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Exercise-induced neuroprotection in a hemiparkinsonian 6-hydroxydopamine rat model

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EXERCISE-INDUCED NEUROPROTECTION IN A HEMIPARKINSONIAN

6-HYDROXYDOPAMINE RAT MODEL

By

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ABSTRACT

Exercise-induced neuroprotection in a hemiparkinsonian 6-hydroxydopamine rat model

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Outside of finding a cure, one of the preeminent goals of research in Parkinson's disease (PD) is finding a neuroprotective treatment that when applied prior to the onset of the disease will decrease the risk and severity of the subsequent disease. One such treatment that has potential as a neuroprotective agent in PD is exercise. Several studies have found forced exercise to be protective of Parkinson's disease in adult rodent models; however, few of these studies have used a design wherein voluntary exercise was evaluated. Moreover, no study has used a true neuroprotective design in which exercise was applied prior to disease onset and throughout the life of the animal. Therefore, the focus of this dissertation was to further explore the role of a preconditioning of exercise, both forced and voluntary, in a 6-hydroxydopamine-induced hemiparkinsonian rat model. The research approach adopted in this dissertation included a randomized control design of forced and voluntary exercise (i.e., treadmill and running wheel) applied before and after neurotoxin-induced hemiparkinsonism. Motor behavior tests were used to assess the outcomes. The findings from this dissertation provide evidence that exercise, either forced or

voluntary, does not provide any neuroprotective benefit in terms of protection from Parkinsonian motor signs and forelimb asymmetry. The collective results of this dissertation cast doubt on the current body of the literature on exercise neuroprotection in PD and suggest that the notion that exercise is neuroprotective is premature.

Keywords: voluntary, forced, exercise, neuroplasticity, Parkinson's disease, dopamine, stress

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CHAPTER 1

Parkinson's disease

Parkinson's disease (PD), named after the first clinical description by James Parkinson in 1817, is the most common movement disorder and the second most common neurodegenerative disease after Alzheimer's disease (Fahn & Sulzer, 2004). The age-adjusted annual incidence in the US between the ages of 50 and 99 is 114.7 cases per 100,000 and steeply increases as one ages (Bower et al., 1999). Since PD is more common with increasing age, its prevalence is expected to triple over the next 50 years due to the aging US population.

PD manifests typically in the sixth decade of life and begins with relatively modest limitations that progressively cause disability over the next few decades. PD is characterized by four slowly progressive cardinal signs: bradykinesia (slowness of movement), rigidity (stiffness), resting tremor, and postural instability (balance impairment). The cumulative effects of these impairments are often very troubling and can lead to considerable disability. While the motor deficits associated with PD are not usually directly life threatening, they contribute to falling which in turn can lead to severe injuries (e.g., head injuries, fractures, etc.) and hospitalization. Eventually those with PD may be confined to a wheelchair and may socially isolate themselves from their regular activities, which stimulates a vicious cycle of decreased physical conditioning and subsequent increased potential for aspiration pneumonia and falling, which are the most common causes of mortality in PD. Not surprisingly, patients with PD have an

increased risk of death as compared to age and gender matched persons (Uitti et al., 1993). Studies have demonstrated a two to three-fold risk for death in PD as compared to those without PD, which corresponded to an 8 year decrease in life expectancy (Morens et al., 1996).

Treatment has not been directed at the causative agent of PD because it is unknown. Therefore, most treatment has focused on ameliorating the symptoms of the PD. Unfortunately, these treatment strategies do not stop the progressive degenerative nature of the disease. Treatment is typically classified into three main categories: nonpharmacologic, pharmacologic, and surgical. There is no single approach to the management of PD; however, the most acceptable approach is pharmacologic therapy using one or more of the following classes of medications: dopamine precursors (levodopa), dopamine agonists, anticholinergics, catechol-O-methyl transferase inhibitors (blocks dopamine metabolism), monoamine oxidase B inhibitors (blocks central dopamine metabolism), and amantadine.

The most effective pharmacologic treatment for PD, levodopa, has a limited period of effectiveness (7-12 years) and is associated with irreversible dyskinesias and motor fluctuations (Poewe et al., 1986; Schrag & Quinn, 2000). Moreover, it is theorized that its oxidative metabolites may cause free radical formation which theoretically potentiate the neurodegeneration (Galvin, 2006; Hastings & Zigmond, 1997; J. Xu et al., 2002; Yu et al., 2005). Because of these reasons, it is generally not given as a first line therapy even though it is the most effective at reducing the symptoms. Most patients typically begin with dopamine agonists and usually one or more of the other

medications as adjuncts. Because of the monoamine oxidase B inhibitor Deprenyl has demonstrated a neuroprotective delay of disease onset by 9 months, it has become an attractive neuroprotective agent (LeWitt, 1991). Unfortunately, no long term positive effects have been demonstrated so the neuroprotective ability of the drug has been a source of contention in the literature (Shoulson, 1998).

Aside from the continual search for the neuropathologic etiology of PD and treatment efficacy studies, attention on neuroprotective strategies in PD has gained considerable traction (Biglan & Ravina, 2007; Fahn & Sulzer, 2004; Hauser & Zesiewicz, 2006; Kieburtz, 2006; LeWitt, 2004, 2006; Poewe, 2006; Simpkins & Jankovic, 2003) with as many as 59 potential neuroprotective therapies in various stages of investigation (Ravina et al., 2003). Some of these putative neuroprotective strategies range from pharmacologic agents, like pramipexole (Joyce et al., 2004) and Ropinirole (Whone et al., 2003), to behavioral manipulations like smoking (Papapetropoulos et al., 2005) and dietary restriction (Mattson et al., 2004). It is hoped that these neuroprotective strategies will eventually either prevent the disease for those at risk, increase the age of disease onset for those at risk, decrease the severity of disease at onset, and/or slow the rate of disease progression once the disease has been acquired.

Etiopathogenesis

PD is characterized by the progressive loss of dopamine producing cells in the substantia nigra of the midbrain. These dopaminergic neurons project to the basal ganglia where they release their dopamine into the striatum of the basal ganglia. This is

commonly referred to as the nigrostriatal pathway. It is the paucity of dopamine in the striatum secondary to nigrostriatal attrition that causes the imbalance of motor facilitation and inhibition resulting in the characteristic features of the disease. Despite considerable research, it is not known what the etiological trigger of the disease is; hence, it is commonly referred to as “idiopathic.”

The current etiological theory is that an unknown environmental toxin or toxins act on genetically susceptible individuals to cause the disease. Unfortunately, the environmental toxin or toxins remain elusive. In addition, there are only a small minority of cases that have a known genetic cause. Currently, there are thirteen different familial types of PD (Table 1) representing a small minority of the cases of PD (Abeliovich & Flint Beal, 2006). While the link between the environmental toxin and the genetic susceptibility lacks direct evidence, it represents the leading etiological theory for the disease.

Table 1. Known genetic causes of PD (Biskup et al., 2008; Gasser, 2005, 2007).

Locus	Inheritance	Gene	Gene product	Map position	Comments
PARK1	Dominant	SNCA	α -synuclein	4q21	First identified, 3 point mutations have been found
PARK2	Recessive	Parkin	465 RING finger protein, a ubiquitin E3 ligase	6q25	Most common cause of early onset PD (18%)
PARK3	Dominant	SPR?	?	2p13	
PARK4	Dominant	α -synuclein	α -synuclein triplications duplications	4q21	Excessive α -synuclein
PARK5	Dominant	UCH L1	Ubiquitin C-terminal hydroxylase L1	4p14	
PARK6	Recessive	PINK1	PTEN-induced putative kinase 1	1p35-37	Second most common cause of early onset PD
PARK7	Recessive	DJ-1	DJ-1	1p38	DJ-1 knockdown leads to oxidative stress susceptibility
PARK8	Dominant	LRRK2 (leucine rich repeat kinase 2)	Dardarin	12 cen	Most common genetic cause of PD (1-6% of US and Euro PD studies; 29.7% of PD in Ashkenazi Jews)
PARK9	AR	ATP13A2	?	1p36	
PARK10	Dominant?	?	?	1p32	
PARK11	Dominant?	GIGYF2?	?	2q34	
PARK12	X-linked	?	?	Zq21	
PARK13	Dominant?	OMI/HTRA2	Serine protease	2p12	

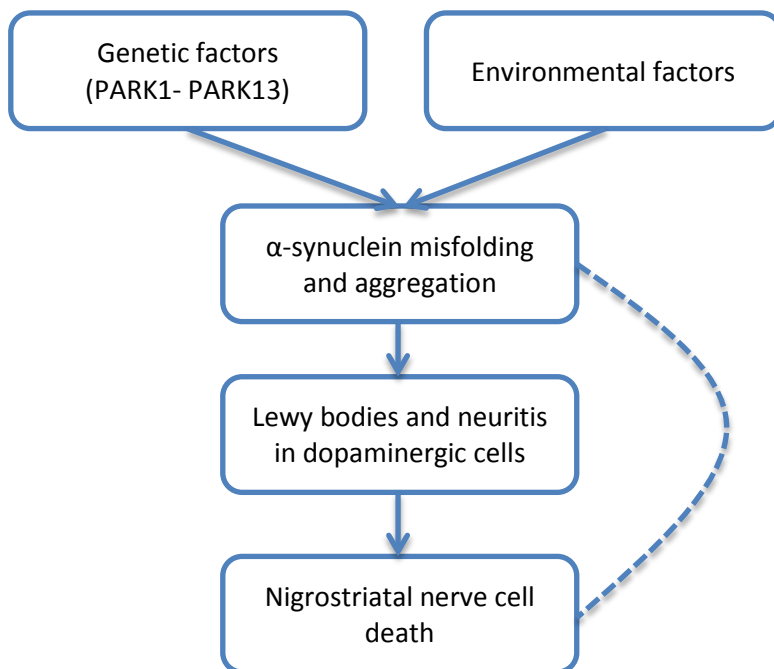
The progressive loss of the dopaminergic nigrostriatal neurons in PD underlies the disease pathogenesis. What triggers this progressive neurodegeneration is not currently known. However, research suggests that α -synuclein, an intrinsically unstructured, presynaptic terminal protein, plays a prominent role (Eriksen et al., 2003; Goedert, 2001). α -synuclein binds to fatty acids and is thought to play a role as a molecular chaperone and as a regulator of dopaminergic synaptic vesicles (Dev et al., 2003). α -synuclein is also theorized to play a role in the filling and refilling of dopaminergic synaptic vesicles (Abeliovich et al., 2000) and in support of this notion has been shown to be closely associated with synaptic vesicles through immunohistochemistry (Clayton & George, 1999). Interestingly, absence or inactivation of α -synuclein in mice models produces only mild, benign behavioral changes (Dev et al., 2003; Goedert, 2001).

Aggregations of α -synuclein are found in cytoplasmic inclusions of affected nigrostriatal neurons in those with PD; these inclusions are called Lewy bodies and Lewy neurites. These Lewy bodies and neuritis are considered the pathological hallmark of the disease (Figure 1). The presence of these inclusion bodies in nigrostriatal neuron soma (Lewy bodies) and axons/dendrites (Lewy neuritis) is highly characteristic of the disease. There are other diseases that also have Lewy body inclusions (e.g., diffuse Lewy body disease, dementia with Lewy bodies); however, these are found in different brain regions.

Lewy bodies and Lewy neurites contain high quantities of misfolded and aggregated α -synuclein, a molecular chaperone, and another protein, ubiquitin, a

marker for protein degradation. In normal states, there is a balance between misfolded proteins and destruction/repair via the ubiquitin-proteosomal system. In PD, it is theorized that increased rates of misfolded α -synuclein and/or an inefficient ubiquitin-proteosomal system are possible mechanisms for the abnormal aggregation and development of Lewy inclusion bodies. Unfortunately, there is no direct evidence on what triggers the high rate of α -synuclein aggregation.

Figure 1. Model of disease pathway in PD. Adapted from Goedert (Goedert, 2001).



It is also not known whether these Lewy structures are an adaptive or maladaptive cellular response or even if they are the cause of nigrostriatal cell attrition. Some studies have demonstrated that it is not the Lewy inclusion bodies that are the problem but rather it is the constituents of these inclusions (i.e., intermediate forms of α -synuclein) that are the culprits. Whatever the case, all current evidence points to a

disease process that consists of a combination of genetic and environmental factors that triggers a cascade of events (likely via α -synuclein dysfunction and Lewy body formation) that ultimately results in the loss of dopamine producing nigrostriatal neurons.

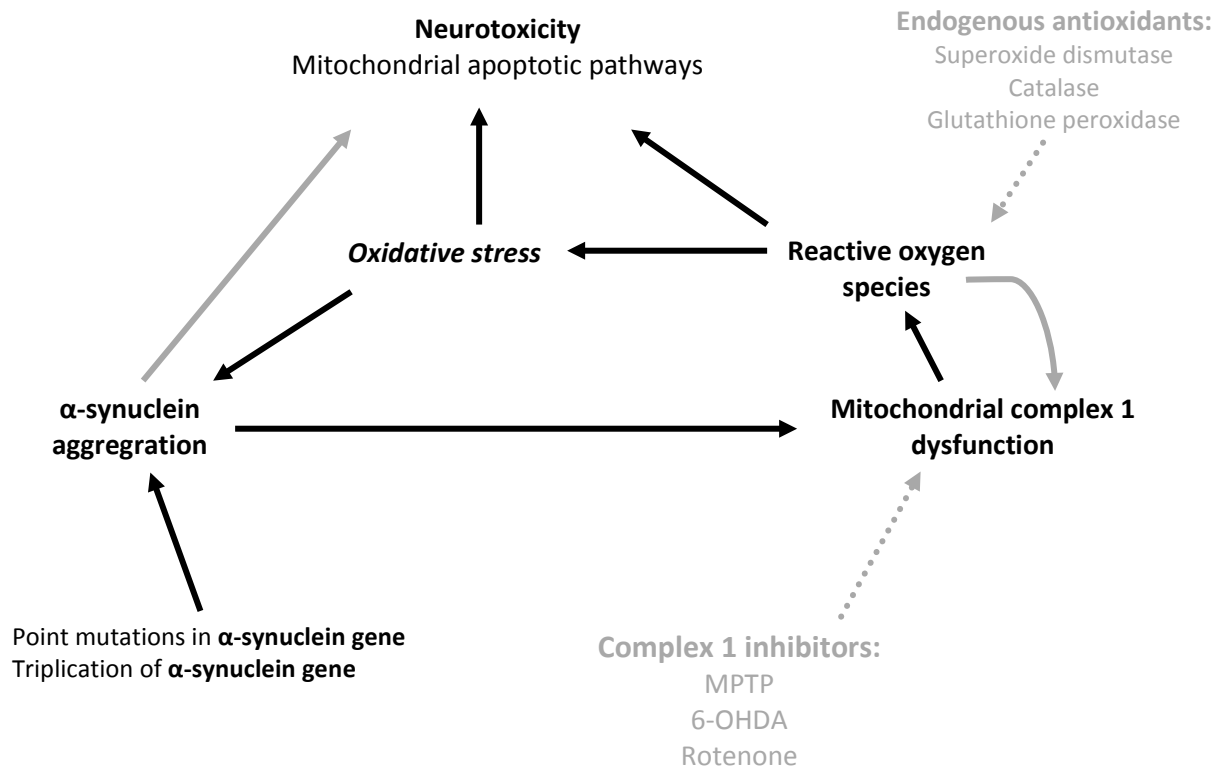
Mitochondrial dysfunction in PD

Some studies have demonstrated that α -synuclein promotes mitochondrial alterations that may result in oxidative stress and subsequent cell death (Hsu et al., 2000). Others have shown that intermediate, oligomeric forms (i.e. misfolded) of α -synuclein are toxic to the cells and, therefore, underlie the death of these nigrostriatal neurons by inhibiting complex 1 mitochondrial respiratory function (Devi et al., 2008). Mitochondrial dysfunction is thought to be central to the pathogenesis of PD and is typically complex 1 related (i.e., nicotinamide adenine dinucleotide phosphate (NADH):ubiquinone oxidoreductase) (Emerit et al., 2004; Mounsey & Teismann, 2010).

Complex 1 respiratory dysfunction, in turn, increases the release of reactive oxygen species (ROS) (e.g., hydrogen peroxide and superoxide radicals), thereby increasing the oxidative stress on these cells. Several studies have demonstrated that cellular oxidative stress plays a prominent role in cellular destruction in PD, likely by increasing cytosolic cytochrome C and subsequently initiating caspase-mediated apoptotic signaling pathways (Mounsey & Teismann, 2010; Perier et al., 2007). In addition, defects in complex 1 respiration lower the threshold for mitochondrial-dependent apoptosis, a crucial event in nigrostriatal degeneration (Perier et al., 2005).

Increasing oxidative stress, in turn, can increase the susceptibility of α -synuclein to misfolding (S. Xu et al., 2006). Thus, a vicious cycle of oxidative stress, α -synuclein misfolding and subsequent α -synuclein aggregation, complex 1 mitochondrial dysfunction, and nigrostriatal cell death is proposed (Figure 2). This vicious cycle implicates any potential endogenous or exogenous complex 1 inhibitor as a potential contributor to the cascade of events that leads to cell death. In addition, it suggests that any endogenous or exogenous agent that can reduce reactive oxygen species or oxidative stress may afford neuroprotection.

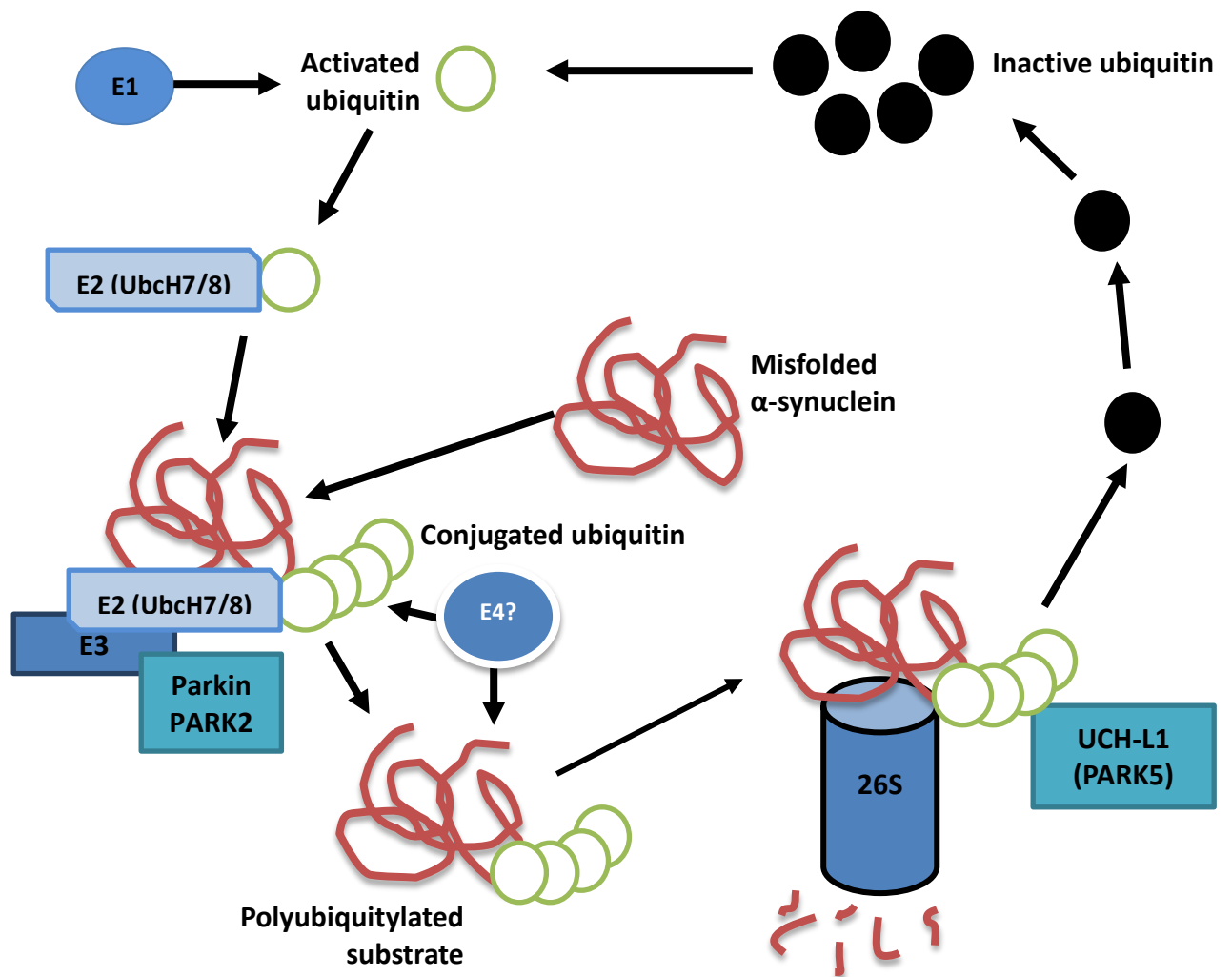
Figure 2. A vicious cycle of dopaminergic cell death in PD.



It is not known what initiates this proposed cycle in PD or whether mitochondrial dysfunction precedes α -synuclein aggregation or vice versa. However, there is no doubt that they are intrinsically linked. It is logical to consider that increased dysfunction at any of the steps in the vicious cycle could trigger or potentiate this cycle. ROS like superoxide radicals and hydrogen peroxide are the normal byproducts of mitochondrial respiration and they are typically controlled by a range of antioxidants (e.g., superoxide dismutase, glutathione peroxidase, catalase); however, in certain pathological states, like PD, the amount of ROS produced swamps the antioxidant protection mechanisms. In addition, there is considerable evidence that neuroinflammatory processes may also play a role by increasing oxidative stress (E. C. Hirsch & Hunot, 2009).

Point mutations in the gene encoding α -synuclein (i.e., *PARK1*) or abnormally high levels of α -synuclein caused by *PARK4* overexpression may trigger the development of PD; either of these mechanisms would be sufficient to increase the rate of misfolding which would then presumably overwhelm the removal capacity of the ubiquitin-proteosomal system (Figure 3). However, only a minority of individuals with PD have these genetic characteristics. In the cases of unknown cause or idiopathic PD, there are a few possibilities: 1. complex Mendelian inheritance has hindered genetic links (i.e., complex inheritance patterns would be incomplete dominance, co-dominance, polygenic traits); 2. undiscovered environmental triggers have remained elusive; and, 3. a combination of the previous two.

Figure 3. Ubiquitin-proteosomal removal of misfolded α -synuclein. Adapted from Pahwa (Pahwa, Lyons, & Koller, 2003).



Environmental toxins have become the target of considerable research in PD because they are able to produce oxidative stress, a prominent factor in PD, in animal models of the disease (Friedrich, 2005). Environmental toxins, like 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 6-hydroxydopamine (6-OHDA), and rotenone cause Parkinson-like symptoms in primates and rodents; these neurotoxins are also all

complex 1 inhibitors (Mounsey & Teismann, 2010; Perier et al., 2005). Additionally, rotenone can produce inclusion bodies with similar characteristics as the human Lewy inclusion body (Betarbet et al., 2000). Moreover, these inclusions are immunoreactive to α -synuclein. Thus, these neurotoxins may enter the cycle downstream or as the initial precipitating factor of the disease (depending on your frame of reference) of the α -synuclein aggregation and may eventually cause α -synuclein aggregation by increasing cellular oxidative stress (Figure 2). In support of this notion is indirect preliminary evidence that rotenone may cause accumulation of α -synuclein *in vivo* and *in vitro* (Betarbet et al., 2006).

Theoretically, any intervention that can slow or stop any of the elements/steps of this proposed vicious neurodegenerative cycle in PD would logically decrease the cascade of events that leads to the death of these dopaminergic neurons. Not surprisingly, anti-oxidant therapies, used to decrease the levels of reactive oxygen species, have been a PD research focus for many years (LeWitt, 2006; Mounsey & Teismann, 2010). While no definitive conclusions for any such therapy have been reached, there has been some progress and some attractive therapies including the following (Mounsey & Teismann, 2010): Coenzyme Q10 (ubiquinone), creatine, SS peptides, green tea polyphenols, mulberry wood polyphenol (oxyresveratrol), uric acid, *Cyperus rotundus* rhizome (Cyperus rhizome), *Uncaria rhynchophylla*, Mito-Q10 (Ghosh et al., 2010). Another promising antioxidant therapy for PD is exercise.

Exercise in rodent models of PD

Recent rodent research on PD has indicated that exercise may offer robust neuroprotection to the dopaminergic nigrostriatal neurons after the onset of toxin-induced PD. In these studies, exercise not only significantly improved motor function, but also had a positive impact on the underlying neurochemical disorder of PD (i.e., loss of nigrostriatal dopamine) (Cohen et al., 2003; Howells et al., 2005; Tillerson et al., 2003; Tillerson et al., 2001). Tillerson et al (2001) demonstrated that forced exercise of rats with PD, induced by 6-hydroxydopamine (6-OHDA), significantly improved motor function (Tillerson et al., 2001). Moreover, this motor improvement was coupled with significant sparing of the concentration of striatal dopamine and its metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). In addition, a relatively high concentration of a protein responsible for sequestering dopamine in its synaptic vesicles, vesicular monoamine transporter (VMAT2), was consistent with the high concentrations of dopamine and its metabolites. VMAT2 levels are considered to be a highly reliable marker of the integrity of dopamine terminals (Kilbourn et al., 1993; Kilbourn et al., 1996; Miller et al., 1999a; Miller et al., 1999b). In a similar study, Tillerson et al (2003) demonstrated similar findings using a forced treadmill running protocol on a 6-OHDA rat model and a MPTP mouse model (Tillerson et al., 2003). Again, treadmill exercise improved motor function and increased levels of dopamine, DOPAC, HVA and VMAT2. In addition, levels of several presynaptic proteins of dopaminergic neurons were also improved by exercise, including dopamine transporter (DAT) and tyrosine hydroxylase (TH), which is the rate limiting enzyme in dopamine

biosynthesis. Work by Poulton and Muir (2005) and Howells et al (2005) confirmed previous findings that exercise in the 6-OHDA rat model is neuroprotective (Howells et al., 2005; Poulton & Muir, 2005). Collectively, these findings suggest that exercise may have a direct protective effect on the neurochemical pathophysiology of PD rather than simply ameliorating its signs and symptoms through improved musculoskeletal fitness.

