The effects of treadmill training in hemi-Parkinsonian rats.

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By

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Abstract

The purpose of this study was to investigate whether locomotor training, in the form of treadmill training, could ameliorate neurochemical changes and behavioural deficits in the 6-hydroxydopamine (6-OHDA) rat model of Parkinson's disease. It has been recently demonstrated that rehabilitative forelimb motor training can attenuate dopamine loss and some deficits in forelimb movements in this animal model. In addition, brief locomotor treadmill training has been shown to attenuate forelimb deficits in 6-OHDA treated rats. However, it is not known whether locomotor training could result in an amelioration of locomotor deficits in these animals. Rats were lesioned with 6-OHDA injected intracerebrally. Lesioned rats were randomly assigned to one of 3 groups: early treadmill trained, late treadmill trained and untrained. Animals in the treadmill groups were trained to trot on a moving treadmill for 2 x 20 minute sessions daily for 30 days, beginning either 24 hours or 7 days after 6-OHDA injection. Untrained animals were exposed to a stationary treadmill for the same time periods. All animals were assessed on their abilities to perform several behavioural tasks designed to test locomotor and forelimb movement abilities prior to 6-OHDA injection and again at 3 and 6 weeks post-injection. These tests included measurement of ground reaction forces during overground locomotion, paw placements during a ladder crossing task, forelimb useage during exploratory behaviour and ability to initiate forelimb stepping movements. In addition, assessments of dopamine depletion in the striatum were carried out first *in vivo*, by measuring apomorphine-induced rotations at 2 weeks post 6-OHDA injection, and subsequently by post-mortem analysis of dopamine levels in the striatum using HPLC at the conclusion of the study. Treadmill training resulted in attenuation of dopamine depletion compared to non-treadmill trained animals, as measured by both apomorphine injection and HPLC. However, treadmill training produced no difference in behavioural deficits on a variety of tests compared to untrained animals. In some cases, early treadmill trained animals tended to display more severe behavioural deficits compared to untrained animals. Late treadmill training had a similar but smaller effect compared to early treadmill training. We conclude that treadmill training does not ameliorate locomotor deficits, in the 6-OHDA model of Parkinson's disease, even though this same training results in attenuation of dopamine loss in the striatum.

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List of abbreviations

6-OHDA	6-hydroxydopamine
ANOVA	Analysis of Variance
DA	Dopamine
DOPAC	3, 4-dihydroxyphenylacetic acid
GRF	Ground reaction forces
GPe	External segment of the globus pallidus.
GPi	Internal segment of the globus pallidus
HP	hemi-Parkinsonian
HPLC	High performance liquid chromatography
LED	Light emitting diode
L-DOPA	L-3,4-dihydroxyphenylalanine
NE	Norepinephrine
PD	Parkinson's disease
PRE	Pre-surgery
PS3	Post-surgery 3 weeks
PS6	Post-surgery 6 weeks
SE	Standard error of the mean
SNC	Substantia nigra pars compata
SNR	Substantia nigra pars reticulate
STN	Subthalamic nucleus
S-VHS	Super-video home system

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Literature review

1.1   **Parkinson's disease**

Parkinson's disease (PD) is a common neurodegenerative disease affecting 300 out of every 100,000 people in North America (Rajput 1992). Dr. James Parkinson first described this neurological disease in 1817 as a shaking palsy. Today this disease is characterized by rigidity, slowness of movements (bradykinesia), stooped posture, difficulty with balance and walking (altered gait), difficulty with fine motor movements and a resting involuntary tremor. The primary pathology in PD is a degeneration of dopamine producing neurons in a midbrain nucleus called the substantia nigra. This nucleus has extensive connections to the striatum, which is part of the basal ganglia system in the forebrain (Foley & Riederer 1999; Hirsch 1999). The underlying neuropathy of PD is dominated by the progressive degeneration of the nigrostriatal dopamine system. This degeneration will give rise to clinical symptoms when there is approximately 50% dopaminergic cell loss in the substantia nigra (Foley & Riederer 1999). This corresponds to an 80% depletion of striatal dopamine (Foley & Riederer 1999).

The clinical symptoms of PD are dominated by difficulty initiating voluntary movement. As a result, patients with severe dopamine loss often demonstrate little or no facial expression, are unable to perform daily tasks such as getting out of bed or walking even short distances, and at rest they have a tremor. Patients with moderate

dopamine loss in the early stages of the degeneration and patients that have been

medicated with L-DOPA, are often able to move around but they have a characteristic

shuffling gait, that is, they take short choppy steps and fail to pick up their feet during

the swing phase of the stride. This abnormal gait arises because the striatum, as part of

the basal ganglia, is an important component of the neural circuitry involved in the

control of locomotion.

1.2   Neural control of locomotion

Every animal must perform some sort of coordinated purposeful movement to

travel through their environment to explore, obtain food and escape from harm. There

are many ways in which an animal can accomplish this task of purposeful movement,

i.e. swim, crawl, fly, walk, trot, gallop or hop. Each form of movement that the animal

is physiologically capable of can be adopted and used as needed when moving through

the environment. Humans have adapted many forms of locomotion to move through

their environment, including walking, climbing, and swimming. Neural control of

locomotion can be voluntary or involuntary. Involuntary locomotion can occur using

only the spinal cord circuitry, as in reflex movements, or stereotyped behaviours. In

fact, spinal circuitry is able to produce functional locomotor movements and can be

utilized to aid in mobility after spinal cord injury (for review see Fouad & Pearson

2004). However, voluntary locomotion, similar to any voluntary movement, requires

cortical interaction with the basal ganglia. In particular, any type of planned motor

output or movement involves activation of the basal ganglia prior to the movement(s)

being executed. Therefore if any part of the basal ganglia are not functioning properly,

the motor commands will not be produced in the intended way or possibly will not be produced at all (DeLong 2000). These pathways, many of which are dopaminergic, are highly conserved among mammals (DeLong 2000).

The use of mammalian animal models to study human locomotor disorders, such as those occurring in Parkinson's disease, present some interesting challenges with respect to differing modes of locomotion in bipeds and quadrupeds. Rats, like all quadrupeds adopt several different gaits for locomoting overground, such as walking, trotting, galloping, and hopping. They are able to compensate for locomotor disorders in ways that are not available to humans. Nevertheless, one common characteristic between bipedal gaits and many quadrupedal gaits is bilateral symmetry. Examination of locomotion overground in quadrupeds that are trotting, for example, reveals that a trot is comprised of alternating diagonal limb pairs in contact with the ground during each stride. This gait is symmetrical and alternating as is the bi-pedal walking gait. Although results of examinations of movement disorders in rats are not directly translatable to humans, valid and valuable comparisons can be made as a result of the conservation of the neural circuitry involved in control of locomotion and the components of symmetry in the gaits.

1.3   Basal ganglia

To further examine the movement disorders produced in Parkinson's disease, the basic workings of the basal ganglia must be understood.

NORMAL
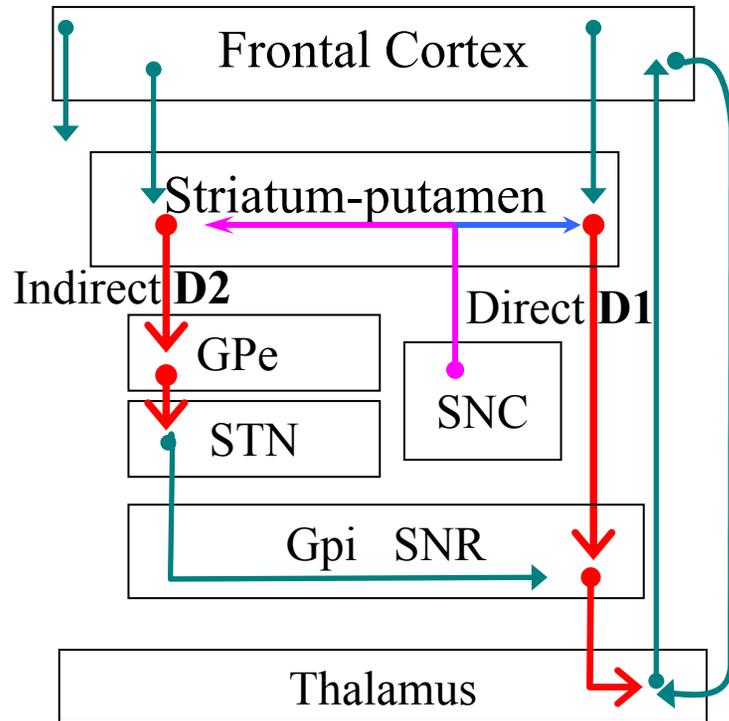
PARKINSONIAN
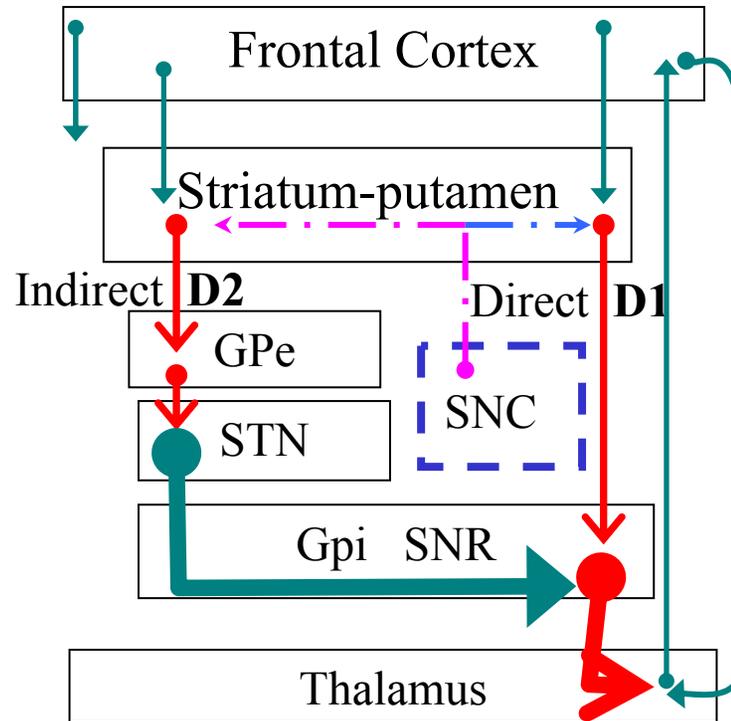
Green → Glutamate excitatory

Red → GABA inhibitory

→ Dopamine D2 receptors (inhibitory)

→ Dopamine D1 receptors (excitatory)

Figure 1. Diagrammatic representation of the workings of the basal ganglia in a normal and Parkinsonian brain. See text for description. The 2 types of dopamine receptors (D1, D2) are located on 2 types of output pathways. Internal segment of the globus palludus (GPi); External segment of the globus pallidus (GPe); Substantia Nigra pars compacta (SNC); Substantia nigra pars reticulate (SNR); Subthalamic nucleus (STN).

The striatum, the globus pallidus, the substantia nigra (pars compacta and pars reticulata), and the subthalamic nucleus are the structures that make up the basal ganglia (Figure 1). These four nuclei are located throughout the forebrain and midbrain and play a major role in the initiation of voluntary movement (DeLong 2000). The striatum, often referred to as the striatum-putamen, consists of the caudate nucleus, the putamen, and the ventral striatum (nucleus accumbens). The striatum is the main input target of the basal ganglia. These inputs come from the cerebral cortex, thalamus and brainstem. The main output of the basal ganglia arises from the substantia nigra (SNR) and the globus pallidus and is inhibitory to the thalamus. The internal circuitry of the basal ganglia is illustrated in Figure 1, as follows. The neurons of the striatum project to the globus pallidus and the substantia nigra which in turn project to the thalamus and brainstem, via two pathways, termed the direct and the indirect pathways. The direct pathway facilitates movement. It projects directly from the striatum to the output nuclei, i.e. globus pallidus (Gpi) and substantia nigra (SNR). This pathway allows the momentary activation of the thalamus and brainstem pathways by inhibiting the tonically active inhibitory neurons which project to the thalamus and brainstem. The indirect pathway, which inhibits movement, also begins at the putamen and runs through the external segment of the globus pallidus (GPE) and secondly from the subthalamic nucleus (STN) to the output nuclei via a rare glutaminergic excitatory pathway. Activation of this pathway ultimately results in the inhibition of the thalamus and brainstem (DeLong 2000).

These two pathways of the striatum are differentially acted upon by the dopaminergic input from the substantia nigra pars compacta (SNC). Striatal neurons of

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the direct pathway normally receive excitatory input from dopaminergic neurons in the substantia nigra pars compacta. This activity acts through D1 type dopaminergic receptors. Conversely, dopaminergic input from the substantia nigra pars compacta inhibits the indirect pathway, by acting through D2 receptors on striatal neurons. This input also facilitates movement by inhibiting the inhibitory indirect pathway. In Parkinson's disease, the lack of dopaminergic input from the substantia nigra (SNC) due to reduced numbers of dopaminergic neurons results in reduced excitation of the excitatory direct pathway and reduced inhibition of the inhibitory indirect pathway, causing reduced movement. In summary, Parkinson's disease results in an overactivity of the basal ganglia output nuclei, resulting in net decrease in movement.

1.4   Current Therapies

Current pharmacological therapies available to people with PD are limited to alleviating the symptoms by increasing the amount of dopamine available in the striatum. This is accomplished in most cases by using L-DOPA, a dopamine precursor that works by increasing the amount of dopamine released from remaining dopaminergic terminals in the striatum. Unfortunately, drugs such as L-dopa have debilitating side effects and are by no means a cure for progressive degeneration of dopaminergic neurons (Grimes et al. 1999). Invasive therapies such as pallidotomy (lesioning of the D2 pathway) often have good outcomes but also do not prevent the further loss of dopamine neurons in the substantia nigra (Lozano & Lang 2001). Another attempted therapeutic strategy as of late is the transplantation of stem cells into many locations within the basal ganglia (Isacson et al. 2001). Stem cell transplantation

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has shown some promising results but it is invasive and does not seem to improve the problem of the neurotoxic brain environment and often times leaves the patient at risk for brain tumor formation (Freed 1999). Less invasive therapies, such as body weight supported treadmill walking or in home physiotherapy, tend not to have as profound effects on alleviation of symptoms or attenuation of dopamine loss (Miyai et al. 2001; Mayai et al. 2002; Nieuwboer et al. 2002)

There have been some positive results after rehabilitative body weight supported treadmill training, in combination with medicinal therapy, on improving the short-step gait and the reported general well-being in Parkinson patients (Miyai et al. 2000; Miyai et al. 2002). Physiotherapy or rehabilitative exercise could potentially improve flexibility and ease rigidity in Parkinsonian patients as well as possibly slow down the progressive loss of dopamine neurons in early stages of Parkinson's disease.

1.5   6-OHDA model of Parkinson's disease

The 6-hydroxydopamine (6-OHDA) induced hemi-Parkinsonian rat has proven to be a useful tool in the study of both the basic biology of PD as well as for testing novel therapeutic strategies (Ungerstedt & Arbuthnott 1970; Bjorklund et al. 2002; Olsson et al. 1995; Dunnett & Bjorklund 1999; Schallert et al. 2000; Metz & Whishaw 2002a; Metz & Whishaw 2002b). The 6-OHDA model has been used to examine various components of PD including basic pathology and neuronal degeneration pathways (Glinka et al.1997; McNaught et al. 2001; McNaught & Jenner 2001; Roedter et al. 2001; Elkon et al. 2001). It has also been used to test therapies such as neural stem cell transplantation, embryonic tissue transplantation, growth factor therapies, and novel

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drug treatment (Winkler et al. 1999; Mukhinda et al. 2001; Armstrong et al. 2002; Dobrossy & Dunnett 2003). This model involves the stereotaxic injection of the catecholinergic-selective neurotoxin 6-OHDA into the medial forebrain bundle of dopaminergic neurons that project from the substantia nigra pars compacta to the striatum (Ungerstedt & Arbuthnott 1970).

