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Effects of Moderate-Intensity Treadmill Exercise on Motor Function and Striatal Neuronal Activity in a Severe Rat Model of Parkinson's Disease

Yoichi Ohno[1*], Yusuke Chigira[1], Yuha Izawa[2], Minami Obinata[3], Miki Kawakubo[3], Moeka Kobayashi[4], Ibuki Karasawa[5]

[1]Department of Physical Therapy, Takasaki University of Health and Welfare, Takasaki, Japan
[2]Department of Rehabilitation, Tsunoda Hospital, Jushinkai Medical Corporation, Tamamura, Japan
[3]Department of Rehabilitation, Kitahara International Hospital, KNI Medical Corporation, Hachioji, Japan
[4]Department of Rehabilitation, Saitama Misato Rehabilitation Hospital, Sanaikai Medical Corporation, Misato, Japan
[5]Formerly Department of Rehabilitation, Sato Hospital, Seihokai Medical Corporation, Nakano, Japan
Email: *ohno@takasaki-u.ac.jp

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Abstract

Background: The effects of exercise on patients with severe Parkinson's disease (PD) with marked dopaminergic loss remain unclear. This study examined whether moderate treadmill exercise improves motor function and striatal neuronal activity in a rat model of severe Parkinson's disease. **Methods:** Male rats with unilateral medial forebrain bundle lesions were assigned to the exercise (EX) or sedentary (SED) groups. The EX group performed moderate treadmill training five times a week for four weeks. Motor function was assessed using beam-walking tests with DeepLabCut-based gait and posture analyses. Dopaminergic integrity was evaluated using tyrosine hydroxylase (TH) immunostaining, and neuronal activity was assessed using c-Fos immunostaining. Apomorphine-induced rotation was also analyzed. **Results:** No significant differences were observed in body weight, motor performance, gait parameters, TH-positive areas, or rotational behavior between the groups. However, c-Fos-positive cells in the lesioned striatum were significantly higher in the EX group than in the SED group ($p < 0.05$), whereas no difference was found in the intact hemisphere. **Discussion:** Moderate-intensity exercise did not improve motor deficits or dopaminergic loss in this severe model but increased c-Fos expression in the lesioned striatum. These findings suggest that neural responsiveness to exercise is preserved even under advanced dopaminergic degeneration, although longer intervention periods or higher exercise doses may be required to achieve functional recovery.

Keywords

Exercise Therapy, Gait Analysis, Neurorehabilitation, Parkinson's Disease

1. Introduction

Driven by global population growth and aging, the prevalence of Parkinson's disease (PD) is projected to increase by 112% between 2021 and 2050. The World Health Organization predicts that neurodegenerative diseases, including PD and Alzheimer's disease, will surpass cancer as the second leading cause of death worldwide by 2040 [1].

The treatments for PD include pharmacological and surgical interventions. Exercise therapy has been used to address secondary complications, such as disuse syndrome, resulting from reduced physical activity in patients with PD. Clinical studies have demonstrated the therapeutic effects of moderate exercise in patients with PD. A 6-month aerobic exercise program improved gait function and motor symptoms associated with PD [2]. We also reported that a 2-month moderate exercise intervention improved Unified PD Rating Scale scores and gait function in patients with PD [3]. These findings highlight the clinical utility of exercise interventions in patients with PD.

The foundation of these clinical findings lies in numerous studies investigating the effects of moderate exercise in animal models of PD. Studies using a rat model of PD induced by 6-hydroxydopamine (6-OHDA)-mediated striatal dopaminergic neuron destruction (striatal lesion model; STR) have reported increased striatal expression of glial cell line-derived neurotrophic factor after a 14-day exercise intervention. This increase is associated with enhanced neuroprotective and reparative effects against 6-OHDA-induced neuronal loss, resulting in reduced cell death [4]. Dopaminergic neurons are particularly vulnerable to oxidative stress [5] [6]. Another study demonstrated that a 14-day exercise intervention in rats with STR increased brain-derived neurotrophic factor levels and reduced oxidative stress in the striatum, thereby attenuating dopaminergic neuronal cell death [7].

Since PD is characterized by distinctive gait and postural impairments, evaluating exercise-induced effects requires a detailed assessment of dopaminergic neuronal changes and motor function. We previously characterized movement patterns in PD model rats by analyzing beam-walking behavior data recorded from multiple directions using a deep neural network-based approach [8].

