Treadmill exercise modifies dopamine receptor expression in the prefrontal cortex of the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned mouse model of Parkinson's disease

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Parkinson's disease (PD) is the second most common neurodegenerative disorder for which there is no cure. PD is a dopamine (DA)-deficit disorder marked by progressive motor and nonmotor disturbances, including cognitive impairment. Executive function (EF) is the most common subtype of cognitive impairment in PD and consists of deficits in number of processes including behavioral flexibility. The prefrontal cortex (PFC) is an important brain region subserving EF. Furthermore, DA plays a key neuromodulatory role in the PFC and altered DA neurotransmission is believed to contribute toward EF deficits in PD. The mechanisms underlying PFC dysfunction are not fully understood and there are no effective treatments for EF deficits in PD. Exercise is a promising therapeutic strategy that may exert beneficial effects on PFC function in PD. Our previous work suggests that exercise improves motor function and restores striatal DA-D_2 receptor (DA-D_2R) expression after DA depletion. This study builds upon our previous work by exploring whether exercise modulates PFC function, specifically DA levels and DA receptor expression in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-mouse model of DA depletion. We found that exercise restores PFC DA levels, reverses the MPTP-induced increase in DA-D_1R and decrease in DA-D_4R, and exerts differential effects on D_2Rs. The modest effect of exercise in PFC function may suggest that other types of exercise, such as those that are more skill based, would be required to target these cognitive behavioral circuits. *NeuroReport* **28:987–995**

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Introduction

Parkinson's disease (PD) is a common age-related neurodegenerative disorder for which there is no disease-modifying therapy or cure. PD is characterized as a dopamine (DA)-deficit disorder, marked by a number of progressive motor and nonmotor features, including cognitive impairment. Executive function (EF) is the most common subtype of cognitive impairment in PD, which transitions to dementia, increased fall risk, and poor quality of life. EF disturbances in PD consist of deficits in working memory, behavioral flexibility, planning, and attention. The prefrontal cortex (PFC) along with its connections to the striatum is an important brain region subserving EF. Furthermore, DA plays a key neuromodulatory role in the PFC and aberrant DA neurotransmission is believed to contribute toward executive function deficits observed in the DA-depleted brain [1,2]. There remains, however, an important need to better understand the mechanisms underlying PFC dysfunction in PD and to develop more effective treatment modalities targeting cognition.

Exercise has been shown to improve motor function in individuals with PD and may also be a promising therapeutic strategy for cognitive impairment. For example, several studies in healthy aging individuals have shown a significant beneficial effect of exercise on cognition in physically fit individuals [3]. Research also suggests that exercise improves cognitive function in PD, as shown by reduced perseverative deficits, an indicator of behavioral flexibility, using the Wisconsin Card Sorting Task [4]. However, the mechanisms underlying the beneficial effects of exercise are not fully understood. Previous work from our lab suggests that exercise improves motor performance and restores striatal DA-D_2 receptor (DA-D_2R) expression in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-mouse model of DA depletion and in patients with PD [5,6]. The current study builds upon our previous work by investigating whether treadmill exercise modulates PFC function, specifically DA receptor expression, in the MPTP-lesioned mouse. Changes in DA levels and DA-D_1R, DA-D_2R, and DA-D_4R protein expressions, the most abundantly expressed DA receptors in the PFC, were assessed by high-performance liquid chromatography (HPLC) and western immunoblotting, respectively, at 1 and 6 weeks after the start of exercise. Understanding

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the effects of exercise on EF and DA signaling in the DA-depleted PFC may provide key insight into new therapeutic targets for the improved treatment of the cognitive disturbances observed in PD.

Materials and methods

Animals

Studies used a total of 84 young adult (8–10 weeks old) male C57BL/6J mice (Jackson Laboratory, Bar Harbor, Maine, USA) in four treatment groups: (i) saline, (ii) saline + exercise, (iii) MPTP, and (iv) MPTP + exercise. Mice were housed five to a cage and acclimated to a 12-h shift in a light/dark cycle and exercise occurred during the normal awake period. All experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23, revised 1996) and approved by the University of Southern California Institutional Animal Care and Use Committee.

