Contributions of the Prelimbic Cortex and Basal Ganglia Circuitry to Proactive Behavioral Switching

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THESIS

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This thesis is dedicated to my wife, Laura. My friend, partner, and companion through life.

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5-CSRT	5-choice serial reaction task
ANOVA	analysis of variance
A-P	anterior-posterior
AP5	(2R)-amino-5-phosphonopentanoate
bac	baclofen
contra	contralateral
CPP	(3-[(R)-2-carboxypiperazin-4-yl]-prop-2-enyl-1-phosphonic acid)
D-AP5	d-(2R)-amino-5-phosphonopentanoate
DMStr	dorsomedial striatum
D-V	dorsal-ventral
g	gram
GABA	ɣ-aminobutyric acid
ip	intraperitoneal
ipsi	ipsilateral
kg	kilogram
M-L	medial-lateral
mL	milliliter
mg	milligram
mm	millimeter
mus	muscimol
n	number
NMDA	N-methyl-D-aspartate

PL	prelimbic cortex
STN	subthalamic nucleus
µg	micrograms
µL	microliters
µM	micromolar
Veh	vehicle
WCST	Wisconsin card sorting task

SUMMARY

Fundamental to survival is the ability to learn associations among stimuli, actions, and outcomes, as well as being able to switch between learned associations as the environment changes. There are two fundamental conditions that initiate a switch in choice patterns. One condition is retroactive switching in which a recent change in outcomes, e.g. positive to negative feedback, indicates a shift in choice patterns should occur. In these conditions, the prior outcome information, e.g. absence of reward, should be used to initiate a switch in choice patterns for the current trial. The other condition, termed proactive switching, involves a change in cue information indicating that a switch in choice patterns should occur for an upcoming decision. In these conditions, presentation of a cue can accurately guide a decision by indicating which choice pattern should be selected. There is substantial evidence that frontal cortex-basal ganglia circuitry supports behavioral switching when a response pattern is no longer reinforced. Less is known about whether specific frontal cortex-basal ganglia circuitry supports proactive switching when explicit cues signal that a change in a behavioral response should occur.

The present experiments investigated the role of the prelimbic cortex, subthalamic nucleus, and the dorsomedial striatum in male Long-Evans rats during proactive switching between learned visual cue-place associations. Additional experiments examined the connections between the prelimbic cortex and its projections to both the subthalamic nucleus and dorsomedial striatum by employing a disconnection design. In a cross-maze, rats learned a conditional discrimination in which a start arm cue (black or white) signaled which one of two maze arms to enter for a food reward. The cue was

switched every 3-6 trials. Baclofen and muscimol infused into the prelimbic cortex significantly impaired performance by causing rats to adopt an inappropriate egocentric turn bias. N-methyl-d-aspartate (NMDA) receptor blockade in the subthalamic nucleus significantly impaired performance by increasing switch errors and errors immediately following the switch. Contralateral disconnection of these areas matched the effects observed with subthalamic NMDA receptor blockade. These findings suggest that the prelimbic area and subthalamic nucleus support the use of cue information to facilitate inhibition of an ongoing choice pattern.

Additional experiments examined the role of NMDA receptors within the dorsomedial striatum and the connection between the prelimbic cortex and dorsomedial striatum in the same cue – place conditional discrimination task. Results confirmed those observed with prelimbic cortex inactivation in the previous set of experiments. Further, they revealed that dorsomedial striatum NMDA receptor blockade caused in impairment in performance by increasing the likelihood of a rat to miss an entire block of trials for one of the discriminations. Similarly, disconnection of the prelimbic cortex and dorsomedial striatum also caused in increased propensity to miss an entire trial block. These results indicate that the connection between the prelimbic cortex and dorsomedial striatum is critical for selection of the appropriate behavior when a rat is required to switch between conditions repeatedly within a single session.

Overall this set of experiments reveals some of the neural circuitry involved in switching between strategies when cues are present to guide behavior. Specifically,

they suggest that the prelimbic cortex through its connections with the subthalamic nucleus and dorsomedial striatum is critical for the generation and selection of appropriate strategies when required to switch between multiple options. The prelimbic cortex is required for the generation of appropriate strategies based on cue information. When the prelimbic – subthalamic projection dominates, this allows for the inhibition of an ongoing behavior. When the prelimbic – dorsomedial striatum connection is active, it facilitates selection of the appropriate strategy. Together, these connections are critical for the execution of switching behavior when cues can be used to change choice patterns.

Chapter I

Introduction

A. Behavioral Adaptations to Changes in Environmental Demands

The environmental conditions of a wide variety of animals can change in predictable and unpredictable ways. These changes may require an animal to adapt new or different strategies in order to successfully adjust to the new environmental contingencies. For example, driving into work an unexpected accident ahead on the highway could require switching to a different route than is normally taken. Seeing the accident and switching from the normal route before getting stuck in a traffic jam could save being late into the office. Likewise, a squirrel may encounter a lack of food resources in an area where it normally finds plenty. This lack of reinforcement informs the squirrel to switch to a new area of foraging in order to survive. In the human animal and non-human animal learning literature, the term cognitive flexibility is often used when describing the capacity to switch a strategy or choice pattern with a change in environmental contingencies (Foreman et al., 1990; Boutet et al., 2005; Ebbesson and Braithwaite, 2012). In the examples described above, animals may use changes in outcome information to flexibly switch choice patterns or use cue information to proactively switch choice patterns.

Wise and colleagues (1996) have proposed that the frontal cortex and basal ganglia are specialized in a cooperative manner to inhibit using learned rules and to acquire new rules when environmental contingencies change. Their proposal is principally based on how separate frontal cortex and basal ganglia areas differentially contribute to

cognitive flexibility under two different learning conditions. One condition requires learning to a make a choice based on particular attribute or stimulus information, e.g. always entering one of two spatial locations and then reversing what spatial location is entered. Thus, the rule is to always choose based on particular attribute information, but the specific choices for that attribute may change. A second condition also requires learning to a make a choice based on particular attribute or stimulus information, but now a subject must reject a choice based on the originally learned attribute information and must instead make a choice based on different attribute information. For example, a subject may switch from always choose the object on the right (choice based on location), to always choose the blue object (choice based on object color). This learning condition is called strategy switching or set-shifting. Various paradigms have been employed to study these two types of cognitive flexibility. Commonly in these tasks, a change in outcome information, e.g. lack of reinforcement, has been manipulated to signal that a change in choice patterns should occur. Overall, there is significant evidence to suggest that separate frontal cortex and basal ganglia regions support cognitive flexibility under these two conditions (Beckwith and Tinius, 1985; Gorrindo et al., 2005; Shafritz et al., 2005; Clarke et al., 2008; Weed et al., 2008; Brown et al., 2010; Castane et al., 2010; Rygula et al., 2010; Baker et al., 2011).

To further understand what processes different brain circuitry supports to enable various forms of cognitive flexibility, several studies have also investigated the error pattern that occurs in reversal learning and set-shifting tests following an experimental manipulation. These studies have provided insight into the processes that different neural regions support to enable cognitive flexibility. In both reversal learning and set-

shifting tasks, one possibility is that an impairment results from the inability of a subject to *initially* abandon the previous response pattern in favor of a new one. This type of error pattern is commonly referred to as perseveration (Lawrence et al., 1999; Kim and Ragozzino, 2005; Castane et al., 2010). Although different operational definitions have been used to define perseveration, the various definitions all focus on errors made before the subject demonstrates an abandonment of the previously choice response pattern (Kim and Ragozzino, 2005; Castane et al., 2010; Baker et al., 2011; Dalton et al., 2011). If manipulation of a brain area, e.g. pharmacological inactivation, leads to increased perseverative responding, it is commonly interpreted as the area being important for the ability to abandon a previously relevant choice pattern and/or the ability to generate a new choice pattern (Kim and Ragozzino, 2005; Block et al., 2007). Alternatively, subjects could fail to reliably execute the new contingencies later in the task after beginning to choose the new correct choice pattern or strategy. This type of error is commonly termed regressive or maintenance errors (McCool et al., 2008; Brown et al., 2010). In this case, increased maintenance errors after a brain manipulation is usually interpreted as the area being critical for the maintenance or reliable execution of the newly relevant response (McCool et al., 2008; Brown et al., 2010). Therefore, although reversal learning and strategy switching test two different types of cognitive flexibility, both require the ability to abandon the previous contingencies in favor of a new one. They also commonly rely on reward feedback from previous trials to guide future responses. Furthermore, insights into how a brain area might be involved in cognitive flexibility (e.g. perseveration on the previous strategy vs. inability to reliably execute the new one) can be achieved by analyzing the types of errors in both tasks.

B. The Contributions of the Frontal Cortex to Cognitive Flexibility

Located anterior to the central sulcus and dorsal to the lateral sulcus in the front of the brain, the frontal lobe has been connected with various forms of cognitive function "since ancient times" (Bianchi, 1895).Toward the end of the 19th century, however, more controlled experiments were beginning to quantify specific changes in a variety of cognitive functions after loss of the frontal lobes. Leonardo Bianchi, the Italian neuropathologist, conducted a series of studies examining lesions of the frontal lobe in both monkeys and dogs. Through these experiments, he concluded that memory, attention, the ability to formulate a plan, and focal consciousness relied on the frontal lobe (Bianchi, 1895). In an attempt to summarize his findings, Bianchi concluded that the frontal lobe was the seat of intelligence, emotion and higher order cognitive functions in which the formation of a plan is required and cannot be accomplished through instinct (Bianchi, 1895). The frontal lobe, however, is not a homogenous region and separate subregions may support various functions. Since the time of Leo Bianchi's book there has been increasing interest in how specific frontal cortex subregions may differentially contribute to a variety of functions. In the 20th century, as there was a greater understanding of brain anatomy and more experimental tools available, studies further refined which frontal cortex areas are important for specific cognitive functions. In particular, there became a focus on the more rostral areas of the frontal cortex, referred to as prefrontal cortex, as an area critical for cognitive flexibility.

The prefrontal cortex is a heterogeneous region of the neocortex which has lacked a clear definition from the beginning. In 1909, Brodmann extensively divided the entirety of the cortex into distinct areas (Brodmann's areas) based on morphological differences

observed in serial stained sections of human brains (Brodmann, 1909). He defined the prefrontal cortex as areas which are granular in nature. However, later definitions of the prefrontal cortex were developed as agranular prefrontal cortex areas which seemed to share functionality with the granular prefrontal cortex were identified. For example, Rose and Woolsey (1948) defined the prefrontal cortex as the frontal lobe region which receives thalamic input exclusively from the mediodorsal thalamus. Specifically, based on the delineation of projections originating in various areas of the mediodorsal thalamus, the prefrontal cortex was divided into the dorsomedial area located medially and anterior to the corpus callosum, and the ventrolateral area located dorsal to the rhinal sulcus (Leonard, 1969). As retrograde and anterograde tracing techniques improved, the former definition of the prefrontal cortex became insufficient due to other thalamic areas showing projections to areas originally thought to exclusively receive mediodorsal input such as the midline and intralaminar thalamic nuclei (Jones and Leavitt, 1974; Berendse and Groenewegen, 1991; Shibata, 1992). The definition of the prefrontal cortex now largely agreed upon stipulates that inclusion requires a prominent connection with the mediodorsal thalamus as wells as receives multimodal input from other cortical areas (Preuss, 1995; Uylings et al., 2003).

The importance of the prefrontal cortex for cognitive flexibility became apparent with the rise in prefrontal lobotomies for the treatment of psychiatric disorders such as schizophrenia in the 1930's. By the mid 1940's a consensus was growing that in both monkeys and humans, lesions of the frontal lobes led to deficits in the ability to maintain attention, plan future actions, and switch from one behavioral plan to another (Jacobsen and Nissen, 1937; Carmichael and Carmichael Jr, 1942; Robinson, 1946). For example,

set-shifting, through the use of the Wisconsin Card Sorting Task (WCST) which requires sorting cards based on number, color, or shape, found impairments in humans and monkeys lacking a prefrontal cortex (Stanley and Jaynes, 1949; Milner, 1963). Gross lesions of the prefrontal cortex were also found to disrupt reversal learning while not affecting the learning of an initial discrimination in monkeys (Harlow and Dagnon, 1943; Settlage et al., 1948). However, it became clear that the sub-regions of the prefrontal cortex were differentially involved in various types of cognitive flexibility. This was found through investigations in which lesions to some areas but not others led to deficits observed with whole prefrontal cortex lesions (Goldman and Rosvold, 1970; Mishkin and Manning, 1978; Rosenkilde, 1979).

Studies in non-human primates revealed that reversal learning deficits occurred most consistently with orbitofrontal cortex lesions, but not dorsolateral prefrontal cortex lesions (Mishkin, 1964). Further, these deficits were found to be multimodal in nature with the impairments observed with both object and place reversals (Jones and Mishkin, 1972). In humans, orbitofrontal cortex damage did not impair set-shifting tests, but dorsolateral prefrontal cortex damage did impair set-shifting (Milner, 1963). This dissociation between these areas in reversal learning and set shifting has been confirmed in later studies. Specifically, lesions of the medial portion of the prefrontal cortex in monkeys led to impairments in set-shifting while orbitofrontal lesions did not affect performance (Dias et al., 1996, 1997). On the other hand, reversal learning was unaffected by medial prefrontal lesions but was impaired by orbitofrontal lesions (Dias et al., 1996, 1997). These studies have served to offer insight into how various brain areas differentially contribute to cognitive flexibility.

The dissociation of function between prefrontal areas such as those described above has been advanced through studies in rodents. Early work in rats led to several discoveries about the role of the frontal lobes in learning and cognitive flexibility. For example, Karl Lashley (1921) reported that even extensive frontal lobe lesions did not affect the performance of well-learned discriminations in maze tasks. Subsequent studies reported that frontal lobe lesions also do not affect learning and retention of various discrimination tests, but do impair reversal learning or extinction of a learned discrimination (Hamilton and Ellis, 1933; Bourke, 1954; Thompson and Langer, 1963; Divac, 1971). These studies across the first half of the 20th century and leading into the 2nd half of the 20th century performed non-specific lesions that commonly damaged multiple subregions of the rodent prefrontal cortex. Thus, unknown from these early studies was whether specific prefrontal cortex subregions support specific types of cognitive flexibility.

Like the primate brain, the rodent prefrontal cortex can be subdivided based on structure and connectivity. The medial area consists of the infralimbic cortex, the prelimbic cortex (PL), the anterior cingulate cortex, and the medial precentral areas (Figure 1). These delineations are based on the architectural makeup of the cortical layers as well as the thalamic projections that each area receives. Located centrally is the PL which is comparable to Brodmann's areas area 24 and 32 (Uylings and van Eden, 1990). The PL is densely interconnected with other areas of the prefrontal cortex (Eden et al., 1992; Heidbreder and Groenewegen, 2003). It also sends projections to the dorsomedial striatum (DMStr) as well as the subthalamic nucleus [STN] (Sesack et al., 1989; Gabbott et al., 2005). Additionally, the PL is one of the few brain areas that

has reciprocal connections with the majority of the neuromodulatory neurotransmitter systems of the brain. Specifically, it has reciprocal projections with the ventral tegmental nucleus and substantia nigra pars compacta, the major dopaminergic neurons of the brain; the dorsal and median raphe nuclei, the serotonergic cells of the brain; the locus coerleous, the primary source of noradrenergic input to the brain; and the nucleus basalis as well as the brainstem cholinergic nuclei, two major acetylcholine systems (Vertes, 2004; Boix-Trelis et al., 2006; Hoover and Vertes, 2007). The connections of the PL with limbic and motor areas of the brain as well as its interconnections with the majority of the neuromodulatory systems of the brain suggest that it may play a critical role in the coordination of complex behavior such as is required in cognitive flexibility.

As neurotoxic lesions and intracranial drug injections began to be used more commonly, studies began to systematically examine the role of the PL in cognitive flexibility. Ragozzino and colleagues (1999a), infusing a local anesthetic aimed at the prelimbic area demonstrated that PL inactivation did not impair initial learning of a place or egocentric response discrimination, but did impair performance when rats had to switch between using these strategies. Furthermore, PL inactivation did not impair place or response reversal learning. Subsequent studies demonstrated that PL lesions or inactivation impaired strategy switching, but not reversal learning across a variety of different stimulus attributes (Ragozzino et al., 1999c; Birrell and Brown, 2000; Ragozzino et al., 2003).

PL inactivation leading to strategy switching deficits resulted from perseveration of the previously learned strategy, but did not affect the maintenance of the new strategy after initially selected. (Ragozzino et al., 1999b; Ragozzino et al., 1999c; Ragozzino et

al., 2003). Thus, in set-shifting tests in which there is a change in outcome information to signal that a learned strategy is no longer reinforced and another strategy should be selected findings suggest that the PL supports the initial inhibition of a previously learned strategy and selection of the currently, relevant strategy.

C. Proactive vs. Retroactive forms of switching

The cognitive flexibility tests described above in humans, non-human primates, and rodents required a subject to use a change in outcome information, e.g. positive reinforcement or negative reinforcement, to switch a choice pattern or maintain a choice pattern. Hikosaka and Isoda (2010) have described these tests as requiring retroactive behavioral switching. This is because prior outcome information, e.g. absence of reward, should be used to initiate a switch in response for the current trial. Hikosaka and Isoda (2010) have also proposed that there is a second basic condition that requires behavioral switching. This is referred to as proactive behavioral switching. In these conditions, cue information is presented in each trial that accurately informs which choice pattern should be selected. The cue information can switch across trials indicating that a behavioral switch should occur. Proactive switching, therefore, involves a change in cue information indicating that a switch in response patterns should occur for an upcoming decision.

D. Role of the Prefrontal Cortex in Proactive Switching

One type of task involving a proactive switch is a conditional discrimination test which was used in some early rodent learning studies by Karl Lashley (1938). In conditional discrimination tests, there are at least two different contingencies that require different responses. This may involve the presentation of an auditory stimulus in

a two lever choice test that requires pressing a left lever for a food reinforcement while the presentation of a different auditory stimulus requires pressing the right lever. In these tasks, the stimulus is switched pseudo-randomly commonly after 1-3 trials. More recent work has modified the conditional discrimination task to feature a less predictable switch from one condition to the other. Instead of the switches occurring at most after two or three consecutive trials, switches occur less often with the same stimulus presented up to 10 consecutive trials (Isoda and Hikosaka, 2007). When proactive switches are conducted in this manner, evidence suggests that a behavioral set is formed and switch trials are more difficult than non-switch trials due to repeating a given response for several trials (Sudevan and Taylor, 1987; Meiran, 1996; Monsell et al., 2003).

To date, there is not significant evidence that the PL supports proactive behavioral switching in conditional discrimination tests. However, studies examining contributions of the prefrontal cortex to conditional discrimination performance in rats have largely utilized neurotoxic lesions prior to training with performance measured during acquisition, retention and reversal of the task (Bussey et al., 1996, 1997; Chudasama et al., 2001). Furthermore, all of these experiments have used brief trial blocks, e.g. 1-3 trials, before switching the cues. Under these conditions, lesions of the PL have not been found to affect learning or performance of a conditional discrimination task (Delatour and Gisquet-Verrier, 1999; Chudasama et al., 2001).

An alternative is that the PL does support proactive behavioral switching, but only when longer trials blocks occur before a behavioral switch is required. None of the previous rodent work has sought to examine switch costs associated with going from a

block of one discrimination type to the other. Based on evidence that the PL is critical for switching from one strategy to another when reinforcement contingencies change (Ragozzino et al., 1999c; Ragozzino et al., 2003; Young and Shapiro, 2009), it may also be important for switching between discriminations when cued to do so in a repeated manner. Furthermore, as described earlier, Wise and colleagues (1996) have proposed that the prefrontal cortex and basal ganglia act as part of a larger neural system to support cognitive flexibility. As the PL has major projections to different basal ganglia areas, it may functionally interact with specific basal ganglia areas to support proactive switching. The central aim of these experiments is to investigate whether the PL functionally interacts with different basal ganglia areas to enable proactive switching.

