Corticostriatal Plasticity, Learning and Choice

by

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Dedication

This thesis is dedicated to the author's father and mother Harold and Nancy Stoetzner for all of the love, care and personal sacrifice that it took to make his education and personal development the highest priority.

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Abstract

The striatum, the entry point for a wide range of information into the basal ganglia, is perhaps the most critical information processing region in this brain system. In addition to playing a role in the facilitation and selection of many kinds of movement, the connections from the cortex to the dorsal striatum may also be a critical site for the learning and storage of a wide range of behavioral routines that allow us to perform well-learned complex actions without having to think through every step. Synaptic plasticity, a prevalent neural phenomenon, has been reported in the corticostriatal pathway and may be a neural correlate of the learning that takes place in the striatum. However, corticostriatal plasticity is a complex process and one that is inherently difficult to investigate in an intact corticostriatal network because of the broad connectivity of the basal ganglia system. Here we investigate the basic rules of this plasticity in the corticostriatal system in awake, freely behaving animals, and report that long term depression is the normal form of synaptic change in response to repeated activation of excitatory corticostriatal inputs. However, the outcome of plasticity is reversed when conducted under conditions of anesthesia, indicating that the state of the basal ganglia network can affect normal plasticity rules. Dopamine is also a critical factor in the processing of information in the striatum. Altering striatal dopamine signaling can direct synaptic plasticity in the corticostriatal pathway and block or enhance some forms of learning that are dependent upon the striatum. Dopamine signaling in the striatum may

also affect the performance of actions by altering motivation selectively, or generally, in addition to regulating some aspects of the decision making. We investigated the role of striatal dopamine signaling in learning and choice and found that enhanced dopamine tone in a region of the striatum that is critical for selecting a contralaterally directed choice can broadly alter the threshold for action and invigorate all chosen actions. In contrast, strongly decreasing dopamine signaling can selectively affect choice and reaction time in a coordinated fashion that reflects the storage of task related information. While effects of dopamine antagonism may represent learning or selective changes in motivation, the persistent nature of these effects are dependent upon the magnitude of a decrease in dopamine signaling and may require coordinated changes in dopamine signaling across two internal striatal pathways thought to alternatively facilitate desired movements and suppress undesired movements. These results suggest a role for corticostriatal plasticity in experience-dependent changes in instrumental behavior and prompt additional questions about models that best explain the role of the basal ganglia in learning, performance and motivation.

Chapter 1:

Introduction

The basal ganglia are a brain system once thought to be primarily a reflexive and unconscious motor system (Mink, 1996); this idea has persisted, largely, because of the difficulties with posture, muscle tone, and involuntary movement control that are observed in clinical patients with damage to portions of the basal ganglia (Marsden, 1982). Indeed, many diseases and disorders that are characterized by a loss of voluntary control such as Huntington's, Parkinson's, Tourette's have a clear or proposed relationship to basal ganglia dysfunction.

The term basal ganglia typically refers to five groups of nuclei: the striatum, globus pallidus pars externa (GPe), the globus pallidus pars interna/substantia nigra pars reticulata (GPi/SNr), the subthalamic nucleus (STN) and the substantia nigra pars compacta (SNc). The cortex and thalamus send excitatory input to the striatum, which is the principal input nucleus of the basal ganglia. The striatum, in turn, sends inhibitory projections to the output nuclei of the basal ganglia, the GPi/SNr. The output nuclei project to motor areas of the brainstem, such as the superior colliculus, and to the cortex via the thalamus. Because output nuclei are themselves inhibitory, activity in these regions can inhibit motor areas of the cortex and brainstem. Some projections from the striatum go directly to basal ganglia output nuclei; activity in direct pathway projection

neurons can inhibit firing in the GPI/SNr and allow cortical and brainstem targets to generate action. A separate subset of striatal projections forming the indirect pathway, send inhibitory projections to the GPe (Albin et al., 1989). The GPe sends inhibitory projections to the STN which sends excitatory projections to output nuclei of the basal ganglia such as the GPi. Since STN projections are excitatory, the usual tonic activity in this nucleus will increase the inhibitory hold of output nuclei over their motor targets. Firing of indirect pathway cells of the striatum that inhibit the GPe will transiently release the STN from inhibition and allow the STN to excite the GPi/SNr resulting in inhibition of targets of basal ganglia output (Smith et al., 1998). Recently, Kravitz *et al.* (2010) were able to provide direct functional evidence which confirms that activity in neurons of the direct and indirect pathway can facilitate and inhibit movement, respectively. Their group excited direct or indirect pathway neurons, selectively, using optogenetic techniques and found that direct pathway activation facilitated movement (exploratory locomotion in an open field) while indirect pathway activation decreased movement to the point of freezing.

Activity in the direct and indirect pathway can potentially exert a complex pattern of inhibitory control over motor regions of the cortex and the brainstem and strongly influence action selection. This organizational scheme is applied to a series of partially segregated parallel loops with diverse functions. Each parallel loop is defined by an output region of the prefrontal or motor cortex that receives inhibitory output from the basal ganglia via the thalamus. The sensorimotor loop, for example, sends output projections to the supplementary motor area (Alexander et al., 1986). The sensorimotor loop will serve here as a model for basal ganglia function with the assumption that many

core features of information processing are shared across basal ganglia loops despite differences in input and output connections.

Striatum and Stimulus-Response Learning

In addition to a role in motor control, the basal ganglia are now considered to be a critical site of habitual and procedural learning. This expanded role for the basal ganglia as a learning system was strongly influenced by Mishkin *et al.* (1984) who suggested that the striatum might be a key part of a habit learning system distinct from an episodic memory system. This hypothesis was offered after observing that monkeys with temporal lobe lesions retained good performance on an object discrimination task that involved repeated object-reward pairings. In contrast, the same animals performed poorly on object recognition, which required identification of a familiar object after a short delay, indicating a deficit in episodic memory formation. Because lesions of the striatum and connected regions of the cortex can produce deficits in similar visual discrimination tasks (Divac et al., 1967; Buerger et al., 1974), the corticostriatal pathway was considered a particularly likely substrate of the habit learning system (Mishkin et al., 1984). Using the term habit Mishkin *et al.* (1984) emphasized that the connections between the cortex and the striatum were the substrate of the association in S-R learning; cortical input provided stimulus representations to the basal ganglia which selected the appropriate response.

Subsequent experiments (Packard et al., 1989; Kesner et al., 1993; McDonald and White, 1993) demonstrated that a striatum-dependent learning system competes for control over limited motor resources under circumstances where a reward predicting

stimulus can be identified. In these experiments, animals with either striatal or hippocampal lesions were free to explore an 8-arm radial maze where food rewards were distributed according to one of two patterns. In the *win-shift* version of the task each arm was baited with one piece of food reward. In the *win-stay* version of the task four of eight arms were baited with food and each arm could be visited twice for reward. Maze arms that contained a reward in the *win-stay* version of the task were marked with a visual cue. Performance was measured as efficiency in the collection of possible rewards, by counting the number of maze arms that were entered that did not contain a reward. Control animals rapidly learned the *win-shift* version of the task, but the *win-stay* version of the task was learned more slowly. Lesions of the hippocampus selectively impaired learning the *win-shift* version of the task while lesions of the striatum selectively impaired learning of the *win-stay* version of the task. Furthermore, lesions of the hippocampus improve learning on the *win-stay* version of the task (Packard et al., 1989) indicating that a striatum-dependent learning system must seize control of the body's limited motor resources when there is competition from other brain systems.

S-R learning theory would suggest that a striatum-dependent learning system would gain control over the body in the *win-stay* task because of a gradual strengthening of an S-R associations. According to the proposed function of Mishkin *et al.* (1984) cortical representations of the visual cue would become more strongly connected to output regions of the basal ganglia that represent the reward procuring response of running down a specific maze arm. Since the striatum and the hippocampus both receive a sampling of representations from a wide range of the neocortex, the circumstances under which the striatum gains behavioral control over the hippocampus may

characterize how the striatum processes input sensory representations (Squire, 2004). Since the striatum gradually gains control after repeated task experiences that contain a consistent sensory representation, the striatum might process all of the incoming sensory input from the cortex in a way that highlights common sensory features (such as a cue light) across a series of individual events (such as every time a reward is obtained). This process of pattern detection or classification might be related to a running average that gets updated after every event (Figure 1.1) and could, in principal, be calculated by plasticity rules and stored as the strength of sets of connections between the cortex and the striatum (Houk and Wise, 1995). After sufficient training, every time that input from the cortex matches a stored pattern, a specific response would be triggered.

A strict interpretation of S-R learning requires that responses be elicited by sensory input and relatively insensitive to changes in the outcome of the action. In humans, habits have the additional property of being initiated unconsciously (Graybiel, 1998; Yin and Knowlton, 2006). The idea that corticostriatal connections are the substrate of S-R learning is appealing in its simplicity and has strongly influenced some current hypotheses of learning in the basal ganglia that go beyond strict S-R definitions, but share some similar properties (Graybiel, 1998; Packard and Knowlton, 2002; Yin and Knowlton, 2006; Horvitz, 2009). For example, motor skill learning is impaired in patients recovering from unilateral stroke involving basal ganglia damage (Platz et al., 1994). These patients were asked to trace an imaginary triangle using their affected hand, but could not learn to do so effectively. This sort of motor skill is similar to a habit in the sense that it is acquired slowly and performed automatically. But motor skills aren't necessarily triggered automatically by sensory cues, like a habit, though once started they

do continue automatically. Furthermore, some instrumental actions that appear to be triggered by an environmental stimulus must be driven in part by an internal representation of a goal or outcome (Yin and Knowlton, 2006). The role of the basal ganglia in learning motor skills and goal-directed instrumental actions may be served by corticostriatal connections with homologous architecture (Figure 1.2) but processing different input representations (Horvitz, 2009). Experimental evidence suggests that regions of the dorsal striatum may independently support stimulus-response and outcome-response forms of learning (Yin and Knowlton, 2006) but for the purposes of this dissertation we use the term S-R learning to represent the mechanism of forming and storing learned associations to specific responses.

Dopamine, Learning and Motivation

The striatum is densely populated with dopamine receptors and dopamine signaling is a critical part of the striatal circuit. Exactly how dopamine signaling in the striatum plays a role in behavior is a subject of much active debate. A widely accepted theory is that dopamine plays a critical role in associative learning of instrumental behavior in the form of a "stamping in" of S-R associations (Schultz et al., 1997; Berke and Hyman, 2000; Everitt et al., 2001; Wise, 2004).

Pharmacological manipulation of dopamine signaling in the dorsal striatum can also impair or enhance instrumental learning in several paradigms. Selective dopamine depletion of the dorsolateral striatum will impair learning of a variety of instrumental tasks (Beninger, 1983; Salamone, 1992; Faure et al., 2005). Increased dopamine levels through infusion of amphetamine will also increase the rate of learning in paradigms such

as learning of how to swim to a hidden platform when guided by a cue (Packard et al., 1994), or an lever pressing task which requires a sustained lever press for a fixed duration (Grilly, 1975) as well as instrumental avoidance where an animal must learn to run to a lit chamber to avoid a shock (Doty and Doty, 1966). Dopamine also seems to have a causal role in directing instrumental behavior as drugs and electrical stimulation that result in increased activation of dopamine receptors can both be used to shape an animal's behavior towards the approach and manipulation of various devices used in operant tasks (Wise, 1996).

Theories of the role of dopamine in learning are also supported by recordings of dopamine releasing neurons in the midbrain of the primate (Schultz, 1998). Neurons of the substantia nigra pars compacta (SNc), which release dopamine into the striatum, respond with phasic bursts of firing to rewards and reward predicting stimuli. In behavioral tasks where cues are repeatedly paired with delivery of reward dopamine neurons display a pattern of firing that shifts characteristically as the animal learns to associate the cue with the reward. Initially, dopamine neurons will fire in response to reward delivery. After repeated pairings, the firing patterns of dopamine neurons will respond exclusively to cues which predict a reward (Ljungberg et al., 1992; Schultz et al., 1993). However, if a predicted reward is omitted, a pause or dip in firing occurs at the time of anticipated reward receipt (Schultz et al., 1993).

These firing patterns are strikingly similar to reward prediction error used in computational reinforcement learning systems (Montague et al., 2004). According to computational theories the dopamine signal from the midbrain reports an error concerning the difference between an expected and an actual reward (Schultz et al.,

1997). This error signal is used to teach target circuitry, including the striatum, by directing synaptic plasticity.

The changes in firing patterns that occur in the striatum during instrumental learning support the idea that dopamine acts as a teaching signal. In the case of occulomotor cells of the caudate, the response properties of cells will rapidly modulate their preferred firing pattern based on changes in the expected reward magnitude of a target direction, and this change in firing pattern occurs rapidly along with an increase in the speed of the eye movement and a decrease in the latency to respond (Kawagoe et al., 1998). In neurons of the dorsal striatum of animals learning a simple motor routine, such a running through a maze, striatal cell firing will be altered based upon the sequence of movement. In this task striatal cells initially begin to fire at each stage in a movement sequence. After extended training striatal neurons developing a pattern firing that increases transiently during the beginning and the end of the movement sequence (Jog et al., 1999). This process of retuning firing patterns to reflect chunking of an action sequence has been suggested to be a critical feature of the ability of the striatum to help learn complex habits and motor skills (Graybiel, 1998).

If firing patterns of midbrain dopaminergic neurons represent a teaching signal, this teaching signal may be related specifically to the value of an action (Morris et al., 2006). Firing patterns of neurons of the striatum reflect the value of an action (Samejima et al., 2005) so it is possible that this value information is communicated in part through dopamine input, although other value related information may come from other inputs to the striatum, such as the cortex (Kable and Glimcher, 2009).

Dopamine firing patterns may reflect a teaching signal that directs neurobiological change in target areas but does dopamine cause the rest of the brain to learn specific S-R associations, or does learning elsewhere in the brain drive firing patterns in dopamine neurons (Berridge, 2007)? Some evidence suggests that dopamine is not sufficient or necessary for associative learning, but rather suggests a role in motivation.

Some studies suggest that elevated dopamine levels are not sufficient to promote learning. Mice which express decreased levels the of dopamine transporter (DAT) protein, which normally clears dopamine from the extracellular space, have extracellular dopamine levels that are nearly twice as high as control animals (Zhuang et al., 2001). Higher tonic levels of dopamine do not result in an increased rate in instrumental learning, as both wild type and DAT knockdown mice acquire lever pressing performance with the same amount of training. DAT knockdown mice do appear more motivated in instrumental responding as they are willing to put more effort, in terms of a greater number of lever presses, into attaining each reward (Cagniard et al., 2006). Some evidence also suggests that dopamine does not alter the persistence of learning. Well-learned instrumental behaviors are no more persistent in DAT knockdown mice when compared to a control (Yin et al., 2006).

Likewise, it has been shown that normal dopamine levels are not necessary for new learning. Animals with dopamine depletion from 6-OHDA infusion are able to learn about aversive taste pairings (Berridge and Robinson, 1998). In a study testing goal directed approach behavior, mice bred to lack the gene for tyrosine hydroxylase (and therefore could not make dopamine form precursors) exhibited normal learning (Robinson et al., 2005). In addition, this study indicated that animals deficient in

dopamine production approached rewards more slowly than control animals, suggesting that low dopamine levels decreased motivation.

Some evidence previously mentioned as support for the idea of a learning role for dopamine may be equally interpreted as changes in motivation. For example, when learning is assessed by an increase in the rate of an action, such as the time taken to swim to a target (Packard et al., 1994) or to run to a chamber to avoid shock (Doty and Doty, 1966), one cannot separate motivational effects from a change in the process of associative "stamping in".

An alternative to the learning theory of dopamine posits that dopamine converts neutral stimuli into attractive, wanted stimuli capable of motivating approach responses, and that dopamine is not necessary for associative learning (Robinson and Berridge, 1993; Berridge and Robinson, 1998). The authors introduce a term, incentive-salience, that describes a psychological process that captures this change in the motivational properties of a once neutral stimulus. Incentive salience can be sensitized by repeated administration of dopamine releasing drugs. Behavioral changes related to incentive sensitization included increased psychomotor activation, evidenced by increases frequency and vigor of exploratory movements when dopamine releasing drugs are repeatedly administered in the same environment or in the enhanced instrumental pursuit of a reward, under extinction, when animals are presented with a Pavlovian conditioned cue (Robinson and Berridge, 2003). This type of definition of motivation has similar properties to that of learning as incentive motivational properties of neutral stimuli must be acquired and stored (Robbins and Everitt, 2007). Indeed, recent experiments by Flagel *et al.* (2011) present evidence that dopamine is required for a certain kind of learning,

where reward predicting stimuli take on incentive motivational properties and elicit approach behavior, but that dopamine is not required for goals to acquire the same approach responses.

Other theories of dopamine function attempt explain dopamine's effects in terms of the performance of movements, taking into account general movement facilitation, arousal and energetic constructs. These theories overlap with the expression of motivation, especially in terms of the role of time and effort that goes into each specific action, but in general, do not explicitly take past experience into account and do not address issues of associative learning. Many such accounts of dopamine's effects on performance are derived from reports of the Parkinsonian condition which results in tremor at rest, rigidity, slowness or absence of voluntary and involuntary movement, postural instability and freezing (Dauer and Przedborski, 2003).

Parkinsonian-like symptoms have been observed in animal models with depleted striatal dopamine where akinesia results in a failure to feed potentially resulting in starvation and death (Ungerstedt, 1971). This inactivity is not due to a true motor deficiency, such as paralysis. Rather, a residual response capacity is preserved since certain stimuli such as handling, being placed in cold water, being placed among conspecifics or given a small tail pinch can temporarily reverse symptoms of akinesia (Marshall et al., 1976). Instead dopamine depletion seems to affect a motivational component of movement. While dopamine was previously addressed in terms of the motivational properties of specific cues, dopamine also appears to affect a separate, non-directional component of motivation that reveals itself in the promotion of certain actions or "behavioral activation" (Salamone et al., 2007). For example, the effort placed on

certain instrumental behaviors is sensitive to administration of dopamine antagonists. For example, animals trained to lever press on an easy fixed response schedule (FR1) are not deterred from lever pressing due to dopamine depletion, however, animals responding on more difficult response ratio schedules (FR5, FR16, FR 64) make significantly fewer lever presses when dopamine antagonists are administered (Aberman and Salamone, 1999; Ishiwari et al., 2004). In addition, animals given a choice between working for a preferred food such as a sugar pellet and freely available lab chow will tend to work for a preferred food if the response ratio is low, but administration of dopamine antagonists such as flupenthixol, raclopride and SCH23390 will abolish this effect resulting in animals that will only consume freely available lab chow (Salamone et al., 1997, 2007)

This findings of dopamine effects on behavioral activation have led to the idea that dopamine controls the effort put into instrumental tasks (Salamone et al., 2007). One possibility is that low frequency or tonic components of the dopamine signal communicate effort or cost related information, perhaps through a representation of the average available reward for a given period of time (Niv et al., 2007).

These findings place the two proposed roles of dopamine in the striatum at odds. On the one hand dopamine appears to be a critical teaching signal, binding together representations of stimuli with the most appropriate response, by storing results of past trial and error experience. On the other hand, dopamine is playing a key role in the ability of a stimulus to activate a response, perhaps taking into account current conditions of the task. In one recent example it was shown that motivational effects of dopamine depletion are acquired in the same fashion as an instrumental response might be reinforced. Animals given unilateral 6-OHDA lesions of the dorsal striatum will fail to perform

contralateral movements; however, this failure to respond is gradually acquired over the course of several training sessions, and can be considered a learning effect (Dowd and Dunnett, 2007). Given the overlap between the motivational or behavioral activation effects of dopamine and the role in strengthening instrumental learning, an outstanding question is precisely what role dopamine is playing in the dorsal striatum, do performance effects exclusively result from learning?

Striatum and Decision Making

The field of decision making has taken a recent interest in the potential role of the basal ganglia in the decision making process (Roitman and Shadlen, 2002; Kable and Glimcher, 2009; Bogacz et al., 2010). This involvement comes in two forms: the first is as a role in generating action value representations (Samejima et al., 2005; Kable and Glimcher, 2009). The second involvement is as a neural mechanism that is responsible for the speed accuracy tradeoff, as a variable threshold that can cut off the time allotted to a choice process based on the speed or accuracy demands of the task (Bogacz et al., 2010). Here we will briefly discuss a proposed role for the striatum in the speed-accuracy tradeoff.

The idea of a decision threshold is one that has been predicted by mathematical models that attempt to explain the tradeoffs between the amount of time spent in the decision process and the accuracy of the choice (Smith and Ratcliff, 2004). Variability in the time that animals take to make a decision is related systematically to the accuracy of the choice (Wickelgren, 1977). Choices that are made more rapidly are less accurate, which suggests that the choice process is based upon a steady stream of information that

builds over time as evidence for one choice or the other. Tradeoffs from the conditions of the decision, however, require that an organism find a balance between the cost of time spent deliberating options and the benefit for accuracy gained from increased evidence supporting a choice.

Some evidence suggests that the striatum plays a role the process of trading off speed and accuracy in a decision. First, cortical areas which send input to the striatum demonstrate neural activity that indicates a process of evidence accumulation over time, similar to what is predicted by a diffusion model. In a reaching task that requires discrimination of movement direction across a field of moving pixels, recordings from the lateral interparietal cortex indicate a proportional buildup of neuronal firing in a population of directionally selective cells. This firing increases over time as the cells receive more evidence. Then firing drops and action occurs. The relative firing rate of directionally selective cells predicts an upcoming action with great accuracy (Roitman and Shadlen, 2002). But the process that indicates to that there is sufficient discrimination to proceed is unknown. Experiments studying decision making under time pressure indicate that, in humans, the striatum and the pre-SMA, the output of the sensorimotor loop, are activated specifically when increased time pressure is applied to a decision making process (Forstmann et al., 2008). Lo and Wang (2006) have suggested a model based on the occulomotor circuit of the basal ganglia that implements this sort of variable threshold decision process using the strength of corticostriatal synapses, modified by dopamine, as an implementation of the threshold required for action. It is possible that a similar plasticity based threshold process is implemented in the sensorimotor loop of the basal ganglia, in order to emphasize speed in the decision process.

Corticostriatal Circuit and Synaptic Plasticity

Though the ultimate role in behavior is still a matter of considerable debate, a detailed understanding of the rules of plasticity in the network of corticostriatal synapses is a critical for developing detailed models of how the basal ganglia processes information. The striatum is not homogenous. Subregions of the dorsal striatum have been divided by the character of their input-output relationships with other regions of the cortex (Alexander et al., 1986), the pattern of reciprocal projections with the midbrain dopamine system (Haber, 2003) and have been shown to have distinct roles in learning and behavioral control (Yin and Knowlton, 2006), however, it is likely that there are many core similarities in information processing in the canonical striatal circuit. Here, for simplicity, we will focus on features of a canonical dorsal striatal circuit.

Organization of inputs

The canonical striatal circuit takes wide ranging input from the cortex and integrates this information in a single layer of output neurons that project to the rest of the basal ganglia. Nearly the entire neocortex sends projections to the striatum that are excitatory and release glutamate (McGeer et al., 1977). A majority of this input occurs on medium spiny neurons (MSNs) which make up 95% of all striatal cells. In addition to MSNs there are at least four other classes of interneurons including tonically active giant aspiny cholinergic neurons (TANs), as well as three varieties of GABAergic interneurons: fast spiking interneurons (FSIs) that express parvalbumin, and two other varieties that express somatostatin-NOS and calretinin, respectively. Two primary

neuronal classes in the layers of the cortex form inputs onto the corticostriatal system which may serve different functions (Wilson, 1987; Cowan and Wilson, 1994). The pyramidal tract fibers descend ipsilaterally and pass through the striatum on the way to the spinal cord, presumably carrying a copy of information about current ongoing motor commands (Redgrave et al., 2008). The intertelencephalic pathway sends bilateral projections throughout the forebrain and may transmit sensory and contextual input necessary for the preparation of an action. Each MSN receives input from a wide range of cortical cells; one estimate suggests that projections from over 5000 cortical cells may converge upon a single MSN (Kincaid et al., 1998). Though widely distributed across the cortex, convergent inputs from functionally related both sensory and motor areas of the cortex project onto overlapping zones within the striatum (Parthasarathy et al., 1992). A given region of the striatum also receives common input from functionally related bodily regions for example, cortical sensory input from the proximal and distal portions of a limb are more likely to converge than a forelimb and hindlimb representation (Hoover et al., 2003). Despite the convergence of functionally related inputs to a region of the striatum, the precise set of cortical inputs onto neighboring MSNs is likely to be substantially different as neighbors share few synapses from the same cortical axon (Kincaid et al., 1998). Likewise, pairs of simultaneously recorded adjacent MSNs have independent firing patterns (Stern et al., 1998).

Though the precise timing of firing patterns from adjacent MSNs are quite different, striatal cells share similarities in subthreshold membrane potential fluctuations between up and down states (Stern et al., 1998) which may synchronize some aspects of their function. Strong inwardly rectifying K^+ currents mediated by G_{IRK} channels activate

when the membrane potential of the MSN is low and may keep MSNs hyperpolarized (Wilson and Kawaguchi, 1996; Mahon et al., 2004). Synchronous input can bring a MSN into a stable depolarized "up" state facilitating action potential firing. Together with several forms of plasticity expressed by MSNs, these features of overlapping functionally related input, and state dependent excitability may help to select and stamp in specific sensorimotor representations and perform pattern classification functions necessary for the context dependent and learning related firing patterns observed in striatal neurons (Houk and Wise, 1995; Graybiel, 1998).

Dopamine fibers from the midbrain terminate throughout the dendritic arbor of an MSN and form synapses on dendrite shafts and directly on necks of dendritic spines (Arbuthnott and Wickens, 2007). In contrast to glutamatergic inputs to the striatum, dopaminergic inputs are highly divergent. In the rat roughly 7,000 dopaminergic cells of the SNc project to 2,000,000 neurons of the striatum, each striatal cell receives approximately 1,000 synapses from dopamine neurons (Arbuthnott and Wickens 2007). Divergence in dopamine signaling may be enhanced by diffusion from synaptic terminals into the extracellular space. In addition, when more rapid changes in dopamine release occur, as in response to behaviorally salient events (Schultz 2002), a large percentage of dopamine cells fire at a high rate for a short period of time. This pattern of firing likely results in a signal that is temporally varying but spatially constant (Arbuthnott and Wickens 2007). In this way the DA signal is more like a broadcast event with a low information content compared to the highly convergent independent inputs from the cortex onto a single striatal neuron (Berke and Hyman, 2000). Divergent dopaminergic projections may have two main functions: first, low frequency changes in the rate of

dopamine release can set and adjust a baseline level of extracellular dopamine throughout the striatum. A second function of the dopamine signal may be transmitted in patterns of rapid phasic bursts of firing that contain precise temporal information that interacts with complex patterns of excitatory input from the cortex released coincidentally onto adjacent synapses of MSNs.

Effects of dopamine are heterogeneous in the striatum. Two main families of dopamine receptors are expressed in the dorsal striatum, D1 and D2 receptors. The expression pattern of these receptors is segregated, direct pathway striatonigral MSNs express D1 receptors while indirect pathway striatopallidal MSNs express D2 receptors (Le Moine and Bloch, 1995). Lesions of the striatal dopamine system, capable of producing the akinesia associated with Parkinson's disease will differentially regulate D1 and D2 gene expression, with direct pathway cells down regulating D1 receptor mRNA expression and indirect pathway cells upregulate D2 receptor expression (Gerfen et al., 1990). D2 autoreceptors are also expressed on dopamine terminals themselves and are part of a critical feedback loop regulating dopamine release (Khan et al., 1998). A balance exists between inhibitory output pathways of the striatum that make up the direct and indirect pathways (Albin et al., 1989) the differential effects of dopamine receptors on striatal spiny neurons may be a critical in both the maintenance of appropriate balance across the network (Di Filippo et al., 2009), and in order to differentially adjust firing patterns in direct and indirect pathways during the learning process.

Varieties of Synaptic Plasticity

Plasticity of the corticostriatal pathway is a potential physiological correlate of learning in the basal ganglia. Multiple forms of plasticity have been demonstrated in the corticostriatal pathway. Both long term potentiation (LTP) and long term depression (LTD) have been demonstrated (Reynolds and Wickens, 2002; Berretta et al., 2008; Di Filippo et al., 2009). However, the rules of plasticity are not entirely clear and may vary depending on several factors, including experimental preparation.