MPTP lesioning

MPTP (Sigma Inc., St. Louis, Missouri, USA) was administered in a series of four intraperitoneal injections of 20 mg/kg (free-base) at 2-h intervals for a total administration of 80 mg/kg. This regimen leads to 60–70% loss of nigrostriatal neurons as determined by unbiased stereological techniques and an 80–90% depletion of striatal dopamine levels [7]. Nigrostriatal cell loss is complete by day 3 after MPTP administration as determined by histological techniques [8].

Treadmill exercise

The treadmill exercise protocol was performed as described previously [9]. Briefly, exercise was initiated 5 days after saline or MPTP lesioning (when cell death is complete) and continued for 5 days/week for 6 weeks starting at a velocity of 10.0 ± 1.5 m/min, increasing to 24.0 ± 0.5 m/min by the final week. As we have reported previously, there was a significant difference in velocity at weeks 1–4 between the saline + exercise and the MPTP + exercise groups ($P < 0.05$) [9]. This difference was eliminated with further training and completion of the treadmill running regimen.

Tissue collection

Tissue was collected from all treatment groups at 1 and 6 weeks after the start of exercise. Mice were killed by cervical dislocation for fresh tissue (HPLC and western immunoblotting). PFC tissue was dissected in a block corresponding to anatomical landmarks: (i) bregma + 1.4 to + 2.0 mm (rostral to the corpus callosum), (ii) 1 mm lateral from the midline to the corpus callosum, and (iii) dorsal–ventral: 1.5–3.5 (inferior to the motor cortex and superior to the lateral ventricles). Dorsal striatal (dStr) tissue was dissected in a block from a coronal slice: (i) bregma + 1.20 to + 0.60 mm, (ii) 2.5 mm lateral from the midline, and (iii) dorsal–ventral (inferior to the corpus callosum and superior to the anterior commissure). All tissue was flash frozen and stored at − 80°C until analysis.

HPLC analysis of dopamine and its metabolites

Dopamine concentration in the PFC and dStr ($N = 4$–7 animals/group/time point) along with its metabolites, homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC), and turnover ratio [(DOPAC + HVA)/dopamine] were determined as described [10]. HPLC data are presented as mean DA (ng)/protein (mg) ± SD.

Western immunoblotting

Western immunoblotting was used to determine the relative expression of proteins within the PFC ($N = 5$–7 animals/group/time point) and dStr tissue ($N = 4$/group/time point) to further validate MPTP lesioning by tyrosine hydroxylase (TH) levels. Tissue samples were disrupted by sonication in homogenization buffer (50 mM Tris pH 7.4, 150 mM NaCl, 1 mM EDTA, 0.1% SDS, and protease inhibitor cocktail (Cat#: 539134; Calbiochem/EMD Millipore, Billerica, Massachusetts, USA). Protein concentration was determined using the BCA method (Pierce, Rockford, Illinois, USA). The immunoblotting technique used has been described previously [5,7,11]. Briefly, samples were separated on a precast Tris-glycine gels, transferred to nitrocellulose membranes in Towbin buffer, washed with PBS, and blocked in LI-COR blocking buffer (LI-COR Inc.; Lincoln, Nebraska, USA). Membranes were exposed to the primary antibody solution overnight at 4°C including TH (1 : 1000, Cat#: MAB318, EMD Millipore); D_1R (1 : 500, Cat#: SC-14001; Santa Cruz Biotechnology, Dallas, Texas, USA); D_2R (1 : 500, Cat#: SC-5303; Santa Cruz Biotechnology); and D_4R (1 : 300, Cat#: SC-136139; Santa Cruz Biotechnology). The secondary antibodies used included Goat-anti-mouse IgG $IRDye_{800}$ CW (1 : 10, 000, Cat#: 926-32210; LI-COR Inc.); Goat-anti-Rabbit IgG $IRDye_{800}$ CW (1 : 10, 000, Cat#: 926-32211; LI-COR Inc); Goat-anti-Mouse IgG $IRDye_{680}$ LT (1 : 15 000, Cat#: 926-68020; LI-COR Inc); and Goat-anti-Rabbit IgG $IRDye_{680}$ LT (1 : 15 000, Cat#: 926-68021; LI-COR Inc). Nitrocellulose membranes were scanned using an Odyssey Infrared Imaging System 3.0 (LI-COR Inc.). Densiometric quantification was performed using Image Studio Software, version 3.1.4 (LI-COR Inc.) and expressed as relative optical density (OD). Each gel contained samples from two to three independent animals from each experimental group. The OD of each band was quantified relative to the OD. of β-actin protein, serving as the loading control. For comparison across groups, the relative OD levels for each sample were compared with the averaged value of saline mice analyzed on the same blot, which was normalized to 10 000 OD units. Data are presented as mean ± SD.