E. Prefrontal Cortex Projections to the Basal Ganglia

One prominent projection of the PL is to the striatum which is the main input area of the basal ganglia (Sesack et al., 1989; Conde et al., 1995; Gabbott et al., 2005). In the striatum 90%-95% of the neurons are GABAergic projection neurons known as medium spiny neurons based on their morphology (Kemp and Powell, 1971b, a; Graybiel, 1990; Smith et al., 1998). The cortex, midbrain and thalamus project topographically to basal ganglia. The heterogeneity of this area based on these inputs has led to its division into several distinct regions including the dorsomedial and dorsolateral portions as well as the ventral regions; the nucleus accumbens shell located medial and ventral to the nucleus accumbens core. The DMStr in particular, receives the majority of projections from the PL to the basal ganglia (Sesack et al., 1989; Conde et al., 1995; Gabbott et al., 2005). Medium spiny neurons in turn project to the output regions of the basal ganglia which play an important role in motor behavior (Bateup et al., 2010). Based on its

position between the PL and motor output, the DMStr represents one area that may be critical as an integrator of PL cortical signals with other thalamic and midbrain input to control goal directed behavior when conditions require flexible responding.

The other main projection from the PL to the basal ganglia is to the STN (Berendse and Groenewegen, 1989; Maurice et al., 1998). Projections from the PL have a profound influence on the properties of the STN (Ryan and Clark, 1991; Maurice et al., 1998). A tri-phasic response in the STN is observed in rats when the cortex is stimulated consisting of a strong short latency excitation, a brief inhibitory phase, and a second broader excitation (Kitai and Deniau, 1981; Ryan and Clark, 1991; Maurice et al., 1998; Nambu et al., 2000; Magill et al., 2006; Bosch et al., 2012). Maurice and colleagues (1998) found that the direct ipsilateral projection from the PL to the STN is responsible for the early excitation in the STN, as stimulation of other afferent input did not produce this early excitation signal. A brief inhibitory response follows the early excitation that results from inhibitory input from the ventral pallidum, while the second excitation emanates from medial parts of the striatum (Maurice et al., 1998). The nature of the excitatory input from the frontal cortex into the STN has also been examined. In monkeys, application of the N-methyl-d-aspartate (NMDA) receptor antagonist 3-((R)-2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) into the STN results in abolition of the early excitatory phases of the response in the substantia nigra pars reticulata, a downstream target of the STN (Nambu et al., 2000). Taken together, the results suggest that there is a direct projection from the PL to the STN, stimulation of the PL produces a characteristic excitatory response and direct frontal cortex input to this area is in part

mediated by NMDA receptors. This suggests that blockade of the NMDA receptor may represent one method for examining cortical input into the STN.

F. The Role of the Dorsomedial Striatum in Cognitive Flexibility

Because the PL prominently projects to the DMStr, one possibility is that the PL and DMStr functionally interact to support cognitive flexibility. Using retroactive switch tests in which a change in outcome information signals a shift in choice patterns should occur, several studies have demonstrated that the DMStr is important for cognitive flexibility. Comparable to findings with the PL, DMStr inactivation impairs performance when required to switch strategies between an egocentric and or visual cue based discrimination (Ragozzino et al., 2002b). Unlike PL lesions, DMStr lesions or inactivation also impairs reversal learning (Pisa and Cyr, 1990; Ragozzino and Choi, 2004). Because NMDA receptors support synaptic plasticity (Spencer and Murphy, 2000a; Boettiger and Doupe, 2001; Akopian and Walsh, 2002b; Dang et al., 2006), the role of these receptors in the DMStr related to cognitive flexibility has been examined. As was observed with DMStr inactivation or lesions, infusion of the NMDA receptor antagonist (2R)-amino-5-phosphonovaleric acid (AP5) into the DMStr impaired reversal learning but not acquisition (Palencia and Ragozzino, 2004). In contrast to PL inactivation effects on cognitive flexibility tests, DMStr manipulations do not increase perseveration, but lead to a selective deficit in maintaining the new strategy or choice pattern after being initially selected (Ragozzino et al., 2002b; Palencia and Ragozzino, 2004; Ragozzino and Choi, 2004). These results suggest that the DMStr acts in a distinct and possibly complementary manner to support cognitive flexibility when

changes in outcome information informs the subject that the response pattern must be changed (Ragozzino, 2007).

Although there is substantial evidence that the DMStr supports cognitive flexibility based on retroactive switching tests, less clear is whether this area is involved in proactive switching. As in studies of conditional discrimination performance examining the PL, DMStr examination has occurred using lesions performed before acquisition of a discrimination which is switched every 1-3 trials (Featherstone and McDonald, 2004, 2005). Tasks using this procedure have not examined the effect of switch costs on performance and have failed to find an effect on conditional discrimination performance with lesions of the DMStr (Featherstone and McDonald, 2004, 2005). One possibility is that like with tasks examining the PL in a conditional discrimination, the DMStr is similarly selectively engaged when switches in behavior are administered in longer blocks of trials in which a switch cost is incurred. This is based on evidence from retroactive switching tests which have found that DMStr manipulation results in an inability to reliably perform a new choice pattern when contingencies change (Ragozzino et al., 2002b; Palencia and Ragozzino, 2004; O'Neill and Brown, 2007). Furthermore, because the PL projects to the DMStr (Sesack et al., 1989; Conde et al., 1995; Gabbott et al., 2005), the PL – DMStr projection may also be critical for proactive switching when conditions likely lead to an establishment of a response set.

G. The Role of the Subthalamic Nucleus in Cognitive Flexibility

Another basal ganglia area that the PL projects to is the STN (Berendse and Groenewegen, 1989; Maurice et al., 1998). There have been almost no studies in rodents investigating the STN in cognitive flexibility. One found that STN lesions do not

impair acquisition or reversal learning in a lever press task that used a Go/NoGo procedure (El Massioui et al., 2007). Despite the paucity of STN studies in rodents, there is evidence from studying Parkinson's disease patients suggesting that the STN supports cognitive flexibility. Specifically, Parkinson's disease patients who have undergone implantation surgery of stimulating electrodes in the STN either improved on the WCST following STN stimulation (Page and Jahanshahi, 2007; Herzog et al., 2009), or worsen following STN stimulation (York et al., 2008) . The conflicting results from these studies are most likely due to differences in stimulation parameters as well as electrode location (McIntyre et al., 2009; York et al., 2009; Daniels et al., 2010). They do, however, suggest that the STN may play a role in cognitive flexibility during a retroactive switching condition.

Recent findings in non-human primates suggest that the STN may also play a role in proactive switching. In particular, recording from STN neurons in a proactive saccade switching paradigm revealed that STN neurons increased their firing rates when the monkey was required to switch from an ongoing block of trials to a new one (Isoda and Hikosaka, 2008). Additionally, a majority of responding neurons changed their patterns before initiation of the new response pattern suggesting that they may inhibit the previous strategy and allow the proper action to be selected. One possibility is that the PL and STN functionally interact to inhibit an ongoing choice pattern when cue information indicates a switch in choice patterns should occur for an upcoming trial.

H. Contralateral Disconnection Method to Investigate Prefrontal Cortex and Basal Ganglia Structures

The findings described above suggest that both the PL and basal ganglia structures support cognitive flexibility when a retroactive behavioral switch is required. Less is known about whether these brain areas support proactive switching when behavioral sets are established. Moreover, there is not a clear understanding whether the PL - DMStr, and the PL - STN may act together to support proactive switching. One method used to determine whether two brain areas are necessary to support a particular behavioral function is the contralateral disconnection approach (Gaffan et al., 1988; Everitt et al., 1991). In this design, a unilateral lesion occurs in one brain area and a unilateral lesion is performed in a second brain area, but in the contralateral hemisphere. With these conditions, if projections from one area to another area are ipsilateral, then the connection between the two areas is effectively disrupted in both hemispheres. Performance in a group which receives lesions in the same hemisphere to both areas is also conducted, termed an ipsilateral disconnection. If this group is unaffected, then the effects of the contralateral disconnection are not likely due to loss of function in either site with a unilateral lesion, or due to mass action of two sites being unilaterally lesioned. More recently, disconnections have been performed using temporary inactivation of the target areas (Jo and Lee, 2010; Gilmartin et al., 2012). This offers the advantage of an acute disconnection and the ability to perform repeated testing within the same animal.

This design has been used to test medial prefrontal connections with both the DMStr and the STN during a task of sustained attention (Christakou et al., 2001; Chudasama et al., 2003). The projections from the PL to both the STN and DMStr are ipsilateral in nature (Afsharpour, 1985; Berendse and Groenewegen, 1989; Sesack et al., 1989;

Canteras et al., 1990; Conde et al., 1995; Gabbott et al., 2005). Therefore, the disconnection design offers an ideal method by which to examine if these areas are functionally connected during behavior. Investigating the effects of a contralateral disconnection of the PL and STN and the PL and DMStr in a conditional discrimination test that requires a rat to establish a choice pattern before a behavioral switch can provide novel insights into how prefrontal cortex and basal ganglia areas interact to affect proactive switching.

I. Experimental Aims

The goal of the experiments was to determine whether the PL and STN areas functionally interact to support proactive switching (Chapter 2) and whether the PL and DMStr functionally interact to support proactive switching (Chapter 3). This was addressed by employing a contralateral disconnection of the brain areas and investigating the effect in a visual cue – place conditional discrimination task. As a comparison to the contralateral disconnection of the two brain areas, a bilateral manipulation of each brain area was performed in the same conditional discrimination test. The use of the conditional discrimination test required multiple behavioral switches within a session and thus the constant monitoring of cue information provided in each trial. A behavioral switch occurred after every 3-6 trials allowing for the formation of a behavioral set prior to switching to the alternative. This behavioral paradigm also permitted an analysis of errors committed when switching between blocks and within a block of trials to determine what process or processes a particular brain manipulation affected.

One aim was to determine whether the PL and STN support proactive behavioral switching by investigating the effects of PL inactivation, NMDA receptor blockade in the STN, and disconnection of these areas in a conditional discrimination test. Previous experiments have not examined the PL during conditional discriminations when each condition is given in blocks of trials. If the PL is important for monitoring of task demands on a trial by trial basis, then an increase in errors should be observed on both switch trials as well as trials later in a block. If, however, the PL is serving a similar role in proactive switching as in strategy switching under retroactive conditions, then a selective increase in switch and trials immediately after a switch (perseverative) should be observed. More evidence supports the STN in proactive forms of switching when trials are given in blocks (Isoda and Hikosaka, 2008). If this is also true for a visual cue – spatial discrimination then an increase in switch errors should be observed. If the STN is important for overriding of an ongoing behavior on a longer time frame than just rapid switches, then perseveration after the switch error should also be observed. Finally, the contralateral disconnection will also provide insight into whether the connection between these areas is also important for proactive switching, or if these areas function is independent of one another.

A second aim was to determine whether the PL and DMStr support proactive switching during the conditional discrimination task. The DMStr is involved in both set-shifting as well as reversal learning when retroactive information must be used to guide behavior, although the error pattern is dissociated from prefrontal areas (e.g. maintenance instead of perseverative errors). Less support exists for a role of this area in proactive switching, although it has not been examined when trials are given in longer

blocks. If the DMStr is engaged in proactive switching in a similar manner to its role in retroactive forms of switching, then an increase in maintenance errors should be observed. If, on the other hand, it is instead involved in monitoring of cues to guide switches in an ongoing behavior, then an increase in switching errors and errors throughout the block should be observed. As with PL – STN disconnection, if the PL and DMStr are functionally connected, impairments in performance should be observed, revealing the contribution of this connection to proactive switching performance.

Figure 1

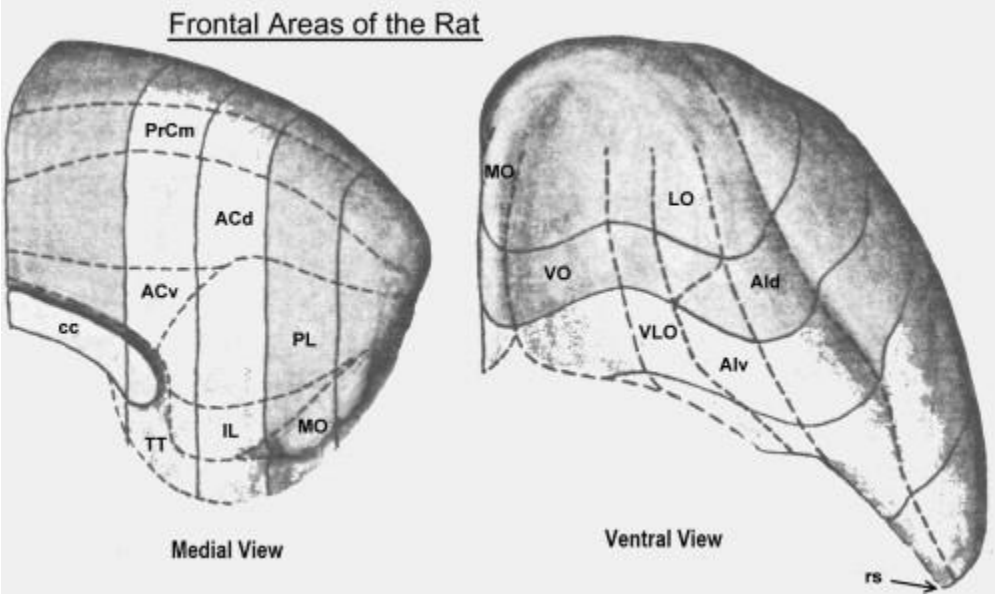

Figure 1. Frontal areas of the rat: A. Medial view. B. Ventral view. Abbreviations: PrCm – precentral cortex; AC – dorsal and ventral anterior cingulate; PL–IL – prelimbic and infralimbic cortex; MO – medial orbital cortex; AI – dorsal and ventral agranular insular cortex; LO – lateral orbital cortex; VO – ventral orbital cortex; VLO – ventrolateral orbital cortex. Reprinted with permission from Elsevier (Kesner and Churchwell, 2011).

Chapter II

The Prelimbic Cortex and Subthalamic Nucleus Support Proactive Behavioral Switching

A. Introduction

Hikosaka and Isoda (2010) have proposed that proactive and retroactive switching represent two fundamental conditions that initiate a change in response patterns. There is significant evidence in non-human primates and rodents that the PL supports behavioral switching when a learned response pattern is no longer reinforced and must be supplanted by an alternative one [retroactive switching] (Stefani et al., 2003; Rudebeck et al., 2008; Young and Shapiro, 2009; Oualian and Gisquet-Verrier, 2010). In particular, infusion of local anesthetics, GABA agonists, or NMDA receptor antagonists into the rodent PL impair strategy switches when a response pattern based on particular stimulus information e.g. spatial, is no longer reinforced and a different pattern based on other stimulus information, e.g. odor, is now reinforced (Ragozzino et al., 1999c; Birrell and Brown, 2000; Ragozzino et al., 2003; Stefani et al., 2003). However, the PL does not appear to be broadly involved in retroactive switching, as lesions or temporary inactivation of the area does not affect reversal learning (Ragozzino et al., 1999b; Birrell and Brown, 2000; Ragozzino et al., 2003).

A common procedure in retroactive switching tests requires a subject to learn a discrimination across a large number of trials within a session or across several sessions. Subsequently, the learned response is no longer associated with a positive outcome and a subject must now learn to select an alternative response pattern to

receive a positive reinforcement. These procedures have not only allowed a determination of whether particular brain areas contribute to retroactive switching, but also affords an analysis of error patterns to reveal the processes that a brain area supports to enable behavioral switching. In the case of the PL, several studies have demonstrated that manipulations of the PL impair strategy switches, as described above, by increasing perseveration but not an inability to maintain a currently relevant response (Ragozzino et al., 1999b; Ragozzino et al., 1999c; Dias and Aggleton, 2000; Ragozzino, 2002; Ragozzino et al., 2003). Taken together, the findings from past studies indicate that when retroactive information about outcomes must be used to enable a behavioral switch, the prelimbic cortex selectively supports strategy switching by initially inhibiting the previously learned response pattern.

In contrast to retroactive switching, there has been significantly less examination of whether the PL supports behavioral switching when stimulus or contextual information can be used proactively to shift responses. A previous study found that neurotoxic lesions of the prelimbic cortex do not affect acquisition or reversal learning of a visual conditional discrimination test in which visual cue information must be used to proactively select the correct response (Chudasama et al., 2001). However, a study by Dunn and Killcross (2007) employing temporary inactivation of the PL in a conditional discrimination test led to findings suggesting that the PL may support proactive switching. In this study, rats learned that different visual or auditory cues were associated with particular operant responses in distinct contexts. During extinction testing, rats were presented with combined visual and auditory cues in which the cues were associated with incongruent responses and a rat needed to use contextual

information to select the appropriate response. PL inactivation selectively impaired performance only when rats had to use contextual information to select the correct operant response. This finding suggests that the PL is critical for using contextual cue information to proactively select a response pattern. However, this study did not determine whether the PL is important for using cue information proactively to allow a behavioral switch when allowed to form a set during a block of trials. In a related manner, unknown is whether the PL supports a similar process, e.g. inhibiting perseveration of a previously relevant response pattern, to enable proactive switching.

The PL has extensive projections to basal ganglia structures and may act in a cooperative manner to facilitate behavioral switching (Chudasama and Robbins, 2006; Kehagia et al., 2010; Jahfari et al., 2011). The STN is one basal ganglia area that receives direct excitatory input from the PL that is mediated, at least in part, by NMDA receptors (Maurice et al., 1998; Nambu et al., 2000; Magill et al., 2006). Related to proactive switching, individual neurons in the primate STN show increased activity in response to a cue that signals when a switch from one behavioral response to another will be rewarded (Isoda and Hikosaka, 2008). In this task, cues were given in blocks ranging in length from 1-10 trials. Thus, the STN may enable rapid switches when cue information is to be used proactively to go from an ongoing or automatic, to an alternative response. Because past studies indicate that the prelimbic cortex projects directly to the subthalamic nucleus and both brain areas are suggested to be involved in behavioral switching, I hypothesized that a contralateral disconnection of these two brain areas would impair performance in a conditional discrimination by increasing errors on switch trials when cues are switched every few trials. The prelimbic

projections to the subthalamic nucleus are predominantly ipsilateral (Canteras et al., 1990). Therefore, altering activity in one hemisphere of the prelimbic cortex and the contralateral hemisphere of the subthalamic nucleus should prevent intact input from the prelimbic cortex to subthalamic nucleus in both hemispheres.

To determine the contributions of the PL and STN to proactive switching, rats were tested in a conditional cue-place association test to evaluate 1) the effect of the GABA agonists, baclofen and muscimol infused into the PL; 2) the effect of the NMDA receptor antagonist, D-AP5 infused into the STN; 3) whether a contralateral disconnection of the PL and STN disrupts proactive switching and 4) whether pharmacological manipulations of the PL and STN affect discrimination performance that does not require behavioral switching within a session.

B. Materials and Methods

1. Subjects

Adult, male Long–Evans rats weighing between 300 and 350 g at the time of testing served as subjects (n = 49). Rats were individually housed in plastic cages (26.5 X 50 X 20 cm) in a temperature (22°C) and humidity (30%) controlled environment and placed on a 12 h light/dark cycle (lights on at 7:00 A.M.). Rats were food restricted to 85–90% of their *ad libitum* body weight during the experiment, and water was available *ad libitum*. Animal care and use was in accordance with the National Institutes for Health Guide for the Care and Use of Laboratory Animals and approved by the University of Illinois at Chicago Institutional Laboratory Animal Care and Use Committee.