The first description of plasticity in the cortiocostriatal pathway indicated that LTD was the primary form of plasticity (Calabresi et al., 1992a). This experiment was conducted using both intracellular and extracellular recordings in a slice preparation and used tetanic stimulation of cortical input fibers at 100 Hz. LTD was shown to be independent of NMDA receptor and $GABA_A$ receptor activation, but required postsynaptic depolarization or action potential firing and activation of metabotropic glutamate receptors (mGluRs) as well as both D1 and D2 receptor activation. Subsequent investigations of the mechanisms of striatal LTD using the same induction protocol indicated the importance of L-type Ca^{++} channels (Calabresi et al., 1994) and mGluR1 receptors (Gubellini et al., 2001) in LTD induction. Using the same preparation, it was demonstrated that disinhibition of NMDA receptors using a low [Mg^{++}] medium is capable of producing LTP (Calabresi et al., 1992b) and that this form of LTP could occur without AMPA receptor activation, though AMPA currents were a critical component of EPSPs generated in conditions of regular [Mg^{++}]. This form of LTP, however, is dependent upon dopamine, as taking slices from a brain treated with 6-OHDA can abolish LTP using the same preparation. Also, while using a 100 Hz tetanic stimulation

protocol, retrograde cannabinoid signaling was shown to be critical for LTD induction and is thought to be controlled in part by D2 receptor signaling (Gerdeman et al., 2002). More recently, it was demonstrated that this version of D2 receptor mediated retrograde cannabinoid signaling dependent LTD was specific to indirect pathway cells (Kreitzer and Malenka, 2007).

Despite this relatively consistent story of striatal LTD in response to tetanic stimulation under standard conditions, the first *in vivo* report of striatal plasticity using 100 Hz tetanic stimulation resulted in LTP, which was also calcium dependent and required post synaptic depolarization (Charpier and Deniau, 1997). Again using an *in vivo* preparation this group was able to show that LTP could be induced without postsynaptic depolarization if stimulation occurred at a frequency that matched the intrinsic oscillatory pattern at 5 Hz observed in the barbiturate anesthetized rat (Charpier et al., 1999). These oscillatory patterns detected from intracellular recording in striatal neurons were found to be synchronized to similar cortical oscillations. Corticostriatal synchronization may drive LTP through a process of coincidence detection when excitatory input arrives under conditions of a barbiturate spindle induced oscillation. In addition, this study indicated two additional critical points: first, 5 Hz stimulation could result in LTP regardless of whether stimulation was of sufficient intensity to drive firing, provided that the striatal cells were entrained to the stimulation and regularly produced EPSPs following stimulation. Next, this study indicated that in cells where LTP was not induced, stimulation usually failed to synchronize striatal EPSPs. Therefore, the timing of input with respect to coincident depolarization is critical in the determination of plasticity.

Do striatal cells normally express both LTP and LTD? Using a slice preparation, Fino *et al.* (2005) have compared two varieties of tetanic stimulation protocol, low (1 Hz) and high (100 Hz) frequency stimulation and found the outcome of a plasticity experiment depends critically on the frequency of input. At a lower frequency, stimulation resulted in LTD while the higher frequency stimulation resulted in LTP. This was the case regardless of whether postsynaptic potentials were held at sub-threshold or super-threshold levels. Next they compared the results of altering the timing between presynaptic activation and postsynaptic depolarization a protocol referred to as spike-timing dependent plasticity (STDP). Rather than reporting a categorical outcome, the results of such an experiment describe changes in synaptic strength as a function of the latency between presynaptic stimulation and post-synaptic action-potential firing. Numerous studies have described this relationship like a reciprocal function: $y = 1/x$, where a change in synapse strength (y) is inversely related to the difference in time between postsynaptic spiking and presynaptic activation (x), and the greatest amplitude changes occurring when absolute value of latency is small and the polarity of charge determined by the sign of the latency. A categorical report of experimental outcome then must, in the least, include positive differences in timing and negative differences in timing (positive and negative timing respectively). Returning to the previously discussed study, Fino *et al.* (2005) described the first STDP function for the corticostriatal pathway which resulted in LTD for positive timing and LTP for negative timing. In general, this result was in agreement with the work of reported by Calabresi *et al.* (1992a) using high frequency tetanic stimulation, if you assume that tetanic stimulation results in a condition at the synapse that is more closely related to a positive timing protocol. In contrast, two

additional studies of STDP functions revealed the inverse, positive timing lead to LTP while negative timing lead to LTD (Pawlak and Kerr, 2008; Shen et al., 2008).

To summarize: striatal cells are capable of both LTP and LTD, although the experimental conditions may play a deterministic role. When 100 Hz stimulation is used, LTP seems to be NMDA dependent, while LTP is dependent on retrograde cannabinoid signaling. Both forms of plasticity appear to require activity of dopamine receptors. However, when using an intact animal, 100 Hz stimulation or 5 Hz stimulation will lead to LTP rather than LTD. Likewise there are other cases of slice preparation in which 100 Hz stimulation does not tend to lead to LTD, such as location along the medial to lateral extent of the striatum and whether the animal is very young (Berretta et al., 2008). Thus, there is a great disagreement about precisely what the rules of plasticity are under normal conditions. This information is critical as a functional description of the basal ganglia will require the assembly of detailed computational models that incorporate known rules of plasticity. Detailed modeling is underway and has yielded fruitful insight (Frank et al., 2004; Frank, 2005), but an accurate model of the basal ganglia cannot be built when basic rules of plasticity are still greatly disputed.

Aside from experimental preparation and input timing, signaling from other neurotransmitters play an important role in the control of plasticity in the striatum. It was already mentioned that dopamine is necessary for some forms of LTD and LTP, however dopamine receptor expression in the striatum is heterogeneous and many of the previously mentioned studies did not discriminate between D1 and D2 expressing MSNs. Shen *et al.* (2008) have thoroughly explored the selective contributions of D1 and D2 receptor signaling to the expression of both LTP and LTD. Previous work had suggested

that plasticity might occur unidirectionally in response to dopamine receptor stimulation, so that D1 receptor expressing direct pathway MSNs could only undergo LTP (Reynolds et al., 2001) and D2 receptor expressing indirect pathway MSNs could only undergo LTD (Gerdeman et al., 2002; Kreitzer and Malenka, 2007). Shen *et al.* (2008) have demonstrated that while dopamine dependent LTP and LTD are segregated across MSN subpopulations, each type is capable of expressing both LTP and LTD.

Plasticity in MSNs can also be influenced by interneurons, either through release of modulatory neurotransmitters, or by altering the timing of integration within MSNs of excitatory input from the cortex. While there are four known striatal interneuron types we will focus here to the role of GABAergic fast spiking interneurons (FSIs) and cholinergic tonically active neurons (TANs). Though MSNs are the most prevalent cell type within the striatum, the relatively more sparsely distributed FSIs and TANs can have a widespread effect through extensive network of synapses onto MSNs. TANs have been shown to play a critical role in learning, rapidly and permanently acquiring a pause response that occurs during the presentation of a conditioned stimulus (Aosaki et al., 1994). TANs send feed forward input to MSNs, from the thalamus, and release acetylcholine that reacts with M1 muscarinic receptors on MSN spines. M1 signaling inhibits LTD at corticostriatal synapses onto both direct and indirect pathway cells by blocking the activity of L-type Ca^{++} channels and the resulting cannabinoid retrograde signaling cascade (Di Filippo et al., 2009). A blockade of M1 receptor activation is critical for the DA dependent expression of LTD in direct pathway cells through the process of D2 receptor driven enhancement of acetylcholine release onto MSN spines, via cholinergic interneurons that express D2 receptors (Wang et al., 2006).

Fast spiking interneurons receive input from cortical afferents and exert a powerful feed-forward inhibition on to MSNs. A single action potential from a FSI is capable of preventing firing in a MSN (Koós and Tepper, 1999), and FSIs have a wide local axonal arbor which contact many MSNs and may control plasticity through the adjustment of spike timing or by generating permissive windows for postsynaptic activation in MSNs (Mallet et al., 2006). Experimental depletion of dopamine may have compound effects on corticostriatal plasticity through feed forward inhibition from FSIs. In rats with 6-OHDA lesions, feed forward inhibition from FSI's becomes more severe in direct pathway neurons by allowing a narrower window for post synaptic firing. In contrast, in indirect pathway cells, FSIs may facilitate spiking by regulating a much larger window of inhibition (Mallet et al., 2006). These circuit level effects may have serious consequences on plasticity in direct and indirect pathway cells in the Parkinsonian state and, as other experiments have demonstrated, the direction of plastic change is tightly regulated by post synaptic timing (Fino et al., 2005; Pawlak and Kerr, 2008; Shen et al., 2008). In addition to adjusting spike timing windows, the presence of a strong GABA signal may force the STDP function to result in LTD with positive timing because of selective engagement of cannabinoid mediated retrograde signaling (Fino et al., 2010).

In summary, plasticity in MSNs is regulated by a range of factors including the timing of excitatory input, modulatory signals from dopamine and acetylcholine and feedforward inhibition from FSIs. Each MSN appears to be capable of bidirectional plasticity, but some basic questions persist about normal forms of plasticity in MSNs.

Plasticity and Disease

In addition to its proposed role in learning and decision making, abnormal plasticity may play a critical role in disease. For example, some aspects of addiction are increasingly being framed as maladaptive associative learning driven through effects of dopamine, particularly the long term susceptibility to relapse when presented with drug related cues and context which may result from the enhanced incentive-motivational properties of drug related cues (Robinson and Berridge, 1993) and the compulsive nature of drug consuming behavior driven by automatic habitual behavior (Berke and Hyman 2000). In addition, some neurological disorders that interfere with the execution or inhibition of normal voluntary movement as well as psychiatric conditions which involve repetitive or involuntary patterns of thought are being considered as having some relation to maladaptive learning or plasticity function mediated by the basal ganglia (Graybiel, 2008; Peterson et al., 2010).

Experiments contained within the upcoming chapters:

The experiments presented in this dissertation are intended to explore mechanisms of learning and performance in the striatum. Synaptic plasticity is a potential mechanism for learning in the striatum. Despite a wealth of knowledge concerning plasticity in the corticostriatal pathway, there is still much dispute concerning the basic rules of plasticity in this system. Some evidence suggests that the outcome of a plasticity experiment depends upon the experimental conditions used test the corticostriatal network. The experiments in the second chapter we hope will resolve some contradictory evidence concerning the normal rules of plasticity in the corticostriatal pathway. We report a comparison of plasticity rules in a completely intact, unanesthetized, unrestrained rat, to

what is observed in a previously described anesthetized preparation (Charpier et al., 1999).

Dopamine signaling is a critical source of input to the striatum which relays feedback information on the outcome of choices that an organism makes and it is likely that mechanisms of plasticity are used to store this feedback information. There is much controversy over precisely what information is being stored in striatal circuits. However, a separate issue persists over whether dopamine's effects in the dorsal striatum primarily drive experience-dependent changes (such as the stamping in of a habit) or have general effects on the performance of an action (such as the akinesia observed in patients with Parkinson's disease). The third and fourth chapters describe the effects on learning and performance in a choice task where dopamine tone in the striatal circuitry is modified pharmacologically. In the third chapter we use a commonly abused drug, amphetamine, that drives an increase in dopamine release and in the fourth chapter we describe the results of selective and nonselective dopamine antagonists on the same behavioral protocol. In the final chapter we discuss additional questions and observations from all three of our main experiments.

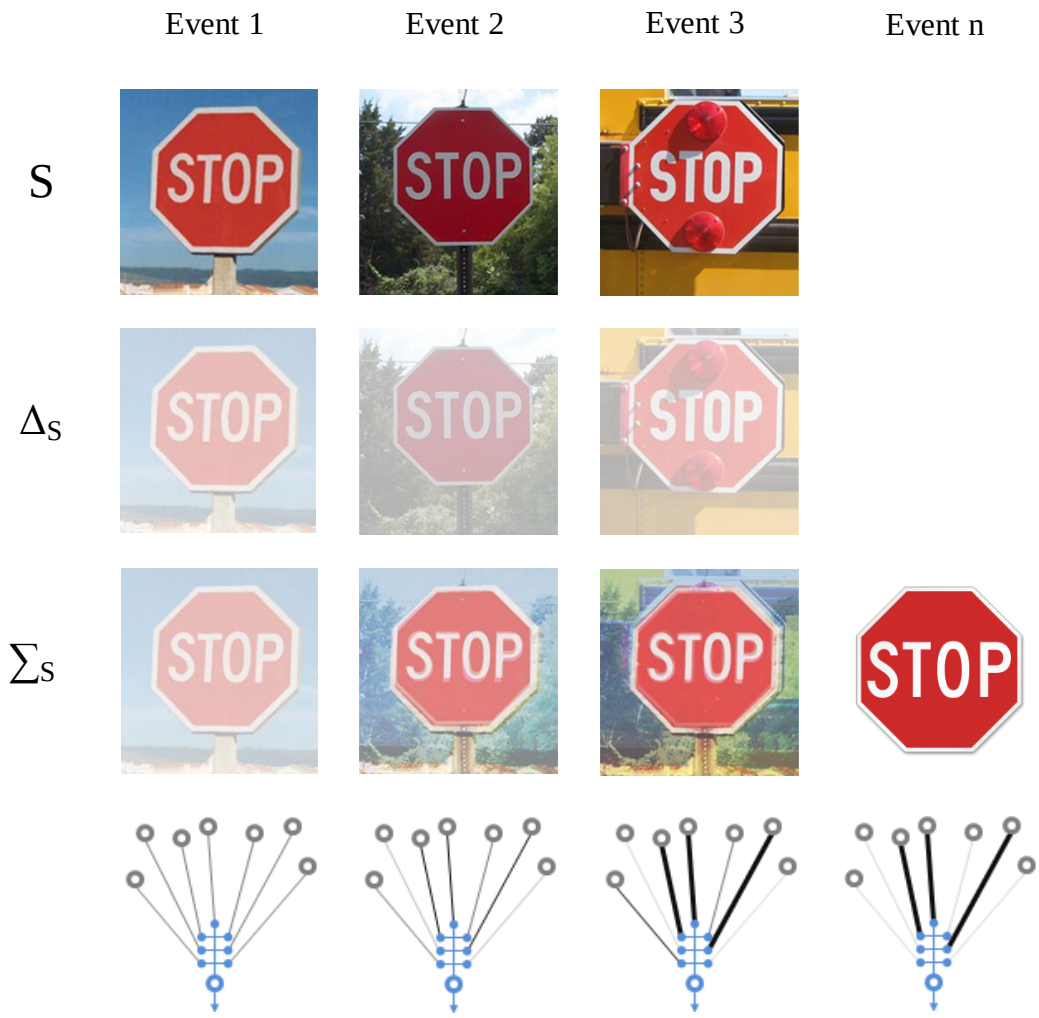

Figure 1.1 An illustration of how a running average of sensory space ∑S, across a series of events, could extract a template that matches features common to the series of events. In this case an image of a stop sign present against a variety of backgrounds serves to represent a sensory cue that might be presented across a series of trials. Each tile represents all input from the cortex to the striatum, indicated below by a simplified circuit representing a single striatal neuron in blue receiving input from cortical neurons in grey. For each relevant event an impression of sensory space ΔS is extracted from sensory space S and is added to the running average ∑S by the adjustment of the strength of

corticostriatal connections. Connections representing the common features of S, the stop sign, result in an increase in connection strength between a subset of cortical inputs and the striatal neuron. After a series of n events, the stop sign is represented by a pattern of strengthened synaptic connections which have been “stamped in” across a set of corticostriatal connections.

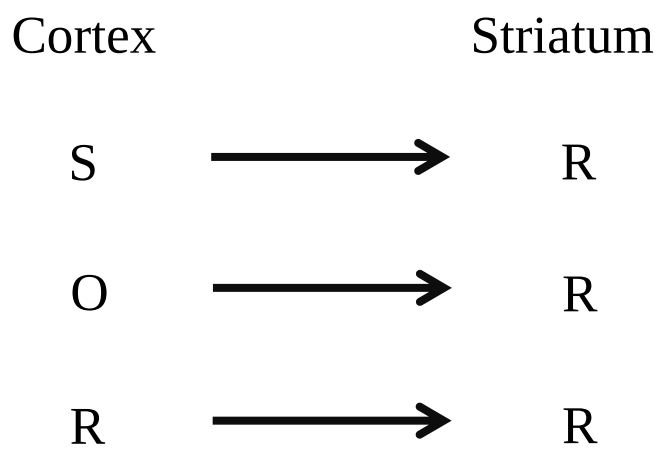

Figure 1.2 Schematic illustrating the concept of a homologous associational architecture potentially formed between the cortex and the striatum for S-R habits, outcome dependent instrumental actions (O-R) and motor skills or action plan (R-R).

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Chapter 2:

State-dependent plasticity of the corticostriatal pathway.

Abstract: Plasticity at corticostriatal synapses is thought to underlie both normal and aberrant forms of reinforcement-driven learning. Studies in brain slices have found bidirectional, spike-timing dependent plasticity in striatum; however it is not known whether similar rules govern corticostriatal plasticity in awake behaving animals. To assess whether behavioral state is a key regulator of plasticity in this pathway, we examined the effects of 5Hz cortical stimulation trains on evoked striatal field potentials, in either anesthetized or awake, unrestrained rats. Consistent with prior studies we observed long-term potentiation in intact, barbiturate-anesthetized animals. However, when an identical stimulation pattern was applied to the same animals while awake, long-term depression was observed instead. Our results demonstrate that the rules governing corticostriatal plasticity depend critically on behavioral state, and suggest that the dynamic context of cortical-basal ganglia loops must be considered while investigating synaptic mechanisms underlying reinforcement learning and neurological disorders.

Introduction

Synaptic plasticity in cortical-basal ganglia circuits is a likely core mechanism by which animals learn context-dependent action sequences leading to rewards (e.g. Houk and Wise, 1995, Schultz 1998). Dysregulation of plasticity may be critically involved in drug addiction (Berke and Hyman, 2000), dyskinesias (Graybiel et al., 2000) and Parkinson's Disease (Shen *et al.*, 2008). Understanding the rules governing striatal plasticity is thus essential for determining how the basal ganglia contribute to both normal and pathological behaviors.

Most information flow into the basal ganglia occurs at glutamatergic synapses onto striatal medium spiny neurons (MSNs), whose spines provide a major locus of synaptic change (e.g. Robinson & Kolb 2004). Corticostriatal plasticity has been extensively investigated in brain slices, and both long-term potentiation (LTP) and long-term depression (LTD) have been observed depending on experimental conditions (for review see DiFillipo et al. 2009). Recent studies that varied the precise timing between synaptic input and post synaptic spiking revealed bidirectional spike-timing-dependent plasticity at synapses onto MSNs (STDP; Fino *et al.*, 2005; Pawlak and Kerr, 2008; Shen *et al.*, 2008), but different groups have obtained very different STDP functions, again possibly due to distinct experimental conditions. Studies in intact animals have also found both LTD and LTP in striatum, and a relationship to dopamine modulation (e.g. Charpier and Deniau, 1997; Charpier *et al.*, 1999; Reynolds and Wickens, 2000; Reynolds *et al.*, 2001; Goto & Grace 2005). However these studies were performed in anesthetized animals, and striatal physiology is very different in awake states (e.g. West 1998, Mahon et al. 2006).

We therefore directly compared corticostriatal plasticity under awake versus anesthetized conditions, using rats with chronically implanted electrodes. To facilitate investigation in freely moving animals, we made use of a cortical stimulation protocol

previously shown to produce LTP in barbiturate-anesthetized animals without requiring current injection into the post-synaptic cell (Charpier *et al.*, 1999).

Methods

Electrophysiology:

All procedures were approved by the University of Michigan's University Committee on Use and Care of Animals. We used 15 adult male Long-Evans rats (350-550g). Thirteen rats were implanted with a chronic microdrive assembly containing 6 independently moveable tetrodes, each consisting of four strands of 12.5µm nichrome wire (Kanthal Palm Coast, FL, USA). Target coordinates in striatum were AP +1.0mm (from bregma), ML 2.0-4.0 mm, DV 3.5-5.5mm (from brain surface). Two rats were implanted with a 4x8 array of microwires (Tucker Davis Technology, Alachua, FL, USA). Array target coordinates were AP +0.5-2.5mm, ML 2.75-4.5 mm, DV 3.5-5.0mm (see Supplemental Figure 2.2). Each animal was also implanted with a bipolar stimulating electrode (either SNEX 200 obtained from David Kopf Instruments, Tujunga, CA or Plastics1 item # 303/3, Roanoke, VA) into contralateral motor cortex (AP +3.2mm, ML +3.3mm DV 1.6mm). Electrophysiologically-guided electrode positioning began immediately following surgery, and continued during post-surgical recovery (minimum 10 days).

Animals were housed on a 12:12 (light:dark) hour cycle and tested during the light phase. Each conditioning stimulation session was conducted with the rat placed on a familiar, elevated octagonal platform (16" diameter, walls 5.5" high). For awake sessions, animals were free to move during the course of the experiment, but tended to rest quietly. For experiments under anesthesia, animals were given an initial dose of sodium pentobarbital (66mg/kg, IP), with supplements (22mg/kg) if an animal twitched in response to hind paw pinch. Awake and anesthetized experiments were conducted on the same day, separated by 1 hour if the animal was tested first under the awake condition or

a minimum of 4 hours if the animal was first tested under anesthesia. Electrodes were not moved between testing sessions.

Electrical stimulation used single, 0.1ms, biphasic square wave pulses (1mA), from an analog stimulus isolator (A-M Systems, Sequim, WA). The experimental sequence consisted of 30 minutes of baseline measurements with stimulation at 0.1Hz (180 pulses), "conditioning stimulation" at 5Hz (1000 pulses), and 60 minutes of post conditioning pulses at 0.1Hz (360 pulses). Single test pulses did not result in any overt movement or vocalization. Occasionally, 5Hz stimulation would result in repetitive neck movements that ceased immediately with stimulation offset. Electrophysiological signals were wide-band filtered (1-9000 Hz) and sampled at 25-31.25kHz**.** Recording, visualization and stimulation were controlled and synchronized using LabVIEW software (National Instruments, Austin TX). After completion of experiments on each rat, current (20μA, 10s) was passed through each recording electrode to create marker lesions. Recording sites were not included in analyses if located outside striatum, or if the noise pattern suggested a non-functional contact.

Data Analysis:

Evoked field potentials were averaged in successive sets of 10 stimulation events. For each such average, the highest amplitude positive peak within 22ms of stimulation was detected ("P1"). Electrodes were excluded if there was no P1 peak (within 22ms). Next, the most negative peak that preceded P1 (excluding 0-2ms to avoid the stimulation artifact) was detected ("N1"). The N1-P1 voltage difference was our estimate of synaptic strength. Just one single wire of each tetrode was used for field potential analyses.

For each electrode estimates of synaptic strength were compared between baseline (30min epoch just prior to conditioning stimulation) and post-conditioning (30min epoch, beginning 30min after conditioning stimulation). The null hypothesis of no change in

synaptic strength was rejected if the means were significantly different (t-test, alpha=0.01). If, in a given animal, no electrodes had a significant change in synaptic strength then we reported the result as no change (nc). Otherwise we reported LTP if the mean normalized post conditioning synaptic strength was above 100% and LTD if below 100%. In no cases did an individual animal show a significant increase in synaptic strength on one tetrode or electrode but a significant decrease on another.

Results

Anesthesia reverses the direction of corticostriatal plasticity.

We assessed the effects of 5Hz cortical stimulation on corticostriatal synaptic strength in fifteen animals, tested while awake and unrestrained, under barbiturate anesthesia, or both (Figures 2.1,2.2; Table 2.1). The shape of the evoked potential was similar in both states, although we occasionally observed lower amplitude and variance in baseline measurements of synaptic strength when under anesthesia (Supplementary Figure 2.1.)

Of eleven animals that received the conditioning stimulation under barbiturate anesthesia, seven showed LTP and four showed no change; in no case did we observe LTD. These observations are consistent with prior findings of corticostriatal LTP in barbiturate-anesthetized rats (Charpier and Deniau, 1997; Charpier et al. 1999), even though experimental conditions were not identical (e.g. they used intracellular recording and ipsilateral stimulation). Results were very different under the awake condition, with the majority of animals (9/15) showing LTD instead; in no case did we observe LTP. The result of the experiment did not depend on the order of treatment, since the three animals which the anesthesia session was performed first displayed the same pattern of results as animals who received stimulation under the awake condition first (Figure 2.2).

Direction of plasticity does not strongly reflect intrastriatal location

Reports in brain slices have found evidence that synaptic plasticity may operate differently in distinct striatal subregions (Partridge et al., 2000; Smith et al., 2001). We therefore examined the contribution of intra-striatal location to our results (Figure 2.3). Although some possible weak trends were apparent, location was not a major factor – our overall finding of LTD in the awake state, LTP under anesthesia held across a wide range of antero-posterior, medio-lateral, and dorso-ventral coordinates.

Evoked corticostriatal field potential originates from within the striatum.

Although evoked potentials are often used to assess synaptic strength they are an indirect measure, and the origin of field potentials measured in striatum is not fully clear (Berke 2005). To gain increased confidence in our measure we examined the spatiotemporal pattern of evoked potentials in two animals implanted with a three-dimensional, fixed-geometry array of 32 individual recording electrodes (Supplementary Figure 2.2). The largest amplitude P1 response was measured from electrodes located deep within the striatum. This is consistent with our evoked potential measure corresponding to striatal physiological events (Ryan et al. 1986), rather than (for example) volume conduction from the overlaying cerebral cortex.

Discussion

This study provides the first description, to our knowledge, of corticostriatal plasticity in intact, unrestrained animals. We found that such plasticity was strikingly dependent on the state of the animal. We confirmed observations of LTP under barbiturate anesthesia (Charpier *et al.*, 1999), but found LTD in the same animals when awake. This finding has broad implications for the study of neural plasticity, and in particular for investigations into relationships between corticostriatal synapses and reinforcement-based learning.

Evoked potentials have been widely used to study synaptic plasticity both in other brain regions and in other areas of striatum (e.g. Goto and Grace 2005). We cannot be certain which intrastriatal processes are contributing to the observed evoked potential in our experiments, although synaptic and postsynaptic currents, close to the recording electrode, are believed to be the dominant factor (Mitzdorf, 1985; Berke, 2005; Katzner *et al.*, 2009). Strong evidence that our evoked potential measure mirrors excitatory post-synaptic potentials (EPSPs) comes from combined intracellular and extracellular recordings in striatum (Ryan *et al.* 1986). They observed cortically-evoked striatal potentials with a very similar or identical time course to ours, and found that the extracellular P1 peak corresponded to intracellular EPSPs. The short latency of this peak makes it very likely to reflect monosynaptically evoked EPSPs, though we cannot entirely rule out the possibility that fast polysynaptic events in cortex are involved.

The reversal of synaptic plasticity with barbiturate anesthesia may reflect either the actions of this drug locally within the striatum, and/or drug-induced changes in the overall patterns of synaptic input to this structure. We consider the former possibility first. Barbiturate is a sedative-hypnotic drug that tends to prolong the opening of $GABA_A$ channels (D'Hulst et al. 2009) and it is worth noting that related manipulations have previously been shown to have a profound effect on striatal plasticity. For example, Yin

et al (2007) observed in brain slices that another sedative-hypnotic, ethanol, can reverse the direction of striatal plasticity in dorsomedial striatum (although this reversal was from LTP to LTD). Alterations in striatal $GABA_A$ transmission may also be involved in the very different STDP functions described for striatum *in vitro*. Two groups (Pawlak and Kerr, 2008; Shen et al., 2008) reported a Hebbian plasticity function in the presence of $GABA_A$ inhibitors, while a third study (Fino et al., 2005) omitted this treatment and reported an anti-Hebbian plasticity function.

Under barbiturate anesthesia cortical activity patterns are quite different to the awake state, and can drive spontaneous ~5Hz membrane potential oscillations in striatal MSNs (Charpier et al. 1999). Successful induction of corticostriatal LTP with artificial 5Hz stimulation correlates with the entrainment of large-scale cortical spindle oscillations (Charpier et al. 1999). This would result in more broadly coherent inputs to striatum, and a higher probability of postsynaptic spiking in synchrony with the directly stimulated inputs. In addition, cortical stimulation results in feed-forward inhibitory input from fast spiking interneurons (FSIs), via $GABA_A$ receptors, onto MSNs. FSIs show marked paired pulse depression at ~100-200ms intervals (e.g. Mallet et al. 2005), so coordinated 5Hz stimulation may result in enhanced postsynaptic MSN activity via disinhibition. Further work is needed to explore the contributions of interneurons to striatal synaptic plasticity, both as coordinators of MSN timing and as a site of plasticity themselves (Fino et al. 2008).

Overall, our results support the general idea that the background patterns of cortical activity are a critical determinant of the direction of synaptic change (Mahon et al. 2004). In contrast to prior *in vivo* results they are more consistent with LTD as a predominant form of plasticity in the awake, behaving striatum. However, our experiments have significant limitations: we also used a highly artificial form of cortical stimulation in our experiments, and cannot directly control the timing of postsynaptic

spiking. Despite this, there are interesting parallels between the striatum and other structures involved in procedural learning that employ an anti-Hebbian plasticity algorithm, including the dorsal cochlear nucleus (Tzounopoulos and Kraus 2009) and cerebellum. Anti-Hebbian rules can support the formation of a "negative image" of one's own actions, that enable their sensory consequences to be distinguished from other events (Bell et al., 2008). This may also be of great importance in striatum, which receives efference copy of corticospinal outputs as part of an overall algorithm in which unexpected outcomes drive reinforcement learning (Redgrave and Gurney, 2006).

