6-1-2015

Assessing motor and cognitive function to detect shifts in brain function in two models of Parkinson's disease

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ABSTRACT

Cognitive changes accompany and often precede the onset of classic motor deficits typical of Parkinson's disease. A current focus of Parkinson's research has become understanding the development and progression of pre-motor cognitive changes. Based on previous research showing that hippocampus-sensitive spatial learning can be enhanced at the cost of impaired striatum-sensitive response learning, we hypothesized that changes in the balance between these two cognitive systems could be used as a proxy for the relative strength or health of their associated brain regions. Because non-motor symptoms of Parkinson's disease can precede the onset of the diagnostic motor dysfunction, changes in the balance between distinct learning strategies may represent an early marker of Parkinson's-related neurodegeneration. Two rat models were used to assess the relationship between Parkinson's disease-related motor dysfunction and changes in cognition. In the first study, a 6-OHDA rat model of Parkinson's disease was used to generate a partial lesion of dopaminergic neurons in the nigrostriatal pathway. Despite the probable depletion of dopamine in the nigrostriatal pathway of lesioned rats that presented as impairment in two motor tasks, rats showed enhanced performance on the cognitive spontaneous alternation task, a test of spatial working memory. However, recent data suggest that multiple brain regions, including both the hippocampus and striatum, are activated during performance of the spontaneous alternation task; Parkinson's-induced enhancements on this task may not be due solely to a shift in cognitive balance. Previous data show that inactivation of the hippocampus can enhance striatum-sensitive learning; however, it is unclear if inactivation of the striatum enhances hippocampus-sensitive functions. Prior to determining the effect of a 6-OHDA-induced lesion on hippocampus-sensitive learning, we wanted first to assess how impairing striatum function modulated place learning to determine cognitive shifts in rats

with an intact brain. The second study uses two single-solution cognitive tasks that may link more closely to activation of separate neural systems. Temporary inhibition of the dorsal striatum by the $GABA_A$ receptor agonist, muscimol, produced deficits in motor function similar to those seen in the 6-OHDA model of Parkinson's. Intrastriatal muscimol also impaired learning on a striatum-sensitive response learning task, suggesting that striatum-sensitive motor processes may overlap with striatum-sensitive cognitive processes. However, muscimol-induced striatum dysregulation did not produce enhancements on a hippocampus-sensitive spatial learning task. It is possible that the cognitive enhancements in hippocampus-sensitive processes are maximized when only specific neurotransmitter systems are dampened, such as the loss of dopaminergic signaling seen in Parkinson's disease. Unlike 6-OHDA, which targets dopaminergic neurons, muscimol activates $GABA_A$ receptors, leading to the opening of Cl^- channels, altering membrane potentials, and changing the likelihood of neurotransmitter release. Thus, activation of $GABA_A$ receptors by muscimol will alter neuron activity regardless of neurotransmitter system while 6-OHDA must initially affect dopaminergic neurons. Consequently, it is possible that muscimol decreases activity in neurotransmitter systems that play a compensatory role following 6-OHDA-induced dopaminergic degeneration. As such, a generalized inhibitor of neural activity like muscimol, may disrupt neural processes that are integral for seeing the Parkinson's disease-related cognitive enhancements.

Assessing motor and cognitive function to detect shifts in brain function in two models of Parkinson's disease

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A.B. Neuroscience, Bowdoin College, 2006

THESIS

Submitted in partial fulfillment of the requirements for the degree of
Master of Science in Biology.

Syracuse University
June 2015

Acknowledgments

First and foremost, I would like to thank my advisor, Donna L. Korol, without whom this thesis would not have been possible. I am grateful for the research experience and career development I have received during my time in her laboratory. I have learned a wider array of techniques and skills than expected and have become a better, more thoughtful researcher under her guidance.

I also grateful for the feedback and guidance I received from my committee members, Samuel Chan, Paul Gold, James Hewett, and Melissa Pepling.

I would also like to thank my lab mates, Frances Batarse, Mike Dash, Wayne Hawley, Ken Morris, Lori Newman, Samantha Pisani, Jamie Richards, Claire Scavuzzo, Tumay Tunur, and Wei Wang, for their help and support over the years. I am especially thankful for having them as a sounding board while analyzing data and trying to make sense of my experiments. I would especially like to thank Ken Morris for his help on the 6-OHDA project.

I owe my deepest gratitude to my parents, Richard and Martha, for their love and support throughout my time in graduate school. Without their continuous encouragement, I would not have been able to achieve my goals. I am also extremely grateful for my husband, Rob, for his love, support, and (most importantly) the many delicious home-cooked meals after long days in the lab.

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CHAPTER 1

Introduction

Parkinson's disease (PD) is the second most common age-related neurodegenerative disease next to Alzheimer's disease. Anatomically, PD is characterized by a degeneration of the nigrostriatal pathway. A probable diagnosis is determined by the presence of at least two of the following cardinal motor symptoms: resting tremor, bradykinesia/akinesia, muscle rigidity, and/or postural imbalance; a positive diagnosis can be made only upon postmortem examination of the brain for the presence of Lewy bodies and Lewy neurites (Braak et al., 2003). However, motor symptoms used to diagnosis the disease are not exhibited until ~60% of the dopaminergic neurons in the substantia nigra have been lost, representing ~80% loss of dopaminergic input to the striatum (Zigmond et al., 1984; Dauer and Przedborski, 2003; de Lau and Breteler, 2006). If an early diagnostic marker were found, it might be possible to implement treatments that slow or halt disease progression before the onset of debilitating symptoms.

Although traditionally associated with motor dysfunction, growing evidence suggests that there are debilitating non-motor symptoms of PD, including autonomic dysfunction, gastro-intestinal disturbances, poor sleep, low affect, and cognitive deficits (Dubois and Pillon, 1997; Tolosa et al., 2007; Beeler, 2011) that may precede the motor dysfunction symptoms characteristic of PD (Tolosa et al., 2007; Tolosa et al., 2009; Foerde and Shohamy, 2011a; Tolosa and Pont-Sunyer, 2011). Change in cognitive function reflects one such pre-motor symptom associated with PD (Lees and Smith, 1983; Branchi et al., 2008). Additionally, because many of the non-motor dysfunctions in early stages of PD present before motor dysregulation, it is possible that the non-motor symptoms of PD are more sensitive to changes in dopamine signaling (Tolosa et al., 2009). Consequently, changes in cognitive function or learning strategy

preference may represent a more sensitive diagnostic tool for the disease than tests of motor dysfunction.

While it is easy to associate only impairments with degenerative processes like PD, evidence from the multiple memory systems field suggests that some cognitive domains may remain unaffected or even show enhanced functioning as a consequence of the neurodegeneration seen in PD. Previous work from our lab and others has shown that hippocampus-based learning strategies have a competitive relationship with striatum-based learning strategies (Packard et al., 1989; Packard and White, 1991; McDonald and White, 1993, 1994; Korol and Kolo, 2002; Chang and Gold, 2003a, b; Gold, 2004; McDonald et al., 2006; Atallah et al., 2008). Additionally, changes in learning strategy preference, learning speed, and/or accuracy using a certain learning strategy can reflect changes in the functionality of the hippocampus or striatum (Chang and Gold, 2003b). However, many of these dissociation studies suggest that the hippocampus normally outcompetes the striatum for strategy selection.

While impairing hippocampus function leads to decreased spatial learning and enhanced striatum-sensitive response learning, there has been limited evidence that decreasing striatum function can bias hippocampus-sensitive cognitive strategies or even enhance hippocampus function (Packard and McGaugh, 1996; Atallah et al., 2008). In a study by Packard and McGaugh (1996), rats were trained over 16 days to find a food reward on a dual solution task that allows rats to use either a striatum-sensitive egocentric turning strategy or a hippocampus-sensitive spatial strategy to remember the location of a food reward on a T-shaped maze. While use of a hippocampus-sensitive spatial strategy is predominant on day 8 of testing, rats given intrastriatal infusions of lidocaine maintain this strategy through day 16. In contrast, control rats given intrastriatal infusions of saline switch to a striatum-sensitive turning strategy. These data

suggest that impairment of the striatum via lidocaine prevents a training-induced shift in cognitive strategy, allowing the initial hippocampus-sensitive spatial strategy to persist as the primary navigational strategy.

Subsequent research by Chang and Gold (2003b) showed that rats will shift navigational behavior from a hippocampus-sensitive strategy to a striatum-sensitive response strategy with prolonged training on this task, possibly due to a transition from declarative learning to a procedural behavior. The data from Packard and McGaugh (1996) support the theory that blockade of a striatum-sensitive strategy permits a previously used hippocampus-sensitive spatial strategy. Atallah et al. (2008) used a probabilistic odor discrimination task to examine the interaction between striatum- and hippocampus-sensitive learning systems. Human fMRI studies have shown activation of the striatum during probabilistic learning (Delgado et al., 2005; Dickerson et al., 2011). Additionally, people with PD show impaired performance on probabilistic learning tasks, suggesting that these tasks may utilize brain regions that are altered in PD (Wilkinson and Jahanshahi, 2007; Foerde et al., 2013). In their study, Atallah et al. (2008) showed that temporary dysregulation of the hippocampus with muscimol (MUSC) leads to enhanced performance on a striatum-sensitive probabilistic odor discrimination task: rats given intrahippocampal infusions of MUSC chose the rewarded odor at higher rates than did control saline-infused rats (Delgado et al., 2005). These data suggest that interference from the hippocampus can negatively affect performance on a task that is best solved using a striatum-sensitive strategy. More frequently, dysregulation of the striatum has been shown to have no effect on hippocampus-sensitive behaviors. For instance, in a study by Zurkovsky et al. (2007), rats show impairments on tasks that tap into striatum-sensitive cognitive behavior, but show normal performance on tasks that assess hippocampus-sensitive behaviors.

While studies in both human and non-human animals suggest that some cognitive systems are unaffected by PD, there is little research regarding changes in the *balance* of cognitive systems during the genesis and progression of the disease. Recent data from Foerde et al. (2013) show that people with PD have cognitive deficits in striatum-sensitive incremental learning while also showing concurrent enhancements in hippocampus-sensitive episodic memory. While the study by Foerde et al. (2013) can serve as "proof of concept" that PD-related degeneration can shift the balance between cognitive systems, the mechanism underlying this shift is still uncertain. It seems likely that decreases in striatum function are caused by the PD-related damage to the substantia nigra and subsequent decrease in dopamine release into the striatum. PD-related dysregulation of the striatum may allow other brain regions to increase their activity due to decreased competition for energy substrates. McNay et al. (2000) found that hippocampal glucose concentrations decreased when rats were tested using the hippocampus-sensitive spatial working memory spontaneous alternation task, suggesting that increased energy substrate uptake was required to perform the task. Additionally, when systemic glucose was administered 30 min prior to testing, task-induced decreases in glucose concentration were blocked and rats showed enhanced performance on this task (McNay et al., 2000). In a similar vein, degeneration of a brain area may reduce metabolic load and allow for additional energy substrate provision to active brain regions, thereby decreasing competition in interacting systems. However, there is some evidence that dopamine production is upregulated in the early stages of the degeneration of dopaminergic neurons in PD (Zigmond, 1997) and that changes in dopaminergic signaling in the striatum may impact other neurotransmitter systems, such as acetylcholine. It is possible that PD-related neurodegeneration causes a rewiring of various

neurotransmitter systems in ways that benefit some cognitive strategies more than others (Ragozzino et al., 1996; Chang and Gold, 2003b; McIntyre et al., 2003a, b).

Two different rat models will be used to characterize the change in cognitive balance in PD. In the first study, a 6-hydroxydopamine (6-OHDA) toxin model of PD was used to examine how a partial lesion of the dopaminergic neurons in the nigrostriatal pathway affects hippocampus-sensitive spatial working memory. 6-OHDA is an analogue of the catecholaminergic neurotransmitter dopamine and can be transported into catecholaminergic neurons via high affinity catecholamine transporters (Zigmond, 1997). When oxidized, 6-OHDA can generate reactive oxygen species, such as H_2O_2, leading to DNA and protein damage that may induce apoptosis (Decker et al., 1993). Additionally, 6-OHDA can inhibit mitochondrial respiratory complexes I and IV, leading to decreased ATP production and potential degeneration (Glinka et al., 1997). In addition to causing oxidative damage, 6-OHDA has also been shown to block reuptake of dopamine (Decker et al., 1993), limiting dopamine recycling in the catecholaminergic neurons that do not degenerate. Although 6-OHDA can induce neuronal degeneration, it is unable to cross the blood-brain barrier and must be applied intracerebrally (Gerlach and Riederer, 1996). Direct application of 6-OHDA to a specific brain region, the approach taken here, allows for a more specific dopaminergic lesion. Because of its structural similarity to dopamine, the ability of 6-OHDA to target dopaminergic neurons makes it valuable for modeling PD. Our study using a 6-OHDA rat model of PD will be presented in chapter 2. In the second study, muscimol (MUSC), a $GABA_A$ receptor agonist, will be used to examine how increased inhibition in the striatum affects the balance between striatum-sensitive response learning and hippocampus-sensitive place learning. Unlike 6-OHDA, which specifically targets dopaminergic neurons, MUSC activates $GABA_A$ receptors, leading to non-specific

hyperpolarization of neurons, which in turn may dysregulate neurotransmitter release. The ability of MUSC to disrupt normal striatum function will allow us to examine whether the enhancements in hippocampus-sensitive cognitive functions in PD is due to changes in dopamine signaling specifically or due to general dysfunction of the striatum. This work will be presented in chapter 3.

CHAPTER 2

Bilateral intrastriatal infusions of 6-OHDA improve spatial working memory in rats: implications for Parkinson's disease

ABSTRACT

Despite being characterized by motor dysfunction, many people with PD have cognitive changes that are concomitant with or precede the onset of the classic motor deficits. Because many neural systems interact through collaboration or competition, some cognitive functions may benefit from Parkinson's-related damage. To examine whether a partial lesion of the nigrostriatal pathway can enhance learning that is not dependent on normal striatum function, we used a rat 6-OHDA model of PD. Young adult male Long-Evans rats received bilateral intrastriatal infusions of vehicle or a moderate (12 μg) or high (20 μg) dose of 6-OHDA. Motor function was assessed weekly for four weeks with the vibrissae-elicited forelimb placing task and forelimb stepping tasks. Compared to vehicle controls, rats that received 6-OHDA showed impairments in motor function during the first two weeks post-infusion; however, rats in the moderate 6-OHDA group attained partial or full recovery of function by the end of testing. Spontaneous alternation testing to assess spatial working memory was performed at post-infusion weeks one and four. Despite having motor deficits, rats treated with 6-OHDA showed enhanced performance on the spontaneous alternation task. At the end of testing, rats were euthanized to assess tyrosine hydroxylase staining. Rats exposed to 6-OHDA showed significant reductions in tyrosine hydroxylase staining in the striatum and substantia nigra pars compacta. Consequently, PD-associated enhancements in spatial working memory may be due to decreased striatal competition with non-striatal memory systems.

INTRODUCTION

While classically characterized by its cardinal motor deficits, more research is coming to light on the non-motor, cognitive changes associated with PD. Retrospective and prospective studies are beginning to elucidate that many non-motor dysfunctions precede the onset of the classic motor symptoms (Lees and Smith, 1983; Dubois and Pillon, 1997; Tolosa et al., 2007; Tolosa et al., 2009). Early alterations in dopamine, serotonin, and noradrenergic signaling may underlie the pre-motor cognitive change experienced by many people with PD (Zigmond et al., 1984; Zigmond, 1997; Taylor et al., 2009; Vernon, 2009; Politis et al., 2012). Classification of early pre-motor symptoms and their underlying neurological changes may be important for PD diagnosis prior to the substantial neural degeneration and neuronal loss that has occurred by the time of diagnosis using motor dysfunction assessments. Early diagnosis may enable people with PD to receive treatment prior to significant degeneration and may slow, stop, or reverse the progression of the disease.

Changes in cognitive function may represent a practical diagnostic tool. Unlike some procedures, cognitive testing is a non-invasive procedure that can usually be done with minimal resources, making it optimal for use as a wide-scaled screening device. Cognitive impairments have been documented in both humans and non-human animal models. People with PD tend to have cognitive deficits in processes associated with executive function, planning, and cognitive flexibility and most likely reflect dysfunctions in the basal ganglia and frontal lobe (Owen et al., 1992; Packard and Knowlton, 2002; Foerde and Shohamy, 2011b). Despite showing deficits in some cognitive domains, many people with PD maintain normal function in other cognitive areas, such as episodic memory (Knowlton et al., 1996; Foerde et al., 2013). The pattern of

cognitive deficits and sparing in PD is interesting because its specificity may help distinguish the cognitive changes of PD from those of other neurodegenerative diseases, such as amnesia and early stages of Alzheimer's disease (Killiany et al., 2002; Myers et al., 2003).

Some measures of cognitive dysfunction seen in non-human animal models of PD parallel those seen in humans. Rats and mice with 6-hydroxydopamine- (6-OHDA) or MPTP-induced nigrostriatal dopamine lesions show problems with visuospatial ability, habit formation, and cognitive flexibility (Lindner et al., 1999; De Leonibus et al., 2007; Haik et al., 2008). Additionally, rats with nigrostriatal dopamine depletion show deficits in route- or cue-based navigation (Braun et al., 2012). Previous multiple memory systems research has shown that cued learning systems often compete with other neural systems, such as hippocampus-related spatial learning. Additionally, direct brain applications of dopamine receptor agonists can modulate learning and memory in a structure to function-specific manner (Packard and White, 1991). For example, intrastriatal infusions of dopamine receptor agonists enhanced striatum-sensitive win-stay behavior while intrahippocampal infusions did not affect performance on this task. In contrast, intrahippocampal infusions of dopamine agonists enhanced hippocampus-sensitive win-shift behavior while intrastriatal infusions did not affect win-shift performance. These data from Packard and White (1991) further strengthen the hypothesis that loss of normal dopamine signaling will impair performance on striatum-mediated cognitive functions. Consequently, it seems highly likely that 6-OHDA-induced deficits in striatum-sensitive cognitive functions will lead to enhancements on hippocampus-sensitive tasks.

We used a 6-OHDA toxin model of PD to examine whether impaired striatum function due to loss of dopamine enhances hippocampus-sensitive processes assessed by testing in a land-maze working memory task. As previously stated, 6-OHDA specifically targets

catecholaminergic neurons via uptake by catecholamine transporters (Zigmond, 1997). Once inside neurons, 6-OHDA can induce degeneration by generating reactive oxygen species via oxidation (Decker et al., 1993) and inhibiting ATP production through blockade of mitochondria respiratory complexes I and IV (Glinka et al., 1997). 6-OHDA further alters dopamine signaling by irreversibly blocking reuptake of dopamine from synapses (Decker et al., 1993). Blockade of reuptake will increase the concentration of dopamine in the synaptic cleft; however, blocking dopaminergic reuptake receptors decreases tissue levels of dopamine and may reduce the amount of dopamine released upon neuronal activation (Tadaiesky et al., 2008). Thus, blocking dopamine reuptake receptors may affect dopamine signaling by increasing the dopamine "noise" within the synaptic cleft and decreasing the activity-dependent dopamine signal. A moderate dose (12 μg) and a high dose (20 μg) of 6-OHDA were used in this study to create partial dopaminergic lesions that respectively model a pre-motor stage and a post-motor stage of PD. Intrastriatal infusions of 6-OHDA yield dopamine loss that is generally confined to the striatum and substantia nigra pars compacta (SNc; Deumens et al., 2002; Tadaiesky et al., 2008). Additionally, intrastriatal infusions produce decreased mortality and health problems compared to other approaches that deliver the toxin directly to the substantia nigra pars compacta (SNc) or the medial forebrain bundle and model early, post-motor dysfunction stages of PD (Lee et al., 1996; Schallert et al., 2000; Ogura et al., 2005). Though less common in PD models, we chose bilateral 6-OHDA infusions because enhanced performance on the cognitive task used in our study could be achieved by preferentially making the same body turn. As such, a unilateral lesion-induced turning bias could confound the cognitive data, masking real changes in cognitive function due to a surgery-induced turning bias.

