The Combined Effects of Exercise and Levodopa in a Unilateral 6-OHDA Rat Model of Parkinson's Disease

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Abstract

Parkinson's disease (PD), a neurodegenerative disorder affecting the control of voluntary movement, is primarily treated pharmaceutically with oral levodopa (L-dopa). However, within a few short years, PD patient develop devastating side effects including motor response complications (MRC) and a diminished duration with chronic use. Although the mechanisms are not widely understood, emerging studies have shown the beneficial effects of exercise at restoring or maintaining motor skills traditionally lost in PD patients. Despite the extensive research on both L-dopa and exercise, there has been limited research about the combined effects of both treatments. This study aims to better understand the interaction effects between exercise and L-dopa, especially regarding exercising as a means to relieve some of the motor side effects associated with L-dopa. Eight male Sprague-Dawley rats were used for the experimental procedure, and all were given unilateral injections into the left medial forebrain bundle of 6-hyrdroxydopamine to induce hemi-parkinsonian symptoms. After two weeks, half of these animals received L-dopa twice daily while the other half received phosphate-buffered saline. Of these divided groups, half underwent exercise twice daily after injection for two hours. The other half was simply placed back into their home cages. It was hypothesized that the combined treatment of L-dopa and exercise would prove to be the most beneficial PD treatment currently available, especially for the treatment of MRC. Exercise as the sole treatment showed beneficial motor recovery. L-dopa alone also helped alleviate PD symptoms, but in time, the rats developed MRC including dyskinesia. The two combined treatments were effective in mediating voluntary movements, and increased the drug's duration of effectiveness. However, the combined treatment resulted in more abnormal involuntary movements than those rats treated solely with L-dopa. These results are promising for PD patients looking to increase their

treatment duration and quality of life; however it is prudent to seek the consultation of a clinician to better meet individualized needs.

1. Introduction

Parkinson's disease (PD) is a prevalent degenerative neurological disorder that affects approximately 1% of the population over 50 years [2]. This disease affects pigmented neurons of the substantia nigra of the basal ganglia system, and consequently, the nigrostriatal pathway – both important for controlling voluntary movement. PD is largely characterized by bradykinesia, a slowness or poverty in movement, to akinesia, a complete loss of movement. Other symptoms include a resting tremor, rigidity, difficulties maintaining limb and body postures, a shuffling gait, and cognitive decline. Most cases of PD are idiopathic and have no known cure.

Treatment options for PD patients are limited in their abilities. The major forms of pharmaceutical treatment simply relieve some PD symptoms, and are not curative. The difficultly in finding a cure for PD lays in the difficulty of finding a cause for idiopathic cases [5]. The most popular pharmaceutical therapy for PD patients is oral levodopa (L-dopa), a precursor of the neurotransmitter dopamine. Although initially useful in treating the motor symptoms associated with PD, motor response complications (MRC) arise as side effects of the drug within a few short years after treatment. These symptoms include various forms of dyskinesia – including choreatic, ballistic, and dystonic movements, on-off periods of effectiveness that often require an increase in dosage, and diminished effectiveness ultimately leading to complete ineffectiveness [2,3,5]. Despite its limitations, L-dopa is nevertheless considered the most effective option for PD patients.

Recently, exercise has been shown to produce beneficial effects in PD patients and animals. It has been demonstrated that the brain has the capacity to change in response to insult or injury molecular level, chemical level, synaptic level, and also at the cellular level. However, these changes must be induced by usage of the implicated structures [1,7]. Various studies

involving the use of both 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in both rats and mice have demonstrated midbrain dopaminergic (DA) sparing and motor improvements after treadmill exercise as a result of increased glial cell-line neurotrophic factor (GDNF) expression [6,11].

Tillerson et al. further demonstrated the ameliorative effects of exercise in a series of experiments. After a unilateral 6-OHDA administration in rats, the unimpaired front forelimb was casted, forcing the animals to rely solely on the parkinsonian side. If casted during a critical period after 6-OHDA, the animals exhibited behavioral improvements as well as DA sparing. Furthermore, if animals were casted on the parkinsonian side, there was a significant increase in DA loss [9,10]. However, casting undoubtedly caused stress to the animals, and it has been shown that stress can diminish the neuroprotective effects of exercise [2]. With the stressful element of casting, the potential benefits of exercise may not have been fully demonstrated.