Regenerative therapies for PD using induced pluripotent stem cells are being actively investigated [9] [10], and treatment strategies for PD are expected to advance substantially in the future. Therefore, elucidating the beneficial effects of exercise interventions on dopaminergic neurons is crucial, as these effects may influence the maturation and functional integration of transplanted dopaminergic neurons.

In this study, we investigated the changes in gait and posture during beam-walking tests and alterations in dopaminergic neuronal activity following moderate exercise loading in a rat model of PD. Herein, we aimed to elucidate the effects of moderate exercise on motor and brain function in a PD rat model.

2. Methods

2.1. Study Design

In this study, we employed a between-group experimental design to examine the effects of moderate exercise on motor and brain function in a PD rat model. Behavioral analyses, ROI selection, and c-Fos/TH quantification were performed without blinding to group allocation.

2.2. Animals

Fifteen-week-old male unilateral PD rats with medial forebrain bundle (MFB) lesions (MFB model) were used. Following confirmation of MFB lesions by the apomorphine-induced rotation test, ten rats were randomly assigned to either the exercise group (EX; n = 5) or the sedentary control group (SED; n = 5).

The animals were housed individually in cages under a 12-h light/dark cycle. Water was provided ad libitum. Food intake was restricted to maintain body weight at 90% - 95% of the initial body weight to enhance learning and task performance during the behavioral experiments.

2.3. Surgical Procedures

Unilateral 6-OHDA-lesioned Wistar rats were purchased from Japan SLC Inc. (Shizuoka, Japan). The lesions had been produced by stereotaxic injection of 4 μL of 6-OHDA (2.25 mg/mL) into the right medial forebrain bundle after intraperitoneal administration of desipramine (25 mg/kg), a neuroprotective noradrenergic agent. The stereotaxic coordinates were 4.4 mm posterior to bregma, 1.5 mm lateral to the midline, and 7.8 mm ventral to the skull surface.

In all animals, the right hemisphere was designated as the lesioned side. Apomorphine-induced rotation tests were performed at 15 weeks of age. Rats exhibiting ≥7 contralateral rotations per minute were considered to have successful MFB lesions, and only these rats were included in the study.

2.4. Exercise Protocol

Rats in the EX group performed treadmill exercise on a small-animal treadmill (KN-37; Natsume Seisakusho Co., Ltd., Tokyo, Japan) five times per week for four consecutive weeks (each session lasted 30 min). The exercise intensity gradually increased as follows: 5 - 10 m/min in week 1, 10 - 15 m/min in weeks 2 - 3, and 15 m/min in week 4. Rats in the SED group were placed on a treadmill for the same duration, with the belt speed set at 0 m/min.

The treadmill speed of 15 m/min was selected based on previous studies

demonstrating that this workload is below the lactate threshold in rats and is generally considered to represent moderate-intensity exercise [11].

2.5. Outcome Measures

2.5.1. General Conditions

To assess exercise-induced excessive workload and stress, changes in body weight were calculated by subtracting the body weight measured on the day before the intervention from that measured on the final day of the intervention.

2.5.2. Behavioral Assessment Methods

1) Beam-Walking Test

Beam-walking tests were conducted as previously described for PD rat models [8]. Briefly, behavioral performance during the task was recorded simultaneously in four directions (frontal, left lateral, right lateral, and top) using a web camera. Video recordings were acquired at 720 p resolution and 60 frames/s. Measurements were obtained before the exercise intervention and on the day after its completion, and were analyzed for differences between post- and pre-intervention values.

a) Number of Footslips

The number of slips of the fore and hind limbs within the measurement zone was visually counted from the lateral-view recordings.

b) Task Completion Time.

The time required to traverse the 500-mm measurement zone was measured using lateral-view recordings.

c) Image analysis.

i) Frontal View

Head inclination angle was calculated using the line connecting both eyes (1) and a reference line connecting two points on the beam projected on the screen (2) (**Figure 1(a)**). Mean and maximum head inclination angles within the measurement zone were recorded.

ii) Lateral View (Lesioned Side)

Head inclination angle was calculated using the line connecting the nose and ear canal (1) and a reference line connecting two points on the tape attached to the beam (2) (**Figure 1(b)**). The stride length was calculated based on forelimb heel coordinates (3). The mean, maximum, and minimum head inclination angles were analyzed, and the stride length was calculated as the average of two consecutive steps (**Figure 1(c)**).

iii) Top View

Head inclination angle was calculated using the line connecting the midpoint between the nose and both ears (1) and the beam reference line (3) (**Figure 1(d)**). Trunk inclination angle was calculated using the line connecting the midpoint between both ears and the base of the tail (2) together with the same beam reference line (3). The mean and maximum values of the head and trunk inclination angles were used in the analysis.