Two assessments of motor function were used: the vibrissae-elicited forelimb placing task, which measures sensory-motor integration, and the forelimb stepping test, which examines forelimb akinesia and postural adjustment (Olsson et al., 1995; Schallert et al., 2000; Meredith and Kang, 2006). Both tasks have been used previously and appear to have validity in PD models (Schallert et al., 2000; Fleming et al., 2005; Meredith and Kang, 2006). Additional motor tests were not included to avoid excess use or training that may influence recovery of function (Tillerson et al., 2001). In addition to assessing motor dysfunction in the 6-OHDA model of PD, we also assessed whether shifts in cognitive function are concomitant. Previous research has focused on elucidating PD-associated motor and/or cognitive deficits (Lees and Smith, 1983; Owen et al., 1992; Lee et al., 1996; Kirik et al., 1998; Bruck et al., 2004; De Leonibus et al., 2007; Branchi et al., 2008; Ibarretxe-Bilbao et al., 2011; Alafuzoff and Parkkinen, 2014); however, a multiple memory systems approach to PD would suggest that disease-induced deficits in nigrostriatal function could lead to cognitive enhancements in other cognitive areas. Development of a cognitive profile of PD that includes cognitive benefits could yield a more comprehensive diagnostic profile as well as potentially novel therapies that compensate for impairments during disease progression by tapping into spared functions.

Immunohistochemical analysis of tyrosine hydroxylase (TH) was used to assess 6-OHDA-induced degeneration of the nigrostriatal pathway. TH is an enzyme that catalyzes the rate-limiting step for catecholamine biosynthesis and has been used previously as a marker of dopaminergic neurons (Castaneda et al., 1990; Gerfen et al., 1995; Olsson et al., 1995; Lee et al., 1996; Kirik et al., 1998; Dowd and Dunnett, 2005; Ferro et al., 2005; Anstrom et al., 2007). In this study, we use TH staining as a proxy for assessing dopamine production. Lastly, we examined the effect of dopamine depletion severity on both motor and cognitive function. Based

on previous data from the PD and multiple memory systems fields, we hypothesize that 6-OHDA-treated rats will show partial depletion of dopamine in the striatum and SNc, leading to impaired motor function on the vibrissae-elicited forelimb placing and forelimb stepping tasks. If decreased striatum function decreases competition with the hippocampus for behavioral strategy control, we also hypothesize that 6-OHDA-treated rats will show enhanced spatial learning memory as assessed by the hippocampus-sensitive spontaneous alternation task.

MATERIALS AND METHODS

Subjects. Young adult (90-100 days) male Long-Evans rats (N = 25; Harlan Laboratories, Indianapolis, IN) were housed individually in translucent cages on a 12:12 hr light:dark cycle and *ad libitum* access to food and water. Rats were given at least 48 hr to acclimate to the vivarium before any procedures were performed. All rats received baseline testing of motor function All behavioral testing occurred 5 to 9 hr after lights on. Animal pain and discomfort were minimized. These experiments were conducted at the University of Illinois, an AAALAC accredited institution, in accordance with animal care guidelines established by the National Institute of Health and were approved by the University of Illinois IACUC.

Preparation of drugs. 6-hydroxydopamine hydrobromide (6-OHDA) containing ascorbic acid (Sigma-Aldrich; St. Louis, MO) was dissolved in artificial cerebral spinal fluid (aCSF) composed of (in mM): 128 NaCl, 2.5 KCl, 1.3 $CaCl_2$, 2.1 $MgCl_2$, 0.9 NaH_2PO_4, 2.0 Na_2HPO_4, 0.7 glucose, pH = 7.4. The 0.7 mM glucose concentration in the aCSF approximates the baseline level seen in striatal cerebral spinal fluid (McNay et al., 2001). Two concentrations of 6-OHDA were prepared: 1) 6 mg/ml + 0.024% ascorbic acid; 2) 10 mg/ml + 0.036% ascorbic acid. aCSF +

0.024% ascorbic acid was prepared as a vehicle solution. All solutions were protected from light and stored at -20°C until use.

Surgery and bilateral intrastriatal drug infusions. Rats were deeply anesthetized with isoflurane and placed in a stereotaxic apparatus (Kopf; Tujunga, CA). Drug infusions were made into the dorsolateral striatum [coordinates: +0.4 mm from bregma; lateral ± 3.4 mm; -4.2 mm from dura] using a 7 mm long, 28 gauge infusion cannula (Plastics One, Inc.; Roanoke, VA). After the infusion cannula was lowered into one hemisphere, 6-OHDA or aCSF was infused at a rate of 0.5 µl/min for 4 min. A total of either 12 µg (moderate dose; n = 8) or 20 µg (high dose; n = 8) of 6-OHDA per hemisphere was infused. The infusion cannula remained in place for 1 min following the infusion to allow the solution to diffuse away from the cannula tip. The infusion cannula was then removed and the infusion procedure was repeated for the other hemisphere. Rats received daily health checks until euthanasia. During the first two weeks after surgery, all rats were provided with moist food in their cages in addition to their normal dry food to encourage eating and hydration.

Vibrissae-elicited forelimb placing task. The vibrissae-elicited forelimb placing task was modified from Schallert et al. (2000). Rats were hand-held by the torso, with their limbs and tail hanging freely. When muscle relaxation was attained, the vibrissae on one side of the head were gently brushed against the side of a table and the movement of the ipsilateral limb was recorded (Figure 1A). Limb movement was scored on a scale of 0 to 3 using the following criteria: 0 – no movement; 1 – slight movement; 2 – limb movement, but failure to grasp table; 3 – limb movement with full grasp of table (Anstrom et al., 2007). Rats received two blocks of five trials on each side, with a 30 sec rest period between sets. The maximum score across all four blocks is 60 points. Prior to intrastriatal infusion, most rats performed at or near perfect levels, with a

mean score of 97.1% of the 60 point maximum. One rat in the aCSF group was excluded from forelimb placing analyses because its pre-infusion score (76.7%) was greater than 3 standard deviations below the pre-infusion mean.

Forelimb stepping task. Rats were placed on a table at a common start location and lifted by their hindquarters, shifting their weight to be supported solely by their forelimbs. Rats were gently pulled backwards along the tabletop for 1 meter in 5-7 sec (Figure 1B). This procedure is similar to and adapted from the forelimb adjusting step task (Schallert et al., 1992; Olsson et al., 1995). This task is usually performed to measure motor function asymmetries in unilateral models of PD. Since we used a bilateral 6-OHDA lesion, pulling the rats backwards allowed us to record movement in both forelimbs simultaneously. Each rat received three trials, with a 30 sec rest between trials. All sessions were video recorded and the number of adjusting steps for the left and right forelimbs were counted and summed.

Working memory: spontaneous alternation testing. Spontaneous alternation performance was measured in a 4-arm plus-shaped black Plexiglass® maze with an open ceiling. The dimensions of each arm were 45 cm L x 13 cm W x 7 cm H. The central square-shaped area was 13 cm L x 13 cm W x 7 cm H. Numerous extra-maze visual cues in the form of objects and wall decorations were place throughout the training room. Rats were placed in a random start arm and were allowed to traverse freely through the maze for 20 min. The number and sequence of arm entries were recorded and used for alternation performance calculations. An alternation was experimentally defined as visiting all 4 arms within overlapping sets of five arm visits. Using this method, the total number of possible alternations was equal to the total number of arm entries minus 4. The percent alternation score was equal to (actual alternations/possible alternations) x 100. Chance performance on this task is 44%. Additional behavioral measures were collected,

including number of arms entered and turning bias (i.e. left-turn or right-turn preference). Turning bias was calculated using the formula: A-B/A+B, where A represents the most prevalent turn direction, B the turn made less often, and A+B is the total number of left and right turns.

Only data from rats that made at least 10 arm choices (6 possible alternations) were included in the analyses. One rat in the moderate dose group and two rats in the high dose group were excluded from the week one analysis of spontaneous alternation performance because they failed to visit the minimum number of arms. Because of these exclusions, we were unable to use repeated measures analyses when measuring changes in cognitive behavior from week 1 to week 4.

Perfusion and histology. Rats were deeply anesthetized with an overdose of sodium pentobarbital (i.p.; Sigma-Aldrich, St. Louis, MO) and then perfused transcardially with 80 ml of 0.1 M phosphate-buffered saline (PBS) follow by 80 ml of 4% paraformaldehyde in 0.1M phosphate buffer (PB). Rats were decapitated and the brains were removed and post-fixed in 4% paraformaldehyde in 0.1 M PB for ~48 hr. The brains were transferred to 20% glycerol in 0.1 M PBS for ~24 hr. Coronal section (40 μm) through the striatum and SNc were collected at -30°C with a Leica 1800 cryostat (Leica Microsystems, Wetzlar, Germany). Every third section was mounted on a gelatin-coated slide and stained with cresyl violet, a histological Nissl stain that binds to nucleic acids. The remaining sections were stored in cryopreservative solution (250 mM 40 KD polyvinylpyrrolidone, 880 mM sucrose, 30% v/v ethylene glycol, 40 mM sodium phosphate) at -20°C until use for immunohistochemical staining.

Immunohistochemistry. For each rat, three sections through the striatum (~0.5 mm apart; +1.3 to +0.3 mm relative to bregma) and three sections through the SNc (~0.25 mm apart; -4.75 to -5.25 mm relative to bregma) were processed for TH immunoreactivity. Adjacent sections

were stained with cresyl violet to assess cell density within the striatum and SNc. TH immunolabeling was performed using a rabbit polyclonal primary antibody (AB152; Millipore, Billerica, MA). All immunohistochemistry procedures occurred at room temperature with gentle agitation. Free-floating sections were washed three times for 10 min each in 0.05 M PBS initially and between all subsequent steps. Sections were first incubated in quenching solution (1% H_2O_2, 1% normal goat serum [NGS], 0.02% triton x-100, 0.05 M PBS) for 10 min. They were transferred to a blocking solution (2% NGS, 0.4% triton x-100, 0.05 M PBS) for 20 min. Sections were incubated overnight in a TH primary antibody solution (1:4000 dilution in 1% NGS, 0.4% triton x-100, 0.05 M PBS). The following day, sections were placed in a secondary antibody solution (1% NGS, 0.4% triton x-100, 0.05 M PBS) containing a goat anti-rabbit biotinylated secondary antibody (1:400; Santa Cruz, Santa Cruz, CA). They were then incubated for 30 min with ABC reagent (Vector, Burlingame, CA) in 0.05 M PBS, followed by incubation with DAB substrate (Vector) for 2 min. Sections were mounted onto gelatin-coated slides and allowed to dry overnight. The tissue was dehydrated with a graded ethanol series of washes followed by HistoChoice® Clearing Agent (Sigma-Aldrich), then coverslipped using DPX mounting medium (Sigma-Aldrich).

Image acquisition and analysis. Sections were imaged using a Leica DM 6000B/CTR6000 light microscope and a Leica DFC350 FX video camera, which was interfaced to a PC computer. This system was used in conjunction with Image-Pro software (Media Cybernetics, Inc., Bethesda, MD) for image acquisition and for correction of unevenness in illumination across images. ImageJ software (NIH, Bethesda, MD) was used to analyze the extent of both TH-positive (TH+) staining and cresyl violet staining in the striatum and SNc.

To assess TH+ staining in the striatum and SNc, the area and intensity of TH+ immunoreactivity were measured. For each image, a background level of staining was determined by measuring a nearby area with no or little TH+ staining (i.e. corpus callosum for striatal sections and thalamus for SNc sections). A threshold for analysis was set at 2 (striatum) or 3 (SNc) standard deviations above the mean background level of staining to ensure that only specifically labeled areas were measured. By thresholding the image, we were able to limit analysis to stained tissue, decreasing the likelihood that non-specific background staining would be included in our measurements. Optical densities were used to quantify the intensity level of staining above threshold. Percent area measures were used to analyze the proportion of a selected region of interest with TH+ staining over threshold levels. Because the TH+ staining area is normalized to a specific rat's anatomy, percent area measures can help minimize inter-animal differences in brain size or histological section orientation.

To assess approximate cell density in the striatum and SNc, the area of cresyl violet staining within these regions was measured. In order capture the entire striatum for analysis, low magnification had to be used. Consequently, the images had lower resolution than could be used for accurate manual cell counts. While the particle count function of ImageJ can perform cell counts, it requires discrete puncta. If the stained cells are touching or overlapping, the software is unable to distinguish the individual cells and will count it as a single, continuous cell. Although neurons in the striatum are less densely packed than other brain regions, the thickness of our tissue yielded overlapping puncta. Thus, using the particle count function could artificially decrease the cell count. Due to the high number of cresyl violet stained puncta, stereology would be the best method to estimate cell counts; however, our laboratory currently lacks the resources to perform unbiased stereological counts. The default/IsoData Auto Threshold setting (striatum)

or the Yen Auto Threshold setting (SNc) was used to ensure that only specifically labeled areas were measured. Only percent area measures were used to assess approximate cell density in the regions of interest.

Data analyses and statistics. All analyses were performed using Statview software. To assess changes over the study duration, body weights and motor tests were analyzed using two-way repeated measures ANOVAs (treatment x time) with planned post hoc Fisher PLSD tests to examine differences among treatment groups at each time point. At each week of testing, data for individual rats were compiled in the following manner: for vibrissae-elicited forelimb placing, the cumulative score across four blocks of trials (two blocks each on the left and right sides; max score = 60) was used for analysis; for forelimb stepping, the average number of total forelimb steps across three trials was used for analysis. The percent of pre-injection baseline at each post-injection week was calculated and used to analyze the vibrissae-elicited forelimb placing and forelimb stepping data. To ensure that there were no significant group differences in pre-infusion motor function, one-way ANOVAs were performed on the raw data for the pre-surgery forelimb stepping (average number of steps across three trials) and vibrissae-elicited forelimb placing (cumulative score across four blocks of trials) tasks. Recovery of function in the forelimb placing and stepping tasks was assessed using one-way repeated measures ANOVAs with planned post hoc Fisher PLSD tests to examine differences among time points. We have experimentally defined recovery of function as motor function that is not statistically different from pre-infusion performance. To assess cognitive changes, percent alternation scores ([actual alternations/possible alternations] x 100), arm entries, and turning bias were analyzed using one-way ANOVAs within each time point because a subset of rats was excluded based on low arm entry numbers on week one. Planned post hoc Fisher PLSD tests assessed difference between

treatment groups. TH+ staining in the striatum and SNc were analyzed using Spearman rank correlations. For all comparisons, $\alpha = 0.05$. Analyses with $p < 0.10$ were considered non-significant trends.

Timeline for experiments. The timeline for the motor and cognitive testing is summarized in Figure 2. All rats were handled for 3-4 min each day for seven consecutive days prior to behavioral tests. Rats were familiarized with the specialized handling used in the motor tasks during the last five days of handling. Motor testing for the vibrissae-elicited forelimb placing and the forelimb stepping tasks was performed one week prior to infusion, and repeated weekly for four weeks post-infusion. Spontaneous alternation testing was performed on post-infusion weeks one and four. Rats were euthanized 30 min after the conclusion of final behavioral testing on week four.

RESULTS

General health:

Treatment with either dose of 6-OHDA did not have significant effects on the rats' survival or health. All rats survived for the duration of the experiment. Aside from rigidity in a few of the rats that received the high dose of 6-OHDA, daily health checks did not reveal any significant qualitative differences between groups. Moreover, there were no significant differences in body weight among groups ($F_{(2,22)} = 0.21$, $p > 0.05$); however, weights were slightly reduced following surgery, but gradually recovered and continued to increase (Figure 3).

Forelimb placing task:

6-OHDA treatments produced a dose-dependent impairment in performance in the vibrissae-elicited forelimb placing task (Figure 4). During post-infusion weeks 1 and 2, the

deficits in forelimb placing behavior seen in the moderate and high 6-OHDA treatment groups represented a 40-50% decrease in function compared to pre-surgery levels. By week 4 of testing, the moderate 6-OHDA group showed a ~22% decrease in function while the high 6-OHDA group maintained a ~50% deficit compared to pre-surgery scores. In contrast, aCSF-treated rats show a modest ~10% decrease in performance at post-surgery week 1, but had returned to pre-surgery performance by week 2 of testing. A two-way repeated measures ANOVA revealed a main effect of treatment on forelimb placing scores ($F_{(2,21)} = 5.880$, $p < 0.01$) and a main effect of time ($F_{(4,84)} = 16.373$, $p < 0.0001$). Additionally, there was a significant interaction between time and treatment ($F_{(8,84)} = 3.628$, $p < 0.01$). Importantly, pre-infusion scores on the vibrissae-elicited forelimb placing test were similar across all groups ($F_{(2,21)} = 0.202$, $p > 0.05$). In the aCSF-treated rats, there was a non-significant trend for an effect of time on forelimb placing performance ($F_{(4.35)} = 2.418$, $p = 0.0669$). This trend was most likely driven by the 10% decrease in performance at week 1 post-surgery; however, this difference was not statistically significant when compared to pre-surgery performance ($t_{(7)} = 1.861$, $p > 0.05$). In post-infusion weeks one and two, rats that received the moderate dose of 6-OHDA showed significant impairments compared to control aCSF-treated rats ($p < 0.05$ for both). However, rats in the moderate 6-OHDA treatment group showed improvements on this task as time post-infusion increased and were not significantly different from aCSF-treated rats in weeks three and four ($p > 0.05$ for both). In contrast, rats that were treated with the high dose of 6-OHDA showed significant impairments on the vibrissae-elicited forelimb placing task compared to aCSF-treated controls at all of the post-infusion tests ($p < 0.01$ at each week).

One-way repeated measures ANOVAs were conducted to assess recovery of function in the vibrissae-elicited forelimb placing task. Rats treated with aCSF showed a trend for a change

in performance on the vibrissae-elicited forelimb placing task over the four weeks post-surgery ($F_{(4,35)} = 2.418$, $p = 0.0669$). This trend was most likely due to the ~10% decrease in performance in week one compared to the other three weeks ($p < 0.05$ vs. all three weeks). There was a significant effect of time in both the moderate 6-OHDA ($F_{(4,35)} = 2.854$, $p < 0.05$) and high 6-OHDA groups ($F_{(3,35)} = 3.212$, $p < 0.05$). In the moderate 6-OHDA group, vibrissae-elicited forelimb placing scores were significantly reduced at weeks 1 ($p < 0.01$) and 2 ($p < 0.05$) compared to pre-surgery levels. By week 3, the forelimb placing scores for the moderate 6-OHDA group's forelimb placing score showed a trend for decreased function compared to pre-infusion performance ($p = 0.0505$) and was no longer significantly different at week 4 ($p > 0.1$). In contrast, rats in the high 6-OHDA group showed persistent deficits in forelimb placing behavior compared to pre-infusion performance ($p < 0.01$ for weeks 1-3, $p < 0.05$ for week 4).