2. Apparatus

Training and testing occurred in a four arm cross maze made of black acrylic. Maze arms contained a base that was 10 cm wide x 55 cm long, two side walls that were 15 cm high by 55 cm long and a back wall that was 8 cm wide and 15 cm high. A 10 x 10 cm square base piece connected all four arms together. A circular food well (3.2 cm diameter and 1.6 cm deep) was located 3 cm away from the end of each arm. The maze was elevated 72 cm above the floor in a room with various extra-maze cues.

3. Surgery

Prior to behavioral training, all rats underwent stereotaxic surgery for bilateral implantation of guide cannulae aimed at both the PL and STN. Thus, each rat had a total of 4 guide cannulae implanted. Although rats in Experiments 1 and 2 (see below) only received infusions into the either the PL or STN, 4 guide cannulae were implanted in all rats to control for the possibility that effects observed in the contralateral disconnection were partially due to the number of cannulae implanted. For surgery, rats received 0.2 mL atropine sulfate (250ug/mL solution) 20 min prior to injection of sodium pentobarbital (50mg/kg, i.p.). Twenty-two gauge stainless steel guide cannulae (Plastics One, Roanoke, VA) were implanted into the PL at a 15° angle. The stereotaxic coordinates were A-P +3.0; M-L ± 1.8; D-V -3.0 (mm). For the STN, cannulae were implanted at a 10° angle. Cannula were implanted at an angle because both the PL and STN are located relatively medial allowing sufficient space for accurate placements. The stereotaxic coordinates were A-P -3.6; M-L ± 4.0; D-V -6.7. The coordinates were based on the stereotaxic atlas by Paxinos and Watson (1998). Four jeweler screws were positioned in the skull surrounding the cannulae and secured with dental acrylic (Stoetling, Wood Dale, IL). Stylets were placed into the guide cannulae to prevent

clogging. During the surgical procedure, Meloxicam (1mg/kg) was administered to manage pain post-operatively. Rats recovered for 7 days after surgery before commencing behavioral training. For 5 days following surgery, rats were fed ad libitum and subsequently food restricted as described above. Following this period, subjects were handled approximately 10 minutes per day.

4. Training

One week after surgery, behavioral training commenced. Each rat received a training procedure in multiple phases. In the first phase, a rat was allowed to consume a quarter piece of Froot Loops cereal (Kelloggs, Battle Creek, MI) in each food well. A rat was also picked up after consuming cereal pieces to acclimate being handled in the maze as in past studies (Ragozzino 2002; Baker et al., 2011). This stage of training lasted 3-7 sessions.

In the second training phase, each rat learned to use a visual cue in the stem arm to guide which one of two choice arms to enter for a cereal reinforcement. In this procedure, a black plastic block was placed in one maze arm giving the maze a T-shape. The stem arm served as the start arm and the other two arms served as choice arms. The choice arms always remained the same throughout training and testing. The other two arms served as start arms and were switched pseudorandomly such that the same arm was used a maximum of 2 consecutive trials. The visual cues were acrylic inserts that covered the walls and floor of the stem arm. Both a black and white insert were used as visual cues. Each visual cue color was always associated with one maze arm containing a cereal reinforcement. For example, if the cue was black, the reinforcement would be in the north arm, while a white cue indicated the reinforcement

would be in the south arm. The location of reinforcement for a given cue was counterbalanced across rats. Once a rat made a choice, it was allowed to travel down to the end of the maze arm and explore the food well. If the choice was correct, it was allowed to consume the cereal piece after which it was picked up and placed on top of its home cage. The home cage was placed on a table adjacent to the maze. If an incorrect choice was made, a rat was allowed to proceed to the food well and examine it after which it was picked up and returned to the top of the home cage. In this second phase of training, a rat was exposed to a single cue for 28 trials each session. One session was given every day thus a rat saw the same visual cue every other day/session. Visual cues were alternated each session until a rat achieved at least 80% reinforcement on two consecutive days. This training phase lasted 5-9 sessions.

In the third phase of training, rats received both cues on a single daily session. Rats were trained for 40 trials with each cue presented for 10 consecutive trials in alternating blocks. Across sessions, the cue that was presented first in a session was randomized. After a rat achieved at least 80% correct for both black and white cue trials in a session, each visual cue block was reduced to 5 consecutive trials over a total of 40 trials (or eight blocks of alternating cues). A rat had to achieve a minimum of 80% correct for each cue type to advance to the final training phase. Rat required 7-16 sessions to reach criterion in this phase.

In the fourth and final training phase, a rat was tested for 57 trials in which a cue was switched every 3 to 6 trials. This involved a total of 12 switches in a session and each rat received three blocks each of 3, 4, 5, or 6 consecutive trials with an extra 3 trials at the end for the 12^{th} switch. A 57 trial session contained approximately an equal number

of presentations for each visual cue (28 or 29). A rat achieved criterion when it accurately discriminated 80% or greater for each visual cue trial type across a 57 trial session. This phase required 1-3 sessions for rats to reach criterion. After achieving criterion, the test phase began.

In the conditional cue-place association, the visual cue was changed every 3-6 trials indicating that a behavioral switch should occur for the upcoming response. The relatively short block length was chosen in order to emphasize the need to monitor task cues on every trial while also facilitating the ability for a rat to establish a pattern of responses prior to a switch. This is commonly done in proactive switching task in order to incur a switch cost on switch trials (Konishi et al., 2005; Hyafil et al., 2009; Hikosaka and Isoda, 2010).

5. Microinfusion Procedure

Five minutes prior to a test session, a rat received an intracranial infusion. Infusions were delivered via 28 gauge injection cannulae which extended 1 mm below the guide cannulae. The injection cannulae were connected by polyethylene tubing to a 10uL syringe (Hamilton Company, Reno, NV). An infusion into the PL consisted of either saline or GABA agonists baclofen and muscimol (Sigma Aldrich, St. Louis, MO). An infusion into the STN consisted of either saline or the NMDA antagonist, D-AP5 (Tocris, Ellisville, MO). An infusion into the PL or STN alone occurred bilaterally with a total volume of 0.25uL at a rate of 0.15 uL/min by a microinfusion pump (74900 Series Cole Palmer, Vernon Hills, IL). Injection cannulae were left in place for an additional minute following the injection to allow for diffusion. A similar procedure was used for the contralateral and ipsilateral injection procedure except that a unilateral infusion was

made in each brain region. Prior to testing, rats remained in their home cages for five minutes after completion of the injection procedure. As in past studies (McCool et al., 2008; Brown et al., 2010), the day prior to the first test procedure, an injection cannula was lowered into each guide cannula and left in place for two minutes. This ensured that any effects observed on the first test day of testing were not due to the initial acute damage caused by the injection cannulae extending 1mm beyond the guide cannulae.

6. Switch Costs in Visual Cue – Place Conditional Discrimination

The conditional discrimination test required rats to establish a response based on learned visual cue – place associations and use cue information proactively to switch a response choice. If the procedure led rats to establish a response pattern within a block, then this should lead to a greater switch cost as displayed by a larger percentage of switch errors compared to non-switch errors. To determine this, performance in the visual cue-place discrimination test was examined in the vehicle treatment for the percentage of switch trial errors committed vs. the percentage of non switch trial errors across experiments. In a test session, the switch error percentage was based on a total of 12 switch trials and the non switch error percentage was based on a total of 45 trials.

7. Experiment 1: The effect of PL inactivation on performance of a visual cue-place conditional discrimination

The proactive switch test was the same as in the final phase of training. Five minutes prior to a test session, a rat received a bilateral infusion of either saline (Veh), baclofen 0.005uM-muscimol 0.018uM (Low dose) or baclofen 0.05uM-muscimol 0.18uM (High dose). Doses were selected based on previous studies in which baclofen and muscimol were administered intracranially to affect retroactive switching in a dose-

dependent manner (Floresco et al., 2006a; Brown et al., 2010). The order of treatments administered was counterbalanced across rats. There were a total of 8 rats included in the analysis for this experiment. Each rat received each treatment with a minimum of two days between test sessions. The day after testing, a rat received no testing. The following day, each rat received a test session, but did not receive an intracranial infusion prior to the test. This procedure was carried out to ensure that there were no lasting effects of a given treatment on the rat's ability to discriminate between the cues. If a rat was unable to perform the discrimination with at least 80% accuracy on each cue, additional sessions were given until criterion was achieved (This occurred only twice with one rat. One time a rat required a single additional session and the other time required two additional sessions to reach criterion). Once a rat had demonstrated the ability to discriminate accurately, the next day another test was performed. This procedure continued until a rat received all three treatments.

In each test session the percent correct was calculated. Similar to past behavioral switching studies (Dias and Aggleton, 2000; Floresco et al., 2006b; Baker et al., 2011), an analysis of the errors committed during each block of trials was calculated to determine whether a treatment affected the initial switch, perseveration of the previously correct response after the switch and/or inability to maintain the currently correct response. Errors were separated into switch, perseverative, and maintenance errors similar to that as in past studies (Baker et al., 2011; Mohler et al., 2011). A switch error was defined as a rat failing to initially switch to the currently relevant response when the visual cue changed (Figure 1A). Perseverative errors were only committed in a block in which an initial switch error occurred. Specifically, perseverative errors were committed

when any subsequent errors were made after a switch error and prior to making a correct response in that block (Figure 1B). Once a rat successfully switched from the previous response to the currently relevant one, it was no longer possible to commit a perseverative error. However, if a rat made a correct response in a block and reverted back to the other response choice in that same block, then this constituted a maintenance error (Figure 1C).

One possibility is that PL inactivation produces a conditional discrimination deficit principally unrelated to switch, perseveration or maintenance errors, but alternatively biases a rat to preferentially use an egocentric response strategy (e.g. always turn right) or an allocentric place strategy that was largely independent of the relevant cue-place response. To determine this, egocentric turn bias and place bias scores were measured for each treatment. Turn bias scores were calculated by determining a percentage of the number of errors committed to the more common egocentric response divided by the total number of errors. For example, if a rat made a total of 12 errors and 9 resulted because a rat turned left when it should have turned right into the correct location, then it would have a percent bias score of 75%. Likewise, a place bias score was calculated by determining a percentage of the number of errors for the more common place location divided by the total number of errors. For example, if a rat made a total of 10 errors and 7 resulted because a rat entered the south arm when it should have entered the north arm, then it would have a percent bias score of 70%.

On occasion it was observed that a rat might make errors on an entire block of trials. To determine whether this differentially occurred following a particular pharmacological

manipulation the number of blocks in which a rat failed to make any correct responses across the different treatments was calculated.

8. Experiment 2: The effect of NMDA receptor blockade into the STN on performance in a visual cue-place conditional discrimination

The proactive switch test was the same as described in Experiment 1. A separate group of rats (n = 7) were tested in this experiment. Five minutes prior to a test session, a rat received a bilateral infusion of either saline (Veh), D-AP5 0.2uM (Low dose), and D-AP5 10uM (High dose) into the STN. Doses were based on previous studies in which NMDA receptor blockade in the STN or striatum were shown to disrupt behavior (Baunez and Robbins, 1999; Palencia and Ragozzino, 2004). All other aspects of this experiment were as described in Experiment 1.

9. Experiment 3: The effect of contralateral disconnection and ipsilateral disconnection of the PL and STN on performance of a visual cue-place conditional discrimination

To determine whether a bilaterally intact PL and STN are necessary for proactive switching, a contralateral disconnection of the two brain areas was carried out. As a control, the effect of an ipsilateral disconnection of the PL and STN was also investigated. The test procedure was the same as described in Experiment 1. A separate group of rats was tested in this experiment with a total of 7 rats included in the final analysis. For this study there were six test sessions that involved intracranial infusions. The injections were counterbalanced for hemisphere injected as well as treatment received across rats. This design led to a maximum of four injections through any one cannula for a rat. The contralateral disconnection manipulation involved a

unilateral infusion into the PL and a unilateral infusion into the opposite hemisphere of the STN. Doses for each brain area remained the same as in Experiments 1 and 2 (e.g. PL low dose was the same as the low dose of baclofen and muscimol injected during ipsilateral and contralateral treatments). The three contralateral disconnection treatments were as follows: 1) Contralateral vehicle injection of saline (Contra Veh); 2) Contralateral low doses of baclofen/muscimol into the PL and D-AP5 into the STN (Contra Low) and 3) PL baclofen/muscimol and STN high doses (Contra High). The ipsilateral disconnection manipulation involved a unilateral infusion into the PL and a unilateral infusion into the same hemisphere of the STN. The three ipsilateral disconnection treatments were as follows: 1) PL–STN injection of saline (Ipsi Veh); 2) Ipislateral injection of the PL and STN low doses (Ipsi Low) and 3) high doses of drug into the PL and STN (Ipsi High). All aspects of the testing procedure were the same as in Experiments 1 and 2.

10. Experiment 4: The effect of PL inactivation, NMDA receptor blockade of the STN, or contralateral disconnection of the PL-STN in a non-switch cued-association test

If pharmacological manipulation of the PL, STN or contralateral disconnection of these structures impairs proactive switching, this may result because of a basic impairment in discrimination performance. To determine this, another group of rats were tested in a discrimination task in which only one of the cues was presented throughout a given session. The training procedure was similar as described above except that training was limited to the procedure in which rats receive a single visual cue per session. Thus, rats were trained to discriminate between the different visual cues but

this occurred across sessions and not within a session. Once rats completed two consecutive days of training at 80% or higher accuracy, they were advanced to the test phase. The test was identical to the training phase in that rats were tested on a single visual cue discrimination for 28 trials. Rats received a total of six intracranial injections in this experiment with a total of 7 rats included in the final analysis. Each visual cue was used for three test sessions. The order of treatments was pseudorandomly administered across rats. Each rat received the following treatments: 1) bilateral saline infusion into the PL (PL Veh); 2) bilateral baclofen/muscimol high dose infusion into the PL (PL High); 3) bilateral saline infusion into the STN (STN Veh); 4) bilateral D-AP5 high dose infusion into the STN(STN High); 5) contralateral saline infusion into the PL and STN (Contra Veh), and 6) contralateral baclofen/muscimol high dose infusion into the PL and D-AP5 high dose infusion into the STN (Contra High). The same procedure was employed for the interval between test sessions as described previously.

11. Histology

After completion of behavioral testing, rats were given an overdose of sodium pentobarbital. Rats were intracardially perfused with 0.9% phosphate buffered saline followed by 4% formaldehyde solution. The brain was removed and stored in formaldehyde until sectioning. Brains were frozen and cut into 50-µm coronal sections on a cryostat. Sections were immediately mounted on slides, dried, and then stained with cresyl violet. Placements were then verified with reference to the stereotaxic atlas of Paxinos and Watson (1998).

12. Statistical Analysis

In experiments 1-4 repeated measures ANOVAs were used to test the effects of drug treatments on performance accuracy, switch errors, perseverative errors, and maintenance errors. Turn bias and place bias scores, as well as the number of missed blocks were analyzed with repeated measures ANOVAs as well. A significant treatment effect was followed by Tukey's post hoc tests to determine significant differences between treatments. Switch cost analysis was carried out by using paired student's t-test comparing percent error rates on switch vs. non switch trials.

C. Results

1. Histology

Rats included in the behavioral analysis were restricted to those who had cannulae placements in the PL and STN. Figure 2 shows placements of cannula tip locations for the PL (Figure 2A) and STN (Figure 2B) across the different experiments. PL cannula placements were primarily located 2.7-3.8mm anterior to bregma. STN cannulae were principally located in the portion of the nucleus located 3.6-4.2mm posterior to bregma.

Twenty rats were excluded from the analyses because of misplacements. In Experiments 1-4, four rats were excluded due to placements outside the PL. Three misplacements were anterior to the PL located in the medial orbital subregion and one rat had ventral cannula placements located in the infralimbic cortex. One rat who had an anterior cannula placement exhibited motor deficits when infused with the high dose of baclofen/muscimol and could not complete testing. There was a total of 16 rats excluded from analyses in Experiments 1-4 because of cannula placements outside the STN. Five rats had unilateral (n = 2) or bilateral placements (n = 3) anterior to the STN in the internal capsule/subincertal nucleus. Six rats had unilateral (n = 2) or bilateral (n =

4) placements dorsal to the STN in the zona incerta. One rat had a unilateral placement in the substantia nigra pars reticulate that exhibited motor problems under the highest dose of D-AP5 and could not complete testing. Four rats had bilateral placements ventral to the STN located in the ventromedial internal capsule.

2. Switch Costs in Visual Cue – Place Conditional Discrimination

The percent error rate for switch vs. non switch trials was examined in vehicle treated rats during the visual cue-place conditional discrimination (see Figure 3). The results from vehicle treatments were collapsed across experiments. A paired t-test revealed that rats were almost twice as likely to commit an error on a switch trial (25.86% ± 2.35 error rate) vs. a non-switch trial (14.38% ± 1.09) [$t_{(28)} = 5.01$, $p < 0.01$].

3. Experiment 1: The effect of PL inactivation on performance of a visual cue-place conditional discrimination

Behavioral performance following PL inactivation is shown in Figure 4A. Vehicle-treated rats made the correct choice on 84.25 ± 1.67% of trials (mean ± SEM). The low dose of baclofen/muscimol led to a similar accuracy (mean = 81.38 ± 1.58%) as vehicle controls. However, the high dose, of baclofen/muscimol infused into the PL reduced performance to a mean of 60.50 ± 2.77% correct. A repeated measures ANOVA revealed a significant effect of treatment on performance accuracy ($F_{(2,23)} = 61.90$, $p < 0.01$). Tukey's post hoc tests indicated that the high dose of baclofen/muscimol led to a significant reduction in performance accuracy compared to that of vehicle or the low dose of baclofen/muscimol (p values < 0.01).

An analysis of the errors committed in the switch test (Figure 4B-D) revealed that there was a significant difference in switch errors among the treatment conditions ($F_{(2,23)}$ = 18.38, $p < 0.01$). The high dose of baclofen/muscimol significantly increased switch errors compared to that of the vehicle and the low dose treatments (p values < 0.01). In addition to an effect of switch errors, there was an effect of treatment on perseverative errors ($F_{(2, 23)}$ = 4.66, $p < 0.05$). The high dose of baclofen/muscimol increased perseveration compared to the vehicle treatment ($p < 0.05$). The low dose was not significantly different from any other treatment. Comparable to switch and perseverative errors, there was also a significant treatment effect for maintenance errors ($F_{(2,23)}$ = 37.00, $p < 0.01$). The high dose treatment significantly elevated maintenance errors compared to that of the vehicle and low dose treatments (p values < 0.01). Thus, PL inactivation at the high dose impaired performance by increasing switch, perseverative, and maintenance errors.

An analysis was carried out to determine whether a treatment biased a rat to preferentially use an egocentric response strategy (e.g. always turn right) or an allocentric place strategy that was largely independent of the relevant cue-place association. A repeated measures ANOVA revealed a significant effect of treatment on turn bias ($F_{(2, 23)}$ = 3.77, $p < 0.05$). Specifically, the high dose treatment (mean = 0.83 ± 0.06) had a significantly higher error ratio when required to turn in one egocentric direction than the low dose (mean = 0.67 ± 0.03) [$p < 0.05$]. The difference in the turn bias score between the high dose and vehicle treatment (mean = 0.71 ± 0.04) approached significance (p = 0.07). In contrast, there was not a treatment effect for the

place bias scores ($F_{(2, 23)} = 2.70$, $p > 0.05$). There was also no effect of treatment on the number of missed blocks within a session ($F_{(2, 23)} = 1.00$, $p > 0.05$).

4. Experiment 2: The effect of NMDA receptor blockade into the STN on performance in a visual cue-place conditional discrimination

The results on D-AP5 infusions into the STN are shown in Figure 5. An analysis on percent correct trials revealed that there was a significant effect of drug treatment ($F_{(2,20)} = 21.95$, $p < 0.01$). Post-hoc tests revealed that the high dose of D-AP5 (mean = 66.29% ± 2.77) significantly reduced behavioral performance compared to that of vehicle (mean = 82.86% ± 1.71) or the low dose of D-AP5 (mean = 80.86% ± 1.88) [p values < 0.01].