Exercise may not only be beneficial in the disease state of PD (i.e., after disease onset), but may also be protective to dopaminergic neurons and the development of PD. Cohen et al (2003) demonstrated that exercise prior to toxin-induced PD (6-OHDA) in rats produced profound neuroprotection of nigrostriatal neurons (Cohen et al., 2003). In addition, exercised rats, in contrast to control rats, had less in the way of motor deficits that are characteristic of this PD model. Thus, forced exercise prior to 6-OHDA lesioning resulted in a milder PD symptom profile compared to rats that were not forced to exercise. Three conclusions can be drawn from these results: 1. forced exercise prevents the development of behavior deficits characteristic of the 6-OHDA lesion model; 2. forced exercise attenuates the loss of striatal dopamine; and, 3. forced exercise increases striatal glial-derived neurotrophic factor (GDNF). This last finding was important because it provided evidence that GDNF may be an underlying neuroprotective mechanism for dopaminergic cells from 6-OHDA insult. This is consistent with evidence from other studies which have demonstrated GDNF as a potent protector of dopaminergic neurons (Akerud et al., 1999; Choi-Lundberg et al., 1998; Gong et al., 1999; Hoffer et al., 1994; Jaumotte & Zigmond, 2005; Kearns & Gash, 1995; Kramer et al., 1999; Lin et al., 1993; Schatz et al., 1999). GDNF is thought to

protect neurons by acting as a neurotrophic factor in providing nourishment to neurons. Unfortunately, there are big gaps in how GDNF accomplishes the protection. Moreover, there is no other evidence in the literature about the effect of exercise on the actual etiological processes of the disease (e.g., oxidative stress). Results from the aforementioned studies certainly warrant further investigation into the physiologic processes at the cellular level.

Enriched environments, which have a voluntary exercise component to go along with social interaction and learning, have also been shown to improve motor function and to resist MPTP insult in mice PD models. These studies have demonstrated decreased loss of striatal dopamine and dopamine-related transporters (i.e., DAT and VMAT2) (Bezard et al., 2003; Faherty et al., 2005; Jadavji et al., 2006). In addition, Faherty et al (2005) also demonstrated a concomitant upregulation of GDNF (Faherty et al., 2005). Interestingly, social rats (i.e., housed with several other rats) without a running wheel also exhibited good improvement.

Enriched environments (Bezard et al., 2003) and exercise (Neeper et al., 1995; Russo-Neustadt et al., 2000; Widenfalk et al., 1999) have also been shown to significantly increase another possible neuroprotective agent, brain-derived neurotrophic factor (BDNF). In many other studies, neuronal growth factors, such as GDNF and BDNF, are capable of protecting cells against oxidative stress (Williams, 1995), which has been consistently documented as a potential mechanism behind protein misfolding and dopaminergic cell depletion in PD.

Forced inactivity (i.e., restricted movement) prior to or after neurotoxin-induced PD has resulted in an exacerbation in motor function deficits and neurochemical loss (Tillerson et al., 2002b). Forced inactivity prior to a mild dose of 6-OHDA, which would not normally produce behavioral or neurochemical deficits, not only led to significant behavioral deficits but also resulted in an exacerbation of striatal dopamine, DOPAC and HVA loss as well as significant decreases in DAT, VMAT2, and TH. Thus, by forcing a rat to be inactive (i.e., restricted movement) it causes an appearance of more severe PD symptoms with an associated worsening of nigrostriatal neurochemical function. In the control rats (no movement restriction and same mild 6-OHDA dose) there were no observable PD symptoms and nigrostriatal neurochemistry was not distinguishable from rats that had had a sham neurotoxin. Thus, forced inactivity turns a very mild 6-OHDA lesion, which produces no PD symptoms and does not affect nigrostriatal chemistry, into a symptom and PD profile that is consistent with a much larger lesion.

Following severe 6-OHDA exposure and forced recovery, forced inactivity caused a return of motor behavior deficits and a decrease in dopamine, DOPAC, HVA, DAT, VMAT2, and TH (Tillerson et al., 2002b). The implication of these findings is that exercise salvages these dopamine cells, but the neuroprotection is fragile and needs to be maintained by regular exercise. Recently, Caudel et al (2007) demonstrated that decreased physical activity or movement during the process of nigrostriatal damage is not merely a symptom of dopamine loss but may contribute to an exacerbation in neurochemical and behavioral asymmetry in MPTP mice (Caudle et al., 2007). Some authors have reported that an overall decrease in physical activity precedes the onset of

the cardinal symptoms in human PD (Comella et al., 1994; Fertl et al., 1993; Mazzoni & Ford, 1999; Toth et al., 1997). This led Tillerson et al (2002) to hypothesize that decreased physical activity is not only a symptom of PD, but perhaps a catalyst in the neurodegenerative process of PD (Tillerson et al., 2002b).

There is not a perfect animal model for PD (Ceravolo et al., 2006; Lang, 2006). There are some issues with the sudden onset of PD symptoms produced by neurotoxins in rat and mouse models. In human PD, the neurodegenerative process is comparatively slow and progressive, whereas, in the toxic rodent model the neurodegeneration is acute and sudden (Galati & Di Giovanni, 2010; Lang, 2006). Also, neurodevelopment, plasticity, life span and degeneration are also on different trajectories in the rodent models compared to human PD. However, the MPTP (mouse and primate) and 6-OHDA (rat) models of PD are the most commonly used models of PD and have shed some important light on the many different putative neuroprotective therapies. Translating these neuroprotective therapies into human clinical research has not yet proved fruitful at this time. Therefore, clinicians and researchers should cautiously generalize the aforementioned animal findings on exercise to human PD. However, it should be remembered that rodent models of PD are of merit because they offer important direct data on the shared neurobiology of the nigrostriatal pathways that are affected in PD. This cannot be done to the same extent in human PD.

Exercise in non-pathologic rodent models that relates to PD

Exercise causes a plethora of benefits in rodent models, some of which are relevant to PD. Exercise has been shown to improve performance in learning and memory tasks in rat models (Radak et al., 2001) and enhance brain plasticity (Cotman & Berchtold, 2002; Cotman & Engesser-Cesar, 2002). It has been shown to increase serum calcium levels which is then transported to the brain where it stimulates dopamine through a calcium/calmodulin-dependent system (Sutoo & Akiyama, 2003). Exercise is also associated with Hsp70 induction in the muscles of treadmill running rats (Noble et al., 2006).

Exercise has been shown to increase levels of antioxidant enzymes in rodent muscle (Ji, 1993; Powers et al., 1993) and brain (Liu et al., 2000; Somani et al., 1995; Teixeira et al., 2008). However, none of these researchers investigated the antioxidant levels in the substantia nigra where nigrostriatal cells degenerate in PD. Therefore, the next logical step would be to determine if, in fact, the antioxidant levels would increase as a result of exercise in a brain region immediately relevant to the neurodegeneration of PD. Linking an increase in exercise-induced antioxidants to the proposed vicious cycle of dopaminergic cell death in PD would be an important neurobiological finding for PD and other neurodegenerative diseases that have similar molecular neuropathology (i.e., Alzheimer's disease). Such a link would also provide an attractive, evidenced-based rationale for ameliorating the underlying disease course in PD.

Exercise in human PD

At present, there is no direct evidence that exercise is neuroprotective in human PD. However, there is some downstream evidence of the neuroprotective potential of exercise in human PD. First, physical exercise has been shown to be beneficial in persons with PD (Crizzle & Newhouse, 2006; de Goede et al., 2001; Suchowersky et al., 2006b). This benefit is mostly in the form of motor function improvements. In addition, recent epidemiologic research suggests that exercise may offer some protection against PD. Based on their study of 48,574 men and 77,254 women, Chen et al (2005) reported that physical activity or strenuous exercise in early adult life was inversely related to PD risk in men (Chen et al., 2005). Strenuous exercise was associated with a 60% lower PD risk in men. It was also found that men with PD were consistently less physically active than men without PD up to 12 years prior to diagnosis. However, this inverse relationship was not demonstrated with statistical significance ($p = 0.06$) in women. Sasco et al (1992) also reported a slight protective effect of moderate to heavy sports participation in adulthood on the risk for PD in a study of 50,002 men (Sasco et al., 1992). It has also been reported that in young onset PD "...early exposure to well water drinking and head trauma may trigger and expedite the appearance of PD features, but such acceleration may be prevented through regular exercise" (Tsai et al., 2002). Taken together, these studies seem to support the data from rodent PD models of exercise in that exercise may offer neuroprotection to relevant pathways in PD.

Summary

Parkinson's disease is a common neurodegenerative that causes a gradual but profound loss of functioning in the life of those affected. Treatment is mostly palliative and does little to disrupt the degenerative process. In lieu of the elusive cure, some researchers have focused their attention on discovery of neuroprotective treatments that can affect the underlying disease course by preventing the onset of the disease or by slowing it down. However, discovering neuroprotection in PD has proven difficult and at present there has been little success (Antonini, 2011; Biglan & Ravina, 2007; Galati & Di Giovanni, 2010; Hauser & Zesiewicz, 2006; Kieburtz, 2006, 2010; Lang, 2006; LeWitt, 2006; Poewe, 2006). So far, no treatment has been shown to significantly slow the progression of the disease (Suchowersky et al., 2006a). However, it remains an important goal in PD research and warrants investigation.

In this dissertation, the overarching goal that guided the designs was to verify the role exercise as a viable and valid neuroprotective agent in a toxin-induced hemiparkinsonian rat model. To accomplish this goal, three different aims were analyzed using comparative-control designs. The first aim (Chapter 2) addressed the role of 4 weeks of forced exercise and no exercise in rats after a toxin-induced hemiparkinsonian. The intent of this aim and subsequent design was to determine if exercise prevented motor asymmetry after experimental lesioning. The second aim (Chapter 3) involved a 4-week preconditioning of exercise prior to induction of hemiparkinsonism. The hypothesis of this aim was that exercise prior to toxin-induced hemiparkinsonism would prevent motor asymmetry relative to no exercise. The third

aim (Chapter 4) was to compare 6 weeks of forced and voluntary exercise compared to a control group and a sham lesioned group. This design was cast to determine if exercise was protective against behavioral motor asymmetry; however, more importantly, it was cast to determine if a stressful running condition (forced exercise) was more protective than a non-stressed running condition (voluntary exercise).

CHAPTER 2

Forced exercise after induction of 6-OHDA-mediated nigrostriatal insult: neuroplasticity in an adult hemiparkinsonian rat model

Abstract: Forced exercise has been shown to ameliorate behavioral symptoms in an adult rat model of Parkinson's disease (PD). Importantly, forced exercise has been shown to increase dopamine and nerve growth factors; this suggests that it might affect the underlying disease course. However, there are a few studies that have found mixed results in the rodent PD model after PD induction with neurotoxin. Therefore, the purpose of this study was to explore the role of a forced exercise after toxin-induced PD in rats in decreasing behavioral asymmetry. Hemiparkinsonian symptoms were induced using a unilateral injection of 6-OHDA in the right medial forebrain bundle. Rats were then randomized into two 4-week experimental conditions, an exercise condition and a non-exercise control condition. Parkinsonian behavioral tests (i.e., apomorphine rotations, forelimb placement asymmetry, exploratory rearing) did not show any significant differences over time between the two groups. These results are not consistent with previous research that has found a positive treatment effect with exercise and cast doubt on the claims that exercise after neurotoxin induced-PD recovers hemiparkinsonian symptoms.

INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disease due to attrition of dopaminergic nigrostriatal fibers. It characteristically produces the following cardinal signs: bradykinesia, resting tremor, postural instability and rigidity. Many of the behavioral and neurochemical alterations in PD can be modeled in rats by using a unilateral infusion of the dopaminergic neurotoxin 6-hydroxydopamine (6-OHDA) into the nigrostriatal fibers. In this rat hemiparkinsonian model, the unilateral infusion of 6-OHDA will cause an asymmetry of movement (hemiparkinsonism) in the rat forelimb that can be used to determine the severity of parkinsonian symptoms. Importantly, 6-OHDA animals with severe destruction of the nigrostriatal system (>95%) will not display any behavioral recovery; however, a less severe lesion (>80%) will demonstrate some behavioral recovery-associated neuroadaptation (e.g., increased dopamine synthesis) in the remaining dopaminergic neurons (Tillerson & Miller, 2002).

Decreased physical activity during the degenerative process has been shown to exacerbate behavioral and neurochemical loss in unilateral parkinsonian rats. Tillerson et al (2002) found that forced-nonuse, which is akin to decreased activity, during the first 7 days post-lesion caused a marked increase in impairment compared to rats that were not restrained (Tillerson et al., 2002b). While this is not the same as implementing exercise after neurotoxin-induced hemiparkinsonism, it does highlight the fact that there may be a dose dependent relationship to physical activity during the neurodegenerative process of the disease.

Much like animal models of cortical injury, forced exercise in the unilateral 6-OHDA rat model has been shown to decrease the behavioral and neurochemical deficits associated with the disease. Tillerson et al (2001) found that moderate exercise initiated soon after unilateral insult resulted in an attenuation of dopaminergic loss and a sparing of behavioral impairment (Tillerson et al., 2001). The characteristic limb-use asymmetry was absent in rats that were forced to run, whereas those rats that were in the sedentary control maintained a marked limb-use asymmetry throughout the course of the experiment. In another study by Tillerson et al, exercise following nigrostriatal damage reduced motor symptoms and increased dopamine and its metabolites in the 6-OHDA hemiparkinsonian model (Tillerson et al., 2003). Tajiri et al (2010) found that forced exercise initiated immediately after a 6-OHDA lesion was better than sedentary rats in terms of motor recovery; using bromodeoxyuridine (BrdU) to label proliferating cells, they demonstrated that exercised rats had a significant preservation of tyrosine hydroxylase positive cells in the striatum and substantia nigra (Tajiri et al., 2010). Exercise has also driven similar findings in the MPTP (1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine) mice model of PD (Fisher et al., 2004).

While previous studies have shown improvements in the motor recovery of the Parkinsonian rat by forcing them to exercise, a more important finding was that the exercise improved the levels of dopamine in relevant brain areas. This suggests that forced exercise may actually affect the underlying disease course by preserving dopaminergic neurons. While this provides some indirect information that exercise may mitigate the damage to dopaminergic neurons, there is no direct evidence to explain

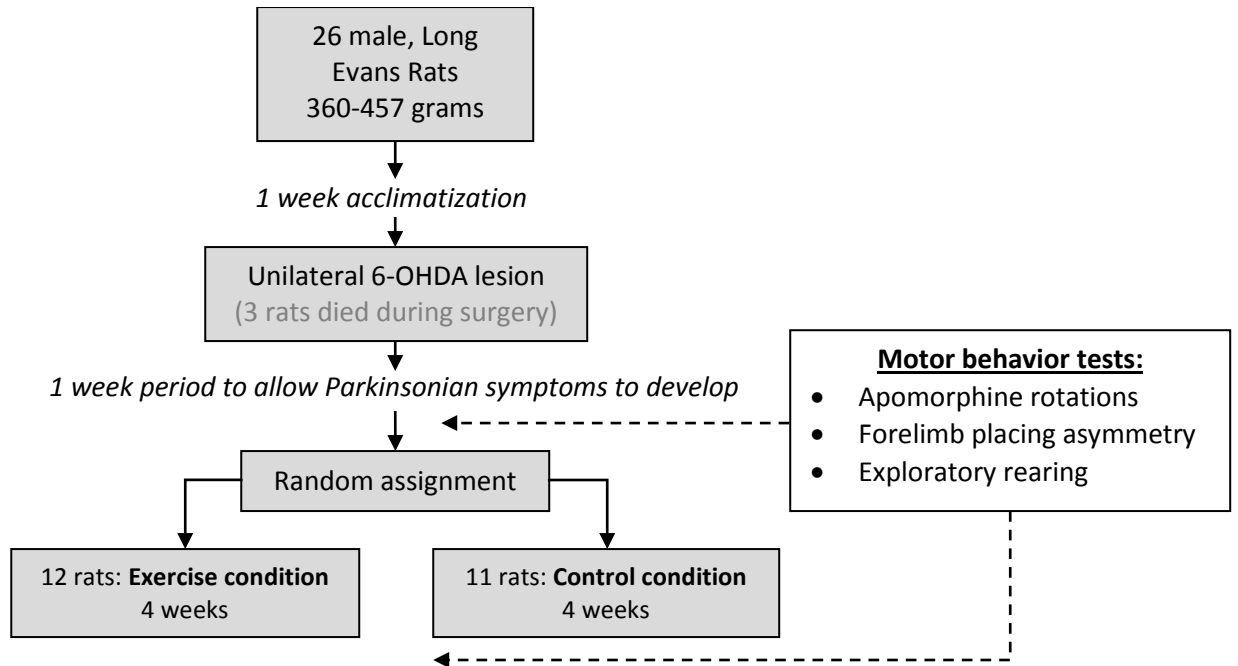
why these dopaminergic neurons are preserved and, importantly, what influence forced exercise has on the underlying molecular causes of dopaminergic cell loss (i.e., apoptosis). Therefore, the primary purpose of this study was to explore the role of forced exercise after 6-OHDA-induced nigrostriatal dysfunction in a hemiparkinsonian rat model.

METHODS

Overall study design

Twenty-six male Long Evans rats (Charles River Laboratories, Hollister, California, USA) aged 91-100 days were used for this experiment. All handling and procedures were in accord with NIH Guide for the Care and Use of Laboratory Animals and approved by the University of Nevada, Las Vegas Institutional Animal Care and Use Committee. The rats first received a unilateral Parkinsonian lesion as described below and then were randomly assigned into one of two 4-week treatment conditions: exercise and non-exercise control (Figure 4). These rats, housed individually and fed ad libitum, weighed between 360 and 457 grams (mean=400.4 grams, SD=24.8) at the time of randomization. Rats were tested for PD severity using behavioral tests prior to and after the treatment conditions. Three different behavioral tests were used to create a comprehensive assessment of the PD severity (Metz & Whishaw, 2002).

Figure 4. Overall study design.



Surgical procedures

After a one-week acclimatization period, animals were anesthetized using an intraperitoneal injection of 75 mg/kg Ketamine and 0.25 mg/kg dexmedetomidine. In order to block noradrenergic uptake sites and thereby prevent noradrenergic toxicity, 15 mg/kg desipramine hydrochloride dissolved in saline was injected intraperitoneally 30 minutes before the neurotoxin was injected (Anstrom et al., 2007; O'Dell et al., 2007). The rats were then placed in a stereotactic frame (Kopf 900, David Kopf Instruments, Tujunga, California, USA).

The scalp was shaved, swabbed with povidone-iodine topical antiseptic and a central incision was made to expose the skull and bregma. A 1 mm burr hole was then drilled through the cranium to gain exposure to the cranial cavity at the following stereotactic coordinates: 3.3 mm posterior to bregma, 1.9 mm lateral, 8.2 mm ventral to skull surface (Paxinos & Watson, 1998; Paxinos et al., 1985; Tillerson et al., 2003; Tillerson et al., 2002b). A unilateral Parkinsonian lesion was induced with 10 µg of 6-hydroxydopamine hydrobromide in 0.1% ascorbic acid (6-OHDA; Sigma, St. Louis, USA) or 2,4,5-trihydroxyphenethylamine in 4 µl of 0.9% saline injected into the right nigrostriatal fibers that make their way to the striatum via the medial forebrain bundle (Mabandla et al., 2004; Moroz et al., 2004; Tillerson et al., 2003; Tillerson et al., 2002b; Tillerson et al., 2001). This infusion would equate to roughly an 80% loss of dopaminergic cells (Tillerson et al., 2003). A 28-gauge 5 µl-microliter syringe (Hamilton, Reno, Nevada) was used to slowly inject the 6-OHDA at a rate of 1 µl/min for 4 minutes into the right medial forebrain bundle.

Following injection of the neurotoxin, the burr hole was closed using bone wax and standard suturing techniques were used to close the incision. During the surgery and recovery, rats were kept warm using a warm heating pad and heating lamp. Rats were brought out of surgical analgesia using 1ml/kg atipamezole (subcutaneous injection into the flank). Rats received postoperative analgesia daily for three days using a 0.01mg/kg subcutaneous injection of buprenorphine into the flank. During surgery, three rats died. Therefore, the remaining 23 rats were randomized into the two experimental conditions.

Parkinsonian behavioral testing

After a one week post-surgical recovery period, the rats were tested to determine their motor behavioral severity of PD prior to entry into the experimental conditions. After the 4-week experimental period, the rats were tested again with the same tests of PD motor severity. This was done using apomorphine-induced rotations, forelimb placement asymmetry, and exploratory rearing.

Due to neurotoxin-induced denervation, the dopamine receptors on the affected side (i.e., injected side) become hypersensitive in the hemiparkinsonian rat. Apomorphine, a dopamine agonist, stimulates these receptors causing an asymmetrical increase in striatal activity on that side relative to the non-affected side. This causes a rapid, repetitive turning of the body away or contralateral from the lesioned side (Da Cunha et al., 2008). The number of complete 360° turns within 30 minutes in a round bowl has been shown to correlate well with lesion severity in terms of dopaminergic cell loss (Hudson et al., 1993; Metz & Whishaw, 2002). Thus, it was felt that apomorphine-induced rotations would be a good indicator of the severity of the hemiparkinsonian lesion. Animals were injected with 0.5mg/kg apomorphine hydrochloride (Sigma, St. Louis, USA) dissolved in saline subcutaneously and then immediately videotaped from above (Joghataie et al., 2004; Mabandla et al., 2004; Roghani & Behzadi, 2001; Tillerson et al., 2002b). The number of times the animal rotated 360° in each direction over a 30-minute period was counted using the videotape at a later time. The number of ipsilateral rotations subtracted from the number of contralateral rotations resulted in a net number of contralateral rotations (Poulton & Muir, 2005).

Forelimb placement asymmetry while rearing in a cylinder was evaluated using similar protocols established in previous studies (Moroz et al., 2004; O'Dell et al., 2007; Poulton & Muir, 2005; Schallert & Tillerson, 1999; Tillerson et al., 2003; Tillerson et al., 2002b). Each rat was placed in a 22 cm diameter by 26 cm high cylinder for 10 minutes and videotaped from above. As the rats explored the walls of the cylinder during rearing, they used one or both forelimbs to contact the wall for support during exploration. Independent use of the impaired, which would be the left forelimb since the contralateral nigrostriatal fibers were lesioned, and unimpaired (right forelimb) forelimbs were counted for each rearing episode. These values were then scored using the following asymmetry ratio: $(\text{Right} - \text{Left}) / (\text{Right} + \text{Left} + \text{Both})$ (O'Dell et al., 2007). Scores on the asymmetry ratio range from -1 to 1 with a high positive ratio being suggestive of a greater use of the unimpaired forelimb over the impaired forelimb. A negative asymmetry ratio would be indicative of greater use of the impaired forelimb relative to the unimpaired forelimb. Thus, a high positive asymmetry ratio would be consistent with a hemiparkinsonian lesion.

The number of times that a rat reared during a 10 minute videotaped period was referred to as exploratory rearing. While this has not been documented previously in the literature, it was felt that exploratory rearing would be a potential new behavioral test for hypokinesia which is a common symptom of PD.

Exercise protocol

Immediately after behavioral testing, the 23 rats were randomly assigned into one of two 4-week experimental conditions: exercise and control. The control condition consisted of 11 rats that were housed individually with no exercise equipment and were not exposed to any exercise for 4 weeks. The exercise condition consisted of 12 rats, one of which became ill and died (cause unknown per veterinarian) during the 4-week forced exercise regimen. This exercise condition consisted of forced exercise using a rat treadmill (Exer 3/6 Treadmill from Columbus Instruments, Columbus, Ohio, USA) five days per week for 4 weeks during the light cycle. The rats received 2 bouts of 15 minute running periods (separated by at least 1 hour) per day at a speed of 15 meters per minute (Tillerson et al., 2003). The Exer 3/6 Treadmill is equipped with an electrical grid on the posterior portion of the treadmill. If the rats' tail or hind limb made contact with the grid, an electrical stimulus strong enough to elicit an aversive reaction would serve as a negative reinforcer for running noncompliance. The electrical stimulus was set at 200 millisecond pulses with an adjustable pulse repetition rate of 1 to 4 per second. The intensity was adjustable from 0.35 mA to 3.4 mA and depended on the responsiveness of the particular rat to the stimulus.

RESULTS

Apomorphine rotations. A 2 (time: pre and post) X 2 (condition: exercise and non-exercise control) mixed factorial ANOVA was conducted to determine if there was an interaction between time and condition on apomorphine rotations. There was no

interaction between the variables for apomorphine rotations, $P = 0.121$ (power = 0.338; Table 2, Figure 5). There was, however, a significant main effect for time ($P < 0.001$; Table 3) suggesting that, regardless of experimental condition, there was an increase in apomorphine rotations from the pre (mean=188.4, SD=71.4) to the post (mean=284.6, SD=71.5). There was no main effect for condition ($p=.906$; Table 4).

Table 2. Descriptive statistics of apomorphine rotations.

	Condition	Mean	Std. Deviation	N
Pre	Runners	179.6	53.1	11
	Non-runners	197.2	87.8	11
Post	Runner	296.8	73.7	11
	Non-runners	272.5	70.5	11

Figure 5. Interaction of time and condition on apomorphine rotations.

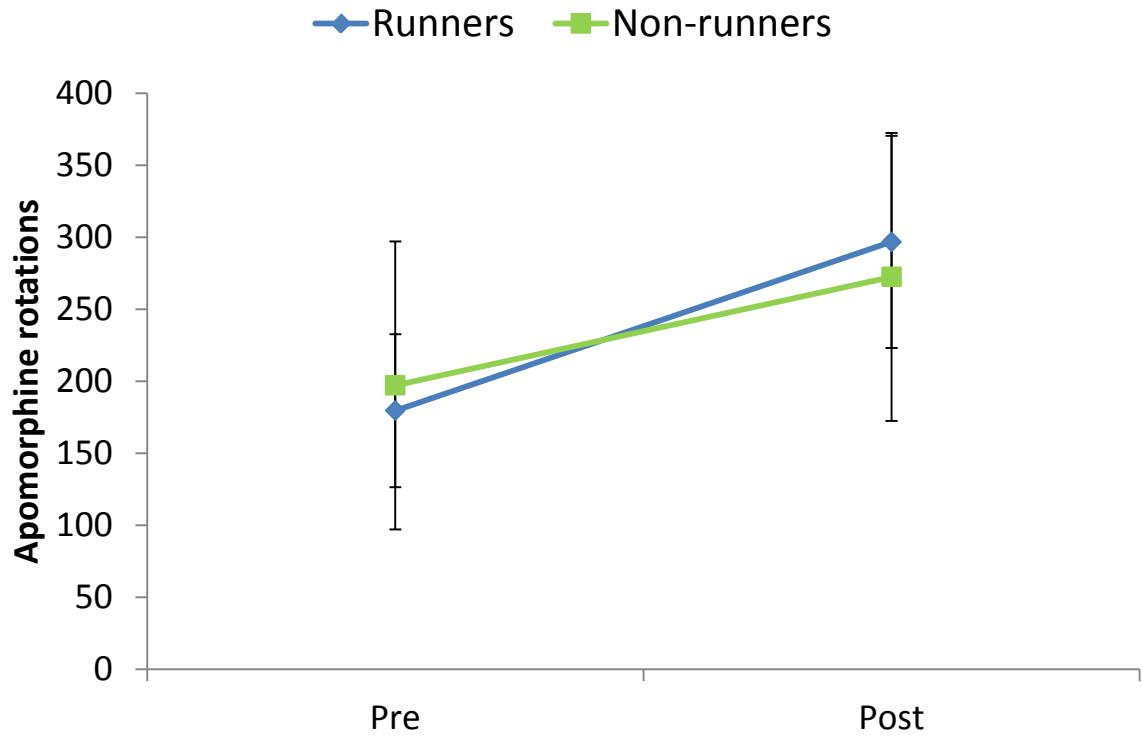


Figure 5. Means and standard deviations for the number of apomorphine-induced contralateral rotations during a 30-minute testing period for runners and non-runners before and after 4 weeks of experimental treatment.