Forelimb adjusting steps:

The effects of 6-OHDA treatment on the total number of forelimb adjusting steps showed dose-dependent impairments similar to the results seen in the vibrissae-elicited forelimb placing task (Figure 5). At week one of post-surgery testing, moderate 6-OHDA-treated rats showed a 25% decrease in the number of adjusting steps compared to their pre-surgery levels; however, their performance had returned to baseline levels by post-surgery week 3. Rats treated with the high dose of 6-OHDA showed a sustained ~35% decrease from pre-surgery performance across all weeks of post-surgery testing. There were no changes in performance in the aCSF-treated group. A two-way repeated measures ANOVA revealed significant main effects of treatment ($F_{(2,90)} = 14.243$, $p < 0.0001$) and time ($F_{(3,90)} = 7.955$, $p < 0.0001$) on the total number of adjusting steps. Additionally, there was a significant treatment x time interaction ($F_{(6,90)} = 5.126$, $p < 0.0001$). There was not a significant effect of treatment on pre-infusion forelimb stepping

scores ($F_{(2,22)} = 0.255$, $p > 0.05$). Post hoc analyses revealed that rats treated with a moderate dose were significantly impaired on this task when compared to aCSF-treated rats during post-infusion weeks one and two ($p < 0.01$ and $p < 0.05$, respectively), but not at weeks three or four ($p > 0.05$ for both). In contrast, rats treated with the high dose of 6-OHDA showed impairments compared to controls at all post-infusion tests ($p < 0.001$ for week one; and $p < 0.0001$ for weeks two through four). Additionally, rats treated with the high dose of 6-OHDA were significantly impaired at weeks three and four ($p < 0.01$ and $p < 0.0001$, respectively) compared to rats treated with the moderate dose.

One-way repeated measures ANOVA were conducted to assess recovery of function in the forelimb adjusting step task. Rats treated with the moderate dose of 6-OHDA showed an effect of time on the number of adjusting steps ($F_{(4,35)} = 7.277$, $p < 0.001$). Compared to pre-surgery performance, the moderate 6-OHDA-treated rats showed a significant impairment on the number of adjusting steps at post-surgery week 1 ($p < 0.001$). There was a trend for a treatment-induced deficit at post-surgery week 2 ($p = 0.0750$); however, performance was indistinguishable from the pre-surgery number of adjusting steps by week 3. Additionally, the moderate 6-OHDA group showed significant increases in performance in post-surgery weeks 2-4 compared to post-surgery week 1 (vs. week 2: $p < 0.05$, week 3: $p< 0.01$, week 4: $p < 0.0001$). Neither the aCSF nor the high 6-OHDA group showed significant change over the four weeks of post-surgery testing. However, the aCSF group maintained pre-surgery level performance during the post-surgery testing whereas the high 6-OHDA group showed no recovery from their treatment-induced performance deficit.

Working memory test:

In contrast to the 6-OHDA-induced impairment seen in the motor tasks, 6-OHDA treatment significantly improved alternation scores (Figure 6A). One-way ANOVA analysis revealed a main effect of treatment on percent alternation scores at post-infusion week one ($F_{(2,19)} = 4.306$, $p < 0.05$) and a trend for an effect of treatment at week four ($F_{(2,22)} = 3.116$, $p = 0.0644$). Treatment with the moderate dose of 6-OHDA significantly enhanced alternation scores compared to controls at week four ($p < 0.05$) and showed a trend for enhancement at week one ($p = 0.06$). Treatment with the high dose of 6-OHDA significantly enhanced spontaneous alternation scores compared to controls at week one ($p < 0.05$), but not at week four ($p = 0.1017$). Rats in all groups performed above chance performance based on 95% confidence interval tests.

While 6-OHDA-treated rats tended to make fewer arm choices (Figure 6B), treatment did not significantly affect the number of arm entries at weeks one ($F_{(2,19)} = 1.041$, $p > 0.05$) or four ($F_{(2,22)} = 1.029$, $p > 0.05$). All rats showed gradual decreases in the number of arm choices across the 20 min testing period. Rats made approximately 50% of all arm entries in the first 5 min, 25% of all entries from 5-10 min, 15% of all entries from 10-15 min, and 10% of all entries from 15-20 min (data not shown). No turning biases were detected for any groups at weeks one ($F_{(2,16)} = 1.211$, $p > 0.05$) or four ($F_{(2,16)} = 0.494$, $p > 0.05$).

Tyrosine hydroxylase measures:

Treatment with 6-OHDA significantly affected several measures of TH+ staining in the dorsolateral striatum (Figure 7). The drug produced obvious lesions that extended over 0.5 mm anteriorly and posteriorly from the tips of the infusion cannulae. Rats treated with 6-OHDA showed significantly lower percentages of striatal areas stained positively for TH (Figure 7B; $F_{(2,22)} = 21.148$, $p < 0.0001$). Both the moderate and the high dose of 6-OHDA showed

significantly smaller percentages of TH+ staining in the striatum compared to aCSF controls ($p < 0.0001$ for both). When compared to tissue from aCSF-treated rats, tissue from rats treated with the moderate dose of 6-OHDA showed a 20% decrease in staining area. Similarly, tissue from rats treated with the high dose of 6-OHDA showed a 24% decrease in staining area compared to aCSF-treated tissue. While 6-OHDA treatment reduced TH+ staining areas, it did not significantly affect TH+ intensity in stained tissue (Figure 7C; $F_{(2,22)} = 1.135$, $p > 0.05$).

Similar to the results for the striatum, 6-OHDA treatment significantly affected TH+ staining in the SNc (Figure 8; $F_{(2,22)} = 17.227$, $p < 0.0001$). The moderate dose of 6-OHDA showed a 13% decrease in TH+ staining area in the SNc compared to aCSF controls ($p < 0.01$). The high dose of 6-OHDA yielded even larger deficits in TH+ staining areas compared to aCSF controls ($p < 0.0001$) and showed a trend for having lower areas compared to the moderate dose ($p = 0.0677$). Tissue from rats treated with the high dose of 6-OHDA showed a 20% decrease in staining area compared to aCSF-treated control tissue. Unlike the striatum, 6-OHDA treatment affected staining intensity in the SNc ($F_{(2,22)} = 5.972$, $p < 0.01$). The high dose of 6-OHDA significantly decreased the intensity of staining by 12.5% compared to aCSF controls ($p < 0.01$) and showed a trend for having reduced levels compared to the moderate dose ($p = 0.0808$).

Loss of TH+ staining in the striatum and SNc was closely linked. Across all groups, the percentage of TH+ staining in the striatum correlated significantly with measures in the SNc (Figure 9A; $N = 25$, $r_s = 0.681$, $p < 0.001$). Additionally, staining intensity in the striatum correlated significantly with levels in the SNc across all groups (Figure 9B; $N = 25$, $r_s = 0.574$, $p < 0.05$).

Analysis of cresyl violet staining in the striatum revealed no significant differences in the percent area measures between groups (Figure 7B; $F_{(2,22)} = 0.756$, $p > 0.05$). In contrast, there

was a significant effect of treatment on cresyl violet staining percent areas in the SNc (Figure 8B; $F_{(2,22)} = 5.917$, $p < 0.01$). Post hoc analysis revealed that treatment with the high dose of 6-OHDA significantly decreased cresyl violet percent area measures compared to treatment with aCSF ($p < 0.01$). Tissue from rats treated with the high dose of 6-OHDA showed a 20% decrease in cresyl violet staining area compared to tissue from aCSF-treated rats. Additionally, the area of cresyl violet staining in tissue from rats treated with the moderate dose of 6-OHDA was intermediate to those from aCSF- and high 6-OHDA-treated rats, representing a trend for a decrease in cresyl violet percent area measures in the moderate 6-OHDA treatment group when compared to aCSF-treated rats ($p = 0.0553$). Decreased cresyl violet percent area staining was also correlated with a decrease in TH+ staining in the SNc (Figure 9C; $N = 25$, $r_s = 0.464$, $p < 0.05$).

Summary

The main results of this study suggest that bilateral infusions of 6-OHDA into the dorsolateral striatum significantly impaired motor function while significantly enhancing spatial working memory. Rats showed partial or full recovery of motor function following a moderate, but not a high dose of 6-OHDA. In contrast, both doses of 6-OHDA conferred enhanced cognitive performance on the spontaneous alternation task.

DISCUSSION

Consistent with previous studies, we found that lesions of the nigrostriatal dopamine pathway via bilateral intrastriatal 6-OHDA infusions elicited deficits in sensory-motor integration and limb akinesia, two rodent motor tasks that model some of the classic PD symptoms (Schallert et al., 2000; Fleming et al., 2005). Despite showing impaired motor

function, rats treated with 6-OHDA showed *enhanced* spatial working memory on a task shown to rely on intact hippocampal function and able to reflect modulation of hippocampal function (Ragozzino et al., 1996; McNay et al., 2000; Newman et al., 2011).

As expected, TH+ immunohistochemical staining was decreased in the striatum and SNc of 6-OHDA-treated rats in a dose-dependent manner. While the use of our staining area and optical density measures may not precisely detect the number of dopaminergic SNc cells lost, the changes in both of these measures can be used to characterize the lesion. The positive correlations between the striatum and SNc immunoreactivity suggests that the 6-OHDA was taken up in the dorsolateral striatum by dopamine transporters on the axon terminals of neurons originating in the SNc. Once inside the neuron, 6-OHDA causes damage by producing free radicals and uncoupling oxidative phosphorylation in the mitochondria, leading to cell damage and reduced ATP production (Glinka et al., 1997). Depending on the severity of the 6-OHDA-induced oxidative damage, impaired cell function may eventually lead to apoptosis (Ichitani et al., 1994). Decreases in TH+ staining area and intensity in the SNc may reflect degeneration of dopaminergic neurons and/or decreased capacity of those neurons to produce dopamine, either of which support our theories that loss of dopamine contributes to cognitive shifts with PD. However, a loss of neurons as opposed to loss of dopamine production might point to different therapeutic targets. Concomitant decreases in cresyl violet staining suggest that the decrease in TH+ immunoreactivity may be due to dopaminergic neuron cell loss, especially in the high 6-OHDA-treated group. However, cresyl violet is a general cell stain and changes in cresyl violet staining may be due to changes in neuronal or non-neuronal cells.

We did not see compensatory upregulation of TH-immunoreactivity in the striatum that has been reported by others (Zigmond et al., 1984). In fact, the intensity of TH staining in the

SNc was significantly *decreased* in tissue from rats treated with the high dose of 6-OHDA, most likely due to a decrease in SNc dopaminergic neurons. However, it is possible that the decrease in TH staining intensity reflects a decreased capacity for dopamine production in the SNc of rats that received the high dose of 6-OHDA. In contrast, rats treated with the moderate dose of 6-OHDA did not have significant decreases in TH+ intensity in stained tissue despite having decreases in TH+ area, implying that the remaining TH+ cells have a normal capacity for dopamine production. Thus, it seems likely that the severe impairment in dopamine production may underlie the chronic motor deficits seen in the rats treated with the high dose of 6-OHDA.

When tested at one week post-infusion, both the moderate and high 6-OHDA-treated rats showed similar motor deficits; however, recovery of function was substantially different between the two groups. In the vibrissae-elicited forelimb placing task, both groups showed robust 40-50% loss of function in the first week of testing. Starting at post-infusion week 3, rats in the moderate 6-OHDA group started to show improvement of function, which continued through week 4. In contrast, rats treated with the high dose of 6-OHDA failed to show any recovery of function on this task.

Similarly, rats treated with 6-OHDA showed a ~30% deficit in the number of adjusting steps compared to aCSF-treated rats at post-infusion week one. Rats in the moderate 6-OHDA group showed full recovery of motor function on the adjusting steps task. However, rats that received the high dose did not show any improvement on the number of adjusting steps taken, supporting the hypothesis that loss of nigrostriatal dopamine signaling below a threshold causes a persistent, and possibly continued deterioration, of motor function.

Performance in both motor tasks was consistent across pre- and post-surgery testing in the aCSF-treated rats. Thus, these rats did not show non-specific surgery-induced deficits in

motor function. In contrast, the moderate 6-OHDA group shows a severe drop in performance followed by a slow improvement whereas the high 6-OHDA group shows a sudden decrease in performance between pre-surgery and post-surgery testing without any increase in performance. Together, the results suggest a lack of recovery of function in the high-dose toxin group and partial or full recovery, depending upon task, in the moderate group.

Partial lesions to the striatum frequently lead to recovery of movement, while more severe lesions, such as toxin infusion to the medial forebrain bundle or SNc, are more resistant to recovery of function (Schallert et al., 2000; Ogura et al., 2005). The magnitude of functional recovery after toxin-induced lesion can be affected by many factors, including the timing of the assessment, physical activity (before and after lesion induction), dosing and timing of toxin, and lesion size (Lindner et al., 1997; Kirik et al., 1998; Tillerson et al., 2001; Deumens et al., 2002; Dowd and Dunnett, 2005; Meredith and Kang, 2006; Ahmad et al., 2009; Gerecke et al., 2010; Beeler, 2011). While unlikely, the improvement in the moderate group may reflect a practice effect because performance on these tasks was so high in aCSF-treated rats, ceiling-level performance may preclude our ability to detect improvement over repeated measures.

The 6-OHDA dose used in this study produced significant, yet moderate losses of TH+ staining in the dorsal striatum and the SNc. Decreases in the area of TH+ staining in the moderate group was approximately 20% in the striatum and 13% in the SNc. Treatment with the high dose of 6-OHDA yielded losses in TH+ staining areas of 24% in the striatum and 20% in the SNc, coupled with a 12.5% decrease in staining intensity in the SNc. While Zigmond et al. (1984) found an upregulation in TH activity following 6-OHDA-induced lesions, we did not find increases in TH+ staining intensity, suggesting that TH protein levels were not upregulated in our study. However, it is possible that increased TH activity went undetected because our

immunohistochemical staining assesses protein levels not enzyme activity. In addition to decreased TH+ staining, tissue from rats that received 6-OHDA shows decreased cresyl violet staining in the SNc, which may reflect degeneration of TH+ neurons. Cresyl violet staining in the SNc decreased by ~12% in the moderate 6-OHDA group and by 20% in the high 6-OHDA group, possibly due to degeneration of dopaminergic neurons. In contrast, cresyl violet staining in the striatum was not affected by 6-OHDA treatment. These data suggest that the 6-OHDA was primarily taken up by axon terminals and retrogradely transported to the SNc, limiting cell loss to this brain region.

The extent of the lesions produced in this study is comparable to the damage observed by others using striatal administration of dopaminergic toxins and are more moderate than lesions caused by toxin infusions into the SNc or medial forebrain bundle. Thus, intrastriatal infusions of varying doses of toxins may enable titration of dopamine depletion, creating models of early stages of PD where loss of dopamine signaling may be more modest (Fleming et al., 2005). Additionally, repeated, subclinical infusions of toxins into the striatum could allow for the creation of a rat model of PD that mimics the disease progression seen in humans.

We found that sustained deficits in sensory-motor integration and forelimb akinesia seen in our high dose were coincident with 20-24% reductions in the area of TH+ staining in the striatum and SNc. These decreases in TH+ staining are smaller in magnitude compared to the amount of dopamine loss measured using other assessments of dopamine signaling. When cell counts of TH+ cells in the SNc are used to assess dopamine, 60-95% reductions are needed to produce motor symptoms such as slowed reaction times, poor limb adjustments, and disequilibrium (Lee et al., 1996; Kirik et al., 1998; Dowd and Dunnett, 2005). The data from rodent models of PD parallel findings from clinical studies of Parkinson's patients. Previous

studies using a variety of infusion regimens and toxins suggest that 50% of nigrostriatal dopaminergic neurons and even larger decreases in dopamine release in the striatum are needed for the emergence of motor symptoms (Castaneda et al., 1990; Lee et al., 1996; Ogura et al., 2005; De Leonibus et al., 2007; Richter et al., 2008). However, limb use asymmetries have been detected following lower doses (10 μg) of intrastriatal 6-OHDA (Moroz et al., 2004), suggesting that changes in motor function can be observed with small lesions. The use of the area and optical density for assessing TH+ staining may overestimate the number of nigrostriatal dopaminergic neurons and may be augmented by compensatory increases in TH levels during the 4-week interval between 6-OHDA infusion and death (for review see Zigmond, 1997). Additionally, the vibrissae-elicited motor task used in this study could be more sensitive to partial striatal lesions than are measures of overall locomotor behavior or gross postural instability, as supported by the subtle decline in performance seen in aCSF-treated rats at one week post-infusion and lower recovery in rats with moderate toxin doses. In contrast, aCSF-treated rats showed no post-surgery loss of function in the adjusting footsteps task and moderate 6-OHDA-treated rats showed full recovery of function by post-surgery week 3.

Despite showing motor dysfunction, 6-OHDA-treated rats showed increased alternation scores, reflecting improvements in spatial working memory. An increase in the bias to make the same body turn can also lead to an increase in alternation scores without any change in spatial working memory. Importantly, no significant treatment-related changes in turning biases were found, supporting the interpretation that the increase in alternation behavior reflects memory improvements. All 6-OHDA-treated rats survived the lesion, showed no significant treatment-induced effects on body weight gain, and did not exhibit qualitative signs of malaise, suggesting toxin-induced health problems that may affect motivation are most likely minimal. While it is

possible that the intrastriatal infusion of 6-OHDA may affect reward evaluation, the rat's ability to correctly appraise an award should not affect spontaneous alternation performance as this task relies on the natural exploration tendencies of rodents and does not use any experimentally applied appetitive or aversive reinforcement.

Although not statistically significant, 6-OHDA rats tended to make fewer arm choices across spontaneous alternation testing, reflecting slower movement on the maze and the motor dysfunction measured in the two motor tasks. Because the number of arm entries determines the number of possible alternations, total alternation scores are converted to percent alternation scores, normalizing the data to the number of arm entries. Consequently, the number of arm entries will not necessarily limit the rat's performance on the spontaneous alternation task. In fact, slower movement on the maze may increase working memory load by increasing the amount of time over which the rats need to remember the location of recently visited arms. Despite this potential increase in working memory load, the 6-OHDA rats showed enhancements in alternation scores, possibly due to changes in the functional balance between different learning and memory systems.

The coincidence of striatum-sensitive motor dysfunction and hippocampus-sensitive working memory enhancement is consistent with the multiple memory system model proposing that neural systems compete for control over the expression or use of different learning and memory processes. Treatments that enhance one cognitive domain often do so at the expense of a different cognitive domain. Previous studies have shown that hippocampal lesions or inactivation impairs performance on hippocampus-sensitive place or win-shift strategy tasks, but can also show enhancements on striatum-sensitive response learning, cued learning, or win-stay strategy tasks (Packard et al., 1989; McDonald and White, 1993, 1994; Packard and McGaugh, 1996;

Chang and Gold, 2003a). In contrast, striatal lesions or inactivation lead to deficits in stimulus-response or cued learning performance (McDonald and White, 1993; Chang and Gold, 2004; Brightwell et al., 2008), while striatum activation with glucose infusions impairs spatial learning (Pych et al., 2006).

In order to further elucidate the nature of the cognitive shifts seen in models of PD, it will be necessary to use behavioral tasks that are more selective for the brain region primarily engaged, such as the hippocampus-sensitive forced place and striatum-sensitive forced response tasks. Regardless, the enhancement of spatial working memory in this study represents a novel diagnostic tool and indicates that cognitive improvements can occur in a diseased brain.

A

B

Figure 1. Photographs of rats performing the motor tasks. (**A**) Vibrissae-elicited forelimb placing. The rat is held with its limbs and tail hanging free until body relaxation is attained (left panel). The rat's whiskers (right panel) are brushed against the side of a table, eliciting the reflex. The rat grasps the table with its ipsilateral forelimb, receiving a score of a 3. (**B**) Forelimb stepping. The rat is held by its hindquarters such that its weight is on its forelimbs. The rat is pulled backward across a tabletop for 1 m in 5-7 seconds. One adjusting step is shown in which the rat lifts its left forelimb off the tabletop.