A further analysis of task performance indicated that there was a significant treatment effect for switch errors ($F_{(2,20)} = 22.20$, $p < 0.01$). The high dose of D-AP5 significantly increased switch errors compared to that of vehicle treatment and the low dose of D-AP5 (p values < 0.01). Likewise, an effect of treatment was observed on perseverative errors ($F_{(2, 20)} = 8.29$, $p < 0.01$). The high dose of D-AP5 significantly increased perseveration compared to that of vehicle ($p < 0.05$) and low dose treatments ($p < 0.01$). In contrast, there was not a significant treatment effect for maintenance errors ($F_{(2,20)} = 1.74$, $p > 0.05$). Additionally, there was no effect of treatment on turn bias scores ($F_{(2, 20)} = 0.04$, $p > 0.05$), place bias scores ($F_{(2, 20)} = 3.41$, $p > 0.05$), or missed blocks ($F_{(2, 20)} = 1.35$, $p > 0.05$).

5. Experiment 3: The effect of contralateral disconnection and ipsilateral disconnection of the PL and STN on performance of a visual cue-place conditional discrimination

The results from contralateral disconnection and ipsiilateral disconnection of the PL and STN are shown in Figure 6. There was a significant treatment effect for percent accuracy ($F_{(5,41)} = 7.75$, $p < 0.01$). The Contra High dose significantly reduced accuracy compared to that of all other contralateral and ipsilateral treatment groups (p values < 0.01).

An analysis of the errors revealed that there was also a significant effect of drug treatment on switch errors ($F_{(5,41)} = 2.73$, $p < 0.05$). Contra High treatment led to significantly more switch errors compared to that of the contra low and vehicle treatment conditions (p values < 0.05). There was also a significant effect of drug treatment on perseverative errors ($F_{(5,41)} = 15.62$, $p < 0.01$). Post-hoc tests revealed that the Contra High treatment led to significantly more perseverative errors compared to that of all other treatment conditions (p values < 0.01).However, no effect of drug treatment was found on maintenance errors ($F_{(5,41)} = 1.68$, $p > 0.05$) [Figure 6C]. Additionally, there was not a significant effect for either turn bias errors ($F_{(5, 41)} = 0.67$, $p > 0.05$), place bias errors ($F_{(5, 41)} = 0.92$, $p > 0.05$), or missed blocks ($F_{(5, 41)} = 2.52$, $p > 0.05$).

6. Initial Block Performance In the Visual Cue – Place Conditional Discrimination

Experiments 1-3 found that PL inactivation, NMDA receptor blockade in the STN, and contralateral disconnection of the PL and STN impaired performance in the conditional discrimination task. One possibility is that the deficits arose because the drug manipulations impaired expression of the learned visual cue-place associations

and/or general discrimination performance as opposed to behavioral switching. If the latter was the case, then a deficit should emerge within the first block before a rat has to switch. To assess this, performance on the first block of trials during Experiments 1-3 were compared among treatments to examine whether performance is affected before the initial switch in a test session. Repeated measures ANOVAs revealed that no effect of drug treatment was observed on the first block of trials for Experiment 1 ($F_{(2, 23)}$ = 0.47, $p > 0.05$), Experiment 2 ($F_{(2,20)} = 2.57$, $p > 0.05$), or Experiment 3 ($F_{(2, 20)} = 1.91$, $p > 0.05$). These data support initial discrimination performance being unaffected by treatments that impaired behavioral switching.

7. Experiment 4: The effect of PL inactivation, NMDA receptor blockade of the STN, or contralateral disconnection of the PL-STN in a non-switch cued-association test

To further examine whether treatment effects in the switch test resulted from a more fundamental deficit in discrimination performance, non-switch discrimination performance was tested under all effective treatments (Figure 7). A repeated measures ANOVA revealed there was no significant effect of treatment on percent accuracy during non-switch discrimination performance ($F_{(5, 41)} = 2.15$, $p > 0.05$). Thus, the high dose of baclofen/muscimol into the PL, high dose of D-AP5 into the STN or the high doses of these drugs to induce a contralateral disconnection had no affect on performance of a learned cue-place association.

8. The effect of drug infusions in rats with cannula misplacement on visual cue-place conditional discrimination performance

In rats which had cannula placements outside of the PL in Experiment 1, the high dose treatment led to a percent accuracy of 79.00 ± 3.61% comparable to that of vehicle treatment which resulted in 80.67 ± 3.53% accuracy. Because seven rats had cannula misplacements outside the STN in Experiment 2 (described above), an ANOVA was carried out to determine whether there was a treatment effect. As a group, the misplaced STN placements did not show an effect of drug treatment on performance ($F_{(2, 20)} = 3.34$, $p > 0.05$) with the high dose treatment leading to performance of 74.00 ± 3.89% compared with vehicle treatment and the low dose treatments (80.71 ± 3.21% and 80.71 ± 1.81% accuracy respectively). Analysis of the six rats which had misplacements in Experiment 3 revealed that as a group, no treatments affected performance of the task ($F_{(5, 25)} = 0.93$, $p > 0.05$) with performance ranging from 77.17 ± 4.19% in the contralateral high dose treatment to 84.14 ± 2.85% with the ipsilateral vehicle treatment. Experiment 4 had four rats in which there were misplaced cannulae. Because performance was unaffected under any treatment, these where not further analyzed.

D. Discussion

In the present studies, I employed a conditional discrimination test that required both an establishment of a response pattern and the use of cue information to produce a proactive switch. Consistent with the task having switch costs, vehicle- treated rats committed a significantly greater percentage of errors on switch trials compared to that of non switch trials. The studies also found that bilateral injections of GABA agonists into the PL or the NMDA receptor antagonist D-AP5 into the STN impaired conditional discrimination performance, in part due to an increase in switch errors. In a similar

manner, Experiment 3 demonstrated that contralateral disconnection of the PL and STN also increased switch errors in the conditional discrimination test. However, the contralateral disconnection also increased perseverative errors leading a rat to continue to choose the previously relevant response after the initial switch trial. Taken together, the findings suggest that both an intact PL and STN is necessary for using cue information to proactively switch and initially inhibit the previously relevant response.

In monkeys, neurons in the presupplementary area and STN exhibit switch-selective activity in a proactive switch test (Hikosaka and Isoda, 2008; Isoda and Hikosaka, 2008). Because the actions of STN neurons appear to mainly suppress an on-going response pattern using a saccade overriding procedure, Isoda and Hikosaka (2008; 2010) have proposed that the STN mediates a signal from the medial frontal cortex that allows inhibition of a response pattern that is no longer correct. The rodent PL may be comparable to the paralimbic cortex and/or anterior cingulate region, as opposed to the presupplementary area in primates (Uylings and van Eden, 1990). However, similar to the presupplementary area, the PL directly projects to the STN (Maurice et al., 1998). These anatomical findings combined with the contralateral disconnection results, raise the possibility that the PL sends a signal to the STN to inhibit an ongoing response pattern and enable a proactive switch.

Comparable to the present experiments, contralateral disconnection of the medial prefrontal cortex and STN using neurotoxic lesions impaired overall performance in the 5 – choice serial reaction time (5-CSRT) task, a measure of sustained attention, due to increased perseveration on a current choice (Chudasama et al., 2003). Unlike the 5-CSRT task that requires maintaining a similar response to a single cue that varies in

spatial location, the conditional discrimination test requires selecting a response based on one of two learned visual cue – place associations and maintaining that response for several trials until a different visual cue signals that an alternative response is necessary. Thus, the present findings extend past results demonstrating that the PL and STN are important for flexible responding in a sustained attention test (Chudasama et al., 2003) to indicate that these areas are part of a neural system involved in behavioral switching when cue information can be used proactively.

Similar to contralateral disconnection of the PL and STN, bilateral PL inactivation also impaired behavioral switching. However, PL inactivation significantly increased switch, perseverative, and maintenance errors. This contrasts with past studies involving a retroactive switch in which PL inactivation selectively increased perseveration of the previously relevant response (Ragozzino et al., 1999c; Dias and Aggleton, 2000; Ragozzino, 2007). Committing errors beyond the switch trial in a block is not likely due to an overall decrease in discrimination performance because PL inactivation did not affect performance in the non-switch discrimination test. The increase in multiple types of errors following PL inactivation may more likely reflect the inability to flexibly apply learned visual cue-place associations that leads to an inappropriate response pattern. More specifically, bilateral PL inactivation in the conditional discrimination test increased a turn bias that was independent of current cue information. Rats, even under saline treatment, exhibited a turn bias in the test, but this was significantly enhanced under the high dose of baclofen/muscimol. However, the exaggerated turn bias is not a necessary consequence of PL inactivation as this did not occur in the non-switch discrimination test. Taken together, the results suggest that the

PL supports the use of cue information to allow the proactive selection of responses under conditions that require behavioral switching.

The conditional discrimination test required a rat to reverse which place it entered every few trials. As described earlier, past studies found that PL inactivation does not impair place reversal learning involving a retroactive switch in which the prior outcome information is used to initiate a behavioral switch (Ragozzino et al., 1999b; Birrell and Brown, 2000; Boulougouris et al., 2007). This is the case even when the level of difficulty is enhanced by increasing the number of maze locations (Ragozzino et al., 2003). The present results suggest that the PL supports a place reversal, but only under conditions in which cue information is to be used proactively to switch. Alternatively, PL inactivation in the present study may have impaired performance not based on requiring the use of cue information to proactively switch, but because multiple switches within the same session were required, as opposed to a single switch as in past reversal learning studies. This is unlikely the case as medial prefrontal cortex lesions that include the PL do not impair multiple reversals within a session that require retroactive switching (Birrell and Brown, 2000; Boulougouris et al., 2007; Rich and Shapiro, 2007).

Past studies have investigated the effects of PL lesions in behavioral switching using conditional discrimination tests somewhat similar to that used in the present studies (Bussey et al., 1997; Delatour and Gisquet-Verrier, 1999; Chudasama et al., 2001). In general, these studies found that PL lesions do not affect behavioral switching in a conditional discrimination test. This is even the case when rats with PL lesions are required to reverse the task contingencies (Chudasama et al., 2001). These findings contrast the present results indicating that PL inactivation impairs behavioral switching

in a conditional discrimination test. One possibility for the conflicting pattern of results is the difference in how the PL was manipulated. More specifically, neurotoxic lesions of the PL may not have led to a behavioral deficit because there was a compensatory mechanism following the lesion that allowed other brain circuitry to support the behavioral function.

NMDA receptors in the STN, in part, mediate excitatory input from the frontal cortex (Nambu et al., 2000) in monkeys. One possibility is that PL input to the STN activates NMDA receptors to enable the initial shift away from a recently applicable, to the currently appropriate response pattern. The present results reveal that, similar to PL inactivation, NMDA receptor blockade in the STN impaired performance in the proactive switch test. However, in contrast to the effects of PL inactivation, NMDA receptor blockade in the STN selectively increased switch and perseverative errors, but did not affect maintenance errors. The findings are comparable to those in which STN lesions impair inhibition of an initiated response in the stop-signal test (Eagle et al., 2008) and further suggest that the STN is critical for inhibiting an ongoing response pattern when cues indicate an alternate response should occur. Because increased STN neuronal activity occurs when a cue presentation signals a behavioral switch (Isoda and Hikosaka, 2008), this area may be critical for inhibiting one response pattern and rapidly switching to an alternative response pattern.

One common set of findings across Experiments 1-3 is that the different pharmacological manipulations, with the exception of ipsilateral disconnection, impaired conditional discrimination performance and increased the likelihood of committing a perseverative error within a trial block. Perseverative errors can only occur after a

switch error. Therefore, following a switch trial error a rat can use both proactive (the visual cue) and retroactive (the immediately preceding outcome) information to guide an upcoming response. The present results indicate that neither retroactive nor proactive information is sufficient to accurately select a response when contralateral disconnection, PL inactivation or NMDA receptor blockade in the STN occurs. Importantly, however, none of the experiments resulted in performance falling to chance levels suggesting that rats were not simply guessing during a testing session.

In conclusion, the present findings suggest that the PL is important for monitoring task cues in order to guide responses under conditions that demand repeated and rapid proactive switching. This may occur by dynamically interacting with multiple basal ganglia structures to allow a fluid and flexible use of various response patterns at appropriate times. The contralateral disconnection findings indicate that the STN represents one brain area that interacts with the PL to enable proactive switching. More specifically, our findings support that the PL–STN circuit may be critical for initial switches between response patterns that allows a proactive switch from one response to an alternative pattern. Overall, the present experiments reveal some of the specific frontal cortex-basal ganglia circuitry that enables behavioral flexibility under conditions that require proactive switching.

Figure 1

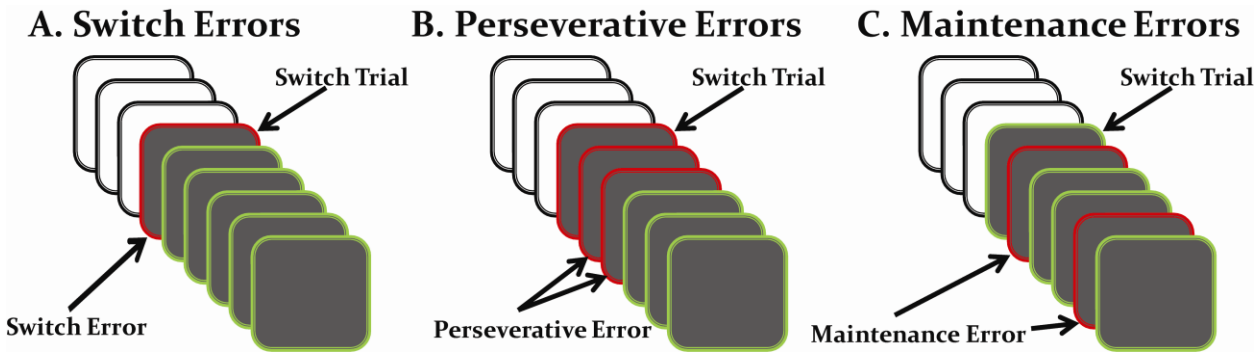

Figure 1 Errors are divided into three types based on when they occur within a block. Correct responses are denoted by a green boarder while an incorrect response has a red boarder. A. Switch errors occur when the first trial of a block is missed. B. Perseverative errors occur when errors immediately follow a switch trial error, until a correct response is made. C. Once a rat makes a correct response in a given block, any errors following that correct response are considered maintenance errors.

Figure 2

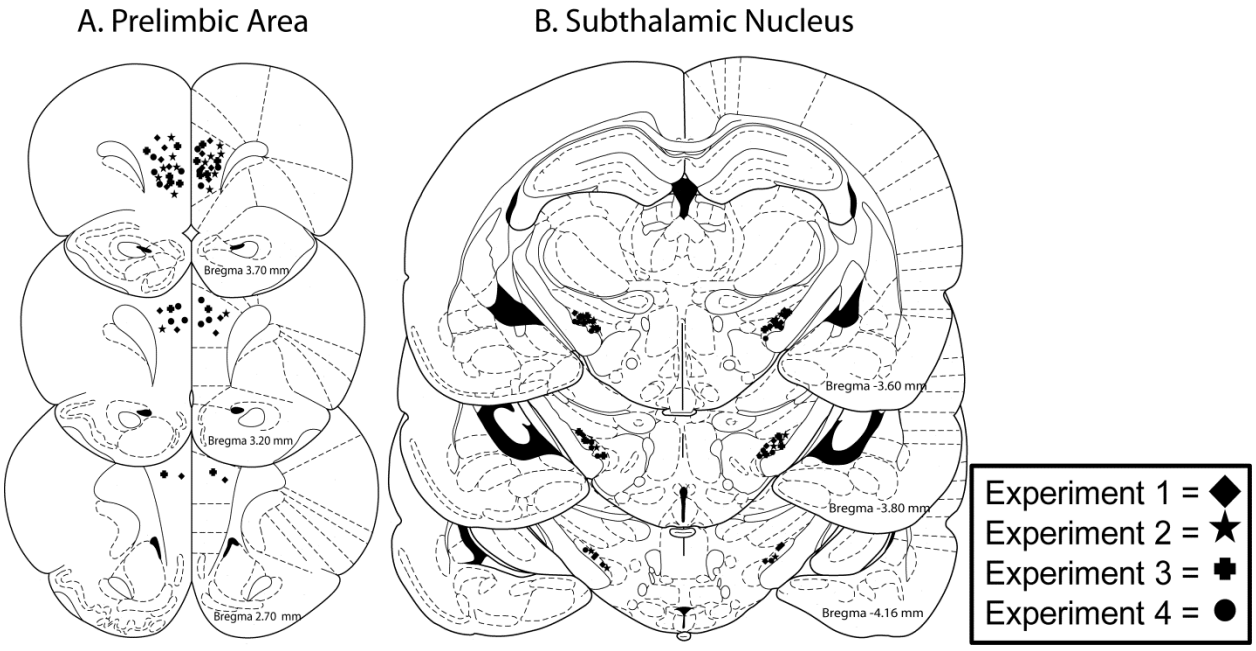

Figure 2. Cannula tip placements in the PL and the STN in experiments 1-4. A. Representation of cannula placements in the PL. B. Representation of cannula placements targeting the STN. Adapted from The Rat Brain in Stereotaxic Coordinates (Paxinos and Watson, 1998).

Figure 3

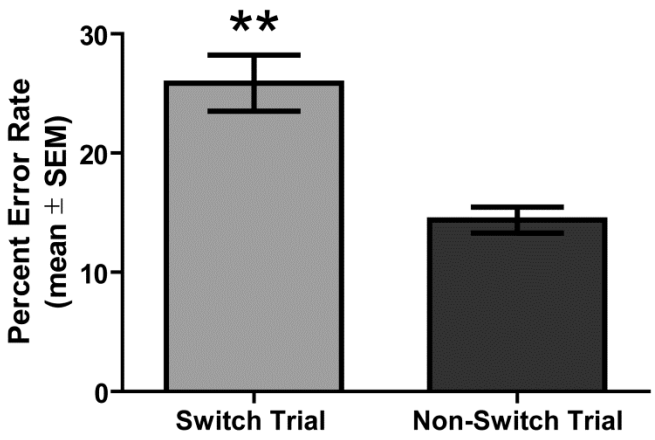

Figure 3. Proactive behavioral switching incurs a switch cost in vehicle treated animals. All saline treatments across experiments 1-3 were collapsed into one group to examine performance (mean ± SEM) on switch vs. non-switch trials. The percent error rate for switch and non-switch trials was calculated based on the number of errors divided by the total number of trials of that type. Vehicle treated animals were more likely to commit an error on switch vs. non-switch trials. ***p* < 0.01.

