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Research Paper

Aerobic exercise improves depressive symptoms in the unilateral 6-OHDA-lesioned rat model of Parkinson's disease

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ABSTRACT

Aerobic exercise has been shown to have established benefits on motor function in Parkinson's disease (PD). However, the impact of exercise on depressive symptoms in PD remains unclear. This study aimed to investigate the effects of regular exercise, specifically using a forced running wheel, on both motor performance and the prevalence of depression in a unilateral 6-OHDA-lesioned rat model of PD. The behavioral outcomes of exercise were assessed through the rotarod test (RT), forelimb adjusting step test (FAST), sucrose consumption test (SCT), and novelty sucrose splash test (NSST). Our data revealed evident depressive symptoms in the PD animals, characterized by reduced sucrose consumption in the SCT and diminished exploratory activity in the NSST compared to the naïve control group. Specifically, after 11 weeks of exercise, the PD exercise group demonstrated the most significant improvements in sucrose consumption in the SCT. Additionally, this group exhibited reduced immobility and increased exploratory behavior compared to the PD control group in the NSST. Furthermore, the PD exercise group displayed the greatest improvement in correcting forelimb stepping bias. Our results suggested that a regimen of running wheel exercise enhances motor abilities and mitigates the occurrence of depressive behaviors caused by 6-OHDA dopamine depletion in the PD rat model.

Introduction

Parkinson's disease (PD) is a neurodegenerative disorder that typically manifests in old age, affecting approximately 1% of individuals over the age of 65 (Aarsland et al., 2021; Tysnes and Storstein, 2017). The primary pathology of PD involves the degeneration of dopaminergic neurons in the substantia nigra pars compacta (Hamani and Lozano, 2003; Raza et al., 2019). PD can lead to severe motor impairments, cognitive dysfunction, and mood disorders (Decourt et al., 2021). While PD is commonly recognized by its characteristic motor symptoms such as bradykinesia, muscle rigidity, resting tremor, and postural instability, non-motor symptoms, including depression, can emerge long before the onset of motor symptoms (Faivre et al., 2019; van der Hoek et al., 2011). It has been estimated that 50% of PD patients experience clinical depression (Marsh, 2013; Ravina et al., 2007; van der Hoek et al., 2011).

The management of PD typically involves pharmaceutical dopamine replacement therapy or deep brain stimulation (DBS). DBS is an established treatment method that utilizes surgically implanted electrodes in specific brain regions to modulate neural activity through electrical stimulation, aiming to alleviate motor symptoms of PD. However, DBS is a highly invasive procedure with limited patient accessibility, and its mechanism of action in PD attenuation and potential cognitive and psychiatric side effects are not yet fully understood (Chiken and Nambu, 2016; Combs et al., 2015; Deuschl et al., 2006). Furthermore, pharmacological interventions yield diminishing returns in the progressive stages of the disease and have shown limited effectiveness in alleviating non-motor symptoms (Hamani and Lozano, 2003; Schapira et al., 2009). It should also be noted that dopamine agonists do not halt the progression of the disease. On the other hand, exercise is widely recognized for its general health benefits. In the past decade, the role of exercise in improving motor performance in PD has been extensively studied (Fisher et al., 2008; Llamas-Velasco et al., 2021; Xu et al., 2010a, 2010b). Epidemiological studies have shown a correlation between regular exercise, reduced risk of developing Parkinson's

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disease, and slowed disease progression, especially in the early stages (Ross et al., 2007; Tsukita et al., 2022; Xu et al., 2010a, 2010b). However, the current focus of exercise as a treatment for PD primarily centers around alleviating motor symptoms. Nonetheless, a few studies have suggested that physical exercise may also provide neuroprotective effects, facilitate neuroplasticity, and improve cognitive function in individuals with PD (Ahlskog, 2011;Hotting and Roder, 2013; Petzinger et al., 2013).

Animal models of PD have been extensively utilized to investigate the pathology and progression of neurodegeneration. Exercise has been shown to improve motor performance in these PD animal models (Fisher et al., 2004; O'Dell et al., 2007; Petzinger et al., 2007;Pothakos et al., 2009; Smith et al., 2011; Tillerson et al., 2003). For instance, in unilateral 6-hydroxydopamine (6-OHDA)-induced hemi-parkinsonian rats, exercise training through treadmill running(Tillerson et al., 2003) or wheel running (O'Dell et al., 2007) has demonstrated improved limb function. Furthermore, running exercise has been widely associated with antidepressant effects (Craft and Perna, 2004; Kvam et al., 2016; McNeil et al., 1991; Mul, 2018). However, the potential of aerobic exercise in ameliorating non-motor depressive symptoms in PD has not yet been thoroughly characterized. Investigating the effects of aerobic exercise as a treatment for PD depressive symptoms can contribute to the development of improved PD treatment strategies.

In this study, we conducted exercise training using a forced running wheel in a unilateral 6-OHDA-lesioned rat model of PD to determine whether regular exercise reduces the prevalence of depression while improving motor performance. We quantified the behavioral effects of exercise on depressive and motor symptoms using four behavioral assays, including the rotarod test (RT), forelimb adjusting step test (FAST), sucrose consumption test (SCT), and novelty sucrose splash test (NSST).

Methods

All animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Michigan Technological University

Unilateral dopamine depletion

A total of 25 adult female Sprague-Dawley rats (6–8 weeks, 250–300 g) were used and split into three groups. Two of these groups (n=14) were unilaterally injected with 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle (MFB) to cause unilateral degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc) and induce hemiparkinsonism. The third group (n=11) was unilaterally injected with saline and served as a control for understanding timing effects on motor and depressive symptoms. Surgery was performed under general anesthesia with isoflurane (induction at 4 % in 2 L/min O2; maintained at 2 % with 2 L/min O2). Dexamethasone (5.0 mg/kg) was injected subcutaneously to reduce potential for brain swelling during surgery. The body temperature was maintained at ~ 37°C with a water heating blanket. Craniotomies were performed over MFB according to a stereotaxic atlas of the rat brain (MFB: AP −2.0 mm, ml 2.0 mm) as previously described (Yu C et al., 2020). The 6-OHDA (6 μL, 2.5 mg/ml in 0.2 % ascorbic acid dissolved with saline, Sigma-Aldrich) was prepared immediately before use and infused into MFB (DV −7.5 mm) through 10 μL Hamilton syringe at a rate of 1μL/min, waiting 5 min after every 2 μL, and the needle was left in the brain for another 10 min after the full injection. The rats were administered 50 mg/kg pargyline (i.p., Sigma-Aldrich) and 5 mg/kg desipramine (i.p., Sigma-Aldrich) to inhibit monoamine oxidase and protect noradrenergic neurons prior to lesioning.After one week of recovery from surgical preparation, eight animals that received a 6-OHDA injection underwent introduction to forced running wheel exercise that continued five times weekly for 10 weeks. All three groups underwent motor and depressive behavioral testing every two weeks starting one week after 6-OHDA lesion (Fig. 1). The drug-induced rotation test was employed to assess lesion effects (Lindgren et al., 2012). One-week post-injection of 6-OHDA in the MFB, circling behavior was induced in the animal via intraperitoneal injection of methamphetamine hydrochloride (1.25–2.5 mg/kg, Salt weight). Rats were monitored within a 25 cm diameter cylinder equipped with a video recording camera in a dark cabinet. The number of full turns (ipsilateral and contralateral to the side of the lesion) was counted over a 120 min period following methamphetamine injection. Previous studies have shown that a turning rate, contralateral to the lesion, exceeding 3 turns per minute indicates a unilateral dopamine depletion of over 95% (Chang et al., 2008).