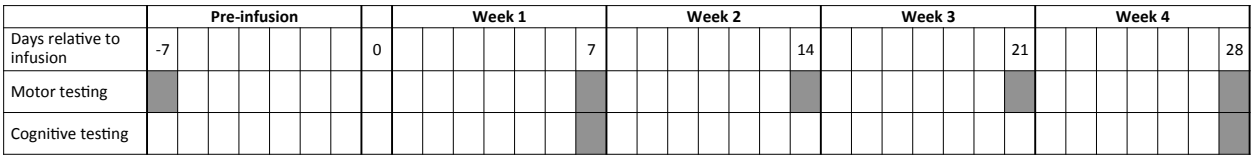

Figure 2. Timeline for behavioral testing. Motor testing included the vibrissae-elicited forelimb placing task and the forelimb stepping task. Motor testing was performed one week prior to 6-OHDA or aCSF infusions and weekly for four weeks post-infusion. Cognitive testing consisted of the spontaneous alternation task, which was performed on weeks one and four after 6-OHDA or aCSF infusions.

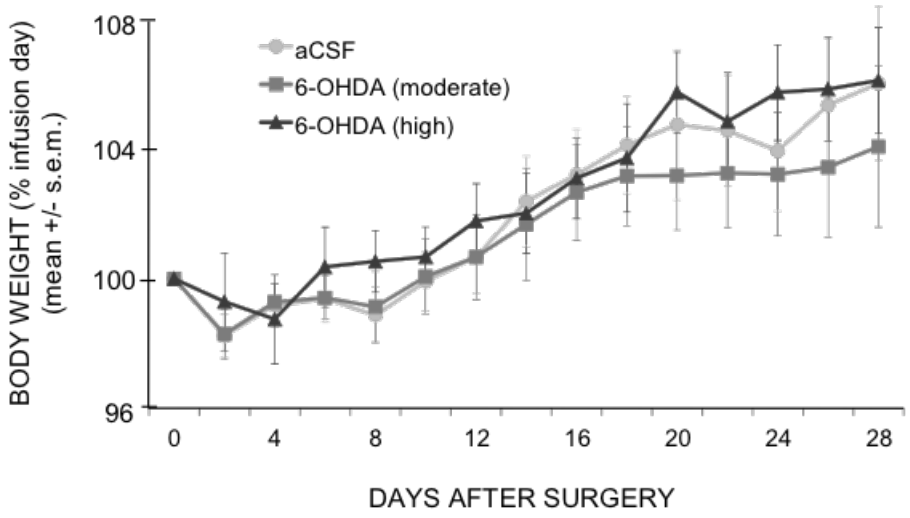

Figure 3. Changes in body weights over time following intrastriatal infusions. Body weights were slightly lower immediately following surgery. Weights gradually increased, eventually reaching and surpassing infusion-day weights. There were no significant differences among treatment groups.

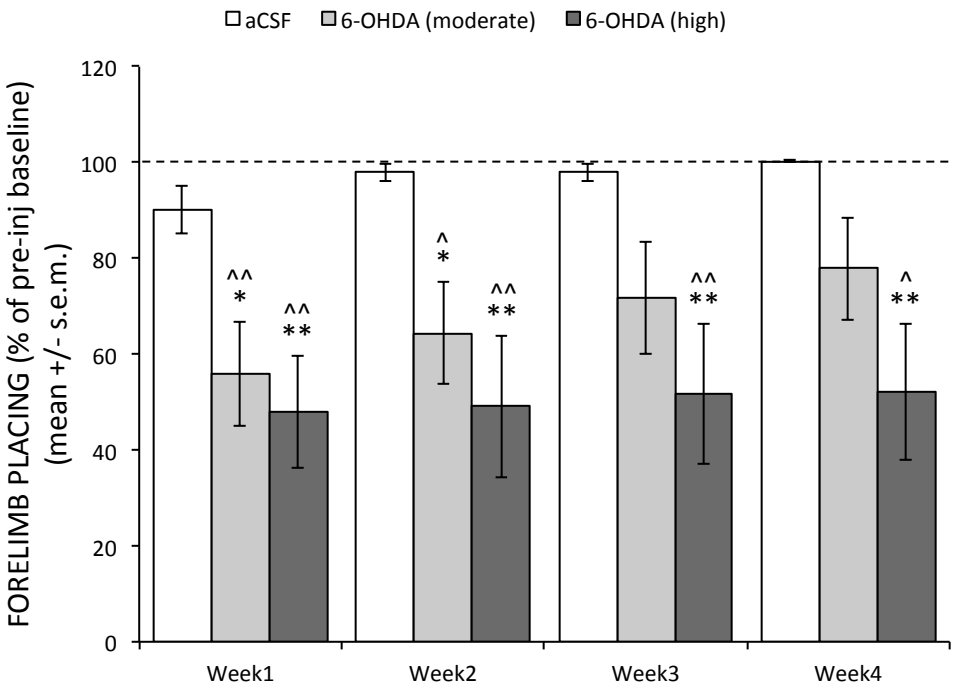

Figure 4. A high dose of 6-OHDA chronically impairs performance on vibrissae-elicited forelimb placing while rats given a moderate dose show recovery of motor function over time. Results are presented as a percentage of pre-injection baseline performance. Rats infused with the moderate dose of 6-OHDA showed deficits in weeks one and two post-infusion compared to aCSF-infused controls, but showed partial recovery of function over time. The high dose of 6-OHDA produced sustained deficits in this task across all four weeks post-infusion compared to pre-infusion performance and to aCSF-infused controls. Dashed line represents pre-infusion baseline performance. Versus aCSF: * $p < 0.05$, ** $p < 0.01$. Versus baseline: ^ $p < 0.05$, ^^ $p < 0.01$. Ns = 8.

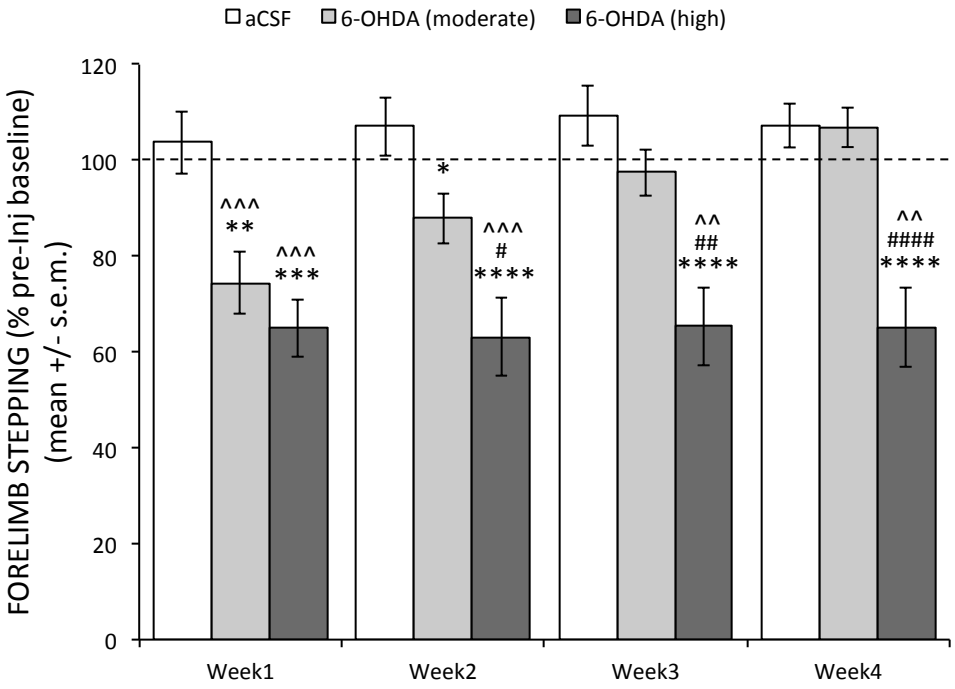

Figure 5. A high dose of 6-OHDA chronically impairs forelimb stepping performance over time while rats given a moderate dose show full recovery by week four. Results are presented as the percentage of pre-infusion baseline performance. The moderate dose of 6-OHDA produced deficits in the first two weeks post-infusion compared to aCSF-infused controls, with scores incrementally increasing over time. Rats in the moderate group appeared to attain full recovery by week four, as they are no longer distinguishable from the aCSF-treated controls and are at 100% of their pre-infusion scores. Rats receiving the high dose of 6-OHDA exhibited deficits that were sustained over all four weeks post-infusion compared to aCSF controls. High dose 6-OHDA-infused rats also show significant impairments compared to moderate dose 6-OHDA-infused rats at weeks two through four. Dashed line represents pre-infusion baseline performance. Versus aCSF: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Versus moderate: # $p < 0.05$, ## $p < 0.01$, #### $p < 0.0001$. Versus baseline: ^^ $p < 0.01$, ^^^ $p < 0.001$ N = 9 for aCSF; Ns = 8 for 6-OHDA groups.

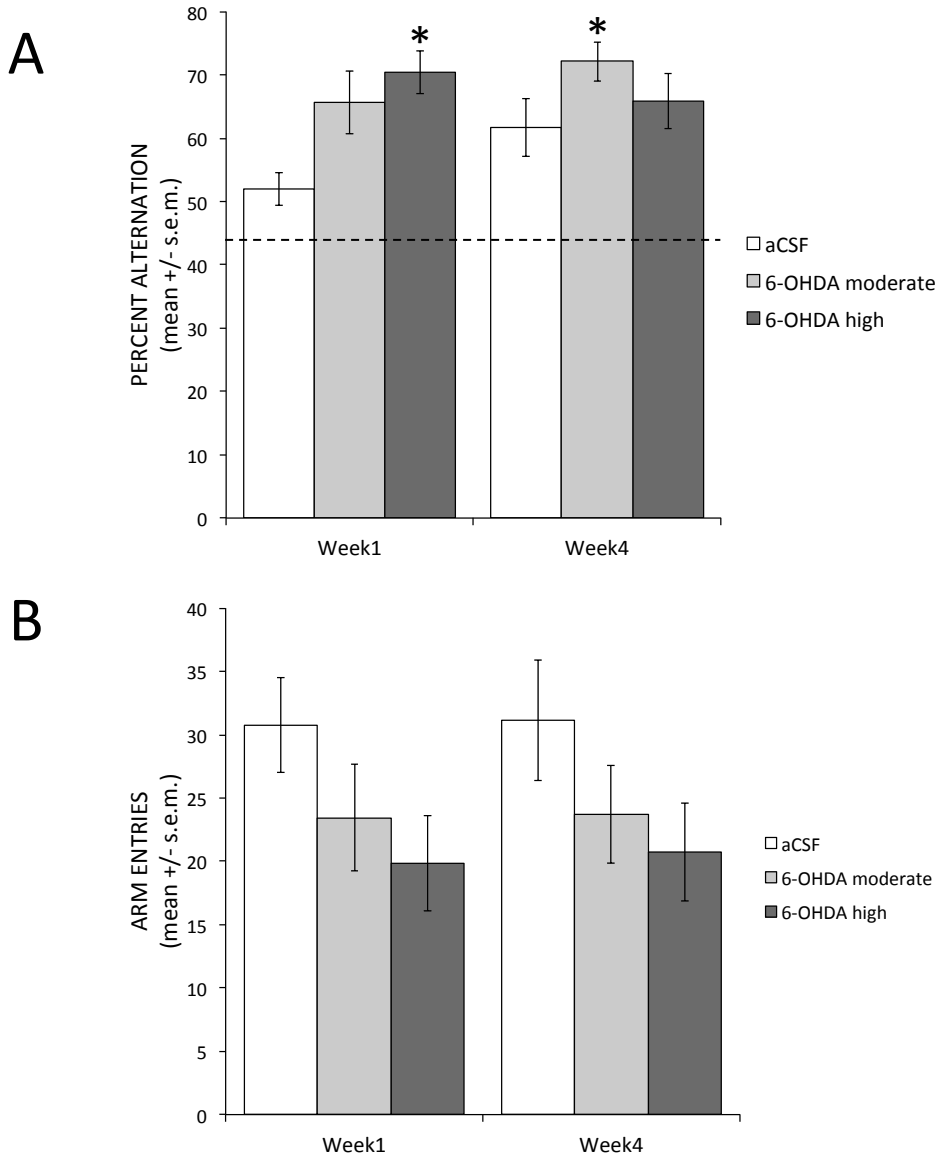

Figure 6. Rats treated with a high dose of 6-OHDA show significant enhancements on spontaneous alternation performance at week one while rats given a moderate dose show significant enhancements at week four. (**A**) The moderate dose of 6-OHDA significantly enhanced alternation scores at four weeks post-infusion, with a trend for enhancement at one week post-infusion compared to aCSF-treated rats. The high dose of 6-OHDA significantly enhanced alternation scores at one week post-infusion compared to aCSF-infused rats. Dashed line indicates chance alternation performance (44%). Versus aCSF: * $p < 0.05$. For aCSF, 6-OHDA (moderate), and 6-OHDA (high), respectively: Ns = 9, 7, and 6. (**B**) While rats in both 6-OHDA groups tended to make fewer arm entries, there was no significant effect of 6-OHDA treatment on the number of arms entered during testing at either week. For aCSF, 6-OHDA (moderate), and 6-OHDA (high), respectively: Ns = 9, 7, and 6 at week one; Ns = 9, 8, and 8 at week four.

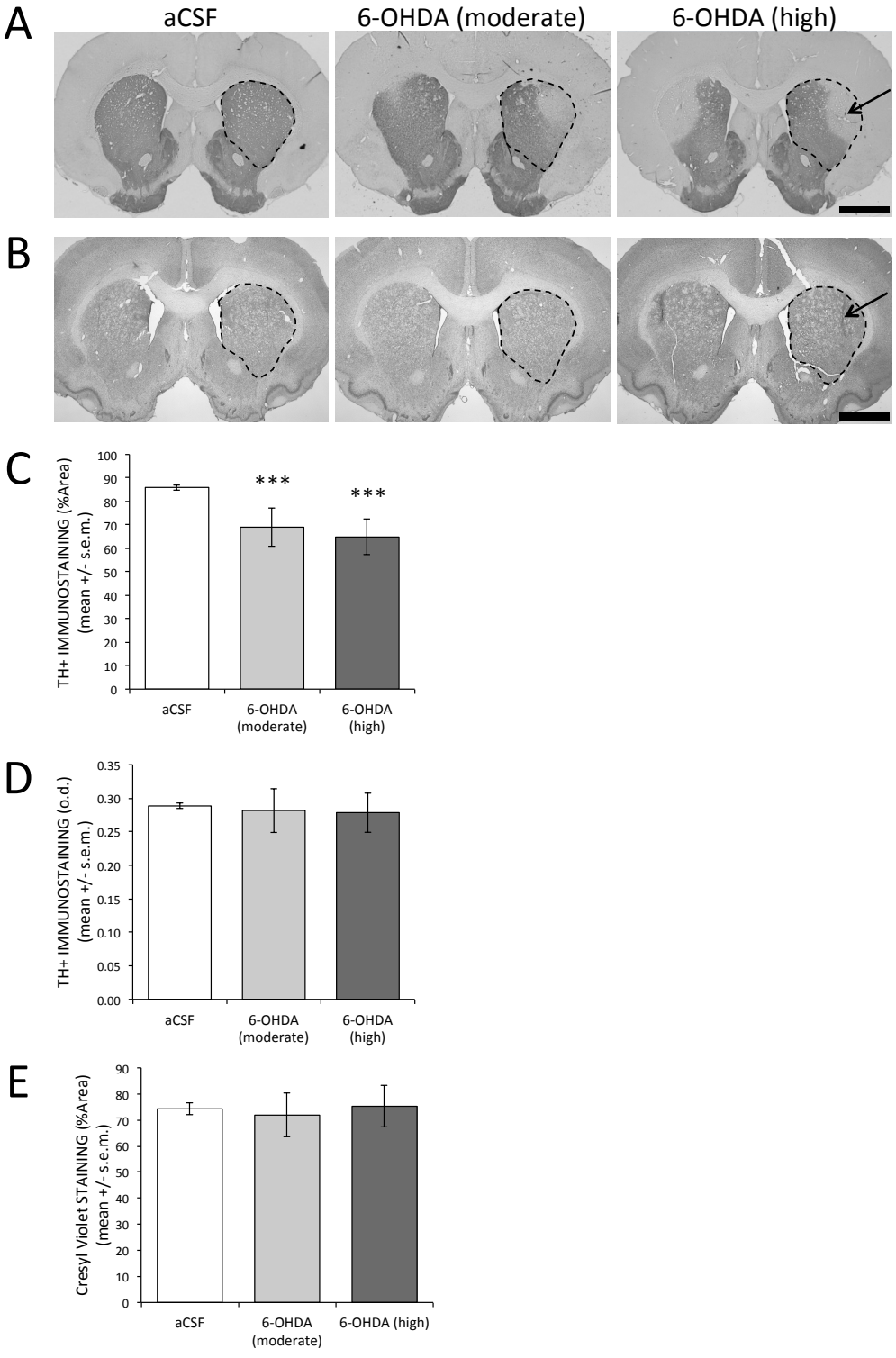

Figure 7. 6-OHDA infusion decreases TH+ staining area, but does not affect TH+ staining intensity or cresyl violet staining area in the striatum at four weeks post-infusion. (**A**) Representative photomicrographs of TH+ staining in the striatum. Loss of TH+ staining most likely represents loss of dopaminergic terminals from the SNc, not cell loss within the striatum.

Arrow indicates the lesioned area on one side. Dashed line indicates the region of interest selected for analysis. Scale bar = 2 mm. (**B**) Representative photomicrographs of cresyl violet staining the striatum. Cresyl violet staining is used to estimate cell density. Images in panel B are adjacent slices to those shown in panel A. Arrow approximates the center of the TH+ lesion seen in figure 7A. Dashed line indicates the region of interest selected for analysis. Scale bar = 2 mm. (**C**) Both doses of 6-OHDA significantly decreased the area of TH+ staining in the striatum at four weeks post-infusion. Versus aCSF: *** $p < 0.001$. (**D**) 6-OHDA treatment did not affect the intensity TH+ staining above background levels in the remaining dopaminergic terminals, as measured by optical densities. (**E**) 6-OHDA treatment had no effect on cell density in the striatum as measured by percent area staining analysis of cresyl violet stained tissue.

