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Effects of Voluntary Exercise on Motor Function in Parkinson's disease Model of Rats

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ABSTRACT

Background. Previous surveys have shown that motor deficits precede the classical motor symptoms seen in Parkinson's disease (PD) and that physical exercise may have beneficial effects on PD. **Objectives.** Here, we evaluated the potential of voluntary exercise to improve motor deficit in experimentally-induced Parkinson's disease (6-OHDA) rats. **Methods.** Forty adult Wistar rats were randomly assigned to four groups: (1) untrained-vehicle (2) untrained-Parkinson's (3) running wheel (RW)-vehicle and (4) RW-Parkinson's. Exercise groups were given free nocturnal access for over four weeks. The motor function, balance and strength were respectively measured by Rotarod and hanging test. **Results.** The data showed that voluntary exercise groups had a significant increase in balance ($p < 0.05$) and strength ($p < 0.05$), when compared to control groups. Running wheel improved motor function in animals induced by 6-OHDA. **Conclusion.** Thus, our results reinforce the potential of voluntary exercise as a useful tool for reducing motor symptoms associated with Parkinson's disease.

KEY WORDS: *Parkinson, Voluntary Exercise, Balance, Strength, Motor Function.*

INTRODUCTION

Parkinson's disease (PD) is a common neurodegenerative disorder that affects people over 50 years (1, 2). Parkinson's disease is characterized by a dopaminergic neuronal loss in the nigrostriatal system and many motor symptoms such as resting tremor, rigidity, akinesia and disturbances of postural reflex and balance impairment.

Previous surveys have shown that physical exercise has many health benefits, improves physical performance, increases neurogenesis in brain and reduces the risk of age-related diseases in the elderly. Recent studies have found a

neuroprotective effect of exercise in CNS disorder patients such as Alzheimer's (3-5). Several studies demonstrated that exercise has its neuroprotective effects (6, 7). However, evidence of the protective effects of voluntary exercise remains uncertain and most of these studies have initiated forced exercise in rodent Parkinson's disease models. Exercise was initiated either before or during neurotoxicant exposure and neurotoxicant rodent models of PD were produced by injecting MPTP in striatum (8, 9).

Since PD is predominantly thought to be a movement disorder (3, 10), this hypothesis that

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exercise could improve motor function in PD patients has been considered in recent years.

On the other hand, the common pharmacological treatment for PD is levodopa, which has a limited time—effectively years—and is associated with many side effects like dyskinesias and motor fluctuations (11, 12). Therefore, limitations and side effects of current treatments and cost of surgical methods strongly suggest that exercise intervention is the most effective in reducing the motor symptoms in PD patients (13).

Understanding the effects of exercise intervention as an easy and available tool for PD symptoms will give information on treatment options aimed at increasing the motor performance and quality of life in PD patients.

Common toxin used in rodent models of PD was either neurotoxin 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) or 6-hydroxydopamine (6-OHDA). In the current study, we used 6-OHDA, which was injected into the median forebrain bundle (MFB) unilaterally.

Therefore, in this study, we want to consider the effects of four weeks of voluntary wheel running on motor function in 6-OHDA rat models with Parkinson's disease.

MATERIALS AND METHODS

Animals. Forty male Wistar rats, weighing 250–300 grams at the beginning of the experiment, were used. Animals were kept in a temperature-controlled room (22±2°C) on a 12-hour light: 12-hour dark cycle, with food and water available ad libitum. The rats were weighed every week. All efforts were made to minimize animal suffering. All experimental protocols and treatments were approved by the Ethical Committee of the Kerman Neuroscience Research Centre (EC: KNRC/94/59), which were completely in agreement with the 'NIH Guide for the Care and Use of Laboratory Animals'. Animals were randomly assigned to four groups: (1) untrained-vehicle (2) untrained-Parkinson's (3) running wheel (RW)-vehicle and (4) RW-Parkinson's.

Exercise. The animals in the voluntary exercise group were individually placed in cages equipped with a running wheel (diameter 31.8 cm; width 9 cm). A magnet attached to a running

wheel triggered a magnetic reed switch that provided input to an electrical counter. The number of revolutions was recorded and wheel revolutions were counted irrespective of the direction of the wheel. The running distance was calculated using the number of wheel revolutions. The animals in the control group were housed four per cage for four weeks. These animals received no specific training. Sham-operated group (sham) received a saline injection instead of 6-OHDA during the stereotactic surgery.