Statistical analysis

Statistical analyses were carried out in SPSS, version 14.0 for Windows (SPSS, Chicago, Illinois, USA), or GraphPad Prism 6 (GraphPad Software, La Jolla, California, USA). For HPLC and western blot analysis, a two-way analysis of variance was used at the 1-week and 6-week time points to compare the effects of MPTP and exercise, and to examine for significant interactions. Post-hoc contrasts were performed using Tukey's multiple comparison test to determine the locus of any significant differences.

Results

Analysis of dopamine levels and tyrosine hydroxylase protein within the prefrontal cortex

HPLC analysis was carried out to examine the effects of exercise on DA and its metabolites within the PFC and the dStr at the 1-week time point (to confirm MPTP lesion) and the 6-week time point after 6 weeks of exercise (Fig. 1a). MPTP significantly reduced PFC DA levels compared with saline mice, and 6 weeks of high-intensity treadmill exercise reversed this effect. At 1 week, MPTP significantly reduced PFC DA levels [$F(1,12) = 12.0$, $P = 0.0047$)] (Fig. 1a) by 59.7% in MPTP mice (3.4 ± 1.1 ng DA/mg protein, $P = 0.0055$) and by 55.2% in MPTP + exercise mice (3.8 ± 1.2 ng DA/mg protein, $P = 0.0096$) compared with saline mice (8.5 ± 2.3 ng DA/mg protein). There was a significant interaction between MPTP and exercise [$F(1,12 = 6.361$, $P = 0.0268$], where exercise reduced PFC DA levels by 45.8% in saline + exercise mice (4.6 ± 1.9 ng DA/mg protein, $P = 0.0319$) compared with saline mice. However, there was no significant difference in the PFC DA levels between MPTP and MPTP + exercise mice ($P = 0.9880$). At 6 weeks, exercise restored the PFC DA levels in MPTP mice (Fig. 1b) and there was a significant interaction between MPTP and exercise [$F(1,24) = 22.72$, $P < 0.001$]. This effect was because of a significant increase in PFC DA levels by 68.4% in MPTP + exercise mice (6.4 ± 1.3 ng DA/mg protein) compared with MPTP mice (3.8 ± 1.3 ng DA/mg protein, $P = 0.0338$), and a significant decrease in PFC DA levels by 43.6% in saline + exercise mice (4.4 ± 1.5 ng DA/mg protein) compared with saline mice (7.8 ± 2.4 ng DA/mg protein, $P = 0.0045$). Furthermore, in the nonexercised groups, MPTP significantly reduced PFC DA levels by 50.7% compared with saline mice ($P = 0.001$). In contrast, there was no significant difference in the PFC DA levels in MPTP + exercise mice compared with the saline groups.