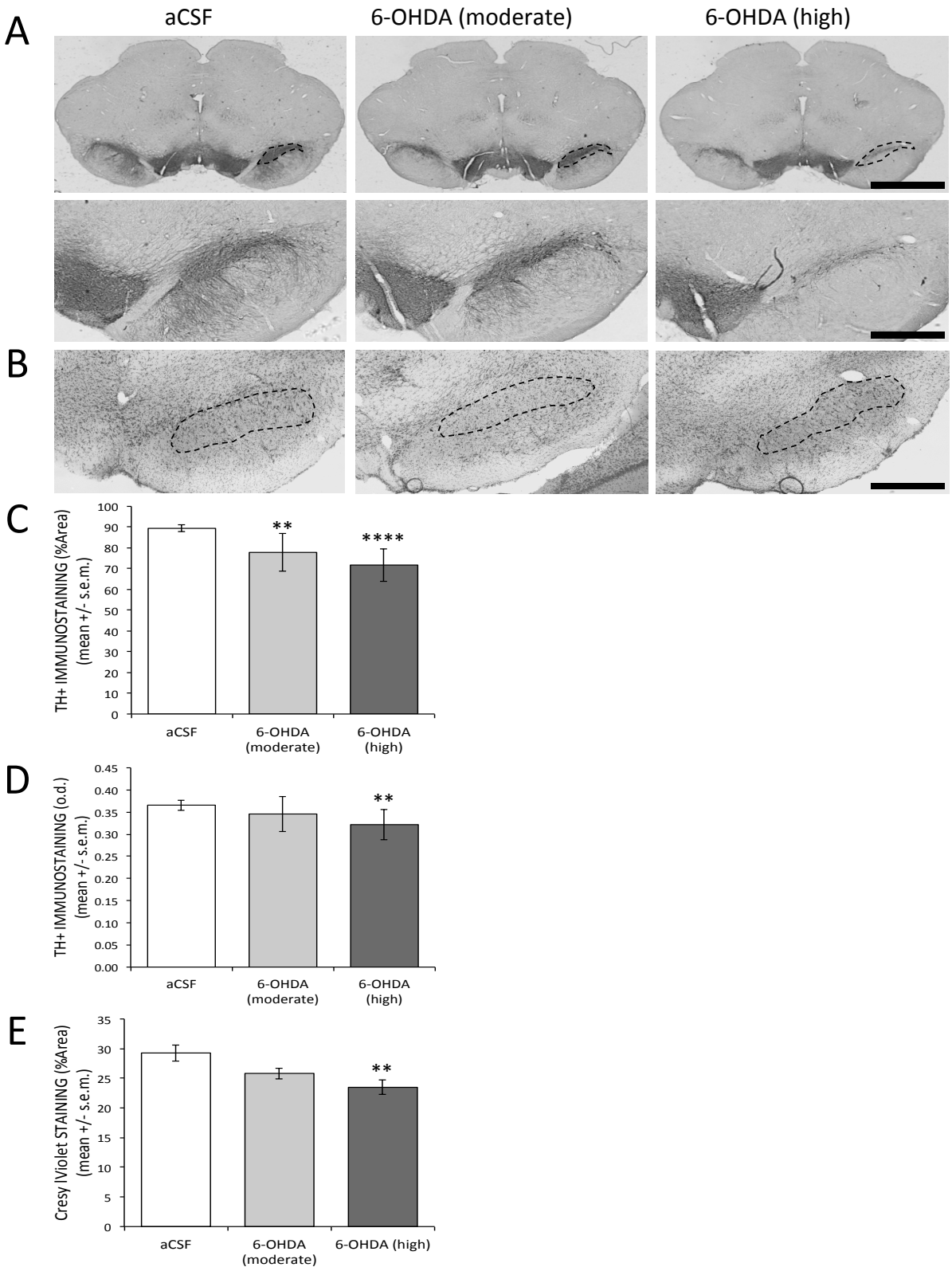

Figure 8. 6-OHDA treatment decreases TH+ staining area and intensity as well as cresyl violet staining area in the SNc at four weeks post-infusion. (**A**) Representative photomicrographs of

TH+ staining in the SNc at two different magnifications. The lower magnification images were used for TH+ staining analysis. Loss of TH+ staining in 6-OHDA-treated rats most likely represents degeneration of dopaminergic neurons and their projections. Dotted line indicates the region of interest selected for analysis. Scale bar = 1 mm for the top row of images and 0.25 mm for the bottom row. (**B**) Representative photomicrographs of cresyl violet staining in the SNc. Cresyl violet staining is used to estimate cell density. Images shown in panel B are adjacent slices to those shown in panel A. Scale bar = 0.25 mm. (**C**) The moderate dose of 6-OHDA significantly decreased the area of TH+ staining in the SNc. Treatment with the high dose of 6-OHDA showed even greater decreases in TH+ staining areas. Versus aCSF: ** $p < 0.01$, **** $p < 0.0001$. (**D**) Treatment with the high dose of 6-OHDA significantly reduced TH+ staining intensity compared to aCSF-treated rats, and showed a trend for having decreased staining intensity compared to rats in the moderate group. Versus aCSF: ** $p < 0.01$. (**E**) Treatment with the high dose of 6-OHDA significantly decreased cresyl violet staining areas compared to aCSF-treated rats. There was a trend for decreased cresyl violet staining areas in the moderate 6-OHDA group compared to aCSF-treated rats.

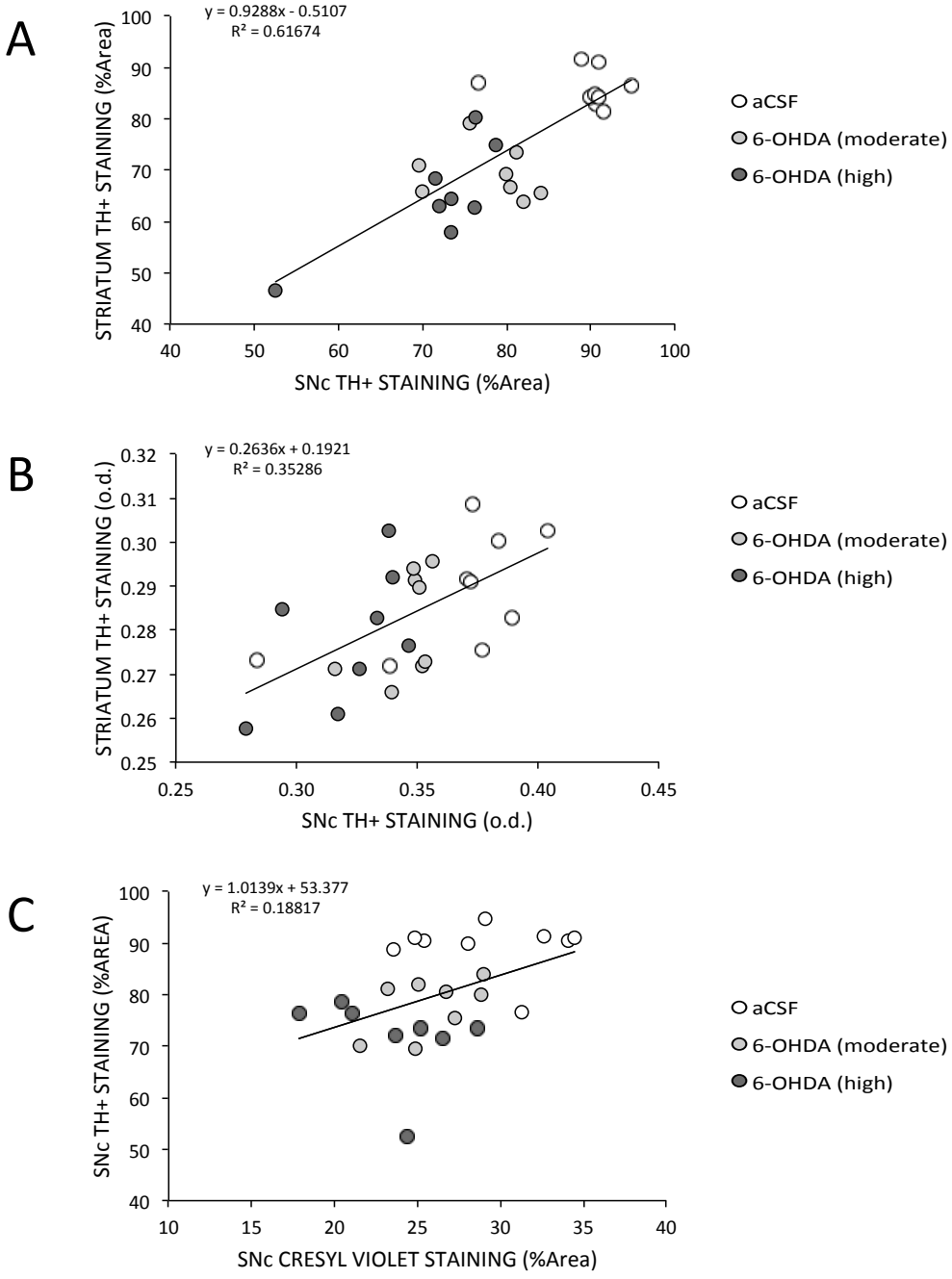

Figure 9. TH+ staining in the SNc is positively correlated with TH+ staining in the striatum as well as cresyl violet staining in the SNc. (**A**) The area of TH+ staining in the striatum was significantly correlated with the area of TH+ staining in the SNc. (**B**) The intensity of TH+ staining in the striatum was significantly correlated with the staining intensity in the SNc. (**C**) The percent area of cresyl violet staining was significantly correlated with the percent area of TH+ staining within the SNc.

CHAPTER 3

Intrastriatal muscimol impairs motor function and striatum-sensitive response learning, without enhancement in hippocampus-sensitive place learning

ABSTRACT

Rats bilaterally infused into the striatum with 6-OHDA show enhanced performance on spontaneous alternation, a test of spatial working memory. While this cognitive enhancement on a hippocampus-sensitive task could be due to lesion-induced decreases in competition from the striatum, recent data from our lab suggests that the hippocampus and striatum both show increased activation when non-lesioned rats are tested on the spontaneous alternation task, suggesting that activation of both the hippocampus and striatum may be needed to perform well on this task. However, if collaboration between the hippocampus and striatum were needed, we would expect that a striatal lesion would impair performance on the spontaneous alternation task. Our findings that the 6-OHDA-induced striatal lesion enhanced performance on the spontaneous alternation task support the theory that PD-associated nigrostriatal damage will enhance hippocampus function. Additionally, the dual activation of the hippocampus and striatum of non-lesioned rats performing the spontaneous alternation task suggests that it may not be the cleanest hippocampus-sensitive task. Because it is unclear how the hippocampus and striatum interact to produce spontaneous alternation behavior, we are unable to determine directly how the 6-OHDA-induced nigrostriatal lesion affects the normal cognitive balance between hippocampus- and striatum-sensitive behavioral strategies. To better elucidate how the balance between these two different cognitive systems may shift in PD, rats were trained on a single solution task that forces them to learn to use either extra-maze spatial cues (hippocampus-sensitive place learning)

or an egocentric body turn (striatum-sensitive response learning) to locate a food reward. Just prior to training on the forced place or forced response task, young adult Long-Evans rats received bilateral intrastriatal infusions of muscimol (MUSC), a $GABA_A$ receptor agonist that mimics the endogenous inhibitory neurotransmitter, GABA, and produces reversible dysregulation of brain region by altering normal neurotransmitter release or neuronal response to neurotransmitter binding (Martin and Ghez, 1999; Olsen and Sieghart, 2008, 2009). Rats were also trained on the vibrissae-elicited forelimb placing and forelimb stepping tasks to assess whether intrastriatal MUSC induced Parkinson's-like motor dysfunction. MUSC-treated rats showed impairment on both motor tasks and on the striatum-sensitive forced response cognitive task, but no change on the hippocampus-sensitive place learning task. However, even the majority of the vehicle-infused control rats failed to learn the place task as well, suggesting that there was a confounding variable that obstructed spatial learning in general not related to striatal inactivation.

INTRODUCTION

Results from the experiments in Chapter 2 support the hypothesis that 6-OHDA-induced deficits in dorsolateral striatum function change the balance between striatum-sensitive and hippocampus-sensitive functions, allowing for enhanced performance on hippocampus-sensitive learning and memory. Recent data suggest that good performance on the spontaneous alternation task may be due to coordinated activation of both the hippocampus and striatum. Using increases in extracellular lactate as a marker of neuronal activation, hippocampus activation can be measured during testing on the spontaneous alternation task (Newman et al., 2011); however, increases in extracellular lactate can also be measured in the striatum during spontaneous

alternation testing (Newman, unpublished data), suggesting that striatal activation can also contribute or respond to spontaneous alternation behavior. Consequently, enhancements on the spontaneous alternation task in our model of PD described in Chapter 2 may not be caused by a decrease in comptetition, but rather by a change in the collaboration between the hippocampus and striatum. Thus, spontaneous alternation may not be the most selective behavioral task for measuring how striatal impairments affect hippocampus-sensitive behavior; we may expect to find even greater cognitive gains from striatal dysfunction in hippocampus-sensitive tasks that rely more heavily on a single memory system, referred to as single-solution tasks.

Evidence from people with PD suggests that degeneration of the nigrostriatal pathway enhances hippocampus-sensitive cognitive functions despite impairments on striatum-sensitive cognitive tasks (Foerde and Shohamy, 2011a, b; Foerde et al., 2013); however, the mechanisms that underlie this shift in cognitive strategy are not well understood. To better examine whether enhancements in hippocampus-sensitive cognitive functions are due to decreased competition from the striatum, rats were trained on either a hippocampus-sensitive spatial learning task or a striatum-sensitive response learning task following intrastriatal infusions of the $GABA_A$ receptor agonist MUSC.

MUSC provides transient dysregulation of surrounding neural tissue by leading to changes in neurotransmitter release and/or cell activation (Yoshida et al., 1997; Login et al., 1998; Levy et al., 2001). $GABA_A$ receptors are heteropentameric, ligand-gated chloride channels found throughout the brain (Ben-Ari, 2002; Mody and Pearce, 2004; Olsen and Sieghart, 2008, 2009). Upon binding GABA, the $GABA_A$ receptor undergoes a conformational change, opening the central ion pore (Olsen and Sieghart, 2008). Because mature mammalian neurons generally have a low intracellular chloride concentration, activation of $GABA_A$ receptors leads to an influx

of chloride, hyperpolarizing the membrane potential, and decreasing the probability of generating an action potential (Ben-Ari, 2002; Chung, 2012). Consequently, activation of $GABA_A$ receptors can lead to a more generalized disruption of neurotransmission compared to 6-OHDA-induced lesions.

Infusions of MUSC into the subthalamic nucleus of people with PD lead to a change in oscillatory activity of surrounding neurons, decreasing the number of neurons firing at ~5 Hz and increasing the number of neurons firing at ~20 Hz (Levy et al., 2001). These changes in frequency could be due to 1) silencing of one neuron population (5 Hz population) and activation of a new neuron population (~20 Hz population); 2) changes to neuron physiology, such as decreasing the firing threshold, to allow neurons to fire at higher frequencies; or 3) a combination of both. Data from the Yoshida et al. (1997) study shows that muscimol infusion into the ventral tegmental area can lead to neuronal activation as measured by increased dopamine release and activation of the immediate early gene cfos. Additionally, administration of MUSC to dissociated striatum cell cultures can cause release of the acetylcholine (Login et al., 1998). Acetylcholine release that does not coincide with cognitive activity may disrupt normal brain function due to aberrant neuronal activation. While readily reversible and far less invasive than a surgical lesion, MUSC-induced changes in neuronal activity can be equally disruptive to normal behavior.

In this study, MUSC will be infused into the dorsal striatum at the level of the dopaminergic inputs from the SNc in order to simulate the nigrostriatal pathway dysregulation seen in PD. If enhancements in hippocampus-sensitive cognitive functions seen in people with PD and models of PD are due to decreased competition from the striatum, I predict that MUSC-infused rats will show impairments on the striatum-sensitive motor tasks and the striatum-

sensitive response learning task. However, rats treated with MUSC should show enhancements in hippocampus-sensitive place learning due to decreased competition from the striatum.

MATERIALS AND METHODS

Subjects. Young adult (90-100 days) male Long-Evans rats (Harlan Laboratories; Indianapolis, IN) were housed individually in translucent cages on a 12:12 hr light:dark cycle and *ad libitum* access to food and water. All rats were given at least 48 hr to acclimate to the vivarium before any procedures were conducted. Animal pain and discomfort were minimized. These experiments were conducted at Syracuse University, an AAALAC accredited institution, in accordance with animal care guidelines established by the National Institute of Health and were approved by the Syracuse University IACUC.

Preparation of drugs. MUSC (Sigma-Aldrich; St. Louis, MO) was dissolved in artificial cerebral spinal fluid (aCSF) composed of (in mM): 128 NaCl, 2.5 KCl, 1.3 $CaCl_2$, 2.1 $MgCl_2$, 0.9 NaH_2PO_4, 2.0 Na_2HPO_4, 0.7 glucose, pH = 7.4. The 0.7 mM glucose concentration in the aCSF approximates the baseline level seen in striatal cerebral spinal fluid (McNay et al., 2001). A 0.125 µg/µl muscimol solution was used for the intrastriatal infusions. aCSF was used as a vehicle control. All solutions were protected from light and stored at -20°C until use.

Surgery and bilateral intrastriatal cannula implantaion. Rats were deeply anesthetized with isoflurane and placed in a stereotaxic apparatus (Kopf; Tujunga, CA). Two sterile, stainless steel cannulae (6 mm, 22 gauge; Plastics One, Inc.; Roanoke, VA) were chronically implanted bilaterally into the dorsolateral striatum [coordinates: +0.4 mm from bregma; lateral ± 3.4 mm; -4.2 mm from dura]. Four sterile, stainless steel jeweler's screws were placed in the skull as anchors and the whole assembly was cemented in place with dental acrylic. To keep the cannulae

patent, sterile 28 gauge stylets (Plastics One, Inc.; Roanoke, VA) cut to the length of the guide cannulae were inserted at the time of surgery and removed only for intrastriatal infusions. All rats were given 7 days to recover from surgery.

Drug infusion. MUSC or aCSF was infused bilaterally through 28 gauge injection needles (Plastics One, Inc.; Roanoke, VA) fit to extend 1 mm beyond the guide cannulae. Infusions (0.6 µl) were made for 2.5 min at a rate of 0.24 µl/min with a CMA microinjection pump (CMA Microdialysis AB; North Chelmsford, MA). Needles were left in place for 1 min post-infusion to allow time for the drug to diffuse from the cannula tips.

Training procedures. Starting 1 week following surgery, rats were food restricted to 80-85% of free feeding weight plus 5 g to account for normal growth for ~10 days prior to training. Daily weights were taken during the food restriction period and feeding was individually titrated. Rats were given a sample of the food reward (Frosted Cheerio®) in the home cage to minimize neophobia during cognitive testing.

During the food restriction period, rats received specialized handling to familiarize them with the holds used during the motor testing. The motor tests detailed in Experiment 1 were also used for this study. Data from one rat was excluded from footstep analysis because the post-infusion video was inadvertently not recorded.

General cognitive training procedure. Training procedures have been used substantially in our lab (adapted from Korol and Kolo, 2002). Each rat's training was completed in a single day. Following intrastriatal infusion, rats were allowed to acclimate to the training room in a clean home cage for 15 min prior to cognitive testing. Ambient lighting was provided by two symmetrically placed floor lamps aimed at the ceiling. A standard house fan was used to mask extraneous noises. An open ceiling, 4-arm, plus-shaped black Plexiglass® maze, configured into

a “T,” was used in both cognitive tasks. The dimensions of each arm were 45 cm L x 13 cm W x 7 cm H. The central square-shaped area was 13 cm L x 13 cm W x 7 cm H. A reward receptacle was anchored at the end of each arm. An inaccessible food reward was placed in the base of the food receptacle to prevent the use of odor cues during training. Each trial began by baiting the goal arm with an accessible food reward (Frosted Cheerio® piece). The rat was then placed in the start arm and allowed to choose a single arm to enter. A choice was recorded when all four paws of the rat crossed into the arm. Rats were allowed to remain in the choice arm for ~10 sec and were then returned to their home cage.

Rats were trained for 100 trials, with a 2 min/trial max and a 30 sec intertrial interval. During the intertrial interval, the maze was randomly rotated to minimize the use of intramaze cues. Criterion was set at 10/10 correct trials. Trials to criterion and percent correct choices over 10-trial blocks were used to assess learning. Trials in which a rat did not make a choice within the two-min/trial max were excluded from trials to criterion and percent correct analyses. Training was terminated early if a rat failed to make a choice on five consecutive trials. If a rat reached criterion before early termination of cognitive testing, its data were used for trials to criterion analysis, but not for learning curve analysis. Rats that failed to reach criterion before early termination of cognitive testing were not included in the cognitive test analysis.