Exercise protocol

After one week of recovery from the surgical preparation, eight animals that received the 6-OHDA injection were introduced to a forced running wheel exercise regimen, which continued for 10 weeks, five times per week. The PD exercise group (n=8) initially underwent a forced running wheel training regimen, beginning with a low speed of 3.5 m/min for 5 min on the first experimental day, following a 30-minute habituation period in the wheel. Subsequently, the rats engaged in daily wheel running sessions, occurring five days per week over a span of 10 weeks. The running speed and exercise duration gradually increased over time, tailored to each animal's running capacity, with increments of approximately 1 m/min in speed and durations ranging from 50 to 70 min. By the seventh week of training, all animals achieved a maximum speed of 8 m/min for a duration of 70 min, which was maintained throughout the remainder of the 10-week training period.

Depressive behavior tests

To assess depressive behaviors in the rats, two tests were conducted: the sucrose consumption test (SCT) and the novelty sucrose splash test (NSST). The SCT evaluated the presence of anhedonia, a symptom of depression characterized by the inability to experience pleasure (Kanner et al., 2012; Klein et al., 2015). Prior to the SCT, the rats were trained to drink a 1% sucrose solution in a two-bottle choice paradigm. This training occurred 72 h before the administration of the test. During the SCT, the rats were exposed to both the 1% sucrose solution and tap water for a duration of 12 h. The volumes of sucrose solution and water consumed by each rat were measured and used to calculate the sucrose preference index (SPI). The sucrose preference index (SPI) was calculated as $\frac{Sucrose\ Consumed}{Sucrose\ Consumed+Water\ Consumed} \times 100$. The SCT was performed overnight for two consecutive nights each test week. On the second night, the positioning of the sucrose and water bottles was swapped to minimize any bias due to bottle positioning.

The NSST assessed the rats' behavior in a novel environment and their response to a sucrose solution. The rats were placed in a rectangular arena (24"x18"x16") that was new and unfamiliar to them. Immediately upon placement in the arena, the rats were sprayed with a 10% sucrose solution on their dorsal coat. The sticky nature of the sucrose solution induced grooming behavior. Two different objects were placed in the arena for the rats to explore during the NSST. The behavior of the rats inside the arena was video recorded for a duration of 15 min after the sucrose solution was sprayed. The following parameters were recorded during the 15-minute period: latency to the first grooming period, time spent exploring the novel arena, and time spent sitting. Decreases in overall locomotor activity in the novel environment were considered indicative of elevated levels of anxiety, which is associated with depressive symptoms. These tests allowed for the assessment of depressive-like behaviors in the rats, including anhedonia, grooming behavior, exploration, and locomotor activity in a novel environment (Fuchikami et al., 2015; Santarelli et al., 2003; Surget et al., 2008).

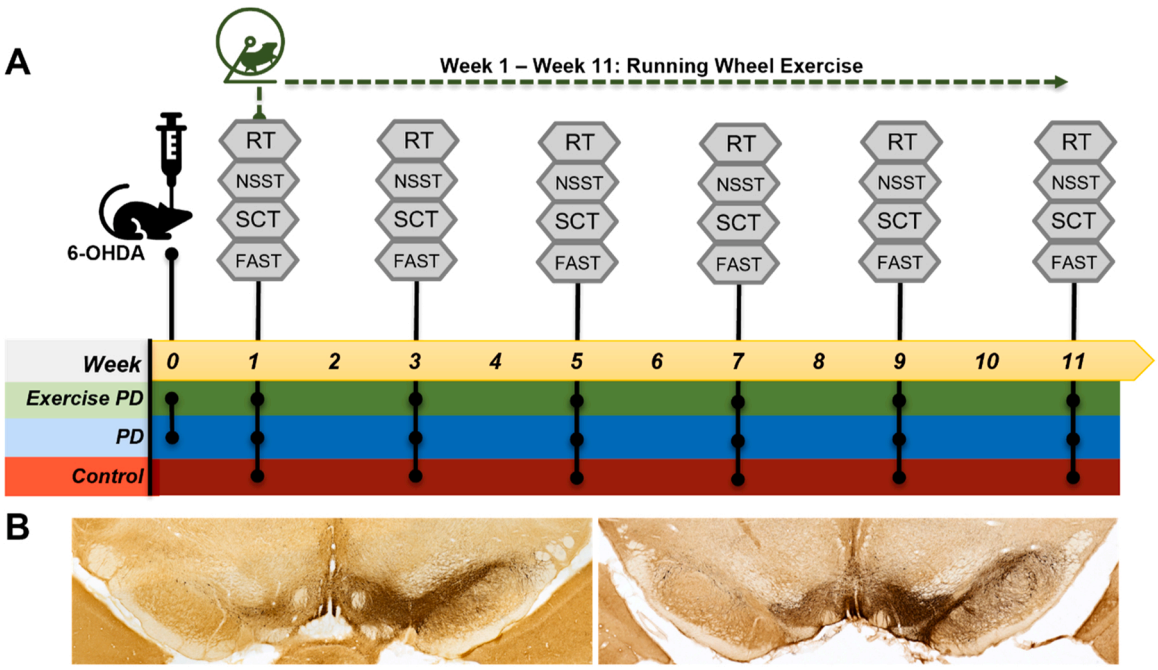

Fig. 1. Experimental Design. A, Fourteen animals were unilaterally lesioned with 6-OHDA. Following one week of recovery from 6-OHDA lesioning, Eight PD animals began forced running wheel exercise that was performed five times per week for 11 weeks post-lesion. All three groups, PD exercise (n=8), PD (n=6), and control (n=11) were subjected to behavioral tests every two weeks, beginning at one-week post-lesion. RT, rotarod test; NSST, novelty sucrose splash test; SCT, sucrose consumption test and FAST, forelimb adjusting step test. B, Two Representative coronal sections immunostained for tyrosine hydroxylase verifies unilateral 6-OHDA lesion producing >90% loss of dopaminergic neurons in the substantia nigra compacta (SNc). Left, PD control. Right, PD exercise.

Motor behavior tests

To assess motor deficits in the hemi-parkinsonian rats, two tests were conducted: the forelimb adjusting steps (FAST) test and the Rotarod latency to fall test (RT). The FAST test is a validated measure of akinesia in rats and is used to assess deficits in contralateral limb use (Glajch et al., 2012; Olsson et al., 1995; Schallert et al., 2000). Each rat was held with their hind limbs elevated and moved backward at a steady rate along a 1-meter glass corridor (Runway, CleverSys) over a duration of approximately 3–4 s. The movement of the rat was videotaped and later analyzed offline. The analysis involved counting the number of steps made by the contralateral and ipsilateral forelimbs of the rat. Three trials were recorded within each session, and a total of six sessions were conducted. The behavioral effects were quantified by calculating the ratio of steps taken by the contralateral limb to the steps taken by the ipsilateral limb. This ratio provided an indicator of the degree of asymmetry in motor abilities, with lower values indicating a higher degree of PD-induced asymmetry.