As TH protein is the rate-limiting step in DA biosynthesis, we examined expression using western immunoblotting in tissues from the PFC. At 1 week, there was a significant effect of MPTP on PFC TH levels [$F(1,22) = 294.0$, $P < 0.001$] (Fig. 1c). TH levels were significantly reduced by 45.3% in MPTP mice (54.6 ± 10.7) and by 46.2% in MPTP + exercise mice (53.7 ± 4.9) compared with saline mice (99.8 ± 26.0). There was no significant difference between the MPTP and the MPTP + exercise groups ($P = 0.995$) or between the saline and the saline + exercise groups (101.9 ± 3.0, $P = 0.949$). At 6 weeks, exercise restored PFC TH levels in MPTP mice (Fig. 1d). Specifically, there was a significant interaction between MPTP and exercise on PFC TH levels [$F(1,24) = 23.12$, $P < 0.001$]. This interaction was because of a significant increase in PFC TH levels in MPTP + exercise mice (96.9 ± 5.5) compared with MPTP mice (81.4 ± 14.3, $P < 0.001$), but there was no significant difference between saline + exercise mice (100.9 ± 1.2) and saline mice (99.4 ± 2.5, $P = 0.9719$). Furthermore, in nonexercised mice, MPTP significantly reduced PFC TH levels by 18.6% compared with saline ($P < 0.001$). Conversely, there was no significant difference in the PFC TH levels in MPTP + exercise mice compared with saline mice ($P = 0.4900$) or saline + exercise (100.9 ± 3.3, $P = 0.2666$).

Analysis of dopamine levels and tyrosine hydroxylase protein in the dorsal striatum

Consistent with our previous work, MPTP significantly reduced DA levels in the dStr at both 1 and 6 weeks, and exercise did not alter this effect (Fig. 2). Specifically, there was a significant effect of MPTP at the 1-week [$F(1,12) = 300.0$, $P < 0.0001$] and 6-week [$F(1,24) = 326.9$, $P < 0.0001$] time points. Furthermore, there was no significant effect of exercise at the 1-week [$F(1,12) = 0.3442$, $P = 0.5692$] or the 6-week [$F(1,24) = 0.7036$, $P = 0.4099$] time point. Our previous work showed no elevation in TH levels after exercise within the dStr of MPTP-lesioned mice [5,10,12]. In the present study, western immunoblotting analysis was carried out to examine the effects of exercise on TH levels within the PFC and dStr at 1 week (to confirm MPTP lesion) and 6 weeks (Fig. 2c and d, respectively). Data from the current study suggest that MPTP significantly reduced PFC TH levels, and 6 weeks of high-intensity treadmill exercise reversed this effect. Consistent with our previous work, MPTP significantly reduced TH levels in the dStr compared with saline, and exercise did not alter this effect (Fig. 2c and d). Specifically, there was a significant effect of MPTP at 1-week [$F(1,12) = 1385$, $P < 0.001$] and 6-week [$F(1,12) = 160.8$, $P < 0.0001$] time points. However, there was no significant effect of exercise at the 1-week [$F(1,12) = 1.277$, $P = 0.2806$] or the 6-week [$F(1,12) = 0.0013$, $P = 0.9721$] time point.

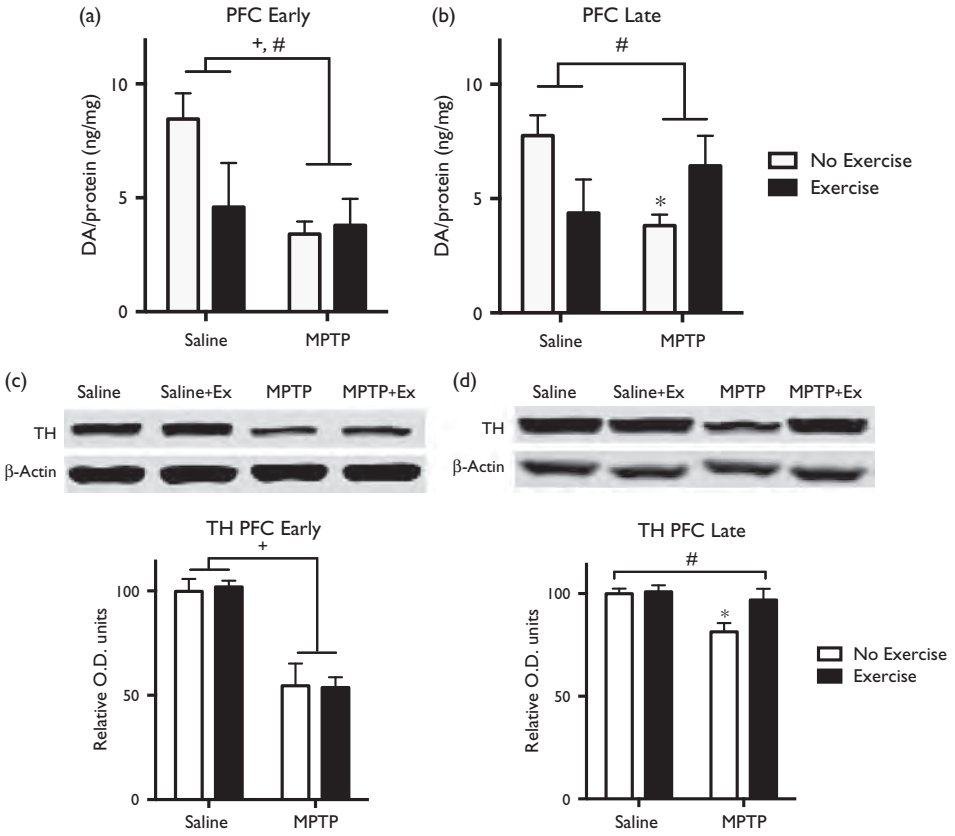
Analysis of dopamine receptor expression in the prefrontal cortex