		Awake State					Barbiturate Anesthesia				
ID#	*note*	*n*	*Min.*	*Max.*	*Mean*		*n*	*Min.*	*Max.*	*Mean*	
92		4/4	76.8	87.3	84.2	**LTD**					
93		0/3	97.8	103.2	100.1	nc					
100		4/4	77.2	87.3	81.3	**LTD**	4/4	110.2	119.9	114.6	**LTP**
107		0/4	105.2	107.2	106.4	nc	4/4	112.3	121.1	115.4	**LTP**
108		0/1	-	-	95.1	nc	0/1	-	-	108.8	nc
109		3/3	83.7	87.6	85.2	**LTD**	0/3	94.0	98.2	95.6	nc
112	*a*	3/3	72.2	81.9	78.5	**LTD**	0/3	96.7	101.5	99.6	nc
113	*a*	0/1	-	-	103.6	nc	0/1	-	-	95.2	nc
115	*a*	1/3	86.6	93.9	91.0	**LTD**	3/3	123.9	155.0	137.7	**LTP**
122	*a*	4/4	79.2	86.1	82.7	**LTD**	3/4	105.6	119.1	114.2	**LTP**
132	*a,b*	1/2	85.9	95.7	90.8	**LTD**	1/2	99.6	112.7	106.1	**LTP**
133	*a,b*	3/3	84.3	90.4	87.4	**LTD**	2/3	101.6	108.6	106.1	**LTP**
136	*a,b*	0/1	-	-	94.4	nc	1/1	-	-	108.0	**LTP**
138	*a,c*	6/14	74.6	92.0	83.8	**LTD**					
139	*a,c*	0/23	88.0	111.0	95.7	nc					

Table 2.1: Summary of plasticity results for all experiments. ID# indicates each animal, and n indicates the fraction of striatal electrodes where a significant change in synaptic strength was observed. The minimum, maximum and mean synaptic strength, following tetanus, is reported as a percentage of baseline for each animal with two or more tetrodes or electrodes, otherwise a single result is presented under mean change. Notes on experimental protocol: a: These animals had a stimulating electrode with a slightly larger tip separation (0.6 mm). b: These animals were tested in the barbiturate anesthetized state first. c: These animals were implanted with a microwire array.

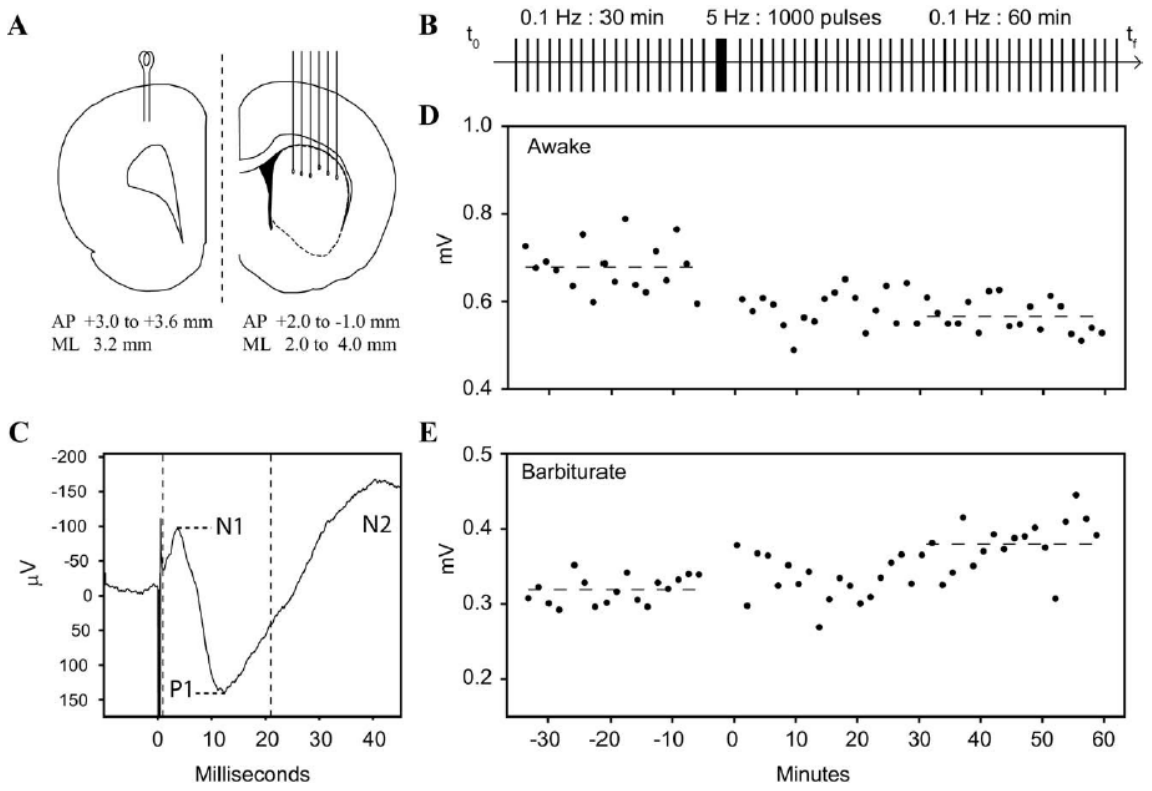


Figure 2.1: Field potential measurement of evoked monosynaptic strength. (A) Electrical stimulation was applied in orofacial motor cortex, and evoked potentials were recorded from multiple electrodes in the contralateral striatum. (B) Illustration of stimulation protocol. (C) Monosynaptic strength was estimated as the voltage difference between the first negative (N1) and positive (P1) peaks. Trace is an average from 10 consecutive stimulation events. Stimulation artifact is at 0 ms. (D, E) Sample measurements of series averaged evoked field potentials from a single electrode recorded under both the awake state (D) and the barbiturate anesthetized state (E). Dashed lines indicate epochs used to calculate synaptic strength at baseline and following conditioning.

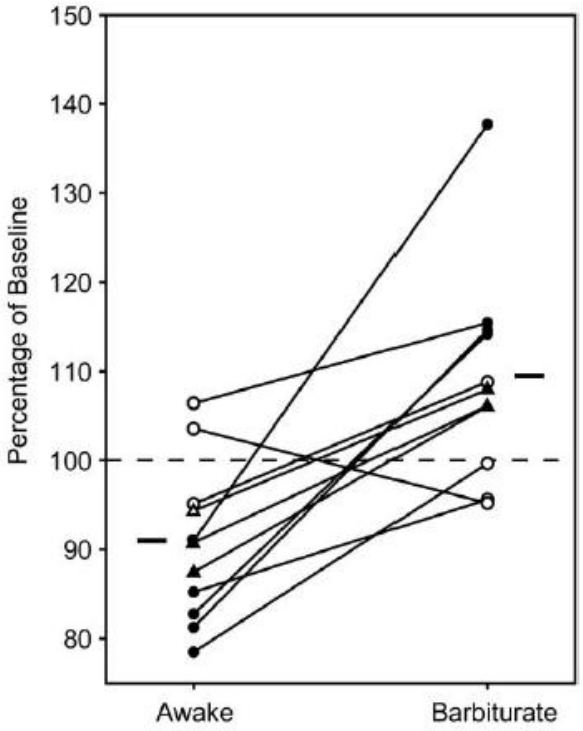


Figure 2.2: Direction of plasticity is dependent upon brain state. Eleven animals received 5 Hz stimulation under two brain states, awake/unrestrained and barbiturate anesthesia. Closed markers indicate significant changes in each state, and horizontal lines indicate state means. A two tailed paired samples t-test reveals a significant effect of brain state on plasticity outcome (P = 0.002). Seven animals were tested in the awake state first (circles) followed by barbiturate anesthesia. In 3 animals the order of testing was reversed (triangles).

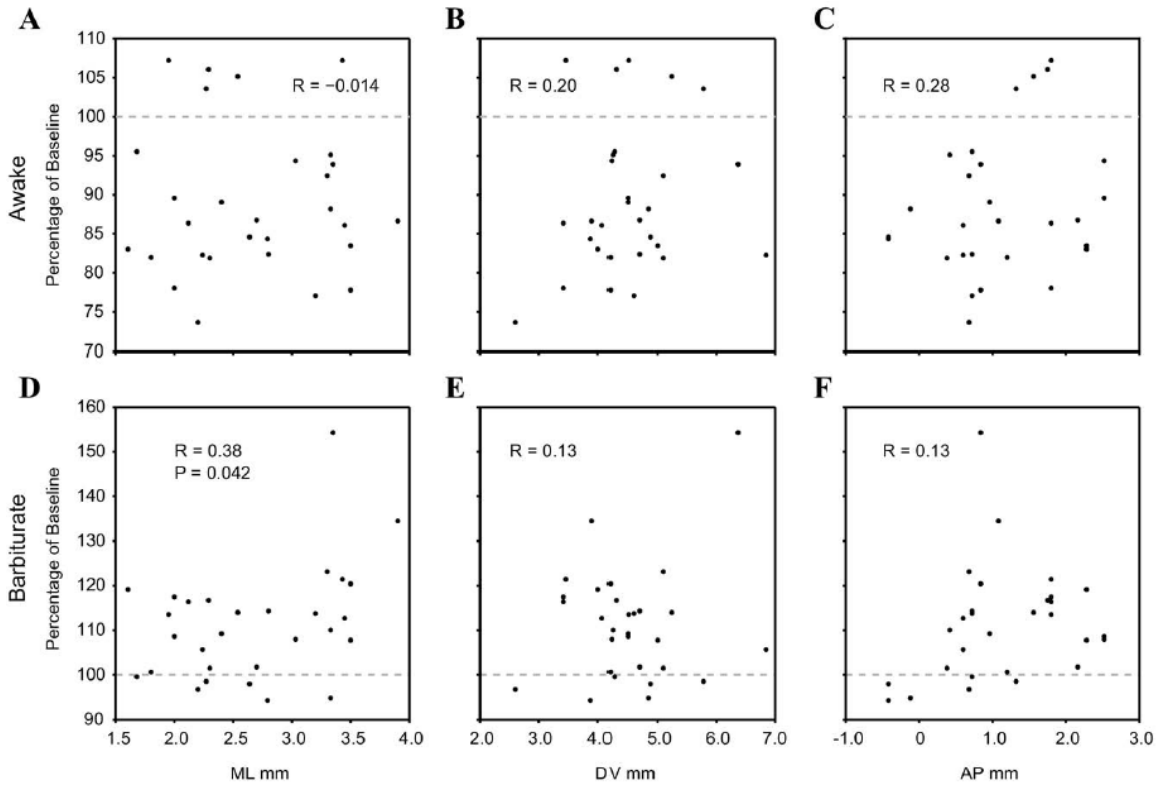
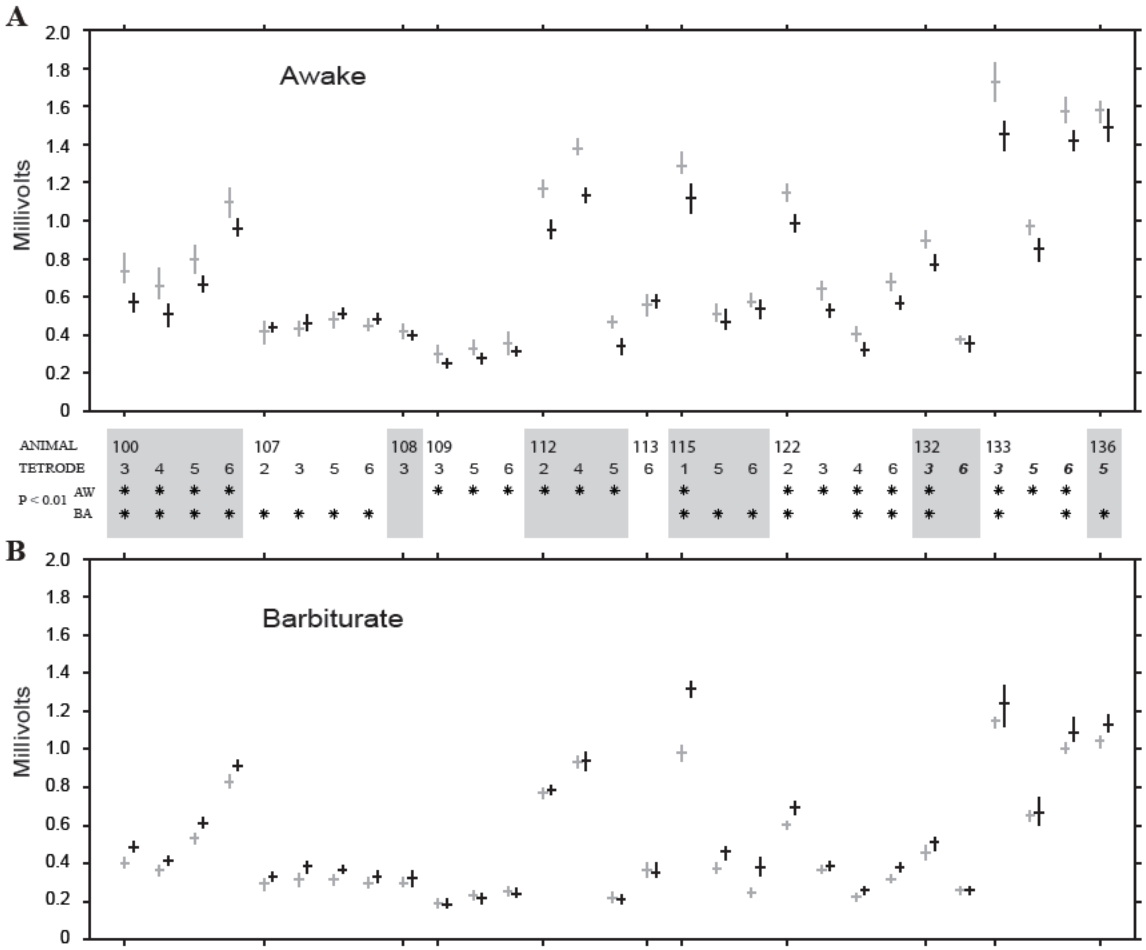
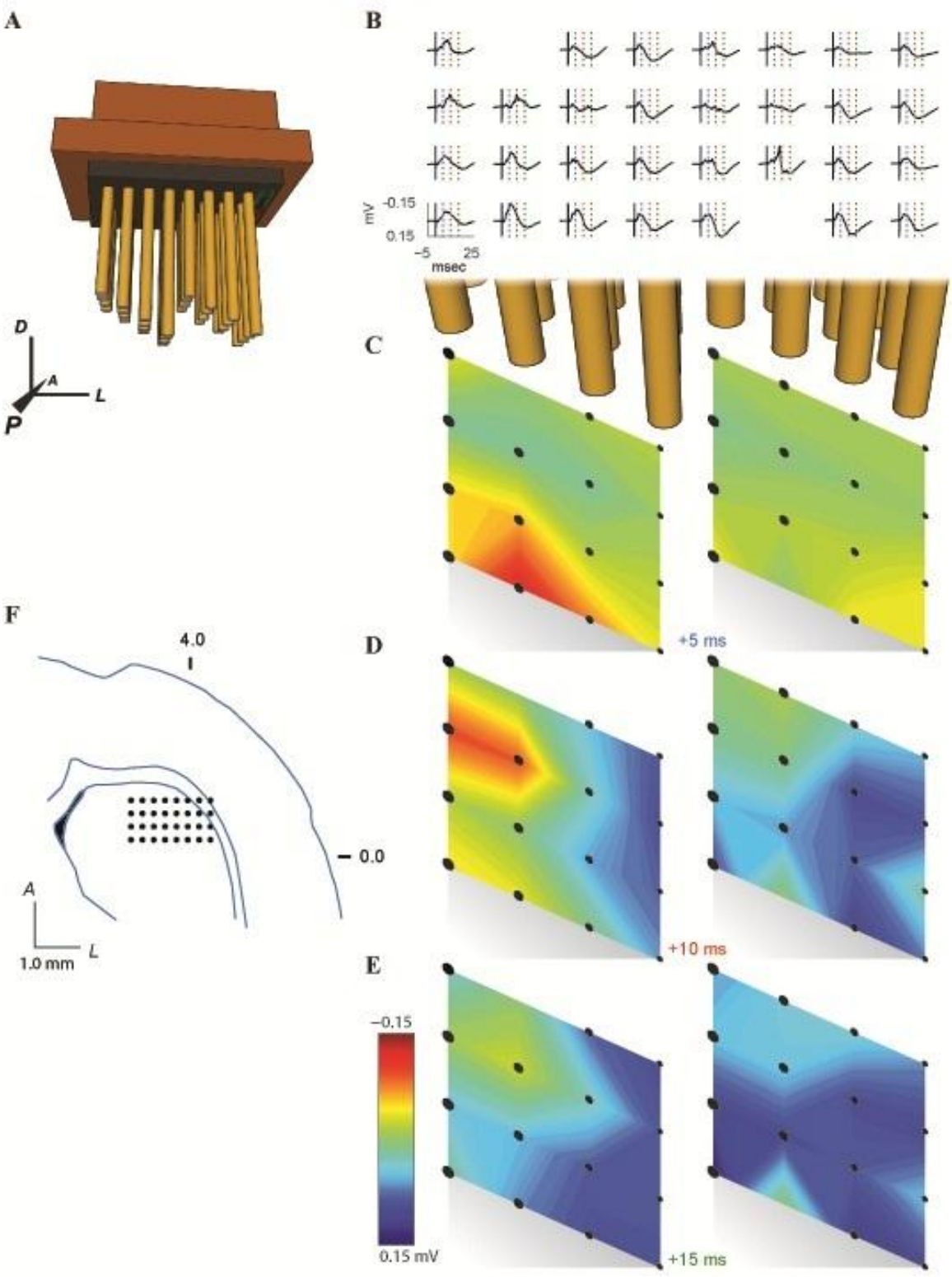


Figure 2.3: No clear relationship between intrastriatal location and plasticity direction. For each striatal tetrode tested under both awake (A-C) and anesthetized (D-F) conditions, post-conditioning synaptic strength is plotted against mediolateral (A,D), dorsoventral (B,E) and anteroposterior (C,F) position. The overall direction of change does not vary with position, although in the anesthetized state (only) there was a weakly significant ($p = 0.042$) linear correlation between percentage change and mediolateral position, if we do not correct for multiple comparisons. This appeared to be largely due to a single outlier point.



Supplementary Figure 2.1: Magnitude and variability of evoked potentials as a function of behavioral state. Crosses show mean and inter-quartile range for baseline (grey) and post-conditioning (black) measurements for all individual tetrodes tested in both awake (A) and anesthetized (B) states. Included tetrodes are grouped together by animal (grey shading). An asterisk indicates a significant difference between baseline and post-conditioning means.


A
B
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E
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D
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L
P
mV
-0.15
0.15
-5
25
msec
+5 ms
+10 ms
+15 ms
4.0
0.0
A
L
1.0 mm
−0.15
0.15 mV

Supplementary Figure 2.2: Evoked corticostriatal field potential originates from within the striatum. (A) 32-site electrode array configuration, showing the two wedge shaped groups with deepest electrodes positioned laterally. (B) Evoked field potential measurements from a representative series average are presented in grid electrode array order (in register with C and D). Vertical black line at 0 ms is the stimulation artifact. Voltage data depicted in B was used to generate contour color maps of voltage at 5, 10 and 15 ms following stimulation (C and D). Interpolated contour maps are shown for each group of 16 coplanar electrodes (black circles, smallest are deepest). Time points were selected to illustrate that the P1 peak appears earliest and at the highest amplitude in deep, striatally-located electrodes.

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Chapter 3:

Disassociation between choice and vigor in a single region of the dorsal striatum.

Abstract: The striatum plays a critical role in the learning, performance and selection of instrumental actions. Dopamine has been suggested to play a critical role in learning relationships between stimuli and beneficial actions and dopamine control over plasticity at corticostriatal synapses is candidate mechanism for this learning process. However, dopamine in the dorsal striatum has often been assigned a role in motor performance, as low levels of dopamine will impair most voluntary movement. Recent studies have shown that motor impairments from unilateral lesions of dopamine projections to the dorsal striatum produce movement deficits in contralaterally directed movements only after task experience, suggesting that motor deficits due to low dopamine levels are in fact acquired through a learning process. Here we explore the relationship between dopamine tone, learning and performance by raising dopamine tone in one striatal hemisphere through amphetamine infusion. We show that amphetamine infusion does not selectively enhance contralateral responding, but does invigorate choice for both contralaterally and ipsilaterally directed actions. Yet, muscimol infusion into the same region produces a clear deficit in contralateral choice indicating that output from this

region is critical for the proper selection of contralaterally directed actions. Together these drug treatments indicate a disassociation between choice and reaction time within a single region of the dorsal striatum and suggest that the dorsal striatum acts as a threshold for the initiation of a choice, and that dopamine tone may act directly on this circuitry to broadly adjust a decision threshold.

Introduction:

Dopamine signaling in the striatum is critical for the acquisition and expression of instrumental behavior (Beninger, 1983; Salamone, 1992). Unilateral dopamine depletion of the nigrostriatal projection can cause impairment in the selection, initiation and execution of a conditioned response directed towards a target located contralateral to the treated hemisphere (Carli et al., 1985; Dowd and Dunnett, 2004). Recently, Dowd et al. (2007) have shown that this deficit in initiation of contralateral movement is not immediately apparent, but develops only after extended experience while performing the task under conditions of dopamine depletion. This experience-dependent decrease in responding argues against a strict "motor" deficit as initial post-lesion performance is normal. Rather, impairment of instrumental responding due to decreased dopamine signaling in the striatum is could be interpreted as a learning effect. Additional experiments with dopamine terminal lesions restricted to the dorsal striatum found a similar experience dependent decrease in responding when a contralateral movement is signaled (Dowd and Dunnett, 2005). It is not known how increased levels of dopamine will affect learning and performance in this task.

Amphetamine can be used to increase dopamine tone as it causes dopamine release from synaptic terminals by driving reverse transportation of dopamine through the dopamine transporter (Jones et al., 1998). While acute amphetamine administration has characteristic motor effects such as increased locomotion and stereotyped movement patterns such as grooming, sniffing or rearing, the enhanced expression of these forms of motor activation after repeated administration is context-dependent and therefore under the control of an associative learning mechanism (Robinson and Berridge, 1993; Berke

and Hyman, 2000). Amphetamine administration can enhance some forms of instrumental learning, for example, direct infusion of amphetamine into the dorsal striatum can enhance learning to swim to platform with the help of a visual cue (Packard et al., 1994), learning to hold a lever down for a fixed duration (Grilly, 1975) and learning to avoid a shock by running to a lit chamber (Doty and Doty, 1966). Likewise, animals will learn to press a lever if amphetamine is administered immediately afterwards (Pickens and Harris, 1968; Wise, 1996).

In this paper we test whether increased dopamine tone via amphetamine infusion into the dorsal striatum can promote the initiation of a specific contralaterally directed conditioned response. We first show that intact output from the infused region of the dorsal striatum is necessary for normal task performance and initiation of contralateral movement. We then explore the experience-dependent effects of amphetamine infusion on response initiation and response speed. Our prediction is that amphetamine infusion will cause an experience-dependent increase in contralateral response initiation coupled with an increase in response speed.

Methods:

Surgery and Animal Care:

All procedures were approved by the University of Michigan's University Committee on Use and Care of Animals. Fifteen adult male Long-Evans rats (350-550g) were implanted with stainless steel guide cannula (obtained from Plastics1 Inc., part # C315G with 11 mm shaft length, Roanoke, VA) into either the left or right hemisphere at coordinates: AP +0.5, ML +/-3.5, DV 3.5 mm, targeting the dorsal striatum. Guide cannula dimensions were 0.46 mm for the outer diameter and 0.24 mm inner diameter. Four to six bone screws were driven into the skull surface and were encased in dental cement, along with a portion of the nylon pedestal of the guide cannula, leaving the top 3-4 mm free from cement. After surgery, each guide cannula was protected with a dummy cannula (obtained from Plastics1 Inc., part # C315DC-SPC) which consists of a length of wire, 0.2 mm in diameter that slides into the guide cannula and protrudes 1.0 mm below the end of the guide (see Figure 3.1, bottom). At the other end is a threaded cap which tightens securely onto the exposed portion of the guide pedestal. Animals were given two weeks to recover from surgery before beginning testing.

Overview of Housing, Behavioral Training and Testing:

Animals were group-housed on a 12/12 hr light/dark cycle, and testing occurred during the light phase, for one hour, at a fixed time for each animal. Animals were fed 15 grams of chow after testing and were tested weekly to ensure that their weight was at least 90% of their free-feeding weight.

Animals were trained and tested in an enclosed operant chamber with 5 horizontally aligned portals on a slightly concave wall. A green LED and an infrared sensor was located inside each portal. On the opposite facing wall was a small well that delivered food reward. Animals were rewarded for correct performance with fruit punch flavored sucrose pellets (45 mg each, obtained from TestDiet, Richmond, IN; Item # 5TUT).

Animals were trained daily to perform a choice reaction time task with a single training session conducted 5 to 6 days of each week. The task required that animals correctly nose-poke into a sequence of two illuminated portals (Figure 3.1). Training advanced through two principal stages where learning was cumulative. The first stage required a single nose poke, and advanced to include a hold period. The second training stage built upon the first requiring a second nose poke following the hold period. The final movement sequence on which animals were trained and then tested consisted of a single nose poke, followed by a hold period, and then a second nose poke. For the second required nose poke, animals were presented with a choice between two lit portals. Only one of two lit portals was rewarded and one of two unique "go" cues played to indicate the end of the hold period also indicated which portal was eligible to earn a reward. Two pure tones at 1000 kHz ("low") and 4000 kHz ("high") were assigned as "go" cues. Tones were played from a single speaker located above the food well. Thus, to an animal working at the array of nose portals, the tone would appear to originate from behind and would not intrinsically contain any allocentric or egocentric information about the location the reward-eligible nose portal.

During each training session, animals would attempt a variable number of trials during a fixed training duration. The total number of trials attempted during a session was partly determined by how quickly the animals worked. Though trials were self-initiated, a variable 15-25 second interval separated individual trials regardless of the outcome of the previous trial. Animals could initiate a trial as soon as the first nose portal was lit. Training sessions were initially 15-30 minutes in duration but increased to 60 minutes prior to surgery.

A brief "pre-training" stage was included to encourage the animals to explore lit nose portals. During the pre-training phase all portals were lit and simply entering any lit portal would earn a reward. Animals remained at this stage until they would perform approximately one trial per minute averaged over the entire session. These sessions were 15-30 minutes in length.

Details of Principal Training and Testing Stages

In the first training phase, a trial was initiated by poking into a single lit portal. The lit portal was selected randomly for each trial. Initially animals were rewarded for simply entering the lit nose portal. Then a hold period was added where animals were required to remain in the portal until a short burst of white noise (250 ms) signaled reward delivery. The hold period was increased to a variable duration selected randomly from the range of 750-1250 ms. Animals that poked into an unlit portal or withdrew prematurely during the hold period were not rewarded, and the error (termed "incorrect attempt" or "aborted attempt", respectively) was signaled by illuminating the house light during the inter-trial interval.

In the second training phase the task was the same as in the first training phase except that a second nose poke was required. Following successful completion of the hold period an instructional tone was played for 250 ms, instead of the white noise burst, and two adjacent portals flanking the initial hold portal were illuminated as the initial hold portal was darkened. Animals choose to poke into the leftward or rightward portal, where only one choice was eligible to earn a reward. Reward eligibility on the left was signaled by a low tone and reward eligibility on the right was signaled by a high tone. If an animal was implanted in the right striatal hemisphere, for example, then a low tone would be a "contralaterally directed trial". After group performance reached 80% correct on directed trials, additional "catch trials" were included (randomly selected on 1/6th of trials) where both tones are played simultaneously and reward eligibility was set at chance for the adjacent portals. After inclusion of catch trials, the task remained unchanged through all post-surgical testing. Following surgery animals received at least two mock infusion sessions.

Additional trial outcomes are defined as follows: trials ended in a "procedure error" if, after the instructional tone was played, the animal poked into a darkened portal or failed to make a second poke within a fixed time period (1000 ms). If a procedure error occurred the trial was terminated, no reward was delivered, and the error was signaled by illuminating the house light during the inter-trial interval. If an animal poked into a lit portal then this was considered a "complete trial", which included both a "correct choice" if the animal chose the direction signaled by the tone, or a "wrong choice" if the animal chose the opposite direction. Animals received one pellet as a reward for a correct choice. No pellets were awarded for a wrong choice, but consequences of a wrong choice differ

from a procedure error, since the house light is not illuminated during the inter-trial interval.