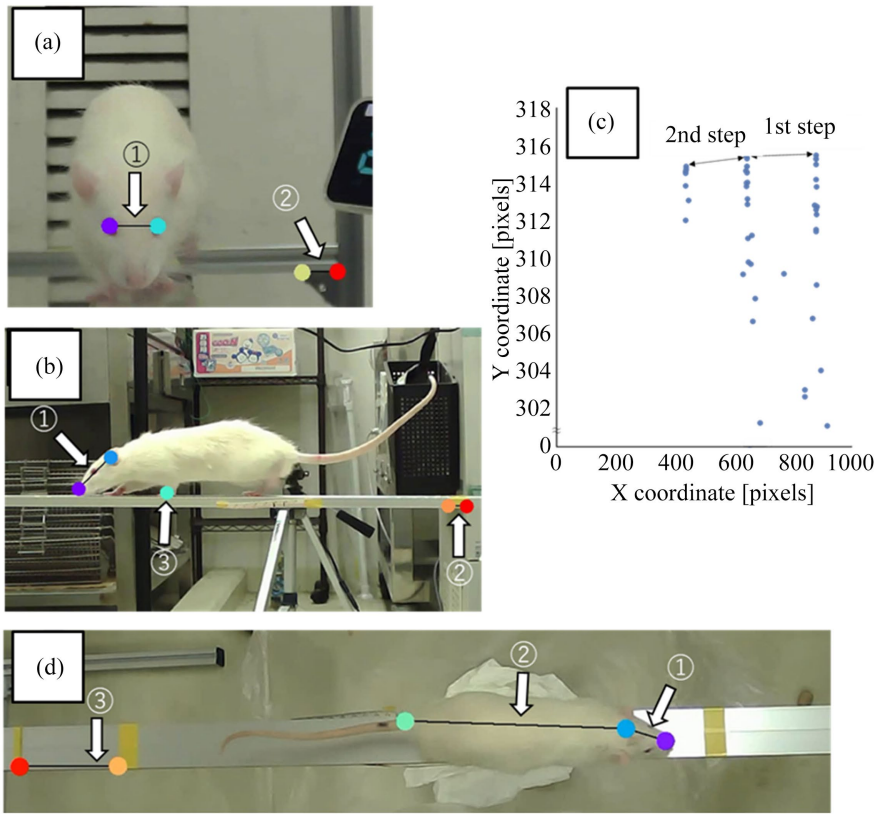

Figure 1. Analysis of gait and posture during beam walking. (a) Frontal view analysis: Head inclination angle was calculated using the line connecting both eyes (1) and a reference line connecting two points on the beam projected on the screen (2). The travel time within the measurement zone was determined using the clock displayed in the frontal and right-lateral images. ((b), (c)) Lateral view: Head inclination angle was calculated using the line connecting the nose and ear canal (1) and a reference line connecting two points on the tape attached to the beam (2) (b). Stride length was calculated based on the DeepLabCut coordinates of the forelimb heel (3) (c). (d) Top view: Head inclination angle was calculated using the line connecting the midpoint between the nose and ears (1) and the beam reference line (3). Trunk inclination angle was calculated using the line connecting the midpoint between ears and the base of the tail (2) together with the same beam reference line (3). Adapted from Ohno and Chigira [8], Open Veterinary Journal, 2025.

2) Apomorphine-Induced Rotation Test

Apomorphine was diluted to 1.0 mg/mL saline and administered intraperitoneally at 1.0 mg/kg. The rotation test was conducted for 30 min in a cylindrical container (28 cm diameter).

The total number of rotations toward the intact hemisphere was counted for 20 min, excluding the first 5 min after rotation onset and the final 5 min of recording. Measurements were conducted the day after the intervention.

2.6. Biochemical Analysis Methods

2.6.1. Tissue Collection and Processing

Kovács reported that Fos protein typically peaks 90 - 120 min after stimulation [12]. Therefore, tissues were collected 90 min after treadmill exercise at 15 m/min,

corresponding to the exercise intensity used in week 4, on day 2 after the completion of the exercise intervention in the EX group. Rats in the SED group did not perform treadmill exercise before perfusion. To minimize the influence of circadian variation on c-Fos expression, the SED group was perfused at the same time of day as the EX group on the following day.