Table 3. Main effect for time on apomorphine rotations.

Time	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Pre	188.4	15.5	156.1	220.6
Post	284.6	15.4	252.6	316.7

Table 4. Main effect for condition on apomorphine rotations.

Condition	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Runners	238.2	19.8	196.9	279.5
Non-runners	234.8	19.8	193.5	276.1

Forelimb placement asymmetry. A 2 (time: pre and post) X 2 (condition: exercise and non-exercise control) mixed factorial ANOVA was conducted to determine if there was an interaction between time and condition on forelimb placements. There was no interaction for forelimb placement asymmetry, $P = 0.497$ (power = 0.101; Table 5, Figure 6). There was a main effect for time ($P < 0.001$; Table 6) which suggests that all of the animals had significant asymmetry at the pre measurement (72.9% unimpaired over impaired forelimb use) which decreased significantly at the post measurement (38.4% unimpaired over impaired forelimb use). There was not a significant main effect for experimental condition ($P = 0.341$, power = 0.153; Table 7).

Table 5. Descriptive statistics for forelimb placement asymmetry.

	Condition	Mean	Std. Deviation	N
Pre	Runners	.758	.304	11
	Non-runners	.700	.272	11
Post	Runners	.460	.334	11
	Non-runners	.309	.273	11

Figure 6. Interaction of time and condition on forelimb placement asymmetry.

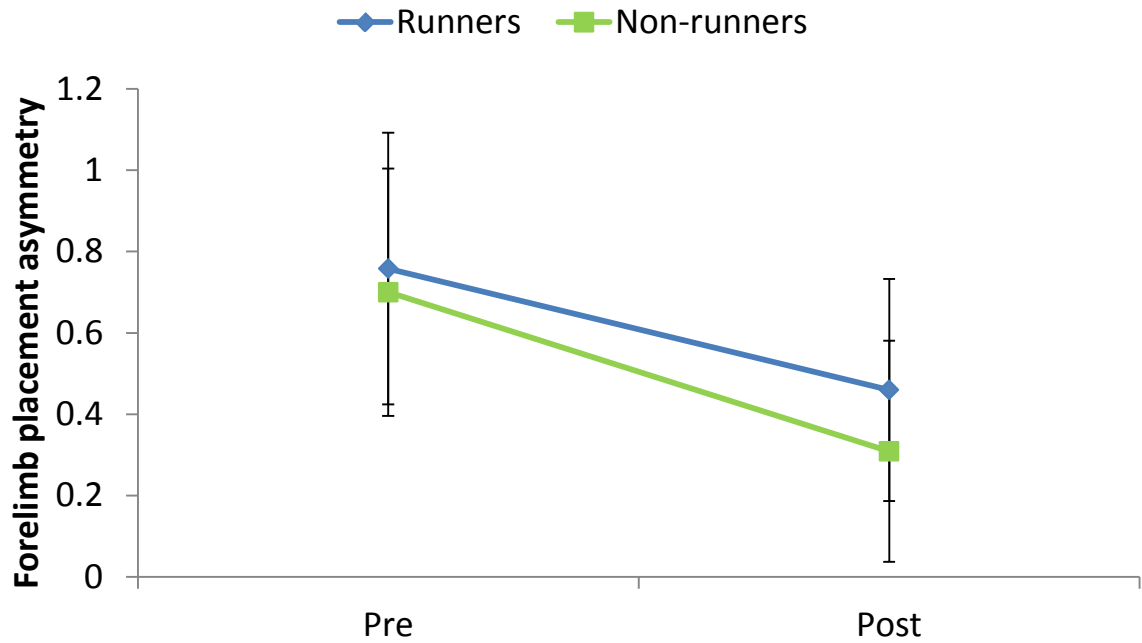


Figure 6. Means and standard deviations of the forelimb placement asymmetry ratio during a 10-minute testing period for runners and non-runners before and after 4 weeks of experimental treatment.

Table 6. Main effect of time for forelimb placement asymmetry.

Time	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Pre	.729	.062	.601	.858
Post	.384	.065	.249	.520

Table 7. Main effect of group for forelimb placement asymmetry.

Condition	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Runners	.609	.076	.451	.767
Non-runners	.504	.076	.346	.663

Exploratory rearing. A 2 (time: pre and post) X 2 (condition: exercise and non-exercise control) mixed factorial ANOVA was also used to determine if there was an interaction for exploratory rearing. There was not a statistically significant interaction between time and condition, $P = 0.315$ (power = 0.166; Table 8, Figure 7). There was not a main effect on condition, $P = 0.260$ (power = 0.166; Table 9); however, there was a main effect on time ($P < 0.001$; Table 10) with less exploratory rears at the pre measurement (mean = 6.6, SD = 0.9) compared to the post measurement (mean = 14.5, SD = 1.3).

Table 8. Descriptive statistics for the number of exploratory rears.

	Condition	Mean	Std. Deviation	N
Pre	Runners	7.0	4.1	11
	Non-runners	6.2	4.3	11
Post	Runners	16.3	6.1	11
	Non-runners	12.8	6.3	11

Figure 7. Interaction between time and condition on exploratory rearing.

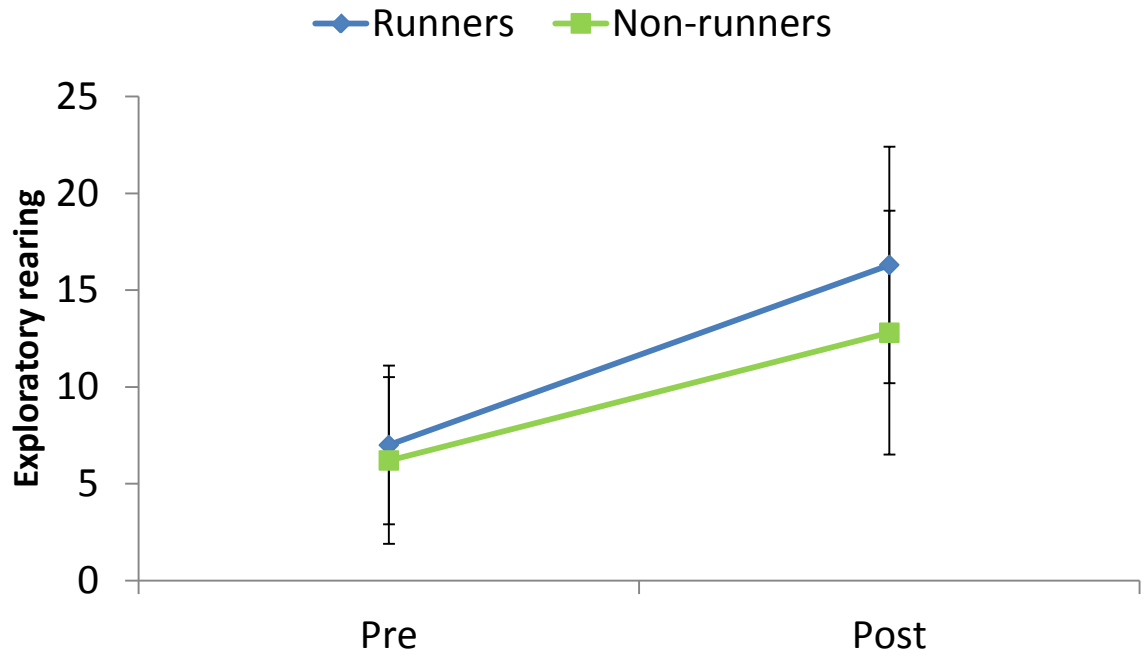


Figure 7. Means and standard deviations of the number of exploratory rearing episodes during a 10-minute testing period for runners and non-runners before and after 4 weeks of treatment.

Table 9. Main effect of condition on exploratory rearing.

Condition	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Runners	11.6	1.3	8.9	14.4
Non-runners	9.5	1.3	6.8	12.2

Table 10. Main effect of time on exploratory rearing.

time	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Pre	6.6	0.9	4.7	8.5
Post	14.5	1.3	11.8	17.3

DISCUSSION

Results of this study do not support the notion that exercise after neurotoxin-induced hemiparkinsonism is beneficial. In general, treadmill trained animals did not improve more than sedentary non-runners in any of the three behavioral tests. Of these tests, all of the rats regardless of group, runner and non-runner, improved in two of the outcome tests (i.e., forelimb placement asymmetry, exploratory rearing) and got worse in one (i.e., apomorphine rotations). The results of this study are not consistent with the literature and cast doubt on previous assertions of the robustness of the neuroprotective treatment effect of exercise in an adult 6-OHDA rat model of PD.

A majority of previous research suggests that exercise ameliorates motor deficits in the 6-OHDA model of PD (Howells et al., 2005; Mabandla et al., 2004; Moroz et al., 2004; O'Dell et al., 2007; Smith & Zigmond, 2003; Tajiri et al., 2010; Tillerson et al., 2003; Tillerson et al., 2001; Vergara-Aragon et al., 2003) and also in other rodent models of PD (Caudle et al., 2007; Fisher et al., 2004). These studies served as the theoretical foundation for the present study. While there were some differences in the modality of the exercise regimen, the overall study designs were fairly consistent. The dosing of the exercise in the present study was consistent with several of the studies (Poulton & Muir, 2005; Tajiri et al., 2010; Tillerson et al., 2003); however, the dosing in the present study was more intense than the Moroz et al study (2 weeks of 15-30 minutes of running in plastic balls) and the Mabandla et al study (2 weeks of exercise in free running wheels (average of 4361 revolutions per day)) (Mabandla et al., 2004; Moroz et al., 2004). Only the O'Dell et al (2007) study had a more intense exercise protocol (4 weeks of voluntary

running wheel for >1000 meters/day plus 10.5 meters per minute for 30 minutes of forced running) (O'Dell et al., 2007). Since the exercise dosing for the present study was consistent with the previous studies that showed a treatment effect with exercise, it is not likely that the lack of treatment effect in the present study had anything to do with the dosing of the treadmill exercise.

While the results of this study were not consistent with the body of the literature on rodent exercise and PD, there was another study that likewise did not find an exercise treatment effect. Poulton and Muir (2005) found that treadmill training did not affect behavioral deficits in the 6-OHDA Parkinsonian rat model (Poulton & Muir, 2005). This study had an exercise protocol (4 weeks of 40 minutes/day at 13 meters/minute) that was similar in duration and intensity. Taken together, the results of the present study and those of Poulton and Muir (2005) study cast doubt on the positive treatment effect of exercise reported after 6-OHDA injection. In light of this lack of consensus in the literature, further research is warranted.

The reason for the worsening (i.e., increase in the number of contralateral rotations) in the apomorphine rotations over time in both of the groups is likely a function of the time it takes for the neurotoxin to cause oxidative damage and eventual apoptosis (Cerruti et al., 1993; Crocker et al., 2001; Decker et al., 1993; Glinka et al., 1997; Hudson et al., 1993). Generally, it is thought that it takes 1-2 weeks in the 6-OHDA model. However, some researchers suggest that the neurotoxic effects of 6-OHDA continue to cause apoptosis well past 2 weeks and even up to 28 days after the injection (Bjorklund et al., 1997). In the present study, the pre measurement was taken

approximately 1 week after the 6-OHDA lesion; this would have been at the early end of the range of dopaminergic toxicity. The post measurement was taken approximately five weeks after the 6-OHDA injection; presumably, the full effect of the neurotoxin would have been realized by that point. Thus, it is logical that the apomorphine rotations had increased over the duration of the study because the toxicity of 6-OHDA on the dopaminergic neurons was protracted.

One of the most logical reasons for a lack of treatment effect was the delay in treadmill treatment following the 6-OHDA lesioning. In the present study, the animals started the exercise regimen 7 days after the lesioning. Tillerson et al (2001) reported that exercise started early in the first week after surgery, but not after the first week, prevented the preferential use of the unimpaired forelimb and reduced contralateral apomorphine rotations (Tillerson et al., 2001). Indeed, in the studies where a positive exercise treatment effect was observed, the start for exercise after 6-OHDA lesioning was very soon after the injection of the neurotoxin: 2-4 hours post-lesioning (Tillerson et al., 2003), and 4-5 hours post-lesioning (Moroz et al., 2004), and 24 hours (Howells et al., 2005; Mabandla et al., 2004; O'Dell et al., 2007; Tajiri et al., 2010). While it appears that early intervention is an important component of neuroprotection, it should be noted that early exercise may have increased blood flow in behaviorally relevant brain locations (i.e., nigrostriatal pathways) and this may have actually flushed neurotoxin from that brain region. Thus, the neuroprotective effect of exercise may not have been mediated by an effect on the underlying disease course. However, this is purely

speculative but could, if true, represent a threat to the validity of exercise as a neuroprotective agent in neurotoxin-induced PD.

Only one study with a positive exercise treatment effect reported an exercise start time beyond the one week window (i.e., 21 days post lesion)(Vergara-Aragon et al., 2003). However, it should be noted that in the Poulton and Muir (2005) study, where no exercise treatment effect was observed, the start time for the early group was 24 hours after surgery and the late group was 1 week after surgery (Poulton & Muir, 2005).

While there is not complete consensus, taken together, it appears that exercise should begin soon after 6-OHDA lesioning because it may interfere with the slowly developing dopaminergic toxicity (i.e., 1-2 weeks). It is possible that an early window of opportunity may have been missed because of the delayed start in the implementation of the forced exercise. This represents a limitation in the design of the present study.

Another logical reason for the lack of a positive exercise treatment effect is that exercise protocol-related stress may have negated the beneficial effects of exercise. Howells et al found that exercise does improve hemiparkinsonian behavioral symptoms; however, mild stressors may cancel the neuroprotection afforded by the voluntary exercise (Howells et al., 2005). Moroz et al (2004) also reported that repeated saline injections (a form of stress) to rats with free access to running wheels cancelled the beneficial effects of exercise (Moroz et al., 2004). In the present study, a “forced” exercise protocol was utilized wherein animals were forced to run using negative reinforcement. Rats in the exercise group ran on a treadmill equipped with an electrical grid on the posterior portion. Noncompliance with the running would cause the tail or

hind limb/s to make contact with an electrical grid causing a stimulus strong enough to elicit an aversive reaction. While this aversive stimulus served as a negative reinforcer for running noncompliance, it may have created a stressful environment for the animal thereby potentially negating the positive treatment effect of exercise. Subsequently, the possibility that stress may have negatively affected the neuroprotective effect of exercise cannot be ruled out. A future study using an exercise modality without an aversive stimulus or reinforcer would be necessary to clear up this issue. In addition, a brief preconditioning trial of the treadmill exercise prior to the surgery may have familiarized the animals with the treadmill and the associated aversive stimulus; this may have decreased the stress experienced during the first few days of treadmill use post injection when the rats were being conditioned to run.

CONCLUSIONS

The results of the present study demonstrated that treadmill training in a 6-OHDA model of PD did not mitigate the behavioral deficits of hemiparkinsonism. This study casts doubt on the previously held assertion that exercise ameliorates behavioral deficits in rodent models of PD. These results are in contrast to several studies that had found a robust treatment effect of exercise after induction of the disease; however, the present findings are consistent with at least one other study. Discrepancies in the results of the present study and those with a positive treatment effects may be accounted for by differences in the type of training and assessment. It is also possible

that stress-inducing electrical shocks for running noncompliance during treadmill training may have negated the benefit afforded by exercise.

CHAPTER 3

Forced exercise before induction of 6-OHDA-mediated nigrostriatal insult: neuroprotection in an adult hemiparkinsonian rat model

Abstract: Recent research has shown that rats preconditioned with exercise are less susceptible to toxin-induced PD in terms of Parkinsonian behavioral symptoms and underlying levels of dopamine and its metabolites. While these promising findings suggest that exercise might protect against dopaminergic attrition, there have been few studies that have utilized a forced exercise regimen prior to the induction of 6-hydroxydopamine (6-OHDA) hemiparkinsonism in rats. Therefore, the purpose of this study was to explore the role of forced exercise before the induction of PD with the intent of supporting these previous findings. Prior to the neurotoxin-induced PD, animals were randomized into two 4-week experimental groups, an exercise group and a non-exercise control group. After participation in the experimental conditions, hemiparkinsonism was induced using a unilateral injection of 6-hydroxydopamine. Parkinsonian behavioral tests (i.e., apomorphine rotations, forelimb placement asymmetry, exploratory rearing) did not show any significant differences between the two groups. These results are in contrast to the literature and suggest that the notion that exercise is neuroprotective may be premature. Further research is warranted.

INTRODUCTION

Exercise early in life may be protective against the development of Parkinson's disease (PD). Chen et al, in a study of 125,828 men and women, found that greater baseline physical activity was associated with a lower risk of PD and that strenuous exercise in early adult life was inversely related to PD in men (Chen et al., 2005). Strenuous exercise resulted in a 60% lower PD risk in men. In another very large epidemiologic study (i.e., 50,002 men who attended Harvard University or Penn University), Sasco et al (1992) found that moderate or heavy sports participation in college was associated with a reduced risk of PD suggesting a slight neuroprotective effect (Sasco et al., 1992). In a case control study by Tsai et al (2002), they found that early exposure to well water drinking and head trauma may trigger and expedite the appearance of PD features, but such acceleration may be prevented through regular exercise (Tsai et al., 2002). Taken together, these studies offer indirect evidence that exercise may somehow afford protection to the brain in areas relevant to PD.

In rodent models of PD, exercise or physical activity has also been shown to be neuroprotective prior to the induction of PD. Cohen et al (2003) found that forced use prior to experimentally induced hemiparkinsonism with 6-hydroxydopamine (6-OHDA) in rats prevented the development of Parkinsonian motor deficits and lessened the loss of striatal dopamine (Cohen et al., 2003). Adult mice preconditioned with exercise (i.e., free access to a running wheel) were found to exhibit outcomes consistent with neuroprotection of the nigrostriatal system (i.e., dopamine transporters) against MPTP-induced Parkinsonism (Faherty et al., 2005). In addition, rats forced to be inactive (i.e.,

no exercise) prior to a mild dose of 6-OHDA, which would not typically result in behavioral asymmetries, actually caused profound, long-term behavioral asymmetry and an exacerbation of the dopaminergic loss from an anticipated 20% loss to 60% loss (Tillerson et al., 2002b). In essence, this study suggests the existence of a dose dependent relationship of physical activity and disease severity prior to PD induction. That is, exercise protects against the disease, whereas, inactivity makes one more susceptible or vulnerable. Anstrom et al (2007) demonstrated that repetitive vibrissae-elicited forelimb placing, which is a sensorimotor exercise modality that is similar to a forced exercise protocol, before 6-OHDA injection also provided a therapeutic behavioral benefit to animals in terms of behavioral performance and sparing of substantia nigra tyrosine hydroxylase immunoreactivity (Anstrom et al., 2007). Metz et al (2004) also found a positive neuroprotective effect to the motor cortex due to a preconditioning of a skilled reaching task prior to unilateral 6-OHDA-injection (Metz et al., 2004). Taken together, these studies suggest, much like the human epidemiologic studies, that exercise or physical activity prior to neurotoxic 6-OHDA insult may provide neuroprotective benefit in terms of behavioral performance and underlying disease processes.

Promising results from the human epidemiologic and rodent PD neuroprotection studies warrant further investigation to determine if preconditioning of exercise can protect the PD brain in terms of motor behavior. Several research groups have found exercise to be neuroprotective in other rodent disease models, like stroke and ischemia (Ang et al., 2003; Cechetti et al., 2008; Ding et al., 2004; Liebelt et al., 2010). The

purpose, therefore, was to explore the role of forced exercise prior to the onset of 6-OHDA hemiparkinsonism in adult rats. Parkinsonian behavioral tests were conducted to determine if forced exercise mitigated behavioral deficits.

METHODS

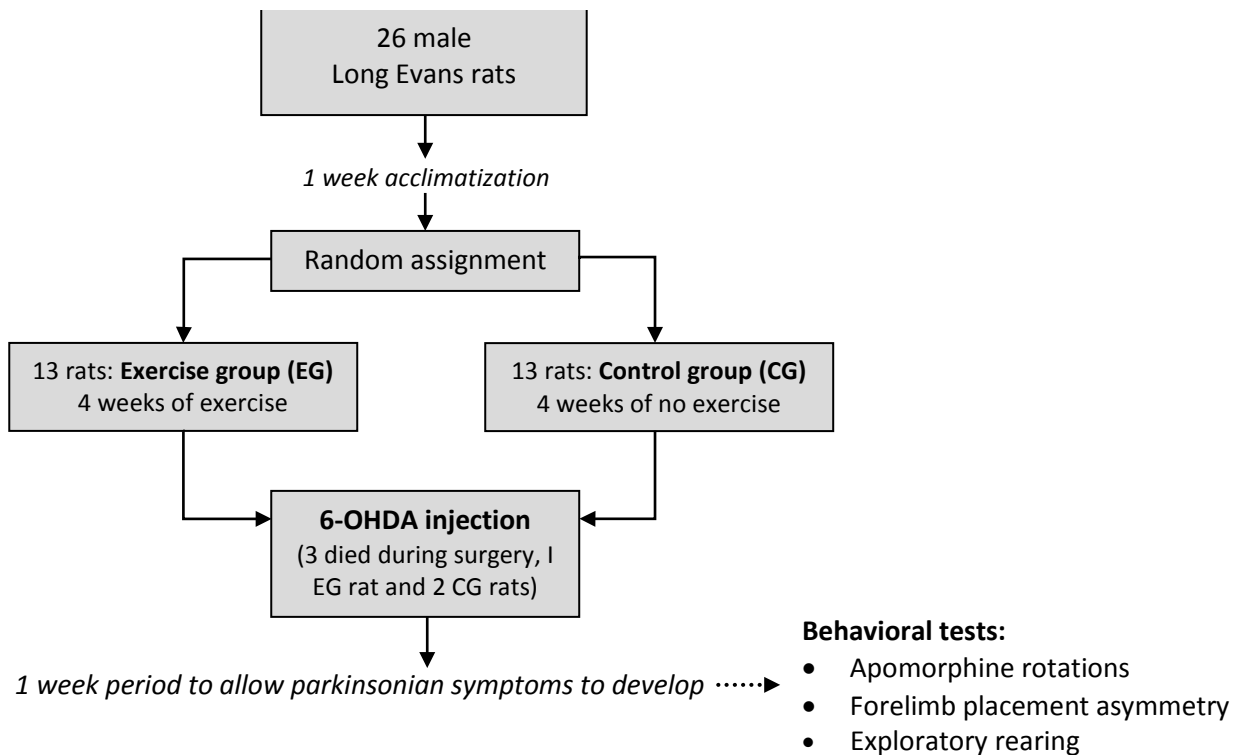
Overall study design

Twenty-six male Long Evans rats (Charles River Laboratories, California, USA) were used in this study. These rats were housed individually under standard laboratory conditions with a 12 hour light/dark cycle and were fed ad libitum throughout the duration of the study. The planning and execution of the experiments in this study were approved by the University of Nevada, Las Vegas Institutional Animal Care and Use Committee and were consistent with the National Institute of Health Guide for the Care and Use of Laboratory Animals. All rats were initially randomized into one of two 4-week treatment groups: forced exercise and non-exercise control (Table 11; Figure 8). Immediately after completing the 4-week treatment protocol, hemiparkinsonism was induced using an injection of the dopaminergic neurotoxin, 6-hydroxydopamine hydrochloride (6-OHDA). Approximately 6-8 days after the neurotoxin injection, rats were tested for motor asymmetry using three behavioral tests: apomorphine rotations, forelimb placement asymmetry, and exploratory rearing.

Table 11. Weight of rats at initial randomization and at 6-OHDA-induced hemiparkinsonism.

	Run status	N	Mean	Std. Deviation	Std. Error	P value
Weight at randomization	Runner	12	392.1	30.8	8.9	.057
	Non-runner	8	420.9	31.5	11.1	
Weight at 6-OHDA injection	Runner	13	436.0	38.6	10.7	.015
	Non-runner	10	471.6	19.3	6.1	

Figure 8. Basic overall study design.



Exercise protocol

After a brief acclimatization period, all 26 rats were randomly assigned into one of two 4-week experimental groups, a forced exercise group and a non-exercise control

group. The non-exercise control group consisted of individually-housed rats with no exercise equipment or access to exercise during the 4-week experimental period. The forced exercise group consisted of forced running on a 3-lane rat running treadmill during the light cycle (Exer 3/6 Treadmill from Columbus Instruments, Columbus, Ohio, USA) for five days per week. Rats were run twice per day at a speed of 15 meters per minute separated by at least one hour; each running bout lasted 15 minutes for a total of 30 minutes per day (Tillerson et al., 2003). An electrical grid at the back end of the treadmill served as an aversive stimulus if the rats came into contact with it due to poor running or noncompliance with the exercise. The electrical grid had adjustable intensity settings (i.e., 0.35 mA to 3.4 mA) at 200 millisecond pulses with an adjustable pulse repetition rate of 1 to 4 per second. This was initially set high and then once the running behavior was reasonably conditioned it was turned down.

Surgical procedures

After the 4-week experimental period, rats were anesthetized using an intraperitoneal injection of 75 mg/kg Ketamine and 0.25mg/kg dexmedetomidine. Desipramine hydrochloride (15 mg/kg) dissolved in saline was then injected intraperitoneally to preserve noradrenergic neurons 30 minutes before injection of 6-OHDA (Anstrom et al., 2007; O'Dell et al., 2007). This was done to mitigate attrition of noradrenergic neurons as 6-OHDA may also be toxic to this class of cells.

Once the rats had achieved surgical anesthesia (i.e., deep and slow breathing, unresponsive to paw-pinch), they were shaved and then immediately moved to the

stereotactic frame (Kopf 900, David Kopf Instruments, Tujunga, California, USA) and placed on the incisor bar. The head was then firmly positioned within the stereotactic frame by means of ear bars which were placed in the ear canals. An eye ointment (GenTeal 0.3% eye gel, USA) was then coated over the eyes. The cranium was then swabbed with povidone-iodine topical antiseptic. A central incision was then made to expose the skull and bregma after which a 1 mm burr hole was drilled through the cranium to gain exposure to the cranial cavity at the following stereotactic coordinates: 3.3 mm posterior to bregma, 1.9 mm lateral, 8.2 mm ventral to skull surface (Paxinos & Watson, 1998; Paxinos et al., 1985; Tillerson et al., 2003; Tillerson et al., 2002b). These coordinates were used to guide the syringe into the right medial forebrain bundle which contains dopaminergic neurons destined for the striatum. A 28-gauge, 5 μ l-microliter syringe (Hamilton, Reno, Nevada, USA) was then used to deliver the neurotoxin to the medial forebrain bundle. A total dose of 10 μ g of 6-hydroxydopamine hydrobromide in 0.1% ascorbic acid (Sigma, St. Louis, USA) in 4 μ l of 0.9% saline was injected at a rate of 1 μ l/min for 4 minutes (Mabandla et al., 2004; Moroz et al., 2004; Tillerson et al., 2003; Tillerson et al., 2002b). After the four minute injection period, the syringe was carefully removed from the burr hole. Following closure of the burr hole using bone wax, the incision was closed with 5-6 sutures using standard simple interrupted pattern suturing techniques.