Despite extensive research on both L-dopa and exercise, there is little direct research about its combined effects. In one particular study of sixteen idiopathic PD patients, patients performed exercise to increase muscle strength and improve flexibility, in addition to exercise that improves quality of movement for a period of 14 weeks. The patients did not alter their pharmaceutical treatments, which mainly consisted of L-dopa and dopamine agonists. They were evaluated during and after the study, and it was serendipitously discovered that the majority of the patients who experienced L-dopa-induced dyskinesias had a reduction in the severity and duration following exercise that lasted even after the study's end [8]. These results, while promising, are based solely on observation and have not been extensively studied and confirmed in animal models. The aim of this study is to investigate the combined effects of L-dopa and exercise in a 6-OHDA rat model. It is hypothesized that L-dopa and exercise will produce an

interaction effect that will help alleviate some of the MRC seen in both PD patients and animal models.

2. Methods and Materials

All procedures were performed in accordance with the Institutional Animal Care and Use Committee at Central Michigan University and the NIH Guide for the Care and Use of Laboratory Animals.

2.1 Animals

Eight male Sprague-Dawley rats were used for the following experiment. The rats were housed in groups of two with a 12:12 hr light/dark cycle with access to food and water *ad libitum*.

2.2 6-hydroxydopamine Administration

All eight animals received a unilateral injection of 6-OHDA HCl (8 μg in 4 μl of saline with 0.02% ascorbate) to the left medial forebrain bundle at coordinates AP +4.0, L 1.3, V 8.4 below the surface of the skull from the lambda while anesthetized under isoflurane.

2.3 Experimental Design

Of the eight animals, half were block randomized depending on baseline exercise activity to either receive L-dopa (5.0 mg/kg with 1.25 mg/kg benserazide I.P.; $n = 4$) or an injection of phosphate-buffered saline I.P. ($n = 4$) twice daily for two weeks following the 6-OHDA surgeries. The animals were further randomized into L-dopa-treated animals undergoing exercise ($n = 2$), L-dopa-treated animals not undergoing exercise ($n = 2$), saline-treated animals undergoing exercise ($n = 2$), and saline-treated animals not undergoing exercise ($n = 2$). The experiment ran for 14 consecutive days.

2.4 Apparatus

Animals assigned to the exercise groups were placed in a cage equipped with a running wheel and a counter in order to quantitatively measure the amount of exercise performed immediately following injections. The animals not participating in exercise were simply placed back into their home cages. Exercising animals learned to associate running on the wheel with a reward prior to 6-OHDA administration, and received rewards based on minimum wheel spins. The non-exercising animals received rewards immediately following injections.

2.5 Behavioral Testing

Two weeks following 6-OHDA administration, behavioral baselines were assessed by quantifying contralateral rotation in the presence of apomorphine rotation test. Contralateral rotation in response to L-dopa in A.M. and P.M. sessions (10 mg/kg and 20 mg/kg, respectively) were also assessed, as were abnormal involuntary movements (AIMS) in the A.M. session. AIMS were measure based on the severity and amplitude of involuntary movements. A.M./P.M. L-dopa-induced contralateral rotations, in addition to AIMS, were reassessed after both 7 and 14 experimental days. Cylinder testing, the drag test (number of removal and replacement of forelimbs when dragged across a carpeted surface), and a paw placement test were also conducted – the first day without using L-dopa, and the next day using a low-dose L-dopa (4 mg/kg).

2.6 Brain Tissue Analysis

After behavioral testing, all animals were sacrificed by rapid decapitation. The brain tissue was then preserved in order to perform immunohistochemical analysis for future studies involving exercise and L-dopa.

3. Results

3.1 Chronic levodopa and exercise effects on drag test performance in hemi-parkinsonian rats

L-dopa given twice daily for 14 days increased the mean right hop percent in the absence of acute L-dopa challenge on day 17 (Fig. 1). L-dopa given twice daily for 14 days in combination with exercise twice daily for two-hour session each also increased mean right hop percent (Fig. 1). The mean right hop percent recovery by chronic L-dopa was much greater in the exercise group than in the no-exercise group (Fig. 1). The mean right hop percent increase in no-exercise animals was 48.1%; the mean right hop percent increase in exercise animals was 77.7%. Although it showed a strong trend, these differences did not reach statistical significance.