Western immunoblotting analysis was carried out to examine the effects of exercise on DA-D_1R, DA-D_2R, and DA-D_4R protein expressions within the PFC of mice from all groups (Fig. 3).

DA-D_1 receptor

At 1 week, there was a significant interaction between MPTP and exercise [$F(1,23) = 4.88$, $P = 0.0375$], where PFC DA-D_1R expression was significantly increased by 5.1% in MPTP mice (104.9 ± 1.8, $P = 0.0219$), but significantly decreased by 10.2% in MPTP + exercise mice

Fig. 1



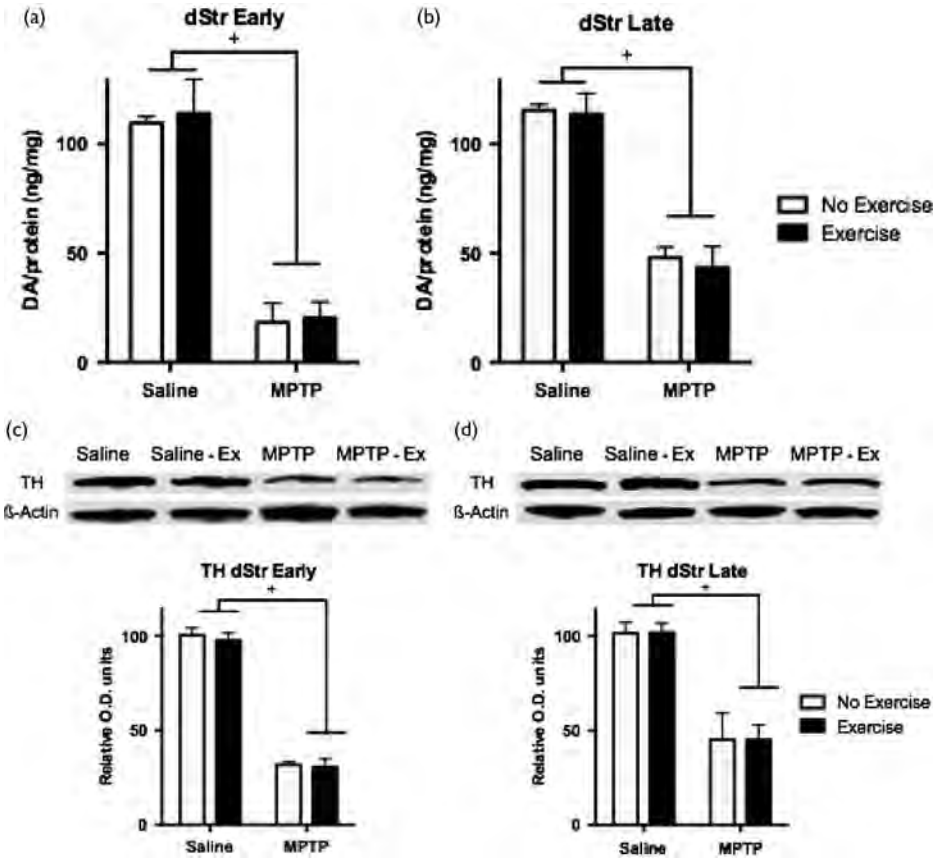
HPLC analysis of dopamine (DA) levels within the prefrontal cortex (PFC). PFC samples were analyzed at 1-week ($N=4$/group) and 6-week ($N=7$/group) time points. Data are expressed as ng of DA per mg of protein (ng/μg). Error bars represent SD. (a) 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) significantly decreased DA levels in the PFC compared with saline mice at the 1-week time point. $^{+}$MPTP effect [$F(1,12)=12.00$, $P=0.0047$]. There was also a significant interaction between MPTP and exercise, where exercise significantly reduced DA levels in saline mice, but not MPTP mice. $^{\#}$Interaction [$F(1,12)=6.361$, $P=0.0268$]. (b) There was a significant interaction between MPTP and exercise on PFC DA levels at 6 weeks, where exercise significantly decreased DA levels in saline mice, but significantly increased DA levels in MPTP mice compared with the sedentary groups. $^{\#}$Interaction [$F(1,24)=22.72$, $P<0.0001$]. In sedentary mice, MPTP significantly reduced PFC DA levels compared with saline mice. *MPTP effect in nonexercised mice ($P=0.0010$). (c, d) Western immunoblot analysis of tyrosine hydroxylase (TH) protein preparations from the PFC. PFC samples were analyzed from five to seven animals/group at 1 and 6 weeks. Data were normalized to the saline group, designated at 100 00 arbitrary optical density (OD) units. Error bars indicate SD. The upper panels are representative scans of western immunoblotting results of PFC tissue samples collected at 1 week (c) and 6 weeks (d) for TH with an antibody against β-actin in the lower bands to