Although 6-OHDA will cause the cell death of any catecholamine neuron (be it dopamine or norepinephrine) it encounters, the precise placement of injection into the medial forebrain bundle of the midbrain allows for the selective death of dopamine neurons, as the medial forebrain bundle contains a discrete population of dopamine neurons (DeLong 2000). This dopaminergic bundle begins at the neuronal somas in the substantia nigra and the axons project toward the forebrain, diverging ventral to the striatum. At this point, about 80% of the neurons in the bundle go to the basal ganglia region while the remainder go on to make other forebrain connections (DeLong 2000). The cell death following unilateral 6-OHDA injection is therefore along the entire ipsilateral forebrain, and not selective for the connections to the striatum. However, due to the divergence of 80 % of the projections from the substantia nigra going to the basal ganglia and only 20% to the remainder of forebrain, the majority of symptoms are attributable to the striatal loss of dopamine.

The 6-OHDA is taken up into the catecholamine neurons by the dopamine transporter and induces oxidative damage. This disrupts normal mitochondrial function, resulting in the selective death of the dopamine neurons in the bundle (for review see Miller et al. 1999). This process takes around 14 days after injection to deplete approximately 90% of the dopamine in the striatum (Ungerstedt & Arbuthnott 1970;

Hudson et al. 1993; Metz & Whishaw 2002b). Importantly, the injection is only placed

on one side of the brain, resulting in dopamine depletion in one striatum only. This

generally produces symptoms on the side of the body contralateral to the lesion.

Animals with these lesions are thus referred to as hemi-Parkinsonian (HP).

1.6   Symptoms in the 6-OHDA model of Parkinson's Disease

Hemi-Parkinsonian rats display particular behavioural deficits in posture,

reaching, and other movements (Miklyaeva et al. 1994; Miklyaeva et al. 1995;

Miklyaeva et al. 1997; Olsson et al. 1995; Johnston et al. 1999; Schallert et al. 2000;

Metz & Whishaw 2002a). In both moving and stationary rats, postural instability

develops by 14 days post-surgery, characterized by a head position bias deviating from

body axis by 10º toward the side of the lesion (Henderson et al. 2003). The limbs on

the impaired side of the body are able aid in the support of posture but do not appear to

partake in adjustments to posture (Miklyaeva et al. 1995). Lesioned rats also

demonstrate a decrease in precision aiming of the forelimb during reaching for a food

pellet, resulting from difficulties in adducting the elbow and bringing the paw to

midline. This deficit requires the compensatory substitution of whole body movement to

manipulate the forelimb towards the food pellet (Metz & Whishaw 2002b). A typical

reaching posture develops in 6-OHDA lesioned rats in which more weight is distributed

on the ipsilateral hindlimbs than in normal rats. This adopted posture appears to affect

reaching success on both the contralateral and the ipsilateral forelimbs (Vergara-Aragon

et al. 2003). 6-OHDA lesioned rats tend to circle toward the side of the lesion when

placed in an open field (Miklyaeva et al. 1995). When forced to walk along a runway,

they often move tangentially compared to normal rats. There is also frequently a slight hop in the gait when freely walking overground (Miklyaeva et al. 1995). Footprint analyses of stepping during walking and trotting in these rats have demonstrated an uneven stride length by each diagonal limb pair (Miklyaeva et al.1995). An essential first work on examination of the ground reaction forces produced by hemi-Parkinsonian rats during overground locomotion using a single force plate demonstrated characteristic deficits during the contact of the impaired forelimb-unimpaired hindlimb during trotting (Muir & Whishaw 1999). The capabilities of the force plates used in the present study exceed those of the early single plate equipment. Thus the current force plate data acquisition system allows for more precise quantification of locomotor deficits effecting hemi-Parkinsonian rats.

1.7   Methods used for assessing motor abilities in rats

Behaviour results from the integration and coordination of sensory and motor information in the central nervous system (CNS). The manifestation of this type of neural information is in locomotion, skilled meaningful movements, and exploratory behaviours. Behaviours like those mentioned can be studied for a variety of reasons, the most pertinent being to determine the specific function of an area or network in the CNS, or to determine whether the behavioural changes (deficits) quantified post-injury have been rectified by a novel treatment. Behavioural tests must be sensitive and quantitative, appropriate to the species being tested, and must utilize robust and reliable motivations or training methods to allow for comparison between individuals with varying degrees of internal motivation.

In our laboratory, we have the ability to precisely assess locomotor characteristics in rats by measuring ground reaction forces (the forces acting through the limb on the ground). We have previously shown that this method is a sensitive technique with which to quantify locomotor deficits in rats after central nervous system injury and disease, including rats with striatal dopamine depletion (Muir & Whishaw 1999; Webb & Muir 2002; Webb & Muir 2003). Kinetic analysis of locomotion has been used extensively in animals to determine the effects of such things as CNS and peripheral damage (Bertram et al. 1997; Webb & Muir 2003; Webb & Muir 2004), ontogeny of bi-pedal locomotion in chicks (Muir et al 1996), voluntary gait modification in cats (Lavoie et al 1995), and changes occurring from cortical spinal tract lesions (Muir & Whishaw 1999), red nucleus lesions (Muir & Whishaw 2000), and unilateral damage to the spinal cord (Webb & Muir 2002).

Kinetic analysis requires the following equipment and facilities: runway (gait-path), force transducers (force plates), signal-conducting electronics, computer soft-ware and hard ware, velocity monitoring electronics, and a camera. It has been suggested that multiple force plates are required for accurate determination of simultaneous measurement of ground reaction forces (Bertram et al. 1997). Ground reaction force determination is precise and can be used to measure a wide range of forces. The analysis of these forces reveals how the limbs are being used during locomotion. Unfortunately, the equipment used for this type of analysis is expensive and elaborate, and requires a trained individual to operate and maintain. However this method of analysis is robust and repeatable between institutions and laboratories.

To assess forelimb use in the 6-OHDA rat model, several labs exploit a naturally occurring exploratory behaviour in rats when placed in a new environment. By examining the vertical placement of the forelimbs on a clear cylinder wall, one can assess asymmetry in the use of the forelimbs that may have occurred due to injury of the CNS or periphery. The range in paw use of intact normal rats has been assessed several times and has demonstrated a natural symmetry in forelimb use during vertical exploratory behaviours (Schallert et al. 2000).

Ability to initiate a step using the forelimbs is traditionally assessed using a stepping test that utilizes a restraint grip on the rat and a table top surface (Olsson, et al. 1995; Schallert & Woodlee 2003). Methods used here are to slowly move the animal across the table and to quantify the time it takes to initiate a step. Although the Parkinsonian rats are significantly slower on this test compared to normal animals, there is a problem of motivation. Even normal animals can display a blatant disregard for their limb during this examination. Due to this potential motivational confound, this test was slightly modified to assess akinesia utilizing a treadmill belt that will move the forelimb in a standardized predictable manner, allowing for each animal to endure the same motivation during the test.

Ladder crossing has been used as a test of fine motor skills as well as an assessment of coordinated gait (Metz & Whishaw 2002). Analysis of the type and frequency of errors produced by 6-OHDA lesioned rats demonstrated a bilateral impairment resulting from the unilateral injury with most errors being produced by the forelimbs (Metz & Whishaw 2002). In addition to assessing error made on the ladder task, the assessment of stride length and speed across the ladder have been quantified in

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the current study as indicators of ability to perform the skill-testing task of ladder crossing.

Treadmill training and voluntary wheel running have been used as attempted remedy and as an inducer of plasticity in many animal models of CNS injury (Fouad et al. 2000; Multon et al. 2003; Yang et al. 2003). Treadmill training after partial spinal cord injury in rats did not improve functional recovery but did increase exploratory behaviours compared to untrained lesioned rats (Fouad et al. 2000). However, treadmill training appears to have accelerated locomotor recovery after spinal cord compressive injury (Multon et al. 2003). Treadmill training has been demonstrated to be neuroprotective prior to ischemia, infarctions and 6-OHDA lesion (Cohen et al. 2003; Wang et al. 2001; Yang et al. 2003). The mechanisms of treadmill trainings effects are as contentious as the effects mentioned earlier. However, several recent reviews have provided some plausible insight in the possible mechanisms of exercises affects on the brain (Miller et al. 1999; Sutoo & Akiyama 2003; Tümer et al. 2001). These mechanisms include increased production of neurotrophic factors (Kleim et al. 2003; Smith & Zigmond 2003), down-regulation of potential transporters of neurotoxins (Miller et al. 1999), up-regulation of vesicle transporters to aid in removal of some neurotoxins (Miller et al. 1999), and increase in circulating calcium that has been found to increase synthesis of neurotransmitters, including dopamine (Sutoo & Akiyama 2003; Tümer et al. 2001).

It is important to avoid complications from extraneous effects of exercise due to exhaustion, and to choose a regime that the animal is both able to complete, and that is consistent. Incomplete recovery after CNS injury with treadmill training maybe due to

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training regimes used or maybe combination therapy is most effective. Examination of the effects of treadmill training on the ground reaction forces of hemi-Parkinsonian rats moving overground will elucidate any improvements in gait as a result of treadmill training in this animal model of Parkinson's disease.

1.8   Rehabilitative motor training in 6-OHDA lesioned rats

Recently, it has been demonstrated that rehabilitative motor training can attenuate some of the behavioural deficits seen in HP rats (Tillerson et al. 2001; Cohen et al. 2003; Vergara-Aragon et al. 2003). In particular, specific motor training in a forelimb-reliance task reduces the forelimb reaching deficits seen in 6-OHDA lesioned rats. The immobilization of the unimpaired forelimb (ipsilateral to the lesion) for one-week post-surgery generated a forced use of the impaired forelimb (contralateral to the lesions) during routine activity. This forced-use resulted in an alleviation of some deficits in the impaired forelimb as well as attenuating the loss of dopamine in the striatum compared to controls (Tillerson et al. 2001). The immobilization of the impaired limb for the same time period exacerbated both the forelimb deficits and striatal dopamine loss in HP rats (Tillerson et al. 2002). Interestingly, the animals that did not receive intervention until one week post-surgery were no different from animals that did not receive intervention in the form of a cast (Tillerson et al. 2001; Tillerson et al. 2002). This indicates that there may be a critical period for use-dependent intervention therapies. In another rehabilitative study that did not involve immobilization, a novel skilled reaching task was used as motor training in HP rats, which resulted in alleviation of reaching deficits in both forelimbs (Vergara-Aragon et

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al. 2003). An interesting study by Döbrössy & Dunnett (2003) explored the idea of using forelimb reaching training in dopamine depleted rats that had just received an intrastriatal transplant of fetal striatal tissue in the form of a graft. They found that this combination therapy to aid in recovery of behavioural performance on forelimb reaching (Montoya staircase) task as well as decrease the lesion size as estimated by apomorphine rotation (Döbrössy & Dunnett 2003).

In addition to specific training in forelimb tasks, brief locomotor treadmill training has recently been shown to attenuate some forelimb deficits in HP rats (Tillerson et al. 2003). However, it is not yet known whether locomotor training could result in a reversal of locomotor deficits in HP rats. Our ability to sensitively quantify locomotor and other behavioural deficits in hemi-Parkinsonian animals prompted us to examine the effects of treadmill training on alleviation of behavioural deficits in this animal model.

1.9   Purpose

The purpose of this study is to examine the behavioral and neurochemical effects of treadmill training in the 6-OHDA rodent model of Parkinson's Disease.

1.9.1   Hypothesis

Locomotor training, in the form of regular treadmill training, will ameliorate striatal dopamine depletion and behavioural deficits, including locomotor deficits, in the 6-OHDA rodent model of Parkinson's disease. The effects of early treadmill training will be more dramatic than late intervention of treadmill training.

2.   Materials and Methods

2.1.   Animals

Twenty-nine (29) female Long Evans rats, weighing 250-330 g (Charles River Laboratories, Quebec, Canada) were used in this study. Animals were housed in groups of 2-4 in Plexiglass cages measuring 45 cm x 24 cm (3-4 per cage) or 28 cm x 35 cm (2 per cage) with a 12 h light/12 h dark photoperiod within the animal care facilities in the Department of Veterinary Biomedical Sciences at the University of Saskatchewan. Starting at 2-3 months of age (250 g), the rats were fed approximately 15 grams of Purina rat chow daily (plus food rewards) to maintain a relatively constant weight. All procedures were approved by the University of Saskatchewan Committee on Animal Care and Supply. Animals were cared for according to the standards set out by the Canadian Council on Animal Care, and were examined weekly by a veterinarian.

2.2.   Behavioural training

Animals were handled daily and weighed weekly. Animals were trained to trot the length of a 1.8 m long x 20 cm wide Plexiglas runway to obtain a food reward. Training began approximately two weeks after arrival at the animal care facility. Training was considered complete when the animals were able to consistently trot back and forth along the runway.

2.2.1. *Treadmill training*

Animals were randomly selected to be in the early treadmill training, late treadmill training or no treadmill training (untrained) group. All animals (n=29) were familiarized with the treadmill for 3 sessions of 20 minutes each, 2 weeks prior to surgery. Untrained (n=11) animals were exposed to the motionless treadmill, and animals in the early (n=9) and late (n=9) treadmill trained groups ran at 13 m/min for these brief exposure session pre-surgery. If animals were not trotting on the treadmill, they were coaxed with a food reward (peanut butter and/or vanilla icing) at the front of the treadmill through a small hole in the clear Plexiglas motivation window, and lightly prodded on the rear by the researchers' hand if necessary. Beginning at 24 hours post-surgery, rats in the early treadmill trained group (n=9) were forced to trot on the treadmill for 20 minutes twice daily at an average speed of 13 m/min. This was continued for 6 days per week for the next 30 days. The late treadmill trained group (n=9) began treadmill training seven days post-surgery, and continued until 37 days post-surgery. The late group followed the same treadmill protocol as the early group. The total treadmill exposure post surgery was 30 days for all groups. All groups, trained and untrained, had equal exposure to the treadmill, prodding and food rewards. Each day of treadmill training, treadmill sessions were performed once in the morning during the rats' light photoperiod and once again in the early evening within the first 3 hours of the rats' dark photoperiod.

2.3. Surgery

Animals were 9-10 months of age at the time of surgery and weighed 250-300 g. All animals were premedicated with subcutaneous injections of buprenorphine (0.05 mg/kg) (Buprenex, Reckitt and Colman Pharmaceuticals, VA, USA) and atropine (0.05 mg/kg) (MTC Pharmaceuticals, Ontario, Canada). Animals were anesthetized using an intraperitoneal injection of 35 mg/kg sodium pentobarbital (Somnotol, MTC Pharmaceuticals). Animals were kept warm with a recirculating warm water blanket and administered 100% oxygen nasally throughout the surgical and recovery periods. Postoperative analgesia was administered (subcutaneous buprenorphine (0.05 mg/kg) 10 hours after the pre-operative injection, ensuring comfort post-surgery. Trimethoprimsulfa (30 mg/kg subcutaneously, Trivetrin, Schering Canada Inc. Quebec, Canada) was also administered daily for 7 days following surgery to prevent infections.

2.3.1. Stereotaxic injections

Rats were placed in a stereotaxic apparatus, and a hole was drilled in the skull to allow for unilateral injections of 10 μg of 6-hydroxydopamine (6-OHDA) in 3 μl of 0.9% NaCl solution with 0.02% ascorbic acid. The solution was infused at a rate of 1μl/min using a 10 μl (701 series) Hamilton syringe. Two injections of 6-OHDA were delivered, the first site at tooth bar -2.4 mm, 4.4 mm posterior and 1.2 mm lateral to bregma, 7.8 mm ventral to dura. The second site was at tooth bar +3.4 mm, 4.0 mm posterior and 0.8 mm lateral to bregma, 8.0mm ventral to dura (Paxinos & Watson, 1998). The total amount of 6-OHDA injected was 20 μg. The cannula was left in place for 2 minutes following each injection prior to removal.

2.4.   *In vivo* verification of DA depletion

Evaluation of the extent of dopamine depletion was examined two weeks post-surgery using apomorphine-induced rotation. Apomorphine stimulates post synaptic dopamine receptors directly, preferentially on the 6-OHDA lesioned side, possibly due to denervation-induced dopamine receptor super-sensitivity in animals with unilateral striatal dopamine depletion (Creese et al. 1977). Apomorphine administration results in increased activity in the lesioned striatum compared to the unlesioned striatum (Hudson et al. 1993). Behaviourally, this produces rapid repetitive turning of the body away from the lesioned side (Ungerstedt 1972; Hudson et al. 1993). The number of complete 360 degree spins contralateral to the lesion has been shown to correlate well with lesion severity, specifically with percent dopamine loss (Ungerstedt 1972; Hudson et al. 1993; Metz & Whishaw 2002b). As such, this test is used not as an assessment of Parkinsonian behavior, but rather as an *in vivo* verification of lesion severity. At 14 days post-surgery, all animals were subcutaneously administered apomorphine (0.05 mg/kg) (Sigma-Aldrich Chemicals, Canada), a dopamine receptor agonist, and placed into a stainless steel bowl. Animals were videotaped (S-VHS) from above. The number of times the animal rotated 360 degrees in each direction over a period of 30 minutes was counted by viewing the videotapes after the test was completed. Data presented in Figure 1 are the number of ipsilateral rotations subtracted from the number of contralateral rotations resulting in the net contralateral rotations induced by the apomorphine in one hour, presented as group mean ± standard error (SE).