The RT is used to evaluate basic motor abilities in hemi-parkinsonian rats and provides a drug-free testing strategy (Monville et al., 2006; Rozas et al., 1998). An initial acclimatization trial was performed for each rat before the first RT. During this trial, all animals were placed individually on the rotarod and run for a duration of 45 s at a slowly increasing speed to acclimatize them to the test. After the acclimatization trial, a five-minute rest period was given to the rats. The rotarod apparatus was set to linearly accelerate from 6 to 40 rpm over a period of 300 s. This acceleration eliminated the need for extensive pre-training as the rats had difficulty staying on the rod at high rotation speeds. If a rat reached a duration of 200 s without falling, the rod would remain at a constant speed of 40 rpm until the animal fell off. The RT was performed three times for each animal with a 10-minute interval between each trial. The average latency to fall over the three trials was recorded for each animal, providing a measure of their maximal gait performance. These motor behavior tests allowed for the assessment of motor deficits in the hemi-parkinsonian rats, specifically the asymmetry in forelimb use and the ability to sustain running on an accelerating rod.

Histology

After completion of experiments, rats were deeply anesthetized with urethane (1.8 g/kg, intraperitoneally) and perfused transcardially with 0.1 M PBS immediately followed by 4% paraformaldehyde in 0.1 M PBS. Brains were post-fixed using 4% paraformaldehyde at 4°C for 24 h and then transferred to 30% sucrose solution at 4°C for approximately 72 h or when the brain ceased to float in solution. Brains were cut into 40 μm coronal sections using a cryostat (Cm3050S, Leica Microsystems). Tyrosine hydroxylase (TH) immunohistochemistry was used to determine the extent of degeneration of dopaminergic neurons in the substantia nigra pars compacta. Briefly, after three rinses in PBS, brain sections were first incubated for 10 min in 3% hydrogen peroxide. The sections were rinsed and blocked for 1 h at 4°C in blocking solution containing 10% goat serum. The sections were then incubated in anti-tyrosine hydroxylase antibody (AB152; 1:1000, Vector Laboratories) overnight at 4°C in solution with 10% goat serum and 0.25% Triton X-100 with PBS. After three rinses in PBS, the sections were incubated with biotinylated goat anti-rabbit secondary antibody (BA-1000, 1:250, Vector Laboratories) with 10% goat serum and 0.25% Trion X-100 in PBS for 1 h at room temperature. After rinsing, the sections were incubated in a VECTASTAIN Elite ABC kit (Vector Laboratories) solution for 1 h and then visualized using DAB solution. The brain sections were examined to verify the extent of DA depletion.

Data analysis

Statistical significance between groups conditions was determined using a one way or two-way repeated measures analysis of variance (ANOVA). Post hoc paired testing was used if the corresponding main effect or the interaction was significant at $p<0.05$. To control for multiple comparisons, pairwise post hoc tests were performed with the Tukey's honestly significant different test with a $p<0.05$ significance

cut-off. For single comparisons, two-sample Mann-Whitney or paired t-test was performed. The results are presented as mean ± standard error (SEM). Data was graphed using GraphPad Prism (version 9.3.1).

Results

We unilaterally injected 6-OHDA into MFB and produced the unilateral loss of nigral dopaminergic neurons confirmed by clear reductions in tyrosine hydroxylase immunoreactivity in the SNc of the lesioned hemisphere (Fig. 1) in all PD rats. Following unilateral MFB lesion, rats reduced the use of limbs on the contralateral side and turned preferentially toward lesioned side.

SCT: The sucrose consumption test was conducted to assess the presence of anhedonia in the rats. One week after the surgery, both the PD and PD exercise groups showed lower sucrose preference indices (SPIs) compared to the control group (PD: 77.86 ± 4.70, PD exercise: 67.47 ± 7.23, control: 89.90 ± 3.45) (ps < 0.012). However, there was no significant difference between the PD and PD exercise groups (p = 0.05). A two-way ANOVA with three testing groups (control, PD, and PD exercise) and testing time (every other week for 11 weeks) as factors revealed a main effect of groups ($F_{2, 132} = 15.9$, $p < 0.0001$), a main effect of time ($F_{5, 132} = 13.67$, $p < 0.0001$), and a significant interaction between group and time ($F_{10, 132} = 2.34$, $p = 0.014$) (Fig. 2A). Post-hoc analysis showed that both the PD and PD exercise groups displayed lower SPIs than the control group throughout the 11-week period (ps < 0.001). Importantly, the SPIs of the PD exercise group significantly increased over time ($p < 0.001$). Furthermore, the normalized SPI (divided by the mean SPI in the first week) of the PD exercise group showed a dramatic increase over the 11 weeks compared to the PD and control groups (main effect of groups, $F_{2, 132} = 15.7$, $p < 0.0001$; main effect of time $F_{5, 132} = 2.78$, p=0.020 and no interaction $F_{10, 132} = 0.74$, p =0.682) (Fig. 2B). Post-hoc analysis revealed significant differences between the PD exercise group and both the control group ($p < 0.001$) and the PD group ($p < 0.001$), but no significant difference between the control and PD groups ($p = 0.571$). Overall, these findings indicate that the 6-OHDA lesioned PD rats displayed depressive symptoms, as indicated by a reduction in sucrose preference. The running wheel exercise regimen facilitated the recovery from 6-OHDA-induced anhedonia symptoms, with the PD exercise group showing the most improvement in sucrose consumption among the three groups.

NSST is a measure of anxiety that is responsive to chronic administration of antidepressants (Fuchikami et al., 2015; Hare et al., 2019; Santarelli et al., 2003; Surget et al., 2008). Decreases in grooming behavior and exploration are indicative of apathy, a symptom of depression. Fig. 3A and B demonstrate that the PD rats exhibited depressive behaviors characterized by increased immobility time and reduced exploration time in the new environment compared to the control group. A two-way ANOVA with group and time as factors revealed a main effect of groups ($F_{2, 132} = 25.7$, $p < 0.0001$), a main effect of time ($F_{5, 132} = 3.52$, p=0.005) and no significant interaction between these two factors ($F_{10, 132} = 1.9$, p =0.05, Fig. 3A). Post-hoc analysis indicated that the PD exercise group exhibited less immobility time than the PD group over the 11-week period ($p < 0.001$). Furthermore, there were significant differences in exploring behavior among the three groups (main effect of groups ($F_{2, 132} = 25.7$, $p < 0.0001$), no effect of time ($F_{5, 132} = 1.33$, p=0.26), and no interaction ($F_{10, 132} = 0.75$, p =0.68) (Fig. 3B). Tukey's multiple comparison test revealed that the PD exercise group spent more time exploring the new environment than the PD group ($p < 0.001$). Moreover, decreases in grooming behavior also indicate apathy, a common symptom of depression. To assess the effect of exercise on grooming behavior, the duration of time between the conclusion of sucrose spraying and the first instance of grooming behavior was analyzed. A two-way ANOVA with group and time as factors revealed a main effect of group ($F_{2, 132} = 7.23$, $p = 0.001$), a main effect of time ($F_{5, 132} = 8.91$, $p < 0.0001$), and no interaction ($F_{10, 132} = 0.70$, $p = 0.72$) (Fig. 3C). Post-hoc analysis indicated that the PD exercise group started grooming with a similar latency as the control group ($p > 0.05$) but shorter than the PD group ($p < 0.001$). These results suggest that the PD exercise group exhibited decreased immobility, increased exploration time, and quicker grooming compared to the PD control group in the NSST test. These observations indicate that 6-OHDA lesioned PD rats displayed depressive symptoms, while the running wheel exercise provided improvement in these depressive symptoms (Fig. 3).

