopamine responses from a specific region.

While electrical stimulation elicited phasic dopamine release in all striatal regions, this response was not uniform. The peak dopamine response was attenuated in the DLS as compared to the Shell, Core and DMS. This could be due to a variety of factors including the placement of the stimulating electrode, presynaptic modifications of evoked release, and regional differences in the regulation of dopamine release by the DAT. First, as discussed in the Discussion in Chapter II, the stimulating electrode was aimed towards the VTA and medial SNpc in all experiments. Therefore, neurons in the lateral SNpc, which primarily project to the DLS, may not have been as robustly stimulated (Bjorklund and Lindvall, 1984; Haber et al., 2000; Voorn et al., 2004). However, it is unknown how far the electrical current from the stimulation spreads and thus how many neurons are excited. Second, dopamine release is modulated at the presynaptic terminal, which could alter evoked dopamine release across striatal regions.
For example, acetylcholine can powerfully regulate phasic dopamine release activity via activity at both muscarinic and nicotinic acetylcholine receptors (Threlfell and Cragg, 2011). The composition of these receptors is different in the dorsal versus ventral striatum which can heterogeneously modulate dopamine activity across striatal regions. Thus, it is possible that electrically-evoked phasic dopamine signaling in the DLS was altered by presynaptic mechanisms such as acetylcholine. Finally, dopamine release is differentially regulated across the striatum by DATs. The density of DATs and resultant functional differences could impact how quickly dopamine is removed from the extracellular space (Cragg and Rice, 2004), thus altering the rising and falling phases of electrically evoked phasic dopamine release. In the DLS, a greater number of DATs could decrease the peak dopamine release and decrease the diffusion of phasic dopamine release events. Taken together, these factors may have contributed to the differentially evoked peak dopamine release across striatal subregions.

The rate of dopamine reuptake also varied across the striatum: fastest in the DLS and slowest in the Shell. Previous studies have demonstrated a gradient of DAT density in the striatum with a higher density of DAT in the DLS and lowest in the Shell (Richfield, 1991; Ciliax et al., 1995; Nirenberg et al., 1997). This gradient of DAT density supports the functional differences in the rate of reuptake shown here. Importantly, the rate of reuptake in striatal regions can alter the temporal and spatial regulation of the lifetime of phasic dopamine release events (Cragg and Rice, 2004). A higher density of DATs and faster reuptake, such as in the DLS, results in dopamine having a shorter active lifetime and sphere of influence on dopamine receptors on both pre- and postsynaptic neurons (Cragg and Rice, 2004). Faster reuptake will have a more
profound influence on the low-affinity D1 receptor as compared to the high-affinity D2 receptor. Given this, a greater reuptake rate in the dorsal striatum would restrict the activation of D1 receptor more than in the ventral striatum where DAT density is lower.

Furthermore, greater spatial and temporal regulation would decrease the diffusion of behaviorally-evoked phasic dopamine release events in the DLS. In Chapters III and IV, no change in phasic dopamine release was recorded in the DLS. One possibility for this is that when release events do occur, they are so tightly regulated that there is not significant diffusion outside of the perisynaptic region. Thus, it is possibly that the lack of phasic dopamine release observed in the DLS is a result of this tight regulation. On the other hand, no change in phasic dopamine release was observed in the Shell, the striatal subregion with high peak dopamine release and the slowest rate of reuptake. This suggests that phasic dopamine release is less regulated in the Shell and thus more diffusion of dopamine can occur, having a larger sphere of influence. Previous studies have demonstrated significant spontaneous release events in Shell that are not time-locked to any behaviorally relevant event (Aragona et al., 2008; Roitman et al., 2008; Aragona et al., 2009; Park et al., 2010). Perhaps the decreased regulation of phasic dopamine release results in the observation of these spontaneous phasic release events. Thus, while a similar response in phasic dopamine release was observe in the Shell and DLS during unpredicted food reward and in response to reward predictive cues, key differences in dopamine regulation may influence dopamine function in these striatal regions.
C. Reward and reward-predictive cues selectively evoke striatal dopamine release

This is the first study to characterize phasic dopamine release to unpredicted food reward and reward predictive cues across four striatal regions. Recordings in the Core replicate previous experiments demonstrating that phasic dopamine release increases to unpredicted reward (Day et al., 2007; Stuber et al., 2008; Zhang et al., 2009) and that the dopamine response shifts in time to the onset of reward predictive cues following conditioning (Roitman et al., 2004; Day et al., 2007; Aragona et al., 2009; Jones et al., 2010). Contrary to electrophysiological recordings, however, the current study found that this phasic dopamine response is not uniform across the striatum. In animals with experience only receiving unpredicted food reward, phasic dopamine release was selectively evoked in the Core, without altering dopamine in the Shell, DMS or DLS.