2.5   Behavioural assessment

All animals were assessed using endpoint and kinetic measurements. Endpoint measurements were: forelimb usage while rearing in a cylinder, forelimb akinesia assessment and foot-faults while crossing a ladder. Kinetic measurements were determined by measuring ground reaction forces during locomotion. All assessments were done prior to surgery, at 3 weeks post-surgery and again at 6 weeks post-surgery.

2.5.1. *Forelimb use asymmetry*

This task tests the animal's relative usage of the impaired and non-impaired forelimbs for weight shifting movements during spontaneous vertical exploration (Schallert et al. 2000). It is commonly used as an index of asymmetry in forelimb usage, and involves scoring the number of contacts of each forelimb while the animal explores inside a clear cylinder (Johnston et al. 1999; Tillerson et al. 2001; Tillerson 2002; Vergara-Aragon et al. 2003). Briefly, each rat was placed in a Plexiglas cylinder (46 cm high x 40 cm diameter) and videotaped (S-VHS) from a ventral perspective through a clear glass floor for 5 minutes. Videotapes were analyzed by an individual blinded to experimental treatment. The number of times that the animal contacted the wall of the cylinder with right, left or both forelimbs was recorded. The number of times that the ipsilateral or contralateral limb was used to contact the wall was divided by the total number of wall contacts. This ratio was then used to produce asymmetry scores for each animal (ipsilateral/total – contralateral/total = asymmetry score). The individual animal asymmetry scores were then averaged and graphed in Figure 2 as group mean ± SE.

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2.5.2. *Forelimb akinesia assessment*

Akinesia, or slowness to initiate movement, can be assessed in the forelimbs by videotaping animals while each forelimb produces stepping movements (Schallert et al. 2000). The hindlimbs and one forelimb are restrained by the experimenter while the forelimb to be tested (the unrestrained forelimb) contacts a horizontal surface. In most studies, stepping movements are produced when the experimenter slowly moves the rat laterally and the number of steps produced per second is used to assess akinesia (Olsson et al. 1995; Schallert & Woodlee 2003). As a modification in the present study, a moving treadmill belt was used to generate a stepping event while the experimenter and rat remain stationary, allowing for more consistency in speed of movements between trials (Figure 2). Utilization of the treadmill belt for this task allows for the quantification of forelimb stepping latency in three directions: extension, adduction and abduction. The treadmill belt speed was set to 9 m/min and a wooden bridge at a height of 3 cm above the belt was used to rest the experimenter's hand while restraining hindlimbs and one forelimb of each rat. The unrestrained limb was placed in contact with the belt so that the belt induced abduction, abduction or extension of the rat's forelimb. The numbers of steps taken per second for each direction were counted using frame-by-frame analysis of the videotape recording. The number of steps per second for each limb were used to identify the asymmetry score [(ipsilateral steps/ipsilateral + contralateral) – (contralateral steps/ ipsilateral + contralateral)]. A score of zero would reflect perfect symmetry in forelimb stepping. Individual scores were then averaged and presented graphically in Figure 3 as group means ± SE.

Figure 2

Figure 2 Assessment of forelimb akinesia. Experimenter is restraining 3 limbs of the rat while using a treadmill belt to induce the stepping event in the unrestrained forelimb. The number of steps taken in a given amount of time during abduction, adduction and extension of the forelimb can be quantified.

2.5.3. *Ladder rung walking test*

The horizontal ladder rung walking test apparatus was a clear Plexiglas runway (1.3 m X 20 cm) with solid platforms (56 cm X 20 cm) on either end and a metal rung floor spanning the middle 18 cm of the runway. Each ladder rung was 3.5 mm in diameter with a distance of 1cm spacing between each rung (Metz &Whishaw 2002a). The ladder test apparatus was suspended above a 45º angled mirrored table to enable simultaneous videotape recording from both a lateral and ventral perspective. A light emitting diode (LED) timer was positioned in the camera field of view to facilitate measurement of movements speed for each run.

Animals were pre-trained to cross the ladder to retrieve a food reward at either end of the runway. For recording sessions, each animal was required to complete 15 acceptable runs at a moderate speed (13-19 cm/sec) across the ladder. A run was considered acceptable if the animal had not stopped or reared prior to entering the field of view of the camera, and if the animal was not galloping or hopping. Videotapes were examined using frame-by-frame analysis at 60 frames/sec to assess limb placement on the rungs of the ladder. Foot fault scoring was performed according to the qualitative scoring system of Metz and Whishaw (2002a). Briefly, each step of each limb was scored as either a correct placement, partial placement, correction, replacement, slight slip, deep slip or total miss (Metz & Whishaw, 2002a). These frequencies were then used to determine the number of foot faults in each category for 12 runs across the ladder per animal. For total correct placements, the frequency counts for the categories of correct and partial placement were combined for left and right limbs [(number correct ipsilateral + contralateral/total steps) + (number partial ipsilateral +

contralateral/total steps)]. Similarly, for total errors, the frequency of slight slips, deep

slips and total misses were summed for the ipsilateral limbs and separately for the

contralateral limbs, and these numbers were divided by the total number of steps for

each limb. Stride length, in cm, was calculated by measuring the horizontal distance

between the position of ipsilateral forelimb in one stride and the position of the same

forelimb in the subsequent stride. Data was averaged from 12 runs for each animal, as

seen on videotape recordings, and individual averages were used to produce group

means ± SE. Speeds across the ladder were calculated by dividing stride length by

stride duration (obtained from the LED timer) over 12 runs for each animal, and

presented as group means in cm/sec ± SE.

2.5.4. *Kinetic measurements of ground reaction forces*

Ground reaction force determination was performed as previously described

(Webb & Muir 2002; Webb & Muir 2003). Briefly, the animals were trained to walk

and trot in a runway in which three force platforms were built into the runway floor

surface. Each force plate (10.5 cm x 11 cm) measured ground reaction forces in the

vertical, fore-aft and mediolateral directions. Analogue output from the force platforms

was amplified and converted to digital and collected on computer (High Speed Imaging

(HSI) Data Acquisition System (Mississauga Ontario, Canada). Digital video was

simultaneously recorded and collected using a high-speed digital camera (Motionscope

1050, Redlake, MASD, Inc.). For each testing session, the weight of the rat was

recorded immediately prior to data collection, and was used to normalize force data to

bodyweight to allow for direct comparison of forces exerted by rats of different weights.

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For a pass to be considered acceptable for analysis, the animal was required to be (1) trotting, (2) moving at a constant velocity, (3) traveling between 40 and 100 cm/sec. The force data were filtered and averaged as previously described using custom writing software (Webb & Muir 2003; Webb & Muir 2004). Limb pairs were separated into left and right, and averaged together at each data collection time point for each rat (minimum of 6 runs per trial). Group averages were obtained by averaging individual averaged forces with others in the same group, generating group mean data with standard error values. Variables of the ground reaction force data that were analyzed statistically were peak vertical, propulsive, and braking forces for both fore and hindlimbs bilaterally.

2.6. Neurochemical analysis

At eight weeks post-surgery, all animals were decapitated, brains were removed, divided into left and right hemispheres and immediately put on an ice cold surface. For each brain, the caudate nucleus was removed bilaterally and placed into a -80 ºC freezer. Catecholamine levels, including dopamine (DA) 3, 4-dihydroxyphenylacetic acid (DOPAC) and norepinephrine (NE), were examined using high-performance liquid chromatography (HPLC) performed in Dr. Peter Yu's Laboratory in the Neuropsychiatry Research Unit, Department of Psychiatry, University of Saskatchewan, Saskatoon, Saskatchewan, Canada. Briefly, caudate tissue was suspended in 0.1 M perchloric acid containing 2.5×10^{-4} M ethylenediamine-tetraacetic acid (EDTA) and 1×10^{-4} M sodium nitrite, homogenized and centrifuged at 14,000 rpm for 10 minutes at 4ºC. The supernatant was removed and the remaining tissue was assessed for dopamine

(DA), 3, 4-dihydroxyphenylacetic acid (DOPAC), and norepinephrine (NE) content by injecting 50 µl aliquot of sample into a Beckman Ultrasphere C-18 reverse phase Column (5 micron, 4.6 x 250 mm Beckman, Toronto, Ontario, Canada). The mobile phase consisted of 1940 ml of HPLC H_2O, 464 mg octyl sodium sulfate (SOS), 20.7 g of NaH_2PO_4, 32.2 mg of EDTA and 175 ml of acetonitrile, pH 2.7. The mobile phase was pumped though the system at 1 ml/min. Molecules were detected electrochemically. Peak heights and standard curves were used to calculate ng/mg tissue. The amount from the ipsilateral hemisphere was divided by the amount on the contralateral hemisphere and presented as percent of intact side by multiplying ratios by 100. Data were square root transformed to generate a normal distribution and grouped by treatment. Statistical analysis was done using SAS (Version 8) to examine differences between treatment groups.

2.7.   Statistical analysis

Statistical assessments for all behavioural tests were done using either repeated measure one-way ANOVA for comparison across testing sessions or one-way ANOVA for group comparisons within testing sessions. The Tukey multiple comparison procedure was used *post hoc* to examine differences between groups. All data are presented as mean ± SE. For HPLC data, a single degree of freedom orthogonal contrast was used to examine differences between groups.

3.   Results

3.1   In vivo estimate of lesion size

Statistical analysis (ANOVA) of the number of contralateral rotations made by animals revealed a significant group effect ($F_{2, 26}$ =3.701; $p <0.05$) (Figure 3). *Post hoc* analysis revealed significantly lower number of rotations in the early treadmill trained group (n=9) compared to untrained (lesion only) group (n=11) (p<0.05). The early treadmill trained group had fewer than 200 contralateral rotations in 30 minutes. There was no significant difference in number of contralateral rotations of the late treadmill trained group (n=9) compared to the untrained group (p= 0.159). There was also no significant difference between the late treadmill and early treadmill groups (p= 0.788).

Figure 3

Apomorphine induced rotation
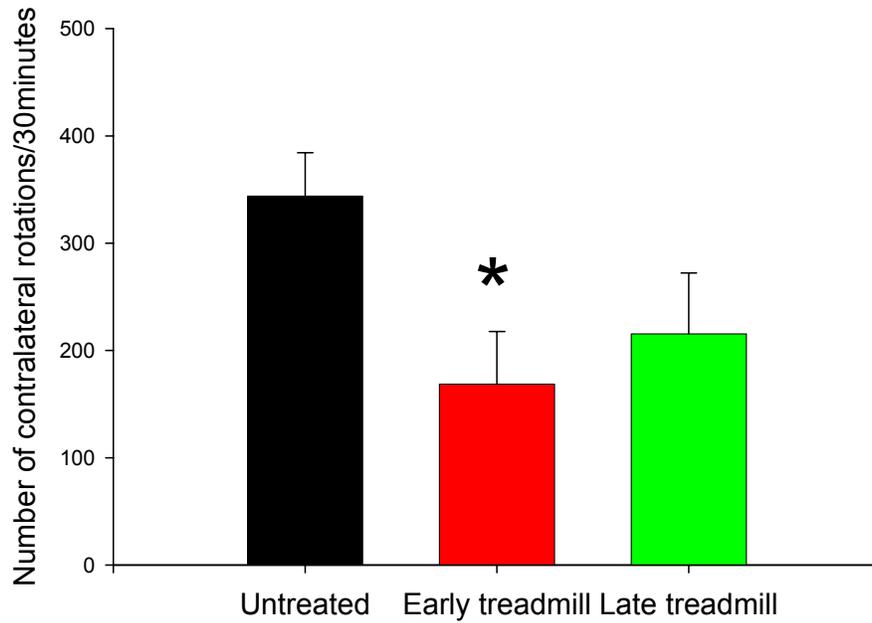
Figure 3 Apomorphine rotation
Effects of treadmill training post-surgery on apomorphine-induced rotation 14 days after 6-OHDA infusion. Early treadmill training significantly attenuated apomorphine-induced rotations compared to those in untrained animals. All values are expressed as mean contralateral rotations in 30 minutes ± standard error. * $p < 0.05$

3.2. Behavioural assessment

3.2.1 Forelimb use asymmetry

Statistical analysis (repeated measures one-way ANOVA) revealed an overall significant effect across testing sessions ($F_{6, 52} = 9.255$; $p < 0.001$) (Figure 4). Post surgery, all groups displayed forelimb use asymmetry favoring the ipsilateral forelimb. *Post hoc* analysis revealed a significant increase in asymmetry at 3 weeks post surgery for all groups (untrained, early and late treadmill) compared to pre-surgical scores ($p < 0.001$). Asymmetry scores for all groups at 6 weeks post surgery also demonstrated a significant increase in asymmetry compared to the pre-surgery scores ($p < 0.001$). There was no significant difference in asymmetry scores between 3 and 6 weeks for any of the groups in this study. There was also no significant effect of treadmill training (early or late) as compared to untrained animals at any of the time points. Thus, treadmill training did not alter the ipsilateral preference in forelimb use in 6 OHDA lesioned animals.

Forelimb use asymmetry

Figure 4
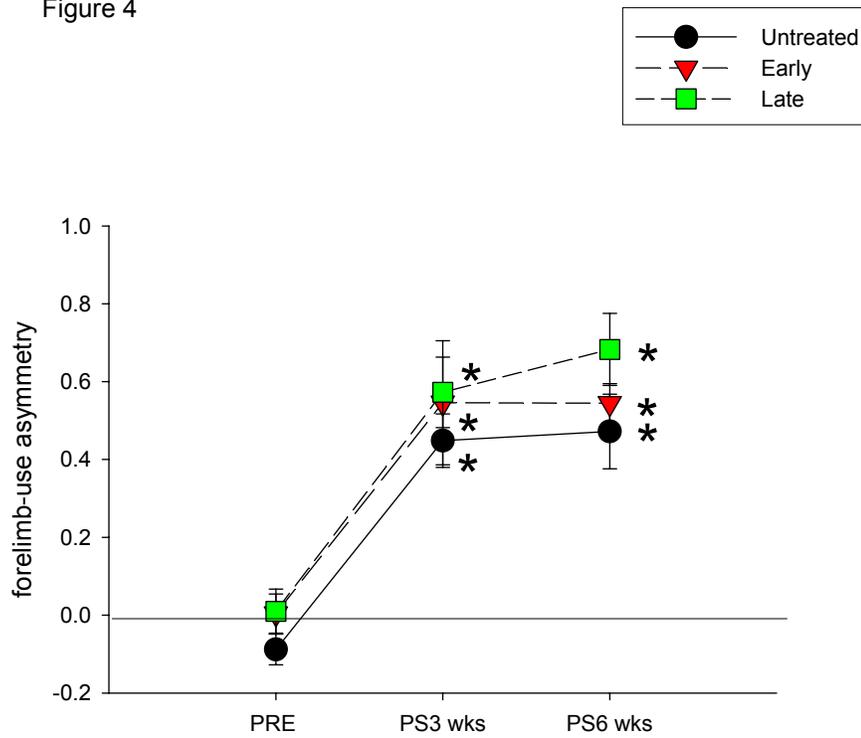
Figure 4 Forelimb use asymmetry

Effects of treadmill training on forelimb use asymmetry after 6-OHDA injection. All animals displayed a significant asymmetry in forelimb preference at 3 (PS3) and 6 (PS6) weeks post-surgery compared to pre-surgical data (p< 0.001). In all cases, animals preferentially used the forelimb ipsilateral to the lesion for exploratory behaviour. No differences could be detected between groups at any time point, indicating that treadmill training did not alter limb use asymmetry. * Denotes significant difference from corresponding pre-surgical values (p<0.001).