Surgical Procedures. The procedure used in this study was based on the modified method previously described (14) and rats were anesthetized with a combination of ketamine/xylazine (60/10 mg/kg, i.p.) and subjected to stereotaxic apparatus (Stoelting, USA). The 6-OHDA (8µg/2 µl normal saline containing 0.01% ascorbic acid) was infused into the right medial forebrain bundle (MFB) using a 5µl Hamilton syringe with a 27-gauge, according to the coordinates in Paxinos and Watson atlas: AP: -4.4 mm, ML: ±1.3 mm from Bregma and DV: -8.2 mm from the skull surface (15).

Following the injection, and before being slowly drawn off, the canal was left on the site for five minutes to allow complete diffusion of the drug. The animals were divided randomly into four groups (n=10): 1) Animals in the control group were housed four per cage for four weeks. These animals received no specific training. Sham-operated group (sham) was essentially the same as control groups in terms of housing and physical exercise; however, sham rats received a saline injection instead of 6-OHDA during the stereotactic surgery. Animals in the treadmill exercise group were forced to run on a motorized treadmill for five days per week for four weeks.

Rotarod Test. Motor coordination was measured by accelerating Rotarod (Hugo Sachs Elektronik, Germany) in the current study. Territory experiment started at a speed of 10 revolutions per minute (RPM) and the maximum speed was 60 RPM. Each rat underwent three trials using maximum time of 300 seconds with a 30-minute integral rest interval. The length of time that each rat was able to maintain its balance walking on top of the moving rod was recorded as a measure of balance (16).

Hanging Wire Grip Test. The hanging wire grip test assays neuromuscular strength and balance of the animals. Each rat was suspended on a horizontal steel wire hanging on both forepaws (80 cm long and 7 mm diameter). While the rat's forepaws were in contact with the steel wire, the rat was placed in a vertical posture and released whenever it grasped the wire. Latency to fall was recorded for each animal. Each rat underwent three trials with a 30-minute integral rest interval.

Statistical Analyses. Results were expressed as mean \pm standard error of mean (SEM). Normality of the data was assessed using the Levene test. All data had a normal distribution and statistical analysis was performed with SPSS Version 19. T-test to compare daily running of two exercised groups and one-way ANOVA, followed by Turkey's post hoc test for variable comparison between groups, were used. A p

value of less than 0.05 was considered a significant difference.

RESULTS

All rats were weighed every week. The weight of rats in PD and sham groups decreased in the week of surgery but increased over the course of the trial; however, the trained rats weighed less than the control rats, but there was no significant difference among the groups after four weeks ($p=0.35$).

The mean average of daily running distance in voluntary exercise groups increased steadily during the study period. The average daily distance run in the sham voluntary group was higher than voluntary PD group, and, as presented in table 1, the t-test showed a significant difference between them ($p=0.001$), (Table 1). After surgery, the running distances dropped dramatically, but in the following days running distances increased continuously.

Table 1. Results of daily running T-test in 6-OHDA treated rats

	group	Mean	Std. Deviation	Std. Error	t	df	p
running	Sham voluntary	976.0114	35.69983	13.49327	8.761	15	0.001
	PD voluntary	721.0513	69.13189	24.44181			

Rotarod Test. We measured the balance and motor coordination skills on an accelerating Rotarod. 6-OHDA lesion produced a comparable deficit in Rotarod performance and post hoc analysis of ANOVA. This determined that PD

groups showed a decreased latency to fall in the Rotarod, when compared with the control and the sham groups ($F=243.17$, $p=0.001$) (Figure 1). All groups remained longer on the Rotarod at low speeds compared to high speed.

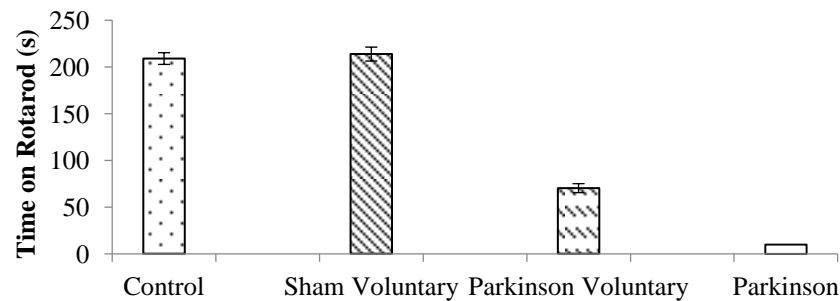


Figure 1. Effect of voluntary exercise on motor coordination in 6-OHDA treated rat assessed by Rotarod test.