The rats were deeply anesthetized with a mixture of ketamine and xylazine and transcardially perfused with heparinized saline at room temperature, followed by perfusion fixation with 4% paraformaldehyde in phosphate-buffered saline. Brains were immediately removed and fixed with 4% paraformaldehyde.

2.6.2. Tyrosine Hydroxylase (TH) and c-Fos Immunohistochemistry

Immunohistochemical staining for TH and c-Fos was performed on paraffin-embedded coronal sections. Brain tissue was collected at the striatal level corresponding to bregma +0.2 mm, fixed in 4% paraformaldehyde, paraffin-embedded, and sectioned at 4 μm.

Sections were deparaffinized in xylene, rehydrated using a graded ethanol series, and washed with Tris-buffered saline (TBS). Antigen retrieval was performed by autoclaving at 120˚C for 10 min. Endogenous peroxidase activity was blocked with 0.3% H_2O_2 in methanol, and nonspecific binding was blocked using skim milk in TBS.

Sections were incubated overnight at 4˚C with rabbit monoclonal anti-TH (E2L6M) or anti-c-Fos (E2I7R) XP primary antibodies (1:100; Cell Signaling Technology, Danvers, MA, USA), washed with TBS, and incubated with SignalStain Boost IHC Detection Reagent (HRP, rabbit; Cell Signaling Technology, Danvers, MA, USA). Immunoreactivity was visualized using 3,3'-diaminobenzidine, and nuclei were counterstained with Mayer's hematoxylin. The sections were dehydrated in a graded ethanol series, cleared in xylene, and coverslipped.

2.6.3. Immunohistochemical Image Analyses

1) TH Immunostaining

For TH staining, we calculated the percentage of TH-positive areas relative to the total area within the region of interest (ROI) in lesioned and intact hemispheres (**Figure 2(a)**). The ROIs were defined using the midline, dorsal, ventral, and lateral striatal boundaries as anatomical landmarks.

Image analysis was performed using the Fiji platform of the open-source software ImageJ (v.2.3.0) [13]. Images were captured under a digital microscope (LPE-06BK; Sanwa Supply Inc., Osaka, Japan).

2) c-Fos Immunostaining

For c-Fos staining, the striatum in each hemisphere was divided into the dorsomedial, dorsolateral, ventromedial, and ventrolateral subregions (**Figure 2(b)**). In each subregion, c-Fos-positive cells were visually counted within a rectangular ROI (100 μm in height × 300 μm in width) positioned near the center of the region, and the total number of positive cells was calculated. Images were acquired using a biological microscope (BA81; Shimadzu Rika Corporation).