L-dopa given twice daily for 14 days increased right mean hop percent in the presence of acute L-dopa challenge on day 18 (Fig. 2). L-dopa given twice daily for 14 days in combination with exercise twice daily for two hour sessions each also increased mean right hop percent (Fig. 2). The mean right hop percent recovery by chronic L-dopa was much greater in the exercise group than in the no-exercise group (Fig. 2). The mean right hop percent increase in no-exercise animals was 2.2%; the mean right hop percent increase in exercise animals was 129.6%. Although it showed a strong trend, these differences did not reach statistical significance.

3.2 Chronic levodopa and exercise effects on mean AIMS severity and amplitude in hemi-parkinsonian rats

On day 16 of L-dopa treatment, animals without chronic L-dopa exposure showed a decrease in mean AIMS severity when combined with exercise (Fig. 3). On day 16 of L-dopa treatment, animals with chronic L-dopa exposure showed an increase in mean AIMS severity when combined with exercise (Fig. 3). The mean AIMS severity of chronic L-dopa was much greater in the exercise group than in the no-exercise group (Fig. 3). The mean AIMS amplitude

increase in no-exercise animals was 22.7%; the mean right hop percent increase in exercise animals was 223.1%. Although it showed a strong trend, these differences did not reach statistical significance.

On day 16 of L-dopa treatment, animals without chronic L-dopa exposure showed a decrease in mean AIMS amplitude when combined with exercise (Fig. 4). On day 16 of L-dopa treatment, animals with chronic L-dopa exposure showed an increase in mean AIMS amplitude when combined with exercise (Fig. 4). The mean AIMS amplitude of chronic L-dopa was much greater in the exercise group than in the no-exercise group (Fig. 4). The mean AIMS amplitude increase in no-exercise animals was 236.0%; the mean right hop percent increase in exercise animals was 350.0%. Although it showed a strong trend, these differences did not reach statistical significance.

3.3 Chronic levodopa and exercise effects on mean AIMS severity and amplitude in hemi-parkinsonian rats

Shortening of the mean A.M. duration of contralateral rotations and the mean P.M. duration of contralateral rotation caused by chronic L-dopa treatment over 14 days was exacerbated with exercise in the absence of L-dopa in both A.M. and P.M. sessions compared to those animals with acute L-dopa challenge on L-dopa day 16 (Fig. 5,6). In the animals that both exercised and received L-dopa, the mean A.M. and P.M. durations were much longer than animals with acute L-dopa challenge on L-dopa day 16 (Fig. 5,6). The mean A.M. duration increase in no-exercise animals was 75.0%; the mean right hop percent increase in exercise animals was 140.0%. The mean P.M. duration increase in no-exercise animals was 41.6%; the mean right hop percent increase in exercise animals was 39.4%. Although both showed a strong trend, these differences did not reach statistical significance.

4. Discussion

Mean right hop percent increased for chronic L-dopa-treated animals both with and without exercise, but the mean right hop percent was much greater with those animals that exercised than those that did not. This effect was more pronounced in the presence of L-dopa. Exercise shortened both mean AIMS severity and mean AIMS amplitude for animals with acute L-dopa challenge. However, with chronic use of L-dopa, mean AIMS severity and mean AIMS amplitude increased when combined with exercise. Exercising animals showed an increase in both mean A.M. duration and mean P.M. duration with chronic L-dopa use. With those animals under acute L-dopa challenge, a decrease in both mean A.M. duration and mean P.M. duration was observed for those animals that exercised. These results are partially consistent with the hypothesis as some, but not all, MRC associated with chronic L-dopa use were ameliorated with exercise.

It is unacceptable and frustrating for those afflicted with the disorder that the best possible drug therapy for PD is one with debilitating side effects that will eventually become ineffective. Therefore, the implications of this study could have dramatic impacts for the Parkinson's community. This study suggests that too much exercise may actually lead to more involuntary movements and dyskinesias. However, the duration of effectiveness was extended with the use of chronic L-dopa and exercise, therefore indicating the potential benefits of L-dopa and exercise on chronic L-dopa-induced motor response fluctuations including minimizing the wearing-off effect of L-dopa. This may indicate that a careful balance of both exercise and pharmaceutical treatment at different stages of PD would be useful. It has been shown that the severity of MRC depends on the progression of PD [3]. Because of this, patients with PD well controlled with the use of L-dopa may choose to exercise less until it becomes necessary as the

treatment becomes less effective. Furthermore, the drag test, which is analogous to voluntary movement, indicates that exercise combined with L-dopa may prove beneficial for these types of movement.