In the discriminative stimulus task, reward predictive cues evoked dopamine release in the Core and DMS. The Core and DMS are both brain regions important for goal-directed behavior. Dopamine signaling in the Core is thought to encode information about reward and reward-associated stimuli (Day et al., 2007; Jones et al., 2010) as well as play a role in mediating the level of effort exerted in motivationally challenging tasks (Aberman et al., 1998; Aberman and Salamone, 1999; Salamone et al., 2001). The DMS, however, is a region proposed to facilitate the ability to select a response (Balleine et al., 2007) such as during instrumental task performance. During the discriminative stimulus task, animals have to pay attention to the predictive cues in order to determine the correct behavioral response. The demands to perform the discriminative stimulus task may recruit dopamine neurons projecting to the DMS. The
current experiment was the first to examine phasic dopamine release in the DMS in response to reward-associated stimuli. Thus, my results suggest that phasic dopamine release in the Core and DMS may play a role in facilitating goal-directed behaviors.

No phasic dopamine response was observed in the Shell during unpredicted food reward and the discriminative stimulus task. Dopamine signaling in the Shell is thought to be involved in signaling novelty as well as the valence of stimuli (Bassareo and Di Chiara, 1999; Roitman et al., 2008). Given that rats had significant training on the task prior to testing, the rewards and associated stimuli were not novel. Phasic changes in dopamine release have been previously observed in the Shell (Roitman et al., 2008). Unexpected intra-oral infusions of sucrose increased phasic dopamine release in naïve rats. These infusions were novel and unexpected, possibly eliciting the phasic dopamine activity observed in the Shell. The reward stimuli used in the current experiments were of similar valence and rats had significant experience with the cues and reward.

Phasic dopamine release was also unchanged in the DLS during unpredicted reward and the discriminative stimulus task. The DLS is thought to facilitate habit formation and habitual responding (Yin et al., 2004, 2006). Dopamine depletion in the DLS leads to impairment in motor behaviors (Evenden and Robbins, 1984; Sabol et al., 1985). Further, dopamine activity in the DLS may be critical for motor movements in a behavioral sequence necessary for habitual responding (Horvitz, 2009). In the current discriminative stimulus task, animals do not seem to be engaged in habitual responding. Based upon personal observations, animals quickly stop lever pressing when reward is omitted (extinction). However, if rats were over-trained on this paradigm and
responding becomes habitual, it is possible that phasic dopamine release may be evoked in the DLS.

Following performance of the discriminative stimulus task, unpredicted reward evoked phasic dopamine release in the DMS. It is this final result that brings up some interesting questions as to why an identical stimulus – unpredicted food – would evoke an increase in the DMS under some circumstances (following the discriminative stimulus paradigm) but not others (in rats just trained with unpredicted food). This recruitment of phasic dopamine signaling in the DMS when reward conditions change could be the result of a change in behavioral strategies. Performance in the discriminative stimulus paradigm required animals to learn about distinct audiovisual cues associated with a response that resulted in a reward or one that was not associated with reward. After several weeks of training on this task, rats were suddenly switched to the unpredicted reward condition. Orienting to the location of the DS+ lever was no longer an optimal strategy, but instead simply orienting toward the food receptacle was a more optimal strategy. Thus, rats had to change their behavioral strategy from attending to the cues and exploring and pressing the appropriate lever to being more selectively positioned at the pellet receptacle to retrieve the sugar pellets. It is possible that over the course of the session, rats engaged in this flexibly shift in behavior, attending to the food receptacle and spent less time orienting toward the lever and cue lights. Importantly, the DMS is a region critical during shifts in behavioral choice patterns. Past studies have demonstrated that neural activity in the DMS is modulated when conditions require a rapid switch or reversal of choice patterns (Ragozzino et al., 2001; Kimchi and Laubach, 2009b). Further, inactivation of the DMS
also impairs a shift in response patterns (Ragozzino et al., 2002; Ragozzino and Choi, 2004). Thus, one possibility is that dopamine input to the DMS may be important for facilitating the flexible use of behavioral strategies during the presentation of the unpredicted pellet following discriminative stimulus task performance.