FAST is a well-established and validated measure of parkinsonian akinesia in rats (Glajch et al., 2012; Olsson et al., 1995; Schallert et al., 2000). The ratio of steps taken by the contralateral and ipsilateral limbs reflects the degree of PD-induced asymmetry in motor abilities, with lower values indicating higher asymmetry. A Two-way ANOVA analysis with group and time as factors revealed a significant main effect of

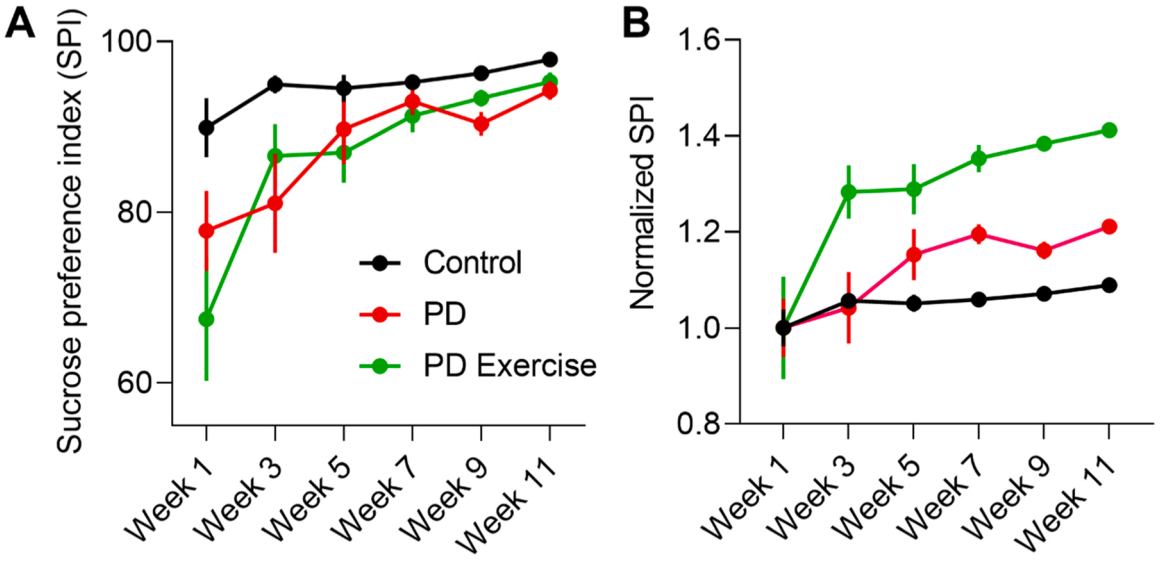

Fig. 2. Behavioral effects of exercise on sucrose consumption behavior. A, Sucrose preference index (SPI) of three groups over time. Throughout the 11-week period, both the PD (n=6) and PD exercise (n=8) groups exhibited lower SPIs compared to the control group (n=11) ($p < 0.001$). B, Normalized SPI (divided by the mean SPI in the first week. The PD exercise group demonstrated a significant increase over the 11 weeks in comparison to the PD and control groups (A two-way ANOVA revealed a main effect of groups, $F_{2, 132} = 15.7$, $p < 0.0001$; main effect of time $F_{5, 132} = 2.78$, p=0.020 and no interaction $F_{10, 132} = 0.74$, p =0.682).

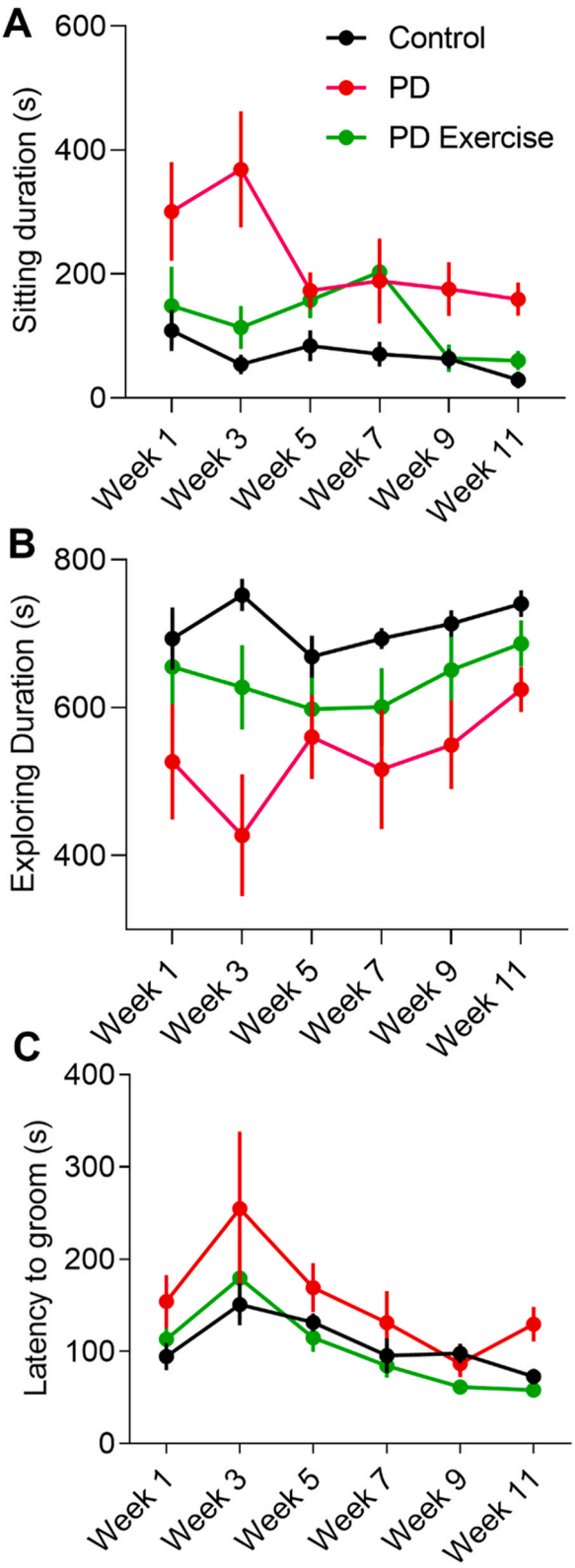

Fig. 3. Behavioral effects of exercise on novelty sucrose splash test. A, Immobility over time. PD exercise group (n=8) exhibited less immobility time compared to the PD group (n=6) throughout the 11-week period ($p<0.001$). B, Exploring activity over time. The PD exercise group spent a significant greater amount of time exploring the new environment compared to the PD group ($p<0.001$). C, Grooming behavior over time. PD exercise group exhibited grooming behavior with a latency similar to that of the control group 9 (n=11) ($p > 0.05$), but significantly shorter latency compared to the PD group ($p < 0.001$).

groups ($F_{2, 87} = 60.4$, $p < 0.0001$), indicating significant differences among the groups. However, there was no effect of time ($F_{5, 87} = 0.27$, $p = 0.92$) and no significant interaction between group and time ($F_{10, 87} = 0.94$, $p = 0.50$) (Fig. 4A). Compared to intact animals, the 6-OHDA lesion led to a significant worsening of forelimb akinesia, particularly in the contralateral forelimb ($p < 0.0001$). However, the exercise intervention ameliorated the dysfunction of the contralateral forelimb in the 6-OHDA-treated animals ($p < 0.0001$). This improvement was further confirmed by the normalized ratio analysis, which showed significant differences among the three groups (Fig. 4B). Specifically, there was a significant difference between the PD group and the control group ($p = 0.0002$), as well as between the PD exercise group and the PD group ($p < 0.0001$). However, there was no significant difference between the control group and the PD group ($p = 0.95$). These findings indicate that the 6-OHDA lesioned PD rats exhibited motor deficits, as evidenced by a reduction in stepping, and the running wheel exercise intervention improved these deficits.