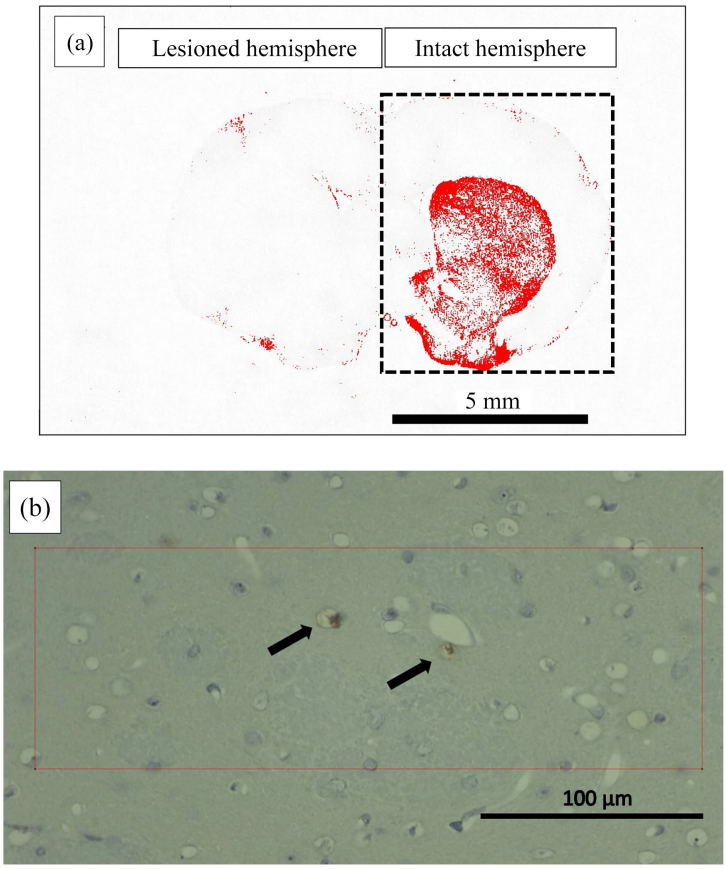

Figure 2. Tyrosine hydroxylase (TH) and c-Fos immunostaining in the striatum. (a) Representative images of TH immunostaining showing coronal brain sections at the striatal level corresponding to bregma +0.2 mm. Regions of interest (ROIs) were defined in lesioned and intact hemispheres, and the percentage of TH-positive areas was calculated relative to the total ROI area. Scale bar = 5 mm. (b) Representative c-Fos immunostaining images are shown. The striatum was divided into dorsomedial, dorsolateral, ventromedial, and ventrolateral subregions. The number of c-Fos-positive cells was counted within a rectangular ROI (100 μm in height × 300 μm in width) placed at the center of each subregion, and the total number of positive cells was calculated. Arrows indicate c-Fos-positive cells. Scale bar = 100 μm.

2.7. Statistical Analyses

Between-group differences in change scores (post-intervention minus pre-intervention values) were analyzed to compare the effects of the exercise intervention between the two groups. The Shapiro-Wilk test was used to assess normality. Levene's test was used to evaluate the homogeneity of variance in normally distributed data. Student's t-test and Welch's t-test were used for normally distributed data with equal and unequal variances, respectively. The Mann-Whitney U test was used for data that were not normally distributed. Statistical significance was set at $p < 0.05$. Data are presented as mean ± standard error.

2.8. Use of AI Tools

Portions of the manuscript were translated from Japanese to English using ChatGPT

(OpenAI, San Francisco, CA, USA). The generated content was carefully reviewed and edited by the authors to ensure accuracy, clarity, and consistency. The authors take full responsibility for the final content of the manuscript.

3. Results

The results of all outcome measures are summarized in Table 1.

Table 1. Effects of moderate exercise on motor and brain functions in a Parkinson's rat model.

				EX	SED	p value
General condition	Body weight (Δg)			13.60 (±4.41)	16.50 (±1.08)	0.54
Behavioral assessments	Foot slips (Δcounts)			0.00	0.00	
	Task completion time (Δsec)			−0.15 (±0.22)	−0.85 (±0.73)	0.39
	Frontal view	Head inclination angle (Δdeg)	Mean	−1.34 (±0.11)	−1.97 (±0.48)	0.23
			Maximum	−0.83 (±0.48)	−0.88 (±1.10)	0.97
	Lateral view	Head inclination angle (Δdeg)	Mean	6.72 (±4.19)	9.79 (±2.47)	0.54
			Maximum	5.92 (±3.93)	5.07 (±3.47)	0.87
			Minimum	12.61 (±5.71)	18.70 (±6.36)	0.49
		Stride length (Δmm)		27.92 (±6.84)	37.02 (±9.21)	0.45
	Top view	Head inclination angle (Δdeg)	Mean	−5.66 (±1.79)	−6.79 (±1.58)	0.66
			Maximum	−8.14 (±2.05)	−3.82 (±5.80)	0.46
		Trunk inclination angle (Δdeg)	Mean	2.66 (±1.06)	3.79 (±1.11)	0.48
			Maximum	2.40 (±3.44)	10.23 (±3.99)	0.18
	Apomorphine-induced rotation test (counts)			286.20 (±27.44)	224.20 (±43.17)	0.26
Biochemical analyses	TH immunohistochemistry (%)		Intact hemisphere	13.74 (±0.39)	14.77 (±2.83)	0.73
			Lesioned hemisphere	0.83 (±0.22)	1.32 (±0.35)	0.27
	c-Fos immunohistochemistry (counts)		Intact hemisphere	0.80 (±0.37)	0.00 (±0.00)	0.15
			Lesioned hemisphere	2.40 (±0.67)	0.40 (±0.40)	<0.05

EX, exercise group; SED, sedentary control group; TH, tyrosine hydroxylase. Values are presented as mean ± standard error. Δ indicates change scores (post-intervention minus pre-intervention values). The general condition of the rats was evaluated based on changes in body weight. Motor functions were assessed during the beam-walking tests based on the number of footslips and task completion time, and on parameters analyzed using DeepLabCut from each camera view, including inclination angles and stride length. The degree of dopaminergic neuronal impairment was evaluated using apomorphine-induced rotation tests. For immunohistochemical analyses, we assessed the percentage of TH-positive areas and the number of c-Fos-positive cells in the striata of the intact and lesioned hemispheres.