Figure 4

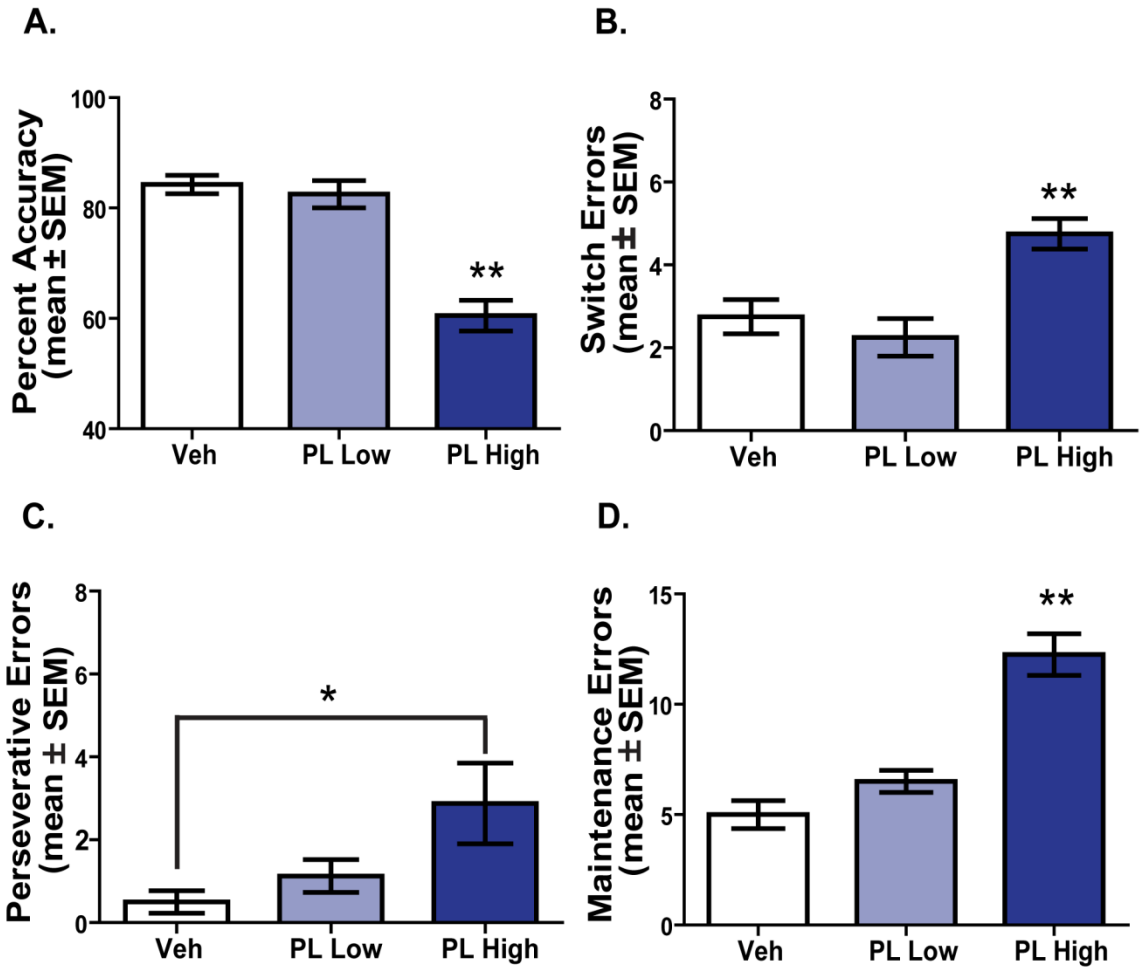

Figure 4. PL inactivation impairs proactive behavioral switching. Each rat (n = 8) received a bilateral injection into the PL of saline (Veh), baclofen 0.005uM-muscimol 0.018uM (PL Low), and baclofen 0.05uM-muscimol 0.18uM (PL High) in a random order 5 min before testing. A. PL High treatment significantly impaired accuracy (mean ± SEM) compared with Veh and PL Low dose. ** $p < 0.01$. B. The number of switch errors (mean ± SEM) increased in the PL High treatment compared to that of all other treatments. ** $p < 0.01$. C. The PL High dose led to significantly more perseverative errors (mean ± SEM) than Veh treatment. * $p < 0.05$. D. PL High dose resulted in significantly more maintenance errors (mean ± SEM) than the PL Low and Veh doses. ** $p < 0.01$.

Figure 5

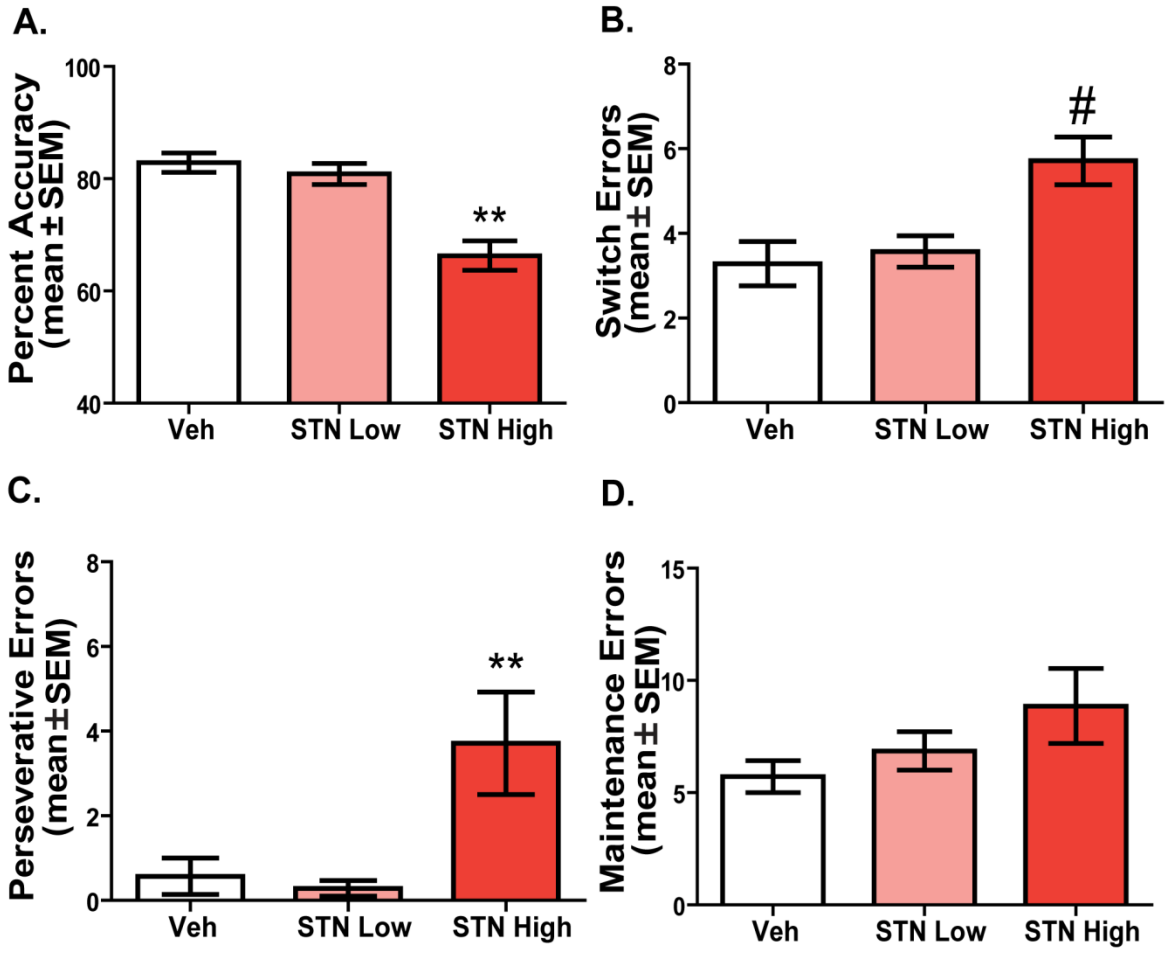

Figure 5. NMDA receptor blockade in the STN impairs proactive behavioral switching. Each rat (n = 7) received a bilateral injection into the STN of saline (Veh), D-AP5 2uM (STN Low), and D-AP5 10uM (STN High) in a random order 5 min prior to testing in the visual cue- place conditional discrimination. A. The STN High condition significantly impaired performance (mean ± SEM) compared to that of Veh and STN Low treatments. #p < 0.05 vs. Veh, *p* < 0.01 vs. STN Low. B. Treatment of the STN High condition led to more switch errors (mean ± SEM) than the STN Low and Veh treatments. ***p* < 0.01. C. The STN High condition significantly increased perseverative errors (mean ± SEM) compared to that of STN Low or Veh treatments. ***p* < 0.01. D. No differences were observed in the number of maintenance errors (mean ± SEM) committed among the treatments.

Figure 6

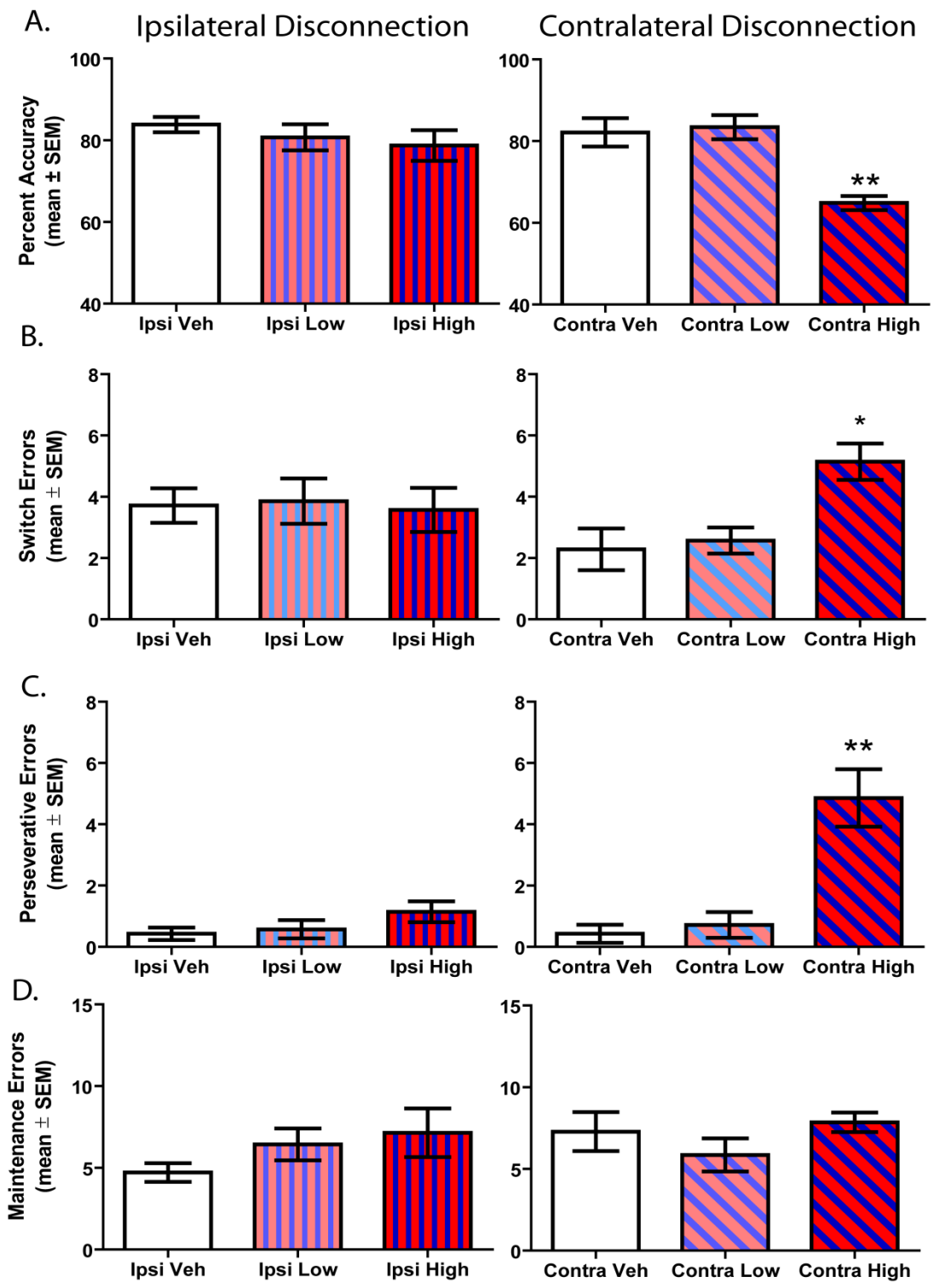
A.
Ipsilateral Disconnection
Contralateral Disconnection
Percent Accuracy (mean ± SEM)
100
80
60
40
**
Ipsi Veh
Ipsi Low
Ipsi High
Contra Veh
Contra Low
Contra High
B.
Switch Errors (mean ± SEM)
8
6
4
2
0
*
C.
Perseverative Errors (mean ± SEM)
**
D.
Maintenance Errors (mean ± SEM)
15
10
5
0

Figure 6. Contralateral disconnection of the PL-STN areas impairs proactive behavioral switching. Each rat (n = 7) received 3 treatments involving ipsilateral injections of saline (Ipsi Veh), combined low dose of baclofen/muscimol in PL and D-AP5 in the STN (Ipsi Low) and combined high dose of baclofen/muscimol in PL and D-AP5 in the STN (Ipsi High). Each rat also received 3 treatments consisting of contralateral injections of saline (Contra Veh), combined low dose of baclofen/muscimol in PL and D-AP5 in the STN (Contra Low) and combined high dose of baclofen/muscimol in PL and D-AP5 in the STN (Contra High). A. The Contra High dose significantly impaired performance (mean ± SEM) on the task compared with that of all other treatments. $^{**}p < 0.01$. B. Contra High dose lead to significantly more switch errors (mean ± SEM) than all other conditions. $^{*}p < 0.05$. C. Treatment with the Contra High dose significantly elevated perseverative errors (mean ± SEM) compared to that of all other treatments. $^{**}p < 0.01$. D. No differences were observed among treatments on the number of maintenance errors (mean ± SEM).

Figure 7

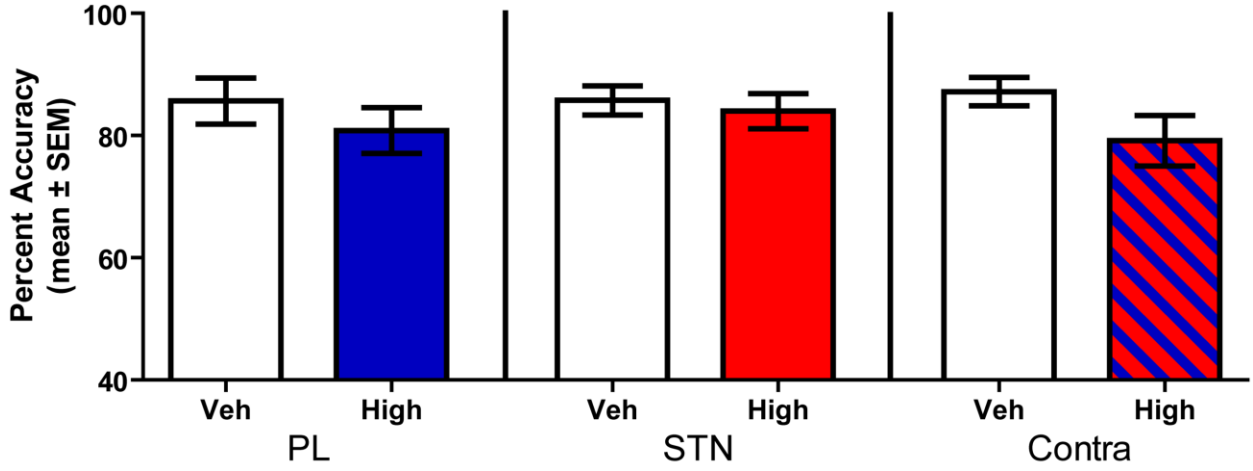

Figure 7. PL inactivation, STN NMDA receptor blockade, and contralateral disconnection of the PL-STN does not affect performance during a non-switch cued-association test. Each rat (n = 7) received 6 treatments during 6 separate non-switch discriminations. Two treatments in the PL, were administered; a saline injection (PL Veh), and baclofen/muscimol (PL High). Two treatments were given in the STN; a saline control (STN Veh), and the NMDA receptor antagonist D-AP5 (STN High). Two treatments were given utilizing a contralateral disconnection of the PL and STN areas; a saline injection (PL-STN Veh), and the previously effective treatment of baclofen/muscimol in the PL and D-AP5 in the STN (PL-STN High). No differences in performance were observed between treatments in a non-switch discrimination.

Chapter III

The Prelimbic Cortex and Dorsomedial Striatum Support Proactive Behavioral Switching

A. Introduction

In the first set of experiments, I demonstrated that contralateral disconnection of the PL and STN impairs performance in a visual cue – place conditional discrimination. The task required rats to proactively utilize cues to switch choice patterns. Bilateral PL inactivation impaired discrimination performance by increasing switch, perseverative, and maintenance errors. This pattern of errors resulted from PL inactivation biasing rats toward using a less effective turn strategy. These results contrast with STN NMDA receptor blockade which impaired conditional discrimination performance by selectively increasing switch and perseverative errors without affecting maintenance errors or a turn bias. The difference in error patterns following pharmacological manipulations of the PL and STN raise the possibility that the PL functionally interacts with other basal ganglia structures to allow maintenance of a currently relevant choice pattern after being initially selected in a proactive behavioral switch test.

The DMStr, which receives input from the PL (Sesack et al., 1989; Conde et al., 1995; Gabbott et al., 2005), may be one basal ganglia region that supports maintenance of a selected choice pattern under proactive behavioral switching conditions. Several studies have investigated the role of the DMStr in cognitive flexibility. Through these studies, the DMStr has been shown to be important for retroactive behavioral switching that involve strategy switching and reversal learning (Pisa and Cyr, 1990; Ragozzino et

al., 2002b; Ragozzino, 2003; Braun and Hauber, 2011). In both reversal learning and strategy switching tests, DMStr inactivation selectively impairs the ability to maintain a currently relevant strategy after being initially selected (Ragozzino et al., 2002a; Ragozzino and Choi, 2004). Past findings suggest that the DMStr supports maintenance of a newly selected choice pattern under retroactive switching conditions. My hypothesis is that the DMStr functionally interacts with the PL to also support maintenance of a newly selected choice pattern under proactive switch conditions.

The PL projection to the DMStr is known to be excitatory and may be, in part, mediated by NMDA receptors (Sesack et al., 1989; Conde et al., 1995; Spencer and Murphy, 2000b; Gabbott et al., 2005).This corticostriatal projection has been demonstrated to cause both long term potentiation and depression of neuronal responses in the DMStr (Charpier and Deniau, 1997; Pisani et al., 2001), two forms of synaptic plasticity. These changes in neuronal responses are due, in part, to NMDA receptors, as their blockade leads to a reduction in potentiation of neuronal responses caused by cortical stimulation (Kita, 1996; Lovinger and Tyler, 1996; Charpier and Deniau, 1997; Akopian and Walsh, 2002a). Furthermore, blockade of NMDA receptors in the DMStr does not impair egocentric response learning, but impairs reversal learning (Palencia and Ragozzino, 2004). As with DMStr inactivation, NMDA receptor blockade in the DMStr impairs reversal learning by increasing maintenance errors (Palencia and Ragozzino, 2004). Thus, NMDA receptors in the DMStr may support cognitive flexibility by enhancing the ability to maintain a recently selected choice pattern through changes in neuronal plasticity. To date, unknown is whether NMDA receptors in the DMStr

support proactive behavioral switching or whether the PL and DMStr functionally interact to enable proactive behavioral switching.

The experiments described in this chapter determined whether the PL and DMStr areas interact to enable proactive behavioral switching, I tested rats in the conditional cue-place association test to evaluate 1) the effect of the GABA agonists, baclofen and muscimol infused into the PL and the effect of the NMDA receptor antagonist, D-AP5 infused into the DMStr; 2) whether a contralateral disconnection of the PL and DMStr disrupts proactive switching and 3) whether pharmacological manipulations of the PL and DMStr affect discrimination performance that does not require behavioral switching within a session.

One possibility is that the DMStr plays a similar role in both proactive and retroactive switching. If this is the case, then I predict that NMDA receptor blockade in the DMStr will selectively increase maintenance errors without affecting switch errors. If, on the other hand, the DMStr is important for an actual proactive switch, then NMDA receptor blockade in this striatal area should significantly increase switch errors.

Materials and Methods

1. Subjects

Adult, male Long–Evans rats weighing between 300 and 350g at the time of testing served as subjects (n = 35). All conditions were the same as in the previous set of experiments.