Intrastriatal Infusions:

All animals received drug infusions in a vehicle of artificial cerebrospinal fluid (ACSF; final ion concentrations, in mM: Na^+ 150; K^+ 3.0; Ca^{2+} 1.4; Mg^{2+} 0.8; PO_4^{3-} 1.0; Cl^- 155, obtained from Harvard Apparatus, item # 59-7316). Prior to testing, animals received infusions into the dorsal striatum. Infusions were conducted in high walled 5-gallon plastic buckets located in a separate room. Injection volume and rate was controlled by a syringe pump (obtained from Harvard Apparatus, Pump 22 Multiple Syringe Pump, part # 55-5920) driving a 10 µl Hamilton syringe (obtained from the Hamilton Company, item # 1701N 10 µl SYR). Attached to each syringe was a length of polyethylene tubing (PE20 tubing, obtained from Becton Dickinson and Company, part # 427406) terminating in an injection cannula (obtained from Plastics1 Inc., part # C315DC-SPC). Tubing dimensions were 1.09 mm for the outer diameter and 0.38 mm for the inner diameter. Injection cannula dimensions were 0.2 mm for outer diameter and 0.1 mm for inner diameter. The cannula were fixed to the tubing with super glue. Slack tubing was counterbalanced with nylon string suspended from a boom arm. Infusions began 15 minutes before testing and lasted for 5 minutes at a rate of 0.1 µl/min for a total injection volume of 0.5 µl. Injection cannula were left in place for at least 1 minute to allow drug to diffuse completely. Following infusion, clean dummy cannula were inserted into the guide cannula and animals were transported to the testing chamber.

During mock infusions animals were placed in infusion chamber for the same duration but injection cannula were not inserted.

Data Analysis and Statistics:

Statistical analysis was performed using a linear mixed model available in commercial software. (SPSS obtained from SPSS Inc., Chicago, Illinois.) *Post-hoc* pair-wise comparisons were made using a Bonferroni correction for multiple tests and, where applicable, referenced to the first session of vehicle infusion. Covariance structure was modeled as first-order autoregressive. All experiments were analyzed for fixed effects of "session" and "cue" where applicable. Testing sessions where an animal did not complete at least 5 of both contralaterally and ipsilaterally directed trials were excluded from analysis. Choice was defined separately for contralaterally and ipsilaterally directed trials as the proportion of correct choice for all completed trials where the tone indicating a contralateral or ipsilateral reward is played (Figure 3.1). Catch trial bias was determined by the proportion of contralateral choice for all completed trials, where the catch trial tone is played. Reaction time and movement time is reported for trials where a correct choice was made. Reaction time represents the duration between the onset of the tone and the removal of the snout from the central portal as indicated by infrared sensor. Movement time represents the duration between removal of the snout form the central portal and entry of the snout into an adjacent portal.

Experiment 1:

Thirteen animals received a series of 3 consecutive daily testing sessions with the following schedule of control and muscimol infusions: Day 1: Vehicle; Day 2: Drug & Vehicle; Day 3: Vehicle. Animals received either 0.05 μg (LOW-MUSC, n=5) or 0.5 μg (HIGH-MUSC, n = 8) of the $GABA_A$ agonist muscimol (obtained from Sigma-Aldrich, St. Louis, MO, item # M1523) on day 2. Dosing for the HIGH-MUSC group was selected based upon the ability of the same dose of muscimol to impair outcome sensitivity in an instrumental task when infused into the striatum (Yin et al., 2005). Dosing for the LOW-MUSC group was determined by a tenfold dose reduction with, respect to the HIGH-MUSC group, after it was observed that animals in the HIGH-MUSC group struggled to complete a sufficient number of trials for analysis.

Experiment 2:

Ten of the 13 animals (5 from the LOW-MUSC group, 5 from the HIGH-MUSC group) received a second series of 6 consecutive daily testing sessions with the following schedule of control and amphetamine infusions: Day 1: Vehicle; Days 2-6: Drug & Vehicle. All animals received 5.0 μg total of the indirect dopamine agonist d-amphetamine (obtained from Sigma-Aldrich, St. Louis, MO, item # A5880) on days 2 through 6. Dosing for the AMPH group was selected based upon improved retention of a tone-shock association (Carr and White, 1984), and improved retention on a cued water maze (Packard et al., 1994) a similar dose of amphetamine is infused into the caudate.

Results:

Experiment 1: Animals given muscimol infusion to the dorsal striatum are less capable of performing the task.

We reversibly inactivated the dorsal striatum in the HIGH-MUSC group to test whether our infusion target coordinates could deliver drug to a region of the dorsal striatum that was necessary for overall ability to perform the task. We measured overall performance in two ways, first by examining the number of times that an animal attempted to initiate a trial and next considering how likely it was that an animal would complete an attempted trial (Figure 3.2A,C). Muscimol infusion significantly affected the mean count of trial attempts [main effect of session, $F(2,11.58) = 126.55$, $p < 0.001$]. When given muscimol, animals attempted significantly fewer trials than when given vehicle on the previous session [mean difference 96 ± 8 attempts, $t(10.07) = 13.49$, $p < 0.001$]. In addition, muscimol infusion significantly affected the mean percentage of completed trials [main effect of session, $F(2,14.37) = 16.80$, $p < 0.001$]. When given muscimol, animals completed a significantly lower percentage of trials than when given vehicle on the previous session [mean difference 35 ± 6%, $t(12.28) = -5.41$, $p < 0.001$] indicating a higher likelihood of committing an error. There was no persistent effect of muscimol on the ability to initiate trials or the likelihood of completing trial; when given vehicle on sessions prior and subsequent to muscimol infusion, animals attempted a similar number of trials and completed a similar percentage of attempts.

Analysis of the choice phase of the task is based on results from complete trials. Animals in the HIGH-MUSC group completed only 15 ± 8 trials when infused with drug because of a combination of fewer attempts and completion of a smaller percentage of

attempted trials. This low count of complete trials makes analysis of choice less precise since choice accuracy is a percentage derived from averaging discrete outcomes. To ensure a minimum resolution of 20% for measurements of accuracy and to exclude potential outliers we limited analysis to sessions where animals completed at least 5 trials for each cue. Additionally we conducted a second set of muscimol infusions using one tenth the dose in the LOW-MUSC group to analyze effects of choice. Animals in the LOW-MUSC completed 52 ± 11 trials suggesting that this group may provide a more precise assessment of accuracy than the HIGH-MUSC group.

In contrast to the HIGH-MUSC group, the significant decrease in the number of completed trials in the LOW-MUSC group was primarily the result of a lower number of attempts (Figure 3.2B, D). Animals in the LOW-MUSC group showed a significant decrease of 50 ± 15 trial attempts [main effect of session, $F(2,7.026) = 10.00$, $p = 0.009$; *post-hoc* comparison $t(5.22) = 3.26$, $p = 0.042$] that was restored after a subsequent session of vehicle infusion.

Experiment 1: Muscimol selectively and reversibly decreases contralateral accuracy.

In the LOW-MUSC group a significant interaction between cue and session was observed for accuracy $F(2,17.17) = 8.35$, $p = 0.003$. To investigate choice bias *post-hoc* comparisons were performed on accuracy between cues within each session. No choice bias was observed in either vehicle infusion session, however, when infused with muscimol, the LOW-MUSC group was 38 ± 8% more accurate on ipsilaterally vs. contralaterally cued trials [$t(16.10) = 4.73$, $p < 0.001$]. We next performed pairwise

comparisons of accuracy across sessions for each cue. Contralaterally directed trials were 26 ± 9% less accurate in the drug infusion session compared to the initial day of vehicle infusion [$t(21.44) = 2.83$, $p = 0.021$]. This effect was reversible as accuracy on the following session of vehicle infusion was not significantly different than the initial day of vehicle infusion. Accuracy on ipsilaterally directed trials did not differ between the initial vehicle infusion session and drug infusion or the subsequent session of vehicle infusion.

We examined choice by considering accuracy independently for each audible cue across control and drug infusion sessions. Muscimol infusion significantly affected accuracy in the HIGH-MUSC group [interaction cue x session, $F(2,21.12) = 14.50$, $p < 0.001$]. To examine the drug's effect on overall choice bias, *post hoc* pairwise comparisons of mean accuracy were first performed across side for each infusion session. Muscimol infusion resulted in a difference in accuracy between cues which indicated an overall bias towards the selection of an ipsilaterally directed movement (Figure 3.3A). The HIGH-MUSC group was more accurate on ipsilaterally targeted trials than contralaterally targeted trials [mean difference in accuracy of 32 ± 10%, $t(17.40) = 3.30$, $p = 0.004$]. In the subsequent testing session where the HIGH-MUSC group was given a vehicle infusion a difference in mean accuracy between cues was present but indicated an overall bias towards the selection of a contralaterally directed movement [mean difference in accuracy mean difference in accuracy 31 ± 8%, $t(17.25) = 4.42$, $p < 0.001$]. This result differed from the initial testing session with vehicle infusion where no difference was observed in accuracy between cues.

We next compared mean accuracy against the initial vehicle infusion, for each cue, in the HIGH-MUSC group (Figure 3.3A). Muscimol infusion resulted in a decrease

in accuracy for contralaterally targeted trials [mean difference of 30 ± 8% in accuracy, $t(34.00) = -3.53$ p = 0.002], which did not persist through the following vehicle infusion. Muscimol infusion did not significantly affect accuracy for ipsilateral targets, however, there was a decrease in accuracy on the following vehicle infusion [mean difference of 24 ± 7% in accuracy, $t(34.00) = 3.47$, p = 0.003].

Experiment 2: Amphetamine does not affect choice but immediately decreases reaction time bilaterally.

To determine whether increasing dopamine tone has an effect on choice, we infused amphetamine into the dorsal striatum prior to behavioral testing (AMPH group). Amphetamine infusions were administered for 5 consecutive days, following a single vehicle infusion session, in order to detect any subtle effects on choice that might develop with task experience under a state of enhanced dopamine tone.

We examined choice for the AMPH group by considering accuracy for each cue across vehicle and amphetamine infusion sessions (Figure 3.4A). Amphetamine did not affect accuracy, however, there was a small initial discrepancy between accuracy on contralaterally cued and ipsilaterally cued trials that was unaffected by 5 subsequent sessions of amphetamine infusion [main effect of cue, mean difference 6 ± 2% greater accuracy on contralaterally cued trials, $F(1,54.38) = 14.32$, $p < 0.001$].

Catch trials potentially provide a more sensitive measure of choice bias since both ipsilateral and contralateral choices are rewarded at chance. Amphetamine did not have a significant effect on directional bias for catch trials as the animals selected contralateral movements at chance across vehicle and amphetamine infusion sessions (Figure 3.4B).

We next investigated the effects of drug infusion on mean reaction time on trials when correct choices were made on ipsilaterally vs. contralaterally directed trials (Figure 3.5A). No significant interaction between session and cue was detected, however, significant main effects of cue [$F(1,112.96) = 6.63$, $p = 0.011$] and session [$F(5,87.98) = 3.89$, $p = 0.003$] were detected independently indicating that amphetamine infusion had an effect on reaction time that was independent from a small difference in reaction time across cues. Reaction time for correct contralateral choice was 10 ± 4 ms faster for all sessions [$t(112.96) = 2.58$, $p = 0.011$]. Amphetamine decreased reaction time by 45 ± 11 ms on the first session of drug infusion as compared to vehicle infusion [$t(110.73) = 4.04$, $p = 0.001$] and mean reaction time remained lower for the remaining amphetamine infusions sessions.

We next considered whether the decrease in reaction time occurred after experience with the task under conditions of elevated dopamine tone by examining reaction time on the first 10 correct trials for each cue (Figure 3.5B). Start of session reaction time was tested for significant fixed effects of factors cue and session. A significant effect on start of session reaction time was attributed to factor session [$F(5,79.36) = 3.07$, $p = 0.002$] and cue [$F(1,101.93) = 10.52$, $p = 0.002$] with reaction time on contralaterally cued trials 16 ± 5 ms more rapid than ipsilaterally cued trials. Comparison of start of session reaction time across sessions revealed a significant decrease of 52 ± 14 ms between the first session of drug infusion and vehicle infusion [$t(101.93) = 3.25$, $p = 0.002$] and remained lower for the following amphetamine infusion sessions. No significant effect of amphetamine on start of session reaction time was seen for the interaction between cue and session. Therefore, amphetamine significantly

affected reaction time without extended experience performing the task under conditions of elevated dopamine tone. Finally, we examined whether there were effects of amphetamine on reaction time would correspond to similar shifts in movement time (Figure 3.6): no significant effects of cue or side were observed for movement time.

Discussion:

It is known that a decrease in tonic dopamine levels in one hemisphere of the dorsal striatum will result in a progressive decrease in the selection and initiation of contralaterally directed movements (Carli et al., 1985; Dowd and Dunnett, 2004). It is also known that an increase in dopamine tone in the dorsal striatum, resulting from amphetamine infusion, can enhance some forms of instrumental learning (Doty and Doty, 1966; Grilly, 1975; Packard et al., 1994). However, it is not known whether an increase in dopamine tone in one hemisphere of the dorsal striatum can facilitate the selection of a contralaterally directed instrumental action, either immediately or through extended testing under conditions of increased dopamine tone. We predicted that increasing dopamine tone in one hemisphere of the dorsal striatum would have a learning effect, specifically, that amphetamine infused animals would, through task experience, change their choice behavior so that contralaterally directed movements would be selected with greater frequency. However, our results do not support this hypothesis as animals given amphetamine did not develop a choice bias immediately or through task experience. Yet, we show that output from this region of the dorsal striatum is necessary for the proper initiation of contralaterally directed movements, and that dopamine tone in this same region is involved with performance. We propose that the function of dopamine tone in the dorsal striatal circuit is to convey information about the temporal parameters of the choice process and invigorate action.

Increasing dopamine tone in the dorsal striatum shortens reaction time on correct trials by approximately 50 ms for both contralaterally and ipsilaterally directed movements. This result is consistent with the idea that dopamine controls the rate of

responding (Niv, 2007) or the level of "behavioral activation" seen for a given instrumental task (Salamone et al., 2007). However, since this decrease in reaction time occurred without change to movement time it must be the process of movement preparation or initiation that is affected. This suggests that the effects of increased dopamine tone specifically invigorated the decision phase of the task. Dopamine acting in the dorsal striatum has often been assigned to a critical role in the decision process, influencing choice by directing plasticity on corticostriatal synapses (Reynolds et al., 2001; Bolam et al., 2006; Shen et al., 2008; Kable and Glimcher, 2009) which are thought to adjust strength incrementally, through training, so that they represent some value or property of the competing choices assigned to patterns of input from the cortex, for example the value of an action (Samejima et al., 2005; Kable and Glimcher, 2009) in a given context (Houk and Wise, 1995). However, our results are not necessarily compatible with the proposed mechanism of dopamine influencing choice by directing corticostriatal plasticity, since the speeding of reaction time is evident from the beginning of the very first testing session with drug administration, rather than developing through an experience-dependent process. The animals do not learn to react more quickly; and correspondingly dopamine is not likely to be acting through an experience-dependent mechanism, rather, dopamine appears to be acting directly on the circuitry of the dorsal striatum to influence the vigor of choice. A potential mechanism for this process is suggested by direct effects of dopamine on a variety of ionic currents in striatal projection neurons (Bolam et al., 2006). Direct effects of dopamine on ionic currents could affect the integration of synaptic input and spiking (Surmeier et al., 1992) immediately affecting the flow of information through striatal circuitry, and support an invigoration of the

choice process. This would imply that integration of synaptic input at striatal projection neurons can potentially be manipulated by dopamine in two ways with an experience-independent mechanism acting directly on membrane currents, and experience-dependent mechanism acting on corticostriatal synapse strength.

Since several studies do suggest that altering dopamine tone can regulate choice through an experience-dependent processes, why then are these mechanisms apparently not engaged during our experiments with amphetamine? One simple explanation is that we did not use a large enough dose of amphetamine to observe these effects, however, this is unlikely as changes in dopamine concentration resulting from local infusion of a similar dose of amphetamine results in increases of dopamine concentration well above what is observed physiologically (Hernandez et al., 1987). Carli *et al.* (1989) have claimed that amphetamine infusion does bias choice towards contralateral targets as well as speed reaction time for contralaterally directed trials when using a similar behavioral assay, although this data was not published. While it is difficult to contrast our results with unpublished work, we will address what methodological details are available where this study was mentioned. In a later report from their group, Robbins *et al.*(1990), referring to the same study, stated that "it is possible to produce significant biasing effects of amphetamine upon a head-movement operant following unilateral striatal infusions of doses as low as 10 µg". This is twice the mass of amphetamine that we used, and was infused into a target that was 0.5 mm medial, 1.0 mm ventral and 1.5 mm anterior to our target (Carli et al., 1989). It would be striking if such similar doses had profoundly different effects on behavior, as their group reports significant biasing effects and we report no effects on bias. We later explored the effects of a higher dose of amphetamine

in a subset of 5 animals which had completed both muscimol and amphetamine infusions. After a short break in daily testing sessions, we resumed daily testing for three additional consecutive sessions with a higher dose of amphetamine (20 µg; 20 µg; 50 µg; respectively). We did not see any clear effects on choice in these animals and reaction time remained steady compared to what was observed with lower doses of amphetamine (not shown). Rather than being due to the degree of increase in dopamine tone, it is possible that the discrepancy between these two experiments may be due to the structure of the task, specifically, the sensory modality most critical in choice discrimination. In their task, Carli *et al.* (1989) used a visual cue to indicate the target that was reward eligible by illuminating only the rewarded nose portal during the second phase of each trial. In our task we illuminate both adjacent nose portals during the second phase of each trial, but play a tone to indicate the rewarded direction. This may be a critical difference in testing because contributions of the striatum to learning appear to be partially segregated into regions which operate on a specific sensory modality (Viaud and White, 1989). It is possible that enhanced dopamine tone might be more effective in driving a choice bias in our task if it is applied to a region of the striatum that is more specialized for processing of auditory information. The caudal portions of the dorsal striatum have been shown to be particularly responsive to auditory input (Arnauld et al., 1996) and it would be of interest to repeat this experiment in a more caudally located infusion site to address this possibility.

While perhaps a satisfying explanation for why our amphetamine infusions did not have an effect on learning or choice, there is still conflict in our results over the apparent scope of action that is influenced by pharmacological manipulation of this

region of the dorsal striatum. We've shown that when amphetamine is infused both ipsilaterally and contralaterally directed choices are equally affected when assessed by reaction time. However, when muscimol is infused into the same region, contralaterally directed choice is selectively impaired. Other studies have indicated directional selectivity in response to unilateral manipulations of the dorsal striatum; this includes electrolytic lesion (Döbrössy and Dunnett, 1997) and application of selective neurotoxin targeting dopaminergic terminals (Carli et al., 1985; Dowd and Dunnett, 2004). Taken as a whole these results give the impression that the striatum specifically encodes for the initiation of contralaterally directed actions. With this framework, the enhancement of ipsilaterally directed reaction time due to amphetamine infusion seems out of place. Even if dopamine is only acting to affect the speed of a choice, amphetamine infusion should still facilitate the selection of any movements encoded downstream from this striatal region. One explanation might be that since the organization of axial movements (both head and trunk) involves bilateral divergence of ipsilateral motor efferents from the forebrain at the level of the brainstem (Kandel et al., 2000), output from the individual hemispheres of the striatum that also project to these brainstem areas (Swanson, 2000), might direct movements towards targets at either hemisphere of egocentric space. However, if it were the case that a single hemisphere of the striatum was able to select a nosepoke direction through the release of inhibition from areas encoding movement towards either hemispheres of egocentric space then muscimol infusion should not be able to selectively impair contralateral movement initiation, rather it should impair movements towards both directions equally. Our results from muscimol argue against this explanation. An alternative model might be that a single hemisphere of the striatum can

select for both contralaterally and ipsilaterally directed movements using separate but complimentary processes where ipsilaterally directed movements are facilitated through the inhibition of contralaterally directed movements. While, selectivity for both contralaterally and ipsilaterally directed movements have been observed in projection neurons recorded from a single hemisphere of the striatum in a nearly identical task (Gage et al., 2010), it is not known whether neurons selective for contralaterally or ipsilaterally directed movements in this study belong to separate populations of projection neurons. Indeed, two populations of striatal projection neurons have been suggested to have opposite effects on action with direct pathway neurons releasing motor centers from inhibition and indirect pathway neurons increasing inhibition onto motor centers (Albin et al., 1989). It is plausible that within a single hemisphere of the striatum, the direct pathway may preferentially encodes initiation of contralateral movements while the indirect pathway encodes the suppression of contralateral movements, allowing a single hemisphere of the striatum to contribute indirectly to the selection of ipsilaterally directed actions. According to this model muscimol infusion would not simply block the initiation of contralaterally directed movements it would also enhance the inhibition contralaterally directed movements through the indirect pathway by disinhibition of the globus pallidus pars externa. Likewise, amphetamine infusion may enhance the rate of choice by speeding signals from both the direct and indirect pathways, enhancing the speed of ipsilaterally directed movements by speeding the inhibition of a contralaterally directed movement.

While the contralateral bias observed for the drug infusion session with both high and low doses of muscimol was anticipated, we did not anticipate that infusion of

muscimol would have a persistent effect on bias. Yet, animals infused with a higher dose of muscimol were less accurate on ipsilaterally directed trials in the following vehicle infusion session. The decrease in ipsilateral accuracy of ~30% is comparable to the ~24% decrease in contralateral accuracy observed during muscimol infusion which had greatly diminished after an additional vehicle infusion session. This temporary rebound effect suggests that a reversible adaptation, sensitive to task experience, was responsible for both ipsilateral and contralateral bias in muscimol and vehicle infusion sessions. The most straightforward mechanistic explanation is that plastic change occurred in the region of the dorsolateral striatum that was infused. Since, following Hebb's proposal (1949), synaptic plasticity is typically thought to require post-synaptic firing, one might suggest that the use of muscimol would preclude plastic change since it is thought to prevent firing by holding the membrane potential of affected cells below threshold. However, in two studies where spiking was monitored by an intracellular electrode implanted into the post-synaptic cell (Charpier et al., 1999; Fino et al., 2005), there have been reports of non-Hebbian plasticity in corticostriatal synapses, where post-synaptic spiking is not required for the change in strength of cortical inputs. Therefore, it is plausible that the contralateral bias seen on the following vehicle infusion session was a result of plastic change at corticostriatal synapses. However, we cannot rule out the possibility that a physiological change responsible for a persistent contralateral bias had occurred in a downstream target from the striatum. Outputs from the basal ganglia return to the prefrontal cortex (Alexander et al., 1986) and plasticity in these cortical targets may depend on inputs from the basal ganglia-thalamic projections as some learning related changes in firing have been shown to occur first in the striatum and then next in the

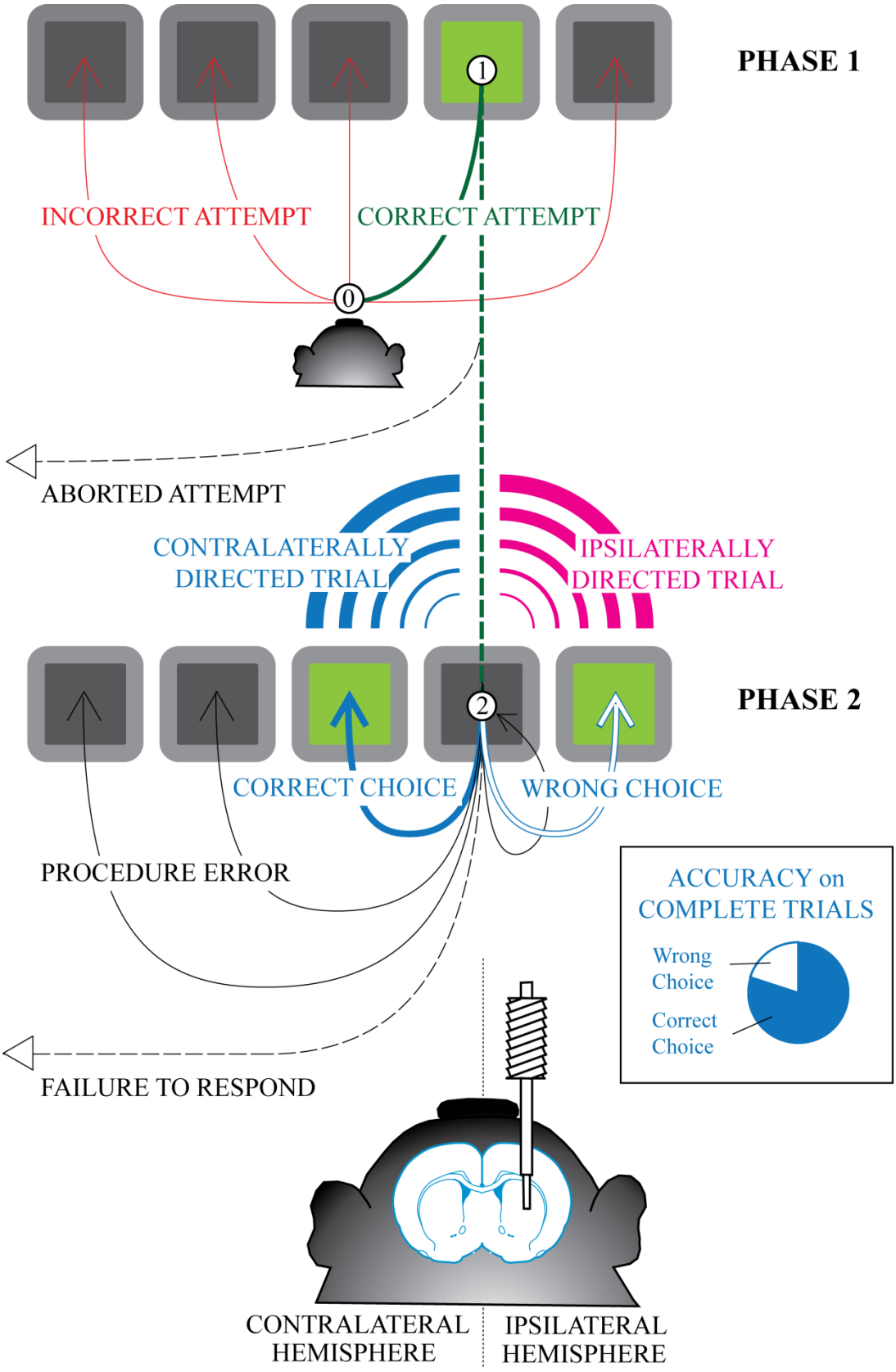
prefrontal cortex (Pasupathy and Miller, 2005). Furthermore, regions of the prefrontal cortex which project to the striatum convey information about the value of an outcome (Yin and Knowlton, 2006) perhaps thalamocortical outputs from the basal ganglia help to update the representation of value in this region. Our experiments were not designed to discriminate between these possibilities, but it is possible that plastic changes in both regions synergize to control choice (Kable and Glimcher, 2009).

Nearly the entire cortex projects to some region of the striatum and the functional characteristics of corticostriatal inputs distinguish the role in learning and decision making among striatal subregions. For example, lesioning a medial region of the dorsal striatum will unmask a pattern of choice that is partially independent of the value of the choice (Yin et al., 2005) which would otherwise be present under the training conditions used. Cortical inputs to some regions of the striatum may already differentiate between potential actions. Studies of firing patterns of cortical cells of the visuomotor system describe competition between individual directionally selective cells that gradually increase their firing rate in a way that predicts choice (Roitman and Shadlen, 2002). And, projections to the basal ganglia are not necessary for information from the visuomotor cortex to reach motor neurons, for example, projections from areas like the frontal eye field go directly to the superior colliculus as well as sending collaterals to the striatum (Hikosaka et al., 2006). The presence of collateral projections implies that striatal cells operate on input that is already partially discriminated for choice. What then do the basal ganglia contribute to choice? The buildup in choice discriminative firing described by Roitman and Shadlen (2002) ends shortly before movement begins. The pattern of firing in these systems is strikingly similar to a theoretical construct utilized in mathematical

models of choice behavior (Smith and Ratcliff, 2004). So-called accumulator models describe decisions that occur in a noisy continuous time environment where choices occur when a sufficient amount of evidence is present (Smith and Ratcliff, 2004). In this circumstance, animals must decide not only what choice to make but when evidence for a choice is sufficient, and this process is modeled by a threshold that accumulated evidence must exceed. Lo and Wang (2006) have suggested that the striatum acts as a similar threshold for the initiation of movement, by inhibiting motor areas in the brain stem that receive differentiated input from the cortex. Again, in this context, plasticity is thought to be the chief operational mechanism. Excitatory corticostriatal synapses may store a threshold value but our experiments indicate that direct effects of dopamine tone are likely to contribute to threshold as well. It may be that both synaptic plasticity and membrane conductances effectively manipulate choice threshold.