3.2.2. Forelimb akinesia

3.2.2.1 Adduction, Figure 5A:

When the animals' forelimb was adducted medially (toward the midline of the body) by the treadmill to produce a stepping event, statistical analysis revealed a significant difference across the testing sessions (Untrained $F_{10, 2}$ =12.750; p<0.001; Early $F_{8, 2}$=7.043; p= 0.006; Late $F_{8, 2}$=4.910; p=0.002). In all groups, asymmetry scores were greater than zero after surgery, indicating that the contralateral limb showed more deficits (i.e. slower step initiation) after surgery compared to before surgery. *Post hoc* analysis revealed a significant increase in forelimb adduction asymmetry score at both 3 and 6 weeks post-surgery compared to pre-surgical scores (p<0.05) for both early and late treadmill trained groups (Early PS3 vs. PRE, p=0.007; Early PS6 vs. PRE, p=0.030; Late PS3 vs. PRE, p=0.024; Late PS6 vs. PRE, p= 0.047). For the untrained group, forelimb asymmetry was not different from pre-surgical values at 3 weeks (p= 0.188) but was different from pre-surgical values at 6 weeks post-surgery (p=0.019). Thus, it appears that treadmill training may accelerate step initiation deficits in the adduction direction, although by 6 weeks there were no differences between trained and untrained groups (p<0.05).

3.2.2.2. Abduction, Figure 5B:

When the animals' forelimb was abducted laterally (away from the body) by the treadmill to produce a stepping event, statistical of asymmetry scores revealed an overall effect across testing session in untrained animals (Untrained $F_{10, 2}$=12.750; p<0.001; Early $F_{8, 2}$=4.748; p=0.024; Late $F_{8, 2}$ =6.316;p=0.010). *Post hoc* analysis

revealed a significant increase in asymmetry in all groups at 3 and 6 weeks post-surgery compared to pre-surgical-scores (p<0.05 for all groups). Again, the increase in asymmetry scores post-surgery indicates that all animals were slower to initiate movements with the forelimb contralateral to the lesion compared to the ipsilateral forelimb. There were no statistically significant differences between groups at any of the data collection sessions (p>0.05 for all groups) and thus treadmill training did not ameliorate stepping akinesia in the abduction direction in 6-OHDA lesioned rats.

3.2.2.3. Extension, Figure 5C:

When the animals' forelimb was moved in a forward stepping motion by the treadmill, (comparable to a forward step during locomotion), statistical analysis revealed a significant effect across testing sessions (Untrained $F_{10, 2}$=16.420; p<0.001; Early $F_{8, 2}$=18.980; p<0.001; Late $F_{8, 2}$=13.954; p<0.001). *Post hoc* analysis revealed a significant increase in forelimb extension asymmetry scores at 3 weeks (PS3) and 6 weeks post-surgery (PS6) compared to pre-surgical scores for both untrained and late treadmill trained groups (p<0.05 for all groups), indicating that the contralateral forelimb was slower to initiate stepping compared to the ipsilateral limb. In contrast, early treadmill training produced forelimb extension asymmetry that was significantly different from pre-surgical values at 3 weeks, but returned to within pre-surgical values by 6 weeks post-surgery (Early PS3 vs. PRE, p<0.001; Early PS6 vs. PRE, p=0.260).. Thus, treadmill training does not appear to ameliorate the forelimb akinesia in the extension direction by 3 weeks post-surgery (PS3), but early treadmill training appears to attenuate akinesia in this direction after 6 weeks.

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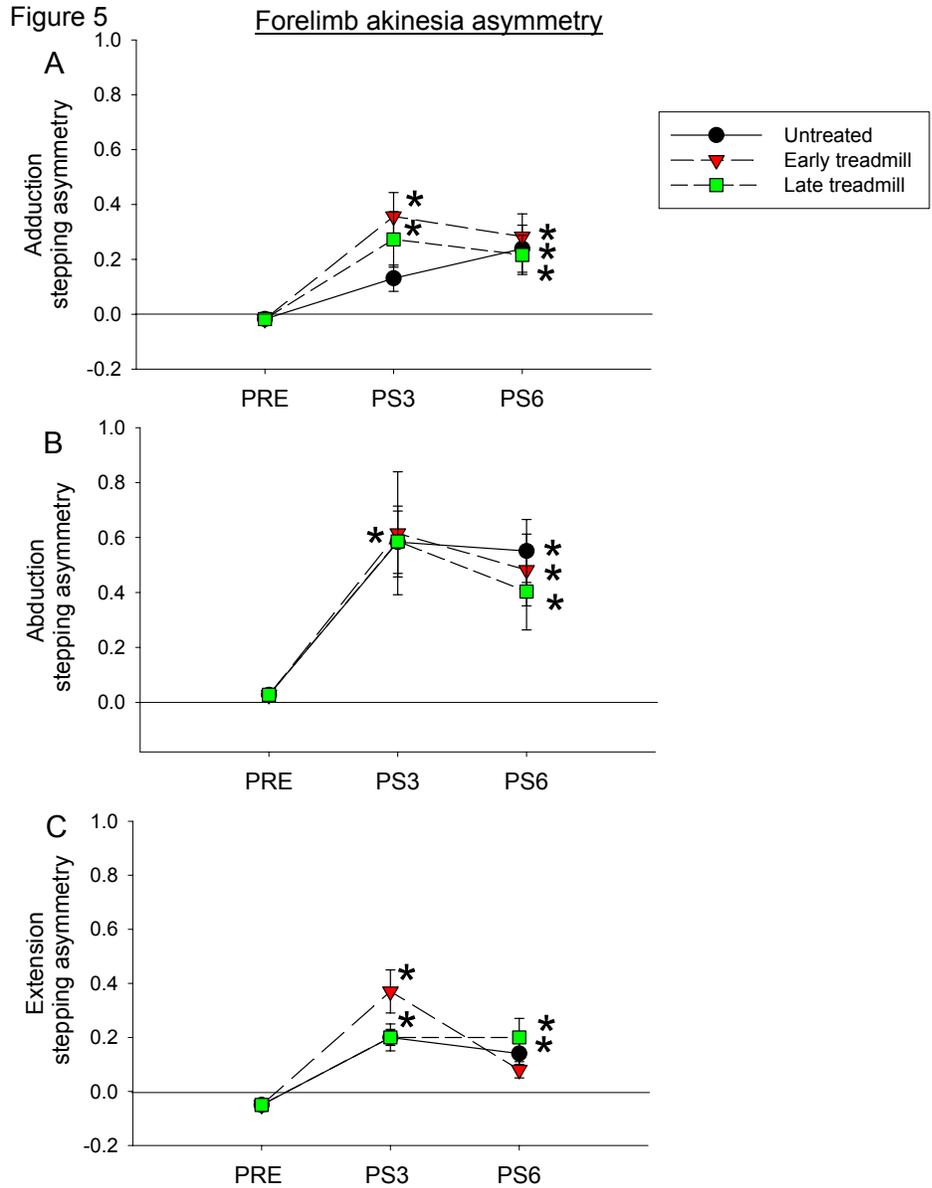
Figure 5 Forelimb akinesia asymmetry

The effect of treadmill training on asymmetries in forelimb stepping initiation in 3 different directions: adduction (A), abduction (B), and extension (C). Behaviour was quantified at 3 different testing sessions once pre-surgical (PRE), 3 weeks post-surgery (PS3) and 6 weeks post-surgery (PS6). In all cases, values greater than 0.0 indicate that the forelimb contralateral to the lesion was slower to initiate stepping movements compared to the ipsilateral forelimb. * Denotes significant difference from pre-surgical values (p<0.05).

3.2.3. Ladder rung walking test

3.2.3.1 Stride length, Figure 6A:

Statistical analysis revealed a significant change in stride length across testing

sessions for all groups (Untrained $F_{10, 2}$=8.683; p=0.002; Early $F_{8, 2}$=30.441; p<0.001;

Late $F_{8, 2}$=13.111; p<0.001). At 3 weeks, all groups demonstrated a significant

decrease in stride length compared to pre-surgical values (p<0.05). Nevertheless, by 6

weeks the stride length of untrained animals increased to pre-surgery levels, while the

stride length of both early and late treadmill trained animals did not recover (Untrained

PS6 vs. PRE, p=0.613; PS6 vs. PRE, p<0.001 for both early and late groups). Thus,

treadmill training of 6-OHDA rats resulted in a sustained decrease in stride length on

the ladder walking task, a change that was not seen in untrained animals.

3.2.3.2 Speed, Figure 6B:

Statistical analysis of average speeds used to cross the ladder revealed a

significant effect across testing sessions in all groups. (Untrained $F_{10, 2}$=7.798; p=0.003;

Early $F_{8, 2}$=8.187; p=0.004; Late $F_{8, 2}$=14.362; p<0.001). *Post hoc* analysis revealed a

significant decrease in speed on the ladder task at 3 weeks post surgery compared to

pre-surgical values in all groups (p<0.05 all groups). However, by 6 weeks post-

surgery, the speeds used by both untrained and early treadmill trained animals showed

no significant difference from pre-surgery values (p>0.05 for both early and untrained),

even though, for the early treadmill trained group, the average speed was slower than

pre-surgery. The lack of statistical significance in the latter group is likely due to the

large variation in speeds used by animals in this group at 6 weeks. Animals in the late

treadmill trained group maintained a slower speed across the ladder at 6 weeks post-surgery compared to pre-surgery values ($p<0.05$). Thus, animals which underwent treadmill training use slower speeds to cross the ladder compared to the speeds used prior surgery. This is consistent with the smaller stride lengths used by these groups described above, as speed is determined partly by stride length.

Figure 6   Stride length and speed on ladder task
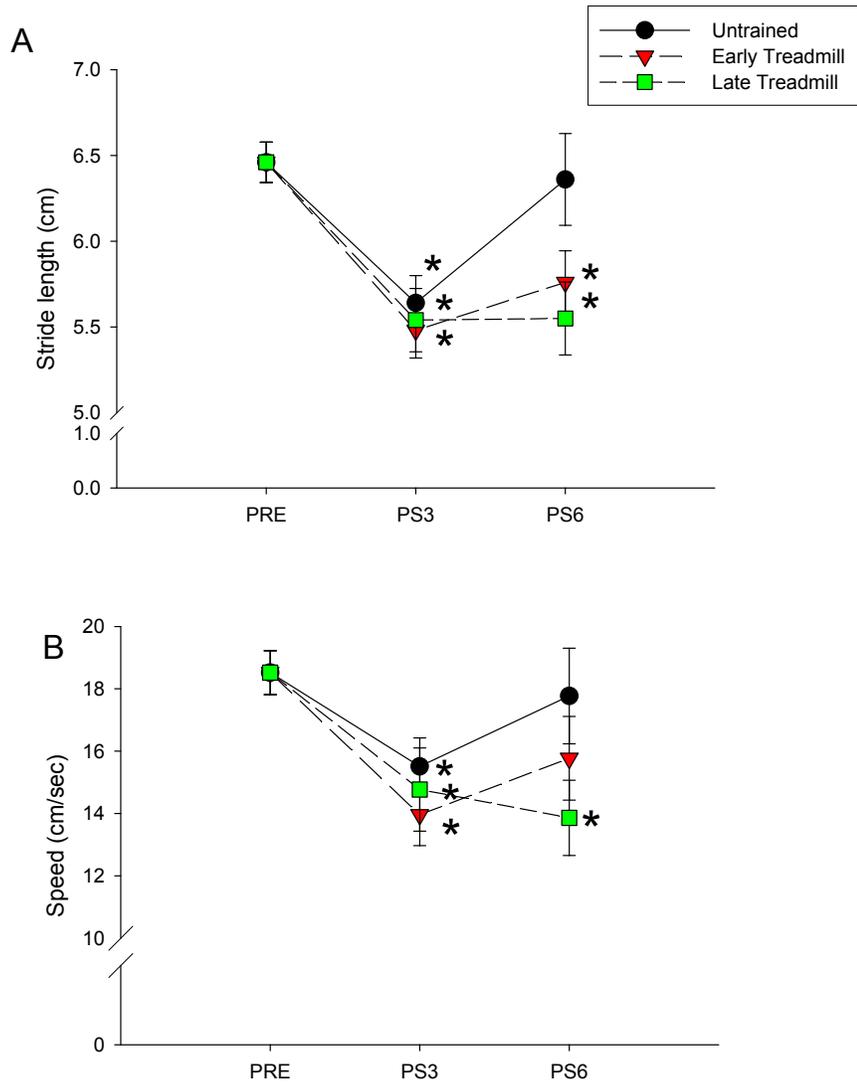
Figure 6 Stride length and speed on ladder task
The effects of treadmill training on stride length and speed on the ladder walking task in 6-OHDA lesioned rats. (A) All groups demonstrate a significant decrease in stride at 3 weeks post surgery compared to pre-surgical values. At 6 weeks post-surgery, there was a natural recovery of stride length in the untrained group that did not occur in either of the treadmill trained groups. (B) All groups demonstrate a significant decrease in stride at 3 weeks post surgery compared to corresponding pre-surgical values, although only speeds used by the late treadmill trained groups were significantly lower than pre-surgical values by 6 weeks. PRE = previous to 6-OHDA surgery, PS3 = 3 weeks post-surgery, PS6 = 6 weeks post-surgery. * Denotes significance from pre-surgical value ($p < 0.05$).

3.2.3.3 Correct placement of forelimbs

Analysis of limb placement during the ladder walking task revealed that there were no differences in the number of correct placements or errors in placement of the hindlimbs between pre-surgery and post-surgery time points for any of the groups ($p > 0.05$ all groups post-surgery 3 and 6 weeks compared to corresponding pre-surgical values) Therefore, the remaining results focus on the placement of the forelimbs only. Prior to surgery, animals tended to make correct forelimb placements on the ladder 95% of the time (Figure 7). At 3 and 6 weeks after the 6-OHDA surgery, all groups made correct forelimb placements only 70 – 75% of the time, which was significantly less than pre-surgical values (Untrained $F_{10, 2} = 60.591$; $p < 0.001$; Early treadmill $F_{8, 2} = 29.299$; $p < 0.001$; Late treadmill $F_{8, 2} = 20.292$; $p < 0.001$). Mean asymmetry scores for correct forelimb placements were consistently less than zero in all groups, indicating that most correct placements occurred in the forelimb ipsilateral to the lesion (i.e. the unimpaired forelimb). There were no differences between any of the groups at any time point, indicating that treadmill training did not affect correct forelimb placement on the ladder rung walking task, (Pre, $F_{2, 26} = 1.859$; $p = 0.176$), (PS3, $F_{2, 26} = 0.502$; $p = 0.611$), (PS6, $F_{2, 26} = 0.136$; $p = 8.74$).

Figure 7

Correct placement of forelimbs on ladder
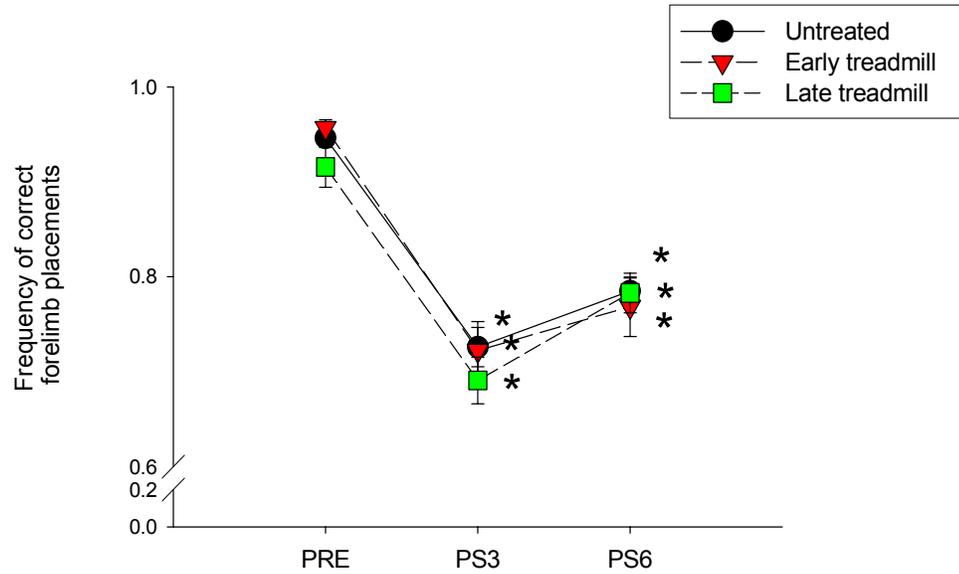
Figure 7 Correct forelimb placements on ladder
All groups made significantly fewer correct forelimb placements on the ladder after 6-OHDA lesion. There was no effect of treadmill training on the number of correct forelimb placements. * Denotes significant difference from corresponding pre-surgical value ($p<0.05$).