3.1. Body Weight

Body weight changes did not differ significantly between the groups. The change in body weight was 13.60 ± 4.41 and 16.50 ± 1.08 g in the EX and SED groups, respectively (Student's t-test).

3.2. Behavioral Assessment Results

3.2.1. Number of Footslips

No footslips were observed in either group within the measurement zone during the beam-walking test.

3.2.2. Task Completion Time

The change in task completion time was −0.15 ± 0.22 s in the EX group and −0.85 ± 0.73 s in the SED group, with no significant differences between them (Student's t-test).

3.2.3. DeepLabCut-Based Gait and Posture Analyses

1) Frontal View (Head Inclination Angle)

The mean head inclination angle was −1.34˚ ± 0.11˚ in the EX group and −1.97˚ ± 0.48˚ in the SED group, with no significant difference between groups (Student's t-test).

The maximum head inclination angle was −0.83˚ ± 0.48˚ in the EX group and −0.88˚ ± 1.10˚ in the SED group, with no significant difference between groups (Student's t-test).

2) Lateral View

a) Head-inclination angles

The mean head inclination angle was 6.72˚ ± 4.19˚ in the EX group and 9.79˚ ± 2.47˚ in the SED group, with no significant difference between groups (Student's t-test).

The maximum head inclination angle was 5.92˚ ± 3.93˚ in the EX group and 5.07˚ ± 3.47˚ in the SED group, with no significant difference between groups (Student's t-test).

The minimum head inclination angle was 12.61˚ ± 5.71˚ in the EX group and 18.70˚ ± 6.36˚ in the SED group, with no significant difference between groups (Student's t-test).

b) Stride Length

Stride length was 27.92 ± 6.84 mm in the EX group and 37.02 ± 9.21 mm in the SED group, with no significant difference between groups (Student's t-test).

3) Top View

a) Head-inclination angles

The mean head inclination angle was −5.66˚ ± 1.79˚ in the EX group and −6.79˚ ± 1.58˚ in the SED group, with no significant difference between groups (Student's t-test).

The maximum head inclination angle was −8.14˚ ± 2.05˚ in the EX group and −3.82˚ ± 5.80˚ in the SED group, with no significant difference between groups (Student's t-test).

b) Trunk Inclination Angle

The mean trunk inclination angle was 2.66˚ ± 1.06˚ in the EX group and 3.79˚ ± 1.11˚ in the SED group, with no significant difference between groups (Student's t-test).

The maximum trunk inclination angle was 2.40˚ ± 3.44˚ in the EX group and 10.23˚ ± 3.99˚ in the SED group, with no significant difference between groups (Student's t-test).

3.2.4. Apomorphine-Induced Rotation Test

The total number of rotations toward the intact side was 286.20 ± 27.44 in the EX group and 224.20 ± 43.17 in the SED group, with no significant difference between groups (Student's t-test).

3.3. Biochemical Analysis Results

3.3.1. TH Immunostaining

In the intact hemisphere, the percentage of TH-positive area relative to the total ROI area was 13.74% ± 0.39% in the EX group and 14.77% ± 2.83% in the SED group, with no significant difference between groups (Welch's t-test).

In the lesioned hemisphere, the percentage of TH-positive area was 0.83% ± 0.22% in the EX group and 1.32% ± 0.35% in the SED group, with no significant difference between groups (Student's t-test).

3.3.2. c-Fos Immunostaining

In the intact hemisphere, the total number of c-Fos-positive cells was 0.80 ± 0.37 in the EX group and 0.00 ± 0.00 in the SED group, with no significant difference between groups (Mann-Whitney U test).

In the lesioned hemisphere, the total number of c-Fos-positive cells was significantly higher in the EX group (2.40 ± 0.67) than in the SED group (0.40 ± 0.40) ($p < 0.05$, Mann-Whitney U test).