Although these results are quite positive, some methodological issues remain. Primarily, the number of animals used in the study was quite low. In the future, it would be better to include more animals in the study to increase statistical validity. Furthermore, different L-dopa doses were not used, nor were varying types of exercise. However, this study gives base reference for such investigations in the future.

In the future, similar studies, including behavioral therapies other than exercise, could also be explored. Behavioral regimens such as physical therapy, aquatic exercise, horse riding, yoga, mild low-impact aerobics, and other related therapies have proven to be useful for some of the patients at all stages of PD. Results from our study are consistent with previous reports advocating these types of behavioral therapies. One of the most important findings from the present study is that behavioral therapy such as exercise can in certain situations worsen some of the MRC including dyskinetic movements. It is possible that exercise or similar behavioral therapies can potentiate the L-dopa effect, but at the same time, potentiate the appearance of dyskinesias including abnormal involuntary movement, dystonia, and choreic movements. Whether or not exercise has an additive effect to potentiate L-dopa pharmical kinetics or exercise has an independent effect on dyskinesias should be further investigated.

Interestingly, exercise in PD patients has been correlated with an increased lifespan [4]. If coupled with L-dopa, this effect could be even more pronounced. In addition, because L-dopa aids in initiating movements, it could facilitate the exercise process for patients as the disease progresses. However, it would be advisable to consult with doctors about how much exercise,

and what types, would be most beneficial on a more individualized basis. Taken together, findings from the present study strongly suggest careful individualized management of symptoms and disease progression in patients with PD especially when pharmical therapy is combined with behavioral therapies such as exercise.

5. References

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6. Figure Captions

Figure 1: Effects of exercise and chronic levodopa on mean right hop percent in the absence of acute levodopa challenge. Chronic levodopa animals received 5.0 mg/kg with 1.25 mg/kg benserazide I.P. twice daily for 14 days. The mean right hop percent is compared on levodopa day 17. On day 17, animals were examined on drag test without levodopa challenge. The results from one animal (no exercise, no levodopa) were omitted due to absence of rotations in the presence of apomorphine.