Rats were kept warm using a warm heating pad and heating lamp throughout the surgery and recovery. Atipamezole at 1 ml/kg (subcutaneous) was used as a reversal agent for dexmedetomidine to bring the rats out of anesthesia. The rats were

injected with this drug immediately after removal from the stereotactic frame. The sutured incision was swabbed again with povidone-iodine topical antiseptic to prevent infection. The entire surgical procedure did not exceed 50 minutes. The rats were observed in recovery for at least one hour. Once they became ambulatory on all four limbs, they received postoperative analgesia using a 0.01mg/kg subcutaneous injection of buprenorphine into the flank and were returned to their plastic housing units. They received this same postoperative analgesia for the next three days. Three of the twenty-six rats died after injection of the anesthesia medications (ketamine/dexmedetomidine) and prior to injection of the neurotoxin 6-OHDA. The reason for these deaths was not known but according to an on-site veterinarian it was thought to be anesthesia related. There were no infections noted for the duration of the study.

Parkinsonian behavioral testing

Approximately one week (6-8 days) after neurotoxin injection, rats were tested to determine motor asymmetry as a result of the injection. The following three behavioral tests were used to create a comprehensive assessment: apomorphine-induced rotations, forelimb placement asymmetry, and exploratory rearing.

Apomorphine rotations were evaluated by injecting 0.5 mg/kg apomorphine hydrochloride subcutaneously in the dorsal right quadrant proximal to the pelvis (Sigma, St. Louis, USA) dissolved in saline subcutaneously (Joghataie et al., 2004; Mabandla et al., 2004; Roghani & Behzadi, 2001; Tillerson et al., 2002b). The number of times the

animal rotated 360° in each direction over a 30-minute period was counted at a later time using a videotaped recording taken from above the animal. Ipsilateral rotations subtracted from the number of contralateral rotations resulted in a net number of contralateral rotations (Poulton & Muir, 2005). Complete 360° rotations within 30 minutes in a round bowl have been shown to correlate with PD lesion severity (Hudson et al., 1993; Metz & Whishaw, 2002). Thus, it was felt that apomorphine-induced rotations would be a good indicator of dopaminergic cell loss.

Forelimb placement asymmetry was evaluated first by placing the animals in a cylinder (22 cm wide by 26 cm high) and then initial forelimb touches to the walls of the cylinder (i.e., impaired left forelimb first, unimpaired right forelimb first, simultaneous) for each individual rearing episode were recorded (Moroz et al., 2004; O'Dell et al., 2007; Poulton & Muir, 2005; Schallert & Tillerson, 1999; Tillerson et al., 2003; Tillerson et al., 2002b). Rats were videotaped for 10 minutes from above and were then scored using the following asymmetry ratio: $(\text{Right} - \text{Left}) / (\text{Right} + \text{Left} + \text{Both})$ (O'Dell et al., 2007). Scores on the forelimb asymmetry ratio range from -1 to 1 with a high positive ratio being consistent with greater use of the unimpaired forelimb over the impaired forelimb. A negative asymmetry ratio would suggest greater use of the impaired forelimb relative to the unimpaired forelimb. Thus, a high positive forelimb placement asymmetry ratio would be consistent with a hemiparkinsonian lesion.

Exploratory rearing was evaluated by observing the 10 minute video tape of the forelimb placement asymmetry test. The number of times that a rat reared on its hind limbs during that 10 minute period was referred to as exploratory rearing. This has not

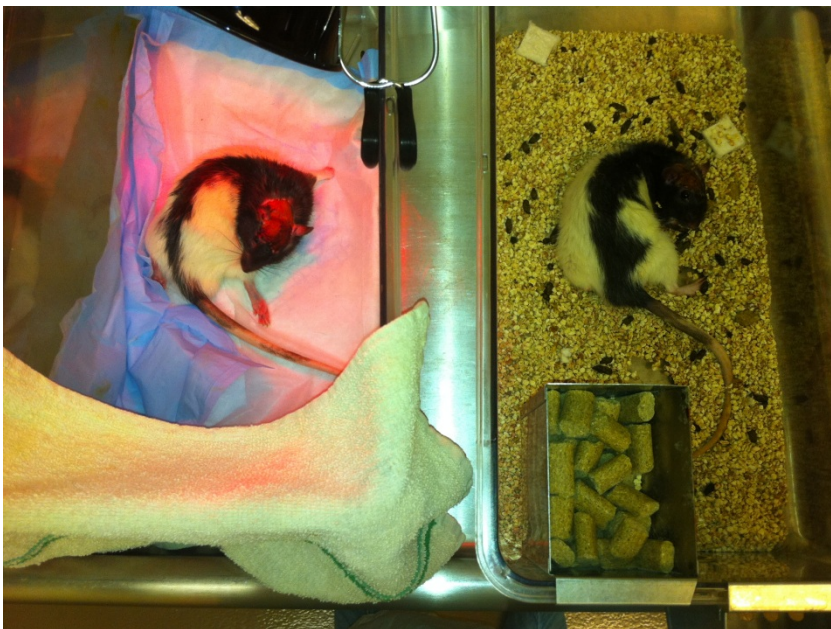
been documented previously in the literature; however, it was felt that exploratory rearing could be a potential new behavioral test for a common symptom in PD, a paucity of movement (i.e., hypokinesia).

Data analysis

All analyses were conducted using SPSS version 19.0 (IBM SPSS, Chicago, Illinois, USA). In order to determine if there was a difference between the forced exercise group and the non-exercise control group, an independent samples t-test was conducted for apomorphine rotations. Apomorphine rotations were only conducted after the 6-OHDA injections. As forelimb placements and exploratory rearing were measured at the start of the study (prior to the 4 week training period) and after at the end of the study (after the 6-OHDA injections), two 2 (time: pre and post) by 2 (group: runner and non-runner) mixed factorial ANOVAs were done, one for forelimb placement asymmetry and one for exploratory rearing. In an exploratory secondary analysis, point biserial correlations were analyzed for the immediate post-surgical observation (i.e., within 20 minutes) of right neck turning (yes or no) to the three other behavioral tests in the study (Figure 9). This has not been previously documented in the literature. It was felt that this immediate neck turning might correlate to the severity of the asymmetry caused by the 6-OHDA injection and could potentially be a way of determining immediate post-operative success in injecting this neurotoxin into the medial forebrain bundle. An additional exploratory analysis was to compare the runners to the non-runners in weight gain throughout the study. It was thought that because of the

difference in activity level between the two groups there was a possibility that weight from the start of the study to the time of surgery may have been differentially affected. A 2 (time: pre and post) by 2 (group: runners and non-runners) mixed factorial ANOVA with weight as the dependent variable was used to explore this possibility.

Figure 9. Profound right neck turning immediately (within 20 minutes) after surgery for two of the rats.



RESULTS

Parkinsonian behavioral tests

There was a statistically significant difference between the runners and non-runners on apomorphine rotations, $t(20) = 3.094$, $P = 0.006$. Animals that had run prior to the 6-OHDA lesion rotated significantly more than the non-runners had (Table 12, Figure 10).

Table 12. Descriptive statistics of runners versus non-runners on apomorphine rotations.

	Run status	N	Mean	Std. Deviation	Std. Error Mean
Apomorphine rotations	Runners	12	306.5	115.772	33.421
	Non-runners	10	139.0	138.320	43.741

Figure 10. Apomorphine rotation box plot of runners versus non-runners.

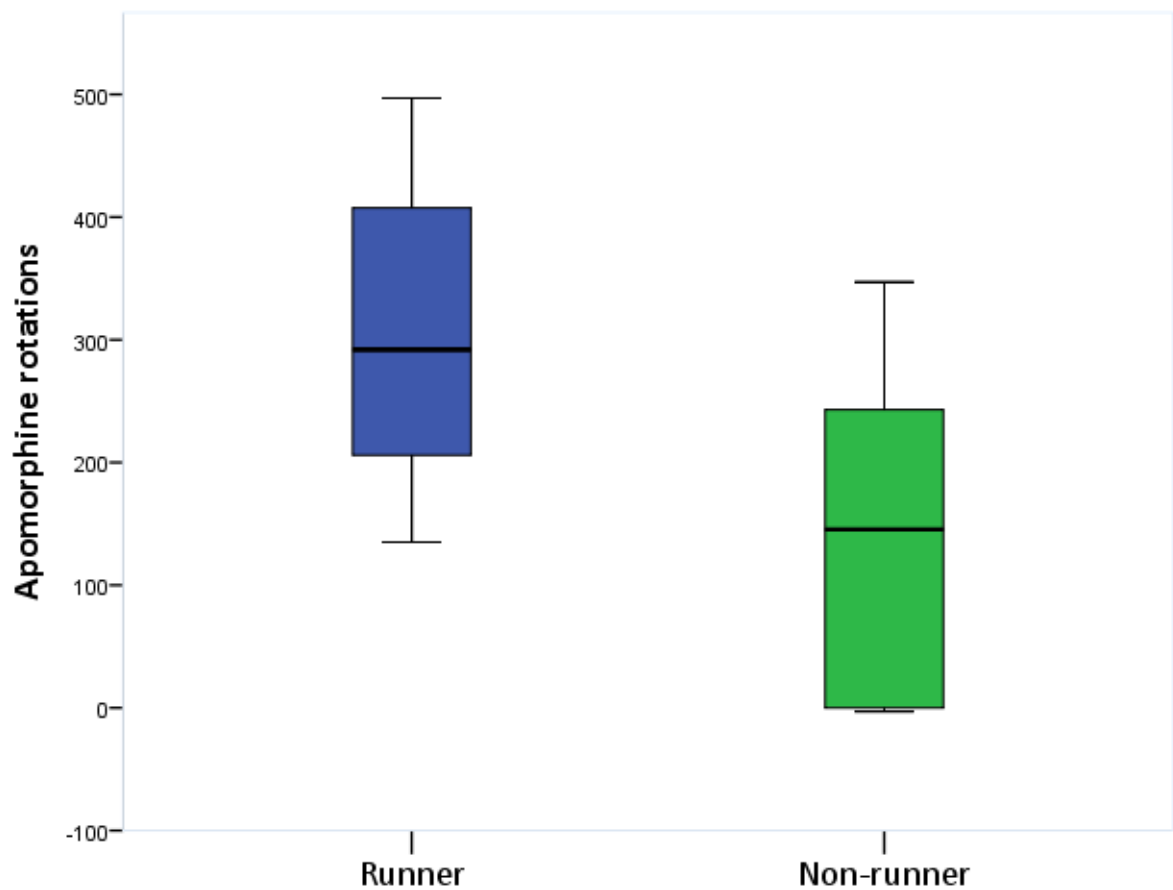


Figure 10. Boxplot with medians, lower and upper quartiles, and smallest and largest observations of runners and non-runners on the number of apomorphine-induced contralateral rotations during a 30-minute testing period after 6-OHDA lesioning.

There was not a statistically significant interaction between group and time for forelimb placement asymmetry, $F(1,16) = 0.680$, $P = 0.422$ ($\eta_p^2 = 0.041$, power = 0.121; Table 13). The main effect of group was not significant ($P = 0.639$); however, the main effect for time was significant ($P < 0.001$). Before the 4-week intervention, the rats were fairly symmetrical in forelimb arm use (runners = -0.02; non-runners = -0.01; Figure 11). One week after the 6-OHDA surgery, they were both fairly asymmetrical in their forelimb use with a preference for wall touches using the unimpaired forelimb regardless of treatment group (runners = 0.670; non-runners = 0.574; Figure 11).

Table 13. Descriptive statistics for forelimb placement asymmetry at the pre and post assessments.

	time	Mean	Std. Deviation	Std. Error	95% Confidence Interval	
					Lower Bound	Upper Bound
Runners	Pre	-.020	.128	.040	-.103	.064
	Post	.670	.230	.080	.502	.839
Non-runners	Pre	-.005	.155	.056	-.123	.114
	Post	.574	.355	.112	.335	.812

Figure 11. Forelimb placement asymmetry at the pre and post measurement times.

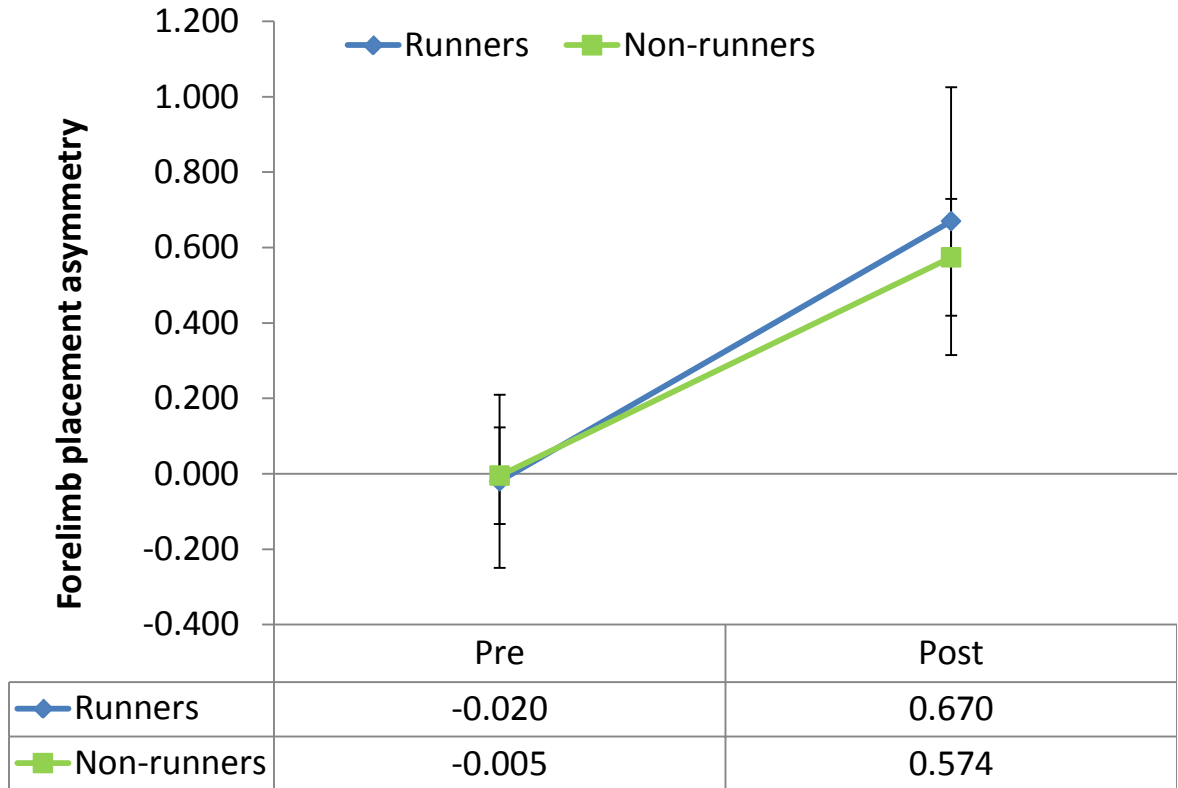


Figure 11. Means and standard deviations of the forelimb placement asymmetry ratio during a 10-minute period for runners and non-runners before and after implementation of the 4-week experimental treatment.

There was not a statistically significant interaction between group and time for exploratory rearing, $F(1,16) = .3.824$, $P = 0.068$ ($\eta_p^2 = 0.193$, power = 0.452; Table 14; Figure 12). The main effect for group was not significant, $p=.914$; however, there was a statistically significant main effect for time with more exploratory rearing at the pre measurement (mean rears = 22.4, SE = 1.6) than the post measurement (mean rears = 13.1, SE = 1.5), regardless of group, $P < 0.001$.

Table 14. Descriptive statistics for exploratory rearing at the pre and post assessments.

	time	Mean	Std. Deviation	Std. Error	95% Confidence Interval	
					Lower Bound	Upper Bound
Runners	Pre	23.8	7.1	1.9	19.9	27.8
	Post	11.4	4.2	1.7	7.8	15.1
Non-runners	Pre	21.0	4.4	2.6	15.4	26.6
	Post	14.8	8.7	2.4	9.7	20.0

Figure 12. Graph of exploratory rearing at the pre and post measurement times.

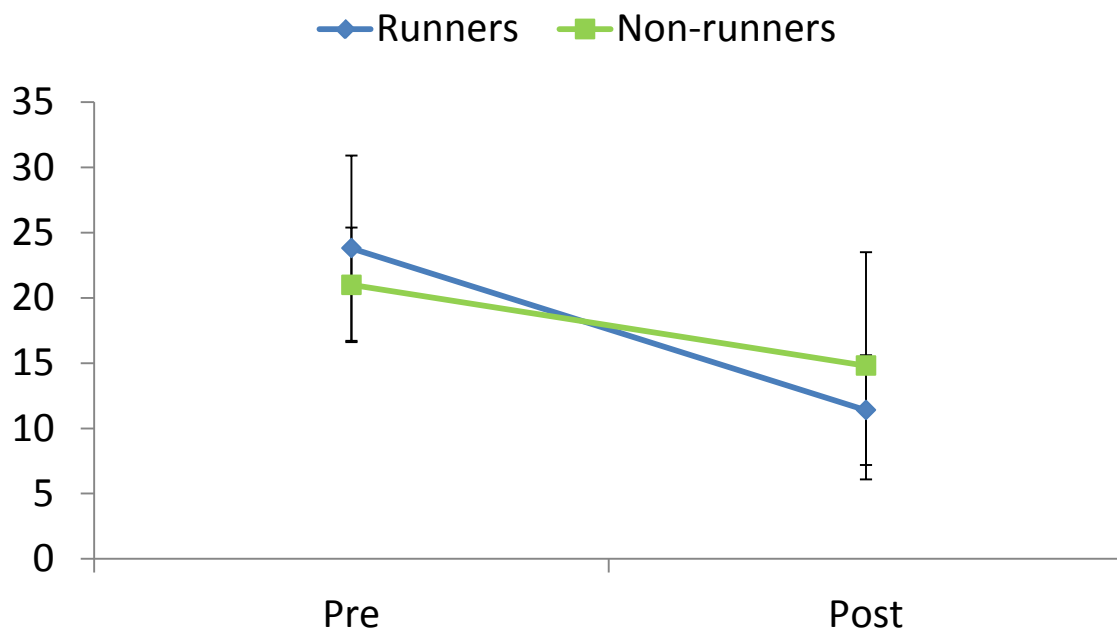


Figure 12. Means and standard deviations of the number of exploratory rearing episodes during a 10-minute testing period for runners and non-runners before and after 4 weeks of treatment.

In the secondary exploratory analysis of the correlation between the other outcome variables and the immediate observation of right neck turning in the first 20 minutes after surgery, there were several moderate to high correlations (Table 15). Of note, the right neck turn after surgery was strongly correlated with apomorphine rotations ($r = 0.734$) and exploratory rearing ($P = -0.749$).

Table 15. Correlations of immediate right neck turning after surgery to the three behavioral tests.

	Right neck turn after surgery	Apo-morphine rotations	Forelimb asymmetry	Exploratory rearing
Right neck turn after surgery	1	.734**	.534*	-.749**
Apomorphine rotations		1	.331	-.589*
Forelimb asymmetry			1	-.511*
Exploratory rearing				1

** Correlation is significant at the 0.01 level (2-tailed)
* Correlation is significant at the 0.05 level (2-tailed)

In the secondary analysis of weight gain throughout the study, there was no significant interaction, which suggests that the running condition did not differentially affect weight gain, $F(1,18) = 0.505$, $P = 0.486$ ($\eta_p^2 = 0.027$, power = 0.103; Table 16; Figure 13). As expected, there was weight gain throughout the study for both groups, $P < 0.001$, from the pre measurement (weight = 406.5 grams, SE = 7.1, 95% CI: 391.6 – 421.4) to the post measurement (weight = 455.1 grams, SE = 7.8, 95% CI: 438.7 – 471.4). There was also a main effect for group, $P = 0.022$, with the running rats weighing less (weight = 414.0, SE = 8.4, 95% CI: 396.3 – 431.8) than the sedentary control rats (weight = 447.5, SE = 10.3, 95% CI: 425.8 – 469.2).

Table 16. Descriptive statistics for secondary analysis of weight gain throughout the study.

	Time	Mean	Std. Deviation	Std. Error	95% Confidence Interval	
					Lower Bound	Upper Bound
Runners	Pre	392.1	30.8	9.0	373.3	410.9
	Post	436.0	40.3	9.8	415.3	456.7
Non-runners	Pre	420.9	31.5	11.0	397.8	443.9
	Post	474.1	20.9	12.0	448.8	499.4

Figure 13. Trajectory of weight gain over time for the runners and non-runners.

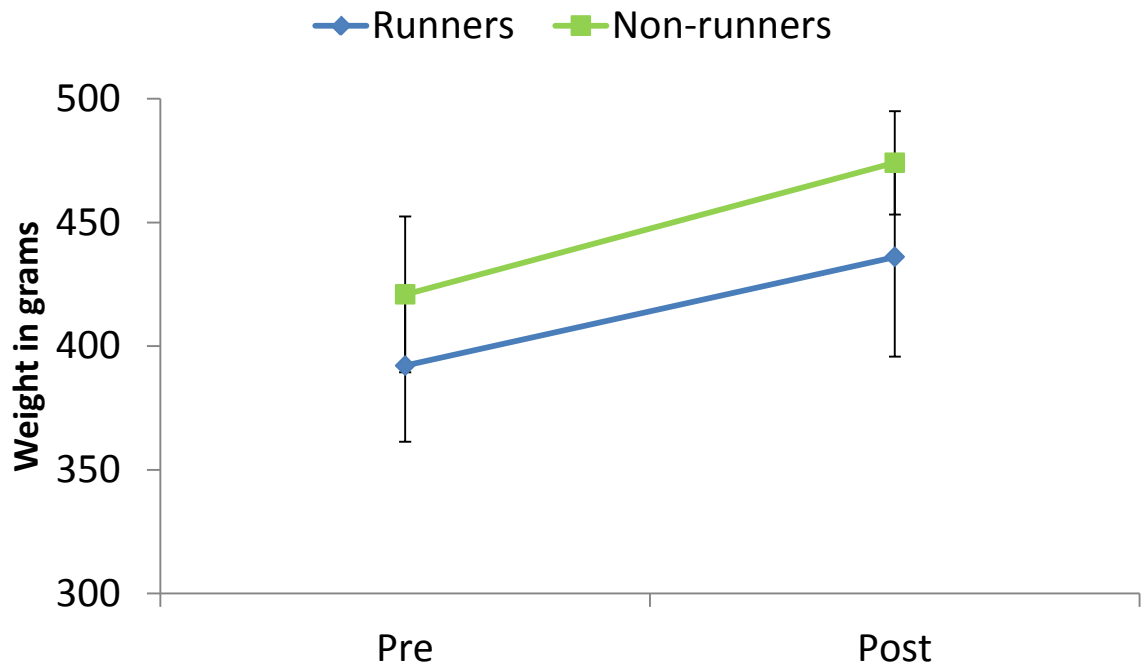


Figure 13. Means and standard deviations of the rats weighted in grams for runners and non-runners before and after 4 weeks of treatment.

DISCUSSION

Forced exercise prior to the onset of 6-OHDA-induced hemiparkinsonism in adult rats did not mitigate behavioral deficits. Thus, rats who engaged in a regular forced running program were not protected from the neurotoxin 6-OHDA more than the sedentary non-runners in any of the behavioral tests. In fact, it could be argued, based on the results of the apomorphine rotations, that non-runners were perhaps more protected than forced runners; however, taken together, these results clearly cast doubt on previous work that has shown a neuroprotective effect on PD (Cohen et al., 2003; Faherty et al., 2005; Metz et al., 2004; Tillerson et al., 2002b). Based solely on the results of the present study, the most logical conclusion is that a preconditioning of exercise is not neuroprotective; nevertheless, in context of the many studies that have demonstrated neuroprotection using this same model, the results of this study warrant a closer look at the methodology and design of this study's exercise parameters and how they compare to the literature.

Neuroprotection in a PD model due to a preconditioning of exercise was first documented by Cohen et al (2003) and Tillerson et al (2002) who found that forced nonuse (i.e., sedentary behavior) prior to 6-OHDA lesioning potentiated behavioral asymmetries (Cohen et al., 2003; Tillerson et al., 2002b). In the Cohen et al (2003) study, which most closely approximates the present study in design, it was found that forced reliance on a forelimb prior to contralateral injection of 6-OHDA prevented behavioral asymmetries. The forced reliance in the Cohen et al (2003) study, which has been equated to exercise, lasted for only 7 days prior to 6-OHDA lesioning and was

executed by casting the unimpaired forelimb in a retracted position to immobilize it, thereby forcing the animal to use the impaired forelimb for mobility, grooming, eating and all other tasks. The dosing of this forced “exercise” was considerably more than the 30 minutes per day for 5 days per week of forced treadmill running even when you consider that the length of treatment in the present study was 4 weeks. Using a different PD model (i.e., mouse model using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)), Faherty et al (2005) used free access to a running wheel for several months (Faherty et al., 2005). In the two studies that most closely resembled the present study, the dosing of exercise was considerably higher. While the results of the present study suggest that preconditioning with exercise may not be neuroprotective, the lack of a positive treatment effect may be explained by the low dose of the exercise. In light of this, further research using a more rigorous precondition of exercise is warranted.

Another possibility for the lack of positive treatment effect from the preconditioning of exercise is deleterious stress. Smith et al (2002) hypothesized that stress might be a key factor in the loss of dopaminergic neurons that underlie the pathology of PD (Smith et al., 2002). They based this hypothesis on the following lines of evidence: 1. stress has been shown to increase PD symptoms in humans; and, 2. animal PD models have shown that stress is deleterious and cancels out the positive treatment effect of exercise. Smith et al (2002) reported that animals subjected to restraint stress and then immediately injected with 6-OHDA demonstrated significantly greater behavioral impairments than animals that were just injected with 6-OHDA. They

argue that this stress may actually increase the vulnerability of dopaminergic neurons to subsequent insult, a phenomenon that Sapolsky termed “neuroendangerment” (Sapolsky, 1992). Howells et al (2005) provided validity to this notion by demonstrating that mild stressors were able to cancel out the neuroprotective effects of voluntary exercise in 6-OHDA-lesioned animals (Howells et al., 2005). In addition, others have demonstrated that stress increases motor impairments in the 6-OHDA model (Snyder et al., 1985). In the present study, rats were exposed to a forced exercise regimen during the light cycle which is not a not a typical time for increased activity and is, therefore, a potential mild stressor. In addition and perhaps more stressful, the rats were forced to run on a treadmill with an aversive electrical stimulus. When the rats would stop running or were non-compliant with running they would receive an unpleasant electrical shock. While most rats learned quickly, the occasional slowing on the treadmill was a quick reminder that a painful shock was ever present. This may have created a stressful milieu and may have negated the neuroprotective effect of exercise. A more sophisticated research design would be necessary to verify this assertion.