D. How to reconcile electrophysiological and electrochemical recordings?

Results from electrophysiological studies strongly suggest that dopamine neurons evoke a global dopamine signal across the striatum to reward and reward predictive cues. However, results from in vivo neurochemical measurements (Di Chiara and Bassareo, 2007; Aragona et al., 2009) or pharmacological (Besson et al., 2010; Ito and Hayen, 2011) or genetic (Palmiter, 2008) manipulations support regional specificity for dopamine action within the striatum. The current set of experiments further support the latter argument – that phasic dopamine release is regionally evoked in response to reward-associated stimuli. Importantly, the current experiments assayed dopamine on a timescale similar to that of electrophysiological recordings. Because of this, the current studies raise the question as to why there is dissociation between electrophysiological and in vivo neurochemical findings. There are multiple possibilities. First, previous electrophysiological recordings may have only sampled a subset of dopamine neurons. Currently, the electrophysiological identification of neurons as dopaminergic relies on a common set of criteria initially proposed by Grace and Bunney (1983). However, recent work has challenged the accuracy of these electrophysiological criteria and suggests that a specific population of dopamine neurons have been excluded based upon these criteria (Margolis et al., 2006; Lammel et al., 2008; Margolis et al., 2008; Margolis et al., 2010; Zhang et al., 2010; Lammel et
al., 2011). These properties have also been suggested to be altered. As such, subpopulations of dopamine neurons with distinct molecular and physiological properties have been identified based on their terminal projection targets. In particular, this research has revealed dopamine subpopulations that, in previous studies, have not been identified as dopaminergic based on signature electrophysiological criteria. The population of neurons that are most often recorded from may, in turn, preferentially project to the Core and DMS (Ikemoto, 2007). However, dopamine neurons projecting to the medial Shell and medial prefrontal cortex have likely been neglected in previous electrophysiological studies.

Another possibility is that dopamine release is heavily modulated by action at striatal dopamine terminals, directly affecting dopamine release. Striatal regions receive a diversity of input from forebrain regions which may differentially affect dopamine terminal release. For example, the makeup and type of acetylcholine receptors located on dopamine axons differ between ventral and dorsal striatum (Threlfell and Cragg, 2011). Acetylcholine has been shown to exert powerful modulatory effects on dopamine signaling (Cragg, 2006) and thus may differentially affect release across subregions. Taken together, phasic dopamine release observed in the Core and DMS, but not other subregions may have a significant influence in reinforcement behaviors and the selective gating of information flow during reward learning and performance of goal-directed behaviors.
E. Implications of the current results for treatment of various disorders

Understanding how dopamine signaling across terminal regions is critical in the development of pharmacotherapies for disorders, such as Parkinson’s disease, depression, schizophrenia and drug addiction. Hallmark to these disorders is altered brain dopamine transmission, indicated as an underlying cause or symptom (Kish et al., 1988; Robinson and Berridge, 1993; Hietala et al., 1995; Laruelle and Abi-Dargham, 1999; Dunlop and Nemeroff, 2007). However, current dopaminergic pharmacotherapies alter global changes in dopamine activity. Based on the results of the current study, this may not be the most effective treatment. For example, one treatment for early stage Parkinson’s disease is pramipexole, which primarily acts as a dopamine D2 and D3 receptor agonist. D2 and D3 receptors are located throughout the brain, with the highest density in the striatum and nucleus accumbens but also have significant distribution in other midbrain and forebrain regions (Boyson et al., 1986). Therefore treatment with pramipexole alters D2 and D3 receptors throughout the brain. The results from the current study demonstrate that dopamine release is not globally evoked, but is released in specific regions in response to behaviorally relevant stimuli. Thus, treatments that alter dopamine release and post-synaptic receptors across all dopamine terminal regions may not be the most effective treatment and may result in significant side effects.

In another example, the atypical antidepressant, bupropion, has partial action as a DAT inhibitor (Feighner, 1999). As previously discussed, DATs are located at a majority of dopamine terminal regions, but with different densities. Thus a drug that acts on the DAT would alter dopamine signaling across the forebrain. However, given
the regional specificity of dopamine release, it may be that, in depressed individuals, altering in dopamine reuptake in a select region, such as the Core or DMS, would be more effective than a global dopamine manipulation. Given that dopamine is not uniformly broadcast, a focus needs to be placed on the development of regionally-selective treatment strategies for these disorders. More directed treatments would possibly lead to a decrease in side-effects and higher efficacy treatment for specific deficits.

F. **Future Directions**

The results of the current studies inspire many new questions that can be addressed in future studies. First, while regional differences in phasic dopamine release were demonstrated in response to reward-associated stimuli, it remains unknown the functional implication of these regionally selective signals. To begin to address this, pharmacological manipulations that selectively alter phasic dopamine release within distinct regions could help to discern functional roles. For example, attenuation of phasic signaling by deletion of the glutamate NMDA receptor on dopamine neurons in the ventral midbrain leads to several impairments in reward-associated behaviors (Zweifel et al., 2009; Parker et al., 2010). If this manipulation could target specific populations of dopamine neurons that project to distinct terminal regions, it would be interesting to see how attenuated phasic dopamine release would affect behavioral responses. Other manipulations could be used to alter phasic dopamine release in specifics subregions such as optogenetic techniques which can artificially drive or inhibit specific populations of neurons on a physiological timescale (Tsai et al., 2009). Another
manipulation to alter phasic dopamine release would be via direct infusions into specific striatal regions to target the presynaptic mechanisms that have been show to alter phasic dopamine signaling (Zhang et al., 2009; Threlfell and Cragg, 2011). Pharmacological manipulation of specific population of dopamine neurons in the ventral midbrain could selectively alter pools of dopamine neurons that project to specific striatal regions (Keath et al., 2007; Tepper and Lee, 2007). Demonstrated in a previous study, Daberkow, Brown et al., (submitted), systemic administration of amphetamine increase the probably of phasic dopamine release in the DMS. At lower doses of amphetamine, behavior on a discriminative stimulus task is unaffected, but at higher does, behavior is completely impaired as animals no longer engage in the task. However, it is unknown whether selective infusion of amphetamine in the DMS, or other brain regions would have a similar effect. This data demonstrates that altering phasic dopamine release has a profound effect on behavior, but future studies are required to fully understand the nature and function of phasic dopamine signaling.