One great success of the accumulator model is their ability to explain the inverse relationship between accuracy and reaction time (speed) which numerous behavioral studies have shown to co-vary across a range of difficulty in cue discrimination (Smith and Ratcliff, 2004). Some unilateral manipulations of dopamine tone in the dorsal striatum do cause a predictable shift in both choice and reaction time for a specific contralateral movement, perhaps because the do act directly on a subset of choice-specific corticostriatal synapses. However, our data suggests that under some circumstances changes in the vigor of a choice occur independently of the choice itself. This could be a critical aspect of adaptive behavior as the appropriate time allotted to a decision will vary depending on the stresses of the environment. Likewise, varying conditions of stress during testing has also been shown to alter the relationship between

speed and accuracy of a choice. It is possible that direct effects of dopamine tone on circuitry of the dorsal striatum are a mechanism for shifting the amount of time allotted to a decision based on the immediate demands of the environment. Dissociation between choice and vigor occurring within a single region of the dorsal striatum suggests that the corticostriatal synapse plays a complex role in the decision process integrating information concerning past task experience with current information about the temporal parameters of the decision process.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IPSILATERAL
HEMISPHERE

Figure 3.1: This diagram illustrates the possible actions that an animal might choose in a contralaterally cued trial, while introducing terminology used to describe task performance. Successful completion of the task requires correct choices in PHASE 1 and PHASE 2. Animals begin a trial in position 0 and may initiate the trial any time after the PHASE 1 portal is illuminated. A correct choice to initiate the trial is illustrated in green as the animal moves from position 0 to position 1 and was called a CORRECT ATTEMPT. Selection of an unlit portal in PHASE 1 is called an INCORRECT ATTEMPT. The animal must hold position 1 for 750-1250 ms, depicted here as a dashed green line connecting position 1 with position 2. Withdrawing from the portal prior the completion of the hold period is referred to as an ABORTED ATTEMPT. The end of the hold period is signaled by a tone, here depicted as semicircular blue or magenta lines indicating a contralaterally or ipsilaterally cued tone, respectively (note that tones originate from a central location and do not implicitly convey directional information). Directional terminology in PHASE 2 is assigned with respect to the implant location, depicted in the right striatal hemisphere at the bottom of the diagram. From position 2 the animal has 1000 ms to respond by entering a lit portal. If the animal enters the portal directed by the tone this is considered a CORRECT CHOICE, shown here as a heavy blue arrow. If the animal enters the opposite lit portal this is considered a WRONG CHOICE. ACCURACY for a given directional tone is computed for only COMPLETED TRIALS, where an animal completes a movement into an adjacent lit portal. Therefore, ACCURACY for a contralaterally cued trial, for example, is equal to the percentage of contralateral choice on for all contralaterally cued trials. Likewise, ACCURACY for ipsilaterally cued trials is equal to the percentage of ipsilateral choice for all ipsilaterally

cued trials. Failing to enter a lit portal results in a PROCEDURE ERROR indicated by thin black arrows, and includes entering an unlit portal. Failing to enter any portals whatsoever is a special case of PROCEDURE ERROR and is referred to as a FAILURE TO RESPOND error. Note the distinction between error terms: INCORRECT ATTEMPTS, ABORTED ATTEMPTS and PROCEDURE ERRORS are all considered to as errors in a trial, while a WRONG CHOICE is not referred to as an error.

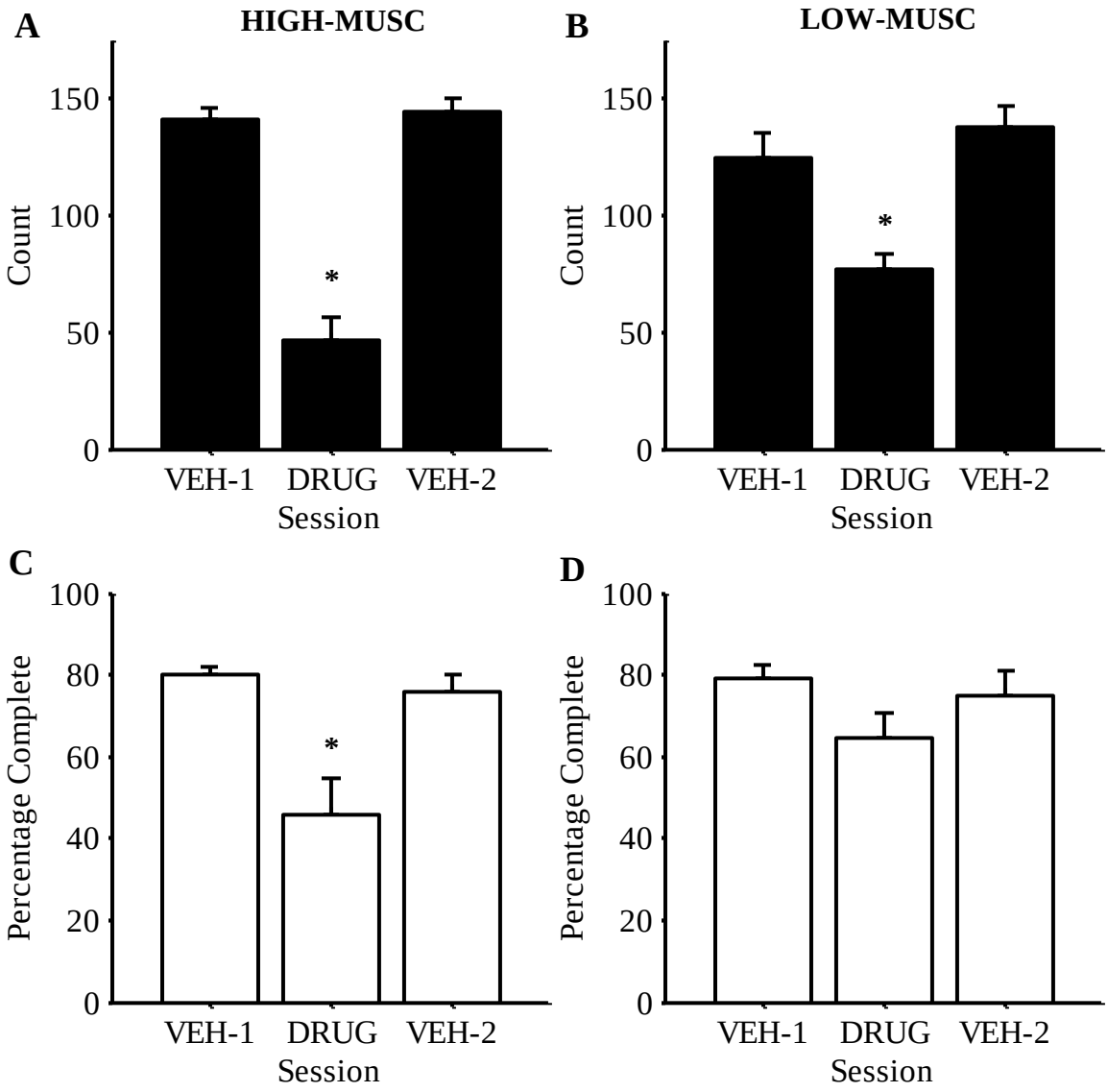

Figure 3.2 Muscimol reversibly degrades task competence in a dose dependent fashion. A high dose of muscimol drastically decreases the total number of completed trials because animals attempt fewer trials (A) and because animals complete a smaller percentage of attempted trials (C) indicating a higher error rate. Animals receiving a lower dose of muscimol complete a lower number of trials as compared to vehicle infusion primarily

because they attempt fewer trials (B) while the percentage of completed trials is not significantly affected by a low dose of muscimol. Error bars are SEM. Table shows mean ± SEM. Asterisks indicate measurements that differ significantly from the initial vehicle infusion session.

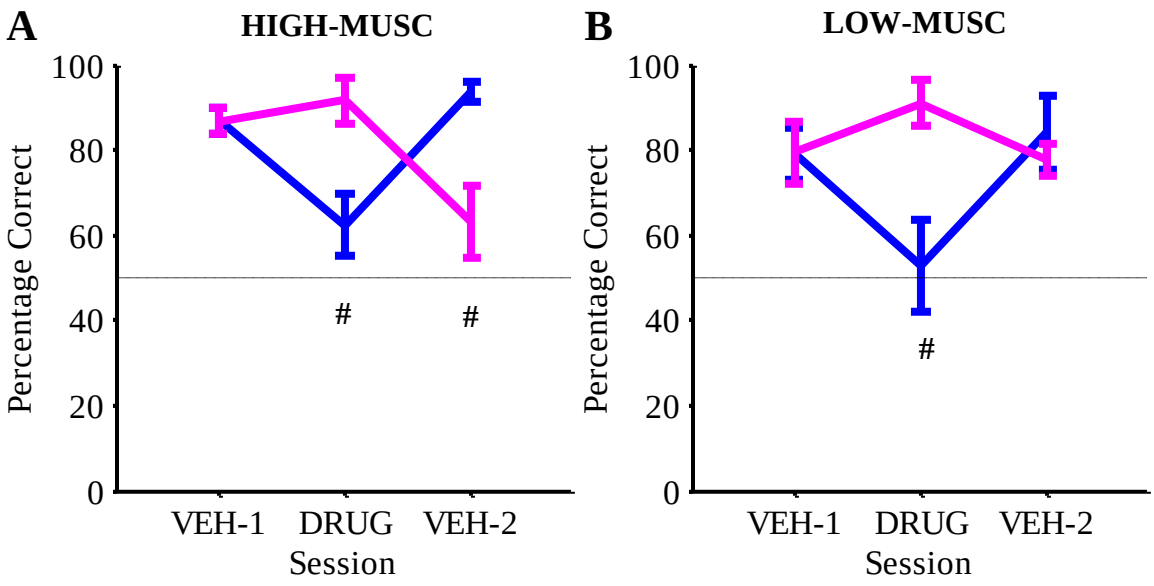

Figure 3.3 Muscimol infusion reversibly lowers accuracy for movements directed towards the contralateral side (blue) but not the ipsilateral side (magenta) for the group receiving a high dose (A, n=8) or low dose (B, n=5) of muscimol. While both doses of muscimol selectively impair accuracy on contralaterally directed trials, accuracy on ipsilaterally directed trials is impaired for session VEH-2 in the HIGH-MUSC group. Plots show the mean and error bars are SEM. Vehicle (VEH-1, VEH-2) and muscimol (DRUG) infusion sessions are conducted across consecutive days. Hash marks indicate both a significant difference in accuracy from VEH-1 and a difference in accuracy between cues for a given session.

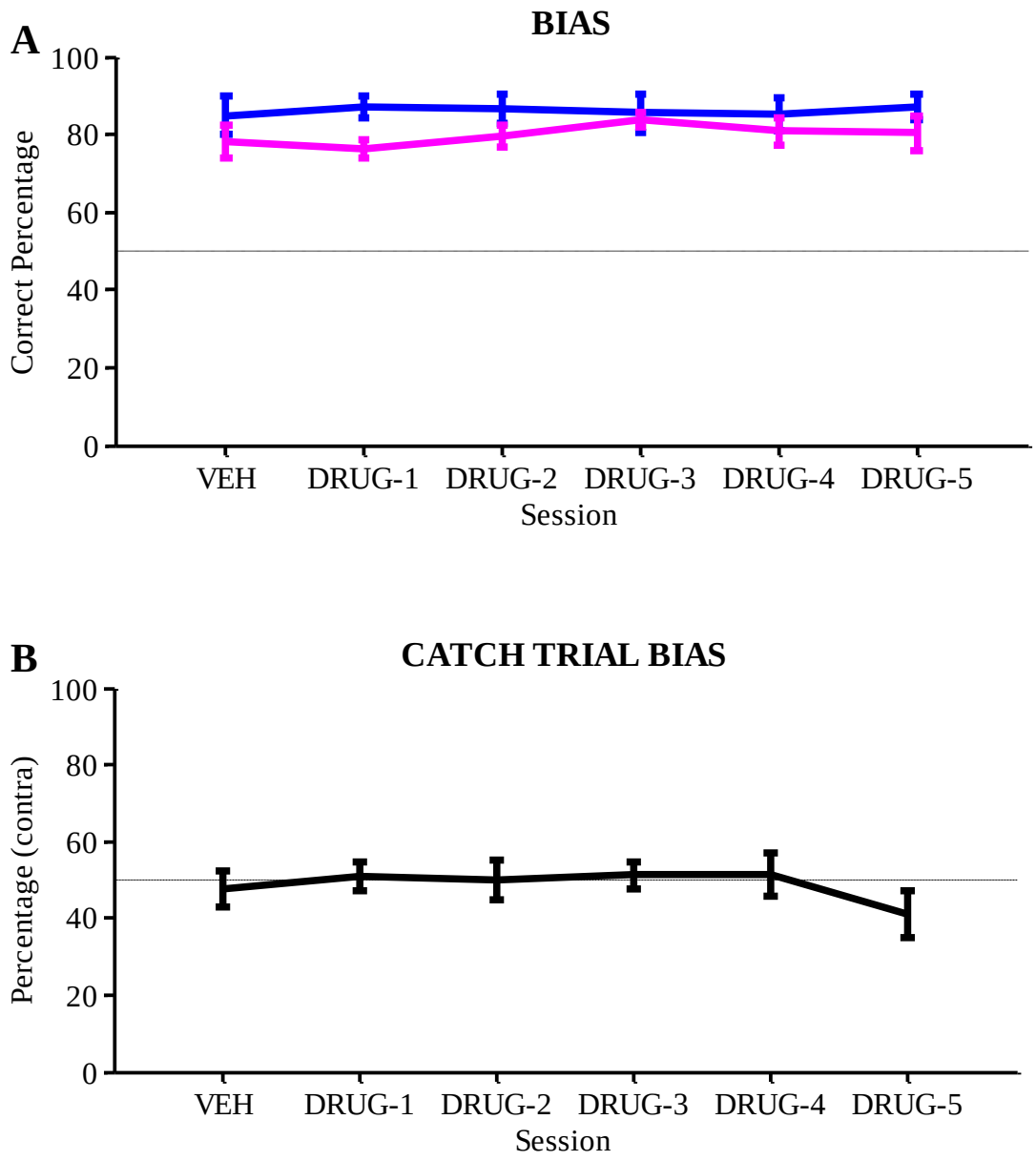

Figure 3.4 Amphetamine infusion does not affect choice on cued trials or catch trials. Contralaterally (blue) and ipsilaterally (magenta) cued trials show no significant difference from vehicle performance through 5 days of drug infusion (A). For catch trials bias is reported as the percentage of times the animal enters the lit portal on the contralateral side when the animal makes a complete trial. Catch trial bias (black) is at chance for vehicle and drug infusion sessions (B). Plots indicate the mean accuracy (n=

10); error bars are SEM. Vehicle (VEH) and amphetamine (DRUG-1-5) infusion sessions are conducted across consecutive days.

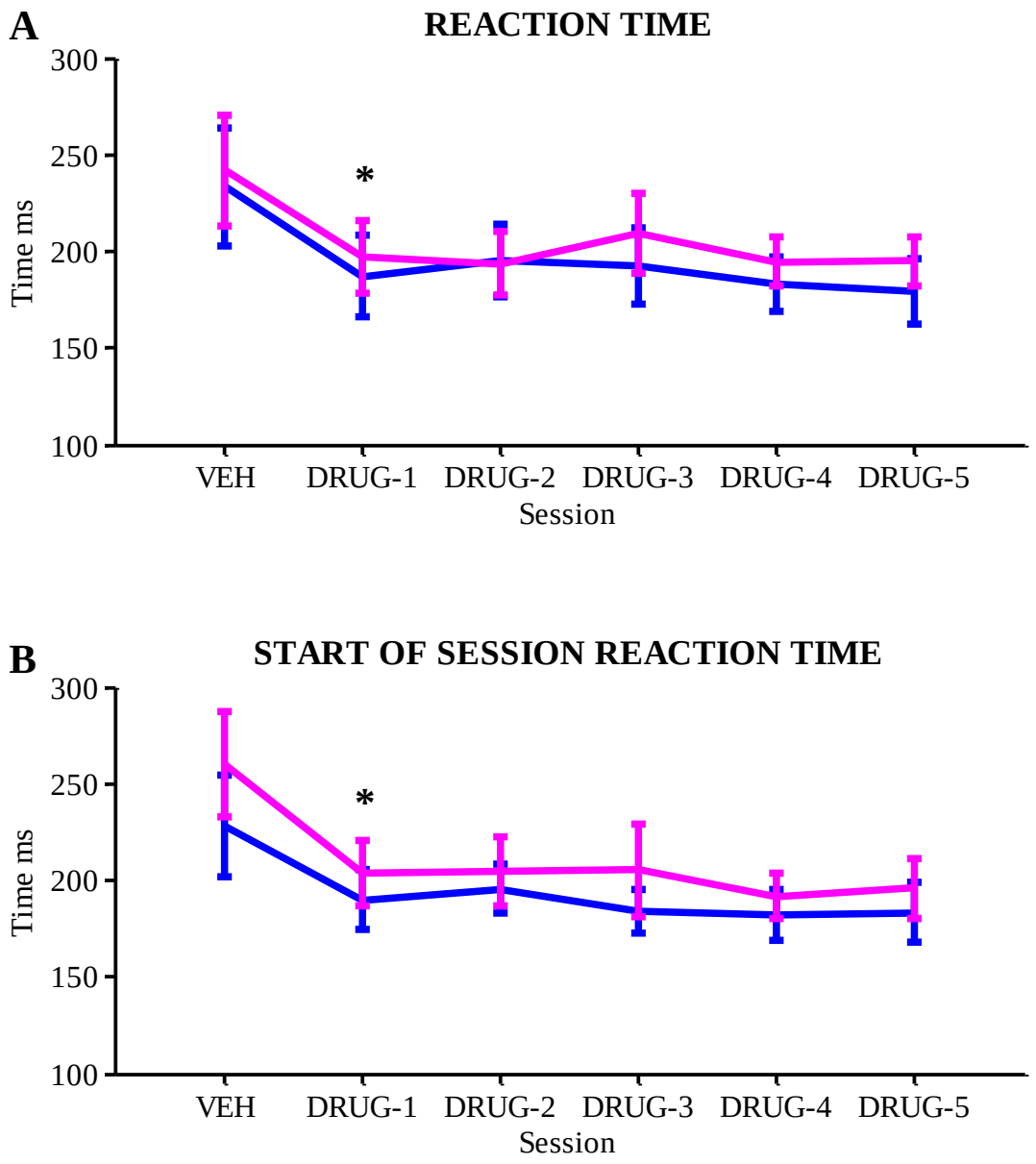

Figure 3.5 Animals make choices more quickly when infused with amphetamine and this increase in choice speed is evident at the start of the testing session. Reaction time decreases are bilateral when amphetamine is infused into a single hemisphere of the striatum. A decrease in reaction time is seen in both contralaterally (blue) and ipsilaterally (magenta) cued trials (A). This decrease in reaction time is evident within

the first 10 correct contralaterally (blue) or ipsilaterally (magenta) cued trials for each session. Plots indicate the mean reaction time (n=10); error bars are SEM. Vehicle (VEH) and amphetamine (DRUG-1-5) infusion sessions are conducted across consecutive days. Asterisks indicate a significant difference in mean reaction time between the first amphetamine infusion session and the vehicle infusion session, for both ipsilaterally and contralaterally cued trials.

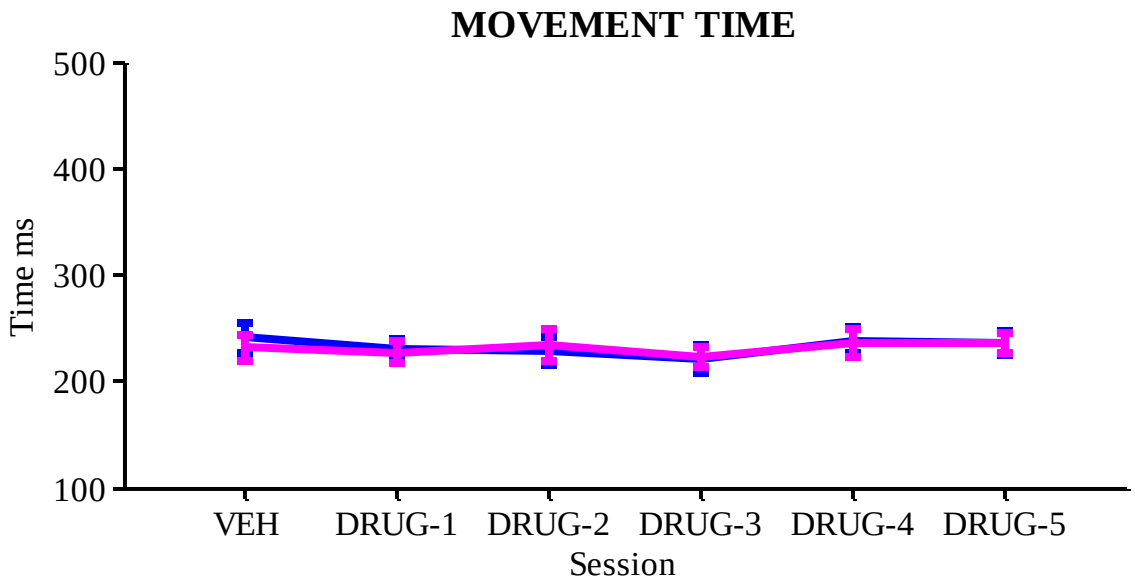

Figure 3.6 Amphetamine does not affect movement time. Plots indicate the mean reaction time for (n=10) for each session for contralaterally (blue) and ipsilaterally (magenta) cued trials, error bars are SEM. Vehicle (VEH) and amphetamine (DRUG-1-5) infusion sessions are conducted across consecutive days.

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Chapter 4:

Coincident unilateral antagonism of dorsal striatal D1 and D2 receptors may be necessary for selective extinction-like effects on contralateral choice.

Abstract: Selective degradation of dopamine terminals from a single hemisphere of the dorsal striatum will result in a deficit in contralateral movement initiation. Recently it was demonstrated that this selective deficit is experience dependent and could be attributed to a learning process. We investigated the effects of dopamine antagonism on the learning and performance of a choice reaction time task using local drug infusion into the dorsal striatum. We were able to reproduce the experience dependent bias against contralateral choice observed in selective lesion studies, however, this effect only occurred under conditions of antagonism of both D1 and D2 family receptors, but not either alone. Learning-like effects were present only at a higher dose of dopamine antagonism suggesting that they were the result of a plasticity mechanism. Selective D1 and D2 receptor antagonism was not without effect however, as each drug significantly increased reaction time bilaterally and decreased the number of times that an animal was willing to attempt the task, perhaps by decreasing a motivational aspect of performance. These results suggest a complex process of integration of direct and indirect pathway

signals within the cortex basal ganglia system in order to select when to execute an action and when to adjust the selection of actions based upon past experience.

Introduction

Intact dopaminergic innervation of the striatum is necessary for proper initiation of contralaterally directed actions (Carli et al., 1985; Brasted et al., 1997, 1998; Dowd and Dunnett, 2004). Lesions of the dopamine terminals in the dorsal striatum using the selective neurotoxin 6-hydroxydopamine (6-OHDA) produce an experience-dependent bias against the choice of contralaterally directed actions (Dowd and Dunnett, 2007), consistent with the idea that dopamine terminals relay a critical feedback signal related to the outcome of a choice (Schultz, 2002). Learning related plasticity in this region is thought to be facilitated by this feedback signal through dopamine dependent plasticity at the corticostriatal synapse. In the previous chapter we were not able to show that increased dopamine tone could affect learning, however, we did illustrate that this region of the striatum was critical for choice. Furthermore, we questioned whether pharmacological manipulation of dopamine in this region of the striatum could have any effects at all on learning, perhaps attributed to a subtle difference in our experimental design. Therefore, we attempted to replicate the results of an experience dependent bias against contralateral choice through pharmacological blockade of dopamine signaling using the nonselective antagonist α-flupenthixol.

After establishing the efficacy of pharmacological blockade of dopamine receptors to produce experience-dependent changes in choice, we conducted a second set of experiments investigating the role of separate subpopulations of striatal neurons in learning. Two subpopulations of striatal cells that form the direct and indirect pathway express different varieties of dopamine receptor (Le Moine and Bloch, 1995) and corticostriatal plasticity in these subpopulations is regulated independently by activity at

D1 and D2 receptors (Shen et al., 2008). Since the direct and indirect pathway are thought to act differently to facilitate and suppress motor plans respectively (Albin et al., 1989; Mink, 1996; Hikosaka et al., 2006), we next investigated whether experience-dependent decreases in contralaterally directed movements can be controlled independently by a decrease in movement facilitation or an increase in movement suppression through the infusion of selective D1 and D2 receptor antagonists.

Methods

Experimental Groups:

Fifty-six animals used in this study were divided into in four experimental groups and received a different drug or dose during testing. Procedures for training, surgical implantation, drug infusion, testing, and data analysis are detailed in the methods of chapter 3 experiment 2, and are replicated here except that five consecutive sessions of drug infusion were both preceded and followed by a single session of vehicle infusion. For each group the complete drug infusion schedule was: day-1, vehicle; days-2 through 6, drug; day-7 vehicle.

Details for individual experimental groups are as follows: in *Experiment 1,* 13 animals in the HIGH-FLU group were infused with a solution of α-flupenthixol (obtained from Sigma-Aldrich, St. Louis, MO, item # F114) in ACSF vehicle at 60 µg/µl, for 30 µg total of drug. In *Experiment 2,* 11 animals in the LOW-FLU group were also infused with solution of α-flupenthixol in vehicle at 12 µg/µl, for 6 µg total of drug. In *Experiment 3,* 16 animals in the RAC group were infused with a solution of raclopride (obtained from Sigma-Aldrich, St. Louis, MO, item # R121) in vehicle at 50 µg/µl, for 25 µg total of drug. In *Experiment 4,* 16 animals in the SCH group were infused with a solution of SCH23390 (obtained from Sigma-Aldrich, St. Louis, MO, item # D054) in vehicle at 4 µg/µl, for 2 µg total of drug.

Doses were selected based upon reported behavioral effects when drug is infused into the striatum. Dosing for the HIGH-FLU group was based upon a similar dose (25 µg) resulting in a gradual decline in operant responding on a VI30 reinforcement schedule (Beninger and Ranaldi, 1993). Dosing for the SCH group was based upon

effective suppression of cocaine self-administration when infused the same dose was infused into the dorsal striatum or the nucleus accumbens (Caine et al., 1995; Bari and Pierce, 2005). Dosing for the RAC group was adjusted to 25 µg after a pilot group receiving 5 µg of drug did not demonstrate any clear behavioral effects. The initial dose used in the pilot group was reported to induce catalepsy when infused into the dorsal striatum (Hauber and Münkle, 1997).

Results

Experiment 1: Repeated infusion of a high concentration of flupenthixol drives an experience-dependent decrease in contralateral choice with a bilateral increase in reaction time which persists into subsequent control testing sessions.

In an attempt to reproduce an experience-dependent bias against contralateral choice we pharmacologically blocked dopamine signaling in the dorsal striatum in the HIGH-FLU group. Flupenthixol infusion did have a significant effect on choice [main effect of session x side, $F(6,110.16) = 3.46$, $p = 0.004$] driving a bias against contralateral choice that did not manifest until after the first drug infusion session (Figure 4.1A). During the initial session of vehicle infusion, animals performed equally well on trials signaled in either direction scoring 85 ± 7% on ipsilaterally directed trials and 83 ± 7% on contralaterally directed trials. Similar results were seen on the first session of drug infusion, however, the following four days of drug infusion showed a progressively larger decrease in accuracy for contralaterally directed trials as compared to ipsilaterally directed trials. On the second day of drug infusion animals performed with 20 ± 10% greater accuracy on ipsilateral trials [$t(102.03) = 1.99$, $p = 0.050$], on the third day 36 ± 11% greater accuracy [$t(102.02) = 3.42$, $p = 0.001$], on the fourth day 39 ± 10% greater accuracy [$t(102.02) = 3.81$, $p < 0.001$] and on the fifth day 52 ± 11% greater accuracy [$t(102.02) = 4.93$, $p < 0.001$]. This response bias persisted when animals were tested with a vehicle injection following drug treatment where a 50 ± 11% greater accuracy on ipsilateral trials was observed [$t(105.19) = 4.57$, $p < 0.001$]. When compared to performance during the initial session of drug infusion animals were less accurate on contralaterally directed trials but not ipsilaterally directed trials. A significant decrease of

31 ± 10% in contralateral accuracy was apparent by the fourth drug infusion session [$t(158) = 2.99$, $p = 0.020$], and a 45 ± 10% decrease in accuracy was seen by the fifth drug infusion session [$t(158) = 4.37$, $p < 0.001$]. A significant 35 ± 11% decrease in contralateral accuracy persisted through the final session of vehicle infusion [$t(158) = 3.31$, $p = 0.007$]. No significant changes in accuracy were seen for ipsilaterally directed trials, though performance did appear to be progressively more accurate for ipsilaterally directed trials across sessions of drug infusion and persisting into the final session of vehicle infusion. Therefore, the ipsilateral bias resulting from flupenthixol infusion takes at least a single session to present itself, and can be largely attributed to a selective decrease in accuracy on contralateral trials, rather than a coincident shift in bias across both contralaterally and ipsilaterally directed trials.