In the secondary analysis on weight gain, all of the rats increased weight from the start of the study to just before the 6-OHDA lesioning. This suggests that the forced running did not differentially slow weight gain compared to the sedentary non-runner group. Since the rats were fed ad libitum, the logical supposition is that the increase in physical activity was met by a concomitant increase in food intake. However, the main effect for group does indicate that the forced runners did not weigh as much as the non-runners. However, closer inspection shows that the runners started out lighter and

finished lighter than the non-runners; the trajectory of their weight gain was the same as the non-runners so it is not likely that differential weight gain may have played a role in the motor impairment (Figure 19). In addition, because the weights of the animals were all within a relatively tight range, the stereotactic coordinates for the 6-OHDA injections were the same and not likely to be affected by differences in animal or head size.

It was noted that during recovery shortly after the 6-OHDA injections (before the rats had achieved full consciousness) that many of the rats would start to turn their neck sharply to the right (Figure 15). Because this was an unusual post-surgical presentation for an animal and because it was ipsilateral to the 6-OHDA lesion, the presence of this right sidebending was recorded for further analysis as a potential indicator of lesion severity. The results of this analysis indicate that this immediate right neck turning was, in fact, related to the severity of the lesion with significant point biserial correlations for all three of the behavioral tests with the highest correlations for apomorphine rotations and exploratory rearing (Table 15). Based on these results, it appears that the immediate right neck turning is an early indicator of the 6-OHDA injection success. In light of these findings, further assessment of right neck turning after 6-OHDA injection as a possible immediate behavioral indicator of successful placement into the medial forebrain bundle is warranted.

CONCLUSION

A preconditioning of regular forced exercise did not protect 6-OHDA-induced hemiparkinsonian behavioral asymmetry prior to the onset of the lesion in adult rats. These results are not consistent with previous data that has shown a neuroprotective effect of forced exercise on subsequent neurotoxic insult and cast doubt about the validity of these previous findings. In light of the present results, further research is warranted.

CHAPTER 4

A comparison of voluntary and forced exercise in driving neuroprotection of nigrostriatal pathways in a juvenile hemiparkinsonian rat model

Abstract: Several studies have found a neuroprotective effect of forced exercise in rodent Parkinson's disease models; however, the evidence for the protective effect of voluntary exercise is mixed. Most of these studies have initiated the exercise after toxin-induced hemiparkinsonism. Few studies have investigated the role of a preconditioning of exercise prior to neurotoxic insult. Therefore, the purpose of this study was to explore the neuroprotective effect of regular forced and voluntary exercise in recently weaned rat pups prior to an adult hemiparkinsonian lesion. Recently weaned rat pups were randomized into four 6-week experimental groups: forced exercise, voluntary exercise, control, and a sham surgery control. After participation in a 6-week experimental condition, hemiparkinsonism was induced using a unilateral injection of 6-hydroxydopamine (6-OHDA). Parkinsonian behavioral tests (i.e., apomorphine rotations, forelimb placement asymmetry, exploratory rearing) demonstrated significant motor asymmetry for all three 6-OHDA group; however, there were no significant differences among them. The sham control rats did not show motor impairment consistent with nigrostriatal motor deficits. Neither a preconditioning of forced nor voluntary exercise was neuroprotective of a future 6-OHDA lesion. These results are in contrast the literature and challenge the validity of these previous studies.

INTRODUCTION

Exercise produces a plethora of benefits in those who have been diagnosed with Parkinson's disease (PD) (Crizzle & Newhouse, 2006; Goodwin et al., 2008; M. A. Hirsch & Farley, 2009). In fact, many national and international panels on the topic have recommended the use of exercise to improve outcomes, activities of daily living and motor performance in those with PD (Horstink et al., 2006; Keus et al., 2007; Suchowersky et al., 2006b). Others have suggested the type of exercise is an important prescriptive consideration in PD (Ridgel et al., 2009). While these studies collectively suggest that functional outcomes are indeed improved with general exercise, they add little knowledge as to how exercise and different exercise parameters influence the underlying disease processes of PD. For this reason, studies using comparative-control designs with animal models of PD are needed to explore these relationships.

In animal models, there are two different exercise modalities, forced exercise and voluntary exercise. While several studies have found a neuroprotective effect on PD with forced exercise (Cohen et al., 2003; Tillerson et al., 2002a, 2003; Tillerson et al., 2001), the results with voluntary exercise are not as well developed. O'Dell et al (2007) found that voluntary wheel running facilitated motor recovery following toxin-induced PD but does so without sparing of dopamine terminals (O'Dell et al., 2007). Mabandla et al (2004) found that voluntary running using the same PD model did prevent apomorphine-induced contralateral rotations suggesting a sparing of dopaminergic neurons (Mabandla et al., 2004). Much like forced exercise, these latter two studies suggest that voluntary exercise may also offer some benefit in rodent models of PD.

One of the key differences between these two exercise modalities is that voluntary exercise allows the animal freedom to choose the timing and dosage of the exercise, whereas forced exercise removes this aspect and usually relies on an aversive stimulus to condition the behavior. In the case of rat forced exercise models, this usually means an electrical grid on the back of a rodent treadmill that administers an electrical shock should the rat slow down or stop running. Unpleasant “encounters” with the electrical grid due to running noncompliance is what conditions running behavior. While this aversive stimulus is effective at conditioning running behavior, it can create a stressful atmosphere for the animal. Voluntary running occurs when the animal decides, usually in the dark cycle; whereas, forced running usually occurs during the light cycle for researcher convenience. The light cycle is typically a time of less activity for the animal and so it is plausible that this may add to the already stressful environment of forced running. Importantly, stress has received traction in the literature as a key factor in the loss of dopaminergic neurons that underlies PD (Smith et al., 2002). In support of this notion, Howells et al (2005) demonstrated that even mildly stressful conditions can negate the neuroprotective effect of exercise in a rat model of PD (Howells et al., 2005).

A recent review from the literature (Ang & Gomez-Pinilla, 2007) suggests that forced exercise and voluntary exercise have different effects on brain neurochemistry and behavior. Leasure and Jones (2008) found that rats in a forced exercise condition ran about the same distance as rats that voluntarily exercised (Leasure & Jones, 2008). However, they report that voluntary exercise rats ran faster and for less total time than

rats that were forced. Additionally, they found that rats in the forced exercise condition exhibited more anxiety-like behaviors compared to the voluntary exercisers despite the fact that there was no difference in corticosterone between the two groups. Leasure and Jones (2008) further suggest that it is imperative that neural and behavioral effects of different forms of exercise using the similar parameters need to be addressed in greater detail scientifically (Leasure & Jones, 2008).

Almost all of the aforementioned studies on the effect of exercise neuroprotection have administered the experimental exercise condition after the onset of toxin-induced PD. Some would argue that this is not technically neuroprotection but rather neuroplasticity of undamaged dopaminergic neurons. While the convention is to use the term “neuroprotection,” it is not technically correct; neuroprotection is technically something that protects nerves from future insult, not a “treatment” administered after the onset of the disease. Few studies have used a true “neuroprotection” design, wherein an exercise program was administered to rats before a 6-OHDA-induced PD (Cohen et al., 2003; Mabandla et al., 2004; O'Dell et al., 2007). While the designs of these studies certainly makes sense from a neuroprotective perspective, they may not capture the true neuroprotective effect of exercise throughout the life of the animal as they were all administered to adult rats. For this reason, the purpose of this study was to explore the neuroprotective effect in terms of parkinsonian behavioral signs of regular forced and voluntary exercise in recently weaned rat pups until they received an adult, neurotoxin-induced PD lesion.

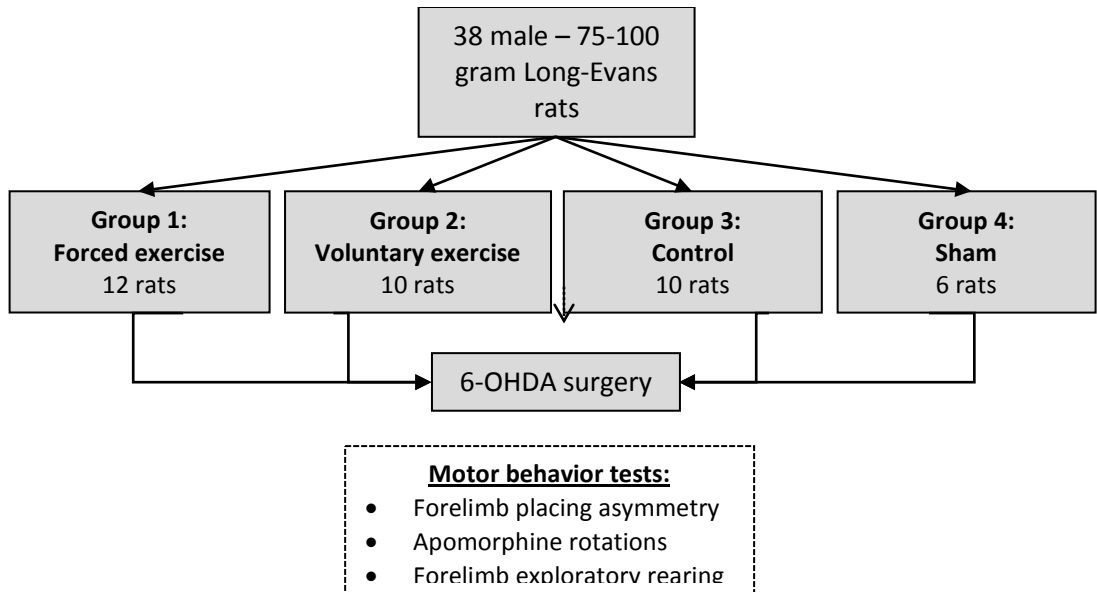
METHODS

Overall study design

Thirty-eight male recently weaned Long Evans rats (Charles River Laboratories, California, USA) were randomized into one of four different experimental groups prior to induction of hemiparkinsonism using an injection of the dopaminergic neurotoxin, 6-hydroxydopamine hydrochloride (6-OHDA). The four experimental groups each lasted 6 weeks and consisted of the following: forced exercise, voluntary exercise, control, and sham surgery control (Figure 14). The original randomization allocation was 10 rats to each of the experimental groups and 6 to the sham group. Because two additional rats were delivered by Charles River Laboratories, randomization was changed to a stratified randomization plan wherein the two additional rats were allocated to the forced exercise group since attrition from non-compliance with forced running has been a concern in previous studies conducted by this investigator; therefore, it was felt that a few extra animals in the forced running group would help in the case of drop out from running non-compliance. After the 6-week experimental treatment and 6-8 days following 6-OHDA lesioning, neuroprotection was tested using three different motor behavioral tests (forelimb asymmetry, apomorphine rotations, exploratory rearing).

Rats were housed individually, fed ad libitum, and housed under standard laboratory conditions with a 12 hour light/dark cycle. All experimental conditions were approved by the University of Nevada, Las Vegas Institutional Animal Care and Use Committee and were consistent with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Figure 14. Overall study design.



Experimental conditions

Group 1 (forced exercise) was a 6-week regimen (5 days per week with 2 bouts of 15 minute periods per day starting at a speed of 8 m/min (Kuo et al., 2010) for two weeks and ultimately progressing to 15 meters/minute for the last 4 weeks (Tillerson et al., 2003) using a rodent treadmill with three running lanes (Exer 3/6 Treadmill, Columbus Instruments, USA); generally, three rats ran concurrently. The treadmill was equipped with an electrical grid at the back end of the belt that would elicit an aversive electrical stimulation upon contact with the tail or hind limbs of rats that had slowed or were noncompliant with running. Once rats were conditioned to the aversive electrical stimulus, the intensity of the stimulus was turned down so that any incidental contacts with the tail would not result in a strong electrical shock. In most cases, the stimulus intensity was turned down to imperceptible levels.

Group 2 (voluntary exercise) was a 6-week regimen of voluntary exercise. These rats were housed singly in a plastic housing container that had an attached running wheel with a revolution counter. Running wheel revolutions were recorded daily to determine the overall dosing of exercise. Group 3 (no exercise) was the non-exercise control group. These control rats were housed singly for six weeks with no running wheel and no other opportunities to exercise or run. Group 4 (sham) was essentially the same as Group 3 in terms of housing and exercise; however, sham rats received a saline injection instead of 6-OHDA during the stereotactic surgery. All other surgical procedures for the sham group were identical to the other three groups.

Surgical procedures

After the 6-week experimental treatment, all rats had a neurosurgical injection into their nigrostriatal fibers. The first three groups (except Group 4) had a 6-OHDA neurotoxin injection into the right medial forebrain bundle. Animals were anesthetized using an intraperitoneal injection of 75 mg/kg Ketamine and 0.25mg/kg dexmedetomidine. After the animal had achieved surgical anesthesia, they were shaved and then moved to the stereotactic frame (Kopf 900, David Kopf Instruments, Tujunga, California, USA). The head was placed into the frame using ear bars which were placed into the ear canals. Once the head was symmetrically placed in the frame, the incisor bar was placed and the nose clamp was tightened. Eye ointment (GenTeal 0.3% eye gel, USA) was placed on the eyes and the incision site on the head was generously swabbed with povidone-iodine topical antiseptic. A heating pad was placed under the rat to keep

in warm during the procedure. Following a longitudinal incision to expose the skull and bregma, a small 1-mm burr hole was drilled through the cranium with a mounted dental drill to gain exposure to the cranial cavity using the following coordinates: 3.3 mm posterior to bregma, 1.9 mm lateral to the midline (Paxinos & Watson, 1998; Paxinos et al., 1985; Tillerson et al., 2003; Tillerson et al., 2002b). A 28-gauge, 5 μ l-microliter syringe (Hamilton, Reno, Nevada, USA) was then plunged into the burr hole 8.2 mm ventral to the skull surface and 10 μ g of 6-hydroxydopamine hydrobromide (10 μ g/4 μ l 0.155 M sterile NaCl containing 0.2 μ g/ μ l ascorbic acid; Sigma, St. Louis, MO)(Howells et al., 2005; Mabandla et al., 2004) in 4 μ l of 0.9% saline was injected at a rate of 1 μ l/min for 4 minutes (Mabandla et al., 2004; Moroz et al., 2004; Tillerson et al., 2003; Tillerson et al., 2002b) into the right medial forebrain bundle which contains the dopaminergic nigrostriatal fibers.

The burr hole was closed using bone wax and the incision was sutured with 5-6 sutures using simple interrupted suture patterning. The incision was swabbed in povidone-iodine antiseptic once more to prevent postsurgical wound infection. The entire neurosurgical procedure did not exceed 45 minutes. After removal from the stereotactic frame, rats were brought out of surgical anesthesia using atipamezole, a dexmedetomidine reversal agent, at 1 ml/kg (subcutaneous). After approximately 10-20 minutes, the rats were out of anesthesia and mobile on all fours. However, they were observed for another hour before they were moved back to their plastic housing units. To manage postoperative pain, rats were then injected with 0.01 mg/kg (subcutaneous) buprenorphine into the flank shortly after ambulatory on all fours. They received this

same injection for the next three days. There were no surgery-related animal deaths or any postsurgical infections in any of the animals in this study.

Motor behavior tests

After 6-8 days post-6-OHDA injection, all of the animals were tested for the severity of the disease (apomorphine rotations), the severity of their motor asymmetry (forelimb placement asymmetry), and the extent of their hypokinesia or paucity of movement (exploratory rearing). During this 6-8 day period, the rats in the two exercise groups continued with their same exercise regimen. The rats in the forced exercise condition began treadmill exercise within 24 hours of the hours of the lesion but were run only once for the first two days after the lesion. For the next 4-6 days, they returned to their twice per day exercise protocol. The voluntary exercise rats were returned to their plastic housing units and were given free access to the running wheels.

Apomorphine rotations. Apomorphine rotations were evaluated by a subcutaneous injection of 0.5 mg/kg apomorphine hydrochloride (Sigma, St. Louis, USA) dissolved in saline in the dorsal right quadrant proximal to the pelvis (Joghataie et al., 2004; Mabandla et al., 2004; Roghani & Behzadi, 2001; Tillerson et al., 2002b). Apomorphine rotations are thought to be due to 6-OHDA-induced denervation of the right medial forebrain bundle; this causes the dopamine receptors in the right striatum of the basal ganglia to become hypersensitive due to the paucity of dopamine relative to the contralateral side. Apomorphine stimulates receptors on both sides (i.e., the affected and unaffected basal ganglia) but because the receptors on the right (affected)

are hypersensitive the outcome is a relative increase of right striatal activity compared to the contralateral side. Since the right striatum of the basal ganglia influences motor behavior on the contralateral side (left side), this causes a rapid, repetitive turning of the body away or contralateral (i.e., to the left) from the lesioned side. The number of times the animal rotated 360° in each direction over a 30-minute period was counted at a later time using a videotaped recording that was taken from above the animal during the 30-minute period. Ipsilateral rotations subtracted from the number of contralateral rotations resulted in a net number of contralateral rotations (Poulton & Muir, 2005). Complete 360° rotations within 30 minutes in a round bowl have been shown to correlate with PD lesion severity (Hudson et al., 1993; Metz & Whishaw, 2002). In addition, it has been reported that a rat which turns more than 200 times in a 30 minute period has had a 95% depletion of striatal dopamine (Schwartzing & Huston, 1996). Since apomorphine rotations are directly related to the severity of the dopaminergic attrition, this test has been recognized as a surrogate for severity of the nigrostriatal neurotoxin-induced damage. It has been used as a motor outcome measure in nearly every exercise study that has used the 6-OHDA PD model.

Forelimb placement asymmetry. Forelimb asymmetry was evaluated first by placing the animals in a plastic cylinder (22cm wide by 26 cm high) for 10 minutes and then by videotaping from above their behavior over that time. As the animal would rear for exploration, the number of initial forelimb touches to the walls of the cylinder (i.e., impaired left forelimb first, unimpaired right forelimb first, simultaneous) for each individual exploratory rearing episode was recorded (Moroz et al., 2004; O'Dell et al.,

2007; Poulton & Muir, 2005; Schallert & Tillerson, 1999; Tillerson et al., 2003; Tillerson et al., 2002b). The wall touches were recorded and the asymmetry ratio was calculated using the following: $(\text{Right}-\text{Left})/(\text{Right}+\text{Left}+\text{Both})$ (O'Dell et al., 2007). Scores on the forelimb asymmetry ratio range from -1 to 1 with a high positive indicative of asymmetry favoring the unimpaired forelimb. A negative asymmetry ratio would be indicative of greater left or impaired forelimb usage. Thus, a high positive forelimb placement asymmetry ratio (i.e., greater right, unimpaired forelimb use) would be consistent with a right 6-OHDA-induced hemiparkinsonian lesion.

Exploratory rearing episodes. Each episode of exploratory rearing was counted over the 10 minute videotaped session that was used for forelimb placement asymmetry. This has not been documented previously in the literature but significant correlations with the other behavioral tests in Chapter 3 suggest that it may offer some value in motor behavior; it was felt that exploratory rearing was a logical test for a common symptom in PD, hypokinesia (i.e., paucity of movement).

Data analysis

All analyses were conducted using SPSS version 19.0 (IBM SPSS, Chicago, Illinois, USA). In order to determine if there was a difference among the four experimental groups (forced exercise, voluntary exercise, control, and sham control), a one way analysis of variance (ANOVA) was conducted for each of the three behavioral tests (apomorphine rotations, forelimb placement asymmetry, and exploratory rearing) that were conducted immediately after the 6-week treatment ended (Figure 14). When

significant violations of assumptions occurred than non-parametric equivalents were used in lieu. In an exploratory secondary analysis, spearman correlations were conducted for the immediate post-surgical observation (i.e., within 20 minutes) of right neck turning (0 = no neck turn, 1 = delayed and mild neck turn, 2 = delayed but profound neck turn, 3 = early and severe neck turn) to the three other behavioral tests in the study (Chapter 3; Figure 9). Significant correlations of this were found in study 2 of this dissertation (Chapter 3; Table 15). As this has been the only line of evidence to suggest that this immediate post-operative phenomenon may be related to 6-OHDA nigrostriatal neurotoxic damage, this observation was again noted in this study for exploratory analysis. Data in the present study were also analyzed using point biserial correlations (similar to Chapter 3 results) to see if the correlations were in the same range as Chapter 3 results; immediate post-surgical right neck turning was dichotomized (1 = no or mild neck turn, 2 = profound neck turn) and reanalyzed using correlations among the three behavioral tests with point biserial correlations. An additional exploratory analysis was to compare the weight of the four groups during the experimental treatment. It was thought that because of the difference in activity level among the four groups there was a possibility that a significant difference in weight/growth may have been a threat to internal validity as the stereotactic coordinates would have been different for different size animals. This analysis was conducted using an 8 (time: 7 preoperative measurements, 1 postoperative measurement at the time of surgery) by 4 (group: forced, voluntary, control, sham) mixed factorial ANOVA. The last exploratory analysis was a comparison between the

two exercise groups on the dosing of exercise. This was done by comparing the average daily distance ran in meters and comparing the preoperative treatment with the postoperative treatment using a 2 (group: forced and voluntary) by 2 (time: preoperative average and postoperative average) mixed factorial ANOVA.

RESULTS

Primary analyses

Because of a violation of homogeneity of variance ($P = 0.05$) coupled with unequal group sizes, a Kruskal-Wallis non-parametric test was run for the apomorphine data. There was a statistically significant difference among the medians of apomorphine rotations, $H(3) = 9.754$, $P = 0.021$. Pairwise comparisons were run using the nonparametric Mann-Whitney test. All three of the groups that had the 6-OHDA injection (i.e., voluntary, forced, control) rotated significantly more than the one group that had a saline injection (i.e., sham), $P_s \leq 0.018$ (Table 17, Figure 15). There were no differences in apomorphine rotations among the three groups that had the 6-OHDA injection, $P_s \geq 0.111$.

Table 17. Apomorphine rotation descriptive statistics for the forced, voluntary, control and sham groups.

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval	
						Lower Bound	Upper Bound
Apomorphine rotations	Forced	12	131.3	93.3	26.9	72.07	190.60
	Voluntary	9	75.0	74.8	24.9	17.50	132.50
	Control	10	90.6	74.3	23.5	37.44	143.76
	Sham	6	-11.5	14.4	5.9	-26.59	3.59

Figure 15. Boxplot of apomorphine rotations for the forced, voluntary, control and sham groups.

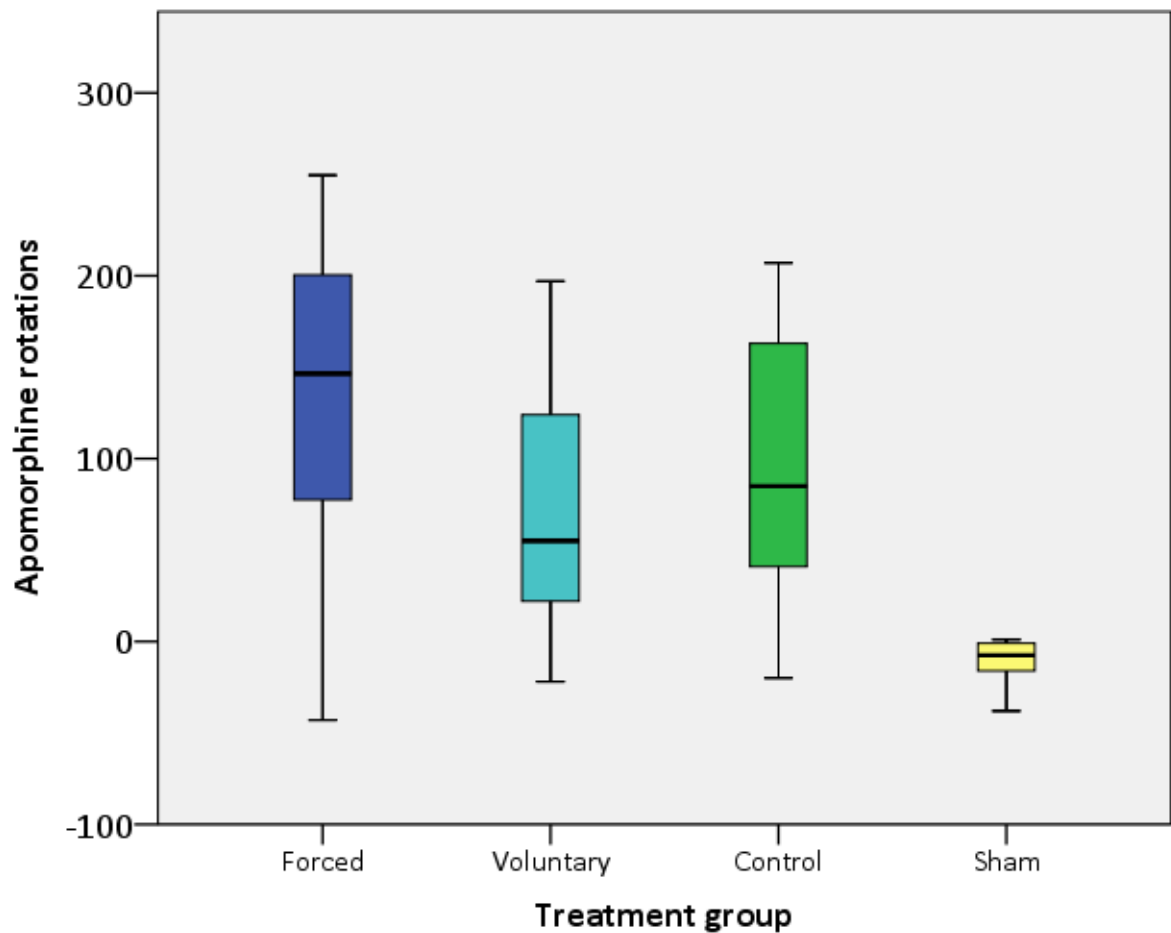


Figure 15. Means and standard deviations for the number of apomorphine-induced contralateral rotations during a 30-minute testing period for runners and non-runners before and after 4 weeks of experimental treatment.

There was a statistically significant difference among the means for the four groups for forelimb placement asymmetry, $F(3,37) = 8.509$, $P < 0.001$. Tukey pairwise comparisons revealed that the three groups that had the 6-OHDA injection had significantly more forelimb asymmetry than the sham group, $P_s \leq 0.001$ (Table 18, Figure 16). The forelimb asymmetry was essentially the same for the three 6-OHDA groups, $P_s \geq 0.909$.