RT was used to assess motor abilities, specifically the latency to fall from the rotating rod. Fig. 5 displays the weekly average latency to fall for each group. The statistical analysis indicated that there were no significant differences between the groups ($F_{2, 132} = 1.75$, $p = 0.18$). Furthermore, the latency to fall remained consistent from week 1 to week 11 ($F_{5, 132} = 0.17$, $p = 0.38$). The interaction between group and time was also not significant ($F_{10, 132} = 0.74$, $p = 0.69$). Similarly, the analysis of normalized latencies did not reveal any significant differences among the groups (group, $F_{2, 132} = 0.09$, $p = 0.91$; time, $F_{5, 132} = 1.11$, $p = 0.36$; interaction, $F_{10, 132} = 0.68$, $p = 0.74$). Therefore, no significant improvement in motor abilities was observed among the groups in the rotarod test.

Discussion

In this study, we investigated the therapeutic effects of aerobic exercise on PD depressive symptoms by utilizing a combination of behavioral assays in a 6-OHDA rat model of PD. Our findings demonstrate that a regimen of forced running wheel exercise accelerates recovery in forelimb motor abilities while reducing the occurrence of depressive behaviors resulting from 6-OHDA-induced dopamine depletion. Exercise is widely recognized for its numerous benefits in the general population. In PD patients, exercise training, such as physical therapy, incorporates goal-based motor skill training, enabling individuals to regain cognitive engagement with movements that were previously automatic and unconscious (Petzinger et al., 2013). Although the beneficial effects of exercise on depression are established, the precise neuronal mechanisms underlying these improvements remain unclear. Several possibilities have been discussed, including increased neuroplasticity, neurogenesis, and enhanced cerebral blood flow. Previous studies have shown that aerobic exercise can enhance neuroplasticity by increasing the expression of brain-derived neurotrophic factor, which promotes neuronal regeneration (Gujral et al., 2017a, 2017b; Kang et al., 2020; Russo-Neustadt et al., 2001; Seo et al., 2019). Other potential mechanisms involve the availability of neurotransmitters such as serotonin and norepinephrine, as well as reduced inflammatory signaling, which have been found to stimulate neurogenesis, increase synaptic connections, and enhance cerebral vasculature in humans (Gujral et al., 2017a, 2017b; Voss et al., 2013). It has also been hypothesized that aerobic exercise activates the nigrostriatal dopaminergic pathways, and repeated activation of these circuits may contribute to exercise-induced stress resistance. Stress resistance, in turn, provides protection against stress-related disorders, including depression and anxiety (Arnold et al., 2020; Greenwood et al., 2013; Herrera et al., 2016). Both voluntary and forced exercise are considered rewarding activities due to the activation of midbrain dopaminergic circuits and the increase in ΔFosB expression in the striatum, which are implicated in reward processing (Arnold et al., 2020; Greenwood et al., 2013; Herrera et al., 2016). Modulating and reinforcing the effects of

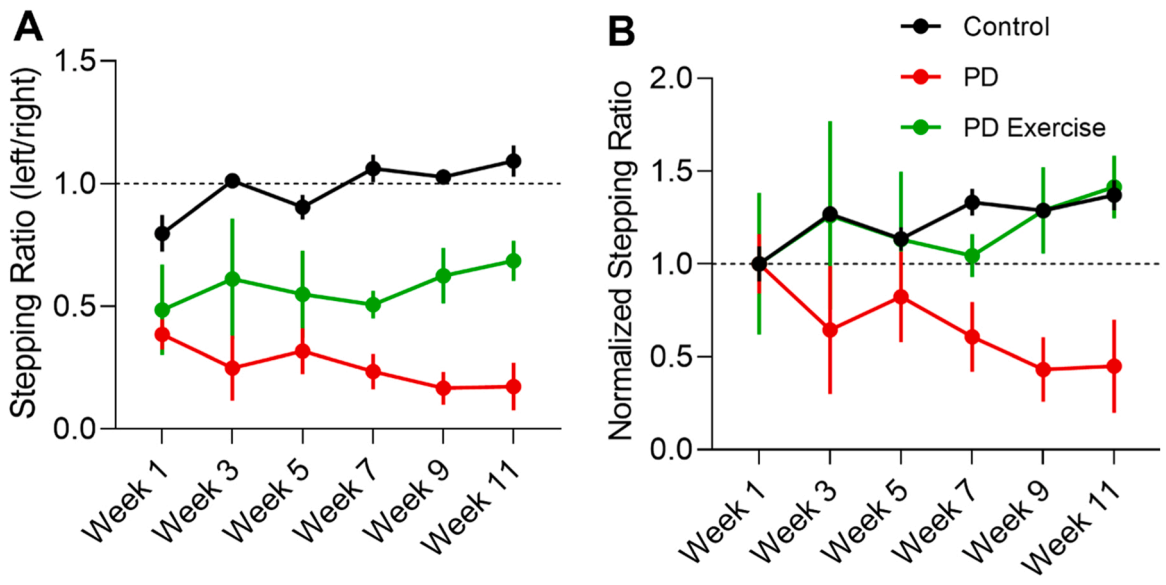

Fig. 4. Behavioral effects of exercise on forelimb stepping. A, PD animals exhibited abnormal use of the contralateral forelimb, as indicated by a reduced stepping ratio. The exercise intervention effectively ameliorated the symptoms in PD animals ($p < 0.0001$). (B) Normalized stepping ratio further confirms that the exercise improved contralateral forelimb use in PD animals ($p<0.0001$). PD exercise (n=8), PD (n=6), and control (n=11).