2. Surgery

Prior to behavioral training, all rats underwent stereotaxic surgery for bilateral implantation of guide cannulae aimed at both the PL and DMStr. Thus, each rat had a total of 4 guide cannulae implanted. For surgery, rats received a mixture of Ketamine (100mg/kg) and Xylazine (10mg/kg). Twenty-two gauge stainless steel guide cannulae (Plastics One, Roanoke, VA) were implanted into the PL at a 15° angle in the dorsal/medial plane. The stereotaxic coordinates were A-P +3.0; M-L ± 1.8; D-V -3.0 (mm). For the DMStr, cannulae were implanted at a 15° angle in the anterior/posterior plane. Cannulae were implanted at an angle to allow for all four cannulae to reach their target areas and allow room for dummy cannulae when not being injected. The stereotaxic coordinates were A-P 0.0; M-L ± 2.0; D-V -3.9. The coordinates were based on the stereotaxic atlas by Paxinos and Watson (1998). Four jeweler screws were positioned in the skull surrounding the cannulae and secured with dental acrylic (Stoetling, Wood Dale, IL). All other procedures were identical to those described previously.

3. Training

Training on the cue-place conditional discrimination is described in Chapter 2.

4. Microinfusion Procedure

The microinfusion procedure was the same as in Chapter 2.

5. Switch Costs in Visual Cue – Place Conditional Discrimination

As in the previous set of experiments (Chapter 2), percent error rate will be examined in relation to switch vs. non-switch trials on the 12 switch trials and 45 non-

switch trials to examine if the task results in a switch cost when cued to go from a block of one of the place strategies to the other.

6. Experiment 1: The effect of bilateral PL inactivation and DMStr NMDA receptor blockade on performance of a visual cue-place conditional discrimination

Upon completion of training, rats in Experiment 1 were given one of four treatments to examine the role of the PL and NMDA receptors within the DMStr in the visual cue – place conditional discrimination. Five minutes prior to a test session, a rat received a bilateral infusion of either saline (Veh), baclofen 0.05uM-muscimol 0.18uM (Bac/Mus) into the PL, or saline (Veh) or D-AP5 10uM (D-AP5) into the DMStr. The drug doses were the same as the high dose concentrations for PL and STN infusions in Chapter 2. The dose of D-AP5 in the DMStr is comparable to that of experiments shown to effect cognitive flexibility in other tasks (Palencia and Ragozzino, 2004, 2006). Only one dose of the drugs was administered as previous experiments have shown that ineffective doses of these drugs do not disrupt behavior (e.g. chapter 2 for the PL and (Palencia and Ragozzino, 2004) for the DMStr). The use of only one drug treatment in each area allowed for the combination of the PL and DMStr treatments into a single group to reduce the number of animals required to address the aims of the experiments. The order of treatments administered was counterbalanced across rats. There were a total of 8 rats included in the analysis for this experiment.

Measures included in the analysis were the same as in the previous set of experiments. Specifically, percent accuracy, as well as switch, perseverative and maintenance errors were examined (Figure 1, Chapter 2). In addition, egocentric turn bias and cue bias were measured by calculating the number of errors made to a specific

side (e.g. right turns) or cue (e.g. white cue block) over the total number of errors as in the previous experiments. In addition, the frequency of a rat missing an entire block of trials was measured for each treatment. There were 13 blocks of trials in total with 12 switches in the task. The number of missed blocks within a given test session was measured.

7. Experiment 2: The effect of contralateral disconnection and ipsilateral disconnection of the PL and DMStr on performance of a visual cue-place conditional discrimination

To determine whether a bilaterally intact PL and DMStr are necessary for proactive switching, a contralateral disconnection of the two brain areas was carried out. An ipsilateral disconnection of the PL and DMStr served as a control for the effects of ipsilateral function of these areas. The test procedure was the same as described in Chapter 2. A separate group of rats was tested in this experiment with a total of 8 rats included in the final analysis. Four injections were used in total for each rat. The injections were counterbalanced for hemisphere injected as well as treatment received across rats. A maximum of two injections through any one cannula was administered for each rat. The contralateral disconnection manipulation involved a unilateral infusion into the PL and a unilateral infusion into the opposite hemisphere of the DMStr. Doses for each brain area remained the same as in Experiment 1. Contralateral disconnection treatments were: 1) Contralateral vehicle injection of saline (Contra Veh); 2) PL baclofen/muscimol and DMStr high doses (Contra High). The ipsilateral disconnection manipulation involved a unilateral infusion into the PL and a unilateral infusion into the same hemisphere of the DMStr. Treatments were as follows: 1) PL–DMStr injection of

saline (Ipsi Veh); 2) Ipislateral injection of the PL and DMStr high doses (Ipsi High). All outcome measurements were the same as in Experiment 1.

8. Experiment 3: The effect of PL inactivation, NMDA receptor blockade of the DMStr, or contralateral disconnection of the PL-DMStr in a non-switch cued-association test

One possible explanation for a resulting impairment in Experiments 1 and 2 could be due to a basic impairment in discrimination performance. To determine this, another group of rats were tested in a discrimination task in which only one of the cues was presented throughout a given session. Rats were trained to discriminate between the different visual cues across sessions and not within a session. Once rats completed two consecutive days of training at 80% or higher accuracy, they were advanced to the test phase. The test was identical to the training phase in that rats were tested on a single visual cue discrimination for 28 trials. Rats received a total of six intracranial injections in this experiment with a total of 7 rats included in the final analysis. Each visual cue was used for three test sessions. The order of treatments was pseudorandomly administered across rats. Each rat received the following treatments: 1) bilateral saline infusion into the PL (PL Veh); 2) bilateral baclofen/muscimol high dose infusion into the PL (PL High); 3) bilateral saline infusion into the DMStr (DMStr Veh); 4) bilateral D-AP5 high dose infusion into the DMStr (DMStr High); 5) contralateral saline infusion into the PL and DMStr (Contra Veh), and 6) contralateral baclofen/muscimol high dose infusion into the PL and D-AP5 high dose infusion into the DMStr (Contra High). The same procedure was employed for the interval between test sessions as described previously.

9. Histology

Histological procedures are the same as described in Chapter 2.

10. Statistical Analysis

In experiments 1-3 repeated measures ANOVAs were used to test the effects of drug treatments on performance accuracy, switch errors, perseverative errors, and maintenance errors. Turn bias scores, place bias and missed block frequency were analyzed with repeated measures ANOVAs as well. A significant treatment effect was followed by Tukey's post hoc tests to determine significant differences between treatments. Switch cost analysis was carried out by using paired student's t-test comparing percent error rates on switch vs. non switch trials.

B. Results

1. Histology

Rats included in the behavioral analysis were restricted to those who had cannulae placements in the PL and DMStr. Figure 1 shows placements of cannula tip locations for the PL (Figure 1A) and STN (Figure 1B) across the three experiments. PL cannula placements were primarily located 2.7-3.8mm anterior to bregma. DMStr cannulae were principally located in the portion of the nucleus located 1.7-0.7mm anterior to bregma.

Thirteen rats were excluded from the analyses because of misplacements. In Experiments 1-3, four rats were excluded due to placements outside the PL. All misplacements were anterior to the PL located in the medial orbital subregion. An additional rat was excluded from analysis due to damage in the prefrontal cortex. There were a total of eight rats excluded from analyses in Experiments 1-3 because of cannula placements outside the DMStr. One rat had a unilateral placement in the

nucleus accumbens core with another rat having bilaterally misplaced cannulae in the nucleus accumbens core. Two rats had bilateral placements ventral to the DMStr in the nucleus accumbens shell. One rat had a bilateral placement in the dorsolateral striatum. Three rats had bilateral placements dorsal to the DMStr located in the corpus callosum.

2. Switch cost in a visual cue – place conditional discrimination

The percent error rate for trials in which there was a switch from one block to another was compared with the error rate on trials in which there was not a switch across saline-treated rats in experiments 1 and 2 (Figure 2). Results of a paired t test revealed that rats were more likely to commit an error on switch trials (26.00% ± 1.71) than on non-switch trials (13.34% ± 0.94) [$t_{(31)} = 6.48$, $p < 0.01$].

3. Experiment 1: The effect of bilateral PL inactivation and DMStr NMDA receptor blockade on performance of a visual cue-place conditional discrimination

Results of the effect of bilateral PL inactivation and DMStr NMDA receptor blockade are shown in Figure 3. A repeated measures ANOVA revealed that performance following PL inactivation (65.75% ± 1.51) or DMStr NMDA receptor blockade (62.87% ± 2.04) significantly impaired performance compared to that of PL or DMStr vehicle treatment (81.37% ± 1.45, and 83.62% ± 1.47 respectively) [$F_{(3, 31)} = 49.38$, $p < 0.01$]. Analysis of errors committed during performance revealed a significant effect of treatment on switch errors ($F_{(3, 31)} = 11.37$, $p < 0.01$) [Figure 3B]. Post hoc analysis revealed that PL inactivation led to more switch errors than DMStr NMDA receptor blockade ($p < 0.05$) or either saline treatment ($p < 0.01$). Additionally, DMStr NMDA receptor blockade led to an increase in switch errors compared to that of vehicle treatments ($p < 0.05$). There was also a significant effect of treatment on the number of

perseverative errors committed during the task ($F_{(3, 31)} = 12.63$, $p < 0.01$). Specifically, NMDA receptor blockade of the DMStr led to an increase in perseveration compared to that of all other treatments (*p's* < 0.01). Finally, there was a significant treatment effect for the number of maintenance errors committed ($F_{(3, 31)} = 10.93$, $p < 0.01$). Both PL inactivation and DMStr NMDA receptor blockade led to an increase in the number of maintenance errors committed compared to that of vehicle treatments ($p < 0.01$ and $p < 0.05$ respectively), but did not significantly differ from one another ($p > 0.05$).

The effect of treatment on turn and place bias was also examined. An effect of treatment on the turn bias was observed ($F_{(3, 31)} = 5.27$, $p < 0.01$). PL inactivation significantly increased turn bias compared to that of saline treatments ($p < 0.01$ for PL Veh and $p < 0.05$ for DMStr Veh) [Figure 4A]. However, there was no effect of treatment on place bias scores ($F_{(3, 31)} = 1.53$, $p > 0.05$).

Observation of rats following D-AP5 infusions into the DMStr suggested that these rats would occasionally make errors for an entire block of trials. To determine whether this was more likely to occur following NMDA receptor blockade in the DMStr then with PL inactivation, a further analysis determined whether there was a significant difference among the treatments in producing errors across an entire block of trials (Figure 4B). The results revealed a significant treatment effect on the number of blocks missed during a session ($F_{(3, 31)} = 9.45$, $p < 0.01$). Specifically, NMDA receptor blockade in the DMStr led to significantly more missed blocks than all other treatments (*p's* < 0.01).

4. <u>Experiment 2: The effect of contralateral disconnection and ipsilateral disconnection of the PL and DMStr on performance of a visual cue-place conditional discrimination</u>

The effect of the ipsilateral and contralateral disconnection of the PL and DMStr areas was examined in the cue place conditional discrimination (Figure 5). The results revealed that there was a significant effect of treatment ($F_{(3,31)} = 27.99$, $p < 0.01$). In particular, contralateral disconnection of the PL – DMStr areas led to a significant decrease in accuracy (65.75% ± 1.84) compared to that of contralateral saline treatment (85.62% ± 1.47) and both ipsilateral saline and drug treatments (83.75% ± 2.16, and 82.12% ± 2.17 respectively). An analysis of errors revealed a significant effect on the number of switch errors committed during performance ($F_{(3, 31)} = 4.08$, $p < 0.05$) such that the contralateral disconnection led to more switch errors than all other treatments (p's < 0.05). There was also a significant effect of treatment on perseverative errors ($F_{(3, 31)} = 17.44$, $p < 0.01$). The contralateral disconnection led to significantly more perseverative errors than the other treatments (p 's< 0.01). Finally, there was also a significant treatment effect on maintenance errors ($F_{(3, 31)} = 5.31$, $p < 0.01$) such that the contralateral disconnection treatment led to more maintenance errors than the contralateral saline treatment ($p < 0.01$) and the ipsilateral saline and drug treatments ($p's < 0.05$).

The effect of ipsilateral and contralateral treatment on turn and place bias scores was examined. No effect of treatment was observed on either a turn ($F_{(3, 31)} = 2.47$, $p > 0.05$) or place bias ($F_{(3, 31)} = 2.07$, $p > 0.05$). However, there was an effect of treatment on the number of missed blocks during the session ($F_{(3, 31)} = 6.86$, $p < 0.01$) [Figure 6]. Specifically, the contralateral disconnection led to more missed blocks than the saline treatments ($p < 0.01$), or ipsilateral drug treatment ($p < 0.05$).

5. Initial Block Performance In the Visual Cue – Place Conditional Discrimination

To test whether the impairments observed with the various treatments was due to a general inability to perform the cue – place discriminations and not due to the difficulty of switching repeatedly between them in blocks of trials, the performance on the initial block of trials was compared between treatments. In experiment 1, no effect of treatment was observed on 1st block performance ($F_{(3, 31)} = 2.91$, $p > 0.05$). Likewise, no effect of treatment was observed on the 1st block of trials in experiment 2 ($F_{(3, 31)} = 0.93$, $p > 0.05$).

6. Experiment 3: The effect of PL inactivation, NMDA receptor blockade of the DMStr, or contralateral disconnection of the PL-DMStr in a non-switch cued-association test

To further examine whether treatment effects resulted from a more fundamental deficit in discrimination performance, non-switch discrimination performance was tested under all effective treatments (Figure 7). Results revealed that all treatments led to a similar level of performance on the non-switch cued-association test ($F_{(5, 41)} = 0.52$, $p > 0.05$).

7. The effect of drug infusions in rats with cannula misplacement on visual cue-place conditional discrimination performance

Of the four rats which had cannula placements outside of the PL in Experiment 1, the high dose treatment led to a percent accuracy of 83.25% ± 3.75 comparable to that of vehicle treatment in animals with accurate placements 81.37% ± 1.45. As a group, the misplaced DMStr placements (n = 8) with the high dose treatment led to performance accuracy of 85.87% ± 1.61 which is comparable to vehicle treatment with

accurate DMStr placements (83.62% ± 1.47). Of the three rats that had misplacements in Experiment 2, performance ranged from 75.33% ± 0.88 in the contralateral high dose treatment to 85.67% ± 4.41 with the ipsilateral vehicle treatment which was also comparable to vehicle treatments with good placements.

C. Discussion

In the present studies, I demonstrated that the PL and DMStr is also part of a neural system that supports proactive switching. This was shown in the same conditional visual cue – place discrimination as with PL and STN pharmacological manipulations. Similar to results observed in the first set of studies, rats exhibited switch costs by committing double the percentage of switch errors compared to non-switch errors. In this set of studies, GABA agonists were again injected into the PL leading to a similar pattern of findings as the first experiments. In this experiment, injection of the NMDA receptor antagonist D-AP5 was infused into the DMStr. NMDA receptor blockade into the DMStr impaired overall conditional discrimination performance comparable to that observed with D-AP5 infusions into the STN. Besides increasing the number of switch errors, D-AP5 injected into the DMStr also significantly elevated the number of perseverative, and maintenance errors. Experiment 2 revealed that contralateral disconnection of the PL and DMStr also impairs conditional discrimination performance by reducing the ability to shift on switch trials, as well as increasing errors on subsequent trials that led to increased perseverative and maintenance errors. The significant increase in all error types following DMStr NMDA receptor blockade or PL-DMStr contralateral disconnection resulted from an increased likelihood of a rat to miss an entire block of trials. Overall, the behavioral deficit following contralateral disconnection of these

structures suggests that the PL and DMStr functionally interact to enable cue – place conditional discrimination performance.

The significant increase in all error types following DMStr NMDA receptor blockade or PL-DMStr contralateral disconnection emerged because these manipulations led a rat to commit errors across an entire block of trials 1-3 times in a session. This was in contrast to saline treatment in which this happened only rarely. The likelihood of missing a block of trials was not due to the length of the previous block or the length of the block which was missed. However, for six of the seven rats in which missed blocks were observed for bilateral DMStr NMDA receptor blockade and five of the six rats in which the PL and DMStr were disconnected, the cue that resulted in a missed block was the second cue encountered in that test session. In other words, rats were more likely to miss a block on the cue opposite to the first cue they received during a test session. These results suggest that the DMStr and its connection with the PL is important for switching from an ongoing response pattern when cued to switch to a new one. Under NMDA receptor blockade or contralateral disconnection, a rat occasionally fails to switch and continues to perform the first choice pattern encountered in that test session.

One explanation for these missed blocks is that the change in cue-reward contingencies fails to update the ongoing choice pattern resulting in the previous choice pattern being carried out. In rats, the DMStr has been implicated in relaying information about the expected value of an action based on recent task demands. For example, during a strategy switching test , neurons in the DMStr reorganize to the new task demands regardless of the type of strategy now required (Yeshenko et al., 2004; Eschenko and Mizumori, 2007). In a recent study, rats were trained in a two-choice

discrimination in which there were different probabilities for reward. The choices were reversed after 35 trials with multiple reversals in a session (Kim et al., 2013). Similar to the current experiments, rats were well-trained in the task in which multiple single-units were recorded during the test. Although the activity of any single neuron only correlated weakly with a choice, there was an ensemble of activity in the DMStr that preceded the actual choice and would change dynamically with a reversal in reward probabilities (Kim et al., 2013). This supports the DMStr being critical for the updating of expected value of an action or strategy.

The findings described above suggest that DMStr neuronal activity can dynamically change when there is a change in reward outcome. The current studies used a test in which a rat had to use cue information to proactively switch its choice pattern. There is evidence based on single-unit recordings that the DMStr may also update the value of certain actions using proactive cue information. Specifically, single-unit recordings in the dorsal striatum during a delay match to sample task revealed that striatal neurons exhibit increased phasic activity prior to a choice on correct but not incorrect trials (Chang et al., 2002) suggesting that the dorsal striatum may play a role in action selection based on recent cue information.

If the DMStr is critical for using cue information to update the accurate selection of actions when environmental contingencies change, then manipulating activity in this area could lead to selecting the incorrect choice pattern for several consecutive trials as observed in the present study. Specifically, on switch trials the change in cue indicates that the previous choice will not lead to a reward while the alternative choice will lead to a reward. If this value signal is critical for the updating of an action by the DMStr, then

one would expect an increased likelihood of failure to switch choice patterns with manipulation of the DMStr. Similarly, because the DMStr is important for reward feedback updating action values for the current choice (Yeshenko et al., 2004; Eschenko and Mizumori, 2007; Kim et al., 2013), this failure to switch should continue to be observed even after both cue and reward feedback information can be used to switch behaviors and the subject could fail to switch for that entire block of trials as was observed in the current experiments. Taken together, the present findings suggest that the DMStr may be important for updating action values when cue information must be used to update an ongoing or previously selected choice pattern.

The current set of experiments also demonstrated that the connection between the PL and DMStr is important for proactive behavioral switching. In addition to previous research implicating the DMStr in action selection, the projection between the prefrontal cortex and the DMStr has also been connected with this process (Seo et al., 2012; Wolfensteller and Ruge, 2012). Additionally, a previous study employing a disconnection of the prefrontal cortex from the striatum showed that when rats were required to recall their previous choice and then choose the opposite of that choice (delayed alternation), disconnection of the prefrontal cortex from the striatum resulted in impairments in performance (Dunnett et al., 2005). The current results extend this to suggest that the ability of the DMStr to correctly switch from an ongoing behavior to a newly relavent one based on cue information and reward feedback is dependent on input from the PL.