Hanging Test. We assessed the strength and motor coordination skills on the hanging wire grip

test. The 6-OHDA injection affected performance in the grip test. The PD groups performed poorly

and ANOVA analysis determined that PD groups showed a decreased latency to fall from the wire

when compared with the control and the sham groups ($F=51.710$, $p=0.001$) (Figure 2).

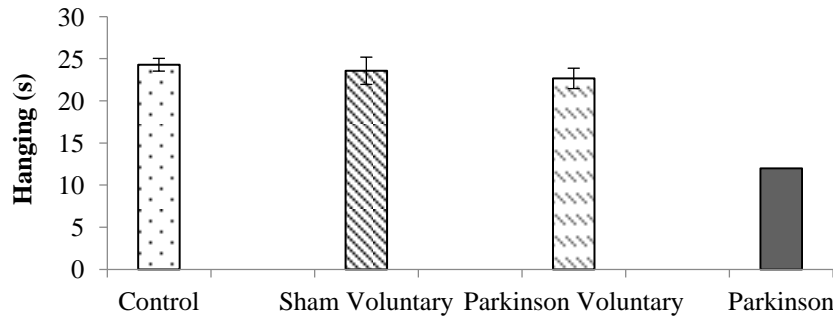


Figure 2. Effect of voluntary exercise on strength in 6-OHDA treated rat assessed by Hanging test.

DISCUSSION

Parkinson's disease is a second most common neurodegenerative disorder after Alzheimer's. Parkinson's is characterized by motor symptoms and predominantly thought of as a movement disorder (17-19). Motor impairments in PD patients affected mortality and the quality of life. So, we evaluated the effect of wheel running exercise on motor function in 6-OHDA lesion rats.

In this study, we employed 6-OHDA infusions into the medial forebrain bundle, which produced large, relatively uniform DA depletions across the caudate putamen (8). These lesions more closely mimic the progressive dopaminergic cell loss and behavioral deficits were seen clinically and caused motor impairment in this model of PD rats (20).

The weight in the lesion group dropped after surgery. This result was similar to those of previous studies (21). This weight loss was caused by the surgical procedure and stress effects, but in a few weeks the weight gain saw a steady increase. We saw no significant difference in weight gain among groups. This is the logical result because all groups had a standard and similar condition in the laboratory as well as food. However, the final weight in the control group is higher than exercise groups. Gorton *et al.* (22) found no difference in body weight among PD and saline groups, but the exercise group showed a significant reduction compared to sedentary controls. This was

because of the fact that exercise groups had more activity.

The average running distance and the duration of daily exercise in all exercise groups increased during the survey period. This finding was similar to previous studies (22-24). In the present study, the average daily running distance that the voluntary rats ran in four weeks in the wheel instrument was approximately between 900 and 1,200 meters, whereas in previous studies the distance that rats ran was 2–3 km in four weeks (23, 25). This difference might be because of variations in lesion place or dose of 6-OHDA used or the variation of wheel running size or kind in different studies. In addition, there is no correlation between running distance and weight, seen also in Howell *et al.*'s (24) study. However, previous studies found a correlation between them (9, 26), suggesting that the weight of the rats and the duration of study were within a range that did not influence their correlation.

The results of Rotarod test showed that PD groups recorded poor performance in the Rotarod in comparison with sham or control groups (27). Published data showed lasting deficits in performance on the Rotarod and balance and gait impairments.

Fewer studies have examined the effects of exercise training on Rotarod in PD rats and indicated improvement in performance by increasing latency time on Rod (22).

The results of the hanging test showed that PD groups presented a poor performance in the

hanging in comparison with sham or control groups (27). Decreased latency to fall from the wire could attenuate hypokinesia and motor weakness (strength and power) in rats with Parkinsonism to some extent; this confirms preliminary findings in previous studies (22) that wheel running exercise significantly improved strength and power in PD rats.

In summary, 6-OHDA lesions do impair motor function and our results strongly suggest that voluntary exercise plays a significant role in motor coordination, reducing strength deficits in an animal model of Parkinson's disease; this result is in accordance with previously published data (28, 29).