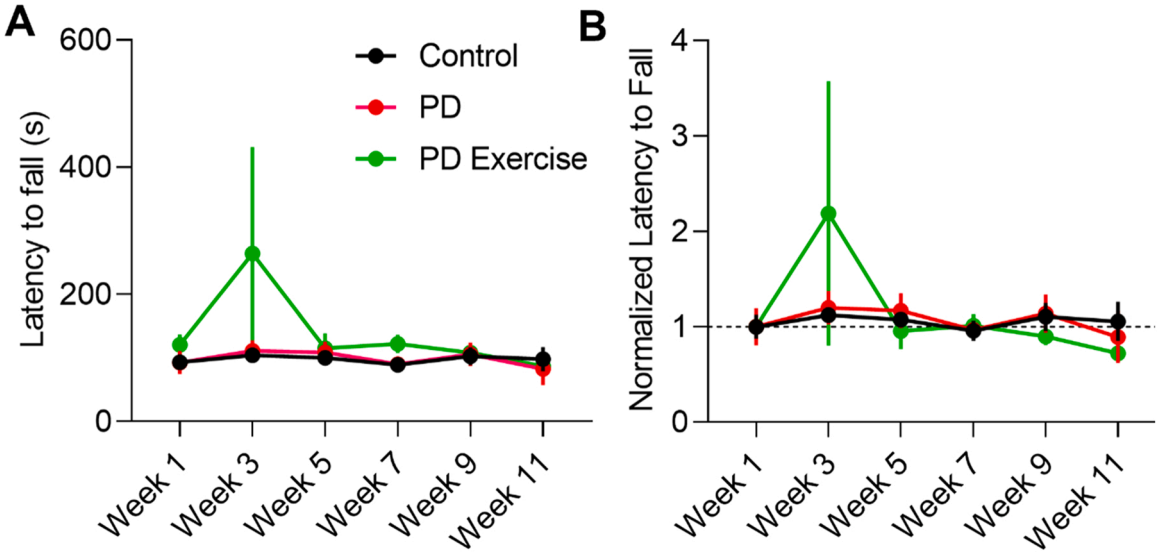

Fig. 5. Behavioral effects of exercise on the latency to fall from the rotating rod. A, On the accelerating rotarod), no significant improvement in motor abilities was observed among the groups (groups: $F_{2,\ 132} = 1.75$, $p = 0.18$; latency to fall; $F_{5,\ 132} = 0.17$, $p = 0.38$ and interaction between group and time: $F_{10,\ 132} = 0.74$, $p = 0.69$). B, normalized latencies did not reveal any significant differences among the groups (group, $F_{2,\ 132} = 0.09$, $p = 0.91$; time, $F_{5,\ 132} = 1.11$, $p = 0.36$; interaction, $F_{10,\ 132} = 0.68$, $p = 0.74$). PD exercise (n=8), PD (n=6), and control (n=11).

exercise and stress resistance through targeting striatal direct pathway neurons hold promise for future interventions (Greenwood et al., 2013; Herrera et al., 2016).

In the context of humans, these findings suggest that midbrain dopaminergic and striatal circuits, involved in reward and stress resistance, are recruited during exercise, even in individuals who perceive the exercise as a 'forced' activity. This observation is particularly relevant in clinical settings when exercise is prescribed and administered to PD patients under the close supervision of healthcare providers (Herrera et al., 2016).

In this study, we evaluated two motor tests, the rotarod test (RT) and the forelimb adjusting steps test (FAST), as well as two depressive tests, the novelty sucrose splash test (NSST) and the sucrose consumption test (SCT), to assess the effects of aerobic exercise on motor and depressive symptoms in a unilateral 6-OHDA rat model of PD. Our findings indicate that a regimen of forced running wheel exercise resulted in expedited recovery of forelimb motor abilities while reducing the occurrence of depressive behaviors associated with 6-OHDA-induced dopamine depletion.

The forelimb adjusting steps test (FAST) is an accepted measure of akinesia in rats, and our testing revealed an accelerated recovery in forelimb motor abilities (Glajch et al., 2012; Yu et al., 2020). FAST has been shown to detect significant behavioral impairments without the need for a pre-training period or chemical agonists, providing a clearer understanding of PD-related impairments (Glajch et al., 2012; Schallert et al., 1978; Yu et al., 2020). Comparing the PD group with the PD exercise group, our results indicate that the PD exercise group exhibited improved motor function, demonstrated by diminished akinesia through

increased spontaneous use of the affected limb. Interestingly, we did not observe significant results in the rotarod test. Animals quickly learned that falling off the rotarod had no consequences, leading them to discontinue their efforts to maintain the rotation speed. We believe that the high number of trials used in each test session allowed the animals to realize that there was no coercive measure to give their best effort.

Regarding our behavioral assays for depressive traits, we observed a decreased prevalence of anhedonia (measured through the sucrose consumption test, SCT) and diminished anxious behavior (measured through the novelty sucrose splash test, NSST) in the PD exercise group compared to the untreated PD group. Both of these depressive tests relied on an element of novelty for their respective measurements, and thus a pre-lesioning baseline test was not performed. A decrease in sucrose preference, as measured in the SCT, has been established as a reliable measure of anhedonia (Kanner et al., 2012; Klein et al., 2015). The sucrose consumption test is widely used to assess anhedonia in rodent models (Hamani et al., 2014; Hamani et al., 2010; Klein et al., 2015). The loss of interest in previously rewarding stimuli is considered a core symptom of clinical depression, disrupting the brain's complex reward processing system. The mesolimbic dopamine circuit, a major component of this system, is implicated in anhedonia due to its dysfunction (Markou et al., 1998; Slattery et al., 2007). The role of dopamine signaling in the mesolimbic system during rewarding conditions is still debated, but in general, dopaminergic activity is known to increase when a preferred stimulus is experienced. In the NSST, the PD exercise group spent significantly more time actively exploring the novel arena. Decreased grooming behavior indicates disturbance in self-care routines and demonstrates apathy (Fuchikami et al., 2015; Hare et al., 2019; Surget et al., 2008).

Conclusions

In conclusion, our study demonstrated that PD animals exhibited clear depressive symptoms, including decreased sucrose consumption indicating anhedonia in the SCT, as well as reduced exploration and increased sitting behaviors in the NSST compared to the control animals. Specifically, the PD exercise group showed the most significant improvement over 11 weeks of exercise among the three groups, as indicated by the highest sucrose preference index (SPI), decreased immobility, increased time spent exploring in the NSST, and the greatest improvement in correcting forelimb stepping bias in the FAST. These findings suggest that a regimen of running wheel exercise accelerates the recovery of motor abilities while reducing the occurrence of depressive behaviors caused by unilateral 6-OHDA dopamine depletion in a rat model of PD. While the existing evidence is robust, ongoing research is needed to continue to explore optimal exercise regimens, mechanisms of action, and the long-term effects of aerobic exercise in PD. Specifically, elucidating the precise mechanism of exercise-induced neuroplasticity will be crucial in understanding the exact benefits of aerobic exercise on PD motor and depressive symptoms. By gaining a better understanding of these underlying mechanisms, we can optimize the use of aerobic exercise as a therapeutic intervention for PD.

CRediT authorship contribution statement

Hannah Loughlin: Conceptualization, Methodology, Investigation, Formal analysis, Writing- Original draft preparation **Jacob Jackson**: Conceptualization, Methodology, Investigation, Formal analysis, Writing- Original draft preparation **Chloe Looman**: Investigation **Alayna Starll**: Investigation **Jeremy Goldman**: Writing- Reviewing and Editing **Zhiying Shan**: Writing- Reviewing and Editing **Chunxiu Yu**: Conceptualization, Methodology, Writing- Reviewing and Editing, Project administration, Funding acquisition, Supervision.

Declaration of Competing Interest

The authors declare no competing financial interests.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions

HL, JJ and CY designed the experiments. HL, JJ, CL, and AS performed the experiments. HL, JJ and CY analysed the experimental data. HL, JJ, JG, SZ and CY wrote the paper.

References

Aarsland, D., Batzu, L., Halliday, G.M., Geurtsen, G.J., Ballard, C., Chaudhuri, K.R., Weintraub, D., 2021. Parkinson disease-associated cognitive impairment. Nat. Rev. Dis. Prim. 7.