3.2.3.4 Errors in forelimb placement on ladder walking task

The frequency of forelimb errors on the ladder (= slight slips + deep slips + misses; see Methods) was increased in all groups after 6-OHDA surgery for both right and left limbs (Figure 8). At 3 weeks post-surgery for all groups, more errors were made by both the right forelimb (the impaired limb – Figure 8A) and the left forelimb (the unimpaired limb – Figure 8B) compared to corresponding pre-surgical values (Untrained group right limb $F_{10,2}=25.497$; Untrained group left limb $F_{10,2}=25.137$; Early group right limb $F_{8,2}=22.038$; Early group left limb $F_{8,2}=25.551$; Late group right limb $F_{8,2}=6.282$; Late group left limb $F_{8,2}=25.551$, $p<0.001$ for all groups, either limb). At 6 weeks post-surgery, untrained animals and those in the early trained group continued to make more errors with the impaired limb compared to errors made prior to surgery, but the errors made by the unimpaired limb had recovered to pre-surgical values by this time (compare PS6 in Figure 8A with Fig 8B; Untrained group right limb $F_{10,2}=25.497$; Untrained group left limb $F_{10,2}=25.137$; Early group right limb $F_{8,2}=22.038$; Early group left limb $F_{8,2}=25.551$; $p<0.001$ for impaired limb, $p=0.50$ for unimpaired limb). Similar results were obtained for the late trained group, except that the errors made by the impaired limb at 6 weeks only approached significant difference from pre-surgical values (Late group right limb $F_{8,2}=6.282$; Late group left limb $F_{8,2}=25.551$, $p=0.059$ for impaired limb, $p=0.60$ for unimpaired limb). Thus, treadmill training does not appear to alter the number of errors made by the forelimbs during ladder crossing.

Figure 8

Figure 8   Forelimb errors on the ladder
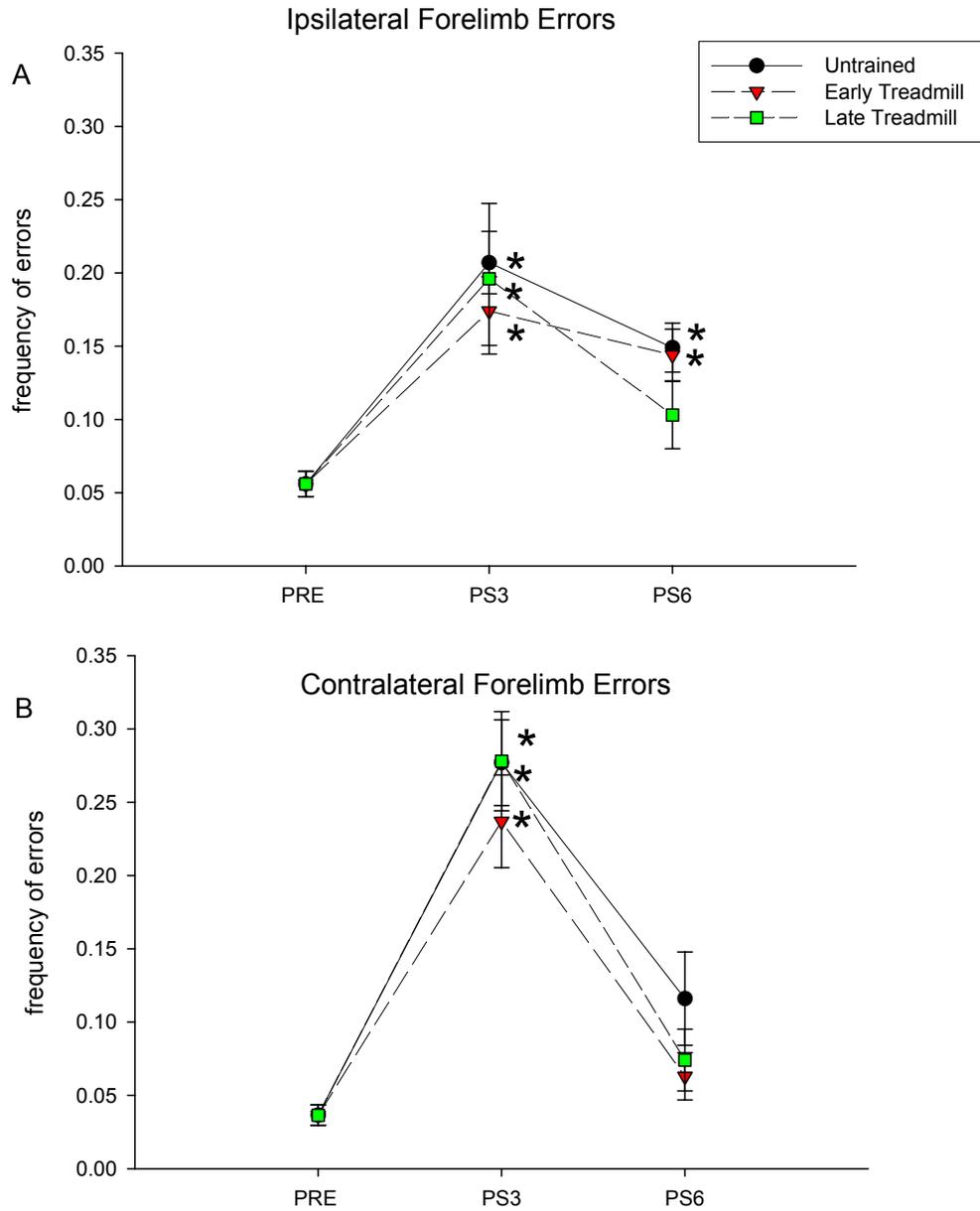
Figure 8 Forelimb errors on the ladder
(A) Frequency of slips and misses made on the ladder by the impaired forelimb
(contralateral to lesion). (B) Frequency of slips and misses resulting from unimpaired
(ipsilateral) forelimb placements on the ladder. * Denotes significant difference from
corresponding pre-surgical values (p<0.05).

3.2.4. Ground reaction forces generated during locomotion

Ground reaction forces produced by all animals prior to 6-OHDA injection were identical to those found previously for normal animals (Figure 9, Muir and Whishaw 1999; Muir and Whishaw 2000; Webb and Muir 2002; Webb and Muir 2003; Webb and Muir 2004). Rats trot overground by weightbearing alternately on diagonal limb pairs. Peak vertical forces produced by the forelimbs are similar to those produced by the hindlimbs, such that rats normally bear equal amounts of weight on fore- and hindlimbs (Figure 9A). Examination of fore-aft forces demonstrates that most of the braking forces (negative fore-aft forces) are produced by the forelimbs, whereas most propulsion (positive fore-aft force) is generated by the hindlimbs (Figure 9B). Lateral forces are small (Figure 9C). Normal animals move symmetrically, with equal forces produced by right and left limbs (Figure 9, compare Right and Left). This symmetry is more apparent when vertical forces are plotted against fore-aft forces for right and left limbs (Figure 10).

After unilateral injection of 6-OHDA, all animals moved with an asymmetric gait characterized by altered forces generated by all limbs (Figures 11 – 17; Muir & Whishaw, 1999). In particular, smaller peak propulsive forces were generated by the impaired forelimb (contralateral to the lesion) compared to pre-surgical values in all groups ($F_{6,80}$ = 31.744, p<0.001; *post-hoc* tests, p <0.05 for all groups). The unimpaired forelimb generated larger peak braking forces compared to pre-surgical values in all groups ($F_{6,80}$ = 6.613, p<0.001; *post-hoc* tests, p<0.05 for all groups). In untrained animals, there was a significant reduction in the peak braking forces

generated by the unimpaired hindlimb compared to pre-surgical values. However, this difference was not seen in the treadmill trained animals ($F_{6,80} = 2.851$, $p<0.05$; *post-hoc* tests, $p< 0.05$ for untrained groups at 3 and 6 weeks post-surgery). There were no other differences in ground reaction forces between trained and untrained animals at any time point.

Force-vector dynamograms in Figure 14 demonstrate the differences in right-left symmetry between pre-surgical animals and those at 3 and 6 weeks after 6-OHDA injection (Figure 16 B – G; compare red and black lines). These dynamograms also illustrate differences in the manner in which the limbs, particularly the impaired forelimb and unimpaired hindlimb, were loaded and unloaded during weightbearing (Figure 16). During the trot, these limbs are weightbearing simultaneously. In both trained and untrained groups, the loading of the impaired forelimb occurred such that braking forces (negative fore-aft forces) were generated only by the time most vertical force was exerted (solid arrows in Fig 16, B-G: impaired forelimb = red solid line). This was in contrast to pre-surgical animals in which braking forces were exerted gradually as the forelimb supported increasing amounts of bodyweight (solid arrowhead in Fig 16A: forelimbs = red and black solid lines). Similarly, the unimpaired hindlimb was unloaded such that propulsive forces (positive fore-aft forces) began to decrease much earlier in the stride compared to those generated by the hindlimbs prior to surgery (open arrows in Fig 16, A-G: black dashed line).
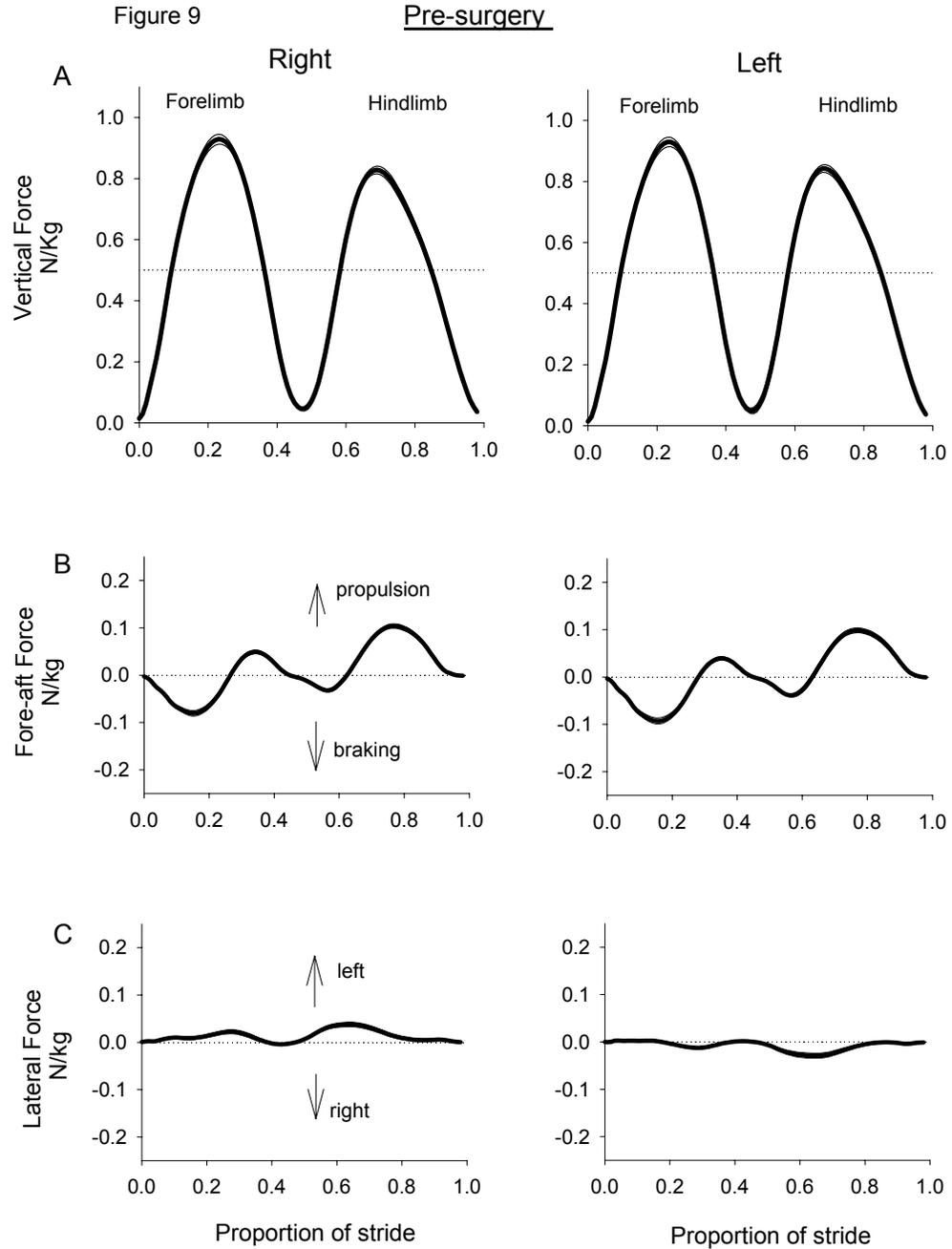
Figure 9 Pre-surgical ground reaction forces
Ground reaction forces for all animals prior to 6-OHDA injection vs. proportion of stride during overground locomotion. Data from right and left limbs are shown separately. Thick lines represent mean forces normalized to bodyweight, thin lines represent ± standard error, n=29. For each graph, data from the forelimb precedes that from the ipsilateral hindlimb (A) Vertical forces (B) Horizontal fore-aft forces, (C) Horizontal lateral forces. Note the normal symmetry between left and right limbs.