Table 18. Forelimb placement asymmetry descriptive statistics for the forced, voluntary, control and sham groups.

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval	
						Lower Bound	Upper Bound
Forelimb placement asymmetry	Forced	12	-.703	.385	.111	-.9477	-.4586
	Voluntary	10	-.793	.194	.061	-.9316	-.6536
	Control	10	-.776	.385	.122	-1.0514	-.5011
	Sham	6	-.057	.084	.034	-.1447	.0305

Figure 16. Boxplot of forelimb placement asymmetry for the forced, voluntary, control and sham groups.

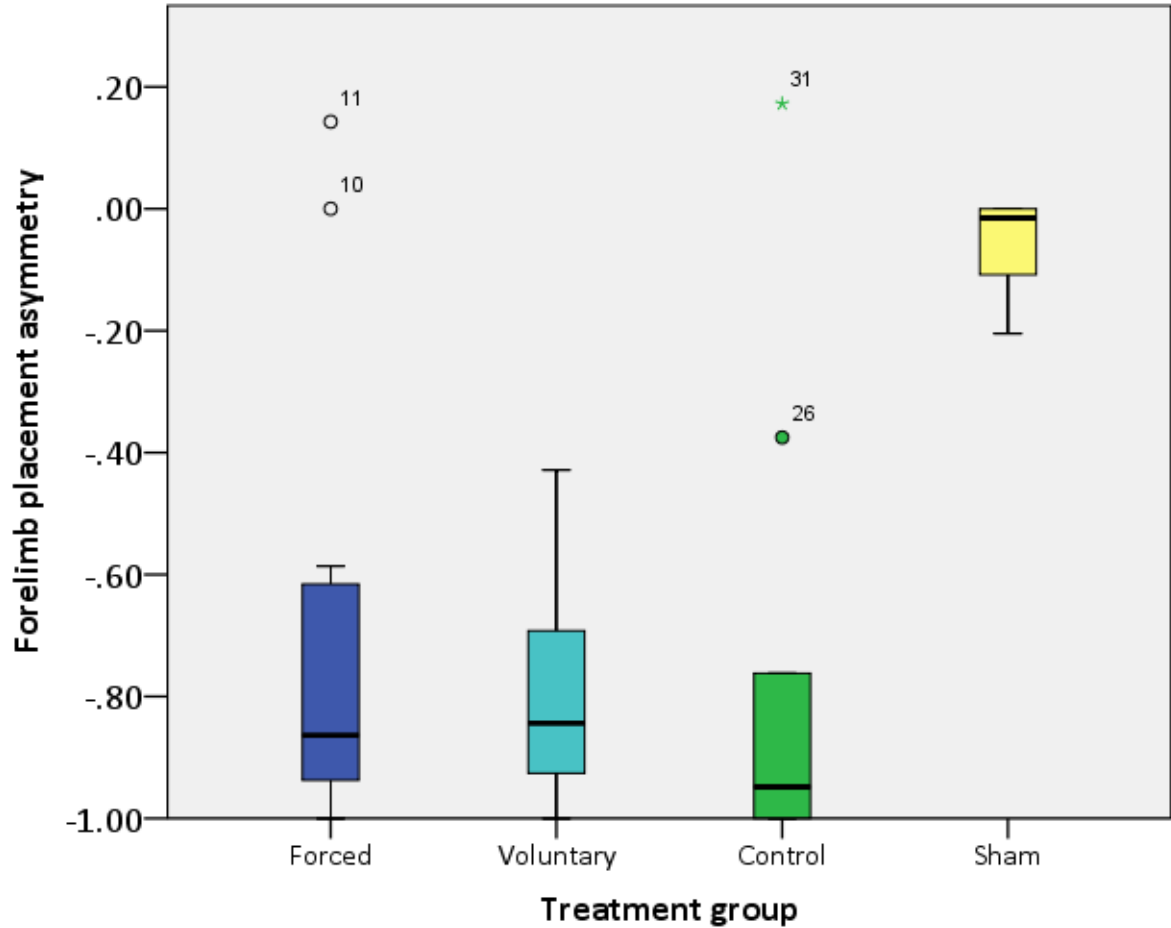


Figure 16. Means and standard deviations for the forelimb placement asymmetry ratio during a 10-minute testing period for runners and non-runners before and after 4 weeks of experimental treatment.

The number of exploratory rears in a 10 minute period was not the same for all four of the experimental groups, $F(3,37) = 6.436$, $P = 0.001$. The number of exploratory rears was similar among the three groups that had the 6-OHDA injections, $P_s \geq 0.842$;

however, each of these three 6-OHDA injection groups had fewer rearing episodes than the sham lesion group, $P_s \leq 0.008$ (Table 19, Figure 17).

Table 19. Exploratory rearing descriptives for the forced, voluntary, control and sham groups.

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval	
						Lower Bound	Upper Bound
Exploratory rearing	Forced	12	23.2	13.9	4.0	14.3634	31.9699
	Voluntary	10	18.7	13.2	4.2	9.2816	28.1184
	Control	10	20.0	9.9	3.1	12.8863	27.1137
	Sham	6	45.0	13.1	5.4	31.2048	58.7952

Figure 17. Boxplot of exploratory rearing for the forced, voluntary, control and sham groups.

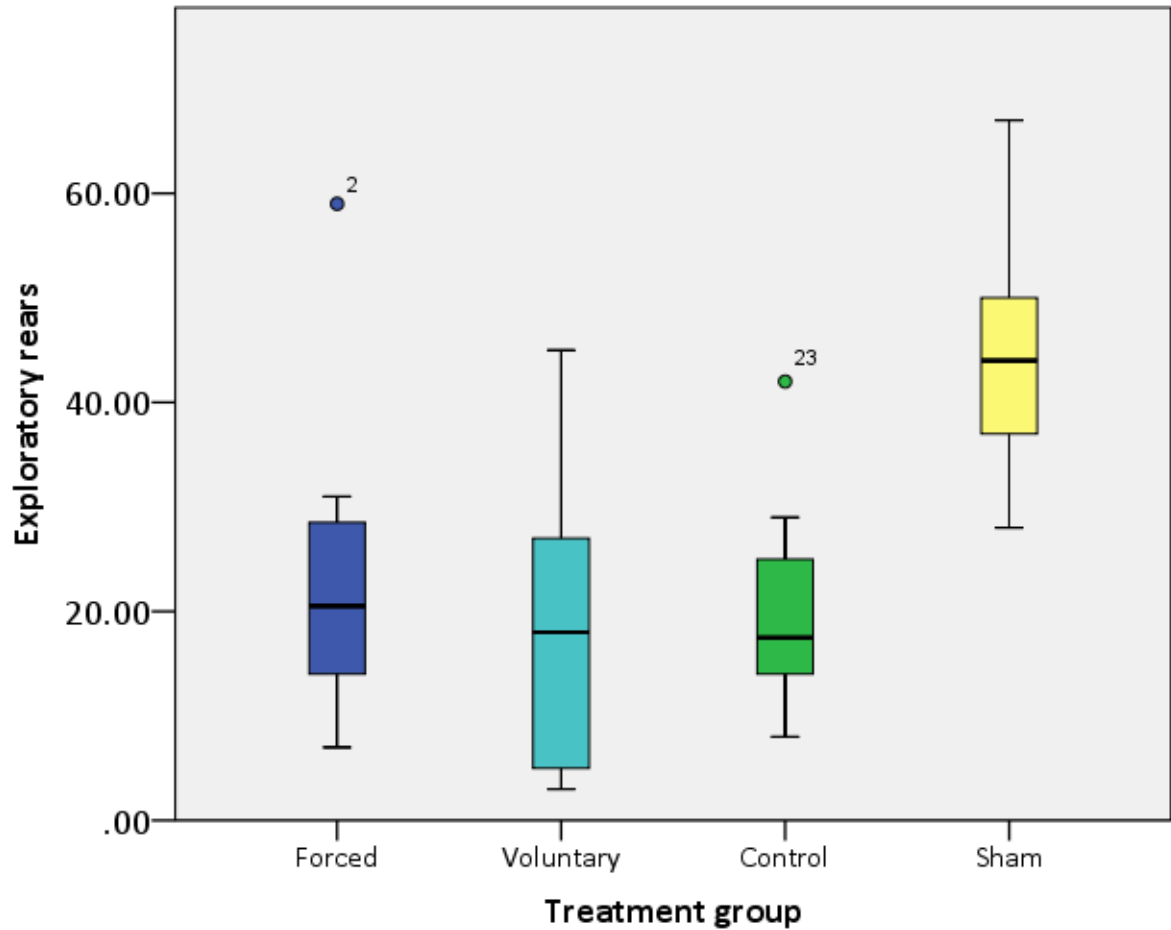


Figure 17. Means and standard deviations of the number of exploratory rearing episodes during a 10-minute testing period for runners and non-runners after 6 weeks of the experimental conditions.

Secondary analyses

Significant Spearman correlations were observed for the immediate post-surgical observation of right neck turning to the three other behavioral tests in the study (Table

20). The values for the correlations among the three behavioral tests in Table 20 are Spearman's correlations. The point biserials of the dichotomized neck turning to the behavioral tests were the following: apomorphine rotations ($r = 0.480$), forelimb placement asymmetry ($r = -0.357$), and exploratory rearing ($r = -0.479$).

Table 20. Spearman correlations for right neck turn after surgery of the three behavioral tests.

	Right neck turn after surgery	Apo-morphine rotations	Forelimb asymmetry	Exploratory rearing
Right neck turn after surgery	1	.492 ^{**}	-.363 ^{**}	-.363 ^{**}
Apomorphine rotations		1	-.633 ^{**}	-.383 [*]
Forelimb asymmetry			1	.444 ^{**}
Exploratory rearing				1

^{**} Correlation is significant at the 0.01 level (2-tailed)
^{*} Correlation is significant at the 0.05 level (2-tailed)

The weight of the recently weaned rats increased significantly over the course of the trial, $p < .001$; however, there was no difference in the rate of weight gain among the four groups over time, $F(21,238) = 1.810$, $P = 0.100$, power = 0.682 (Table 21, Figure 18).

Table 21. Weight gain for all of the rats over the course of the study.

time	Mean	Std. Deviation	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Pre	53.8	6.1	0.9	52.0	55.7
1	117.8	13.1	2.2	113.3	122.2
2	167.7	14.3	2.3	163.0	172.5
3	214.0	18.1	3.0	207.8	220.1
4	264.6	18.5	2.9	258.7	270.5
5	298.6	21.9	3.7	291.1	306.1
6	328.0	25.6	4.0	319.9	336.2
Post	323.8	31.7	5.1	313.4	334.2

Figure 18. Weight gain for each of the four treatment groups over the duration of the study.

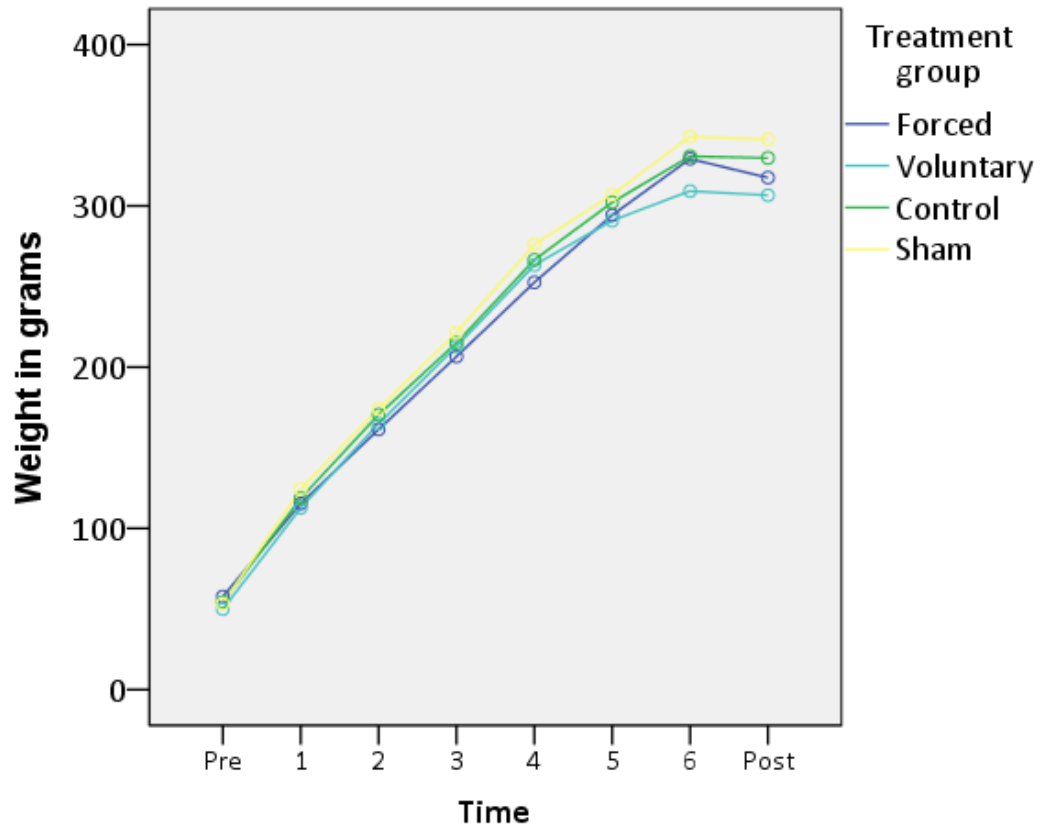


Figure 18. Means and standard deviations of the weight in grams of the rats in the four experimental conditions at each of the 6 weeks preceding the 6-OHDA lesion and one week after the lesion.

In terms of the difference between the two exercise groups, voluntary and forced, in the average number of meters ran per day, the voluntary group ran significantly more than the forced group before and after the surgery, $P < 0.001$ (Figure 19).

Figure 19. Average distance ran in meters for the 6 weeks pre surgery and the 1 week post-surgery for the voluntary and forced exercise groups.

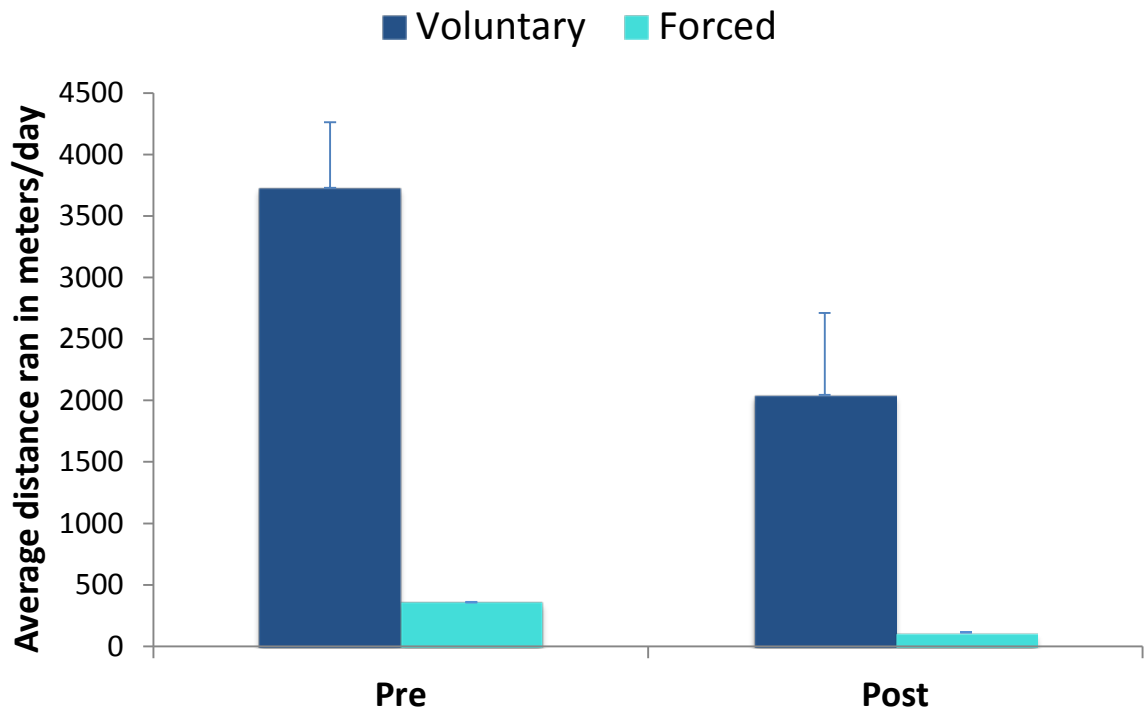


Figure 19. Means and standard deviations of the average distance ran in meters in the forced and voluntary exercise conditions in the 6 weeks before and the one week after the 6-OHDA lesioning.

Figure 20. Average daily distance in meters ran for each rat in the voluntary running group.

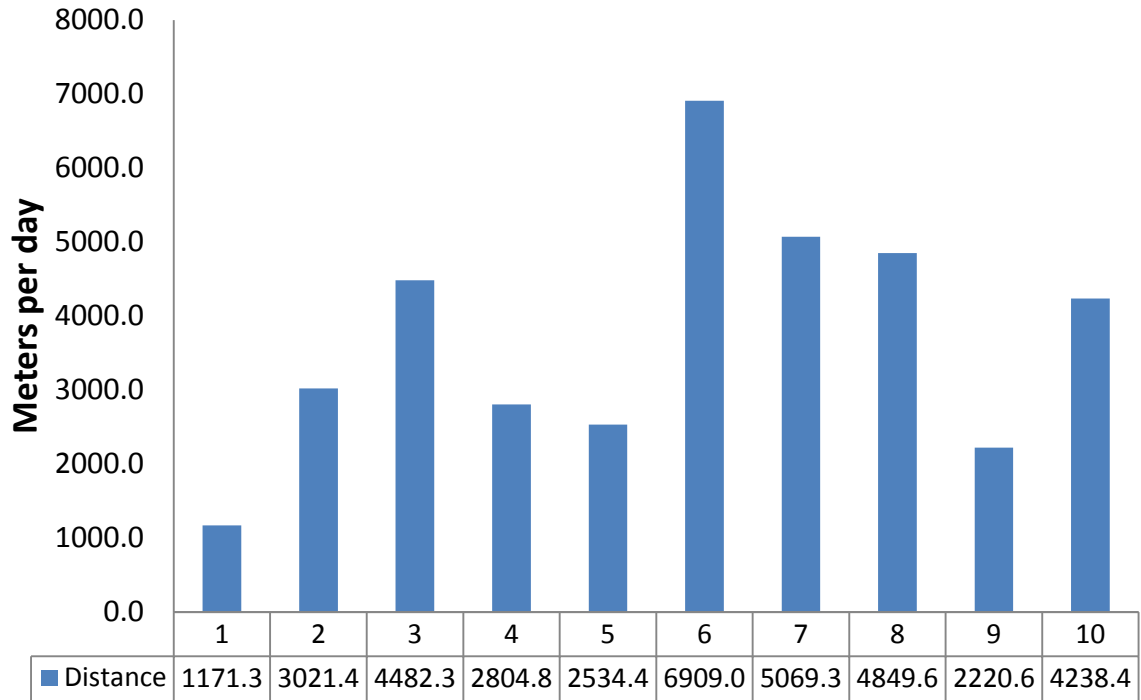


Figure 20. Means of the distance ran in the 6 weeks of voluntary exercise for each of the rats in the voluntary exercise group.

Figure 21. Daily totals in running wheel clicks over the 6 weeks of voluntary running and the one week after running.

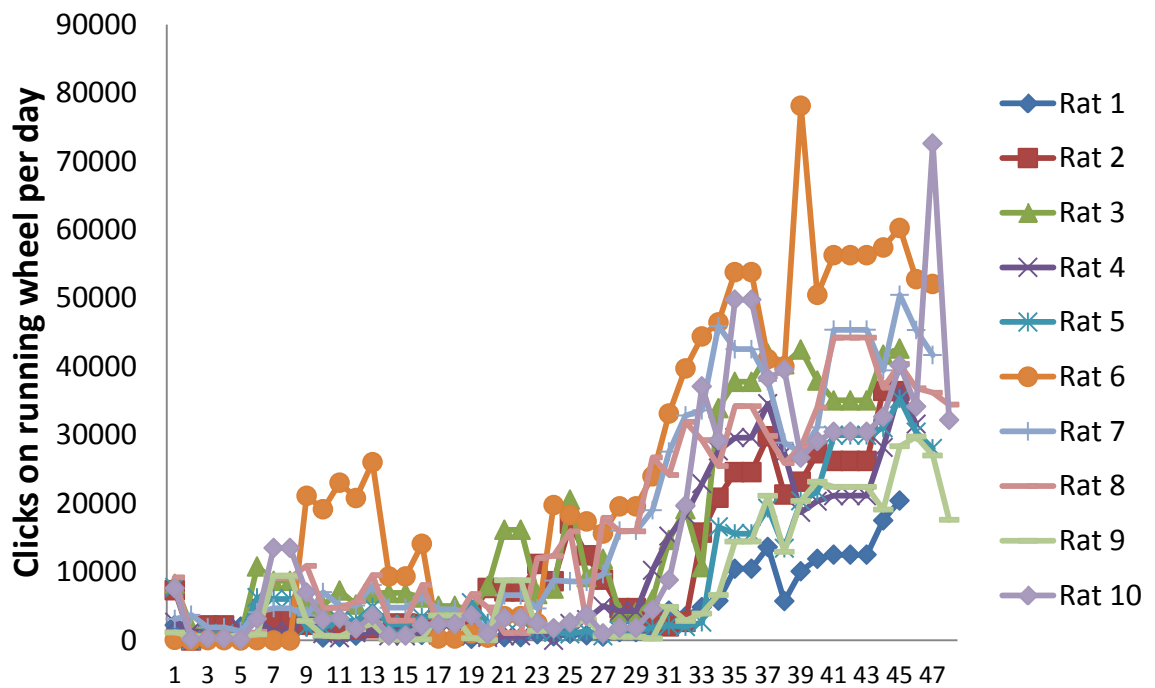


Figure 21. Daily means of the distance ran for each of the rats in the voluntary running group showing the increase in trajectory of running during the 6 weeks prior to 6-OHDA lesioning.

DISCUSSION

While previous research has provided several lines of evidence that exercise in rodent models of PD is neuroprotective, the results of the present study do not support this notion. In addition, it has been demonstrated that neither forced nor voluntary running affords neuroprotection in 6-OHDA-induced hemiparkinsonian rats. Despite the novel design element of introducing the exercise shortly after weaning, which would be akin to exercising throughout life, there was no evidence that exercise provided

neuroprotection from 6-OHDA-induced behavioral asymmetry. These results are in contrast to the body of literature on exercise neuroprotection in PD and cast doubt on the validity of the notion that exercise is neuroprotective in PD.

Several human studies have shown that greater baseline exercise in early life is associated with a lower risk of PD (Chen et al., 2005; Sasco et al., 1992; Tsai et al., 2002). This exercise occurred before PD diagnosis and presumably before the pre-clinical phase had begun. This was an important consideration in the casting of the design of the present study. Namely, that implementation of a putative neuroprotective treatment should happen before the onset of the disease rather than after the fact on while the disease is in process. All of the previous work using a true neuroprotective design (i.e., exercise implemented before the neurotoxic lesion) had staged the implementation of the exercise to rats who were physically mature adults. Since neurodevelopment is most robust in young and adolescent brains, it was felt that an important neuroprotective window of opportunity may have not been appropriately vetted scientifically. Thus, in the present study, exercise was implemented shortly after weaning and continued for 6 weeks, which would be well into the maturity of the animal. From a design perspective, the implementation of the exercise shortly after weaning was novel; however, the results of this study did not show any positive neuroprotective benefit from exercise throughout the post-weaned lifespan of the animal.

One of the premises of the study was that forced running, because of an aversive electrical stimulus, would be traumatic to the animal and that this would create a

stressful milieu that would reduce the neuroprotective effect of exercise as was shown in the Howells et al (2005) study (Howells et al., 2005). In accordance with Howells et al (2005), Smith et al (2002) hypothesized that stress may actually endanger dopaminergic neurons, thereby potentiating dopamine attrition (Smith et al., 2002). Thus, this study was designed with a comparative-control design that would hopefully clear up the hypothesis that exercise was truly neuroprotective; the other main premise of the study. Two exercise modalities, forced (i.e., stressful) and voluntary (i.e., non-stressful), were compared to a no exercise control and a no exercise sham control. If stress was indeed deleterious and if exercise was neuroprotective, then we would have expected to see the voluntary running group with forelimb placement symmetry similar to or close to the sham group and the forced running group with behavioral asymmetry similar to or close to the no exercise control group. Neither of these happened in the present study. Instead, there were no statistically significant differences among the forced running, voluntary running and control for any of the motor behavior tests suggesting no treatment effect of the exercise and no deleterious effect in the “stressful” forced running group. These three groups did have significantly more behavioral asymmetry than the sham group which suggests that they all had significant motor signs and asymmetry consistent with the 6-OHDA-induced PD model. Based on the results of this study, neither of the two main premises (i.e., exercise is neuroprotective, forced exercise is deleterious) of this study were upheld.

Since there appears to be differences in the means of all three behavioral tests (not statistically significant) that are somewhat consistent with exercise being

neuroprotective (Figure 15-17), it is plausible that a type 2 error may have occurred since the study was clearly underpowered. That is, exercise may be neuroprotective but the treatment effect was so small that it would require a much larger sample size to realize a statistically significant difference. Using the effect size indices in the present study (apomorphine rotations $f = 0.309$; forelimb placement asymmetry $f = 0.124$; exploratory rearing $f = 0.161$) and 80% power estimate, it would require the following approximate sample sizes for differences to become statistically significant for the two exercise groups and the control (sham was not factored into the estimate): apomorphine rotations (36 rats per group; 108 rats total); forelimb placement asymmetry (322 rats per group; 966 rats total); and, exploratory rearing (144 rats per group; 432 rats total). Based on the results of the present study, either there is no treatment effect at all (i.e., exercise is not neuroprotective) or there is only a small treatment effect (i.e., mildly neuroprotective). The latter would represent a type 2 error since we did not find a statistically significant difference among the three 6-OHDA lesioned groups; however, powering a study to verify this would be implausible with sample size estimates ranging from 108 to 966 total rats needed. Moreover, the risk-to-benefit ratio in light of animal mortality would not be warranted given the mild treatment effect.