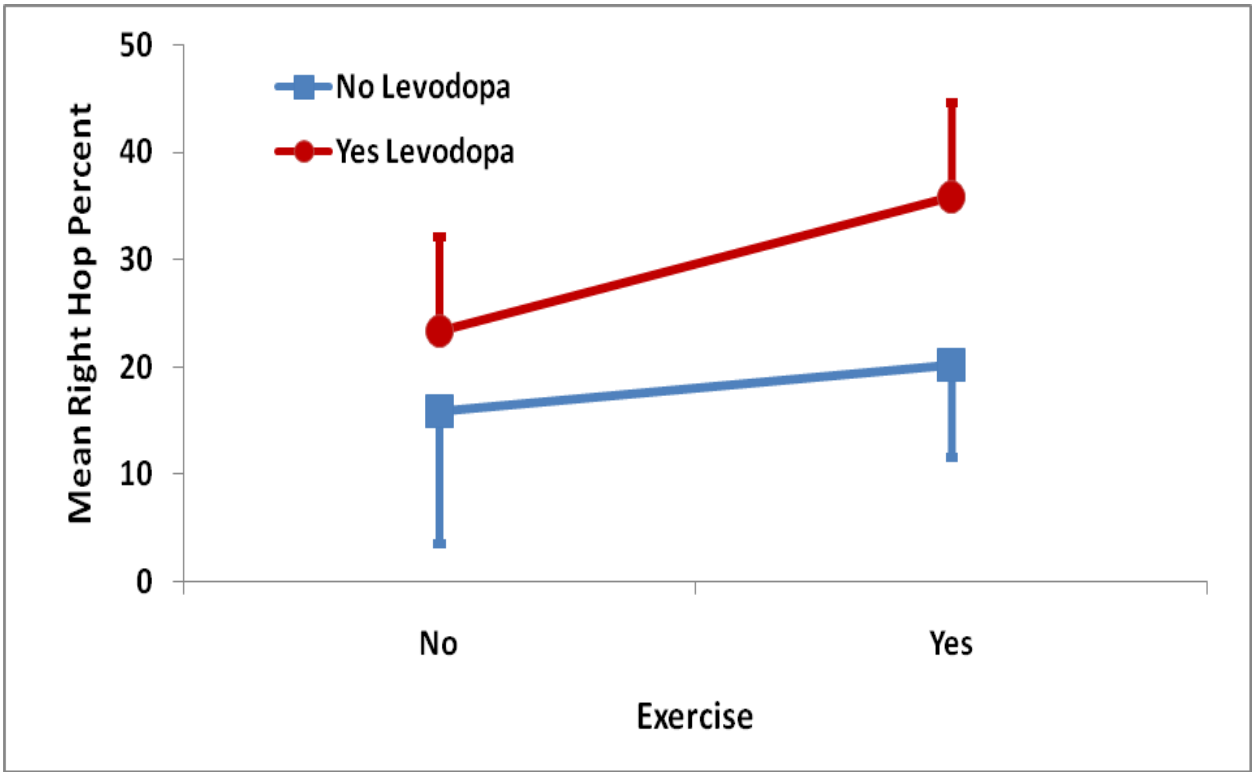

Figure 2: Effects of exercise and chronic levodopa on mean right hop percent in the presence of acute levodopa challenge. Chronic levodopa animals received 5.0 mg/kg with 1.25 mg/kg benserazide I.P. twice daily for 14 days. The mean right hop percent is compared on levodopa day 17. On day 17, animals were examined on drag test with acute levodopa challenge (4.0 mg/kg with 1.25 mg/kg benserazide I.P.). The results from one animal (no exercise, no levodopa) were omitted due to absence of rotations in the presence of apomorphine.