It is also possible that the difference in our result with other studies may have been due to small variations in exercise protocol, intensity and duration of exercise, dosing, place of injection and use of a different design.

As previous studies reported, 6-OHDA lesion caused motor abnormality, and from the results of this study, it is apparent that PD groups presented significant neuromuscular and power impairments compared with the sham and control groups, as indicated by a reduction in the time of falling.

In addition, the distance of running is significant and the voluntary sham rats ran more than voluntary PD rats in the same period. The duration of four weeks used in this study was sufficient to provide the neuroprotection afforded by voluntary wheel running, but future studies with longer time are required to discern the effects of different durations and the lasting effects of exercise.

One limitation of our study is that we did not conduct behavior and cognitive tests. So, future research must focus on the behavioral and

cognitive characters. Studies should take into account the effects of forced exercise or comparison of forced and voluntary exercises on motor impairments in PD diseases.

CONCLUSION

In this study, we showed that after injecting 6-OHDA unilaterally into the MFB, it is possible to achieve a permanent model of PD in rats with motor impairments. Since voluntary exercise improved the motor function and strength in the MFB-lesioned rats by 6-OHDA, it can be concluded that voluntary exercise can be helpful in reducing PD patients' motor symptoms.

APPLICABLE REMARKS

- Injecting 6-OHDA unilaterally into the MFB can be considered a good model of PD in rats.
- Voluntary exercise can improve the motor function in a 6-OHDA-induced rat model of Parkinson's disease.
- Then, voluntary exercise can be helpful in reducing PD patients' motor deficits.

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REFERENCES

1. de Rijk MC, Tzourio C, Breteler MM, Dartigues JF, Amaducci L, Lopez-Pousa S, et al. Prevalence of parkinsonism and Parkinson's disease in Europe: the EUROPARKINSON Collaborative Study. European Community Concerted Action on the Epidemiology of Parkinson's disease. *Journal of neurology, neurosurgery, and psychiatry*. 1997;62(1):10-5.
2. Fahn S. Description of Parkinson's disease as a clinical syndrome. *Annals of the New York Academy of Sciences*. 2003;991:1-14.
3. Ang ET, Gomez-Pinilla F. Potential therapeutic effects of exercise to the brain. *Current medicinal chemistry*. 2007;14(24):2564-71.
4. Lynskey JV, Belanger A, Jung R. Activity-dependent plasticity in spinal cord injury. *Journal of rehabilitation research and development*. 2008;45(2):229-40.