Figure 10

Pre-surgery force-vector dynamogram
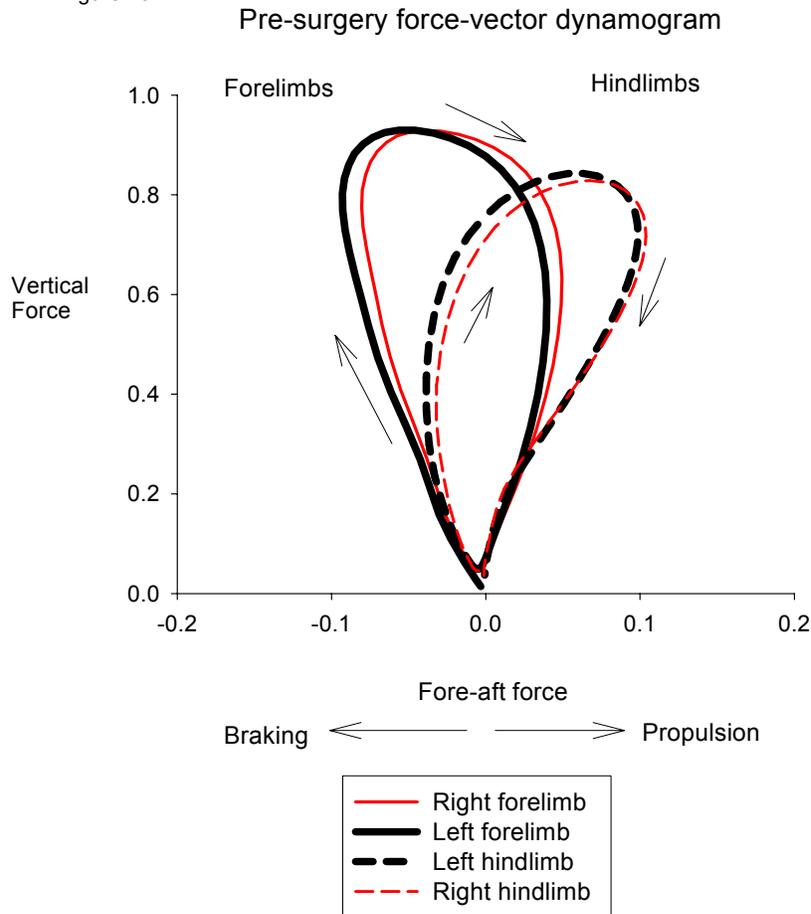
Figure 10 Force vector dynamogram of pre-surgery ground reaction forces
Force vector dynamogram (mean vertical vs. mean fore-aft forces) of pre-surgical
animals (n=29) during overground locomotion. All forces are normalized to
bodyweights. Vertical force indicates the weight supported by a given limb. Fore-aft
force indicates the amount of braking or propulsion produced by a given limb. The
forelimbs produce mainly braking forces (negative fore-aft forces) whereas the
hindlimbs produce mainly propulsive force (positive fore-aft forces). Note the
symmetry between left and right for both fore and hindlimbs. Arrows indicate the
loading of the limbs over time, beginning at the initiation of the stance phase at 0.0.
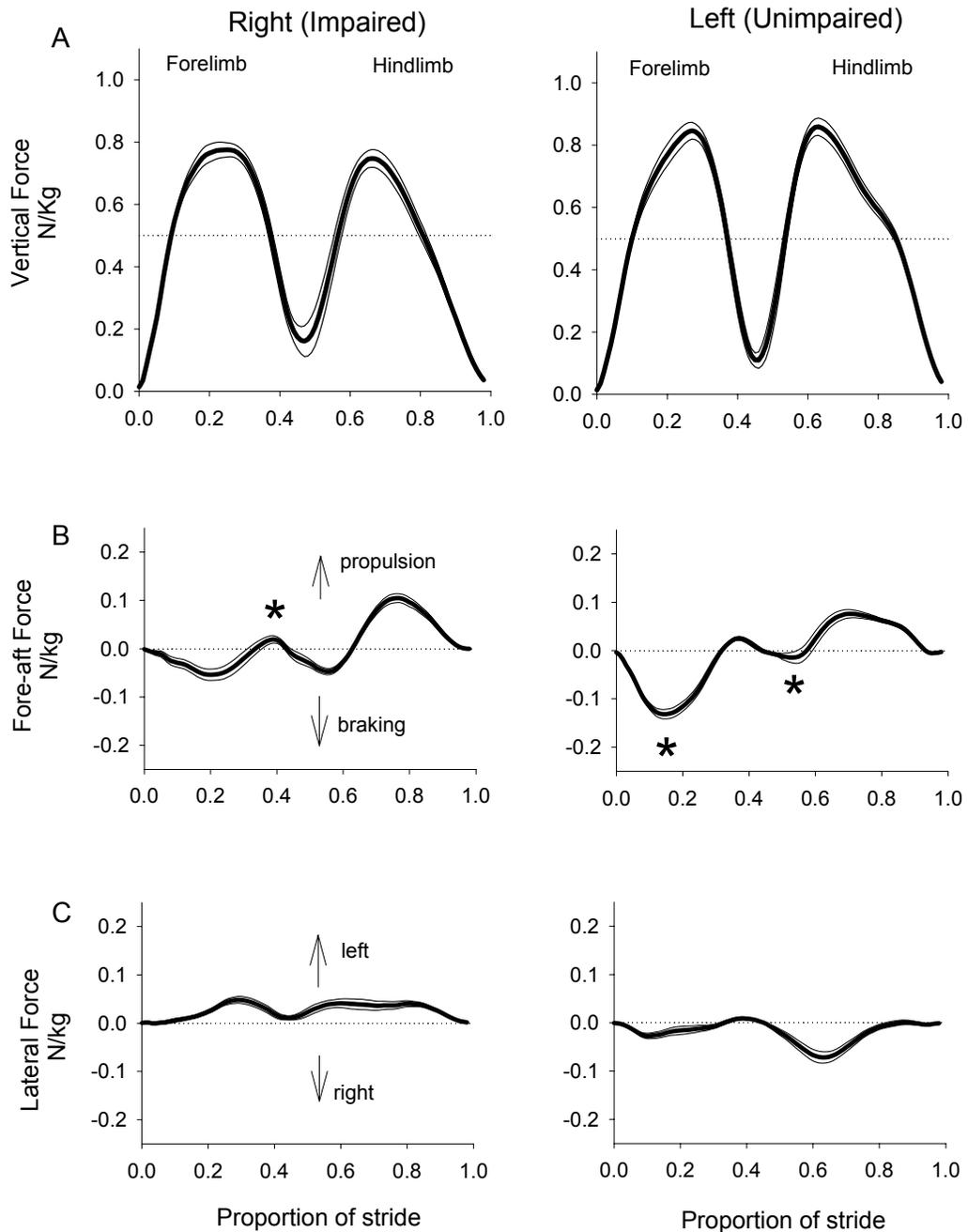
Figure 11 Ground reaction forces in untrained animals, 3 weeks post surgery
Ground reaction forces for untrained animals 3 weeks post 6-OHDA injection vs. proportion of stride during overground locomotion. Data from right and left limbs are shown separately. Thick lines represent mean forces normalized to bodyweight, thin lines represent ± standard error, n=11. For each graph, data from the forelimb precedes that from the ipsilateral hindlimb (A) Vertical forces (B) Horizontal fore-aft forces, (C) Horizontal lateral forces. * Denotes that peak force is significantly different from corresponding pre-surgical values (p<0.05).
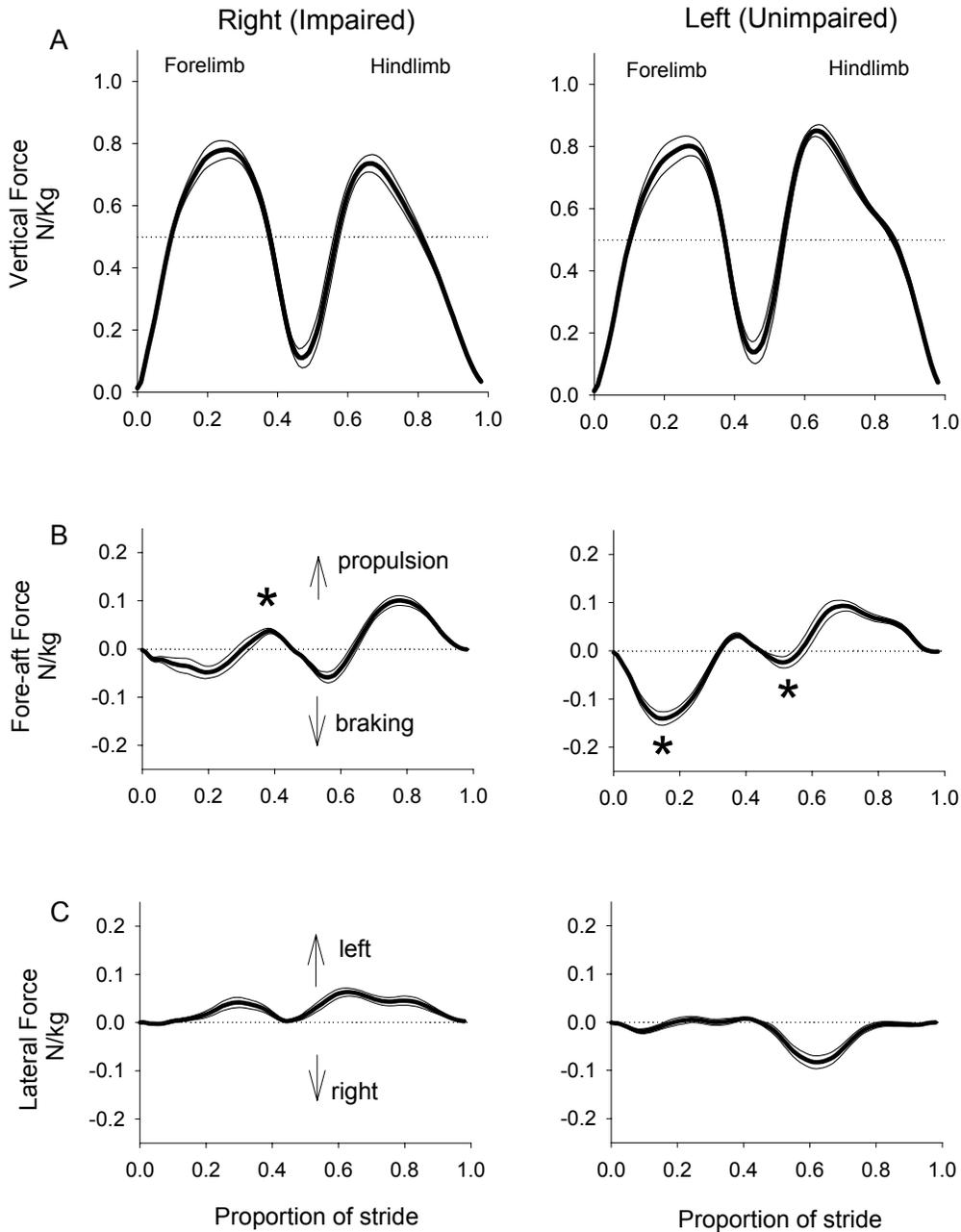
Figure 12 Ground reaction forces in early trained animals, 3 weeks post surgery
Ground reaction forces for early treadmill trained animals 3 weeks post 6-OHDA
injection vs. proportion of stride during overground locomotion. Data from right and
left limbs are shown separately. Thick lines represent mean forces normalized to
bodyweight, thin lines represent ± standard error, n=9. For each graph, data from the
forelimb precedes that from the ipsilateral hindlimb (A) Vertical forces (B) Horizontal
fore-aft forces, (C) Horizontal lateral forces. * Denotes significant difference in peak
force from corresponding pre-surgical values (p<0.05).