Ahlskog, J.E., 2011. Does vigorous exercise have a neuroprotective effect in Parkinson disease. Neurology 77, 288–294.

Arnold, M.R., Greenwood, B.N., McArthur, J.A., Clark, P.J., Fleshner, M., Lowry, C.A., 2020. Effects of repeated voluntary or forced exercise on brainstem serotonergic systems in rats. Behav. Brain Res. 378.

Chang, J.Y., Shi, L.H., Luo, F., Zhang, W.M., Woodward, D.J., 2008. Studies of the neural mechanisms of deep brain stimulation in rodent models of Parkinson's disease. Neurosci. Biobehav. Rev. 32, 352–366.

Chiken, S., Nambu, A., 2016. Mechanism of Deep Brain Stimulation: Inhibition, Excitation, or Disruption? Neurosci. Rev. J. Bringing Neurobiol., Neurol. Psychiatry 22, 313–322.

Combs, H.L., Folley, B.S., Berry, D.T.R., Segerstrom, S.C., Han, D.Y., Anderson-Mooney, A.J., Walls, B.D., van Horne, C., 2015. Cognition and depression following deep brain stimulation of the subthalamic nucleus and globus pallidus pars internus in Parkinson's disease: a meta-analysis. Neuropsychol. Rev. 25, 439–454.

Craft, L.L., Perna, F.M., 2004. The benefits of exercise for the clinically depressed. Prim. Care Companion J. Clin. Psychiatry 6, 104–111.

Decourt, M., Jimenez-Urbieta, H., Benoit-Marand, M., Fernagut, P.O., 2021. Neuropsychiatric and cognitive deficits in Parkinson's disease and their modeling in rodents. Biomedicines 9.

van der Hoek, T.C., Bus, B.A., Matui, P., van der Marck, M.A., Esselink, R.A., Tendolkar, I., 2011. Prevalence of depression in Parkinson's disease: effects of disease stage, motor subtype and gender. J. Neurol. Sci. 310, 220–224.

Deuschl, G., Herzog, J., Kleiner-Fisman, G., Kubu, C., Lozano, A.M., Lyons, K.E., Rodriguez-Oroz, M.C., Tamma, F., et al., 2006. Deep brain stimulation: postoperative issues. Mov. Disord. Off. J. Mov. Disord. Soc. 21 (Suppl 14), S219–S237.

Faivre, F., Joshi, A., Bezard, E., Barrot, M., 2019. The hidden side of Parkinson's disease: studying pain, anxiety and depression in animal models. Neurosci. Biobehav. Rev. 96, 335–352.

Fisher, B.E., Petzinger, G.M., Nixon, K., Hogg, E., Bremmer, S., Meshul, C.K., Jakowec, M. W., 2004. Exercise-induced behavioral recovery and neuroplasticity in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned mouse basal ganglia. J. Neurosci. Res 77, 378–390.

Fisher, B.E., Wu, A.D., Salem, G.J., Song, J., Lin, C.H., Yip, J., Cen, S., Gordon, J., et al., 2008. The effect of exercise training in improving motor performance and corticomotor excitability in people with early Parkinson's disease. Arch. Phys. Med Rehabil. 89, 1221–1229.

Fuchikami, M., Thomas, A., Liu, R., Wohleb, E.S., Land, B.B., DiLeone, R.J., Aghajanian, G.K., Duman, R.S., 2015. Optogenetic stimulation of infralimbic PFC reproduces ketamine's rapid and sustained antidepressant actions. Proc. Natl. Acad. Sci. USA 112, 8106–8111.

Glajch, K.E., Fleming, S.M., Surmeier, D.J., Osten, P., 2012. Sensorimotor assessment of the unilateral 6-hydroxydopamine mouse model of Parkinson's disease. Behav. Brain Res 230, 309–316.

Greenwood, B.N., Spence, K.G., Crevling, D.M., Clark, P.J., Craig, W.C., Fleshner, M., 2013. Exercise-induced stress resistance is independent of exercise controllability and the medial prefrontal cortex. Eur. J. Neurosci. 37, 469–478.

Gujral, S., Aizenstein, H., Reynolds, C.F., Butters, M.A., Erickson, K.I., 2017b. Exercise effects on depression: possible neural mechanisms. Gen. Hosp. Psychiat 49, 2–10.

Gujral, S., Aizenstein, H., Reynolds 3rd, C.F., Butters, M.A., Erickson, K.I., 2017a. Exercise effects on depression: possible neural mechanisms. Gen. Hosp. Psychiatry 49, 2–10.