5. Smith BA, Goldberg NR, Meshul CK. Effects of treadmill exercise on behavioral recovery and neural changes in the substantia nigra and striatum of the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned mouse. *Brain research*. 2011;1386:70-80.
6. Tillerson JL, Caudle WM, Reveron ME, Miller GW. Exercise induces behavioral recovery and attenuates neurochemical deficits in rodent models of Parkinson's disease. *Neuroscience*. 2003;119(3):899-911.
7. van Praag H, Christie BR, Sejnowski TJ, Gage FH. Running enhances neurogenesis, learning, and long-term potentiation in mice. *Proceedings of the National Academy of Sciences of the United States of America*. 1999;96(23):13427-31.
8. Argue J. *Parkinson's Disease & the Art of Moving*: New Harbinger; 2000. 220 p.
9. Tarr BA, Kellaway LA, St Clair Gibson A, Russell VA. Voluntary running distance is negatively correlated with striatal dopamine release in untrained rats. *Behavioural brain research*. 2004;154(2):493-9.
10. Sethi KD. Clinical aspects of Parkinson disease. *Current opinion in neurology*. 2002;15(4):457-60.
11. Julien C, Berthiaume L, Hadj-Tahar A, Rajput AH, Bedard PJ, Di Paolo T, et al. Postmortem brain fatty acid profile of levodopa-treated Parkinson disease patients and parkinsonian monkeys. *Neurochemistry international*. 2006;48(5):404-14.
12. Samadi P, Gregoire L, Rouillard C, Bedard PJ, Di Paolo T, Levesque D. Docosahexaenoic acid reduces levodopa-induced dyskinesias in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine monkeys. *Annals of neurology*. 2006;59(2):282-8.
13. Schapira AH, Bezard E, Brotchie J, Calon F, Collingridge GL, Ferger B, et al. Novel pharmacological targets for the treatment of Parkinson's disease. *Nature reviews Drug discovery*. 2006;5(10):845-54.
14. Farbood Y, Sarkaki A, Dolatshahi M, Taqhi Mansouri SM, Khodadadi A. Ellagic Acid Protects the Brain Against 6-Hydroxydopamine Induced Neuroinflammation in a Rat Model of Parkinson's Disease. *Basic and Clinical Neuroscience*. 2015;6(2):83-9.
15. Watson C, Paxinos G, Kayalioglu G, Heise C. Chapter 15 - Atlas of the Rat Spinal Cord. *The Spinal Cord*. San Diego: Academic Press; 2009. p. 238-306.
16. Shabani M, Larizadeh MH, Parsania S, Asadi Shekaari M, Shahrokhi N. Profound destructive effects of adolescent exposure to vincristine accompanied with some sex differences in motor and memory performance. *Canadian journal of physiology and pharmacology*. 2012;90(4):379-86.
17. Al-Jarrah M, Pothakos K, Novikova L, Smirnova IV, Kurz MJ, Stehno-Bittel L, et al. Endurance exercise promotes cardiorespiratory rehabilitation without neurorestoration in the chronic mouse model of parkinsonism with severe neurodegeneration. *Neuroscience*. 2007;149(1):28-37.
18. Cohen AD, Tillerson JL, Smith AD, Schallert T, Zigmond MJ. Neuroprotective effects of prior limb use in 6-hydroxydopamine-treated rats: possible role of GDNF. *Journal of neurochemistry*. 2003;85(2):299-305.
19. Nieuwboer A, Rochester L, Muncks L, Swinnen SP. Motor learning in Parkinson's disease: limitations and potential for rehabilitation. *Parkinsonism & related disorders*. 2009;15 Suppl 3:S53-8.
20. Deumens R, Blokland A, Prickaerts J. Modeling Parkinson's Disease in Rats: An Evaluation of 6-OHDA Lesions of the Nigrostriatal Pathway. *Experimental Neurology*. 2002;175(2):303-17.
21. Sconce MD, Churchill MJ, Greene RE, Meshul CK. Intervention with exercise restores motor deficits but not nigrostriatal loss in a progressive MPTP mouse model of Parkinson's disease. *Neuroscience*. 2015;299:156-74.
22. Gorton LM, Vuckovic MG, Vertelkina N, Petzinger GM, Jakowec MW, Wood RI. Exercise effects on motor and affective behavior and catecholamine neurochemistry in the MPTP-lesioned mouse. *Behavioural brain research*. 2010;213(2):253-62.
23. Hayes K, Sprague S, Guo M, Davis W, Friedman A, Kumar A, et al. Forced, not voluntary, exercise effectively induces neuroprotection in stroke. *Acta neuropathologica*. 2008;115(3):289-96.
24. Howells FM, Russell VA, Mabandla MV, Kellaway LA. Stress reduces the neuroprotective effect of exercise in a rat model for Parkinson's disease. *Behavioural brain research*. 2005;165(2):210-20.
25. Kinni H, Guo M, Ding JY, Konakondla S, Dornbos D, 3rd, Tran R, et al. Cerebral metabolism after forced or voluntary physical exercise. *Brain research*. 2011;1388:48-55.
26. Lambert MI, Van Zyl C, Jaunky R, Lambert EV, Noakes TD. Tests of running performance do not predict subsequent spontaneous running in rats. *Physiology & behavior*. 1996;60(1):171-6.
27. Razavinasab M, Shamsizadeh A, Shabani M, Nazeri M, Allahtavakoli M, Asadi-Shekaari M, et al. Pharmacological blockade of TRPV1 receptors modulates the effects of 6-OHDA on motor and cognitive functions in a rat model of Parkinson's disease. *Fundamental & Clinical Pharmacology*. 2013;27(6):632-40.
28. Aguiar AS, Jr., Araujo AL, da-Cunha TR, Speck AE, Ignacio ZM, De-Mello N, et al. Physical exercise improves motor and short-term social memory deficits in reserpinized rats. *Brain research bulletin*. 2009;79(6):452-7.
29. Mabandla M, Kellaway L, St Clair Gibson A, Russell VA. Voluntary running provides neuroprotection in rats after 6-hydroxydopamine injection into the medial forebrain bundle. *Metabolic brain disease*. 2004;19(1-2):43-50.