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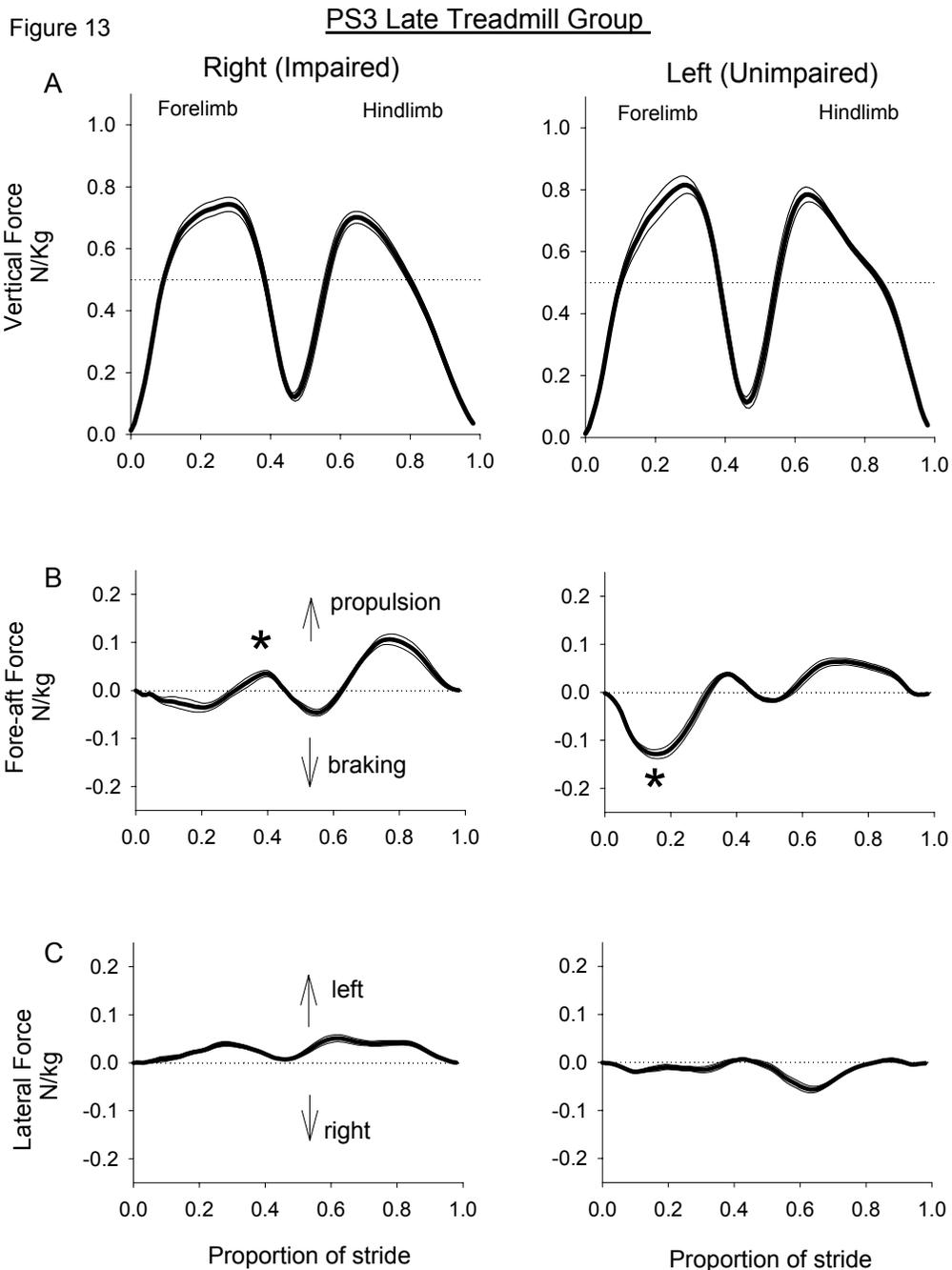
Figure 13 Ground reaction forces in late trained animals, 3 weeks post surgery
Ground reaction forces for late treadmill trained animals 3 weeks post 6-OHDA
injection vs. proportion of stride during overground locomotion. Data from right and
left limbs are shown separately. Thick lines represent mean forces normalized to
bodyweight, thin lines represent ± standard error, n=9. For each graph, data from the
forelimb precedes that from the ipsilateral hindlimb (A) Vertical forces (B) Horizontal
fore-aft forces, (C) Horizontal lateral forces. * Denotes significant difference in peak
force from corresponding pre-surgical values (p<0.05).
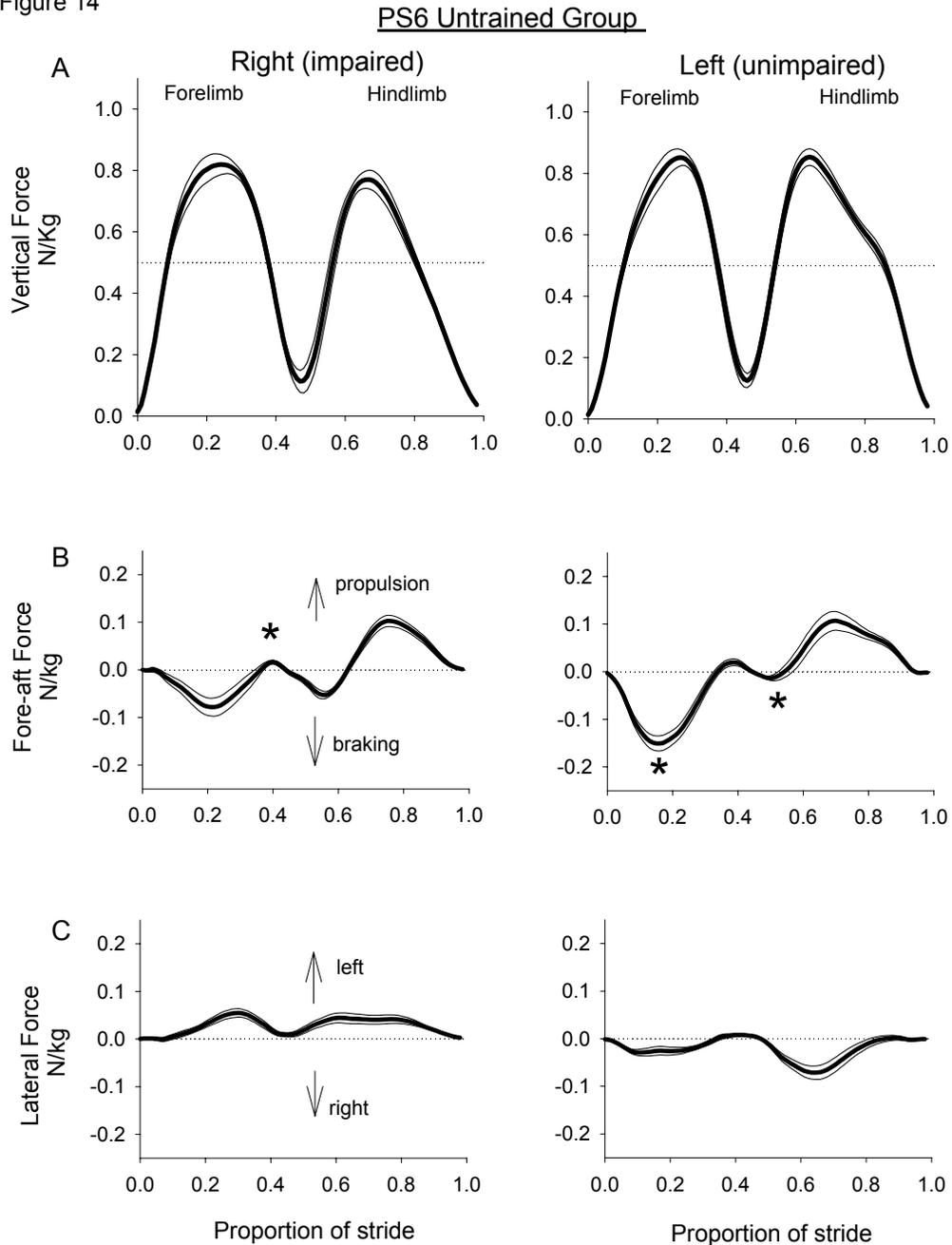
Figure 14 Ground reaction forces in untrained animals, 6 weeks post surgery
Ground reaction forces for untrained animals 6 weeks post 6-OHDA injection vs.
proportion of stride during overground locomotion. Data from right and left limbs are
shown separately. Thick lines represent mean forces normalized to bodyweight, thin
lines represent ± standard error, n=11. For each graph, data from the forelimb precedes
that from the ipsilateral hindlimb (A) Vertical forces (B) Horizontal fore-aft forces, (C)
Horizontal lateral forces. * Denote significant difference in peak force from
corresponding pre-surgical values (p<0.05).
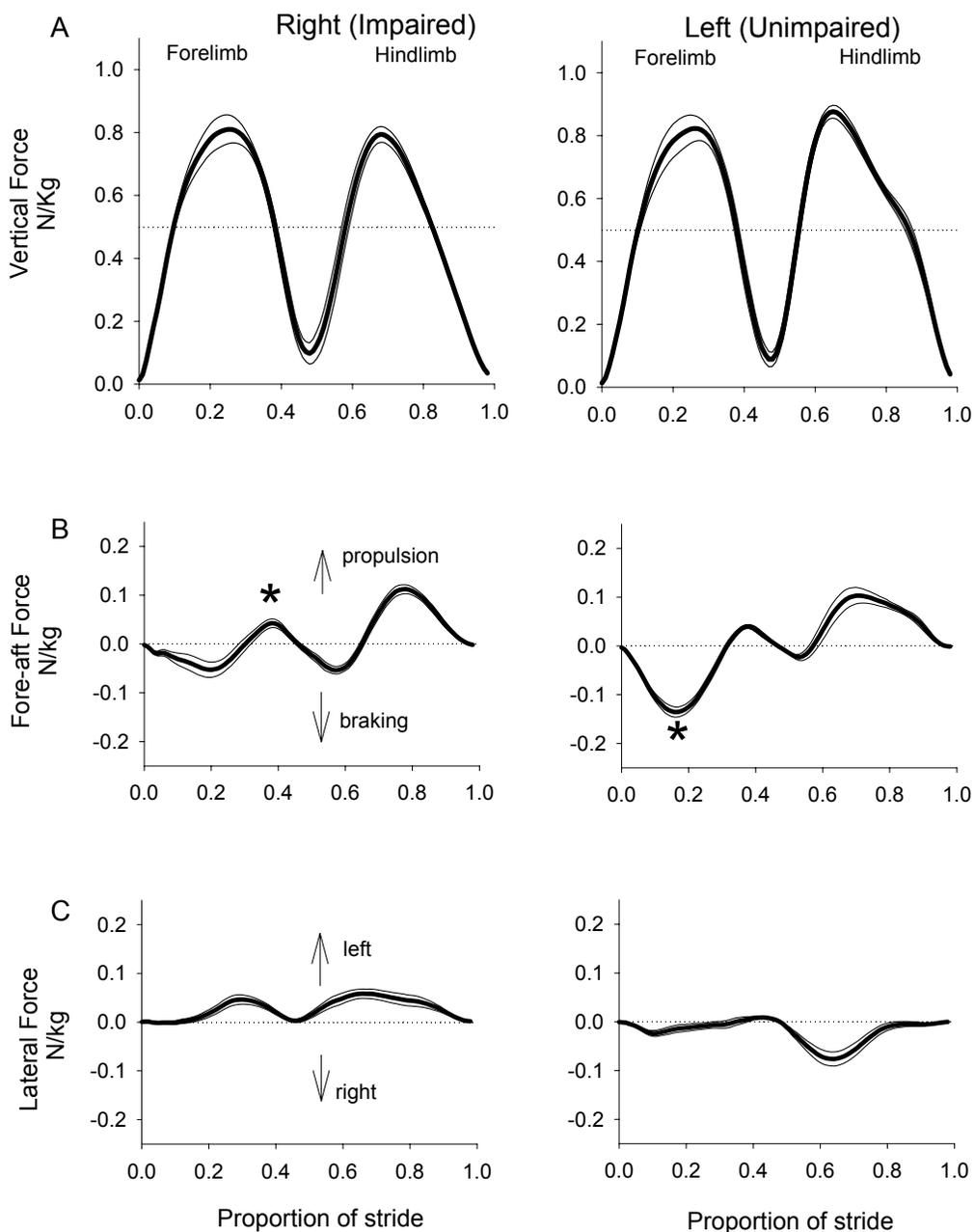
Figure 15 Ground reaction forces in early trained animals, 6 weeks post surgery
Ground reaction forces for early treadmill trained animals 6 weeks post 6-OHDA
injection vs. proportion of stride during overground locomotion. Data from right and
left limbs are shown separately. Thick lines represent mean forces normalized to
bodyweight, thin lines represent ± standard error, n=9. For each graph, data from the
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force from corresponding pre-surgical values (p<0.05).
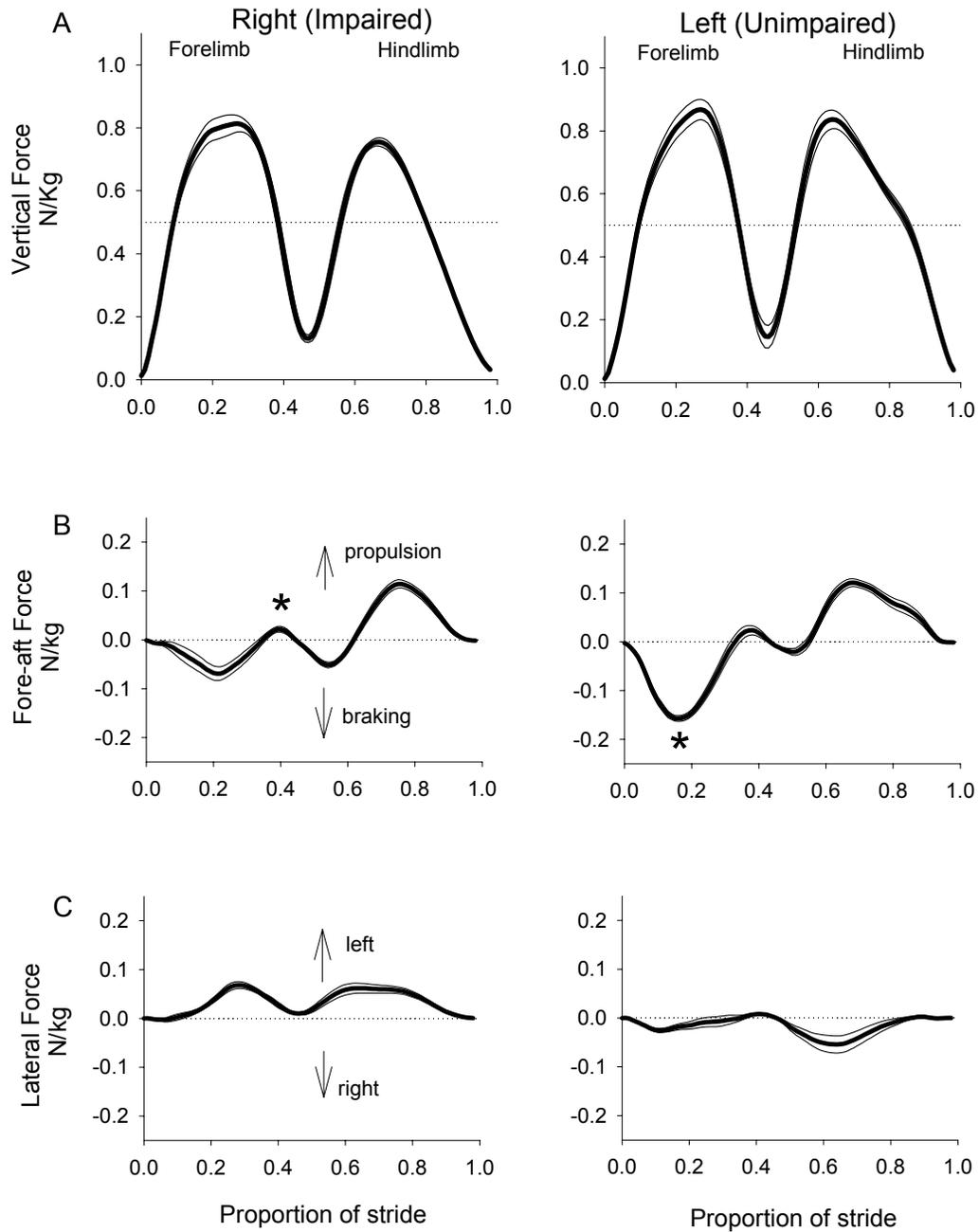
Figure 16 Ground reaction forces in late trained animals, 6 weeks post surgery
Ground reaction forces for late treadmill trained animals 6 weeks post 6-OHDA
injection vs. proportion of stride during overground locomotion. Data from right and
left limbs are shown separately. Thick lines represent mean forces normalized to
bodyweight, thin lines represent ± standard error, n=9. For each graph, data from the
forelimb precedes that from the ipsilateral hindlimb (A) Vertical forces (B) Horizontal
fore-aft forces, (C) Horizontal lateral forces. * Denote significant difference in peak
force from corresponding pre-surgical values (p<0.05).
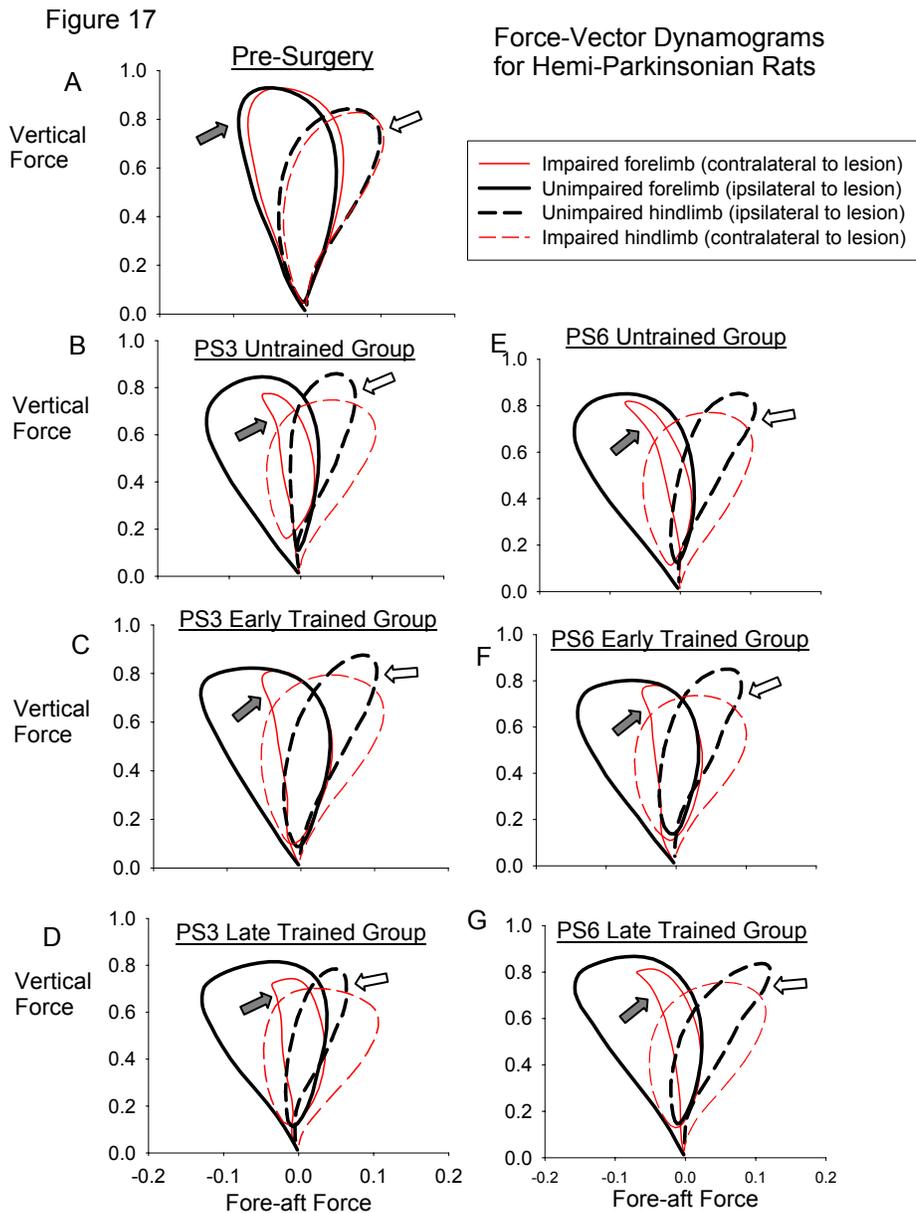
Figure 17 Force-Vector Dynamograms in hemi-Parkinsonian rats
Force-Vector Dynamograms (mean vertical vs. fore-aft forces) illustrate symmetry
between left and right limbs during overground locomotion. All forces are normalized
to bodyweight. (A) Animals have nearly perfect symmetry prior to 6-OHDA lesions.
(B-G) Note the distinct right-left asymmetries in all groups at 3 and 6 weeks post-lesion
(B-D) 3 weeks after 6-OHDA injection. (E-G) 6 weeks after 6-OHDA injection. Solid
arrows indicate differences in impaired forelimb braking in all groups compared to
presurgical forces. Open arrows indicate differences in unimpaired hindlimb propulsion
in all groups compared to corresponding pre-surgical forces.

3.3. Neurochemical analysis

Individual HPLC values for left and right hemispheres were used to calculate a ratio (lesion/intact hemisphere x 100) which indicated the percent of catecholamine remaining in the lesioned hemisphere. Date for both treadmill trained groups were combined, as there was no significant difference between early and late treadmill training on catecholamine levels at 8 weeks post-surgery (DA F=0.04, p=0.8389; DOPAC F =0.28, p=0.5984; NE F=0.00, p=0.9533). A single degree of freedom orthogonal contrast demonstrated a significant difference between the trained and untrained animals for levels of dopamine (p<0.05) and DOPAC (p<0.05) but not for NE (p=0.07) (Figure 18).   Thus, treadmill training attenuated dopamine and DOPAC loss in the striatum compared to that in untrained animals.

Figure 18   Catecholamine content of striatum 8 weeks post-surgery
The effect of treadmill training on catecholamine content in the striatum at 8 weeks post-surgery. (A) Dopamine content of the lesioned caudate/intact caudate after square root transformation (see methods). Treadmill training preserved significant amounts of dopamine on the lesioned side of the brain. (B) DOPAC content of the lesioned caudate/intact caudate after square root transformation (see methods). Treadmill training preserved significant amounts of DOPAC on the lesioned side of the brain. (C) Norepinephrine content of the lesioned caudate/intact caudate after square root transformation (see methods). Treadmill training did not affect the NE levels on the lesioned side of the brain. (* indicates p<0.05)

4. Discussion

In summary, the results demonstrate that treadmill training in a 6-OHDA induced model of Parkinson's disease caused attenuation of dopamine loss in the striatum as assessed by response to apomorphine injection and assessment of post-mortem striatal dopamine levels. In contrast, this same training did not ameliorate behavioural deficits, including locomotor deficits. Treadmill trained animals showed similar behavioural deficits compared to untrained animals on most tests, including forelimb preference during exploration, forelimb akinesia during limb abduction, forelimb placement during ladder crossing, or the ground reaction forces generated during overground locomotion. On several tests, treadmill trained animals tended to display more severe behavioural deficits compared to untrained animals. For example, treadmill trained animals developed forelimb akinesia in the adduction direction at an earlier time point than did untrained animals. Treadmill trained animals also moved across the ladder at slower speeds and with smaller stride lengths compared to untrained animals. There was only one test on which treadmill trained animals appeared to have less severe deficits than untrained animals - early treadmill training appeared to reduce forelimb akinesia when stepping in the extension direction. For most behavioural tests, late treadmill training had a similar effect compared to early treadmill training.