Hamani, C., Lozano, A.M., 2003. Physiology and pathophysiology of Parkinson's disease. Ann. Ny. Acad. Sci. 991, 15–21.
Hamani, C., Diwan, M., Macedo, C.E., Brandao, M.L., Shumake, J., Gonzalez-Lima, F., Raymond, R., Lozano, A.M., et al., 2010. Antidepressant-like effects of medial prefrontal cortex deep brain stimulation in rats. Biol. Psychiatry 67, 117–124.
Hamani, C., Amorim, B.O., Wheeler, A.L., Diwan, M., Driesslein, K., Covolan, L., Butson, C.R., Nobrega, J.N., 2014. Deep brain stimulation in rats: different targets induce similar antidepressant-like effects but influence different circuits. Neurobiol. Dis. 71, 205–214.
Hare, B.D., Shinohara, R., Liu, R.J., Pothula, S., DiLeone, R.J., Duman, R.S., 2019. Optogenetic stimulation of medial prefrontal cortex Drd1 neurons produces rapid and long-lasting antidepressant. Nat. Commun. 10.
Herrera, J.J., Fedynska, S., Ghasem, P.R., Wieman, T., Clark, P.J., Gray, N., Loetz, E., Campeau, S., et al., 2016. Neurochemical and behavioural indices of exercise reward are independent of exercise controllability. Eur. J. Neurosci. 43, 1190–1202.
Hotting, K., Roder, B., 2013. Beneficial effects of physical exercise on neuroplasticity and cognition. Neurosci. Biobehav. Rev. 37, 2243–2257.
Kang, J., Wang, D., Duan, Y., Zhai, L., Shi, L., Guo, F., 2020. Aerobic exercise prevents depression via alleviating hippocampus injury in chronic stressed depression rats. Brain Sci. 11.
Kanner, A.M., Schachter, S.C., Barry, J.J., Hersdorffer, D.C., Mula, M., Trimble, M., Hermann, B., Ettinger, A.E., et al., 2012. Depression and epilepsy: epidemiologic and neurobiologic perspectives that may explain their high comorbid occurrence. Epilepsy Behav. 24, 156–168.
Klein, S., Bankstahl, J.P., Loscher, W., Bankstahl, M., 2015. Sucrose consumption test reveals pharmacoresistant depression-associated behavior in two mouse models of temporal lobe epilepsy. Exp. Neurol. 263, 263–271.
Kvam, S., Kleppe, C.L., Nordhus, I.H., Hovland, A., 2016. Exercise as a treatment for depression: a meta-analysis. J. Affect Disord. 202, 67–86.
Lindgren, H.S., Lelos, M.J., Dunnett, S.B., 2012. Do alpha-synuclein vector injections provide a better model of Parkinson's disease than the classic 6-hydroxydopamine model? Exp. Neurol. 237, 36–42.
Llamas-Velasco, S., Contador, I., Mendez-Guerrero, A., Romero Ferreiro, C., Benito-Leon, J., Villarejo-Galende, A., Bermejo-Pareja, F., 2021. Physical activity and risk of Parkinson's disease and parkinsonism in a prospective population-based study (NEDICES). Prev. Med Rep. 23, 101485.
Markou, A., Kosten, T.R., Koob, G.F., 1998. Neurobiological similarities in depression and drug dependence: a self-medication hypothesis. Neuropsychopharmacol 18, 135–174.
Marsh, L., 2013. Depression and Parkinson's disease: current knowledge. Curr. Neurol. Neurosci. Rep. 13, 409.
McNeil, J.K., LeBlanc, E.M., Joyner, M., 1991. The effect of exercise on depressive symptoms in the moderately depressed elderly. Psychol. Aging 6, 487–488.
Monville, C., Torres, E.M., Dunnett, S.B., 2006. Comparison of incremental and accelerating protocols of the rotarod test for the assessment of motor deficits in the 6-OHDA model. J. Neurosci. Methods 158, 219–223.
Mul, J.D., 2018. Voluntary exercise and depression-like behavior in rodents: are we running in the right direction. J. Mol. Endocrinol. 60, R77–R95.
O'Dell, S.J., Gross, N.B., Fricks, A.N., Casiano, B.D., Nguyen, T.B., Marshall, J.F., 2007. Running wheel exercise enhances recovery from nigrostriatal dopamine injury without inducing neuroprotection. Neuroscience 144, 1141–1151.
Olsson, M., Nikkhah, G., Bentlage, C., Bjorklund, A., 1995. Forelimb akinesia in the rat Parkinson model: differential effects of dopamine agonists and nigral transplants as assessed by a new stepping test. J. Neurosci.: Off. J. Soc. Neurosci. 15, 3863–3875.
Petzinger, G.M., Walsh, J.P., Akopian, G., Hogg, E., Abernathy, A., Arevalo, P., Turnquist, P., Vuckovic, M., et al., 2007. Effects of treadmill exercise on dopaminergic transmission in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned mouse model of basal ganglia injury. J. Neurosci.: Off. J. Soc. Neurosci. 27, 5291–5300.
Petzinger, G.M., Fisher, B.E., McEwen, S., Beeler, J.A., Walsh, J.P., Jakowec, M.W., 2013. Exercise-enhanced neuroplasticity targeting motor and cognitive circuitry in Parkinson's disease. Lancet Neurol. 12, 716–726.
Pothakos, K., Kurz, M.J., Lau, Y.S., 2009. Restorative effect of endurance exercise on behavioral deficits in the chronic mouse model of Parkinson's disease with severe neurodegeneration. BMC Neurosci. 10, 6.
Ravina, B., Camicioli, R., Como, P.G., Marsh, L., Jankovic, J., Weintraub, D., Elm, J., 2007. The impact of depressive symptoms in early Parkinson disease. Neurology 69, 342–347.
Raza, C., Anjum, R., Shakeel, N.U.A., 2019. Parkinson's disease: mechanisms, translational models and management strategies. Life Sci. 226, 77–90.
Ross G.W., Abbott R.D., Petrovitch H., Tanner C.M., Popper J., McMurtray A., Masaki K., Launer L., White L.R. (2007), Association of mid-life physical activity with risk of future Parkinson's disease. Movement Disord 22:Xi-Xii.
Rozas, G., Lopez-Martin, E., Guerra, M.J., Labandeira-Garcia, J.L., 1998. The overall rod performance test in the MPTP-treated-mouse model of Parkinsonism. J. Neurosci. Meth 83, 165–175.
Russo-Neustadt, A., Ha, T., Ramirez, R., Kesslak, J.P., 2001. Physical activity-antidepressant treatment combination: impact on brain-derived neurotrophic factor and behavior in an animal model. Behav. Brain Res 120, 87–95.
Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., Weisstaub, N., Lee, J., et al., 2003. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 301, 805–809.
Schallert, T., Whishaw, I.Q., Ramirez, V.D., Teitelbaum, P., 1978. Compulsive, abnormal walking caused by anticholinergics in akinetic, 6-hydroxydopamine-treated rats. Science 199, 1461–1463.
Schallert, T., Fleming, S.M., Leasure, J.L., Tillerson, J.L., Bland, S.T., 2000. CNS plasticity and assessment of forelimb sensorimotor outcome in unilateral rat models of stroke, cortical ablation, parkinsonism and spinal cord injury. Neuropharmacology 39, 777–787.
Schapira, A.H., Agid, Y., Barone, P., Jenner, P., Lemke, M.R., Poewe, W., Rascol, O., Reichmann, H., Tolosa, E., 2009. Perspectives on recent advances in the understanding and treatment of Parkinson's disease. Eur. J. Neurol. 16, 1090–1099.
Seo, J.H., Park, H.S., Park, S.S., Kim, C.J., Kim, D.H., Kim, T.W., 2019. Physical exercise ameliorates psychiatric disorders and cognitive dysfunctions by hippocampal mitochondrial function and neuroplasticity in post-traumatic stress disorder. Exp. Neurol. 322.
Slattery, D.A., Markou, A., Cryan, J.F., 2007. Evaluation of reward processes in an animal model of depression. Psychopharmacology 190, 555–568.
Smith, B.A., Goldberg, N.R.S., Meshul, C.K., 2011. 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 Res. 1386, 70–80.
Surget, A., Saxe, M., Leman, S., Ibarguen-Vargas, Y., Chalon, S., Griebel, G., Hen, R., Belzung, C., 2008. Drug-dependent requirement of hippocampal neurogenesis in a model of depression and of antidepressant reversal. Biol. Psychiatry 64, 293–301.
Tillerson, J.L., Caudle, W.M., Reveron, M.E., Miller, G.W., 2003. Exercise induces behavioral recovery and attenuates neurochemical deficits in rodent models of Parkinson's disease. Neuroscience 119, 899–911.
Tsukita, K., Sakamaki-Tsukita, H., Takahashi, R., 2022. Long-term effect of regular physical activity and exercise habits in patients with early Parkinson disease. Neurology 98, e859–e871.
Tysnes, O.B., Storstein, A., 2017. Epidemiology of Parkinson's disease. J. Neural Transm. 124, 901–905.
Voss, M.W., Vivar, C., Kramer, A.F., van Praag, H., 2013. Bridging animal and human models of exercise-induced brain plasticity. Trends Cogn. Sci. 17, 525–544.
Xu, Q., Park, Y., Huang, X., Hollenbeck, A., Blair, A., Schatzkin, A., Chen, H., 2010b. Physical activities and future risk of Parkinson disease. Neurology 75, 341–348.
Xu, Q., Park, Y., Huang, X., Hollenbeck, A., Blair, A., Schatzkin, A., Chen, H., 2010a. Physical activities and future risk of Parkinson disease. Neurology 75, 341–348.
Yu, C., Cassar, I.R., Sambangi, J., Grill, W.M., 2020. Frequency-specific optogenetic deep brain stimulation of subthalamic nucleus improves Parkinsonian motor behaviors. J. Neurosci. Off. J. Soc. Neurosci. 40, 4323–4334.