Second, if dopamine truly is a reward prediction error signal, then what happens when the task contingencies are reversed? For example, if the cue that previously predicted reward is no longer reinforced and the non-reward predictive cue is now predictive of reward, does the phasic dopamine signal change? If so, does this occur in particular striatal regions? Based upon previous studies from Ragozzino and colleagues, the DMS is a striatal subregion important for reversal learning (Palencia and Ragozzino, 2006; Ragozzino et al., 2009; Brown et al., 2010). If dopamine signaling serves as a reward prediction error signal in the DMS or other striatal subregions, then phasic dopamine release in response to the reward predictive cues will be altered. My
hypothesis would be that phasic dopamine release will slowly shift from the previously rewarded predictive cue to the new predictive cues as the behavioral response begins to change from pressing the previously rewarded lever to the newly reward lever. It is possibly that the phasic dopamine response to the previously rewarded cue may impair the shift. Modulation by presynaptic mechanism, such as acetylcholine, may help to facilitate this behavioral shift. As suggested earlier, dopamine signaling may be important for a flexible shift in choice patterns, and thus a shift in dopamine to the new reward predictive cue may assist in the selection of the new choice pattern.

Finally, the present experiment examined several behavioral paradigms where phasic dopamine release was evoked in the Core and/or DMS, but no changes were observed in the Shell and DLS. However, past studies have shown that in the Shell and DLS, certain behavioral conditions can evoke dopamine output changes (Bassareo and Di Chiara, 1999; Cheer et al., 2005; Cheer et al., 2007; Aragona et al., 2008; Owesson-White et al., 2008; Roitman et al., 2008; Aragona et al., 2009; Bassareo et al., 2011; Ostlund et al., 2011) and dopamine manipulations in the Shell and DLS can alter behavior (Wyvell and Berridge, 2000; Faure et al., 2005; Vanderschuren et al., 2005). For example, given that dopamine in the DLS is thought to be important for habitual responding (Faure et al., 2005; Vanderschuren et al., 2005), it would be interesting to record phasic dopamine release in the DLS during habit formation. Understanding what types of behavioral paradigms and stimuli evoke dopamine release events in these other areas is critical for building a more comprehensive understanding of dopamine function in the striatum and potentially developing treatments associated with altered dopamine signaling in the striatum.
CITED LITERATURE


Curriculum Vitae

EDUCATION

2001-2005: Bachelor of Science in Psychology (Focus in Biopsychology)  
University of Michigan, Ann Arbor, MI

2006-2011: Ph.D. in Neuroscience (Specialization: Behavioral Neuroscience)  
University of Illinois at Chicago, Chicago, IL  
Advisors: Dr. Michael E. Ragozzino and Dr. Mitchell F. Roitman

HONORS/AWARDS:

- University of Illinois Alumni Association’s Student Leadership Award: 2011
- Chancellor’s Leadership and Service Award: 2010, 2011
- Order of Omega and Delta Epsilon Iota Academic Honor Societies: 2003-2005

FUNDING:

2008-2009: T32 Training Grant from NIMH

RESEARCH EXPERIENCE

January 2007- July 2011: Graduate Research Assistant with Dr. Michael Ragozzino  
and Dr. Mitchell Roitman at the University of Illinois at Chicago, Department of  
Psychology

August 2006 – December 2006: Doctoral Research Rotation with Dr. Sue Carter at the  
University of Illinois at Chicago

May 2005 – June 2006: Research Associate in Department of Psychology with Dr.  
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TEACHING EXPERIENCE:

Spring 2011: Teaching Assistant for Physiological Psychology Laboratory (Psch 363)  
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PROFESSIONAL SOCIETIES

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TECHNICAL SKILLS:
- Experimental analyses of animal behavior
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- In vivo detection of electrically stimulated and behaviorally evoked neurotransmitter release with fast-scan cyclic voltammetry
- Stereotaxic microsurgery
- Intracranial microinfusions
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PUBLICATIONS


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