Along with a decrease in contralateral accuracy, flupenthixol preferentially increases the reaction time of contralaterally directed trials [main effect of cue x session $F(6,115.71) = 2.68$, $p = 0.018$]. During the initial vehicle infusion session animals made correct choices at nearly equal speed for both movement directions with a mean reaction time of 258 ±34 ms on ipsilaterally directed trials and 263 ± 34 ms on contralaterally directed trials (Figure 4.1B). Mean reaction time did not significantly differ between ipsilaterally and contralaterally directed trials across the first three sessions of drug infusion, however, in the fourth drug infusion session mean reaction time was 104 ± 29 ms greater on contralaterally directed trials as compared to ipsilaterally directed trials [$t(115.43) = 3.58$, $p = 0.001$]. This is the only drug infusion session which shows a significant difference in reaction time across trial direction. Following drug infusion, however, reaction time for contralaterally directed trials was again observed to be 93 +-

32 ms slower as compared to ipsilaterally directed trials [$t(115.08) = 2.88$, $p = 0.005$]. We next compared mean reaction time for each cue to the initial session of vehicle infusion. In both contralateral and ipsilaterally directed trials, reaction time appears unaffected, with respect to the initial session of vehicle infusion, for the first two sessions of drug infusion. In the third and fifth drug infusion sessions, contralateral reaction times increased by 84 ± 48 and 68 ± 51 ms, respectively. However, a 161 ± 49 ms increase in contralateral reaction time in the fourth drug infusion session was the only significant change [$t(92.73) = 3.32$, $p = 0.008$] in reaction time observed in contralaterally directed trials. Reaction time appeared to remain high in the final vehicle infusion session, but there was not a significant difference as compared to the initial session if vehicle infusion. No significant increases in ipsilateral reaction time were observed with respect to the initial vehicle infusion session, despite a modest apparent increase during the third and fourth drug infusion sessions. Changes in reaction time occur without significantly affecting movement time (Figure 4.1C). Therefore, flupenthixol infusion increases reaction time primarily on contralaterally directed trials, and this increase is persistent. Though flupenthixol may drive a modest increase in ipsilateral reaction time, these effects were not significant and did not persist through the final vehicle infusion session. Flupenthixol infusion primarily affects both the accuracy and reaction time of contralaterally directed trials, with an increase in reaction time that parallels a gradually developing decrease in contralateral accuracy that persists into the subsequent session of vehicle infusion.

Flupenthixol infusion significantly affected the total number of trials attempted [main effect of session, $F(6,55,78) = 2.69$, $p = 0.023$]. But, in contrast to gradually

developing and persistent effects on accuracy and reaction time, the number of attempts is significantly lower in the first drug infusion session (Figure 4.1D) as compared to the initial vehicle infusion session [t(56.80) = 3.30, p = 0.010]. Following this decrease the number of attempts is gradually restored to the original level.

Experiment 2: Flupenthixol effects on choice accuracy and choice reaction time are dose dependent.

Infusion of a HIGH dose of flupenthixol has a significant effect on contralateral choice accuracy and reaction time which we assume is derived from known mechanisms of antagonism at dopamine receptors, if this is the case, then behavioral effects of flupenthixol infusion should be dose dependent. To test this prediction we infused the LOW-FLU group with the same volume of drug at one fifth the concentration. The LOW-FLU group showed more modest effects of drug on accuracy and reaction time that were related to the side-specific effects seen in the HIGH-FLU group.

The lower dose of flupenthixol only had modest effects on choice (Figure 2.2A). Choice accuracy was 10 ± 3% higher on ipsilateral trials across all sessions [fixed effect of cue, $t(43.61) = 3.34$, $p = 0.002$]. The group appeared to increase accuracy towards ipsilaterally directed trials: during the initial session of vehicle infusion, accuracy was similar on contralaterally and ipsilaterally directed trials, with mean accuracy measurements of 90 ± 5% and 84 ± 5%, respectively, for the group. Drug infusion tended to cause a bias in accuracy against contralaterally directed trials which was as high as 18 ± 7% during the first drug infusion session, but this difference was not significant and the

bias appeared to decrease in magnitude across drug infusion sessions through the final session of vehicle infusion.

Effects of a lower dose of flupenthixol on reaction time were similarly modest (Figure 2.2B). Reaction time was 48 ± 9% ms slower on contralaterally directed rather than ipsilaterally directed trials, across all sessions [main effect of cue, $t(82.113) = 5.05$, $p < 0.001$]. Reaction time appeared to increase across trials directed toward both sides, especially during later drug infusion sessions: drug related increases in contralateral reaction time were, at most, 50-51 ms higher during the third fourth and fifth drug infusion session. Ipsilateral reaction time increased more modestly, with the largest increase of 26 ms observed during the final drug infusion session. In the final vehicle infusion session, reaction time on both contralaterally and ipsilaterally directed trials was increased, by 21 ms and 20 ms respectively. These increases were not significantly different across cues or with respect to the initial session of drug infusion. No changes were observed in movement time or attempts (Figure 4.2C,D).

The effects of repeated drug infusion on the LOW-FLU group appeared similar to the HIGH-FLU group. A lower dose of flupenthixol resulted in a significant bias against contralateral choice and longer contralateral reaction time as compared to ipsilateral reaction time. Despite more modest effects on choice and reaction time in the LOW-FLU group, when compared to the HIGH-FLU group, the nature of drug effects across doses appears qualitatively different. A higher dose of flupenthixol results in progressive changes in choice and reaction time while the lower dose results in transient changes choice and reaction time. Since many of the effects of flupenthixol were attenuated or absent at a lower dose our results support the assumption that behavioral effects of

flupenthixol are mediated by known antagonistic effects at dopamine receptors, however the persistent character of effects resulting from a high dose of flupenthixol suggest that the flupenthixol has an elaborated mechanism of action when administered at high doses.

Experiment 3: Antagonism of D1 receptors drives an increase in accuracy on ipsilaterally directed trials, and bilaterally increases in reaction time.

Having reproduced the result of a selective decrease in contralateral accuracy coupled with an increase in contralateral reaction time, through nonselective antagonism of dopamine receptors, we next infused the SCH group with a selective D1 receptor antagonist in order to determine whether a shift in bias or reaction time could be attributed to dopamine's effects on the direct pathway.

Drug infusion into the SCH group caused a bias against contralateral choice [main effect of session x cue, $F(149.26) = 3.045$, $p = 0.008$] which was evident during the first two sessions of drug infusion with a 15 ± 3% and 8 ± 3% greater accuracy in ipsilaterally directed trials [$t(147.57) = 4.42$, $p < 0.001$; $t(146.75) = 2.24$, $p = 0.028$], respectively (Figure 4.3A). Accuracy on ipsilaterally directed trials was also 10 ± 3% greater on the fifth drug infusion session [$t(147.56) = 3.06$, $p = 0.003$], but accuracy on the final session of vehicle infusion was not significantly different across cues. This bias against contralateral accuracy was the result of an increase in accuracy on ipsilaterally directed trials. Accuracy was significantly greater than the initial session of vehicle infusion on the first, fourth, and fifth sessions of drug infusion with increases of 12 ± 4%, 11 ± 4% and 11 ± 4% observed, respectively [$t(199.89) = 3.19$, $p = 0.011$; $t(184.74) = 2.82$, $p =$

0.032; $t(184.74) = 2.84$, $p = 0.029$]. No significant changes were observed for contralaterally directed trials.

When animals were infused with drug, reaction time increased for correct choices directed towards both contralaterally and ipsilaterally located targets [main effect of session, $F(6,153.99) = 2.76$, $p = 0.014$]. Animals appeared to perform more slowly during all drug infusion sessions (Figure 4.3B), however, reaction time was only significantly slower during the third, fourth and fifth session of drug infusion, with increases of 82 ± 25 ms, 80 ± 27 ms and 82 ± 29 ms respectively [$t(156.50) = 3.37$, $p = 0.006$; $t(116.24) = 2.94$, $p = 0.023$; $t(88.69) = 2.82$, $p = 0.036$]. This increase appeared to be somewhat more pronounced for contralaterally directed trials, but did not show a significant interaction. Still, we observed an overall difference in reaction time across ipsilaterally and contralaterally directed trials of all sessions [main effect of cue, $F(1,201.45) = 16.04$, $p < 0.001$] where animals were 21 ± 5 ms slower on contralaterally directed trials [$t(201.45) = 4.01$, $p < 0.001$]. We interpret these results as evidence for a strong bilateral increase in reaction time resulting from drug infusion. However, it is not clear whether there is a selective slowing on contralaterally directed trials that is due to drug.

Increases in reaction time in the SCH group occurred coincidentally with more modest increases in movement time [main effect of session, $F(6,142.36) = 3.32$, $p = 0.004$]. While only the first drug infusion session was significantly slower than vehicle infusion, subsequent sessions appeared to have slightly slower movement times (Figure 4.3C). Drug induced increases in movement time, like reaction time, were not persistent. Following this pattern, a strong decrease in the number of attempted trials was observed in the SCH group [main effect of session, $F(6,67.96) = 5.63$, $p < 0.001$], with most

individual sessions showing a significant decrease in the number of trials attempted compared to the initial session of vehicle infusion (Figure 4.3D).

Experiment 4: Antagonism of D2 receptors drives a decrease in accuracy on contralateral trials and a bilateral increase in reaction time.

Next, we infused animals in the RAC group with a D2 receptor antagonist in order to determine whether blockaide of the dopamine signal at receptors on indirect pathway neurons would contribute to a bias in choice or affect reaction time. Raclopride infusion had a weakly significant effect on choice that generated a bias against contralateral responding [main effect of cue x session, $F(6,111.00) = 2.132$, $p = 0.055$]. During each session of drug infusion, ipsilateral accuracy was significantly greater than contralateral accuracy with differences of 24 ± 8%, 36 ± 8%, 25 ± 8%, 29 ± 8% and 26 ± 8% for the first through fifth sessions [$t(108.39) = 3.025$, $t(105.15) = 4.62$, $t(103.77) = 3.02$, $t(100.26) = 3.84$, $t(103.76) = 3.39$; $p \leq 0.003$ for all comparisons], respectively (Figure 4.4A). There was no significant difference in accuracy for ipsilaterally and contralaterally directed trials during either vehicle infusion session. This bias appeared to result from a selective decrease in accuracy on contralaterally directed trials. When accuracy for each session was compared to the initial session of vehicle infusion, contralateral accuracy was found to be significantly decreased in the second [26 ± 7%, $t(178,06) = 3.87$, $p = 0.001$] and fourth [16 ± 7%, $t(174.74) = 2.35$, $p = 0.051$] session of drug infusion. Accuracy on ipsilaterally directed trials appeared to increase marginally with respect to the initial session of vehicle infusion but these changes were not significant. Therefore, blockade of D2 receptors drives a bias against contralateral choice

which is primarily derived from a selective decrease in accuracy on contralaterally directed trials, but this decrease in accuracy does not appear to be persistent or experience-dependent.

When animals were infused with drug, reaction time increased for trials directed to both contralaterally and ipsilaterally located targets [main effect of session, $F(141.54) = 2.35$, $p = 0.034$]. Reaction time changes appeared to habituate (Figure 4.4B). Increases of 73 ± 23 ms and 76 ± 28 ms, were observed during the first and second sessions of drug infusion [$t(189.89) = 3.24$, $p = 0.008$; $t(178.13) = 2.72$, $p = 0.042$], respectively. During the remaining drug infusion sessions, reaction time appeared to incrementally return towards levels observed during the initial session of vehicle infusion. Reaction time was significantly slower by 52 ± 7 ms, across all contralaterally directed trials [main effect of cue, $t(149.72) = 6.76$, $p < 0.001$] as compared to ipsilaterally directed trials. Therefore, drug infusion immediately resulted in a large amplitude increase in reaction time across both contralaterally and ipsilaterally directed trials, which appeared to habituate and return to baseline levels by the final session of drug infusion. Contralaterally directed trials appeared to be somewhat slower overall, but the pattern of drug induced increases in reaction time for contralaterally and ipsilaterally directed trials was largely the same.

Similarly to the SCH group, raclopride infusion resulted in modest bilateral increases in movement time [main effect of session, $F(131.22) = 4.55$, $p < 0.001$] as well as pronounced decreases in the total number of trail attempts [main effect of session $F(6,67.71) = 14.59$, $p < 0.001$] where animals attempted significantly fewer trials during all drug infusion sessions but did not persist into subsequent vehicle infusion sessions (Figure 4.4C,D).

Discussion

In order to first establish whether dopamine antagonism in our experimental procedure was capable of producing experience-dependent changes in contralateral responding, we attempted to reproduce the effects of 6-OHDA lesion of the striatum using nonselective antagonism of dopamine receptors using α-flupenthixol. Our results indicated that nonselective pharmacological antagonism is capable of producing an experience dependent effect on contralateral responding that is similar to extinction through reward omission (Dowd and Dunnett, 2007). Animals infused with a high dose of flupenthixol displayed a progressive change in choice that was evident by the second session of vehicle infusion where a significant difference in accuracy on contralaterally and ipsilaterally directed trials and was first observed. Accuracy on contralaterally directed trials was most strongly affected with a decrease of over 40% observed in the final session of drug infusion which largely persisted through a subsequent session of vehicle infusion. Accuracy on ipsilaterally directed trials appeared to increase but this change was not significant, perhaps because of a ceiling effect due to an initially high accuracy on vehicle infusion sessions. Reaction time in these sessions changed predictably with animals taking longer to make correct contralaterally directed choices. Similar results were observed in previous studies where 6-OHDA was used to selectively degrade dopamine fibers in a single hemisphere (Carli et al., 1985; Dowd and Dunnett, 2004; Dowd and Dunnett, 2007).

When animals are trained in an instrumental task that includes a stimulus that predicts when an action will be rewarded, but reward delivery does not occur, a brief

pause in the firing of dopaminergic cells of the substantia nigra is observed (Schultz 2002). This signal is thought to be an error signal related to a prediction made about an upcoming reward. Feedback from this error signal may be used to update striatal circuitry with new information via plastic change in synaptic strength (Schultz, 1998). Some suggest that this error signal drives associative learning by directly modifying the strength of connection between reward predicting cues and rewarded response and "stamping in" of habit (Graybiel, 1998; Berke and Hyman, 2000; Yin and Knowlton, 2006; Horvitz, 2009). While prominent, the learning hypothesis of dopamine is controversial. An alternative proposal is that reward predicting cues take on motivational properties and become attractive and "wanted" to an animal (Robinson and Berridge, 1993; Berridge and Robinson, 1998). Our task was not designed to differentiate between these theories. However, both approaches propose that dopamine helps to promote the storage of cue-specific information whether it is a direct association with a response or a cue-specific incentive value. Synaptic plasticity in the corticostriatal synapse would be a clear candidate mechanism for the storage of either stimulus-response associations or cue specific incentive values.

Results from our high dose infusion of flupenthixol may mimic conditions of an outcome that is worse than expected, such as when a reward is omitted. Midbrain dopamine neurons that project to the striatum fire regularly with a cue that predicts a rewarded response (Schultz, 1998). Following a cue that predicts a reward, if the reward is omitted unexpectedly, a pause in dopamine cell firing will occur at the time of the expected reward (Schultz et al., 1993). It is possible that cumulative and persistent changes in choice and reaction time resulting from administration of a high dose of

flupenthixol implement learning-like behavioral changes by mimicking the pauses observed in omission trials.

Changes in dopamine signaling occur in across two distinct time scales (Hauber, 2010). Behaviorally relevant stimuli can trigger rapid phasic changes in dopamine cell firing that are time-locked to stimulus presentation (Schultz, 1998). These phasic bursts and pauses in dopamine cell firing have been suggested to play a role in reward related learning, but may also relate to selective persistent changes in motivation (Berridge and Robinson, 1998). Low dopamine levels are associated with akinesia, such as in Parkinson's disease, and movement can be restored by treatment with a dopamine precursor, L-DOPA (Marsden and Parkes, 1976), which may facilitate movement by restoring tonic dopamine levels. Administration of dopamine antagonists can decrease performance on instrumental tasks that have a high effort to reward ratio and drive a bias towards less energetically costly behaviors (Salamone et al., 2007). Other behavioral states such as sexual stimulation, novelty, feeding and stress are accompanied by gradual changes in dopamine tone (Hauber, 2010). An outstanding question is whether phasic and tonic dopamine signaling corresponds to learning processes and performance processes.

Comparison of our findings from infusion of a higher and a lower dose of flupenthixol do not permit a direct contrast between behavioral effects of manipulating phasic and tonic dopamine signaling, however, it is possible that a high dose of flupenthixol drives cumulative and persistent effects of choice by engaging mechanisms of plasticity that require a certain absolute level of dopamine signaling (whether very high or very low). This idea is supported by the high magnitude changes in dopamine concentration observed in relation to phasic dopamine release (Hauber, 2010). If

cumulative and persistent changes do not require an absolute level of dopamine signaling to have an effect, then one would predict that a lower dose of flupenthixol would drive cumulative and persistent changes in choice that accrue more slowly, however, this was not observed in our experiments. Rather, a low dose of flupenthixol had modest and transient effects on choice that were restored with further task experience.

In the chapter 3 we proposed that a single hemisphere of the striatum can select for both contralaterally and ipsilaterally directed movements using separate but complimentary processes where ipsilaterally directed movements are facilitated through the inhibition of contralaterally directed movements. Current understanding of basal ganglia anatomy supports this proposal with complimentary roles of movement facilitation and inhibition for the direct and indirect pathways of the striatum (Albin et al., 1989; Kravitz et al., 2010). Activity in direct pathway neurons of the striatum is thought to facilitate movement, while activity in indirect pathway neurons of the striatum is thought to inhibit movement. Neurons in both pathways display synaptic plasticity which is regulated by separate families of dopamine receptors: D1 receptors are primarily expressed by direct pathway cells and D2 receptors are primarily expressed by indirect pathway cells (Shen et al., 2008). Selective degradation of dopamine terminals in the striatum using 6-OHDA drives LTD in the direct pathway and LTP in the indirect pathway (Shen et al., 2008). These effects may work synergistically to decrease movement facilitation and increase movement inhibition in direct and indirect pathways, respectively. Experiments 3 and 4 investigated the contribution of selective dopamine antagonism in direct or indirect pathway neurons to experience dependent changes in choice.

Selective D1 and D2 antagonists resulted in similar changes in learning and performance in the choice task. Both had an effect on choice that decreased the overall frequency of making a contralateral choice, however, the treatments differed in how this was accomplished. The D2 antagonist decreased accuracy on contralaterally directed trials. When animals were instructed to make a contralateral movement, they made incorrect ipsilateral movements more frequently. In contrast, the D1 antagonist increased accuracy on ipsilaterally directed trials. On trials when animals were instructed to make an ipsilateral movement, they did so more frequently. Neither treatment resulted in cumulative and persistent changes in choice, which could suggest a learning effect; rather these results suggest a performance effect.

Direct and indirect pathway neurons within a single hemisphere of the striatum may work in concert to facilitate and inhibit contralaterally directed movements to maximize reward. This concept is supported by results from unilateral 6-OHDA lesions. Animals that have dopamine removed from one hemisphere of their brain will neglect things that occur in the opposite space. But, if cues that indicate when a movement should occur arise from ipsilateral space, animals still fail to initiate contralaterally directed movements (Carli et al., 1985). Likewise, lesions of the striatum disrupt selection of an action in contralateral space even under conditions where both movement choices are presented on the contralateral side. Under these circumstances animals respond more slowly and tend to choose a response that is closer and requires less movement (Brasted et al., 1997). Studies of visually responsive caudate neurons indicate that a majority of recorded neurons have receptive fields in the contralateral hemisphere (Hikosaka et al., 1989). When visually responsive caudate neurons were studied in a task

where cues indicated whether or not a large reward would be received for a specific movement, Kawagoe *et al.* (1998) found that a subset of striatal neurons responded more strongly to cues that indicate the a reward would not occur if a movement was made in the contralateral direction. Hikosaka *et al.* (2006) hypothesized that these neurons were part of the indirect pathway and that dopamine dependent plasticity in this movement-inhibitory pathway was modulated oppositely from direct pathway neurons. If the performance effects that we observed were due to antagonist driven corticostriatal plasticity then it's possible that the D1 antagonist increased accuracy on ipsilaterally directed trials by weakening (through LTD) a go-contralateral signal that would engage during the wrong trials. Likewise, a D2 antagonist could have decreased accuracy on a contralaterally directed trial by strengthening (through LTP) a don't-go-contralateral signal also on the wrong trials.

If plasticity is driving a change in choice by acting selectively on the direct or indirect pathway neurons of the striatum, why would changes driven by selective antagonists not be cumulative and persistent? One possibility is that selective antagonists did not affect plasticity processes because the dose administered was too low but that the higher dose of flupenthixol was sufficient to affect plasticity processes. We can attempt to address this question by comparing binding affinities between the three compounds flupenthixol, raclopride and SCH23390 and adjust these for the molar quantity of drug that was infused. One study of radiolabeled [H^3] SCH23390 displacement directly compared the binding affinities of unlabeled flupenthixol and SCH23390 and found that unlabeled SCH23390 had a roughly 10 fold higher binding of the D1 receptor than flupenthixol (Billard et al., 1984). Correcting our drug infusion quantities for molarity we

find that the molar quantity of flupenthixol dose was 10 fold higher than SCH23390, which suggests that these doses were equally effective at preventing dopamine from binding the D1 receptor. Similarly, two displacement studies of radiolabeled [H^3] raclopride compared binding affinities of unlabeled raclopride (Seeman and Tallerico, 1998) and flupenthixol (Burstein et al., 2005) and found affinity to be extremely close (K_d 0.64 nM vs. 0.5 nM), especially when compared with the calculated molar quantities of drug dosage (0.05 µmol vs. 0.059 µmol), suggesting that both doses were equally effective in displacing dopamine from the D2 receptor. While speculative, the roughly equivalent D1 and D2 receptor binding affinities for flupenthixol suggests that we did equally block D1 and D2 receptors using selective and nonselective antagonists.

Assuming that D1 and D2 receptors were equally prevented from binding dopamine in the HIGH-FLU vs. RAC and HIGH-FLU vs. SCH groups, the lack of a cumulative and experience-dependent change in choice during selective antagonist treatments is not consistent with the proposed mechanism of striatal neurons storing information about past trial experience as an array of weights distributed across corticostriatal inputs (Graybiel, 1998; Yin and Knowlton, 2006; Horvitz, 2009). If this model was correct, then selective antagonism of either the direct or indirect pathway alone should be sufficient to drive some cumulative and persistent change. Perhaps, learning-like changes that depend upon the striatum require neurobiological changes beyond an interaction between dopamine signaling and glutamate receptors in the dendrites of a medium spiny neuron.

Here we suggest possible alternative neurobiological mechanisms of cumulative and persistent changes in choice that may occur within the striatum or in downstream

brain regions. Though plasticity has been shown to be separately regulated by D1 and D2 family receptors in direct and indirect pathway cells (Shen et al., 2008), it is possible that experience-dependent changes are stored in a separate set of neurons that require coincident changes in dopamine signaling to mediate communication between these pathways within the striatum. Dopamine receptors are expressed on a variety of striatal interneurons (Kreitzer and Malenka, 2007; Di Filippo et al., 2009), and while medium spiny neurons may respond independently to dopaminergic drugs when tested using intracellular recording (Shen et al., 2008) the environment of a slice recording might disrupt alternative streams of communication between direct and indirect pathway neurons that require co-activation of both varieties of dopamine receptor. For example, interneurons that release acetylcholine and NOS express D2 and D1 family receptors which play a critical role in the regulation of plasticity in the striatal microcircuit (Di Filippo et al., 2009). It is possible that behaviorally persistent changes in either the direct or indirect pathway require signaling at both D1 and D2 receptors via local interneurons.

Alternatively, dopamine acting on corticostriatal synapses in the direct or indirect pathway independently may trigger short term changes in behavior that manifest within a single testing session, but are consolidated elsewhere. Changes in activity of the direct and indirect pathway alone may not be sufficient for downstream changes to consolidate. The supplementary motor area (SMA) of the cortex is a downstream target of the basal ganglia which could consolidate changes in output from the basal ganglia that occur over a series of testing session. The SMA forms a closed loop with the dorsal striatum (Alexander et al., 1986) and changes in firing during instrumental learning have been shown to occur first in the striatum and then next in the cortex (Pasupathy and Miller,

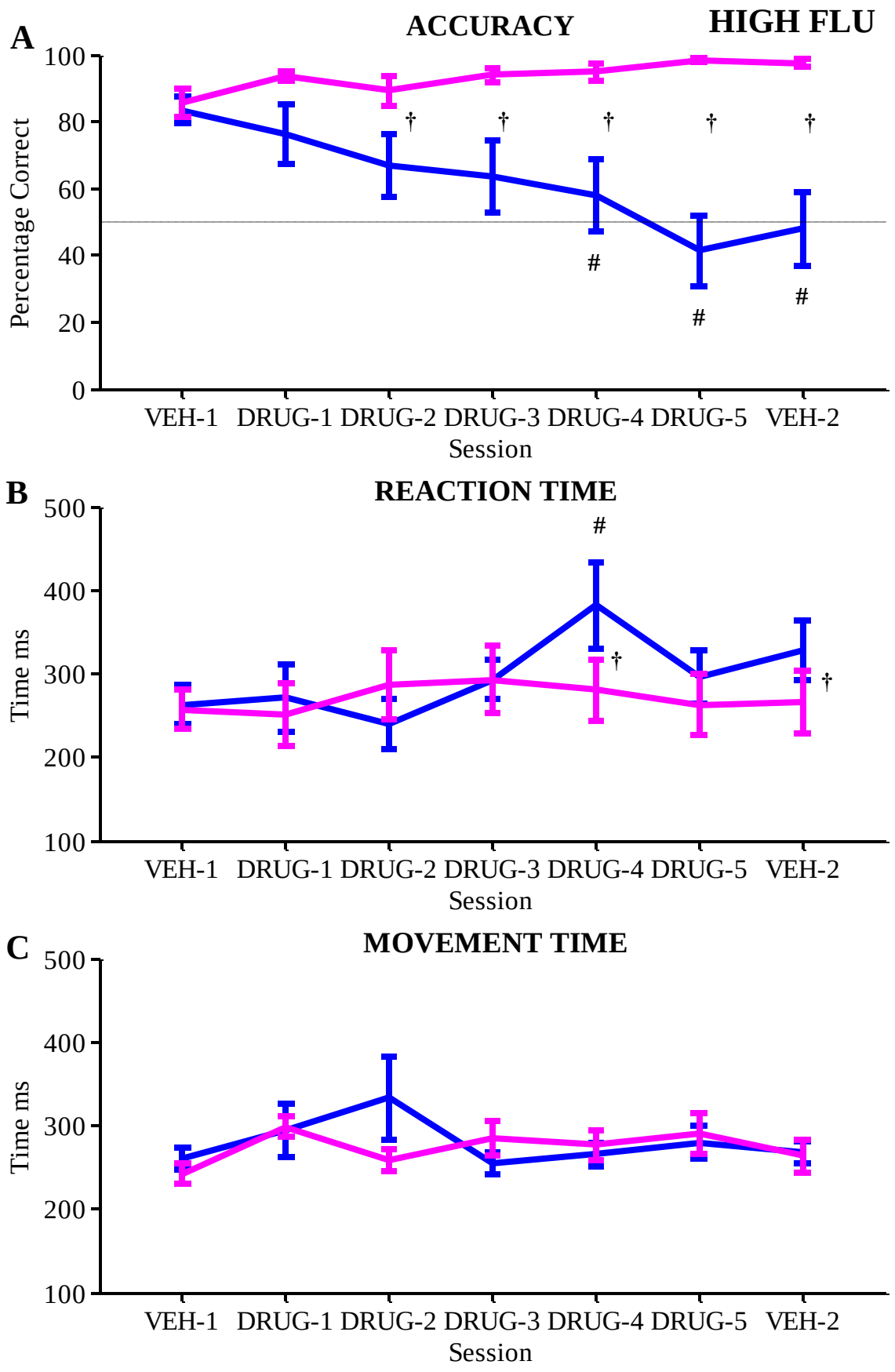
2005). Furthermore, regions of the prefrontal cortex which project to the striatum convey information about the value of an outcome (Yin and Knowlton, 2006; Kable and Glimcher, 2009) perhaps thalamocortical outputs from the basal ganglia help to update the representation of value in this region. Our experiments were not designed to discriminate between these possibilities, but it is possible that the lack of persistent change in behavior following selective antagonist administration is attributed to either local interactions or downstream consolidation of striatal output, or some combination of the two processes.

Results from reaction time and trial attempts resist interpretation under a framework of the striatum implementing associative learning (Graybiel, 1998; Yin and Knowlton, 2006; Horvitz, 2009). If a cue activates a set of cortical inputs to the striatum which is associated with a certain response, then a behavioral change that alters choice so that one action is selected less frequently should also alter reaction time for that choice so that when it is selected that response takes longer to initiate because both pieces of information would be communicated via spatiotemporal summation of excitatory input within striatal MSNs. However, we observed a bilateral increase in reaction time in response to administration of either D1 or D2 antagonists accompanied by a more modest increase in movement time. In addition, we observed a strong decrease in the number of trials attempted when either D1 or D2 antagonist are administered. These changes in reaction time may be interpreted as motivational in the undirected sense (Salamone et al., 2007) with low levels of behavioral activation that slow the initiation of a range of behaviors rather than affecting behaviors related to a specific cue (Berridge and Robinson, 1998), which would also be predicted to persist into a subsequent vehicle

infusion session. A similar pattern of decrease in the number of attempts during selective antagonist infusion sessions also support a role in motivation perhaps indicating that animals are not accurately valuing the opportunity cost of inactivity (Niv et al., 2007). These changes to reaction time and the number of attempts are largely restored in the second session of vehicle infusion and are therefore not likely to result from the permanent changes to striatal circuitry; rather they appear to be changes in performance. This motivational interpretation is complicated by results from flupenthixol infusion. In the case of a high dose of flupenthixol, animals display a cumulative and persistent imbalance in reaction time that is selective to contralateral choices. The number of trial attempts decreases initially but then gradually restores to baseline levels throughout the course of subsequent drug infusion sessions. The animal's behavior is more consistent with a learned decrease in value contralateral choices that an animal adapts to within the context of the entire task and continues to initiate a high number of trials.