Response learning in the single solution response task. Rats (N = 23; aCSF n = 10; MUSC n = 13) were trained to find a food reward by making the same egocentric body turn, i.e. a 90° right or left. Rats were randomly assigned to a rewarded turn (right or left) and were counterbalanced across treatment groups. Curtains were hung around the outer walls to obscure visual cues. The start arm was randomly counterbalanced between the north and south arms of the maze (Figure 10A). One rat in the aCSF-treated group was excluded from cognitive testing

analysis for failing to make a left turn during training. One MUSC-treated rat was found to have pathologically enlarged brain ventricles during post-mortem histological analysis and was excluded from all analyses. Six additional MUSC-treated rats were excluded from cognitive testing analysis due to early termination of testing without reaching criterion.

Spatial learning in the single solution place task. Rats (N = 32; aCSF n = 14; MUSC n = 18) were trained to learn the location of the food reward by using extra-maze cues. Various visual cues were distributed around the perimeter of the room and consisted of items such as tables, a door, high-contrast posters, and shelves. Rats were randomly assigned to a reward location (i.e. the east or west start arm). The location of the goal arm remained constant during testing and the start arm was randomly counterbalanced between the north and south arms of the maze (Figure 10B). One aCSF-treated rat and eight MUSC-treated rats were excluded from cognitive testing analysis for early termination of testing without reaching criterion.

Motor function testing. To assess MUSC-induced changes in motor function, the vibrissae-elicited forelimb placing and forelimb stepping tasks detailed in Chapter 2 were used (Figure 1). Because MUSC treatment provides transient inactivation of the striatum, all motor testing was performed on a single day. Rats were first tested on both tasks to establish a pre-infusion baseline motor function score. The rats then received bilateral intrastriatal infusions of aCSF or MUSC. Following infusion, the rats were returned to their home cage for 15 min to allow time for drug diffusion and interruption of the striatal function. Rats were then retested on both motor tasks. Change from baseline was used to assess motor function.

Cannulae placement verification. At the end of all testing, rats were killed with an overdose of pentobarbital and their brains were collected for verification of cannula placement. Brains were post-fixed overnight in 4% paraformaldehyde in 0.1 M PB and then cryoprotected in

20% glycerol in 0.1 M PBS for at least 48 hr. Coronal sections (40 μm) through the cannula area were collected at -30°C with a Leica 1800 cryostat (Leica Microsystems, Wetzlar, Germany). Every fourth section was mounted on a gelatin-coated slide and stained with cresyl violet for cannulae placement verification.

Data analyses and statistics. All analyses were performed using Statview software. Performance on the cognitive tests was analyzed using non-parametric Mann-Whitney U test to examine differences among treatment groups. The non-parametric Mann-Whitney U test was used because several rats failed to learn either the response or the place task within the 100-trial testing limit, leading to a ceiling effect that produced a non-normal distribution of scores. Repeated measures ANOVA analyses (treatment x training block) were performed on the learning curve data to examine the effects of either treatment or training block on the rate of learning. Motor task data were compiled in a manner similar to that used in Chapter 2: for vibrissae-elicited forelimb placing, the cumulative score across four blocks of trials (two blocks each on the left and right sides) was used for analysis; for forelimb stepping, the average number of total forelimb steps across three trials was used for analysis. The percent of pre-infusion baseline was calculated and used to analyze post-infusion motor performance. Thus, individual differences in baseline motor function were minimized. To ensure that there were no significant group differences in pre-infusion motor function, independent samples t-tests were performed on the raw data for the forelimb stepping (average number of steps across three trials) and vibrissae-elicited forelimb placing (cumulative score across four blocks of trials) tasks. Independent samples t-tests were used to examine differences in motor task performance between aCSF- and MUSC-treated rats. For all comparisons, $\alpha = 0.05$.

RESULTS

The main results of this study showed that intrastriatal MUSC infusions induced impairments on the motor tasks and response learning without coincidental enhancement of place learning. Inactivation of the striatum with MUSC was able to induce the same motor impairments seen in toxin models of PD (Schallert et al., 2000; Fleming et al., 2005). Additionally, the concomitance of impaired motor function and poor response learning in MUSC-treated rats suggests that decreases in striatum-sensitive cognitive functions may be a symptom of PD.

Fourteen MUSC-treated rats were excluded from cognitive testing due to early termination of the training session. The majority of these rats (n = 10) exhibited motor impairments during cognitive testing that included: fine motor loss that rendered the rat unable to handle the food reward; abnormal turning behavior where the rat would traverse the maze in a series of tight circles; and general lack of ambulation. However, no analysis of the abnormal motor behavior was performed, so the severity of these problems is unknown. Post-mortem examination of the brains did not reveal anything abnormal anatomically. Many of the motor abnormalities may be due to a problem with the MUSC infusion, such as uneven flow through the injection needles, asymmetrical diffusion through the striatum, or asymmetrical positioning of the guide cannulae. Additionally, no gross differences in flow rates were observed during microinjection pump set up. During cannula placement verification (Figure 11), no gross asymmetries in cannula placements within an animal were found.

Nissl stained, coronal sections through the cannula area were used to assess cannula placement. All cannulae were contained within the striatum and clustered in the same general area (Figure 11). However, it is important to note that the drugs were infused 1 mm ventral to the

location of the aCSF or 6-OHDA infusions used in Chapter 2. While the ends of the guide cannulae were positioned at the same coordinates as the infusions in experiment 1, this position does not account for the additional 1 mm extension of the injection needle for the aCSF or MUSC infusions performed in this experiment.

Like the 6-OHDA treatment in Chapter 2, intrastriatal MUSC significantly impaired performance on the vibrissae-elicited forelimb placing task, as measured by independent samples t-test ($t_{(32)} = 3.312$, $p < 0.01$; Figure 12). However, pre-infusion vibrissae-elicited forelimb placing scores did not show significant treatment differences ($t_{(31)} = 1078$, $p > 0.05$). Following the intrastriatal infusion, rats that received MUSC had a 20% decrease in performance compared to pre-infusion baseline whereas aCSF-treated controls showed no change in performance.

MUSC-treated rats also showed significant deficits on the forelimb stepping task compared to aCSF-treated controls (Figure 13), as measured by independent samples t-test ($t_{(31)} = 4.968$, $p < 0.0001$). There was no significant effect of treatment on pre-infusion forelimb stepping scores ($t_{(32)} = -1.067$, $p > 0.05$). MUSC-infused rats had a 22.5% decrease in performance on the forelimb stepping task compared to their baseline performance ($p < 0.01$) whereas aCSF-treated controls did not show any significant change in performance ($p > 0.05$).

As expected, rats that received striatum inactivation via MUSC infusion were significantly impaired on the striatum-sensitive forced response learning task (Figure 14). Three rats in the MUSC-treated group failed to learn during the 100-trial testing period, causing a ceiling effect. Differences in number of trials to reach criterion between aCSF-treated ($n = 9$) and MUSC-treated ($n = 6$) rats were compared with a Mann-Whitney U-test. The analysis revealed that MUSC-treated rats were significantly impaired compared aCSF-treated controls ($U = 10$, $p < 0.05$). Two-way ANOVA revealed a main effect of training block on performance of the

response task ($F_{(9,100)} = 5.522$, $p < 0.0001$) and a trend for a treatment effect ($F_{(1,100)} = 3.618$, $p = 0.0863$). Percent correct scores (Figure 14B) increased as training progressed ($ps < 0.05$). These data are consistent with previous studies that inactivation of the striatum leads to deficits on striatum-sensitive cognitive tasks (Packard et al., 1989; Chang and Gold, 2004; Atallah et al., 2008; Braun et al., 2012).

While we predicted that dysregulation of the striatum via central infusions of MUSC would lead to enhanced place learning, MUSC infusion failed to modulate hippocampus-sensitive place learning (Figure 15). More than half of the rats in each treatment group failed to learn the place learning task within the 100-trial maximum (aCSF = 7 of 13 rats; MUSC = 8 of 10 rats), causing both groups to have median trials to criterion (10/10 correct) scores of 100 trials (Figure 15A). Mann-Whitney U-test analysis of trials to criterion did not reveal an effect of treatment on place learning ($U = 57.50$, $p > 0.05$). Although neither group reached criterion, repeated measures ANOVA revealed a main effect of training block on performance of the place task ($F_{(9,210)} = 3.683$, $p < 0.001$) and a trend for a treatment effect ($F_{(1, 210)} = 3.375$, $p = 0.0804$). Across training on the place task, aCSF-treated rats had a 22% increase in performance while MUSC-treated rats showed a 9% increase in performance. One-way repeated measures ANOVA revealed a significant effect of time on place learning in the aCSF-treated group ($F_{(9, 130)} = 4.195$, $p< 0.0001$). In contrast, one-way repeated measures ANOVA showed that there was no effect of time on place learning in the MUSC-treated group ($F_{(9, 80)} = 0.811$, $p > 0.5$). Additionally, there was a significant interaction between training block and treatment ($F_{(9, 210)} = 2.144$, $p < 0.05$), with aCSF-treated rats showing improvement while MUSC-treated rats showed no change in performance.

DISCUSSION

Consistent with previous studies, dysregulation of the striatum via MUSC led to deficits in striatum-sensitive response learning (Packard et al., 1989; Chang and Gold, 2004; Atallah et al., 2008; Braun et al., 2012). Intrastriatal MUSC was found to elicit motor impairments that mimicked those produced using a 6-OHDA model of PD. However, intrastriatal MUSC was not found to enhance hippocampus-sensitive place learning, a finding that is in contrast with previous findings where manipulations of striatal functions were made (Packard and McGaugh, 1996; Atallah et al., 2008).

MUSC-induced striatal dysregulation impaired motor function as assessed by the vibrissae-elicited forelimb placing and forelimb stepping tasks. Unlike 6-OHDA, intrastriatal MUSC disrupts normal release of neurotransmitters within the striatum by acting as an agonist at $GABA_A$ receptors (Yoshida et al., 1997; Levy et al., 2001). MUSC provides a much broader dysregulation of the striatum compared to the 6-OHDA-induced lesion, which specifically targets dopaminergic neurons. Additionally, the ability of the MUSC to induce motor function impairments that were similar to those seen in the 6-OHDA model suggest that the same general brain areas or functions were dysregulated in each study. While the cannulae were implanted 1 mm deeper in the striatum than intended, it seems like the delivery location of the MUSC was adequate for inducing the motor impairments, but potentially too ventral for enhancing place learning (Ferretti et al., 2010; Penner and Mizumori, 2012).

While the striatum has been implicated in response learning and procedural behavior, there is also evidence that the striatum plays a role in strategy selection and outcome evaluation (Penner and Mizumori, 2012; Jo et al., 2013). It is possible that the striatum, like the hippocampus, plays a role in spatial learning. In a study by Eschenko and Mizumori (2007),

electrophysiological recordings from the dorsal hippocampus and dorsal striatum of rats performing a maze-based behavioral task detected place cells (i.e. neurons whose activity is associated with a specific spatial location). When the behavioral strategy was switched, a subset of neurons in both brain regions showed changes in firing patterns, suggesting a strategy-specific reorganization of neural activity. However, it is possible that pattern of striatal neuron activity represents a non-spatial aspect of behavior: increases in firing in the start arm and/or decision point may reflect strategy selection whereas increases at the goal may reflect reward evaluation. Although the striatum is not classically associated with spatial learning, Eschenko and Mizumori (2007) propose that the striatum is constantly assessing behavioral outcomes and will be active regardless of behavioral task. An updated model of hippocampus-striatum interactions is that the hippocampus is a context detector while the striatum is strategy selector and/or outcome evaluator (Eschenko and Mizumori, 2007; Mizumori et al., 2009; Penner and Mizumori, 2012). Mizumori et al. (2009) have proposed a collaborative interaction between these two brain systems where the hippocampus modifies cortical representations based on updated contextual changes while the striatum modifies cortical representations based on recent reinforcements. Additionally, it has been proposed that the dorsomedial striatum mediates associated action-outcome behavior while the dorsolateral striatum mediaties habit formation (Penner and Mizumori, 2012).

Recent findings also suggest that the ventral striatum may be integral for processing spatial and/or contextual information. Dysregulation of the ventral striatum has been shown to inhibit short-term spatial information processing (Coccurello et al., 2012, 2013) and long-term spatial information encoding (Ferretti et al., 2010). Pharmacological manipulations of glutamatergic and dopaminergic neurons in the ventral striatum impaired detection of spatial

novelty in in mice (Coccurello et al., 2012, 2013). Additionally, disrupting gene transcription, protein synthesis, and synaptic remodeling blocked encoding of long-term spatial memory (Ferretti et al., 2010). These data suggest that the ventral striatum may also play a role in learning and remembering spatial information. Although the cannula placements remained within the striatum, it is possible that diffusion of the aCSF or MUSC affected ventral striatum structures and interfered with the cognitive testing (Ferretti et al., 2010; Coccurello et al., 2012; Penner and Mizumori, 2012; Coccurello et al., 2013). Despite being located 1 mm deeper in the striatum than intended, the cannula placements were approximately symmetrical across the two hemispheres, minimizing the possibility that cannulae asymmetry was the cause of the motor abnormalities. However, it is possible that a subtle asymmetry in where the MUSC was delivered could have large effects on motor function, especially on something like induced turning behavior (Lee et al., 1996; Kirik et al., 1998; Lundblad et al., 2002; Fleming et al., 2005).

Intrastriatal MUSC impaired performance on the striatum-sensitive response task, as predicted by previous work from our lab and others (McDonald and White, 1993; Chang and Gold, 2004; Atallah et al., 2008; Brightwell et al., 2008). The ability for the MUSC to impair both the striatum-sensitive response task and the motor tasks suggests that both functions are facilitated by a shared mechanism, such as the same neurotransmitter system or shared neural circuitry. It may be possible to use deficits in striatum-sensitive as a marker of striatum degeneration; however, it will be important to determine the nature of the shared mechanism before applying it as a diagnostic tool. Additionally, it will be important to determine if the extent of dopamine depletion is correlated with deficits on the striatum-sensitive response task. If measures of dopamine are correlated with striatum-sensitive cognition, it may be possible to use performance on striatum-sensitive cognitive tasks to determine a patient's PD stage.

While we were able to measure MUSC-induced deficits in striatum-sensitive response learning, the effects of MUSC on hippocampus-sensitive place learning were negligible. Although the aCSF-treated rats showed a statistically significant increase in place learning across training, the change in the number of correct trials per training block was less than anticipated. Using a more challenging plus-maze version of this task, un-implanted control male Long-Evans rats showed a 33% increase in performance across 75 trials (unpublished data). Additionally, the significant interaction effect is most likely driven by the poor performance (44% correct) in the aCSF-treated group in training block 2 (see Figure 15B). Although both groups showed increases in performance across training, neither group approached the criterion of 10/10 correct trials within the 100-trial limit. While statistically significant, the increase in learning over time and the treatment x time interaction are most likely skewed by the drop in performance of the aCSF-group in training block 4. The inability of the aCSF-treated rats to learn the place task suggests that a confounding factor, such as the arrangement of visual cues in the testing room or damage from the guide cannula implantation, affected the rats' abilities to perform the hippocampus-sensitive place task. There are a number of experiments that could be performed in an attempt to optimize hippocampus-sensitive place learning.

First, it is possible the spatial cues located in the testing room were not configured in an optimal way or may have included an aversive object. Rats in both the aCSF- and MUSC-infused groups showed modest learning on the place learning task; however there was not a significant effect of learning on the number of trials to reach a criterion of 10/10 correct trials. The lack of a treatment effect despite showing a significant effect of time on performance suggests that the configuration of the training room was not optimal to show robust enhancements on place learning. The presence of an aversive object could cause the rats to avoid certain parts of the

maze. Avoidance of an object could lead to poor performance on the place learning task by skewing which arms of the maze the rat will enter, especially if the rats avoid the rewarded arm.

There is evidence from our lab that the presence of non-salient cues can affect performance on cognitive learning tasks (Chang and Gold, 2004; Zurkovsky et al., 2007). In both of these studies, rats trained on the striatum-sensitive response task in a cue-rich environment (i.e. ideal for hippocampus-sensitive place task) lead to slower learning compared to rats trained in a cue-poor environment. It seems likely that the cue-rich environment may lead to improper use of a hippocampus-sensitive spatial strategy, causing impairments on the striatum-sensitive response task. While those studies determined that the presence of unnecessary or distracting spatial cues impairs learning of the striatum-sensitive response task, it is possible that the configuration of extra-maze cues may also affect a rat's ability to learn the hippocampus-sensitive place task. For instance, if cues are placed too close to the arms of the maze, they may be viewed as intra-maze cues, leading to use of an aberrant striatum-sensitive learning strategy (Rice et al., 2015). Additionally, if the cues are too symmetrical, it is possible that the rats are unable to distinguish the spatial differences between the two different start arms (Rice et al., 2015). To test whether the training room was negatively affecting performance on the place task, a small cohort of rats (n = 15 per group) could be trained using either the original training room or a different training room. If rats trained in the new room learn the place task while rats trained in the original room do not, it would suggest that there was a feature about the original training room that was interfering with their learning. However, if they still fail to learn the hippocampus-sensitive place task, it would suggest that either the configuration of the original testing room did interfere with the learning of this task or the new room was not optimally arranged.

Another possibility is that damage from the implanted guide cannula impairs performance on this task. While dorsal striatum is associated with egocentric behavioral strategies, the ventral striatum is associated with reward evaluation (Penner and Mizumori, 2012). Cannula-induced damage to the ventral striatum may have disrupted reward pathways, impairing the rats' abilities to associate the spatial location with the food reward. In order to test whether the guide cannulae contribute to the lack of learning on the forced place task, a cohort of non-implanted rats could be run on the forced response task and compared to a group with bilateral striatal cannula (n = 15 per group). If the guide cannula were contributing the rats' inability to learn the response task, the un-implanted rats should exhibit normal learning and reach criterion in approximately 50 to 60 trials while the implanted rats do not learn.

As mentioned above, the coordinates for the all implanted cannula implantations were one millimeter lower than originally planned, due to a failure to account for the fact that the injection needle extends 1 mm beyond the end of the guide cannula. Consequently, the MUSC may be inactivating brain regions that are important for the acquisition of the hippocampus-sensitive place task. For instance, it may be possible that the lower cannula placement is leading to dysregulation of the ventral striatum, a brain area associated with assessing reward value (Penner and Mizumori, 2012). Additionally, the recent implication that the ventral striatum plays a role in spatial learning (Coccurello et al., 2012, 2013) and memory (Ferretti et al., 2010) suggests that the lower cannula placement may disrupt performance on the place learning task by altering the function of this brain region. It is possible that both aCSF- and MUSC-infused groups failed to learn the place learning task due to cannulae-induced disruption of important neuronal connections in the ventral striatum, rendering rats unable to encode the reward location. To test if the deeper implants impaired learning of the place task, a cohort of rats could be

implanted with guide cannulae using the correct coordinates [coordinates: +0.4 mm from bregma; lateral ± 3.4 mm; -3.2 mm from dura] and compared with a group implanted with the original, deeper coordinates ($n \geq 5$ per group). These rats would receive intrastriatal infusions of aCSF prior to training on the forced place task. While it will be important to determine whether intrastriatal MUSC can enhance spatial learning is important, it may be prudent to first determine whether aCSF-infused controls are capable of learning the task since neither treatment group exhibited learning on the place task. If the lower cannula placements impaired learning due to ventral striatum damage, I would expect that moving the placement 1 mm dorsally will decrease the amount of tissue damage, allowing for learning on this task. If the aberrantly low cannula placement affected learning, rats implanted with the correct, higher coordinates should learn the task in approximately 60 to 70 trials. After verifying that aCSF-treated rats were capable of performing the place learning task, the study could be expanded to include MUSC-treated rats. If application of MUSC to this more dorsal portion of the striatum allows for enhanced place learning, I would expect MUSC-treated rats to learn the task in significantly fewer than 60 trials.