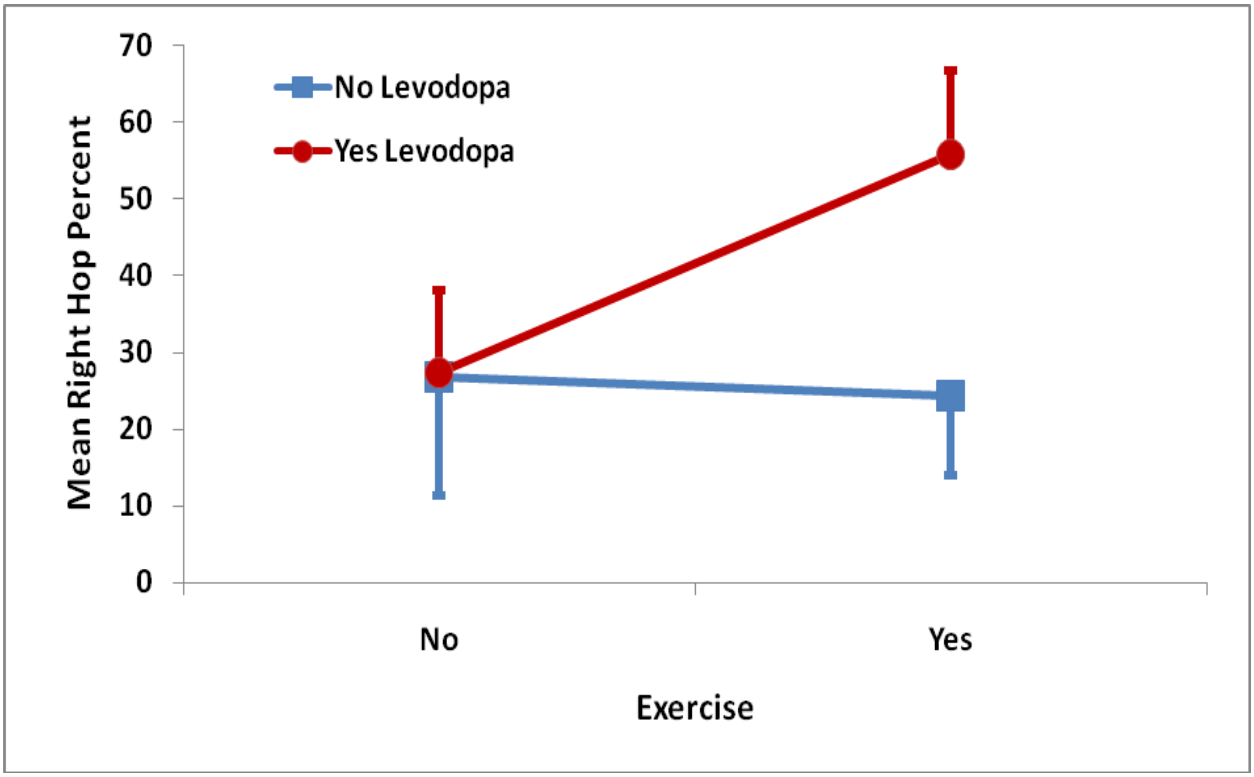

Figure 3: Effects of exercise and chronic levodopa on mean AIMS severity in the presence of acute levodopa challenge. Chronic levodopa animals received 5.0 mg/kg with 1.25 mg/kg benserazide I.P. twice daily for 14 days. The mean AIMS severity is compared on levodopa day 16. On day 16, animals were examined on the severity of AIMS with acute levodopa challenge (10.0 mg/kg with 1.25 mg/kg benserazide I.P.). The results from one animal (no exercise, no levodopa) were omitted due to absence of rotations in the presence of apomorphine. The results of another animal (exercise, levodopa) were omitted due to an inactive response.