Numerous approaches have attempted to explain the contributions of the dorsal striatum to behavior. Our experiments suggest that the storage of past experience, as evidenced by cumulative and persistent changes in choice and reaction time require coincident changes in dopamine signaling in the direct and indirect pathway. This result may be modeled as a change in the associative strength between a cue and a contralaterally directed movement, (Mishkin et al., 1984; Graybiel, 1998; Yin and Knowlton, 2006; Horvitz, 2009) or may equally be interpreted as a change in the incentive value of a specific cue (Berridge and Robinson, 1998). However, details of behavioral changes resulting from selective antagonism do not fit with either model. If the doses of raclopride and SCH23390 are sufficient to induce changes in corticostriatal

synapse strength comparable to what is assumed to occur in the high dose of flupenthixol, then an additional neurobiological mechanism that integrates changes in the direct and indirect pathway must be included as a critical factor persistent learning like behavioral changes.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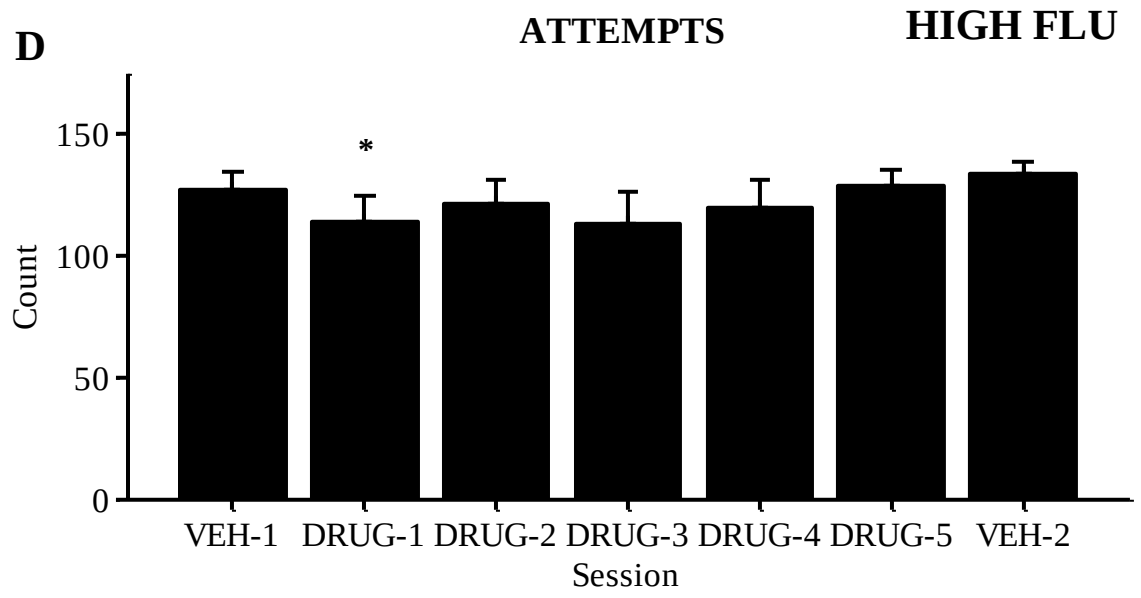
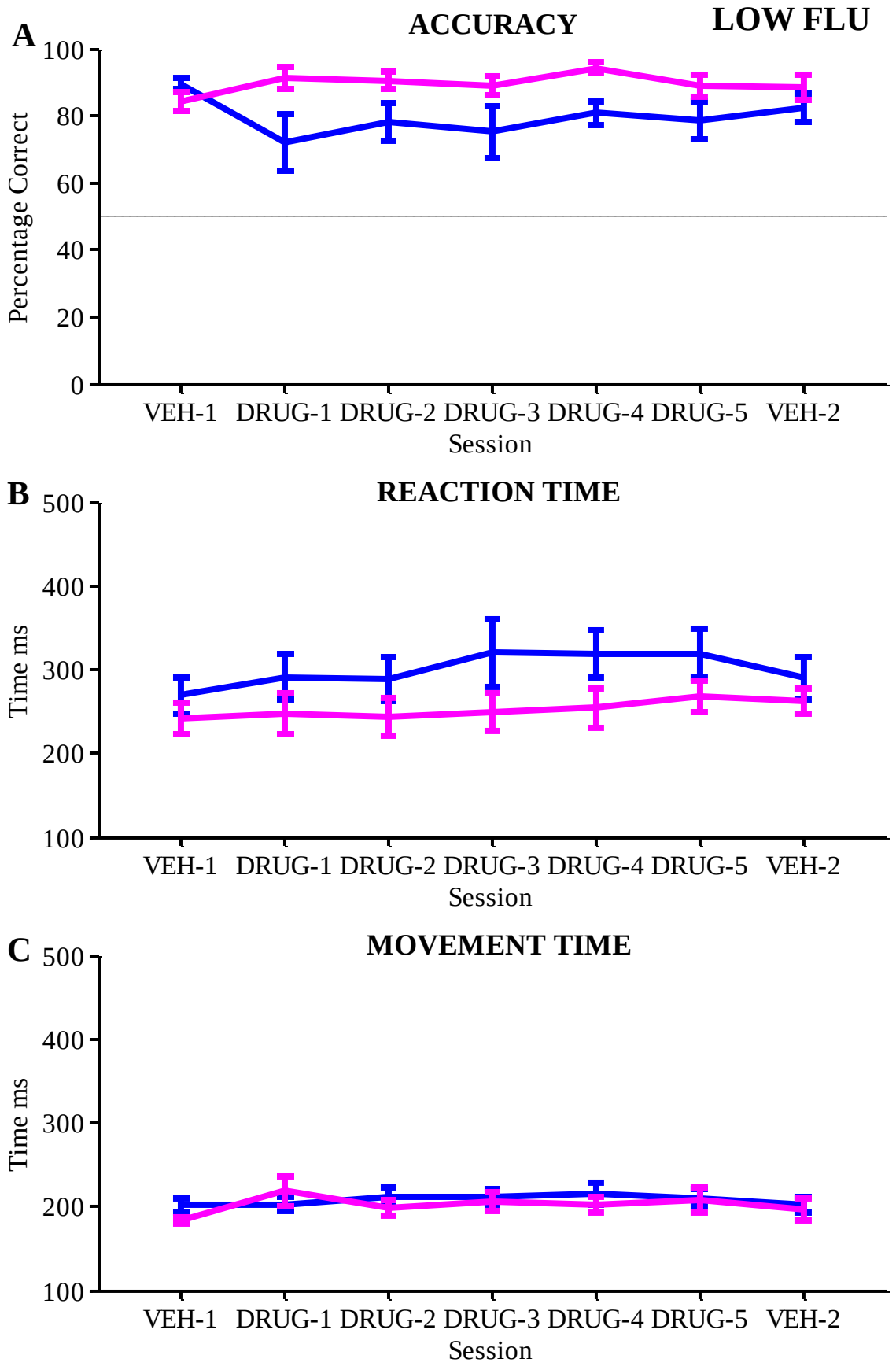


Figure 4.1 A high dose of flupenthixol drives a gradual decrease in contralateral accuracy (A) which persists through a subsequent vehicle infusion session. Reaction time increases in contralaterally directed trials (B), with most prominent changes occurring after several testing sessions with drug. The high dose of flupenthixol did not significantly affect movement time (C). The total number of attempts decreases transiently during the first session of flupenthixol infusion but this effect is restored (D). Plots indicate the mean for n=13 animals; errorbars are SEM. Contralaterally directed trials are plotted in blue, ipsilaterally directed trials in magenta. Hash marks indicate a significant difference in accuracy or reaction time from the initial vehicle infusion session for contralaterally directed trials. Daggers indicate a significant difference in accuracy or reaction time when comparing contralaterally vs. ipsilaterally directed trials within the same session. Asterisks represent a measurement that is significantly different from the initial vehicle infusion session.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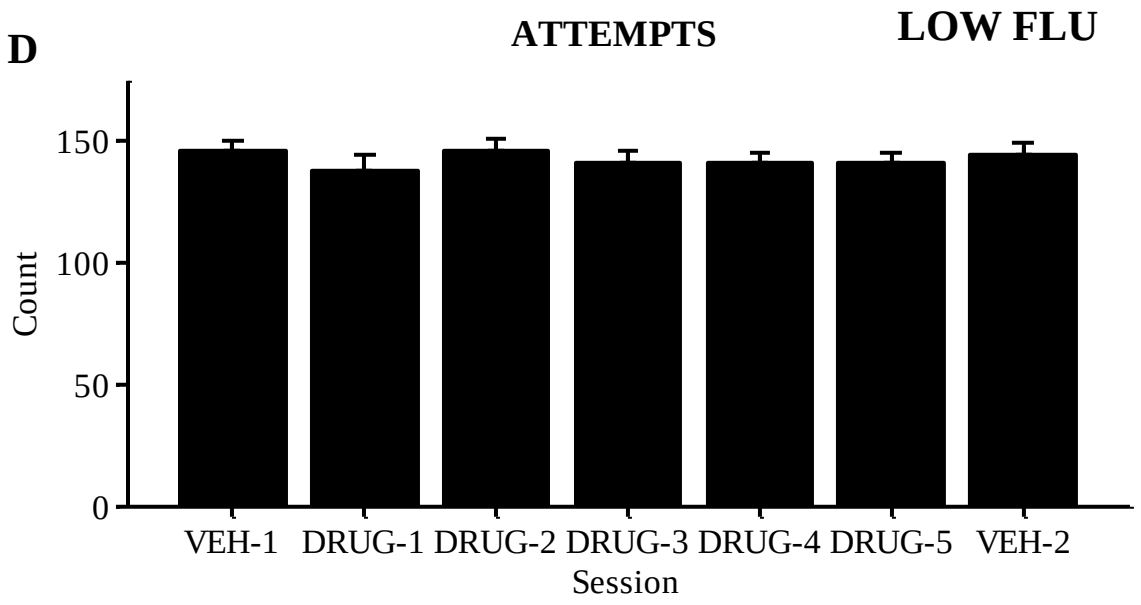
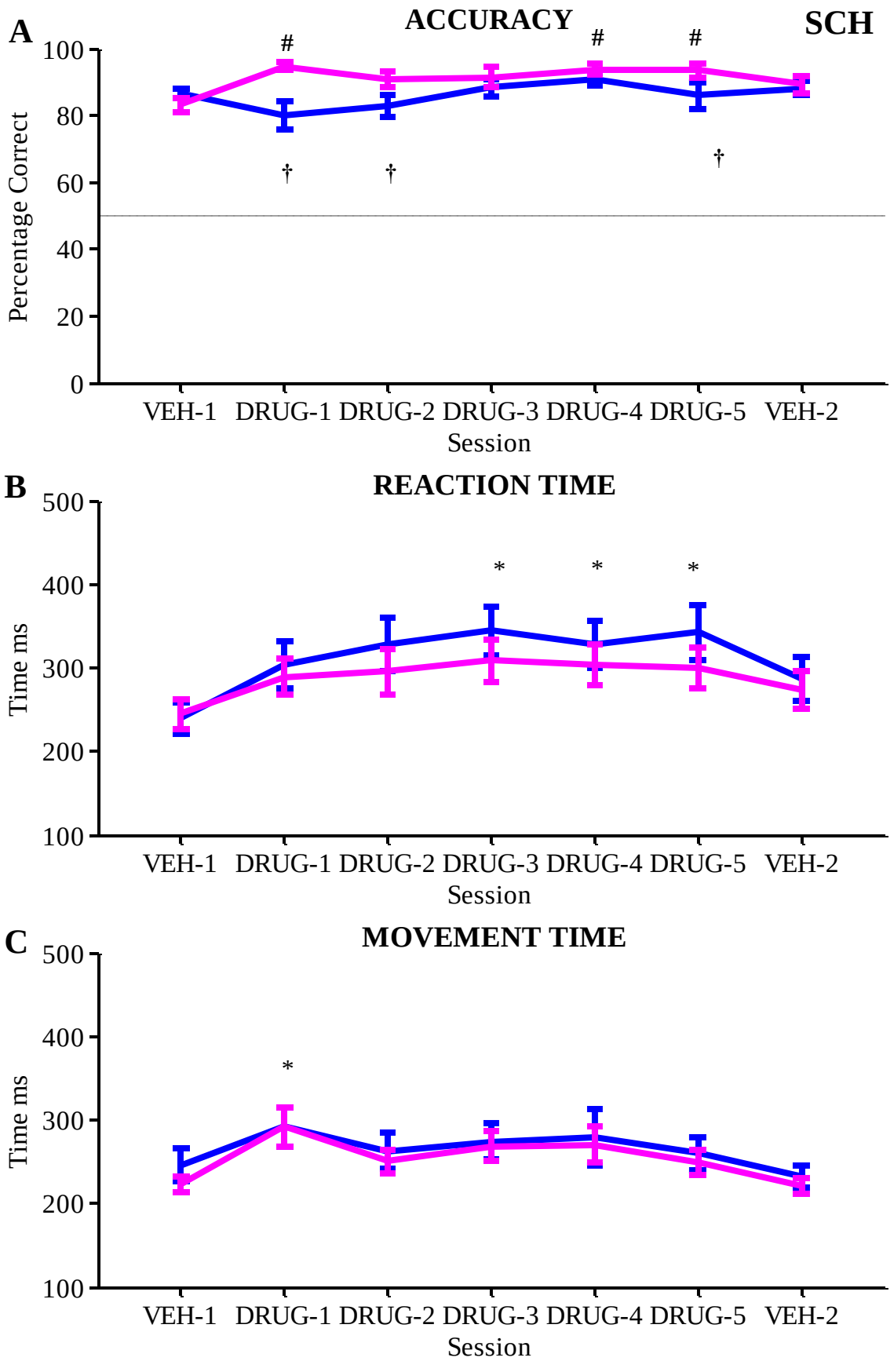


Figure 4.2 A low dose of flupenthixol drives a modest decrease in contralateral accuracy (A) and a similarly modest shift in contralateral reaction time (B). Persistent effects on choice accuracy and choice reaction time are not apparent. A low dose of flupenthixol did not affect movement time (C). Attempts are not significantly affected (D). Data points indicate the mean for n=11 animals; errorbars are SEM. Contralaterally directed trials are plotted in blue, ipsilaterally directed trials in magenta.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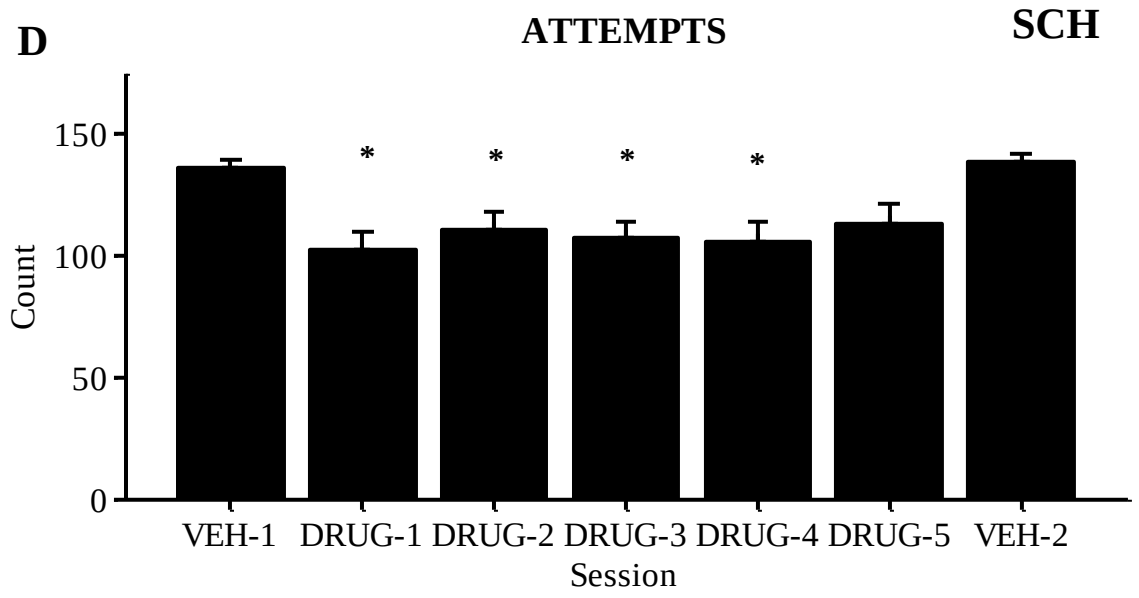

Figure 4.3 Selective antagonism of D1 receptors with SCH-23390 primarily causes an increase in accuracy on ipsilaterally directed trials (A). Reaction time and movement time increases bilaterally with drug administration (B, C). Reaction time is more severely retarded than movement time with drug administration. SCH-23390 significantly decreases the total number of attempts in all drug infusion sessions (D) but does not have an overall effect on trial completion (E). Effects of SCH-23390 do not appear to develop with experience or persistent into a subsequent vehicle infusion session. Plots indicate the mean for n=16 animals; errorbars are SEM. Contralaterally directed trials are plotted in blue, ipsilaterally directed trials in magenta. Hash marks represent a significant difference in accuracy from the initial vehicle infusion session for ipsilaterally directed trials. Daggers indicate a significant difference in accuracy when comparing contralaterally vs. ipsilaterally directed trials within the same session. In (B) and (C), asterisks represent a significant difference in reaction time between the drug infusion session and the initial vehicle infusion session, for both contralaterally and ipsilaterally directed trials. In (D)

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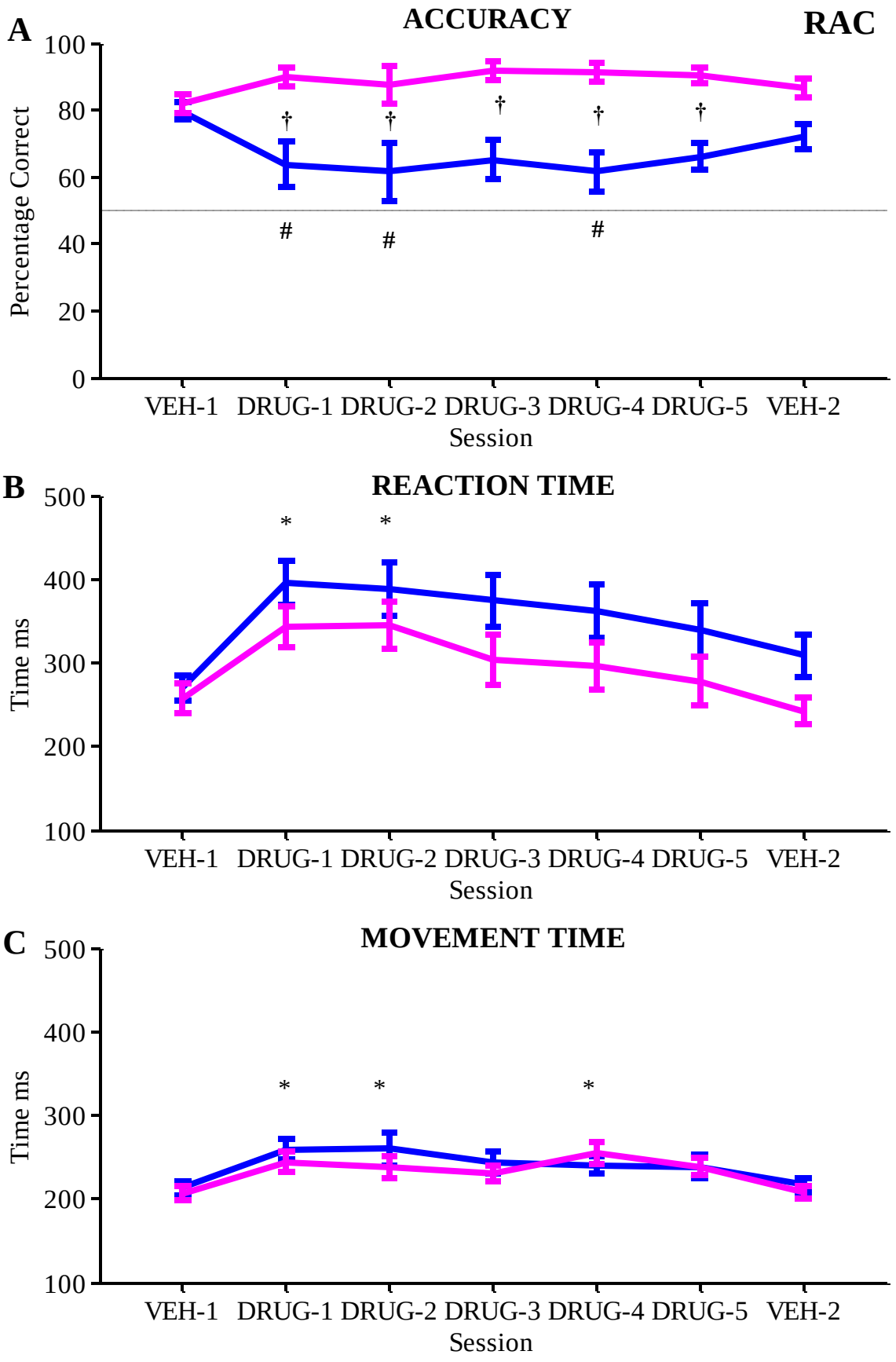
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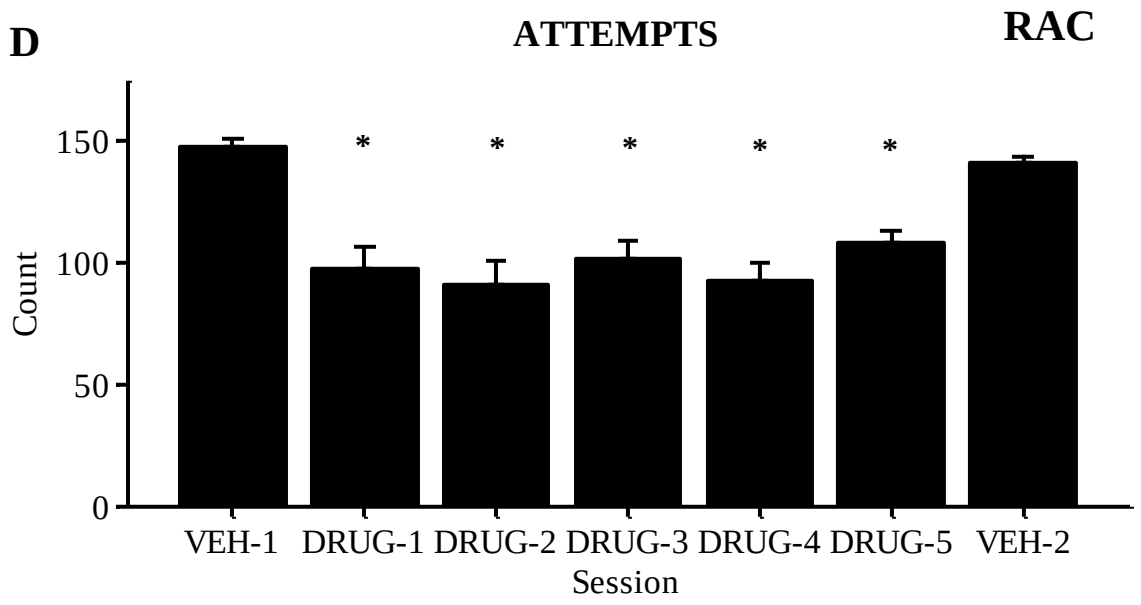

Figure 4.4 Selective antagonism of D2 receptors with raclopride drives a decrease in contralateral accuracy (A). Reaction time increases bilaterally during the first two drug infusion sessions but these effects are diminished in later testing sessions (B). Movement time is slower during some drug infusion sessions but returns to normal levels during the final session of vehicle infusion (C). Animals attempt significantly fewer trials when infused with raclopride (D). Plots indicate the mean for n=16 animals; errorbars are SEM. Contralaterally directed trials are plotted in blue, ipsilaterally directed trials in magenta. Hash marks represent a significant difference in accuracy from the initial vehicle infusion session for contralaterally directed trials. Daggers indicate a significant difference in accuracy when comparing contralaterally vs. ipsilaterally directed trials within the same session. In (B) and (C), asterisks represent a significant difference in reaction time or movement time between the drug infusion session and the initial vehicle infusion session, for both contralaterally and ipsilaterally directed trials. In (D), asterisks represent a measurement that is significantly different from the initial vehicle infusion session.

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Chapter 5:

Discussion

The experiments described in this dissertation represent an attempt to advance understanding of the physiology of the striatum and the contribution of changes in dopamine signaling in the striatum to learning and performance of an instrumental task. In discussion of our experimental results we have attempted to link ideas about striatal plasticity to ideas about instrumental learning. Some results from chapters 3 and 4 are difficult to interpret using a conceptual model of the striatum as a switchboard for the formation of associations between stimulus and response (Figure 5.1A). The idea that the corticostriatal pathway is a substrate for stimulus-response (S-R) associations is an idea that has numerous roots but gained considerable momentum from a report by Mishkin *et al.* (1984) on preserved habit learning in monkeys with temporal lobe lesions. Work from Schultz strengthened this idea when it was reported that phasic changes in dopamine cell firing appear similar to a teaching signal used in computational models of reinforcement learning (Ljungberg et al., 1992; Schultz et al., 1993; Schultz, 1998) and associational strength may be stored in corticostriatal connections (Graybiel, 1998; Yin and Knowlton, 2006; Horvitz, 2009). The incentive-salience hypothesis for dopamine function proposes an alternative role for dopamine in the striatum of attribution of an incentive values to specific cues (Berridge and Robinson, 1998; Berridge, 2007). This hypothesis rejects the

idea that dopamine in the striatum is necessary for forming S-R associations but does retain the idea that a property of a specific cues persists, an idea which is still compatible with dopamine triggering long term changes in corticostriatal synapse strength which presumably carries sensory information to the striatum. Other theories of dopamine function in the striatum do not rely on synaptic plasticity or a storage mechanism and may be focused more generally on movement, such as in movement deficits driven by Parkinson's disease (Dauer and Przedborski, 2003) or may be focused on a motivational construct that affects any potential movement such as the level of effort required (Salamone et al., 2007), or the cost of time (Niv et al., 2007). The later theories focus more on performance and dopamine signals that transmit effort or cost related information are derived from dopamine tone, rather than changes in plasticity; their effects are not necessarily stored, or specific to future contexts. Finally, some have suggested a role for dopamine in the striatum in the context of decision making where dopamine dependent changes in corticostriatal plasticity have performance effects on the decision process, perhaps by representing a limit in terms of how long an organism should spend deliberating before acting (Smith and Ratcliff, 2004; Kable and Glimcher, 2009; Bogacz et al., 2010). These ideas propose a role for plasticity in short term storage where changes in the properties of corticostriatal connections adjust to meet the current task conditions, but do not necessarily reflect long term selective changes like a learning process. We will next attempt to assess theories of striatal and dopamine function based upon our results.

Corticostriatal Plasticity, Dopamine, Learning and Performance

A model of corticostriatal connections acting as a substrate for the formation of associations in instrumental learning implies that striatal neurons identify relevant patterns of input that indicate a specific response and then implement this response. Since cortical inputs come from at least 5000 individual cortical neurons for each medium spiny neuron (Kincaid et al., 1998), there is potential for a wide range of input patterns to sufficiently activate a striatal neuron. Dopamine dependent plasticity may help to tune synapse strength so that only a subset of inputs are capable of exciting a striatal neuron and driving activity (Houk and Wise, 1995). However, in this type of model, activity that is sufficient to recognize a pattern and drive striatal cell firing must also be sufficient to drive a response. Any selection process that is implemented by the striatum must also depend on a process of pattern detection across corticostriatal inputs. Selection would occur because recognition of a stimulus would activate a subset of striatal neurons more strongly than other striatal neurons winning control over behavior. This process of activation of striatal neurons would be based on the magnitude of excitation derived from the input pattern. Properties of spatiotemporal summation across inputs would convert a high magnitude excitatory input into a rapid onset in output activity from the striatum. If output activity represents a specific response, then the measured reaction time of this response would be related to the choice itself; choice and reaction time should not change independently in response to experimental intervention. While a high dose of flupenthixol did lead to coordinated changes in choice and reaction time, because infusion of raclopride and SCH23390 are capable of independently altering choice and

reaction time, we consider these results as potential evidence against a role for the striatum as a substrate for direct S-R associations.