While we were unable to show that intrastriatal MUSC shifts the cognitive balance in the favor of spatial learning, we were able to show that intrastriatal MUSC is capable of inducing Parkinson's-like motor dysfunctions as well as impairments of striatum-sensitive response learning. Currently, it is unclear how much loss of function in the striatum needs to occur before other cognitive systems, such as hippocampus-sensitive place learning, begin to show cognitive enhancements. As such, a decrease in striatum-sensitive cognitive functions, such as response learning, may represent an early marker of degeneration in the striatum.

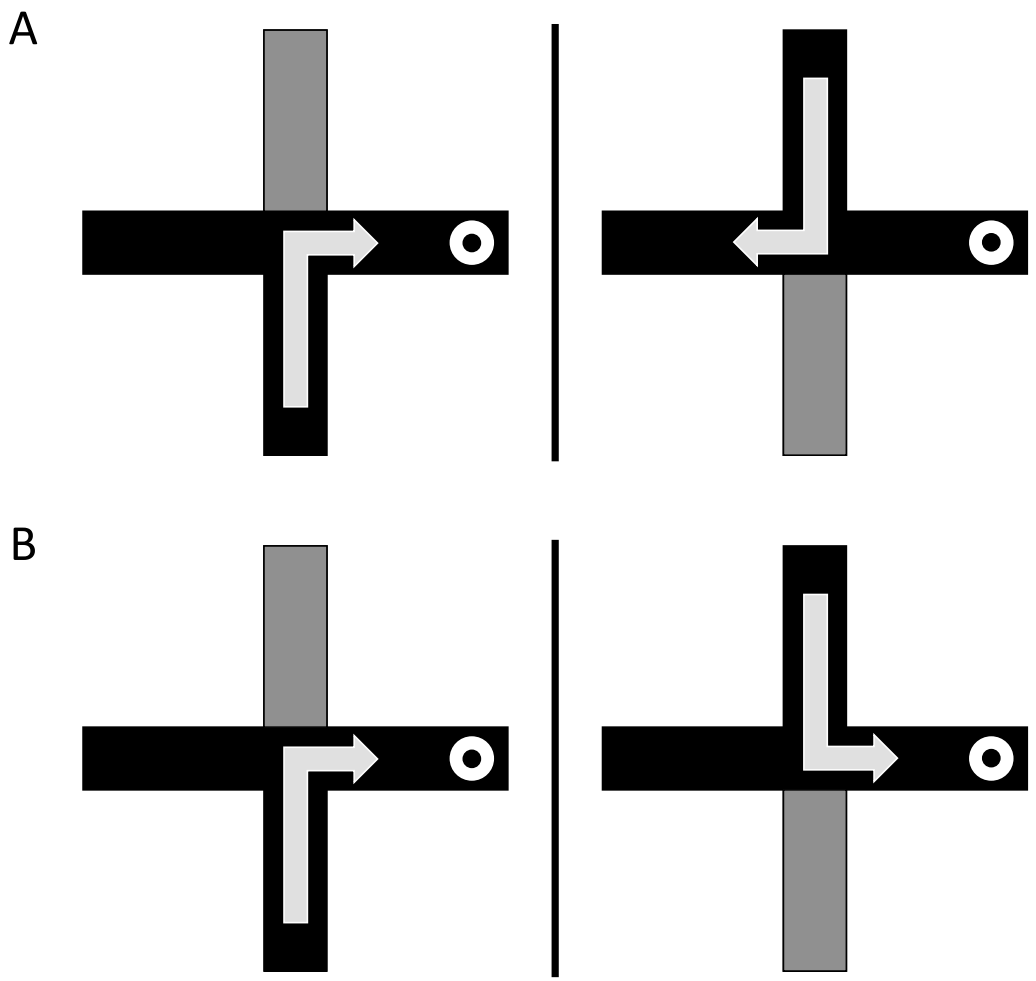

Figure 10. Graphic representations of the (**A**) forced response task and the (**B**) forced place task used to test the effect of MUSC on cognitive function. In the forced response task, rats learn to use the same egocentric body turn to reach the food reward. In the forced place task, rats learn to use extra-maze cues to reach the static goal arm.

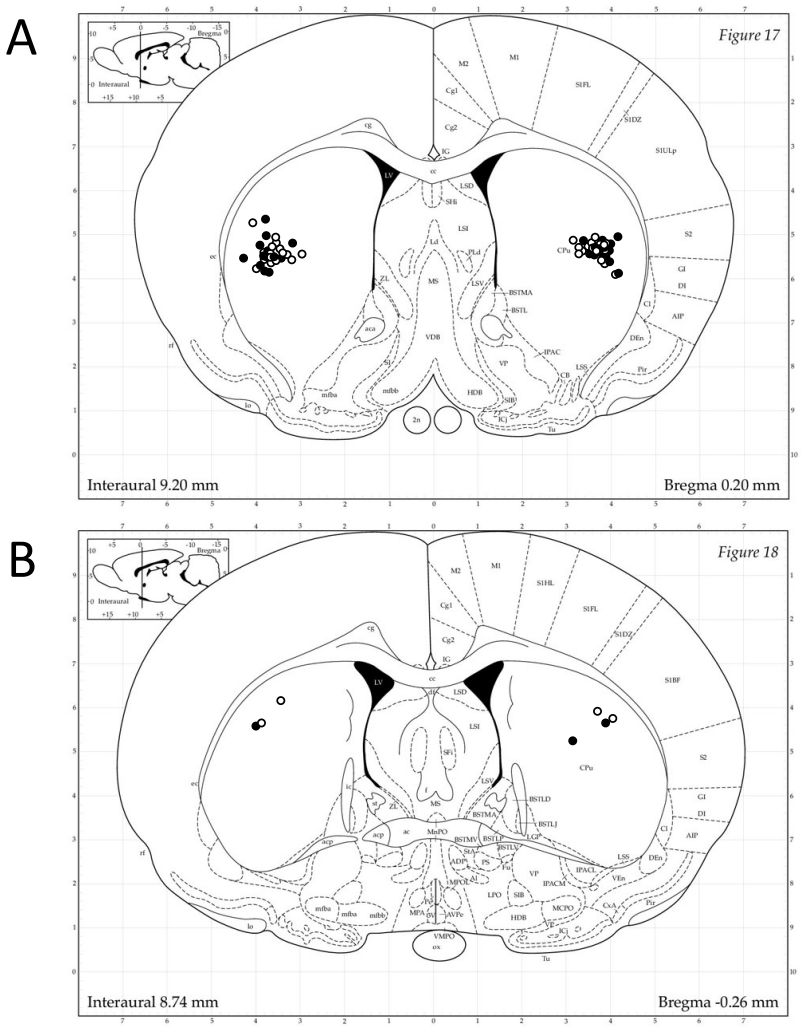

Figure 11. Graphic representations of cannulae placements. All cannulae were located within the striatum and were mostly clustered around the coordinates used for implantation. The majority of the cannulae were approximately +0.2mm from Bregma (figure A) while some were closer to -0.26mm from Bregma (figure B). Adapted from Paxinos and Watson (2005). Open circle = aCSF infusion. Closed circle = MUSC infusion.

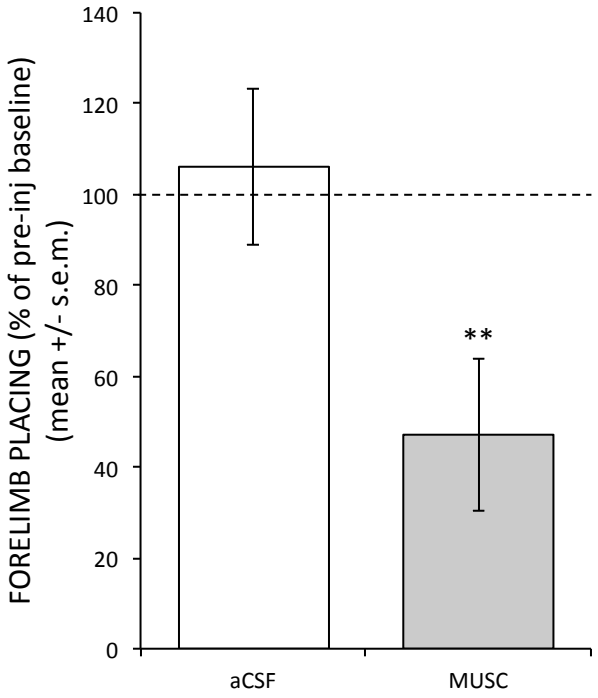

Figure 12. Intrastriatal MUSC significantly impaired performance on the vibrissae-elicited forelimb placing task. Data is presented as a percent of pre-infusion baseline performance. Rats that received an intrastriatal infusion of MUSC had an approximately 20% decrease in performance compared to their pre-infusion baseline scores. Dashed line represents pre-infusion baseline performance. Versus baseline: ** $p < 0.01$.

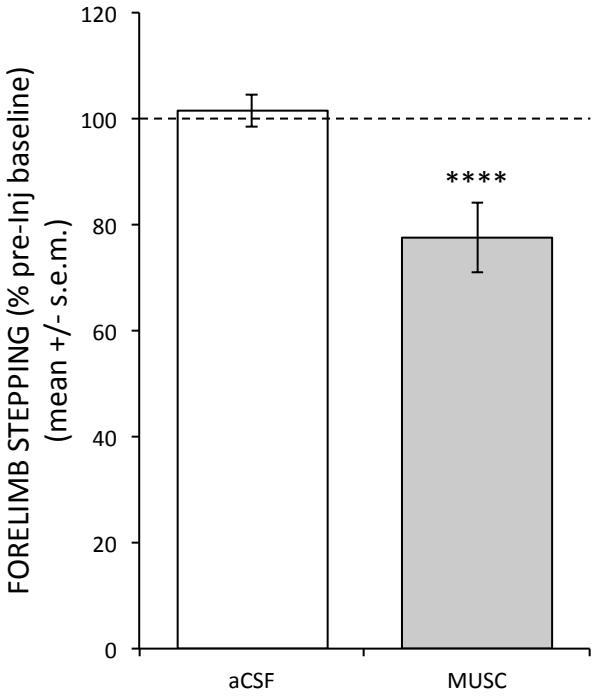

Figure 13. Intrastriatal MUSC significantly impaired performance on the forelimb stepping task. Data is presented as a percent of pre-infusion baseline performance. Rats that received an intrastriatal infusion of MUSC had an approximately 20% decrease in performance compared to their pre-infusion baseline scores. Dashed line represents pre-infusion baseline performance. Versus baseline : **** $p < 0.0001$.

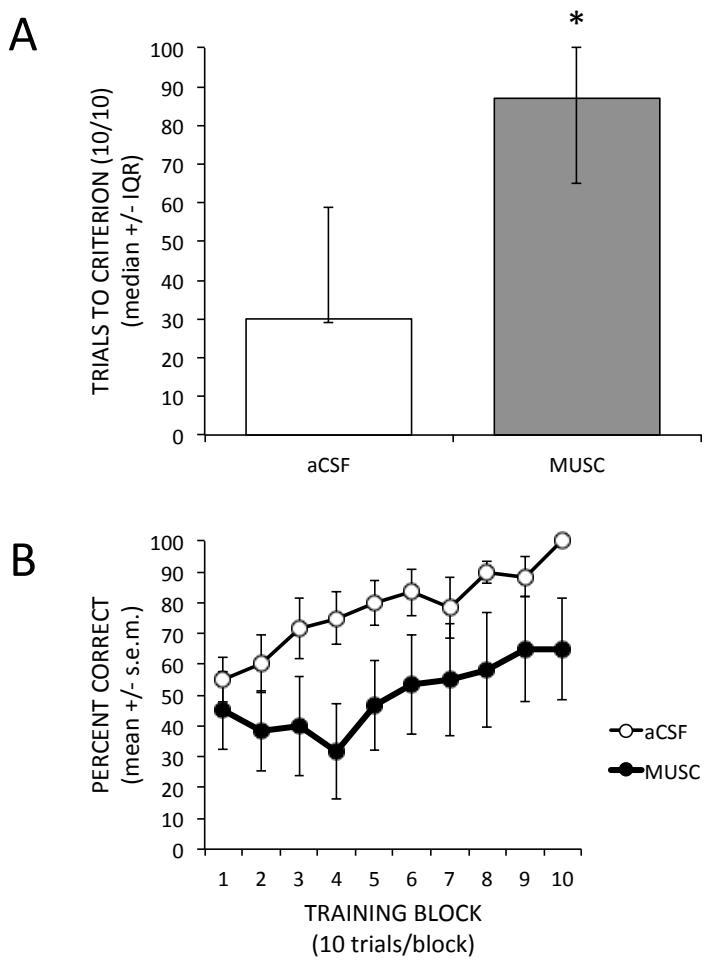

Figure 14. Intrastriatal MUSC significantly impaired striatum-sensitive response learning compared to aCSF-treated controls. (**A**) Treatment with MUSC significantly increased the number of trials needed to reach a criterion of 10/10 correct trials. (**B**) MUSC-treated rats learned the response task at a slower rate compared to the aCSF-treated controls. However, both groups showed learning across the 100 trials of testing, as measured by increases in the percent correct within a 10 trial training block. Versus aCSF: * $p < 0.05$.

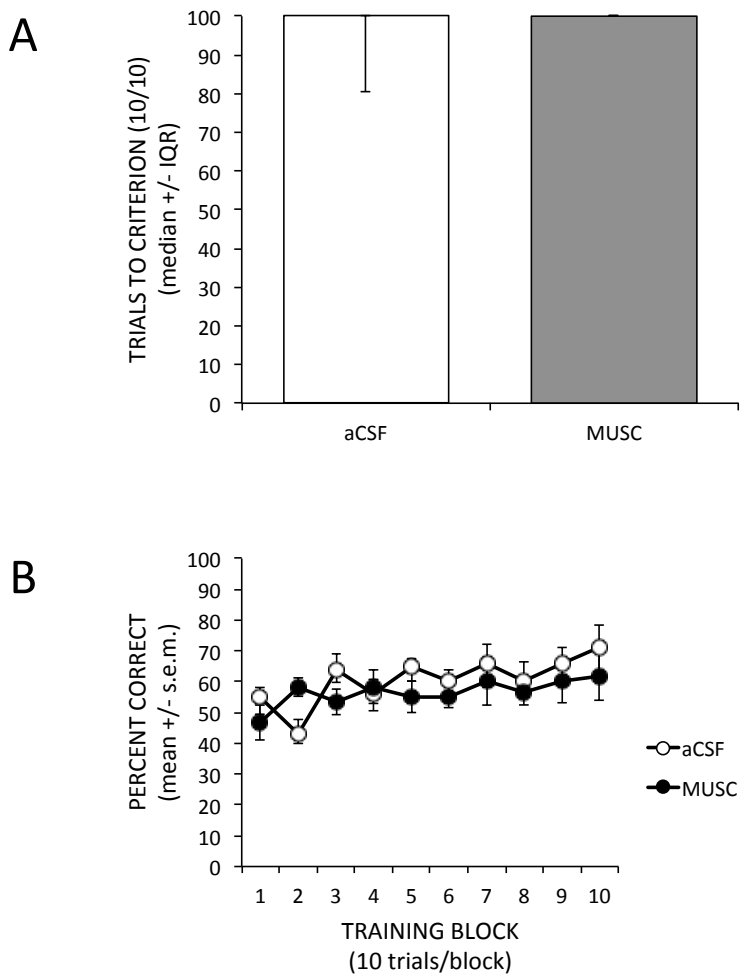

Figure 15. Neither treatment group showed learning on the hippocampus-sensitive place learning task. (**A**) Both the aCSF- and MUSC-treated rats exhibited a ceiling effect due to the large number of individuals that failed to reach criterion within the 100-trial limit. (**B**) Neither aCSF- nor MUSC-treated rats showed learning on the hippocampus-sensitive place task.

CHAPTER 4

CONCLUSIONS

Changes in the balance between striatum-sensitive cognitive functions and non-striatal cognitive processes may represent novel early markers of nigrostriatal degeneration seen in PD. Although cognitive impairment is not a classic symptom of PD, a subset of people with PD show mild cognitive impairments, including deficits memory, visuospatial function, and attention/executive function (Pillon et al., 1989; Owen et al., 1992; Packard and Knowlton, 2002; Aarsland et al., 2010; Foerde and Shohamy, 2011b; Ekman et al., 2014). In the study by Aarsland et al. (2010), mild cognitive impairments were found in approximately 20-30% of Parkinson's disease patients during the first 2.5 years following diagnosis, with levels rising as high as approximately 40% in patients 12 years post-diagnosis. Supporting the hypothesis that cognitive changes accompany the classic motor symptoms of PD, these data demonstrate that 1) PD affects cognition; 2) deficits in cognitive function can occur in early stages of PD; and 3) cognitive impairments in People with PD are measureable.

In contrast, some studies have found cognitive *enhancements* in People with PD (Knowlton et al., 1996; Foerde et al., 2013). The specificity of the cognitive changes suggests that it may be possible to develop a diagnostic cognitive profile; however, it unclear how early in the pre-symptomatic to symptomatic disease spectrum this cognitive profile may emerge. Because inclusion in many of these studies requires a PD diagnosis, pre-motor dysfunction profiles are reliant on past medical history or self-reporting and may not be accurate. Though longitudinal aging studies may eventually provide a cognitive profile for pre-motor dysfunction in people with PD, a preliminary cognitive profile may be generated through data collected from non-human animal studies.

Previous research shows that multiple memory systems may compete for learning and memory strategy choice despite the fact that the brain systems involved may specialize in specific functions. The multiple memory system competition hypothesis proposes that a decrease in the function of one competing brain system may allow enhanced functioning of its competitor. Because the striatum, a brain area important for proper response-based learning, is affected in PD, changes in cognitive function (i.e. deficits in striatum-sensitive functions and/or enhancements in other brain systems) may provide a diagnostic cognitive profile for PD. Despite the abundance of evidence that impairing hippocampus function can enhance striatum function (Packard and White, 1991; McDonald and White, 1993; Packard and McGaugh, 1996; Chang and Gold, 2003a), there has been little research presented about the converse. Recently, it has been shown that people with PD with poor performance on striatum-sensitive incremental learning also have enhanced hippocampus-sensitive episodic memory (Foerde et al., 2013). In our 6-OHDA model of PD, we showed that rats with impaired motor function and lesions of the dopaminergic nigrostriatal pathway exhibited *enhanced* performance on the spontaneous alternation task, a test of spatial working memory. This shift in cognitive balance in people with PD and models of PD is consistent with the multiple memory system perspective in which different neural systems compete for control over learning and memory strategy choice. Additionally, these data are important because they show that animal models of PD not only mimic the motor dysfunction seen in human patients, but also can mirror the changes in cognitive balance seen in motor dysfunction stages of the disease. Thus, the 6-OHDA rat model of PD may be a valid method for creating a preliminary cognitive profile for the various stages of the disease (i.e. pre-motor symptom, early motor symptom, and late stage).