One hypothesis to explain these results is that the prefrontal cortex, including the PL, is important in the generation of strategies during cognitive flexibility tasks

(Ragozzino, 2007). Under this framework, the PL would apply the two possible strategies in the cue – place conditional discrimination (e.g. go north when white, or go south when black) and value would be assigned to these strategies by the DMStr. Evidence supports a role for the PL in this function as increased local field potential coherence, a measure of a brain area's coordination, in the PL has been observed in a task in which an odor determined which of two egocentric responses was to be performed during the odor sampling phase (Gruber et al., 2010). Without the PL, the appropriate strategies would not be generated and possibly ineffective strategies generated leading to selection of an inappropriate choice pattern. This increased propensity of animals to use an inappropriate egocentric strategy without a functional PL has been observed in a previous experiment (Dias and Aggleton, 2000). Therefore, both the PL and DMStr may functionally interact by first generating possible strategies and then selecting the appropriate strategy at the appropriate time.

Figure 1

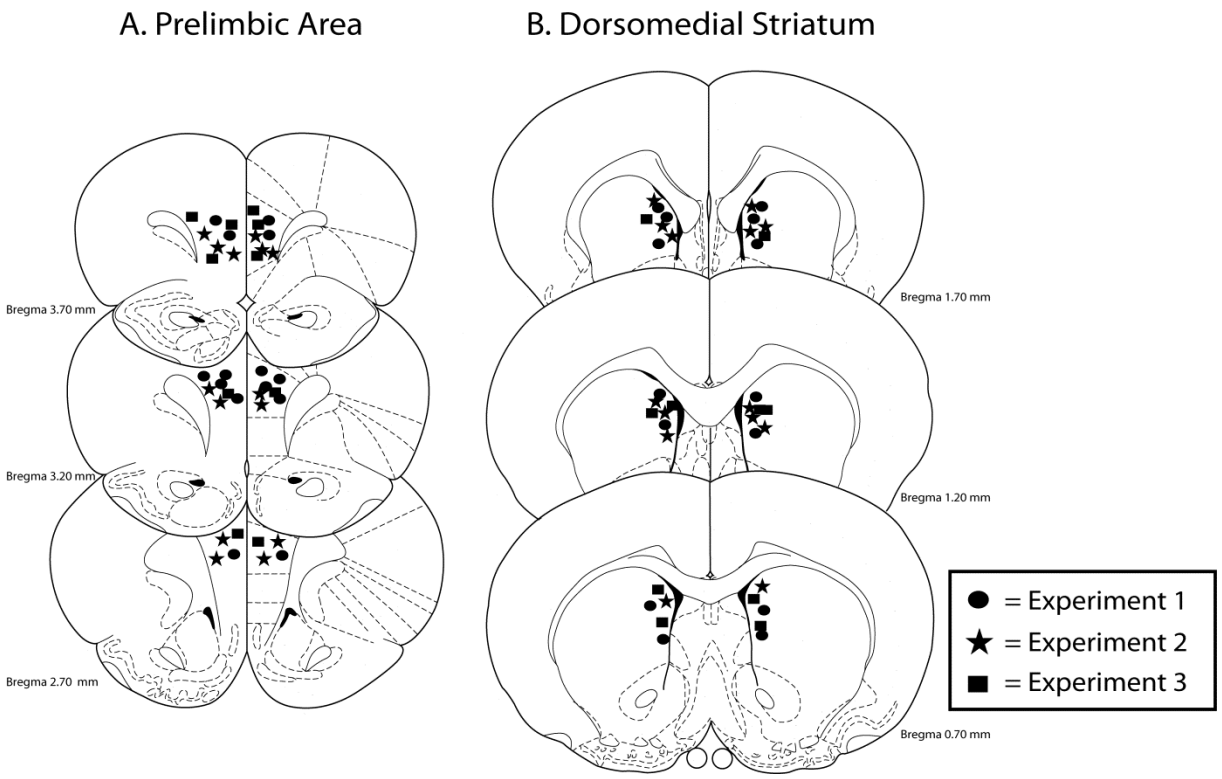

Figure 1. Cannula tip placements in the PL and the DMStr in Experiments 1-3. A. Representation of cannula placements in the PL. B. Representation of cannula placements targeting the DMStr. Adapted from The Rat Brain in Stereotaxic Coordinates (Paxinos and Watson, 1998).

Figure 2

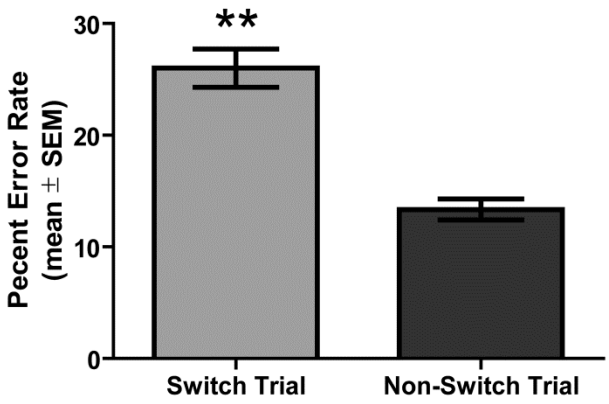

Figure 2. Proactive behavioral switching incurs a switch cost in vehicle treated rats. All saline treatments in experiments 1 and 2 were collapsed into one group to examine performance (mean ± SEM) on switch vs. non-switch trials. The percent error rate for switch and non-switch trials was calculated based on the number of errors divided by the total number of trials of that type. Vehicle treated animals were more likely to commit an error on switch vs. non-switch trials. ** $p < 0.01$.

Figure 3

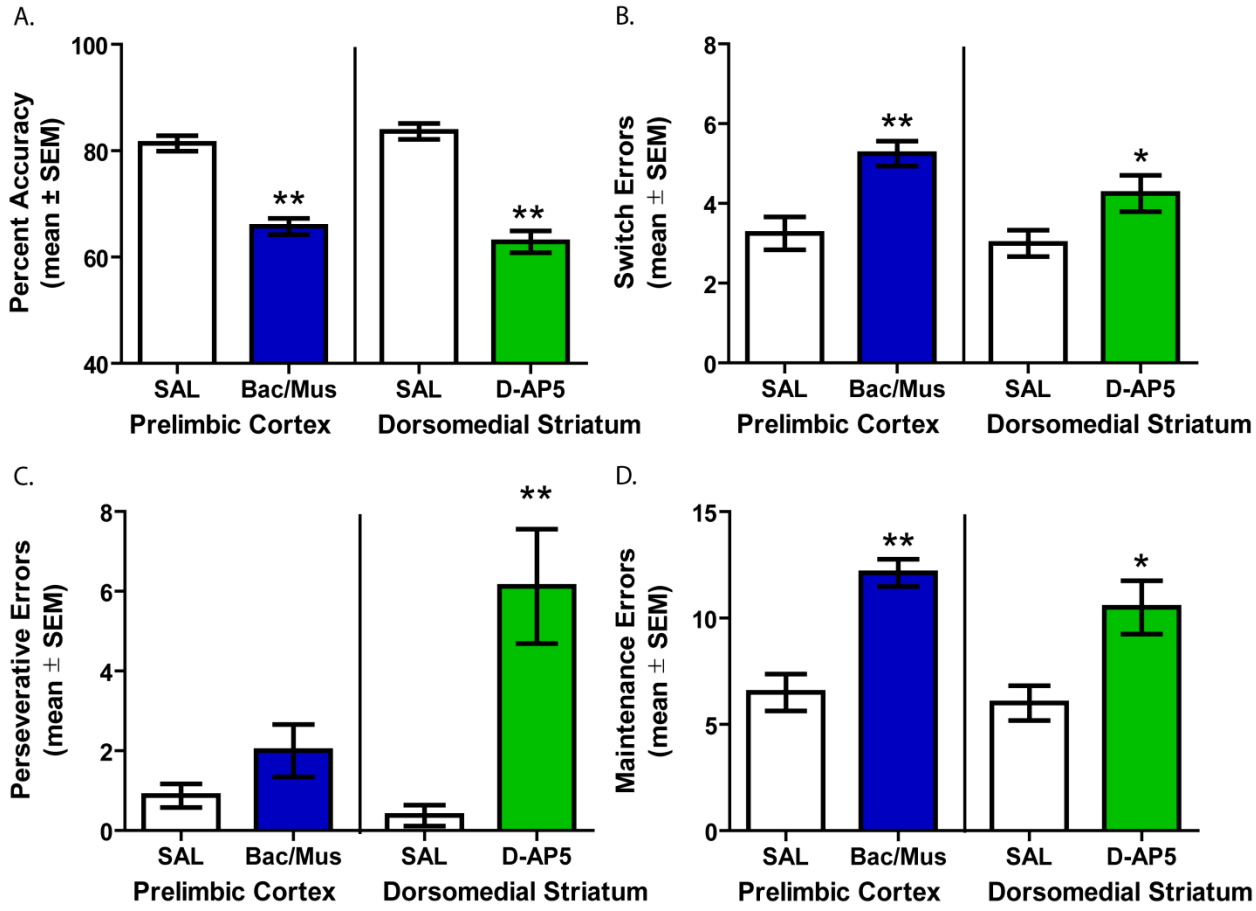

Figure 3 PL inactivation and DMStr NMDA receptor blockade impairs proactive behavioral switching. Each rat (n = 8) received a bilateral injection into the PL of saline (SAL), and baclofen 0.05uM-muscimol 0.18uM (Bac/Mus) or into the DMStr of saline (SAL) or D AP-5 10µM (D-AP5) in a random order 5 min before testing. A. Bac/Mus treatment in the PL or D-AP5 treatment in the DMStr significantly impaired accuracy (mean ± SEM) compared with SAL treatments. $^{**}p < 0.01$. B. The number of switch errors (mean ± SEM) increased in the Bac/Mus and D-AP5 treatments compared to SAL treatments. $^{**}p < 0.01$, $^{*}p < 0.05$. C. The D-AP5 treatment led to significantly more perseverative errors (mean ± SEM) than all other treatments. $^{**}p < 0.01$. D. The number of maintenance errors (mean ± SEM) increased in the Bac/Mus and D-AP5 treatments compared to SAL treatments. $^{**}p < 0.01$, $^{*}p < 0.05$.

Figure 4

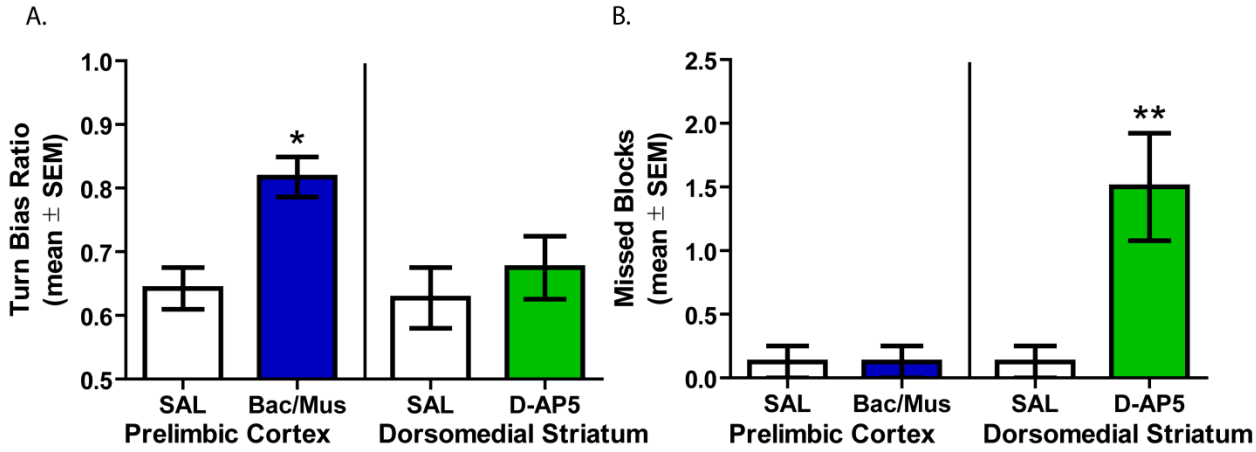

Figure 4. The effect of treatments on turn bias and missed blocks during proactive behavioral switching. A. Turn bias ratio (mean ± SEM) during the visual cue – place conditional discrimination. Bac/Mus treatment in the PL led to a greater likelihood of rats to adopt an egocentric turn bias during the task than all other treatments *$p < 0.05$. B. Missed blocks (mean ± SEM) during the visual cue – place conditional discrimination task. D-AP5 treatment in the DMStr led to more missed blocks during a session then all other treatments. **$p < 0.01$.

Figure 5

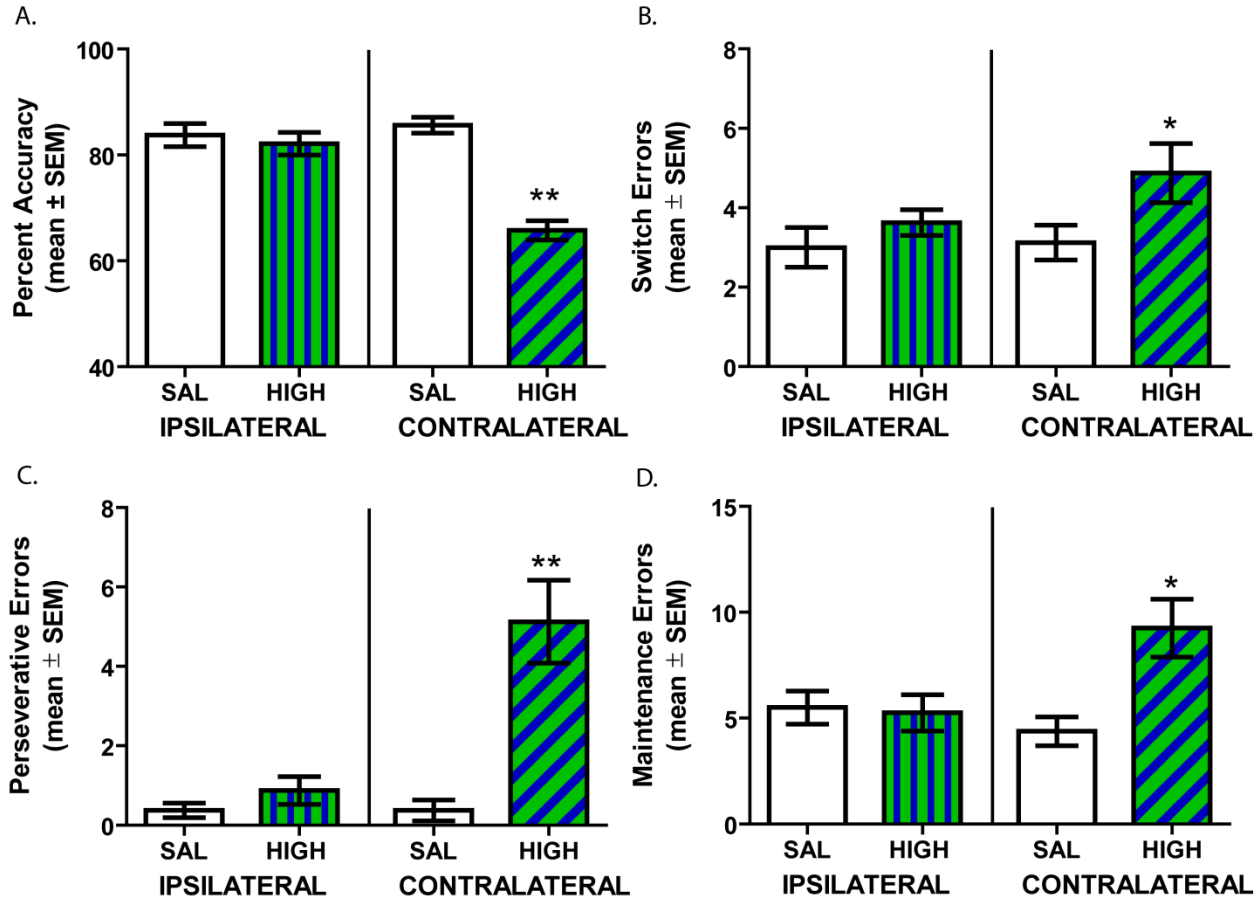

Figure 5. Contralateral but not ipsilateral disconnection of the PL – DMStr areas impairs proactive behavioral switching. Each rat (n = 8) received a unilateral injection into the PL and an injection into the DMStr either in the same or opposite hemisphere in a random order 5 min before testing. Drug doses were the same as in the bilateral treatments for the respective areas. A. Contralateral drug treatment led to a decrease in accuracy (mean ± SEM) compared to all other treatments. ** $p < 0.01$. B. The number of switch errors (mean ± SEM) increased in the contralateral drug treatment compared with other treatments. * $p < 0.05$. C. The contralateral treatment led to significantly more perseverative errors (mean ± SEM) than all other treatments. ** $p < 0.01$. D. The number of maintenance errors (mean ± SEM) increased in the contralateral treatment compared to other treatments. * $p < 0.05$.

Figure 6

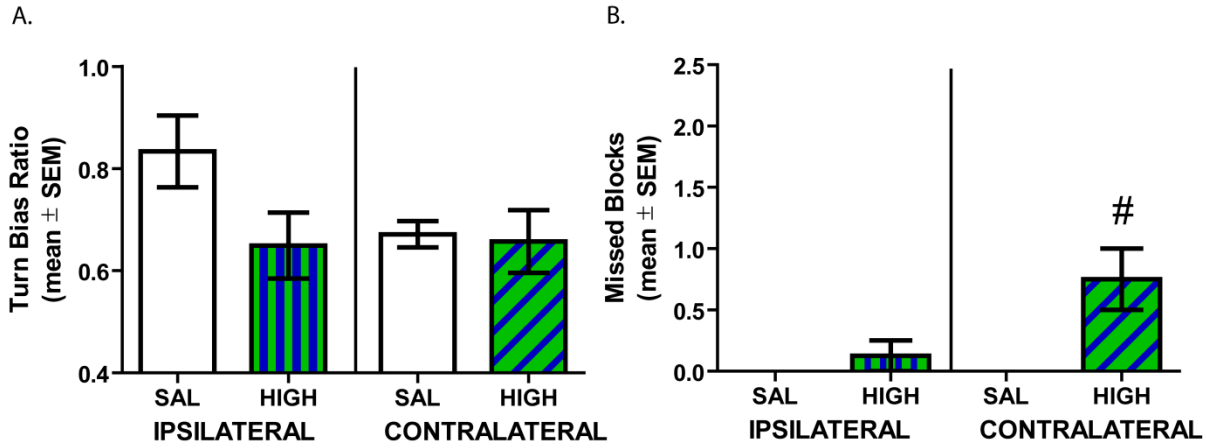

Figure 6. The effect of treatments on turn bias and missed blocks during proactive behavioral switching. A. Turn bias ratio (mean ± SEM) during the visual cue – place conditional discrimination. No effect of treatment was observed on turn bias B. Missed blocks (mean ± SEM) during the visual cue – place conditional discrimination task. contralateral treatment led to more missed blocks during a session then all other treatments. #$p < 0.01$ vs SAL, $p < 0.05$ vs. IPSI HIGH.