The incentive salience theory proposes a role for dopamine in motivation rather than associative learning. Activation of the dopamine system can give certain contexts and stimuli incentive properties which render them able to motivate certain behaviors such as eliciting approach. Incentive stimuli can act as reinforces in their own right as animals will work for presentation of conditioned stimuli alone (Berridge and Robinson, 1998). Because incentive stimuli retain their ability to elicit approach and act as conditioned reinforcers, the process of incentive salience attribution to a specific cue implies the storage of information, though not necessarily in the striatum. A decreased incentive value of a stimulus might be reflected in an increased reaction time when presented with a specific stimulus. In our results, the only treatment that selectively and persistently altered reaction time in response to a specific cue was administration of a high dose of flupenthixol. In selective antagonist experiments we observed changes in reaction time in response to both stimuli and these changes were not persistent. We consider these results as evidence against an S-R learning role for the striatum. However, because the effects of storage of an incentive value for a cue or stimulus does not necessarily mean that a specific response must be elicited, we cannot argue against an incentive salience model based on our results where choice and reaction time diverge.

Since the incentive salience theory of dopamine function allows for other performance changes to occur from dopamine administration (Berridge, 2007) bilateral increases in reaction time may be interpreted a broad change in motivation derived from another function of dopamine. Nevertheless, a bilateral change in reaction time in

response to administration of a selective dopamine antagonist raises questions about the circumstances under which dopamine might cause selective vs. general changes in motivation. We don't know for certain whether our experimental manipulations were affecting corticostriatal plasticity, but we infer this based on the persistent nature of change in the high dose of flupenthixol. Furthermore, we predict that administration of a selective antagonist was equally effective in receptor binding and therefore an equivalently effective antagonist. A role for dopamine in attribution of incentive salience does not necessarily preclude a separate role for dopamine in affecting performance more generally (Berridge, 2007). However, general changes in motivation are assumed to be derived from dopamine tone rather than from phasic dopamine signals (Wickens, 1990; Salamone et al., 2007; Niv et al., 2007). It is possible that selective antagonists affected motivation more generally because of tonic effects. And while it seems inconsistent for a general effect on motivation to be lost with a potentially higher dose of antagonist in flupenthixol infusions, dopamine may have effects on ionic conductances that affect the spatiotemporal summation of excitatory input in ways that conflict with effects on corticostriatal plasticity (Wickens, 1990; Bolam et al., 2006) which could result in different doses having conflicting effects on motivation. One relatively straightforward way to test this possibility would be to repeat flupenthixol infusion in one or more additional groups using drug dosage intermediate to our LOW-FLU and HIGH-FLU groups. The prediction would be that in our two doses of flupenthixol our low dose that was too low to have clear behavioral effects on general motivation by altering ionic conductances within MSNs and our high dose had selective effects on motivation derived from changes in corticostriatal plasticity that masked general motivational effects. An

intermediate dose of flupenthixol may more generally resemble the behavioral effects observed from selective antagonists because the dose was high enough to have robust general motivational effects but low enough to not engage mechanisms of plasticity. If this result were the case then perhaps a hybrid of selective and general effects on motivation may be the best model to explain a range of dopamine's effects on behavior.

Models of the role of the role of the striatum in decision making may also be a useful framework to interpret our results from drug infusions. Results from chapter 4 suggest that the neurobiological substrate of experience-dependent change in instrumental behavior is distinct from plasticity at corticostriatal synapse. An argument for this assertion relies on two predictions, the first being that cumulative and persistent changes in behavior resulting from infusion of a high dose of flupenthixol that affects plasticity mechanisms in the corticostriatal pathway. Next, we predicted that selective dopamine antagonist and a high dose of flupenthixol are equally effective at antagonizing dopamine receptors and therefore equally effective in recruiting corticostriatal plasticity onto the direct and indirect pathway. If these predictions are true, why then would the resulting changes in behavior attributed to selective antagonism not be cumulative and persistent? A potential explanation may arise from analysis of the basal ganglia from using a decision making framework. First, corticostriatal plasticity is not the direct substrate for permanent storage of any property that affects behavior whether motivation or associative learning. Instead changes in corticostriatal plasticity reflect temporal aspects of a decision process limiting the time allotted to deliberation between response alternatives (Lo and Wang, 2006; Bogacz et al., 2010). Next, output from the basal ganglia does not directly encode action and sensory and contextual input to the basal

ganglia does not link to specific responses. Instead, the basal ganglia acts as a central controller to mediate resource conflicts between other brain systems that vie for control over a limited set of motor resources (Redgrave et al., 1999). Output from the basal ganglia can inhibit or disinhibit motor controllers that receive specific movement instructions from elsewhere (Lo and Wang, 2006; Redgrave et al., 2008) and stimulus response associations are formed in parallel to pathways through the basal ganglia (Figure 5.1B). Basal ganglia inputs and stimuli that form stimulus response associations are shared, and basal ganglia outputs target areas that implement the actions specific to stimulus response associations, but responses that are derived from stimulus-response associations form a separate pathway and are not encoded by the basal ganglia. Instead the basal ganglia can take information about the current state of the animal, current conditions of the task and recent information about feedback and adjust the likelihood and timing of expression different behaviors. Relieving theoretical requirements for persistent storage of information in corticostriatal connections allows for plastic change from selective antagonists to have a temporary effect on choice, since, in this view, the basal ganglia implements a choice rather than selecting specific action.

Some evidence suggests that inputs to the basal ganglia may already be clearly discriminated for choice. Shadlen and colleagues have conducted a series studies on perceptual discrimination in the monkey, where animals must choose to make a saccade to one of two targets based on a visual stimulus (Roitman and Shadlen, 2002). The firing rate from individually recorded cells predicted whether an animal would make a correct choice. Cells from all regions of the cortex send collateral projections to the striatum. We do not know if the firing pattern described in this experiment is relayed to the striatum,

but it's likely based on the widespread nature of inputs. If so, these results suggest that input to the striatum already reflects a choice and that choice is already mapped to a specific response. Changes in choice derived from selective antagonist administration would then result from interference with implementation of the choice through the direct or indirect pathway.

The closed loop structure of the basal ganglia position cortical motor areas such as the SMA as both inputs to the basal ganglia and output targets of the basal ganglia (Alexander et al., 1986). If the basal ganglia plays a restricted role in the implementation of an action plan, formed in a region such as the SMA, or elsewhere in the cortex, then long term changes between stimulus and response may also reside in targets of the SMA and be influenced by how the basal ganglia implements a request for an action through feedback from the basal ganglia. Changes in implementation of an action that affect only the direct or indirect pathway may not be sufficient to drive changes in downstream targets.

When a high dose of flupenthixol is administered, animals make contralateral choices with less frequency and take longer to initiate these choices. Initially this results in a decrease in the number of attempts that an animal makes, but the number of attempts is restored as the animal's choice pattern changes. This is consistent with a change in value of the choice and this change may be stored in the cortex (Kable and Glimcher, 2009) and fed back into the striatum in order to be implemented. The number of attempts may increase because the animal no longer views contralaterally directed trials as outcomes that are worse than expected, or with indifference derived from low motivation but rather that contralaterally directed trials are still worth working through to increase

the overall reward rate on ipsilaterally directed trials (Niv et al., 2007). Selective antagonists may alter the implementation of a plan requested by the cortex but not give consistent feedback to the cortex which would otherwise permit long term changes in the value of a choice that could be stored in that location. In addition, selective changes to the direct or indirect pathway may slow both choices because they appear the same to the system as poorly discriminated input needing additional time for clear resolution.

A model of the basal ganglia working in conjunction with the cortex to implement decisions has advantages in explaining our results. This model allows for changes in reaction time to occur both selectively, based on a pattern inputs from the cortex, it also allows for changes in reaction time occur generally based the current conditions of the task in order to allow sufficient time to gather evidence for implementation a choice. Finally, it removes the requirements that corticostriatal plasticity be a source for long term storage of information and suggests a different neural substrate, the cortex, as a source for long term changes that depend on coordinated activity in the direct and indirect pathway. A hybrid of models of selective changes in motivation, such as incentive salience, and general changes in motivation and performance such as behavioral activation are consistent with some aspects of a model based on decision making and control theory, and the behavioral observations (such as changes in reaction time) that fuel an idea of a striatal role in motivation overlap with behavioral observations (reaction time) that suggest a role for implementing a threshold in a decision process. Indeed, motivational models of the dopamine acting in the striatum and models of the striatum implementing a threshold for initiation of action may be referring to the same

physiological mechanism, for example, the pattern and rate of integration of excitatory input from the cortex within striatal medium spiny neurons.

State-Dependent Corticostriatal Plasticity

Regardless of whether corticostriatal plasticity is a substrate for the storage of long term associations, attribution of incentive value to specific cues or monitor the firing rate of a set of cortical inputs during a decision, all of these processes may depend upon the ability to adjust the strength of corticostriatal synapses. In chapter 2 we report that in awake, non-anesthetized animals, repetitive activation of cortical inputs results in LTD. This agrees with the original reports of 100 Hz stimulation in slice physiology (Calabresi et al., 1992), and with one recent study of spike timing dependent plasticity (Fino et al., 2005), however it does not agree with several other studies that suggest that tetanic stimulation (Charpier and Deniau, 1997; Charpier et al., 1999) or positively timed STDP (Pawlak and Kerr, 2008; Shen et al., 2008) could result in LTP. We proposed two mechanisms which may affect plasticity in this brain region, the first suggestion was that the state of the entire cortex-basal ganglia network could alter striatal plasticity by enhancing or suppressing feed-forward inhibition from FSIs onto MSNs. In the processing of olfactory information in the honey bee, the state of activity of the entire olfactory network is strongly influenced by local interneurons which are required to properly coordinate associative learning in this system (Linster and Cleland, 2001; Assisi et al., 2007; Fontanini and Katz, 2008). It's possible that in the striatum, state dependent plasticity rules are also relevant for behavior.

FSIs may be critical in terms of regulating whether different patterns of cortical input can drive firing in MSNs (Mallet et al., 2006). In the striatal system, excitatory inputs from the cortex are thought to be necessary to drive medium spiny neurons into in to a permissive state in order to generate firing (Wilson and Kawaguchi, 1996). Individual medium spiny neurons receive inputs from a wide range of the neocortex and may receive prolonged excitatory input from a region of the cortex. For example, in studies of decision making, associative regions of the cortex represent predictions about the directional information taken from a stimulus as a prolonged buildup of activity (Roitman and Shadlen, 2002). Presumably during these decision phases the striatal MSNs receive steady excitatory input, however, it is not known precisely how widespread and active input must be in order for behaviorally relevant stimuli to activate striatal circuitry.

The activity of FSIs may restrict the conditions under which cortical activity can drive striatal cell firing, perhaps altering the conditions under which action may be initiated during a buildup of excitatory input. In our experiment we only drove activity from a single region of the orofacial motor cortex. Perhaps the natural state of LTD in response to repeated activation of this circuit is to protect striatal cells from being driven (and for behavior to be triggered) unless a similar buildup of sensory information in another region of the cortex. It would be interesting to attempt another experiment where excitatory electrodes were implanted into several regions of the cortex and coincidentally used to drive excitation while recording from striatal regions that receive overlapping inputs. These patterns of stimulation might be more likely to drive LTP and may increase the likelihood that repeated stimulation could drive the release of some form of motor routine.

A second interpretation of our results of state dependent activity is a direct effect on intracellular signaling triggered by $GABA_A$ receptors expressed on MSNs. We have already pointed out the curious inversion of STDP function depending upon the experimental preparation. Two studies reported LTP with positive timing experiments while where synaptic input preceded postsynaptic spiking and LTD if spiking preceded synaptic input (Pawlak and Kerr, 2008; Shen et al., 2008). However, in a different experiment where $GABA_A$ antagonists were omitted, the STDP function was inversed with LTD resulting from presynaptic activation preceding postsynaptic activation. Another recent study from Fino *et al*. (2010) confirmed this precise point by developing STDP functions under conditions normal and antagonized $GABA_A$ signaling and found that antagonism could reverse the STDP function. While this is not entirely consistent with the nature of our results since LTP was found under conditions of $GABA_A$ agonists, it is consistent with the concept of reversal of plasticity associated with the level of GABA tone. While the source of the difference is unclear, these experiments indicate that GABA synapses which are present all along the base of the soma and throughout the dendrites from both FSIs and neighboring MSNs (Tepper et al., 2004) may have a surprising amount of direct control over the long term changes in synaptic strength that are observed in the corticostriatal pathway. Whether the results of dopamine dependent plasticity on individual MSN populations (Shen et al., 2008) is or is not affected by GABA signaling remains to be determined but GABA and dopamine dependent plasticity could potentially work competitively or cooperatively to shape striatal output.

Representation of movement in dual corticostriatal inputs.

It was surprising to see that so little activity was generated from stimulation of the motor cortex during our plasticity experiments. One exception to this rule was the case when animals first received repeated excitatory activation while under the barbiturate influenced state. When the plasticity experiment was repeated afterwards in the awake state, 5 Hz conditioning stimulation initiated repeated head and neck movements in the contralateral direction. This movement was not fully elaborated, in the sense that it was like a twitch, an initiation of contralaterally directed movement that was interrupted and restarted at the rhythm of stimulation. We only saw this in three animals where the order of awake and barbiturate state testing was reversed so that the awake condition occurred second; twitching was always observed in the awake state. It would be interesting to follow up this observation and see if repeated stimulation of the cortex, that normally does not result in movement, can have its movement activation ability potentiated by coincident application of plasticity activating agents in the striatum.

Another issue concerning this point is that movements of an entirely different nature were evoked from stimulation of the same region of the orofacial motor cortex using a higher frequency (100 Hz) stimulus train. These stimulation events were conducted following the plasticity experiment in order to validate the location of electrode placement. The movements that were driven using higher frequency stimulation were of a different nature and seemed to be more relevant to a normally behaving animal. Rather than a neck twitch, which seemed like an interrupted orienting response, the animals would engage in specific head turning and mouth opening movements that resembled the animal turning his head to take an object into his mouth. On occasion this

stimulation would also result in forearm movement to the same location, always directed towards contralateral targets. These results are similar to the ethologically relevant movements elicited by the stimulation of the supplementary motor cortex in the monkey (Graziano and Aflalo, 2007). While our higher frequency stimulation experiments were not conducted in naïve animals notice any difference in excitability at the higher stimulation frequency across animals that were studied in the awake-anesthetized or anesthetized-awake groups. Why then would the nature of movement elicited by stimulation of a single region of the cortex on the one hand seem to be functional yet under other circumstances result in a repetitive orienting movement? One idea is that brief, repeated activation of the cortex at 5 Hz is triggering the beginning of a movement sequence that never finished because it was constantly interrupted. Short trains of high frequency stimulation also evoked brief twitches, but these movements were different from the sort of response elicited from 5 Hz stimulation in the awake animal, following barbiturate treatment. High frequency stimulation elicited subtle mouth and lip movements that were gradually elaborated into extended mouth opening head turning and arm movements with longer duration stimulation trains. This seems to suggest that movement is being activated in a fundamentally different fashion in these two circumstances. It is not surprising that stimulation of the motor cortex would result in movements, or that repeated stimulation would result in more elaborated functional movements, since the cortex is highly interconnected and extended stimulation may be able to recruit more distant networks of motor neurons perhaps even activating pattern generator networks (Yuste et al., 2005). The movements elicited during 5Hz stimulation, however, appear similar to the descriptions of movement activity elicited through direct

simulation of the dorsal striatum alone which are often described as contralateral orienting responses of the head and arms (Murer and Pazo, 1993). One possible explanation is that 5 Hz stimulation of the cortex that results in corticostriatal LTP makes future activation of this region at the same frequency more likely to facilitate some form of movement disinhibition via activation of the basal ganglia. Do movement routines elicited from stimulation of one region of the cortex typically match the movement routines elicited from stimulating regions of the striatum that receive these cortical projections? This could be investigated through studies with pairs of stimulating electrodes placed simultaneously in regions of the cortex and striatum with known connectivity. A potential finding might be that connected regions of the cortex and the striatum only elicit the same variety of movement during stimulation if stimulated at a low frequency and after low frequency stimulation of the cortex under conditions that drive corticostriatal LTP.

Anatomy of corticostriatal connections suggest that orienting movements and more elaborated reaching and grasping movements may be communicated via separate pathways from the cortex to the striatum. A large majority of the inputs from the cortex to the basal ganglia come from intertelencephalic neurons that project diffusely throughout the forebrain and the basal ganglia (Wilson 1987) rather than pyramidal tract neurons. In so far as the basal ganglia might play a role in the preparation of movement, intertelencephalic projections seem to be the most appropriate source of an input as a control signal for movement preparation. While pyramidal tract inputs descend through the striatum and make synapses in passing, their projections continue to brainstem and spinal motor neurons. Information conveyed from the pyramidal tract fibers to the basal

ganglia may deliver movement plan related information too late for the basal ganglia to use these inputs for anything prospective, as the movement signal is presumably already committed (Redgrave et al., 2008). Intertelencephalic projections, however, may play a more critical role in the preparation to move (Turner and DeLong, 2000) and orienting movement is a key component of reward acquiring behavior (Swanson, 2000). It is possible that a single region of the cortex may send two kinds of movement signals to the basal ganglia along different pathways. Preparatory signals to the basal ganglia via the intertelencephalic pathway may trigger orienting responses in order to prepare for more elaborate movement sequences while pyramidal tract projections inform the basal ganglia of what happened next in that movement sequence.

Cortical anatomy may support the proposition of separate coding of orienting and more elaborated movements in intertelencephalic and pyramidal tract neurons respectively. Connections between intertelencephalic neurons and pyramidal tract neurons within the cortex have a hierarchical order of projections, where intertelencephalic neurons send excitatory projections to each other. Pyramidal tract fibers only receive inputs from these regions (Morishima and Kawaguchi, 2006). However, as we discussed in an earlier section, some evidence suggests that the basal ganglia does not directly encode any kind of movement. In contrast we introduced the idea that a separate projection from the cortex projects directly to motor areas of the cortex and brainstem conveying specific information about a movement plan, while connections to the basal ganglia send a signal that facilitates or inhibit these movement centers. For example, Hikosaka *et al.* (2006) has proposed a model for eye movements where projections from the frontal eye field project to the basal ganglia and also send

corticobulbar projections that meet at a common target of the superior colliculus. A movement signal from the cortex attempts to control the superior colliculus but must awaits permission from the basal ganglia to allow a saccade. It was not clear from this model whether precise collaterals of the same neurons or separate projections from the same region activate the basal ganglia and superior colliculus respectively (Hikosaka et al., 2006). It is possible that intertelencephalic neurons projecting to the basal ganglia are responsible for movement facilitation and pyramidal tract neurons contain precise movement codes. Five Hertz stimulation of the cortex may succeed in releasing a movement center from inhibition but fail to relay an actual movement plan to a target region with homologous connectivity to the superior colliculus. One potential experiment that could test the selective effects of stimulation of the cortical and pyramidal tract projection fields may be feasible using optogenetic techniques, where cell type specific expression of optical switches that depolarize a selective class of cells (Airan et al., 2009). Intertelencephalic rather than pyramidal tract can be selectively activated using a laser to trigger activity rather than the less specific activation of passage fibers and cell bodies used by electrical stimulation. Perhaps repeated activation of intertelencephalic cells can broadly disinhibit movements with less specificity to the target or specific nature of the movement.

Future Directions: Persistent changes in behavior and corticostriatal plasticity

One of the challenges of studying dopamine's effects on learning and performance is the difficulty in disassociating effects of dopamine that may result from direct effects on membrane conductances in striatal neurons, and effects on corticostriatal

plasticity. We predicted from our experiments in chapter 4 that dopamine's effects on corticostriatal plasticity drive cumulative and persistent changes in behavior. We also argued that selective antagonists would be equally effective in antagonizing dopamine receptors in the direct and indirect pathway and should therefore be sufficient to drive corticostrial plasticity in these pathways separately. Based on results from Shen *et al.* (2008) we would predict that antagonism of D1 receptor expressing cells in the direct pathway would drive LTD and antagonizing D2 receptor expressing cells in the indirect pathway would drive LTP. In theory, if corticostriatal plasticity is a source of long term storage, the observed effects on choice and motivation should persist, however they did not. A critical experiment to address this issue would be to test an additional group using a combined dose of raclopride and SCH23390 that were used in these experiments. If the behavioral changes are cumulative and persistent then we could have more confidence that treatments with selective antagonists did affect corticostriatal plasticity. This result would strengthen our evidence that a separate downstream process was required as a site for persistent neurobiological change resulting in persistent changes in behavior.

A complementary approach to this question could attempt to isolate effects of a high dose of flupenthixol that are independent of effects on corticostriatal plasticity. Shen *et al.* (2008) also described additional molecular mechanisms other than dopamine receptor signaling that could alter plasticity selectively in direct and indirect pathway cells. Two drugs that they used would be of interest to add to a high dose of flupenthixol in additional groups of animals trained on our choice task. First AM251, an inverse agonist of CB1 receptors, is capable of selectively blocking LTD in D1 receptor expressing cells that have had dopamine depleted with reserpine. This could potentially

correct LTD that is predicted to occur in direct pathway cells in response to flupenthixol administration. Additionally, SCH58261, an adenosine A_{2A} receptor antagonist, could be used to selectively reverse LTP that occurs in D2 receptor expressing cells that have had dopamine depleted with reserpine. Three additional groups could potentially dissect effects of dopamine on corticostriatal plasticity from direct effects on membrane conductances. A group that has flupenthixol and AM251 could permit plasticity in the indirect pathway alone. A group with flupenthixol and SCH58261 could permit plasticity in the direct pathway alone. A final group with flupenthixol, AM251 and SCH58261 could permit only direct effects of dopamine on membrane conductances, blocking all plastic changes in synaptic strength. We would predict that groups that selectively permit plasticity in D1 or D2 receptor expressing cells could result in persistent and cumulative changes in choice that result from a selective increase in accuracy on ipsilaterally directed trials and a selective decrease in accuracy on contralaterally directed trials respectively, as was observed in temporary effects of D1 and D2 receptor antagonism respectively. A group with flupenthixol infused along with AM251 and SCH58261 may only show effects on reaction time which would indicate that these effects could be attributed to nonselective effects of dopamine antagonism.

Future Directions: Recommendations for analysis of individual reaction time distribution

There are several approaches towards the analysis of reaction time that may yield additional insight into the contribution of striatal circuits to psychological processes such as attention (Salamone and Correa, 2002; Brown et al., 2010) Mean reaction time is reported in several studies that provide a foundation for our work including experiments

by Dowd *et al.* (2004; 2007) that we sought to replicate. Reporting mean reaction time facilitates comparison to these studies and is a satisfactory approach to summarizing a reaction time distribution based upon a small number of trials. Ideally, one would want to compare the shape of the reaction time distributions directly for each animal across all testing sessions for each instructional cue. This level of analysis, however, typically requires a greater number of trials; according to one report, accurately fitting a parametric reaction time distribution data requires hundreds of trials for each condition (Ratcliff, 1979; Rouder et al., 2005; Whelan, 2010). In our studies, it was not uncommon for an animal to correctly complete fewer than 20 trials for a given cue when infused with muscimol or a selective dopamine antagonist; in treatments where accuracy and attempts were not strongly affected an exemplary animal could correctly complete 50-60 trials for a given cue within a single testing session.

Ideally, the experiment could be restructured so that more trials were completed within each training session. This adjustment could be made simply by shortening the variable intertrial interval which is currently set at 20 seconds on average (ranging from 15-25 seconds). When infused with vehicle or amphetamine, animals attempted approximately 140 trials within a one hour testing session. This is equivalent to attempting one trial every 26 seconds. While we did not collect data about the latency to initiate after the termination of an intertrial period, it seems reasonable to state that animals were initiating trials promptly and would increase the rate of attempts if the intertrial period was shortened. This would allow retesting for more accurate reaction time distributions and all trials would still be conducted at the same latency following drug infusion.

Two alternative approaches that are worth further investigation could provide a method for analysis of reaction time distribution in a small set of trials. The first technique generates a pooled reaction time distribution by averaging individual quantiles from a group of individual distributions. The procedure is similar in concept to comparing medians of individual reaction times. Ratcliff (1979) describes this method in detail and suggests that it is effective to pool individual distributions as small as 10 measurements but issues a caveat that the pooled distribution might not truly reflect individual distribution shape unless certain conditions are met. A more computationally challenging but potentially more effective approach presented by Rouder *et al.*(2005) estimates reaction time distribution parameters for small individual data sets using a hierarchical Bayseian model. The model generates parameters for scale, shift and shape for each individual distribution and can account for a range of parent distributions across individuals while preserving the shape of the individual distribution, unlike the pooled method. Comparing the shape of individual reaction time distributions is a challenging process, individual animals may have very distinct characteristic distribution shapes which can be lost in small sample sizes and in pooling methods, but may be preserved by using a hierarchical Bayseian model or adjusting experimental structure to accommodate collection of a much larger number of individual trials for each cue in each session, a process that may be directly undermined by testing with muscimol or selective dopamine antagonists. The benefits of accurately modeling a reaction time distribution allows for insight into additional processes that may have selective effects on short or long latency responses (Whelan, 2010). For example, in a study comparing reaction time distributions of children with ADHD to unaffected children, Hervey *et al.* (2006) found that children

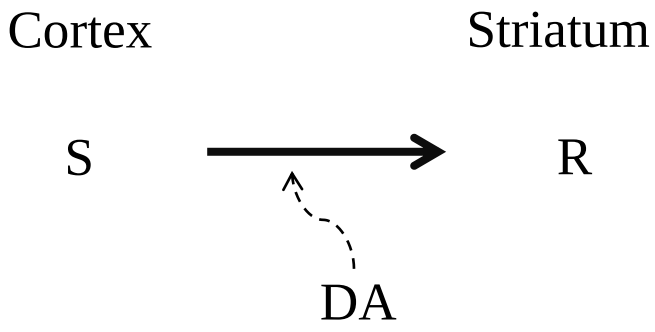
with ADHD respond more slowly on average to a computer based Go-NoGo task. However, after performing an analysis of the shape of individual reaction time distributions, found strikingly different results. ADHD children were actually faster than unaffected children in the normal portion of the distribution but much slower in the tail suggesting a lapse in attention in a subset of trials. It's possible that the shift in reaction time, that we observed, in animals receiving amphetamine infusion may be related to increased attentiveness (Salamone and Correa, 2002) to instructional stimuli which could be revealed through exploration of these analytical techniques.

Closing Remarks

In summary, the experiments described in this dissertation represent an attempt to advance understanding of the physiology of the striatum and the contribution of changes in dopamine signaling in the striatum to learning and performance of an instrumental task with an emphasis on the role of corticostriatal plasticity. We found that repeated activation of the corticostriatal pathway results in LTD, not LTP. This result suggested some insight into a controversy about basic rules of plasticity that has been in place for the last twenty years (Reynolds and Wickens, 2002; Di Filippo et al., 2009) and offers a framework for resolving conflicting results on basic rules of plasticity from STDP experiments (Fino et al., 2005; Pawlak and Kerr, 2008; Shen et al., 2008). Our second sets of experiments have yielded results more challenging to interpret and may require further experimentation before conclusive statements can be made. However, given some directly testable assumptions about the efficacy of our drug treatments, our results have the potential to yield deep insight into the role of dopamine dependent corticostriatal

plasticity in the long term storage of information critical to learning and performance of instrumental tasks. We hope that this line of inquiry might pique the curiosity of the future student, and continue this line of investigation.

A



B

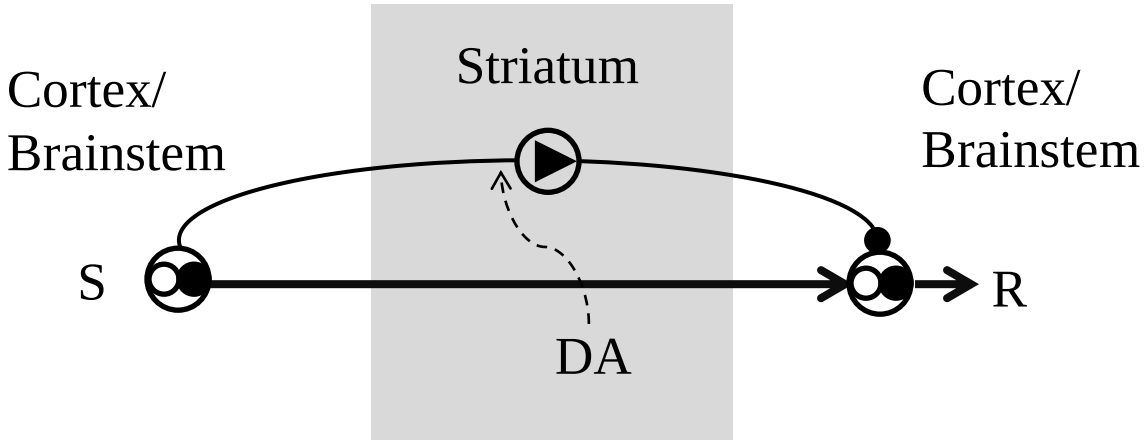


Figure 5.1 In chapter 1 we proposed a model of the striatum that uses dopamine to drive a process of stamping in connections between stimuli and response which may be a neurobiological correlate of associative learning (A). Based on theories of dopamine and corticostriatal plasticity in motivation and decision making we suggest a revised schematic that represents the choice of an S-R relationship that is presented to the striatum but where associative learning occurs outside of the striatum. Presentation of

relevant stimuli to the striatum may inhibit or facilitate motor centers that would implement the selected response. Dopamine acting on the corticostriatal pathway would have the ability to adjust the probability of performance of an S-R association (B).

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