One of the expectations of this study was that the rats in the voluntary running group would run more on their running wheel than the forced running group would on the treadmill. What was not expected, however, was how much more the voluntary running group would run. On average, the voluntary running rats ran more than 3.5

kilometers per day before the 6-OHDA lesion, whereas the forced rats averaged less than 500 meters per day before the 6-OHDA lesion, almost a seven fold difference in the dosing of exercise (Figure 19). Clearly the forced exercise group could have tolerated more exercise; however, this would have been difficult considering the man power necessary to the run the animals for that length of time (i.e., at least 3.5 more daily hours per rat). In addition, after the 6-OHDA lesion in the week before the last motor behavior test, the voluntary rats ran on average 10 times more than the forced rats in the same period, >2,000 meters and <200 meters per day, respectively. If exercise was truly neuroprotective, then a dose dependent neuroprotective relationship in the motor behavior tests would have likely been observed. That is, if exercise was beneficial, it would have been expected that the forced running group would have less motor impairment than the no exercise control group and an even greater amelioration of motor impairment in the voluntary running group. Since this clearly did not happen in the present study, the logical conclusion is that exercise does not afford a neuroprotective benefit.

The trajectory of voluntary running over the course of the study (6 weeks pre-lesion and 1 week post-lesion) show a gradual increase in running over time until the 6-OHDA lesioning and a decrease after surgery (Appendix, Figure 20). Thus, as the rats got older and more acclimatized to the running wheel they began to run more. This is not fully consistent with Peng et al (1980) who demonstrated that rats decrease running activity as they age (Peng et al., 1980); though, it should be noted that the rats in that study were older than the rats in the present study when they started their running.

Whether they were running faster or for longer durations or both in comparison to the forced exercise group is not known as this was not specifically tracked. As rats are nocturnal, it is not surprising that the majority of their running episodes occurred in the dark cycle. Unfortunately, this too was not documented; however, qualitatively, it was the observation of the research team that the rats did not run during the day and this is consistent with the literature which shows running activity is about 80% nocturnal (Peng et al., 1980). The volume of the running in the voluntary group was also remarkable (Appendix, Figure 21). Five of the ten rats averaged more than 4 kilometers of running per day and one of these averaged 7 kilometers per day. The rat that ran the least still ran more than twice the average distance of the forced exercise rats. While the distances in voluntary running seem quite high, rats are known to be fairly prodigious voluntary runners with one documented case as high as 43 kilometers in a 24 hour period (Richter, 1927). Since rats are clearly capable of much greater distances than forced treadmill exercise protocols permit and since forced exercise typically uses aversive stimuli to condition the running behavior, it is recommended that this approach to exercise in rodents become obsolete.

The secondary analysis of the correlation between the immediate post-operative observation of right neck turning and the motor behavioral tests conducted a week later suggests that this observation is indeed related to the severity of the neurotoxic 6-OHDA lesion (Table 20). The more profound the right neck turns immediately after surgery, the more apomorphine rotations, the higher the preference for use of the unimpaired forelimb, and the fewer exploratory rearing episodes. Based on these

findings, this novel observation, that has not been previously reported in the literature, can be used as an immediate indicator of successful placement of the neurotoxin 6-OHDA in the medial forebrain bundle. The fact that this observation begins almost immediately (within 10-15 minutes) post-operatively suggests that this neurotoxin has an almost immediate damaging effect on the nigrostriatal fibers. However, it is known that 6-OHDA toxicity may be quite protracted and does not realize its full neurotoxic potential for up to 28 weeks after injection (Bjorklund et al., 1997); so, clearly it is just an early indicator of 6-OHDA toxicity to the medial forebrain bundle.

The secondary analysis of weight gain throughout the study suggests that all of the rats, regardless of group assignment, had the same trajectory of weight gain over the course of the study (Table 21, Figure 18). The general weight gain trajectory in the present study was consistent with the Kretschmer et al (2005) study (Kretschmer et al., 2005). However, in the Kretschmer et al (2005) study, rats who exercised for 12 minutes daily reduced their body weight over time. This did not happen in the present study. Presumably, the rats in the two groups with an exercise component (i.e., voluntary, forced) must have had a compensatory increase in food consumption to keep up with the increased metabolic demand from the exercise. Likewise, it is likely that the two none exercise groups (i.e., control, sham) did not consume as much because the metabolic demand of sedentary behavior was not as high as the exercise groups. However, these assumptions are speculative as the amount of food and fluid intake was not observed or recorded. All rats had access to standard laboratory chow ad libitum; so, it is logical that food intake may have been compensatory depending on the energy

expenditure and metabolic demand of their daily tasks. The literature is not consistent on this point. Kretschmer et al (2005) showed no compensatory food intake in exercised rats (Kretschmer et al., 2005); however, Levin and Dunn-Meynell (2004) found that food intake was increased in exercising rats and Peng et al (1980) found a compensatory increase in food intake with voluntary running (Levin & Dunn-Meynell, 2004; Peng et al., 1980). The Kretschmer et al (2005) study used a time limited swimming regimen, whereas, the Levin and Dunn-Meynell (2004) study used rats that had free access to a running wheel, much like the voluntary running regimen in the present study (Kretschmer et al., 2005; Levin & Dunn-Meynell, 2004). Monitoring the weight gain was an important consideration in the design of the present study because if there were a significant differential pattern of growth of the animal in the different groups it could mean that the stereotaxic coordinates of the 6-OHDA injection may not have been in the same area of the medial forebrain bundle. Considering the inherent variability of stereotactic injections and variability in the size and shape of head, another source of potential error (i.e., difference size of the animal) would have been a threat to the internal validity of the study.

CONCLUSION

A 6-week preconditioning of forced and voluntary exercise did not afford neuroprotection to recently weaned, 6-OHDA-lesioned rats in terms of motor behavioral tests. Voluntary exercising, which had a 10 fold increase in exercise dosing compared to forced exercising was not better in terms of protection from motor behavioral

asymmetry than forced exercise. In addition, neither was better than a no exercise control. These results are not consistent with the body of literature on neuroprotection and suggest that there is no consensus on the premise that exercise is neuroprotective. Since the present study casts some doubt on the role of exercise as a neuroprotective agent, further research is warranted.

CHAPTER 5 SUMMARY, SIGNIFICANCE, AND FUTURE STUDIES

The concept of neuroprotection in the neurodegenerative diseases (e.g., PD, Alzheimer's disease (AD)) has been at the forefront of the research agenda for neuroscientists for decades. It hinges on the theory that these neurodegenerative diseases have an onset later in life and that the disease course (i.e., neurodegeneration) could possibly be prevented or lessened by employing a treatment before the onset of the disease. Many in the neurodegenerative research community have called the search for a viable neuroprotective agent the "holy grail" of research in PD and AD.

Over the last few decades, PD researchers have investigated many putative neuroprotective agents with limited success. Because PD is thought to cause attrition of dopaminergic neurons through oxidative stress, many of these attempts have focused on agents that have strong antioxidant properties (e.g., Coenzyme Q10, creatine) (LeWitt, 2006; Mounsey & Teismann, 2010). Most of these studies met mild or unremarkable results; yet the search for neuroprotective agents continues. This dissertation was yet another attempt on a promising neuroprotective treatment that is inexpensive, easy to implement, free from serious side effects, and has a multitude of other robust benefits to many systems of the body: exercise.

The overarching premise of this dissertation was that exercise was neuroprotective in a rat model of PD. It was based on several studies from rodent PD models that all reported strong treatment effects from exercise in terms of motor protection and positive changes to the underlying dopaminergic pathways (Cohen et al.,

2003; Howells et al., 2005; Tillerson et al., 2003; Tillerson et al., 2001). Despite this fairly strong and consistent evidence from the literature, the results of the studies in this dissertation do not support these findings. In fact, the findings of the present study cast doubt on the role of exercise as a neuroprotective agent. From a clinical perspective, the results of this study are a disappointment for patients with PD and clinicians who treat patients with PD; however, they do suggest that more research is needed and this could potentially open up new lines of evidence about the underlying disease process of PD. Clearly, the notion that exercise slows the neurodegeneration in PD is premature.

When taking into account the differences in the designs of the three studies in this dissertation to those that this research was based on, it is clear that there were some small variations in design; however, they do not represent significant departures in dosing, duration, intensity and housing (Table 22). Instead, an argument could be made that the designs in this dissertation were more rigorous from an exercise perspective than the majority of studies. While it might be appropriate to dissect and scrutinize the differences between the studies, it is also important to understand that if exercise were truly neuroprotective, minor departures (mostly in the direction of increased dosing and intensity) should not negate the neuroprotective effects of exercise. Thus, if exercise were truly neuroprotective as the body of literature suggests then increasing the exercise regimen should actually produce a more robust neuroprotective effect. On the other hand, it could be argued that the differences in design and protocol may have introduced confounding effects that negated the

protection. In either case, if exercise is truly neuroprotective then its positive protective effect should not be so fragile.

Table 22. Design characteristics of the literature on neuroprotection in 6-OHDA models of Parkinson’s disease.

Study	Design	Comparison	Exercise dose	Exercise duration	Other	Housing
Tajiri et al, 2010	Neuroplasticity	Forced vs. control	5 days/week 30 min/day 11 m/minute	Post: 4 weeks	Exercise group had more BrdU + cells in striatum	Housed two per cage
Tillerson et al, 2003	Neuroplasticity	Forced vs. Sham control vs. lesion control	Daily? 30 min/day 15m/minute	Post: 4 weeks	Running occurred during the dark cycle	
Tillerson et al, 2001	Neuroplasticity	Forced (3 start times) vs. Control vs. Sham (3 start times)	Unimpaired forelimb casting	Post: 1 week?		Housed in groups of 3
Tillerson et al, 2002	Neuroplasticity	Forced non-use vs. Sham vs. Control	Impaired forelimb casting	Post: 1 week		Housed in groups of 3
O’Dell et al, 2007	Neuroprotection and Neuroplasticity	Forced+Voluntary vs. Control	Pre: 10.5 m/min 30 min/day Running wheel Post: 6m/min 30 min/day Running wheel	Pre: 2.5 weeks Post: 4 weeks	No preservation of dopamine terminals Most rats ran >1000 meters/day	Individually
Cohen et al, 2003	Neuroprotection	Forced vs. Sham vs. Control	Impaired forelimb casting	Pre: 1 week		Housed two per cage
Mabandla et al, 2004	Neuroprotection and neuroprotection	Voluntary vs. control	Access to running wheel	Pre: 1 week Post: 2 weeks	Pre: 5388 revolutions/day Post: 4361 revolutions/day	Individually
Howells et al, 2005	Neuroprotection and neuroplasticity	Stressed voluntary vs. Voluntary vs. Control	Access to running wheel	Pre: 1 week Post: 2 weeks	Pre average distance: 2182 meters/day Post: 2140 meters/day	Individually
Aim 1	Neuroplasticity	Forced vs. Control	5 days/week 30 min/day 15 m/minute	Post: 4 weeks	450 meters/day	Individually
Aim 2	Neuroprotection	Forced vs. Control	5 days/week 30 min/day 15 m/minute	Pre: 4 weeks	450 meters/day	Individually
Aim 3	Neuroprotection and neuroplasticity	Forced vs. Voluntary vs. Sham control vs. Lesion control	6 days/week 30 min/day 15 m/minute	Pre: 6 week Post: 1 weeks	Forced: 450 meters/day Voluntary: 3500 meters/day	Individually

As the dissertation evolved, three other premises emerged that helped guide the design of the study in Chapter 4. First, a “true” neuroprotective design would implement exercise before the injection of the neurotoxin (i.e., before disease onset). However, the body of literature on the neuroprotective effect of exercise in PD has almost uniformly been implemented in animals after neurotoxic insult (Cohen et al., 2003; Howells et al., 2005; Tillerson et al., 2003; Tillerson et al., 2001). Technically, this is not “neuroprotection” in any sense but rather “neuroplasticity” or “neurorecovery” because the exercise was implemented after the onset of the disease. While it has been convention to use the term “neuroprotection” in these studies, it is misleading. There have been only a few studies that have used a true “neuroprotection” design, wherein an exercise program was administered prior to neurotoxic insult using PD rodent models (Cohen et al., 2003; Mabandla et al., 2004; O'Dell et al., 2007). Therefore, Chapter 2 was technically a “neuroplasticity” design and Chapters 3 and 4 were designed with “neuroprotection” in mind. Since there are very few true “neuroprotective” designs with exercise and PD in the literature, Chapters 3 and 4 were fairly novel.

Another important premise in the design of the study in Chapter 4 was that implementation of exercise in young, recently weaned rats would potentially produce more robust outcomes. Using rats so young was also a novel design element as this has not been reported in the literature. Almost all of the published studies on the effect of exercise on symptoms and neurobiology in rat PD models have used older rats. The reasons for this are not entirely clear. However, one prominent researcher in the field (Timothy Schallert, personal communication) suggested that younger rats are much

more tolerant to the toxin 6-OHDA the Parkinsonian behavioral symptoms. While using older rats would make sense for a study that has incorporated a “neuroplasticity” design wherein the rats are exercised after neurotoxin-induced PD, it does not make sense in a “neuroprotection” design wherein rats are exercised before neurotoxin-induced PD. Unfortunately, neuroprotection studies in PD have almost uniformly used adult rats. This is akin to having humans start an exercise program later in life with the hopes that it will be protective. Perhaps an important “window of opportunity” may have been missed by not starting exercise earlier during brain development. While implementation of exercise in adult rats certainly makes sense from a neuroprotective perspective, these designs may not capture the true neuroprotective effect of exercise throughout the life of the animal as they were all administered to adult rats as was done in Chapter 3. For this reason, the purpose of the study in Chapter 4 was to explore this potential window of opportunity by implementing exercise to recently weaned rat pups. Since it is conventionally thought that young rats are more resistant to the neurotoxin 6-OHDA, the exercise period was lengthened to 6 weeks so that these recently weaned rats would be adults when they were injected with the neurotoxin. If you consider the three studies in this dissertation, older adult rats (Chapters 2 and 3) had considerably more PD behavioral symptoms than the younger adult rats in Chapter 4. While these results would support the assertion that was made by Timothy Schallert (personal communication), the overall findings of these studies suggest that exercise in either adult or recently weaned rats is not neuroprotective in terms of motor behavioral symptoms.

The third premise that emerged in this dissertation and helped guide the design of the study in Chapter 4 was that forced exercise, because of aversive conditioning (i.e., unpleasant encounters with an electrical grid at the back of the rodent treadmill), might not afford neuroprotection because it stressed the animal. Stress has been implicated as a key factor of neurodegeneration in PD (Smith et al., 2002). In addition, it has been shown to negate the neuroprotective effect of exercise in a rat model of PD (Howells et al., 2005). In order to determine if exercise itself was neuroprotective a novel design would be necessary. First, an additional exercise modality (i.e., voluntary exercise) was added to compare it to exercise within a stressful milieu (i.e., forced exercise). If exercise was indeed neuroprotective and if stressed rats were less likely to benefit from this neuroprotection, then this design element would have allowed some inference into the true relationship between exercise and PD. This was the first study to compare forced and voluntary exercise in the 6-OHDA model. Unfortunately, the results of this study did not support exercise, either forced or voluntary, as a neuroprotective strategy in this model of PD.

Other than the stress component of forced exercise, one of the key differences between voluntary and forced exercise in animal models is that voluntary exercise allows the animal flexibility in the timing and dosage of the exercise. Based on the results of the study in Chapter 4, rats are capable of considerable amounts of running when they are left to run at their own discretion (Table 27, Figures 20-21). This is in contrast to Leasure and Jones (2008) who found that rats in a forced exercise condition ran about the same distance as rats that voluntarily exercised; however, they also found

that voluntary exercise rats ran faster and for less total time than rats that were forced (Leasure & Jones, 2008). Since forced and voluntary exercise are different on so many levels (i.e., dosing of exercise, time of day, aversion stimuli), it is an attractive area of comparison. Leasure and Jones (2008) agree that comparing the neural and behavioral effects of different forms of exercise, like forced and voluntary exercise, needs more scientific attention (Leasure & Jones, 2008). From an internal validity perspective, voluntary exercise is a more attractive modality for future exercise studies because it is free from potential confounding variables that plague forced exercise, like exercising in the less active cycle (i.e., light cycle), aversive stimuli (i.e., electrical shocks), and animal handling variations associated with transfer to and from the treadmill.

One novel element of this dissertation was the analysis of exploratory rearing as a behavioral surrogate of hypokinesia, which is a paucity of movement or abnormally diminished motor activity. It was felt that the number of times an animal would rear for exploration during a 10 minute time frame might possibly be an accurate indicator of this common, human PD symptom (Dunnewold et al., 1998; Hoshiyama et al., 1994; van Hilten et al., 1995; van Hilten et al., 1998). Hypokinesia, observed in this way, has not previously been explored in the literature in rodent PD models. Only one study has explored hypokinesia in 6-OHDA rats; however, they did so by measuring motor activity for one minute using a photocell activity apparatus in rats with bilateral 6-OHDA lesioning (Jolicoeur et al., 1991). These researchers did find hypokinesia to be a prominent feature of the 6-OHDA symptom profile. This lends validity to the use of exploratory rearing in this dissertation as a behavioral surrogate of hypokinesia.

Moreover, the collective findings in this dissertation of this motor test suggest that this test might be useful in future studies using the 6-OHDA model even in cases with only a unilateral neurotoxic injection.

The results of this dissertation clearly show that exploratory rearing is a prominent feature of 6-OHDA lesioning. In Chapter 2, exploratory rearing improved over time after 6-OHDA lesioning (Figure 7, Table 8). In Chapter 3, exploratory rearing decreased immediately after the 6-OHDA lesion (Figure 12, Table 14). In Chapter 4, exploratory rearing was significantly higher in the sham lesion group compared to the three groups that had the 6-OHDA lesion (Table 19, Figure 17). In addition, in Chapters 3 and 4, exploratory rearing was significantly correlated to two of the most commonly used behavioral tests in rat PD model research, apomorphine rotations and forelimb placement asymmetry (Tables 15 and 20). Taken together, these results lend validity to exploratory rearing as a useful assessment tool for hypokinetic behavior in rat models of PD.

Another novel observation in this study was the presence of an immediate right neck turn after 6-OHDA lesioning before the animal had regained full consciousness. This was transient and went away after an hour or so. This has not been documented in the literature. During the first study (Chapter 2), it seemed that many of the rats had a right neck turn within about 10 minutes after surgery (Figure 22). Since all of the rats had a 6-OHDA injection into the region of the right medial forebrain bundle, it was logical that this right neck turn could be related to the surgery. From a neurophysiologic perspective, this might suggest that the neurotoxin is immediately taken up by

nigrostriatal fibers destined for the right striatum. Because the right striatum reinforces motor facilitation in the contralateral half of the body, toxic insult to this pathway would logically cause a decrease in striatal-reinforced motor facilitation in the left side of the animal, including the neck sidebending musculature that would cause the neck to move to the left. Concomitantly, a decrease in muscular facilitation on the left would be met by a relative increase in motor facilitation on the right causing the right neck turn. Over time, the animal, when fully conscious, would be able to make corrective centering of the head during routine behavioral activities and, thus, would be able to overcome the imbalanced motor facilitation through volitional control.

It was felt that the immediate but transient right neck turn could possibly be an early indicator of successful neurotoxin placement into the right medial forebrain bundle. Since some of the rats did not have this right neck turn, it was thought that perhaps the stereotactic injection of the neurotoxin did not reach its mark. Since this was just a theory, observation of it was standardized in Chapters 3 and 4. In Chapter 3, moderate to strong correlations were found with this immediate postoperative observation to apomorphine rotations, forelimb placement asymmetry and exploratory rearing (point biserials = 0.734, 0.534, and -0.749, respectively). Thus, this immediate right neck turn was indeed related to the severity of the Parkinsonian behavioral asymmetries. Moderate correlations, consistent with Chapter 3 results, were also observed in Chapter 4. Taken together, the immediate observation of postoperative right neck turn is an early sign of successful neurotoxin injection into the medial forebrain bundle. While it is a fairly well-established fact that 6-OHDA has a protracted

neurotoxicity (Bjorklund et al., 1997), based on the results of the studies in this dissertation, 6-OHDA has in the least an immediate neurotoxic effect on motor facilitation.

There were several limitations in the design of this dissertation that may have influenced the results. First, the experimental control of the designs could have been improved. For instance, all of the rats should be exposed to the same time, type and duration of handling procedures and other design related variations regardless of experimental group. Consider the design of Chapter 4. The voluntary rats were housed in different housing units than the other rats (i.e., housing units with free access to an attached running wheel). Also, the forced rats were handled twice daily during their forced running protocol. The voluntary, sham and control rats were not handled at all except for weighing and cleaning housing units. Ideally, every rat should have been housed in the same type of housing unit; the housing unit with the running wheel would have been ideal for all of the rats in all of the conditions. However, in the conditions where there were no running expectations then the wheel could have been immobilized. In addition, all of the rats should have been exposed to the running treadmill with the same frequency and duration as the forced runners; however, the treadmill belt would not have been turned on for the other conditions. Second, housing the rats individually could have been a potential stressful confounding factor. Rats could have been housed in groups and then, in the case of the voluntary exercise, radiofrequency identification technology could have been utilized to identify which rats were running, how long, and how intense. Third, rats should be exercised during their more active cycle (i.e., the

dark cycle). Since the rats in the voluntary exercise clearly exercised more during the dark cycle it would stand to reason that the forced exercise should have taken place during that same time.

Future designs investigating the effects of exercise in rodent model of PD should consider some of the new transgenic and knockout models (i.e., rat and mouse) that have recently emerged on the market. The majority of these mostly untested models were not available at the time of this dissertation; however, they represent an important development in rodent PD models and might afford a closer approximation to human PD. In addition, rather than using a bolus delivery protocol for neurotoxin, as was done in this dissertation, researchers should consider a slower delivery method (e.g., Alzet pumps) to more closely approximate the slow, progressive neurodegeneration seen in human PD (Alvarez-Fischer et al., 2008).

In summary, the results of the studies in this dissertation cast doubt on the neuroprotective properties of voluntary and forced exercise in a 6-OHDA-induced hemiparkinsonian model. Since the findings of this dissertation are clearly in contrast to the body of literature on this topic, there is a need to further develop these hypotheses using more rigorous designs. In the least, the results of this dissertation should add some caution to the commonly held assumption in the neurodegenerative community that exercise is neuroprotective in rat models of PD.

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- Xu, S., Zhou, M., Yu, S., Cai, Y., Zhang, A., Ueda, K., & Chan, P. (2006). Oxidative stress induces nuclear translocation of C-terminus of alpha-synuclein in dopaminergic cells. *Biochem Biophys Res Commun*, *342*(1), 330-335.

Yu, S., Ueda, K., & Chan, P. (2005). Alpha-synuclein and dopamine metabolism. *Mol Neurobiol*, 31(1-3), 243-254.

CURRICULUM VITAE

Merrill Russen Landers, PT, DPT, OCS

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EDUCATION

- Brigham Young University - Provo, Utah
 - Bachelor of Science in Exercise Science – 1993
- Creighton University - Omaha, Nebraska
 - Doctor of Physical Therapy - 1997 (Cum Laude)

ACADEMIC APPOINTMENTS

- Chair – Department of Physical Therapy, School of Allied Health Sciences, Division of Health Sciences, University of Nevada, Las Vegas (7/2011 to present)
- Associate Professor – Department of Physical Therapy, School of Allied Health Sciences, Division of Health Sciences, University of Nevada, Las Vegas (4/2007 to present)
- Adjunct Assistant Professor – Department of Health Physics, School of Health and Human Sciences, Division of Health Sciences, University of Nevada, Las Vegas (8/2005 to present)
- Associate Professor – Doctor of Physical Therapy Program (Entry-Level), Rocky Mountain University of Health Professions, Provo, Utah (6/2010 to 12/2011)
- Assistant Professor – Department of Physical Therapy, School of Health and Human Sciences, Division of Health Sciences, University of Nevada, Las Vegas (8/2001 to 4/2007).

UNIVERSITY SERVICE

- Chair, Faculty Review Committee, School of Allied Health Sciences – 2007-2011
- Chair, Academic Review Committee, Department of Physical Therapy – 2004-2011
- Faculty Senator, School of Allied Health Sciences, 2003-2005

PROFESSIONAL WORK EXPERIENCE

- Nevada State Board of Physical Therapy Examiners License #1158
- Vice-Chairman, Continuing Education Advisory Committee, State of Nevada Board of Physical Therapy Examiners (6/2000 to 2010)
- Medicolegal consultation to Proconsul, Inc. (2002 to 2005)
- Adjunct Faculty – Physical Therapist Assistant Program, Community College of Southern Nevada (2001)
- Clinician II Physical Therapist – Healthsouth Sports Science and Rehabilitation Institute, 321 N Buffalo, Las Vegas, Nevada 89145 (12/2000 to 8/2001)
- Senior Clinician Physical Therapist – Healthsouth Sports Medicine and Rehabilitation,

1341 S Rainbow, Suite 201, Las Vegas, Nevada 89146 (9/1997 to 12/2000)

PEER-REVIEWED PUBLICATIONS

- Puentedura EL, March J, Anders J, Perez A, Landers MR, Wallmann HW, Cleland JA. Safety of cervical spine manipulation: are severe adverse events preventable and are manipulations being performed appropriately? a review of 134 case reports. *Journal of Manual & Manipulative Therapy*. 2012, in print
- Bungum T, Landers MR, Azzarelli M, Moonie S. Perceived environmental physical activity correlates among Asian Pacific Islander Americans. *Journal of Physical Activity & Health*. 2012, in print
- Young DL, Shen JJ, Estocado N, Landers MR. Financial impact of improved pressure ulcer staging in the acute hospital with the use of a new tool, the NE1 Wound Assessment Tool. *Advances in Skin & Wound Care*. 2012;25:158-166.
- McWhorter JW, Landers MR, Young D, Puentedura EL, Hickman RA, Brooksby C, Liveratti M, Taylor L. Knee extension isometric torque production differences based on verbal motivation given to introverted and extroverted female children. *Physiotherapy Theory and Practice*. 2011;27:422-428.
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- Puentedura EJ, In A, Huijbregts P, Celeste S, Edwards D, In A, Landers MR, Fernandez-de-las-Penas F. Immediate effects of quantified hamstring stretching: hold-relax proprioceptive neuromuscular facilitation versus static stretching. *Physical Therapy in Sport*. 2011;12:122-126.
- Puentedura EJ, Landers MR, Cleland JA, Mintken P, Huijbregts P, Fernandez-de-las Penas C. Thoracic spine thrust manipulation versus cervical spine thrust manipulation in patients with acute neck pain: a randomized clinical trial. *Journal of Orthopaedic & Sports Physical Therapy*. 2011;41:208-220.
- Young DL, Estocado N, Landers MR, Black J. A pilot study providing evidence for the validity of a new tool to improve assignment of NPUAP stage to pressure ulcers. *Advances in Skin & Wound Care*. 2011;24:168-175.
- Warren CD, Brown LE, Landers MR, Stahura KA. Effect of three different between-inning recovery methods on baseball pitching performance. *Journal of Strength and Conditioning Research*. 2011;25:683-688.
- Puentedura EL, Landers MR, Hurt K, Meissner M, Mills J, Young DL. Immediate effects of lumbar spine manipulation on the resting and contraction thickness of transversus abdominis in asymptomatic individuals. *Journal of Orthopaedic & Sports Physical Therapy*. 2011;41:13-21.
- Puentedura E, Wallmann HW, Landers MR. Rehabilitation following lumbosacral percutaneous nucleoplasty: a case report. *Journal of Orthopaedic & Sports Physical Therapy*. 2010;40:214-224.