4. Discussion

The purpose of this study was to investigate the effects of moderate exercise intervention on motor and brain functions in a rat model of PD. There were no significant differences between the two groups in terms of body weight changes, behavioral assessments, or TH immunostaining. These findings suggest that the exercise protocol used in this study did not impose an excessive physical load or stress on the animals. Although no significant difference was observed in the intact hemisphere, c-Fos immunostaining revealed a significantly greater number of c-Fos-positive cells in the striatum of the lesioned hemispheres in the EX group than in the SED group.

One possible reason for the lack of significant differences in motor function and TH immunostaining between groups may be the use of the MFB model. We selected this model because MFB lesions result in severe motor deficits, including gait disturbances, whereas striatal lesion models generally exhibit milder motor deficits and less prominent gait disturbances [14]. In the present study, a model exhibiting clear gait impairment was required because beam-walking tests were used to detect exercise-induced changes in motor function. To observe exercise-induced neuroprotective effects, a certain number of residual dopaminergic neu-

rons is required [15]. Studies using voluntary wheel running for 8 weeks following MFB lesioning reported improvements in gait function after 6 weeks, with no significant differences in striatal dopaminergic neuron preservation between the exercise and sedentary groups [16]. These findings suggest that the changes in motor function and dopaminergic neuronal preservation are not directly correlated.

A study involving 14 days of voluntary running exercise in MFB-lesioned rats, which allowed 24 h/day of voluntary exercise, reported a significant reduction in apomorphine-induced rotations in the exercise group compared with the sedentary group [17]. Additionally, long-term treadmill exercise at a low intensity for 10 weeks (15 or 30 min/day) attenuated both motor deficits and dopaminergic neuronal loss in the striatum [18]. These findings suggest that, in cases of severe dopaminergic neuronal damage, such as that induced by MFB lesions, moderate-intensity exercise may require either longer intervention periods or a higher exercise frequency to produce measurable improvements in motor function and dopaminergic neuronal recovery.

In this study, c-Fos immunostaining was the only outcome measure that exhibited significant differences between groups. Exercise increases c-Fos expression in various brain regions [19]. Increased c-Fos expression in the striatum following exercise has also been documented [20]. Studies using MFB-lesioned models have reported exercise-induced c-Fos expression in both the lesioned and intact hemispheres [21]. The exercise intensity in these studies was higher than that used in the present study. As c-Fos expression has been reported to increase in an intensity-dependent manner [22], the finding that a significant increase was observed only in the lesioned hemisphere in the present study suggests that even moderate exercise intensity is sufficient to increase c-Fos expression in the severely damaged striatum.

The results of the present study indicate that a 4-week moderate exercise intervention increased c-Fos expression in the lesioned striatum, suggesting that responsiveness to exercise may be preserved even under severe dopaminergic degeneration. However, improvements in motor function and dopaminergic neuronal preservation may require longer intervention periods or higher exercise doses.

5. Limitations

This study has the following limitations: 1) the small sample size likely increased susceptibility to individual variability; 2) no footslips were observed in either group during gait assessment, likely because of the experimental setting to avoid excessive deviation of head and trunk inclination angles caused by slips, thereby lowering task difficulty; 3) although the treadmill speed was selected based on previous studies classifying 15 m/min as moderate-intensity exercise in rats, physiological indicators of exercise intensity, such as blood lactate concentration, oxygen consumption, or heart rate, were not measured. Therefore, the actual exercise intensity in this severe PD model was not directly validated and may not

have represented a truly moderate workload for every individual; 4) only striatal c-Fos expression was evaluated, and neuronal activity in other basal ganglia regions was not assessed; and 5) although multiple outcome measures were evaluated, a statistically significant between-group difference was observed only for c-Fos expression. Therefore, this finding should be interpreted with caution and warrants confirmation in larger studies. Future studies should include larger sample sizes, optimized task difficulty, individualized exercise intensities, and physiological validation of exercise intensity to enhance the robustness and precision of our findings.

6. Conclusion

Moderate-intensity treadmill exercise for 4 weeks did not improve motor performance, apomorphine-induced rotational behavior, or striatal dopaminergic preservation in rats with severe Parkinson's disease induced by medial forebrain bundle lesions. However, exercise significantly increased c-Fos expression in the lesioned striatum. These findings suggest that neural responsiveness to exercise remains preserved even under severe dopaminergic degeneration, although longer intervention periods or higher exercise doses may be required to achieve functional recovery.

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Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Conflicts of Interest

The authors declare no conflicts of interest.

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