While the PD-associated motor symptoms have been correlated previously with decreased substantia nigra dopamine levels in humans (Dauer and Przedborski, 2003; Skodda et al., 2013; Dewey et al., 2014; Dijkstra et al., 2014) and non-human animal (Olsson et al., 1995; Lindner et al., 1997; Kirik et al., 1998; Lindner et al., 1999; Deumens et al., 2002; Fleming et al., 2005; Ogura et al., 2005), we show that some types of motor dysfunction may be more sensitive to decreases in nigrostriatal dopamine release than others. In our 6-OHDA model of PD, rats receiving the moderate dose were indistinguishable from pre-infusion performance by week 3 when tested on the adjusting steps task. When treated with the high dose of 6-OHDA, the rats failed to show any recovery of function on the adjusting steps task over the 4 weeks of post-infusion testing. In contrast, rats treated with the moderate dose showed a more modest recovery when tested on the vibrissae-elicited forelimb placing task. Rats given the high dose of 6-OHDA failed to show recovery on the adjusting steps task by week 4 compared to pre-infusion performance, but rats treated with the moderate dose were not statistically different from their pre-infusion performance at weeks 3 and 4. Despite showing recovery, moderate dose-treated rats never fully reached pre-surgery forelimb placing scores, achieving only 71.7% and 77.9% of their baseline scores at weeks 3 and 4, respectively. Additionally, treatments with the moderate 6-OHDA dose yielded an intermediate decrease in TH+ staining in the SNc in terms of both staining area and staining intensity. These data suggest that tests of sensory-motor integration (i.e. vibrissae-elicited forelimb placing) may be more sensitive in detecting changes in dopamine levels than tasks that measure forelimb akinesia or postural adjustments (i.e. adjusting footsteps). It is possible that rats treated with the moderate dose of 6-OHDA would eventually attain the vibrissae-elicited forelimb placing scores of the aCSF-treated controls if allowed more time to recover, but the rate of recovery would most likely remain modest. Importantly, these data also

show that motor function can be *recovered* despite decreases in nigrostriatal dopamine, suggesting that halting dopaminergic SNc neuron degeneration may slow or stop the progression of PD symptoms.

Classically, dopaminergic degeneration has been associated with PD-associated dysfunction, but newer data suggest that noradrenergic degeneration in the locus coeruleus precedes nigrostriatal dopaminergic degeneration (Braak et al., 2003; Zarow et al., 2003; McMillan et al., 2011) and may underlie non-motor symptoms of the disease (Vajda and Solinas, 2005; Sethi, 2008). In fact, the staging guidelines developed by Braak et al. (2003) note that the neuromelanin-containing neurons of the coeruleus-subcoeruleus complex are the first neurons to exhibit Lewy bodies and Lewy neurites, the neuropathological marker used for post-mortem disease confirmation. As neuromelanin is associated with catecholamine synthesis, the co-localization of the Lewy bodies and Lewy neurites with neuromelanin suggests that early PD-associated pathology is limited to catecholaminergic neurons more generally. It should be noted that norepinephrine, not dopamine, is the main neurotransmitter produced in the locus coeruleus (Singewald and Philippu, 1998), suggesting that dopaminergic neurodegeneration may be secondary in disease progression. Non-human animal studies have shown that pre-treatment with a noradrenergic lesion of the locus coeruleus exacerbates later dopaminergic lesions, suggesting that intact locus coeruleus function may be protective against damage to nigrostriatal dopaminergic neurons (Gesi et al., 2000; Srinivasan and Schmidt, 2003; Marien et al., 2004; Fornai et al., 2007).

Data from a study using mice with hyperinnervation of noradrenergic neurons further strengthen the theory that noradrenergic projections from the locus coeruleus protect dopaminergic neurons in the substantia nigra. Following MPTP treatment to induce a

dopaminergic nigrostriatal lesion, transgenic *tottering* mice with hyperinnervation of noradrenergic neurons originating in the locus coeruleus show maintenance of nigrostriatal dopaminergic terminals compared to wild type controls (Kilbourn et al., 1998). Additionally, locus coeruleus noradrenergic lesions exacerbate motor dysfunction induced by nigrostriatal dopaminergic lesions (Gesi et al., 2000; Srinivasan and Schmidt, 2003). When given either a partial nigrostriatal 6-OHDA-induced lesion, a systemic noradrenergic lesion, or dual systemic noradrenergic and partial nigrostriatal 6-OHDA-induced lesions, rats failed to show lesion-induced catalepsy or decreases in voluntary locomotor activity. If the rats were challenged with a subthreshold dose of haloperidol (a D2 dopamine receptor antagonist), the combined of the noradrenergic and dopaminergic lesion had a synergistic effect on performance in both tasks compared to the dysfunction shown in the single lesion groups (Srinivasan and Schmidt, 2003). Collectively, these data suggest that pre-motor dysfunction degeneration of the locus coeruleus noradrenergic neurons in PD hastens the secondary degeneration of nigrostriatal dopaminergic neurons and potentiates the dopaminergic lesion-induced motor dysfunction.

In addition to showing that multiple neurotransmitter systems may be involved in the initiation and progression of PD-associated neurodegeneration, new data suggest that multiple brain regions may underlie some of the cognitive changes seen in PD. The MUSC-induced neuronal dysfunction may also model anatomical changes associated with PD. Previously, PD-related neuropathology has been associated with substantia nigra atrophy (Aquino et al., 2014) and subsequent dysregulation of the nigrostriatal pathway. However, there is growing evidence that people with PD also show astrophy of the hippocampus, a brain region not traditionally associated with PD (Camicioli et al., 2003; Bruck et al., 2004; Bouchard et al., 2008). It has been hypothesized that hippocampal atrophy may underlie PD-associated dementia (Bruck et al.,

2004; Bouchard et al., 2008), suggesting that the dementia may be due to a combination of neurodegenerative diseases. Although hippocampus dysfunction has been associated with dementia, hippocampal atrophy may not be limited to individuals with PD-associated dementia as Camicioli et al. (2003) showed that hippocampus atrophy is also evident in people with PD without dementia. Additionally, data from Bouchard et al. (2008) showed acceleration of normal age-related atrophy in the hippocampus and amygdala of people with PD with dementia, suggesting that the neurodegeneration or neural dysfunction seen in PD may negatively impact the anatomy and/or physiology of brain areas not classically associated with the disease. Because rats in our MUSC study had impaired striatum- and hippocampus-sensitive learning, it is possible that these animals model individuals with both substantia nigra and hippocampus atrophy.

Data from both our 6-OHDA and MUSC studies may provide insights into cognitive changes associated with PD. Even though we failed to show an effect of MUSC on hippocampus-sensitive place learning, we were able to establish that rats with motor impairments associated with PD also show impairments in striatum-sensitive response learning. Because MUSC can broadly affect multiple neurotransmitter systems, it is possible that our MUSC study mimics the dysfunction at a different stage of PD compared to our 6-OHDA model. The data from our 6-OHDA study are noteworthy because we were able to show that a dopaminergic nigrostriatal lesion can lead to enhanced performance on a hippocampus-sensitive working memory task. These data mirror similar findings that people with PD show enhanced performance on hippocampus-sensitive tasks despite showing impairments on striatum-sensitive tasks (Foerde et al., 2013). Taken together, data from both our 6-OHDA and MUSC studies are help to define the cognitive shifts that may occur in PD. Evidence of cognitive enhancements in

models of PD may help shift the research focus from searching for cognitive deficits to instead try to determine shifts in cognitive functions. Moving forward, it will be important to determine degeneration in noradrenergic signaling from the locus coeruleus interacts with nigrostriatal dopaminergic degeneration. It is possible that certain cognitive changes are better correlated with decreased noradrenergic signaling from the locus coeruleus compared to cognitive changes caused by nigrostriatal dopaminergice degeneration. Because noradrenergic degeneration seems to precede dopaminergic degeneration (Zarow et al., 2003; McMillan et al., 2011), determining the cognitive changes correlated with PD-associated noradrenergic degeneration may be important for developing a cognitive profile for pre-motor dysfunction diagnosis of the disease. It is possible that early pharmacological intervention to protect noradrenergic neurons in the locus coeruleus may prevent or slow the degeneration of dopaminergic neurons in the substantia nigra, limiting PD-associated motor dysfunction. Additionally, discovery and classification of memory systems that compete or collaborate with the striatum could yield new behavioral therapies designed to augment cognitive processes that degenerate with PD.

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Zarow C, Lyness SA, Mortimer JA, Chui HC (2003) Neuronal loss is greater in the locus coeruleus than nucleus basalis and substantia nigra in Alzheimer and Parkinson diseases. Arch Neurol 60:337-341.
Zigmond MJ (1997) Do compensatory processes underlie the preclinical phase of neurodegenerative disease? Insights from an animal model of parkinsonism. Neurobiol Dis 4:247-253.
Zigmond MJ, Acheson AL, Stachowiak MK, Stricker EM (1984) Neurochemical compensation after nigrostriatal bundle injury in an animal model of preclinical parkinsonism. Arch Neurol 41:856-861.
Zurkovsky L, Brown SL, Boyd SE, Fell JA, Korol DL (2007) Estrogen modulates learning in female rats by acting directly at distinct memory systems. Neuroscience 144:26-37.

Curriculum Vitae

Katherine L. Mitterling
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EDUCATION

Syracuse University
Biology master's degree student August 2012 – present. Expected graduation date June 2015.

Univeristy of Illinois Urbana-Champaign (UIUC)
Neuroscience Ph.D. program student August 2008 – August 2012.
Formal coursework: 61 credits. GPA 3.77

Bowdoin College in Brunswick, ME
A.B. in Neuroscience with honors May 2006

HONORS

INBRE Summer Research Fellowship, Bowdoin College, June-August 2005

SELECTED WORK AND RESEARCH EXPERIENCE

Estrogen, exercise, and Parkinson's disease research in rats
Graduate student, Syracuse University: Dr. Donna Korol (August 2012-present)
Graduate student, UIUC: Dr. Donna Korol (August 2008-August 2012)

- Performing research examining how estrogen and exercise affect learning, memory, and brain metabolism
- Assessing changes in cognitive and motor function in a toxin-induced model of Parkinson's disease in rats
- Techniques used: immunohistochemistry, light microscopy and densitometry, ovariectomy surgery, rat vaginal smear cytology, ELISAs, cannula surgery, histological enzyme assays, glycogen extraction and assays

Project NEURON (Novel Education for Understanding Research On Neuroscience)
Graduate research assistantship, Univ. Illinois Urbana-Champaign (January 2010-August 2011)

- Science Education Partnership Award (SEPA) funded project to develop high school science curricula based on neuroscience research currently performed at the University of Illinois Urbana-Champaign
- Skills gained: curriculum development, lesson plan writing, assessment item writing, translation of complex science techniques/ideas for grades 9-12 understanding, development of experiments & activities based on current science techniques/ideas for use in grade 9-12 classrooms

Estrogen and progesterone research in rats and mice
Research Assistant: Dr. Bruce McEwen, Rockefeller University, and Dr. Teresa Milner, Weill-Cornell Medical College (July 2006-June 2008)

- Perform immunocytochemistry experiments to detect changes in proteins and receptors at the light and electron microscopy levels.

- Techniques used: immunohistochemistry, light microscopy and densitometry, electron microscopy, rat and mouse vaginal smear cytology, ELISAs for estrogen levels

Differences in hippocampus and septum volumes in storing and non-storing birds across seasons
Honors thesis research: Dr. Seth Ramus, Bowdoin College (September 2005-May 2006) and Dr. Diane Lee, California State University Long Beach (December 2005, June 2006)

- Examined how species and season can affect hippocampus and septum volumes in a correlational study linking food-storing behavior and increased volume in areas of the brain that store spatial memory.
- Techniques used: wild trapping of birds in fall and spring, BrdU injections to detect neuronal birth, tissue slicing with Vibratome, histological staining for volumetric analysis

Episodic memory in the hippocampus: Can rats remember what, where, and when?
INBRE Summer Research Fellowship: Dr. Seth Ramus, Bowdoin College (June-August 2005)
Independent Study in Neuroscience: Dr. Seth Ramus, Bowdoin College (January-May 2005)
Laboratory in Behavioral Neuroscience: Learning & Memory, Bowdoin College (September-December 2004)

- Worked to design a "What, Where, When" task for rats to show that rats have episodic-like memory
- Techniques used: behavioral testing, surgical lesions, tissue slicing with Cryostat, histological staining, lesion analysis

The effects of vasotocin RNA interference on social approach behavior in the goldfish (*C. auratus*)
Laboratory in Behavioral Neuroscience: Social Behavior, Bowdoin College (Spring 2005)
Maine Biological & Medical Sciences Symposium (April 2005)

- Injection of vasotocin siRNA into the third ventricle of the brain increases social approach behavior in goldfish (*C. auratus*); however, *in situ* verification of mRNA silencing was unsuccessful.
- Techniques used: behavioral testing, *in situ* histochemistry, siRNA experiments, gene sequencing

Neuronal regeneration in the cricket: *in vitro* examination using cell cultures
Independent Study in Neuroscience, Bowdoin College (Spring 2004)

- Worked to create a cricket neuron cell culture protocol to use for *in vitro* experiments
- Techniques used: ganglia extraction surgeries, cell culture experiments

Effect of hypoxia and hypoglycemia on neuronal cell death *in vitro*
Summer Laboratory Assistant, University Connecticut Health Center (Summer 2002 and 2003)

- Examined effects of oxygen-glucose deprivation on neuronal and astrocytic cell cultures.
- Techniques used: PCR analysis of endogenous receptors, Western blot, cell culture techniques, use of anoxic chamber

PROFESSIONAL ACTIVITIES

Professional Development

- UIUC Neuroscience Program's Professional Development Program (Spring 2009)

Professional Affiliations

- Society for Neuroscience member (Fall 2005-present)
- Society for Behavioral Neuroendocrinology member (Spring 2010-present)

Outreach

- Brain Awareness
  - 2004-2006 at Bowdoin College; 2007 in New York City with Weill-Cornell Medical College
  - Oct 2008 at the Orpheum Children's Science Museum Halloween open house
  - Spring 2009, Spring 2010 and Spring 2012 with the UIUC Neuroscience Program
- Don Moyers Boys and Girls Club Neuroscience Fair: Spring 2011

MENTORING

Undergraduate students

- Katherine Anderson (Spring 2011 – Spring 2012) Honors thesis: Effects of estrogens and exercise on learning and memory
- Colin Therriault (Summer 2009 – Spring 2011) Senior thesis: Estrogens effects on pCREB/CREB levels 24 hours after injection of 17 β -estradiol.

High school students

- Sunjay Koshy (Summer 2011) UIUC iSTEM summer program

PUBLICATIONS

Published Abstracts

Mitterling KL, Anderson K, Korol DL (2012) The effects of exercise on learning and hippocampal succinate dehydrogenase histochemistry: Sex differences and the interaction of estradiol. Program #916.17. Abstracts of the 42nd Society for Neuroscience Annual Meeting.

Morris KA, Mitterling KL, Rocha Cabrero F, Gold PE, Korol DL (2012) Bilateral injection of 6-OHDA into the dorsolateral striatum improves spatial working memory in rats: implications for Parkinson's disease. Program # 56.03. Abstracts of the 42nd Society for Neuroscience Annual Meeting.

Mitterling KL, Allen A, Allen J, Blattner MS, Brown JW, Lauren H, Morrisette S, Ogrodnik JM, Planey J, Watson PDK, Zengin Bolatkale H, Korol DL, Hug B (2011) Do you see what I see? A novel secondary school curriculum for guiding explorations on the evolution of visual perception. Program #22.12. Abstracts of the 41st Society for Neuroscience Annual Meeting.

Blattner MS, Allen JR, Allen A, Brown J, Lauren H, Mitterling KL, Ogrodnik J, Planey J, Zengin Bolatkale H, Korol DL, Hug B (2011) From the classroom to the community: taking neuroscience into diverse community settings. Program #22.10. Abstracts of the 41st Society for Neuroscience Annual Meeting.

Brown, JW, Blattner MS, Mitterling KL, Morrisette S, Ogrogdnik JM, Watson PDK, Zengin H, Reese GC, Korol DL, Hug B (2011) The cutting edge: integrating contemporary neuroscience and molecular biology to teach about regeneration and the nervous system. Program #22.06. Abstracts of the 41st Society for Neuroscience Annual Meeting.

Mitterling KL, Korol DL (2010) Acute exposure to estradiol *in vivo* enhances CREB activation in the hippocampus. Program #296.14. Abstracts of the 40th Society for Neuroscience Annual Meeting.

Scavuzzo CJ, Mitterling KL, Korol DL (2009) Voluntary exercise enhances response learning in young adult male Sprague-Dawley rats: A role for BDNF. Program #97.16. Abstracts of the 39th Society for Neuroscience Annual Meeting.

Akama KT, Mitterling KL, Milner TA, McEwen BS (2008) PELP1 associates with PSD-95 and localizes to dendrites in hippocampal neurons. Program #279.8. Abstracts of the 38th Society for Neuroscience Annual Meeting.

Gardner RD, Law LM, Mitterling KL, Ramus SJ, Lee DW (2008) Cell proliferation in the septo-hippocampal pathway: Season, lesion, and species effects. Program #122.6. Abstracts of the 38th Society for Neuroscience Annual Meeting.

Gardner RD, Mitterling KL**,** Law LM, Ramus SJ, Lee DW (2008). Evidence for evolved adaptive specialization in the food-storing black-capped chickadee (Poecile atricapillus). Paper presented at the Southern California Animal Behavior Symposium, Long Beach, CA.

Mitterling KL, Law LM, Gardner RD, Ramus SJ, Lee DW (2007) Hippocampus and septum volumes show season, sex, and species differences in black-capped chickadees and dark-eyed juncos. Presentation #751.11. Abstracts of the 37th Society for Neuroscience Annual Meeting.

Waters EM, Mitterling K, Spencer JL, McEwen BS, Milner TA (2007) Estrogen receptor agonists, DPN and PPT, alter synaptophysin immunoreactivity in the hippocampus. Presentation #625.2. Abstracts of the 37th Society for Neuroscience Annual Meeting.

Peer Reviewed Articles

Milner TA, Drake CT, Waters EM, Torres-Reveron A, Graustein B, Mitterling KL, Frys K, Iadecola C (2008) Estrogen and progestin receptors are present in central autonomic regulatory areas and are differentially affected by chronic infusion of angiotensin in female rats. Exp Neurol 212(2):393-406.

Milner TA, Mitterling KL, Iadecola C, Waters EM (2008) Ultrastructural localization of extranuclear progestin receptors relative to C1 neurons in the rostral ventrolateral medulla. Neurosci Lett 431(2):167-72.

Waters EM, Mitterling K, Spencer JL, Mazid S, McEwen BS, Milner TA (2009) Estrogen receptor alpha and beta specific agonists regulate expression of synaptic proteins in rat hippocampus. Brain Research. 1290: 1-11.

Gardner RD, Allen TA, Mitterling KL, Law LM, Ramus S, Lee DW (2009) Cell proliferation in the septo-hippocampal pathway of food-storing and non-storing wild birds: Effects of species, season, and injury. Submitted for review 11/2/09 to Biology Letters.

Mitterling KL, Spencer JL, Dziedzic N, Shenoy S, McCarthy K, Waters EM, McEwen BS, Milner TA (2010). Ultrastructural localization of extranuclear progestin receptor, estrogen receptor-a, and estrogen receptor-b in female mice across the estrus cycle. J Comp Neurol. 518 (14) 2729-2743.

Williams TJ, Mitterling KL, Thompson LI, Torres-Reveron A, Waters EM, McEwen BS, Gore AC, Milner TA (2010) Age- and hormone-regulation of opioid peptides and synaptic proteins in the rat dorsal hippocampal formation. Brain Res 1379: 71-85.

Mitterling KL, Korol DL (in prep) Effects of different estradiol injection protocols on CREB phosphorylation in the CA1 and CA3 of the dorsal hippocampus.

Morris KA, Mitterling KL, Rocha-Cabrero F, Gold PE, Korol DL (in prep) Bilateral intrastriatal infusions of 6-OHDA improve spatial working memory in rats: implications for Parkinson's disease.