- Pike E, Landers MR. Responsiveness of the Physical Mobility Scale in a non-community dwelling geriatric population. *Journal of Geriatric Physical Therapy*. 2010;33:92-98.
- Landers MR, McWhorter JW, Young D, Schuerman S, Hickman R. Employer funding and time off for physical therapists toward formal continuing education in states with and without a mandate for continuing education. *Health Policy and Administration Journal*. 2010;10:J1-J8.
- Landers MR, Adams M, Acosta K, Fox A. Challenge-oriented gait and balance training in sporadic olivopontocerebellar atrophy: a case study. *Journal of Neurologic Physical Therapy*. 2009;33:160-168.
- Wulf G, Landers M, Lewthwaite R, Töllner T. External focus instructions reduce postural instability in individuals with Parkinson's disease. *Phys Ther*. 2009;89:162-168.
- Wulf G, Lewthwaite R, Landers M, Töllner T. Author response to invited commentary on: External focus instructions reduce postural instability in individuals with Parkinson's disease. *Phys Ther*. 2009;89:170-172.
- Landers MR, Backlund A, Davenport J, Fortune J, Schuerman S, Altenburger P. Postural instability in idiopathic Parkinson's disease: discriminating fallers from non-fallers based on standardized clinical measures. *Journal of Neurologic Physical Therapy*. 2008;32:56-61.
- Wallmann HW, Mercer JA, Landers MR. Surface EMG assessment of the effect of dynamic activity and dynamic activity with static stretching of the gastrocnemius on vertical jump performance. *Journal of Strength and Conditioning Research*. 2008;22(3):787-793.
- Landers MR, Creger RV, Baker CL, Stutelberg KS. Reply to "Letter to the editors": Re: Landers M, Creger R, Baker C, Stutelberg K. The use of fear-avoidance beliefs and nonorganic signs in predicting prolonged disability in patients with neck pain. *Manual Therapy*. 2008; *Manual Therapy*. 2008;13:e3-e4.
- Landers MR, Creger RV, Baker CL, Stutelberg KS. The use of fear-avoidance beliefs and nonorganic signs in predicting prolonged disability in patients with neck pain. *Manual Therapy*. 2008;13:239-248.
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- McWhorter JW, Landers M, Wallmann H, Altenburger P, Berry K, Tompkins D, Higbee C. The effects of loaded, unloaded, dynamic, and static activities on foot volumetrics. *Physical Therapy in Sport*. 2006;7:81-86.

- Landers MR, McWhorter JW, LaPorta Krum L, Glovinsky D. Mandatory continuing education in physical therapy: a survey of therapists in states with and without a mandate. *Physical Therapy*. 2005;85:861-871.
- Landers MR, Wulf G, Wallmann HW, Guadagnoli MA. An external focus of attention attenuates balance impairment in patients with Parkinson's disease who have a fall history. *Physiotherapy*. 2005;91(3):152-158.
- McWhorter JW, Landers MR, Wallmann HW. The effects of verbal motivation on maximal isokinetic torque production in children with varying personality types. *Pediatric Exercise Science*. 2005;17:329-333.
- Landers MR, Maguire L. Effects of a work injury prevention program for housekeeping in the hotel industry. *Work: A Journal of Prevention, Assessment, and Rehabilitation*. 2004;22(3):239-246.
- Landers MR. Treatment-induced neuroplasticity following focal injury to the motor cortex. *International Journal of Rehabilitation Research*. 2004;27(1):1-5.
- McWhorter JW, Wallmann HW, Landers M, LaPorta L, Altenburger B. The effects of walking, running, and shoe size on foot volumetrics. *Physical Therapy in Sport*. 2003;4:87-92.
- Landers M, Altenburger P. Peripheral nerve injury. *Advances in Physiotherapy*. 2003;5(2):67-82.
- Landers M, Barker G, Wallentine S, McWhorter JW, Peel C. A comparison of tidal volume, breathing frequency, and minute ventilation between two sitting postures in healthy adults. *Physiotherapy Theory and Practice*. 2003;19:109-119.

CONFERENCE PROCEEDINGS

- Landers MR, Baker CL, Stutelberg KS, Creger RV. Effectiveness of cervical nonorganic signs in predicting disability in patients with neck pain. In: *Proceedings of the 8th Congress of European Federation for Research in Rehabilitation; June 13-17, 2004; Ljubljana, Slovenia*

PUBLISHED ABSTRACTS

- Landers MR, Durand CS, Powell DS, Kuiken AM, Young DL, Hickman R. Balance confidence and fear of falling avoidance behavior are most predictive of falling in older adults: a prospective analysis.
- Landers MR, Kinney JW, Elekonich MM, Allen DN, van Breukelen F. A comparison of voluntary and forced exercise in driving neuroprotection in a hemiparkinsonian rat model. *Movement Disorders*, in press
- Gramly A, Rhodes K, Smith A, Landers MR. Active recovery and electro-muscular stimulation on delayed onset muscle soreness after endurance running: a randomized clinical trial. *Physical Therapy*, 2012, in press
- Landers MR, Blazer R, Richards A, Davis A, Rosenlof L. Attentional focus during balance training in idiopathic Parkinson's disease: a randomized clinical trial. *Journal of Neurologic Physical Therapy*. 2012, in press

- Puentedura E, Landers MR, Cleland J, Mintken P, Huijbregts P, Fernandez-de-las-Penas C. Thoracic spine thrust manipulation versus cervical spine thrust manipulation in patients with acute neck pain: a randomized clinical trial. *Journal of Orthopaedic & Sports Physical Therapy*. 2012, in press
- Puentedura E, Landers MR, McCauley A, Rasmussen Z, Louw A. A population-based survey of lumbar surgery beliefs. *Journal of Orthopaedic & Sports Physical Therapy*. 2012, in press
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- Landers MR, Davenport JM, Puentedura EJ, Wallmann HW. Physical therapists' subjective assessment of overreaction in patients with neck pain is predictive of prolonged disability. *Physical Therapy*. 2009. Available at: http://www.apta.org/AM/abstracts/pt2009/abstractsPt.cfm?m_id=19539
- Puentedura EJ, Brooksby C, Wallmann HW, Landers M. Physical therapy rehabilitation following lumbosacral percutaneous nucleoplasty: a case study. *Physical Therapy*. 2009. Available at: http://www.apta.org/AM/abstracts/pt2009/abstractsPt.cfm?m_id=19538
- Hickman R, McCoy S, Rauh M, Long T, Landers M. Experience as the link between contemporary ecologic theories and movement science in early intervention. *Pediatric Physical Therapy*. 2009;21:113.
- Landers MR, Hickman RA, Young DL, Schuerman SE, McWhorter JM. Time off and employer funding for physical therapists toward formal continuing education in states with and without a mandate for continuing education.

Physical Therapy. 2008. Available at:

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- Landers MR, Wulf G. Cognition in idiopathic Parkinson's disease: correlates with gait and balance. *Parkinsonism and Related Disorders*. 2008;14(1):S31.
- Menzel NN, Bray-Ward P, Landers M. Inflammatory cytokine links to depression and pain intensity in low back pain disability. *Communicating Nursing Research Conference Proceedings*, 2008;41:120.
- Krum LL, Schuerman S, Colton M, George S, Kocher K, Rees M, Alpert P, Landers M. Female collegiate athletes: relationships of quadriceps angle, bone mineral density (BMD), muscular strength, body composition, and calcium intake across sports. *J Women's Health Phys Ther*. 2007;31:26.
- McWhorter JW, Landers MR, Somerhalder A, Altenburger A, Altenburger B. An evaluation of academic variables that predict physical therapy licensure scores. *Physical Therapy*. 2007. Available at: http://www.apta.org/AM/abstracts/pt2007/abstractsPt.cfm?m_id=15011.
- McWhorter JW, Landers MR, Michelman M, Robbins C, Wakefield T. An afterschool health and nutrition program's effect on electronic media and childhood obesity. *Pediatric Physical Therapy*. 2007;19:97-98.
- Landers MR, Acosta K, Fox A, Groves M. Challenge-oriented balance training in Olivopontocerebellar atrophy: a single case study. *Journal of Neurologic Physical Therapy*. 2006;30:221-222.
- Landers MR, Wulf G. Postural instability in idiopathic Parkinson's disease: predicting fallers from non-fallers based on standardized clinical measures. *Movement Disorders*. 2006;21:S555.
- McWhorter JW, Altenburger B, Wallmann HW, Landers MR. The effects of verbal motivation on maximal isokinetic torque production in children. *J Orthop Sports Phys Ther*. 2006;36:A50.
- Nocera JR, Rubley MD, Holcombe WR, Guadagnoli MA, Landers MR. Effects of a single bout of throwing on rotator cuff strength and proprioception. *Journal of Athletic Training, Supplemental*. 2005;40(2):S-22.
- Landers MR, McWhorter JW, Filibeck D, Robinson C. Does sitting posture in chronic obstructive pulmonary disease really matter?: an analysis of two sitting postures and their effect on pulmonary and cardiovascular function. *Journal of Cardiopulmonary Rehabilitation*. 2005;25:305.
- Landers MR, Cheung W, Miller D, Summons T. A prospective investigation of the effects of secondary gain in neck pain. *Manual Therapy, in press*
- Landers MR, Creger R, Baker C, Stutelberg K. An investigation of the predictive ability of fear-avoidance beliefs and nonorganic signs in patients with neck pain. *Manual Therapy, in press*

- McWhorter JW, Berry K, Higbee C, Tompkins D, Altenburger P, Wallmann H, Landers M. The effects of loaded, unloaded, dynamic, and static activities on foot volumetrics. *J Orthop Sports Phys Ther.* 2005;35:A37.
- Landers MR, McWhorter JW, Krum LL, Glovinsky D. A comparison of physical therapist continuing education practices in states with and without mandatory state continuing education requirements. *Journal of Physical Therapy Education*????, 2004, *in press*
- Landers MR, Creger RV, Stutelberg KS, Baker CL. Relationship of fear-avoidance beliefs to functional limitation and secondary gain in a cohort of patients with neck pain. *Physical Therapy.* 2004. Available at: <http://www.ptjournal.org/abstracts/pt2004/abstractsPt2004.cfm?pubNo=PO-RR-58-TH>
- Landers MR, Wallmann HW. Differences between idiopathic Parkinson's disease fallers and non-fallers on computerized dynamic posturography measurements and subscale scores of the Parkinson's Disease Questionnaire (PDQ-39). *Physical Therapy.* 2004. Available at: <http://www.ptjournal.org/abstracts/pt2004/abstractsPt2004.cfm?pubNo=PO-RR-55-TH>
- Landers MR, Baker CL, Stutelberg KS, Creger RV. Effectiveness of cervical nonorganic signs in predicting disability in patients with neck pain. *International Journal of Rehabilitation Research.* 2004;27:89-90.
- Landers M, Wulf G, Wallmann H, Guadagnoli M. An external focus of attention can attenuate balance impairment in Parkinson's disease. *Journal of Sport and Exercise Psychology.* 2003;25:S141.
- Landers M, Wulf G, Wallmann H, Guadagnoli M. The effects of attentional focus on balance in idiopathic Parkinson's disease. *Physical Therapy.* 2003. Available at: <http://www.ptjournal.org/abstracts/pt2003/abstractsPt2003.cfm?pubNo=PL-RR-138-F>
- McWhorter JW, Wallmann H, Landers M, Krum LL, Altenburger P, Altenburger B. The effects of walking, running, and shoe size on foot volumetrics. *Physical Therapy.* 2003. Available at: <http://www.ptjournal.org/abstracts/pt2003/abstractsPt2003.cfm?pubNo=PO-RR-71-F>

BOOK CHAPTERS

- Landers MR, Daubs MD. Rehabilitation Following Orthosis Removal. In: Vaccaro AR, Anderson P, eds. *Cervical Spine Trauma.* Philadelphia: Rothman Institute; 2010.

PROFESSIONAL PRESENTATIONS

- Landers MR, Durand CS, Powell DS, Kuiken AM, Young DL, Hickman R. Balance confidence and fear of falling avoidance behavior are most predictive of falling in older adults: a prospective analysis. Joint World Congress of ISPGR and Gait & Mental Function, Trondheim, Norway, June 24-28, 2012.
- Landers MR, Kinney JW, Elekonich MM, Allen DN, van Breukelen F. A comparison of voluntary and forced exercise in driving neuroprotection in a hemiparkinsonian rat model. The Movement Disorder Society's (MDS) 16th International Congress of Parkinson's Disease and Movement Disorders, Dublin, Ireland, June 17-21, 2012.
- Gramly A, Rhodes K, Smith A, Landers MR. Active recovery and electro-muscular stimulation on delayed onset muscle soreness after endurance running: a randomized clinical trial. PT 2012: APTA's Annual Conference and Exposition. Tampa, FL, June 6-9, 2012.
- Landers MR, Blazer R, Richards A, Davis A, Rosenlof L. Attentional focus during balance training in idiopathic Parkinson's disease: a randomized clinical trial. 2012 APTA Combined Sections Meeting, Chicago, IL, February 8-11, 2012.
- Puentedura E, Landers MR, Cleland J, Mintken P, Huijbregts P, Fernandez-de-las-Penas C. Thoracic spine thrust manipulation versus cervical spine thrust manipulation in patients with acute neck pain: a randomized clinical trial. 2012 APTA Combined Sections Meeting, Chicago, IL, February 8-11, 2012.
- Puentedura E, Landers MR, McCauley A, Rasmussen Z, Louw A. A population-based survey of lumbar surgery beliefs. 2012 APTA Combined Sections Meeting, Chicago, IL, February 8-11, 2012.
- Landers MR, Durand C, Powell S, Dibble L, Young D. Development of a scale to assess fear-avoidance behavior due to falling: the fear of falling avoidance beliefs questionnaire (FFABQ). 2011 APTA Combined Sections Meeting, New Orleans, LA, February 9-12, 2011.
- Young D, Estocado N, Landers MR, Black J. A pilot study providing evidence for the validity of a new tool to improve assignment of NPUAP stage to pressure ulcers. 2011 APTA Combined Sections Meeting, New Orleans, LA, February 9-12, 2011.
- McWhorter JW, Landers MR, Burford C, Fairbanks S, Ingram A. Employment trends of resident and non-resident physical therapy graduates in underserved states. 2011 APTA Combined Sections Meeting, New Orleans, LA, February 9-12, 2011.
- Landers MR, Pike E. The Physical Mobility Scale: Reliability and Responsiveness in Long Term Care Facility Residents. Physical Therapy 2010: Annual Conference & Exposition of the American Physical Therapy Association, Boston, Massachusetts, June 16-19, 2010.
- Puentedura EJ, Hurt KA, Meissner MC, Mills JD, Young DL, Landers MR. A real-time ultrasound examination of the immediate effects of lumbar spine manipulation on the contraction of transversus abdominis in asymptomatic subjects. Physical Therapy 2010: Annual Conference & Exposition of the

American Physical Therapy Association, Boston, Massachusetts, June 16-19, 2010.

- McWhorter JW, Landers MR, Young D, Brooksby C, Liveratti M, Purtell A, Taylor L. The effects of verbal motivation on maximal isometric torque production in 8 to 11 year old females with differing personality types. 2010 APTA Combined Sections Meeting, San Diego, California, February 17-20, 2010.
- Landers MR, Davenport JM, Puentedura EJ, Wallmann HW. Physical therapists' subjective assessment of overreaction in patients with neck pain is predictive of prolonged disability. Physical Therapy 2009: Annual Conference & Exposition of the American Physical Therapy Association, Baltimore, Maryland, June 10-13, 2009.
- Puentedura EJ, Brooksby C, Wallmann HW, Landers M. Physical therapy rehabilitation following lumbosacral percutaneous nucleoplasty: a case study. Physical Therapy 2009: Annual Conference & Exposition of the American Physical Therapy Association, Baltimore, Maryland, June 10-13, 2009.
- Hickman R, McCoy S, Rauh M, Long T, Landers M. Experience as the link between contemporary ecologic theories and movement science in early intervention. 2009 APTA Combined Sections Meeting, Las Vegas, NV, February 9-12, 2009.
- Landers MR, Hickman RA, Young DL, Schuerman SE, McWhorter JM. Time off and employer funding for physical therapists toward formal continuing education in states with and without a mandate for continuing education. Physical Therapy 2008: Annual Conference & Exposition of the American Physical Therapy Association, San Antonio, Texas, June 11-14, 2008.
- Menzel NN, Bray-Ward P, Landers M. Inflammatory cytokine links to depression and pain intensity in low back pain disability. Diverse Roles of Cytokines in Biological and Psychological Phenomena, Western Institute of Nursing's 2008 Annual Communicating Nursing Research Conference, "The Circle of Nursing Knowledge: Education, Practice and Research," Garden Grove, California, April 17-19, 2008.
- Wulf G, Landers MR, Töllner T. Postural instability in Parkinson's disease decreases with an external focus of attention. The 2nd International Congress on Gait & Mental Function: the interplay between walking, behavior, and cognition. Amsterdam, The Netherlands, February 1-3, 2008.
- Landers MR, Wulf G. Cognition in idiopathic Parkinson's disease: correlates with gait and balance. The 2nd International Congress on Gait & Mental Function: the interplay between walking, behavior, and cognition. Amsterdam, The Netherlands, February 1-3, 2008.
- McWhorter JW, Landers MR, Michelman M, Robbins C, Wakefield T. An afterschool health and nutrition program's effect on electronic media and childhood obesity. American Physical Therapy Association Combined Sections Meeting, Boston, Massachusetts, February 14-18, 2007.

- Landers MR, Acosta K, Fox A, Groves M. Challenge-oriented balance training in Olivopontocerebellar atrophy: a single case study. American Physical Therapy Association Combined Sections Meeting, Boston, Massachusetts, February 14-18, 2007.
- Krum L, Schuerman S, Colton M, Fogg M, George S, Kocher K, Alpert P, Landers M. Female college athletes: relationships of quadriceps angle, bone mineral density, muscular strength, body composition, and calcium intake across sports. American Physical Therapy Association Combined Sections Meeting, Boston, Massachusetts, February 14-18, 2007.
- Landers MR, Wulf G. Postural instability in idiopathic Parkinson's disease: predicting fallers from non-fallers based on standardized clinical measures. The Movement Disorder Society's 10th International Congress of Parkinson's Disease and Movement Disorders, Kyoto, Japan, Oct 28 – Nov 2, 2006.
- McWhorter JW, Altenburger B, Wallmann HW, Landers MR. The effects of verbal motivation on maximal isokinetic torque production in children. American Physical Therapy Association Combined Sections Meeting, San Diego, California, Feb 2006.
- Landers MR, McWhorter JW, Filibeck D, Robinson C. Does sitting posture in chronic obstructive pulmonary disease really matter?: an analysis of two sitting postures and their effect on pulmonary and cardiovascular function. 2005 Annual Meeting of the American Association of Cardiovascular and Pulmonary Rehabilitation (AACVPR). Milwaukee, Wisconsin, October 21, 2005.
- Landers MR, Cheung W, Miller D, Summons T. A prospective investigation of the effects of secondary gain in neck pain. 2nd International Conference on Movement Dysfunction. Pain and Performance: Evidence & Effect. Edinburgh, Scotland, United Kingdom, September 23-25, 2005.
- Landers MR, Creger R, Baker C, Stutelberg K. An investigation of the predictive ability of fear-avoidance beliefs and nonorganic signs in patients with neck pain. The 2nd International Conference on Movement Dysfunction. Pain and Performance: Evidence & Effect. Edinburgh, Scotland, United Kingdom, September 23-25, 2005.
- Nocera JR, Rubley MD, Holcombe WR, Guadagnoli MA, Landers MR. Effects of a single bout of throwing on rotator cuff strength and proprioception. NATA Annual Meeting, Indianapolis, Indiana, June 2005.
- McWhorter JW, Berry K, Higbee C, Tompkins D, Altenburger P, Wallmann H, Landers MR. The effects of loaded, unloaded, dynamic, and static activities on foot volumetrics. American Physical Therapy Association Combined Sections Meeting, New Orleans, LA, Feb 2005
- Landers MR, McWhorter JW, Krum LL, Glovinsky D. A comparison of physical therapist continuing education practices in states with and without mandatory state continuing education requirements. American Physical Therapy Association Combined Sections Meeting, Nashville, Tennessee, February 4-8, 2004.

- Landers MR, Baker CL, Stutelberg KS, Creger RV. Effectiveness of cervical nonorganic signs in predicting disability in patients with neck pain. 8th Congress of European Federation for Research in Rehabilitation, Ljubljana, Slovenia, June 13-17, 2004.
- Landers MR, Creger RV, Stutelberg KS, Baker CL. Relationship of fear-avoidance beliefs to functional limitation and secondary gain in a cohort of patients with neck pain. American Physical Therapy Association Annual Conference and Exposition, Chicago, Illinois, June 30 – July 2, 2004.
- Landers MR, Wallmann HW. Differences between idiopathic Parkinson's disease fallers and non-fallers on computerized dynamic posturography measurements and subscale scores of the Parkinson's Disease Questionnaire (PDQ-39). American Physical Therapy Association Annual Conference and Exposition, Chicago, Illinois, June 30 – July 2, 2004.
- Landers M, Wulf G, Wallmann H, Guadagnoli M. The effects of attentional focus on balance in idiopathic Parkinson's disease. Platform presentation. American Physical Therapy Association Combined Sections Meeting, Washington, DC, June 17-22, 2003
- McWhorter JW, Wallmann H, Landers M, Krum LL, Altenburger P, Altenburger B. The effects of walking, running, and shoe size on foot volumetrics. Posture presentation. American Physical Therapy Association Combined Sections Meeting, Washington, DC, June 17-22, 2003.
- Landers M, Wulf G, Wallmann H, Guadagnoli M. An external focus of attention can attenuate balance impairment in Parkinson's disease. Posture presentation. Annual Meeting of the North American Society for the Psychology of Sport and Physical Activity (NASPSA), Savannah, GA, June 5-7, 2003.

INVITED PRESENTATIONS

- Landers MR. Neck pain-related disability: current evidence on clinical correlates and conservative treatment options. 28th Annual National Workers' Compensation and Occupational Medicine Conference. Hyannis, Massachusetts, July 14-17, 2008.
- Landers M. Update on peripheral nerve injury. 41st Annual Conference of the Indian Association of Physiotherapists, Mumbai (Bombay), India, January 1 – February 2, 2003.

RESEARCH GRANTS

- Keil DE, Klaassen JM, Landers MR, Elekonich M, McWhorter JM, Peden-Adams M.. Establishing a clinical research infrastructure. Clinical Research Core (CRC) Laboratory: Support for Interdisciplinary Studies in Health Sciences. Research Infrastructure Award Program. UNLV. 2007, \$100,000, *funded*
- Menzel N, Landers MR, Keil D. Biomarkers for low back pain-related disability. Research development Award. UNLV. 2007. \$9,514, *funded*

- Landers M, Patton P, de Belle S. An investigation of the neuroplastic changes in subcortical brain pathways associated with Parkinson's disease using positron emission tomography. UNLV SITE Grant. 2005. \$7,500, *funded*
- Wulf G, Landers M. Attentional focus training to enhance balance in individuals with Parkinson's disease. American Parkinson's Disease Association. 2004. \$50,000, *funded*
- LaPorta L, McWhorter JW, and Landers M. Acquisition of equipment to support DEXA scan research. UNLV SITE Grant. 2002. \$4,685, *funded*
- Landers M, and McWhorter JW. A Comparison of Continuing Education Practice in States with and without Mandatory Continuing Education Requirements. New Investigator Award, University Research Council, University of Nevada, Las Vegas. 2002. \$4,444.00, *funded*

MANUSCRIPT REVIEWER

- Physical Therapy Journal, 2006 to present
- Physiotherapy Journal, 2008 to present
- Gait & Posture, 2009 to present
- Journal of Neurologic Physical Therapy, 2010 to present
- Parkinson's Disease and Related Disorders, 2011
- Stroke, 2011
- Journal of NeuroEngineering and Rehabilitation, 2011
- Journal of Manipulative and Physiologic Therapeutics, 2009
- Archives of Physical Medicine and Rehabilitation, 2009
- Physiology & Behavior, 2008
- Surgical Laparoscopy, Endoscopy & Percutaneous Techniques, 2007

GRANT REVIEWER

- University of Utah Center on Aging (CoA) Pilot Grant Program, 2011
- National Institute of Neurological Disorders and Stroke (Neurological Sciences and Disorders K Panel (NSD-K)), 2011
- National Institute of Neurological Disorders and Stroke (Neurological Sciences and Disorders K Panel (NSD-K)), 2010
- Parkinson's Disease Society UK, 2008

PROFESSIONAL MEMBERSHIP

- Member American Physical Therapy Association (1994 – present)
- Member Nevada Physical Therapy Association (1997 – present)
- Member Orthopedic Section of the American Physical Therapy Association (1995 – present)
- Member Education Section of the American Physical Therapy Association (2003 – present)
- Member Neurology Section of the American Physical Therapy Association (2004 – present)

- Member Research Section of the American Physical Therapy Association (2004 – present)
- Member International Society for Posture & Gait Research (2011 – present)

PROFESSIONAL SERVICE

- Advisory Board – Pima Medical Institute Physical Therapist Assistant Program, 7/2011
- Awards Committee - Subcommittee on Publications, American Physical Therapy Association, 7/2010 - 6/2014
- Institutional Review Board Member, Lou Ruvo Brain Institute (Alzheimer's disease and movement disorders), Las Vegas, Nevada, 2009
- NPTA Research Committee Chair (2001 to 2010)
- Parkinson's Disease Conference Planning Committee (2002 – 2005)
- Continuing Education Chair Nevada Physical Therapy Association (2000 – 2002)
- Continuing Education Co-Chair Nevada Physical Therapy Association (1999 – 2000)
- Program/Education Committee Southern District Chair (1999 – 2002) NPTA
- NPTA Ethics Committee (2000 to 2002)
- Clinical Instructor for UNLV – 2000
- Clinical instructor for CSN – 1999

AWARDS

- 2011 Cyrus Tang Research Award.
- 2011 Wound Management SIG Research Award (APTA)
- 2010 UNLV Travel Award.
- 2009 eLayne Library Verve Award for Parkinson's disease research.
- 2008 UNLV Travel Award.
- 2006 UNLV Travel Award.
- 2005 Faculty Development Award. Nevada Geriatric Education Center.
- 2005 UNLV Travel Award.
- 2003 Nevada Physical Therapy Association Leadership Award
- 2002 New Investigator Award, University Research Council, University of Nevada, Las Vegas
- Orthopedic Certified Specialist (OCS) by the American Board of Physical Therapy Specialties (2001), #4628
- 2000 Nevada Physical Therapy Association Award of Merit