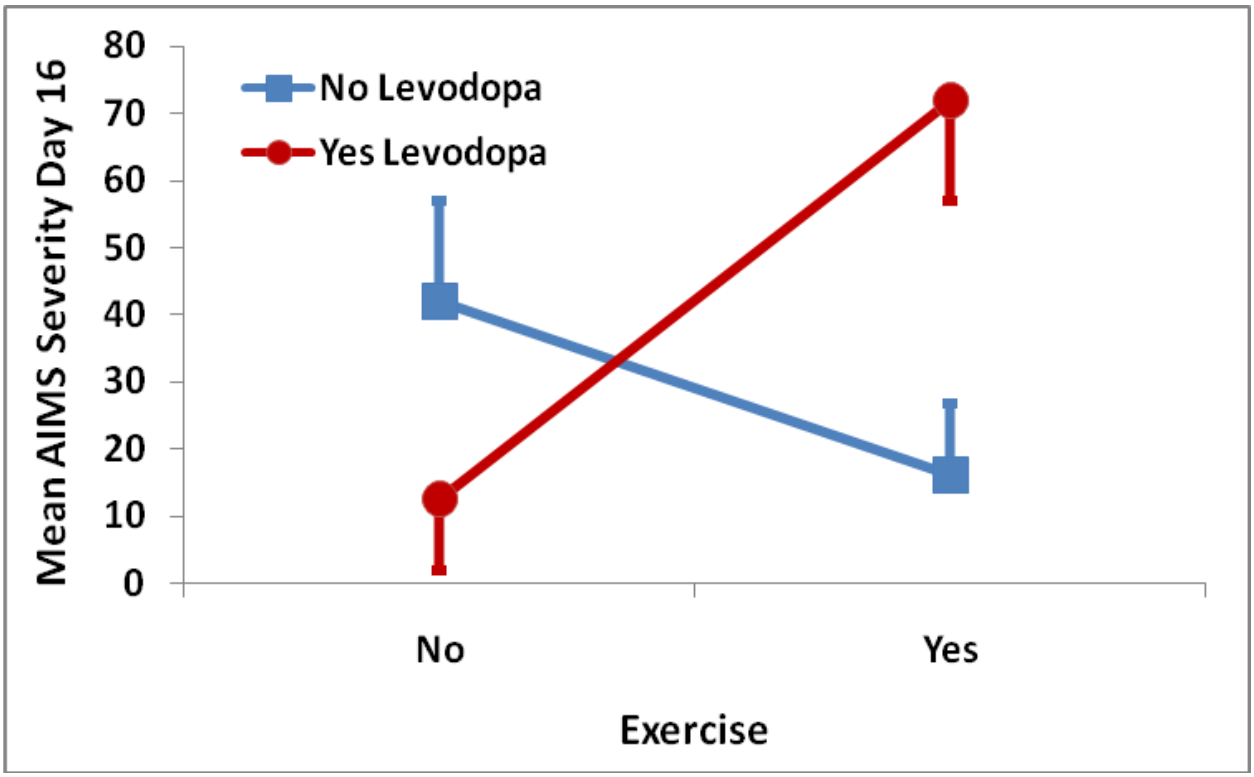

Figure 4: Effects of exercise and chronic levodopa on mean AIMS amplitude in the presence of acute levodopa challenge. Chronic levodopa animals received 5.0 mg/kg with 1.25 mg/kg benserazide I.P. twice daily for 14 days. The mean AIMS amplitude is compared on levodopa day 16. On day 16, animals were examined on the amplitude of AIMS with acute levodopa challenge (10.0 mg/kg with 1.25 mg/kg benserazide I.P.). The results from one animal (no exercise, no levodopa) were omitted due to absence of rotations in the presence of apomorphine. The results of another animal (exercise, levodopa) were omitted due to an inactive response.

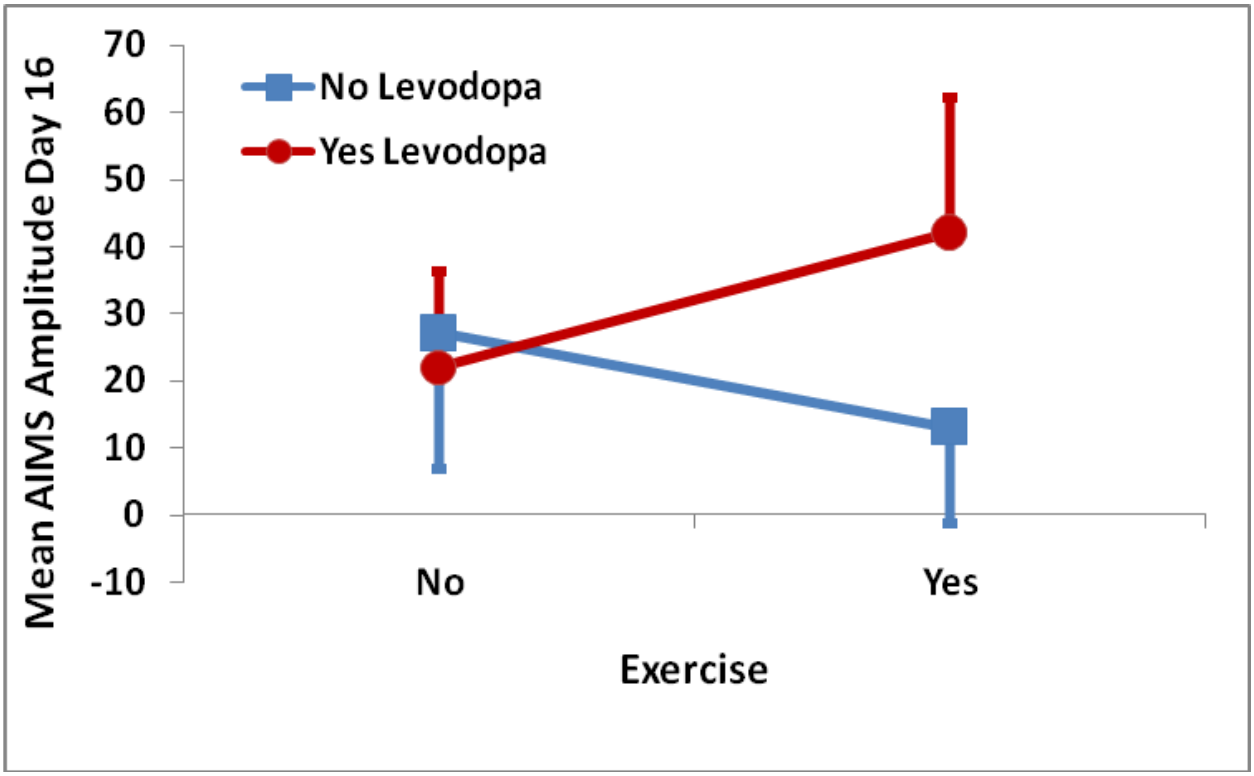

Figure 5: Effects of exercise and chronic levodopa on mean A.M. duration in the presence of acute levodopa challenge. Chronic levodopa animals received 5.0 mg/kg with 1.25 mg/kg benserazide I.P. twice daily for 14 days. The mean AIMS A.M. duration is compared on levodopa day 16. On day 16, animals were examined on the quantity of contralateral rotations with acute levodopa challenge (10.0 mg/kg with 1.25 mg/kg benserazide I.P.). The results from one animal (no exercise, no levodopa) were omitted due to absence of rotations in the presence of apomorphine. The results of another animal (exercise, levodopa) were omitted due to an inactive response.