Figure 7

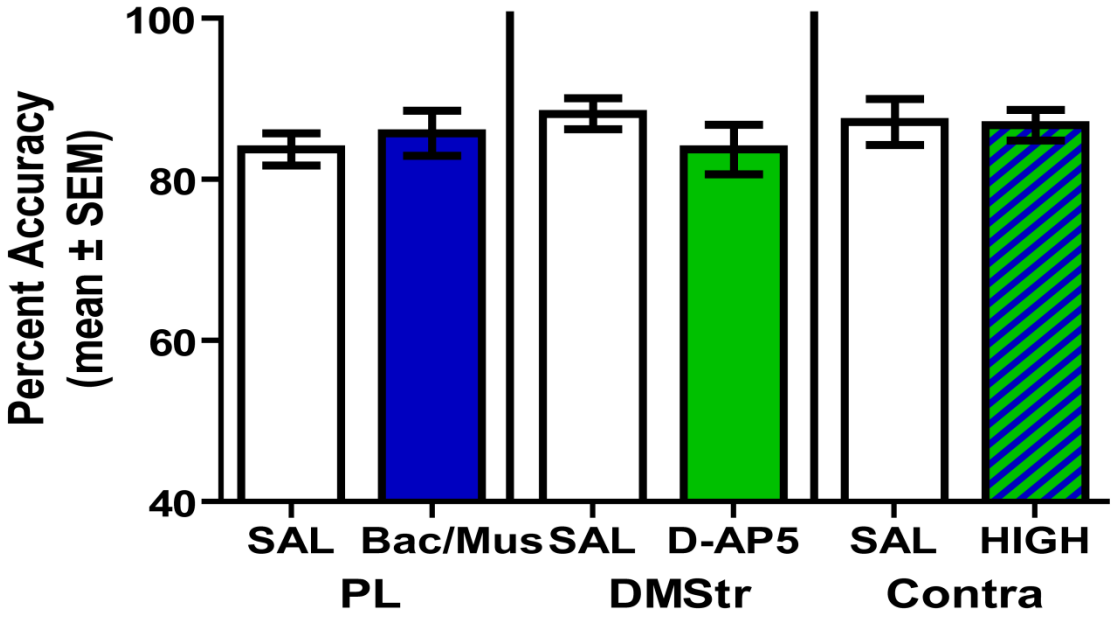

Figure 7. PL inactivation, DMStr NMDA receptor blockade, and contralateral disconnection of the PL-DMStr (Contra) does not affect performance during a non-switch cued-association test. Each rat (n = 7) received 6 treatments during 6 separate non-switch discriminations. Two treatments in the PL, were administered; a saline injection (PL SAL), and baclofen/muscimol (PL Bac/Mus). Two treatments were given in the DMStr; a saline control (DMS SAL), and the NMDA receptor antagonist D-AP5 (DMStr D-AP5). Two treatments were given utilizing a disconnection design to examine the PL-STN areas together; a saline injection (Contra SAL), and the previously effective treatment of baclofen/muscimol in the PL and D-AP5 in the DMStr (Contra High). No differences in performance were observed between treatments in a non-switch discrimination.

Chapter IV

General Discussion

The ability of animals to switch between strategies is critical to survival. Animals across a variety of species confront alterations in environmental demands, e.g. changes in food location, predators in an area, software to complete work tasks, or sudden developments in traffic, that require inhibiting the use of a current strategy and switching to a new or different strategy. These behavioral switches can be brought about by either changes in reinforcement or from cues which indicates that the contingencies will change. For humans, this may occur in a game of poker in which several strategies including bluffing and folding have to change as they variously are successful or not throughout the game to win money. Alternatively, an opponent may be bad at hiding their strategy and may inadvertently give signals which can determine how you bet. In the former case, reward feedback informs switches in strategy from one hand to the next. In the latter case, a cue (the tell) is able to guide a person to use the most beneficial strategy. Hikosaka and Isoda (2010) have proposed that there are two fundamental conditions that lead to behavioral switching. In the first condition, termed proactive switching, cue information is presented in each trial that accurately informs which choice pattern should be selected. In the second, called retroactive switching, prior outcome information, e.g. absence of reward, should be used to initiate a switch in response for the current trial. Understanding the underlying neural substrates of these two forms of behavioral switching can provide insights into how the brain functions to allow adaptations in a changing environment. Understanding this brain-behavior relationship may also offer insights into how to address specific cognitive flexibility

deficits in aging, and diseases where impairments are observed (Verte et al., 2005; Ashendorf and McCaffrey, 2008; Koerts et al., 2009; Thoma et al., 2011).

As past studies investigating the neural basis of cognitive flexibility focused on retroactive switching, the central goal of the present studies was to investigate whether specific rat prefrontal cortex and basal ganglia areas interact to support proactive switching. The present experiments revealed that bilateral PL inactivation and bilateral NMDA receptor blockade in the STN or DMStr impairs proactive switching. In addition, contralateral disconnection of the PL and STN areas, as well as the PL and DMStr also impaired proactive behavioral switching. These latter results suggest that prefrontal cortex and basal ganglia areas functionally interact to enable rapid and repeated switches in choice patterns when cue information is to be used proactively. These studies extend the substantial literature indicating that the PL and DMStr support behavioral switching when retroactive outcome information is to be used by showing that the PL and STN, as well as the PL and DMStr may be part of larger neural systems to enable proactive behavioral switching.

There is substantial evidence that the PL is involved in behavioral switching when retroactive information is to be used for a behavioral switch (Ragozzino et al., 1998; Birrell and Brown, 2000; Ragozzino et al., 2002a; Floresco et al., 2006b; Rich and Shapiro, 2007; Floresco et al., 2008; Enomoto et al., 2011). The present findings with PL inactivation suggest that the PL is also important for cognitive flexibility when cue information can be used proactively to guide behavioral switching. However, when comparing the deficits in proactive switching with those in retroactive switching following PL inactivation, there are distinct differences between the types of errors committed. In

retroactive switching, PL inactivation or NMDA receptor blockade in the PL results in a selective increase in perseverative errors (Ragozzino et al., 1999b; Ragozzino et al., 1999c; Ragozzino et al., 2003). The current results suggest that when cues are present to guide switches, a more general increase in errors due to an increased likelihood of using an inappropriate turn bias results. Taken together these data suggest that the PL may be broadly involved in cognitive flexibility but that the PL may support various processes to enable cognitive flexibility which are based on the specific environmental contingencies.

The present experiments also demonstrated that the PL is not the only forebrain area that supports proactive switching. The STN represents another brain area involved in proactive switching. The mainly ipsilateral projections from the PL to the STN have been previously described (Afsharpour, 1985; Berendse and Groenewegen, 1989; Canteras et al., 1990). These projections utilize excitatory amino acids to relay signals from the PL to the STN on short time scales (Maurice et al., 1998; Magill et al., 2006). Although there is evidence of direct projections from the PL to the STN, less is known of how these brain areas may interact to support various behavioral functions. Investigations of the STN in rodents have suggested that this area is important for response inhibition and sustained attention (Baunez and Robbins, 1999; Eagle et al., 2008), and in non-human primates the STN has been proposed to play a role in proactive switching (Isoda and Hikosaka, 2008). The findings in non-human primates led me to determine whether the rat STN also contributes to proactive switching. Based on NMDA receptor blockade in the STN, this area appears to be required for the inhibition of an ongoing response pattern. This is due to the selective increase in switch

and perseverative errors observed in the conditional discrimination test. Further, contralateral disconnection of the PL and STN resulted in a similar selective increase in switch and perseverative errors. This result supports the idea that the connection between the PL and STN plays an important role in the function of the STN to stop an ongoing response pattern.

Comparable to NMDA receptor blockade in the STN, the same pharmacological manipulation in the DMStr also impaired performance in the cue – place conditional discrimination. The error pattern observed with NMDA receptor blockade in the DMStr, however, differed from both that observed in the PL and STN manipulations. Specifically, a general increase in all error types occurred but was not due to an egocentric turn bias as was observed with PL inactivation. Instead, in the present studies an increase in the likelihood of missing an entire block of trials, which was not observed with PL or STN treatments, accounted for the general increase in errors. This would suggest that, as with the involvement of the PL in cognitive flexibility, the DMStr may play a broad role that manifests in different ways depending on the specific environmental contingencies in a task.

Contralateral disconnection of the PL and DMStr also resulted in an increased frequency of missing an entire block of trials during the cue – place conditional discrimination. Based on past studies, the projection from the PL to the DMStr is excitatory and may be mediated, in part, by NMDA receptors (Sesack et al., 1989; Conde et al., 1995; Spencer and Murphy, 2000b; Gabbott et al., 2005). NMDA receptors within the DMStr can cause either potentiation or depression of post-synaptic responses depending on the specific parameters of cortical stimulation (Partridge et al., 2000;

Spencer and Murphy, 2000b; Paille et al., 2010). The PL projection to the DMStr may facilitate behavioral switching through plastic changes in DMStr neurons that in part is mediated by NMDA receptors (Kita, 1996; Lovinger and Tyler, 1996; Charpier and Deniau, 1997; Akopian and Walsh, 2002a). Reorganization of striatal neuronal responses can occur rapidly during tests of cognitive flexibility (Kimchi and Laubach, 2009). Therefore, NMDA receptors, through changes in synaptic plasticity within the DMStr, could represent a critical mechanism for switching choice patterns. Thus, the PL – DMStr may functionally interact to allow a switch from an ongoing to a currently relevant strategy and maintaining that strategy for period of time.

The following section will offer a possible framework for the role of the PL and its connections with the DMStr and STN in cognitive flexibility.

A. The top down coordination of proactive switching behavior

Because the present findings suggest that the PL connections with different basal ganglia areas act in a somewhat different manner to support proactive behavioral switching, one possibility is that the PL is acting in a top down fashion to control behavioral switching through two different basal ganglia pathways. Narayanan and Laubach (2006, 2009) have proposed that the dorsomedial frontal cortex encodes both prepotent responses and proactive inhibition such that when neurons encoding proactive inhibition predominate, a rat will be less likely to make a premature response in tests that have a delay component. I suggest a somewhat similar top-down model in which the PL encodes both inhibition of an ongoing strategy and generation of relevant strategies in response to specific cues. In this manner, the PL would be critical for the monitoring of task cues in order to guide appropriate behavior on a trial to trial basis.

When excitatory input from the PL to the STN predominates this allows an inhibition of the ongoing response and selection of a different pattern. This hypothesis is supported both from results described in Chapter 2, and in research from other laboratories. Specifically, the frontal cortex – STN circuit has been shown to be important for proactive switching and inhibition of an ongoing response (Aron and Poldrack, 2006; Hikosaka and Isoda, 2010). In this way, the PL and STN together can rapidly terminate an ongoing or prepotent response when no longer relevant.

Physiological evidence suggests that the PL – STN circuit is ideally suited to this function. PL stimulation is followed by a large burst of neuronal firing in the STN after 4-8 ms (Maurice et al., 1998; Magill et al., 2006). This early burst is attributed to the PL – STN projection. A later excitatory burst appears after 21-30 ms which comes from the indirect pathway release of EPN inhibitory input into the STN. Furthermore, recordings in the substantia nigra pars reticulata reveal that input from the STN arrives before that from the direct pathway coming from the striatum (Fujimoto and Kita, 1993; Ryan and Sanders, 1994; Maurice et al., 1999). This is important for a proposed model of PL – STN input in overriding a prepotent or ongoing behavior (Mathai and Smith, 2011). The signal from this pathway arrives at basal ganglia output structures before that of the direct and indirect pathway allowing for modification of the output back to the motor cortex. In this way the PL – STN circuit represents an ideal mechanism for the top down inhibition of an ongoing behavior or strategy when cues indicate the choice pattern should not be used.

PL inactivation not only led to switch errors, but also increased maintenance errors. This would suggest that the PL interacts with other areas to support proactive switching.

The findings from studying the PL – DMStr areas suggest that these areas functionally interact differently than the PL and STN areas to support behavioral switching. This is because contralateral disconnection of the PL – DMStr areas and bilateral DMStr NMDA receptor blockade were the only treatments which increased the likelihood of rats to miss an entire block of trials. One possibility is that the PL input to the DMStr provides information about possible strategies or choice patterns in a context and the DMStr aids in the appropriate selection of a strategy (Kim et al., 2009; Tai et al., 2012). In fact, signals within the DMStr have been shown to encode information about the expected reward value of a given behavioral response based on previous reward feedback from making that choice (Stalnaker et al., 2012; Kim et al., 2013). One possibility is that cue information also can be used proactively by the DMStr to select a strategy. If input from the PL to the DMStr is disrupted, this may decrease information about possible strategies and limit the accuracy of selecting a strategy (Ragozzino, 2007) which could lead on occasion to making errors for an entire block of trials. Thus, in the conditional discrimination test rats may have been unable to generate a different choice pattern appropriate to the cues on a given trial and the previous or original choice pattern is instead carried out. Taken together with the PL-STN findings, one possibility is that when cue information should be used to proactively switch choice patterns that a neural system that includes the PL and STN support the rapid inhibition of an ongoing choice pattern while concomitantly a neural system that includes the PL and DMStr enables selection of an alternative choice pattern. This latter system also continues to be critical for maintaining the alternative choice pattern after being initially selected.

Taken together, evidence from the current experiments and supported by previous work suggests that the PL is involved in at least two top down functions during proactive behavioral switching. The PL – STN circuit is critical to initially inhibit and ongoing action when cues change. The PL – DMStr circuit is critical for switching to an alternative choice pattern and maintaining that choice pattern based on current task contingencies. Without this circuit, rats "lock in" to one of the strategies and fail to reliably execute both of the discriminations.

B. Implications of the current results for the treatment of Parkinson's disease and other disorders

Proactive behavioral switching is a critical ability which we utilize constantly throughout the day. However, in disorders such as Parkinson's disease (PD), impairments are observed in a variety of cognitive measures including proactive switching (Witt et al., 2006). Deficits in cognitive function are observed in a third of patients at initial diagnosis (Foltynie et al., 2004). Furthermore, executive function, which includes proactive switching, is the most significant predictor of quality of life in PD patients (Cahn et al., 1998). Currently, existing treatments for PD do not consistently improve cognitive impairments (Moustafa et al., 2008; Massano and Garrett, 2012). PD is a condition of the basal ganglia and to some extent the prefrontal cortex (Blandini et al., 2000; Weintraub et al., 2011). Early in the disease, dopaminergic input into the striatum degrades (Davis et al., 2003; Jokinen et al., 2009). Changes in basal ganglia due to this disruption of outflow are thought to affect prefrontal cortex functions (Owen et al., 1998; Cools et al., 2002). Normal function in these areas is disrupted causing abnormal physiological function (Schnitzler and Gross, 2005; Kwak et al., 2012). Of

particular note to the current experiments is the effect on the STN. STN neurons in primate models of PD show increases in baseline firing rates as well as increased burst firing in erratic patterns (Bergman et al., 1994). These changes in basal ganglia function could help explain some of the effects of PD on proactive switching.

The current experiments offer support for the development of alternative treatments aimed at improving cognitive symptoms in PD. Specifically, the present results indicate that connections between the PL – STN and PL – DMStr are critical for proactive switching. The STN has become a major target in the treatment of PD through DBS surgery. My results suggest that careful examination of specific cognitive flexibility tasks should be examined when testing the effects of any treatment on PD and other basal ganglia related disorders. Recent work has focused on improving placement of DBS in the STN to minimize negative outcomes on cognitive function by focusing on motor parts of the human STN (York et al., 2009). Generally, these studies have used broad cognitive batteries which may fail to detect extant deficits in specific domains such as proactive switching. More work on ideal stimulation parameters and locations could lead to improvements in cognitive outcomes known to rely on the STN.

C. Future Directions

The results from the current set of experiments inspire a variety of new questions to be examined in future studies. These studies offer a preliminary examination of PL – basal ganglia connections during proactive behavioral switching. Additional excitatory input from the intralaminar thalamic nuclei also reaches both areas (Canteras et al., 1990; Castle et al., 2005). Previous work in our laboratory has shown that the input from the parafascicular nucleus of the thalamus contributes to the role of the DMStr in

reversal learning (Brown et al., 2010). This raises the possibility that the projection from the parafascicular nucleus also contributes to proactive forms of switching. Because it projects to two areas I have shown to be involved in proactive switching, investigation of this connection could lead to a broader understanding the neural mechanisms involved.

The current experiments revealed that the PL projections to both the DMStr and STN contribute to proactive switching. These structures may also have additional roles in proactive switching through their involvement in the indirect pathway. The indirect pathway is part of the larger basal ganglia circuit including the direct pathway, the indirect pathway, and the cortico – STN pathway (Mathai and Smith, 2011). In the indirect pathway, a portion of the DMStr projects to the entopeduncular nucleus which in turn projects to the STN. Several recent studies have sought to understand contributions of this pathway through pharmacological and electrophysiological means (Yu et al., 2009; Tai et al., 2012; Cui et al., 2013). To better understand the role of the STN and DMStr in proactive switching, use of these methods to test indirect pathway contributions could determine if their contribution to performance is specific to PL input or if additional input is also important.

Another interesting future direction for this research is into specific receptor or receptor subunit contributions to proactive switching. Pharmacological manipulations of specific NMDA receptor subunits have revealed differential contributions to behaviors. Specifically, antagonism of the NR2B subunit of the NMDA receptor has been shown to be pro-cognitive during retroactive forms of switching (Kos et al., 2011). Understanding specific contributions of NMDA receptor subunits or other neurotransmitters in these areas could lead to targeted treatments for disorders where impairments in proactive

switching are observed. Additionally, other neurotransmitters such as serotonin or dopamine are known to influence the function of both the STN and DMStr (Parent et al., 2010; Cruz et al., 2011; Lex et al., 2011; Agnoli and Carli, 2012). Investigation of these neurotransmitters during the visual cue – place conditional discrimination task could lead to novel treatment avenues to disorders of the corticostriatal circuit.

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2012-Present C2A Tutor for low income High School students

Service/Administrative

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2004 Student Council Senator, Eastern Mennonite University

Publications

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2011 **Phillip M. Baker**, Jennifer L. Thompson, John A. Sweeney & Michael E. Ragozzino. Differential Effects of 5-HT2A and 5-HT2C Blockade on Strategy-Switching. Behav Brain Res. 2011 May 16; 219: 123-131.

2011 Eric G. Mohler, **Phillip M. Baker**, Sharon Shacham, Kimberly Gannon, John A. Sweeney & Michael E. Ragozzino. PRX-07034, A Novel 5-HT6 Antagonist Enhances Cognitive Flexibility and Working Memory. Psychopharm (Berl). 2012 Apr;220(4):687-96

2012 **Phillip M. Baker**, Michael E. Ragozzino. The Role of the Prelimbic Cortex and Subthalamic Nucleus in Proactive Behavioral Switching. [Submitted]

2012 **Phillip M. Baker**, Michael E. Ragozzino. Prelimbic Cortex Dorsomedial Striatal Contributions to Proactive Behavioral Switching. [In Preparation]

Book Chapters

Michael E. Ragozzino, **Phillip M. Baker**. Frontal Cortex- Basal Ganglia Systems Support of Learning and Memory Functions. Title: The Neurobiological Basis of Memory: A System, Attribute, and Process Analysis. A Festschrift in Honor of Raymond P. Kesner [In Preparation]

Presentations/Abstracts

2009 **Baker PM**, Brown HD, Ragozzino ME. Parafascicular thalamic nucleus inactivation simultaneously modifies dorsomedial striatal acetylcholine output and place reversal learning. Society for Neuroscience Annual Meeting 2009. (abstract)

2010 **Baker PM**, Thompson JM, Sweeney JA, Ragozzino ME. Differential effects of 5HT-2A and 5HT-2C blockade on attentional set shifting. Chicago Chapter of the Society for Neuroscience 2010. (abstract)

2011 **Phillip M. Baker**, Michael E. Ragozzino. Differential Contributions of the Prelimbic Cortex, Subthalamic Nucleus, and Disconnection of the Circuit During a Conditional Discrimination in Rats. Society for Neuroscience Annual Meeting 2011. (abstract)

2012 **Phillip M. Baker**, Gena Grospe, Michael E. Ragozzino. Impairments in Cognitive Flexibility in a Rat Model of Parkinson's Disease. Center for Clinical and Translational Science Annual Pre-doctoral Meeting 2012. (abstract)

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2003-2007 Presidents Scholarship Award, Eastern Mennonite University

2003-2007 Dean's List, Eastern Mennonite University

2003-2007 NCAA Division III Academic All-Star Award in Men's Soccer, Eastern Mennonite University

Research Interests

I am interested in the neural circuitry that underlies decision-making. Specifically I seek to understand how the frontal cortex and the basal ganglia are involved in learning from errors in choice patterns to shape future choices and patterns of behavior. Using animal models offers unique opportunities to examine how critical brain areas and neurotransmitters involved in decision-making respond *in vivo* to a constantly changing external environment. My long term goal is to remain in academia and inspire students to consider a career in research.

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