4.1 Locomotor asymmetry in hemi-Parkinsonian rats

This is the first thorough examination of the ground reaction forces produced during locomotion in 6-OHDA lesioned rats. Untrained lesioned rats moved with a characteristic asymmetric gait. The most abnormalities were seen during the simultaneous diagonal contact of the impaired forelimb (contralateral to the lesion) and the unimpaired hindlimb (ipsilateral to the lesion). The impaired forelimb generated smaller propulsive forces and also produced braking forces much later in the support phase compared to those generated by the forelimbs prior to surgery (Figures 11B-16B, Fig 17). Propulsive forces generated by the unimpaired hindlimb began to decrease much earlier in the stride compared to those generated by the hindlimbs in pre-surgical animals (Figure 17). These changes were compensated in part by the actions of the unimpaired forelimb, which produced increased braking forces much earlier in the stride compared to pre-surgical values (Figures 11B – 16B). Interestingly, many of these changes are similar to those seen in rats with other unilateral central nervous system (CNS) lesions, including unilateral red nucleus ablation and unilateral spinal lesions (Muir & Whishaw 2000; Webb & Muir 2003; Webb & Muir 2004). It may be that rats with unilateral CNS impairment adopt a general compensatory strategy for locomoting overground.

Locomotor training in the form of treadmill training did not induce a return to a symmetric pre-surgical gait. As discussed below, it may be that the amount and type of training was not able to attenuate the loss of DA in this animal model sufficient to affect locomotor behaviour. It is also possible that the asymmetric gait adopted by treadmill trained animals in the present study was actually reinforced by repeated treadmill

training. A third possibility is that, because animals were still able to move overground

effectively using an asymmetric gait, there was no impetus to return to normal

symmetrical movement. In future studies, it would be of interest to administer proven

pharmacological therapies, i.e. L-DOPA, in this animal model to determine whether

lesioned animals would recover normal symmetrical locomotion.

4.2 The effects of rehabilitative training in 6-OHDA treated rats

4.2.1. The effects of forced forelimb use

The results of this study are in contrast to previous work which examined the

forelimb use and forelimb akinesia after forced use of the impaired forelimb in 6-

OHDA treated rats. Specific rehabilitation strategies involving the forelimbs have

resulted in attenuation of deficits on forelimb-specific tests post surgery (Tillerson et al

2001; Vergara-Aragon et al. 2003). Forced use of the impaired forelimb (by casting the

unimpaired forelimb for 7 days starting at 1-3 days post-surgery) resulted in attenuation

of forelimb use asymmetry, forelimb abduction akinesia, and reduced striatal dopamine

loss (Tillerson et al. 2001). If the cast was not in place until 7 days post-surgery, there

was no attenuation of behavioural deficit or dopamine loss (Tillerson et al. 2001).

Interestingly, if the cast was placed on the impaired limb for 7 days post-surgery,

forelimb deficits were exacerbated (Tillerson et al. 2002). Both attenuation and

exacerbation of forelimb deficits correlated well with the amount of dopamine

remaining in the striatum post-lesion (Tillerson et al. 2001; Tillerson et al. 2003).

These studies suggest that there is an opportune window of time in which use of the

forelimb can prevent the death of dopamine neurons in the presence of 6-OHDA. In this

animal model, development of striatal dopamine depletion is thought to occur over a 1-2 week post-injection period through oxidative damage and eventual apoptosis of dopaminergic neurons (Cerruti et al. 1993; Croker et al. 2001; Decker et al 1993; Glinka et al. 1997; Hirsch 1999; Hudson et al 1993; Ungerstedt 1972). Early forced forelimb use may interfere with this process through a variety of mechanisms (see Section 4.2.3), producing animals with less severe dopamine loss.

In a separate study, forelimb reaching rehabilitation that commenced 21 days post-surgery was also demonstrated to have positive behavioural effects in 6-OHDA treated rats (Vergara-Aragon et al. 2003). Rehabilitation training for 14 days in a reaching box, with wire grid construction, beginning 21 days after surgery, resulted in significant improvement on a single pellet reaching task (Vergara-Aragon et al. 2003). During training, the rats were free to use either forelimb, although the unimpaired limb was used preferentially (Vergara-Aragon et al. 2003). Although this task improved reaching success (ability to bring food to their mouth) with the unimpaired limb, detailed movement analysis revealed that subtle deficits in paw supination persisted after training (Vergara-Aragon et al. 2003). It was suggested in this study that the improvement in reaching success was not due to reduction of lesion severity, but resulted from the development of compensatory movements that were specific to reaching behaviour (Vergara-Aragon et al. 2003). It is therefore possible that deficits in other, untested, behavioural tasks would remain unaffected by forelimb rehabilitation training.

4.2.2 The effects of treadmill training

In the present study, we investigated whether locomotor rehabilitation, in the form of treadmill training, would ameliorate locomotor and forelimb behavioural deficits. We found that neither early or late intervention in the form of treadmill training resulted in the amelioration of deficits for most behaviours examined. It is possible that, if reduction in lesion severity is the cause of amelioration of behavioural deficits after early training, as suggested by Tillerson (2001, 2002), then perhaps there was insufficient preservation of DA levels in the present study to produce attenuation of deficits, as discussed further in this section. If, instead, behavioural compensation underlies rehabilitative recovery after late training, as described in Vergara-Aragon et al. (2003), then perhaps treadmill training provided insufficient specific forelimb training than was necessary to improve forelimb behavioural tasks. The exception to this finding was the reduction in forelimb extension akinesia seen in early treadmill trained rats (fig 5C). This apparent attenuation may be a result of the repetition of forelimb extension movement which occurred during treadmill training. In any case, the range and quantification of different behaviours examined in the present study reflect a clear representation of the effects of treadmill training on forelimb and locomotive behaviour in 6-OHDA induced hemi-Parkinsonian rats.

There is only one other study which has examined the effects of early treadmill training on forelimb use and akinesia in hemi-Parkinsonian rats (Tillerson et al. 2003). Surprisingly, complete amelioration of DA depletion, deficits in forelimb use asymmetry and stepping initiation asymmetry were found after brief exposure to very

early treadmill training (Tillerson et al. 2003). As discussed below, several methodological differences may have contributed to these contrasting results.

First, there were differences in the amount of 6-OHDA used to create the lesions. In the present study, we infused a total of 20 μg of 6-OHDA over 2 injections. In the Tillerson (2003) study, a single injection of 10 μg of 6-OHDA was reported. The quantitatively different exposure to the neurotoxin might have produced different severity of lesions in that a larger amount of neurotoxin may have induced more rapid degeneration. In the current study, post-mortem striatal DA analysis showed that, in untrained animals, 95-100% of DA was depleted in the affected striatum, whereas this value was 80% in untrained animals in the Tillerson (2003) study. In contrast, the treadmill trained animals in the Tillerson (2003) study had an astounding 0%-10% loss in dopamine content whereas our findings indicate a 75%-80% loss of dopamine in the treadmill trained groups (Figures 3 & 18). In future studies, it may be fruitful to examine the behavioural effects of treadmill training on animals with mild or moderate 6-OHDA lesions, to determine whether rehabilitation is more effective in animals with less severe lesions.

A second major methodological difference was the time of onset of the treadmill training. In the present study, animals were allowed to recover for 24 hours post-surgery before the first 20 minute treadmill session began (see Methods). Tillerson (2003) reported that treadmill training commencing 2 - 4 hours post-surgery following a forelimb-use asymmetry screening (i.e. only animals that displayed asymmetry were kept in the study). This very early introduction to treadmill running directly following anesthetic and surgery (which involves removal of bone from the skull and an

intracerebral injection), may have had unexpected effects. For example, it may have introduced an increased amount of stress on the animals compared to that involved after a 24 hour recovery period prior to exercise. Stressful experiences in hemi-Parkinsonian animals have been demonstrated to produce a phenomenon of "paradoxical kinesia," in which behavioural deficits are strikingly less severe following exposure to electric shock, submersion in cool water, forced immobilization, etc. (Keefe et al. 1989; Keefe et al. 1990; Keefe et al. 1993; Schallert 1989). Paradoxical kinesia effects are usually transient, however, unlike the long lasting results in the Tillerson (2003) study, but the mechanism of paradoxical kinesia is still not well understood (Keefe et al. 1990; Keefe et al. 1993).

4.2.3 Possible mechanisms for the effect of training

It is difficult to interpret the findings of Tillerson et al (2003) in terms of what is known regarding the development of motor output deficits after 6-OHDA injection. Dopamine cell loss has been reported to occur over 14 days post-injection, as assessed by asymmetrical rotation induced by apomorphine injection (Sauer & Oertel 1994; Metz & Whishaw 2002b). Nevertheless, the animals in Tillerson's study showed forelimb use asymmetry 2 – 4 hours post-injection and then showed complete return to symmetrical forelimb use at 24 hours post-injection, after a 15 minute training session the previous day. This almost immediate recovery of behavioural symmetry and the astounding 90-100% intact dopamine remaining after 6-OHDA injection are indicative of the interruption of lesion formation. This may have occurred by inhibition of 6-OHDA uptake into the neuron and/or by an inhibition of apoptosis after 6-OHDA

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uptake. In future studies, these possibilities could be examined using apoptotic markers in the dopaminergic and other catecholaminergic cell populations surrounding the initial injection site for both trained and untrained groups of animals. It is also possible that the lesion was not formed due to normal physiological events occurring during exercise, (discussed later) which may have induced an unintended dispersion of the 6-OHDA away from the medial forebrain bundle, to an area that did not allow for uptake of the neurotoxin by DA neurons. A simple test of this possibility could be performed by examination of the spread of a labeled 6-OHDA after treadmill running at various times post-surgery.

One specific mechanism suggested for DA neuronal sparing after training in 6-OHDA injected rats was exercise-induced down-regulation of the dopamine transporter (DAT) (Tillerson et al, 2003). The dopamine transporter is thought to be the entrance to the cell for neurotoxins such as 6-OHDA (Decker et al. 1993; Cerruti et al. 1993; for review see Miller et al 1999). Once inside the neuron, the neurotoxin induces a cascade of oxidative pathways that lead to apoptosis of the cell (Sauer & Oertel 1994; Crocker et al. 2001). In normal animals, the dopamine transporter functions to reuptake excess synaptically released dopamine. Upon entering the cell, the re-cycled dopamine is packaged into vesicles by vesicular monoamine transporter (VMAT2) (for review see Miller et al.1999). It is thought that the ratio of DAT to VMAT2 is predictive of susceptibility to Parkinson's disease (for review see Miller et al. 1999). It is possible that treadmill running would induce a change in DAT expression, as exercise is known to alter dopamine synthesis and gene expression of catecholamines (Sutoo & Akiyama 2003; Tümer et al. 2001). If one session of treadmill exposure was able to induce an

effective down-regulation of DAT, thus blocking the uptake of 6-OHDA as suggested by Tillerson et al (2003), and if the uptake of 6-OHDA into the DA neuron occurs only during the first 24 hours post-injection, this may provide an explanation for the marked differences in the results of the present study compared to those of Tillerson et al (2003). Treadmill training in the present study did not begin until 24 hours post-injection and it is possible most of the 6-OHDA was already taken up into dopaminergic neurons by this time, such that further treadmill training was not successful in preventing DA neuron cell death. If this is the case, then a single episode of treadmill training within 24 hours after 6-OHDA injection should ameliorate dopaminergic loss and behavioural deficits. The clearance and/or degradation of 6-OHDA from the extra-cellular space requires further study and may play an important role in the prevention of neurodegeneration in this animal model.

The possibility that very early behavioural training after 6-OHDA injection results in lack of striatal DA depletion raises an important issue. If the ultimate purpose for developing rehabilitation protocols in the 6-OHDA animal model is to apply them to Parkinsonian patients, then rehabilitation interventions should be assessed in animals which have developed Parkinsonian symptoms, i.e. after striatal DA depletion has developed 2 weeks post-injection. The biological constraints of the 6-OHDA model are interesting, of course, and should be further explored, but it is yet unclear how these would eventually have consequences for Parkinson's patients. One potential application is the prevention of further development of symptoms in patients in the early stages of Parkinson's, but there is no evidence that rats within the first 2 weeks after 6-OHDA injection are a model for human patients with early stage Parkinson's. Instead, it may be

more useful to examine the effects of rehabilitation protocols in rats with partial lesions induced by smaller quantities of 6-OHDA, as previously suggested.

4.3   The effects of exercise on catecholamine levels in healthy rats

One important result from the present study which was consistent with previous studies was the reduction in DA depletion in trained 6-OHDA-injected animals. Treadmill exercise therefore has an effect on DA levels in the striatum. This also occurs in normal animals. Normal animals that undergo treadmill training at moderate speeds have an increased level of dopamine in the striatum, above that which is induced by other stresses alone (Hattori et al. 1994). Exercise training in normal rats results in a rise in tyrosine hydroxylase activity (the rate limiting enzyme for catecholamine synthesis), as measured by mRNA levels in various brain regions (Tümer et al. 2001). Catecholamines other than dopamine are also affected by exercise, but importantly, treadmill training has mixed effects on brain catecholamine levels depending on the training regime, which can alter the outcome dramatically (for review see; Dishman 1997). Acute exposure to treadmill training has been found to induce a decrease in NE levels, whereas chronic forced exercise has been shown to increase NE in the hypothalamus, brainstem and amygdala. Interestingly, these changes in brain NE levels apparently attenuate the normal increase in NE following foot shock or immobilization stress in rats (Dishman et al. 2000). In the present study, there were no differences in the NE levels in the striatum between trained and untrained animals, likely due to the 3-4 week time period between the end of treadmill training and analysis for catecholamine content. Moderate treadmill training has also been demonstrated to up-regulate growth

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factors such as brain-derived neurotrophic factor, and growth factor receptors such as TrkB in healthy rats (Skup et al. 2002). The result of such neurochemical changes on the effects of 6-OHDA injection are unclear and require close attention in future studies involving treadmill training.

4.4 Catecholamine levels and behavioural correlates in 6-OHDA treated rats

In the present study, there was essentially no effect of treadmill training on behavioural deficits in 6-OHDA injected animals despite some preservation of DA levels in the striatum in these animals. The discrepancy between striatal dopamine levels and performance on behavioural tests has been previously noted in this animal model (Metz & Whishaw 2002b). It was found that apomorphine-induced rotation, an *in vivo* measure of striatal DA levels, was not correlated with endpoint measures of skilled forelimb reaching or performance on the ladder rung walking task (Metz & Whishaw 2002b). Other studies have found that certain behavioural deficits, such as increased latency during switching from one behaviour to another (disengagement task), body axis asymmetry and some sensorimotor functions showed non-linear correlations to apomorphine-induced rotations and to the amount of dopamine remaining (Henderson et al. 2003). Studies of animals with partial lesions demonstrated that these animals do not rotate after apo-morphine injection yet still have behavioural deficits on motor tasks (Barnéoud et al. 1995). It is possible that there is a threshold level of dopamine that is required to produce functionally correct movements (e.g. >70-80 % reduction in normal levels) and that this threshold is different from that required to produce apomorphine rotations (>90% reduction in normal levels) (Barnéoud et al.

1995; Metz & Whishaw 2002b). Thus, as previously mentioned, the small difference in striatal DA levels between trained and untrained animals in the present study may not have been sufficient to affect their behavioural performances.

5. Conclusion

The results of the present study demonstrate that treadmill training in a 6-OHDA model of Parkinson's disease caused partial attenuation of dopamine loss in the striatum, but did not ameliorate behavioural deficits. These results are in contrast to several studies which have shown positive effects of training on behavioural recovery after 6-OHDA injection, but discrepancies between studies could be accounted for by differences in the type of training and methods of assessment. This study has provided new insights into the manner in which hemi-Parkinsonian animals move overground and highlights several important issues concerning rehabilitation protocols in this animal model.

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