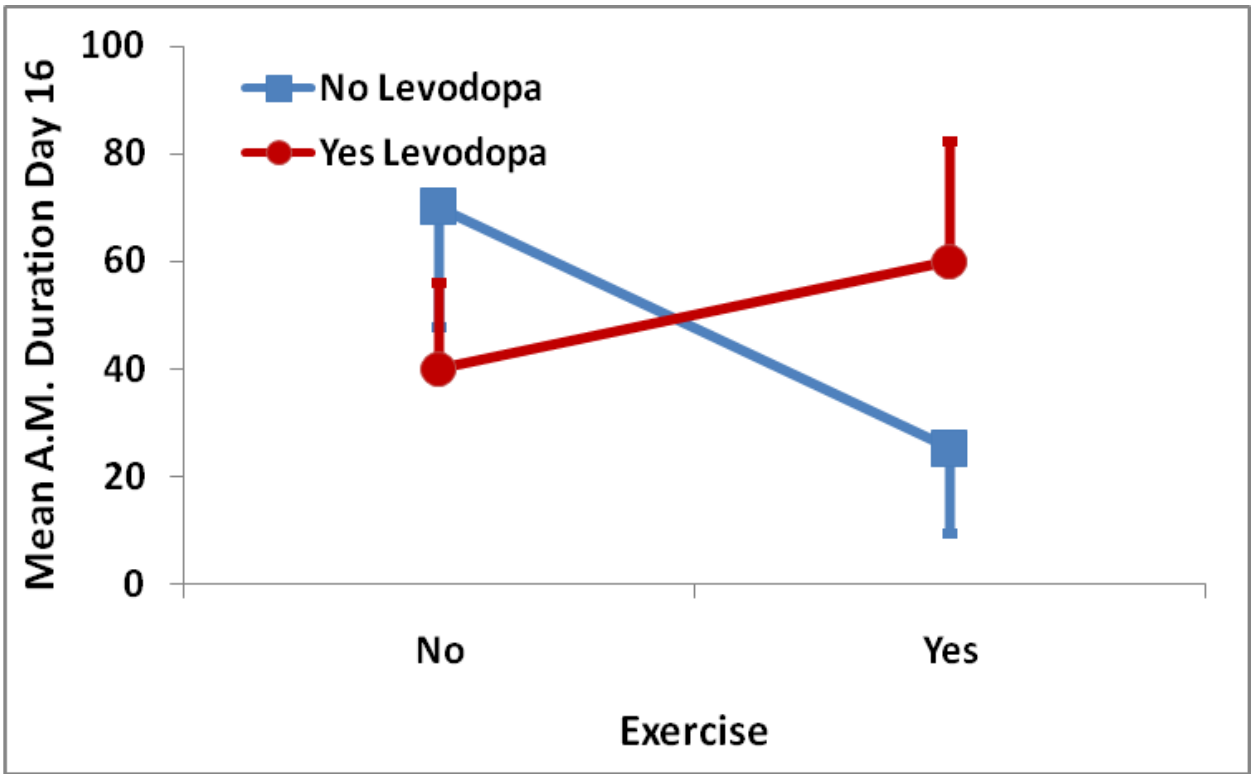

Figure 6: Effects of exercise and chronic levodopa on mean P.M. duration in the presence of acute levodopa challenge. Chronic levodopa animals received 5.0 mg/kg with 1.25 mg/kg benserazide I.P. twice daily for 14 days. The mean AIMS P.M. duration is compared on levodopa day 16. On day 16, animals were examined on the quantity of contralateral rotations with acute levodopa challenge (10.0 mg/kg with 1.25 mg/kg benserazide I.P.). The results from one animal (no exercise, no levodopa) were omitted due to absence of rotations in the presence of apomorphine.