normalize for gel loading. MPTP significantly decreased TH levels in the PFC compared with saline mice at 1 week. $^{+}$MPTP effect [$F(1,22)=294.0$, $P<0.001$]. There is a significant interaction between MPTP and exercise on TH levels in the PFC at 6 weeks. $^{\#}$Interaction [$F(1,24)=23.12$, $P<0.001$]. This interaction was because of a significant increase in TH expression in MPTP + exercise mice compared with MPTP no-exercise mice. In nonexercised mice, MPTP significantly reduced TH levels in the PFC compared with saline. *MPTP effect in nonexercised mice ($P<0.001$). However, there was no significant difference between MPTP + exercise mice compared with saline mice ($P=0.490$) or saline + exercise mice ($P=0.267$).

(89.7 ± 3.1, $P<0.0001$) compared with saline mice (99.8 ± 1.9) (Fig. 3a). Furthermore, exercise significantly reduced PFC DA-D_1R expression by 14.5% in MPTP + exercise mice and by 10.1% in saline + exercise mice (89.8 ± 4.7) compared with their respective nonexercised groups [exercise effect: $F(1,23)=119$, $P<0.001$]. At 6 weeks, DA-D_1R levels remained significantly increased in MPTP mice and significantly reduced in MPTP + exercise mice (Fig. 3b). Specifically, in nonexercised mice, PFC D_1R levels were significantly increased by 7.3% in MPTP mice (106.8 ± 4.3) compared with saline mice (99.5 ± 4.4, $P=0.0110$). Furthermore, exercise significantly reduced PFC DA-D_1R expression by 16.34% in MPTP + exercise mice (89.4 ± 1.9) and by 11.4% in saline + exercise mice (88.2 ± 4.4) compared with the respective nonexercised groups [exercise effect: $F(1,24)=91.57$, $P<0.001$]. These data suggest that MPTP significantly increased PFC DA-D_1R expression, and exercise reversed this effect in MPTP and saline mice at both 1-week and 6-week time points. Although the absolute changes were modest, they were statistically significant.

DA-D_2 receptor

At the 1-week time point, there was a significant interaction between MPTP and exercise on PFC DA-D_2R expression [$F(1,23)=97.68$, $P=<0.001$] (Fig. 3c). This interaction was because of a significant increase in

Fig. 2



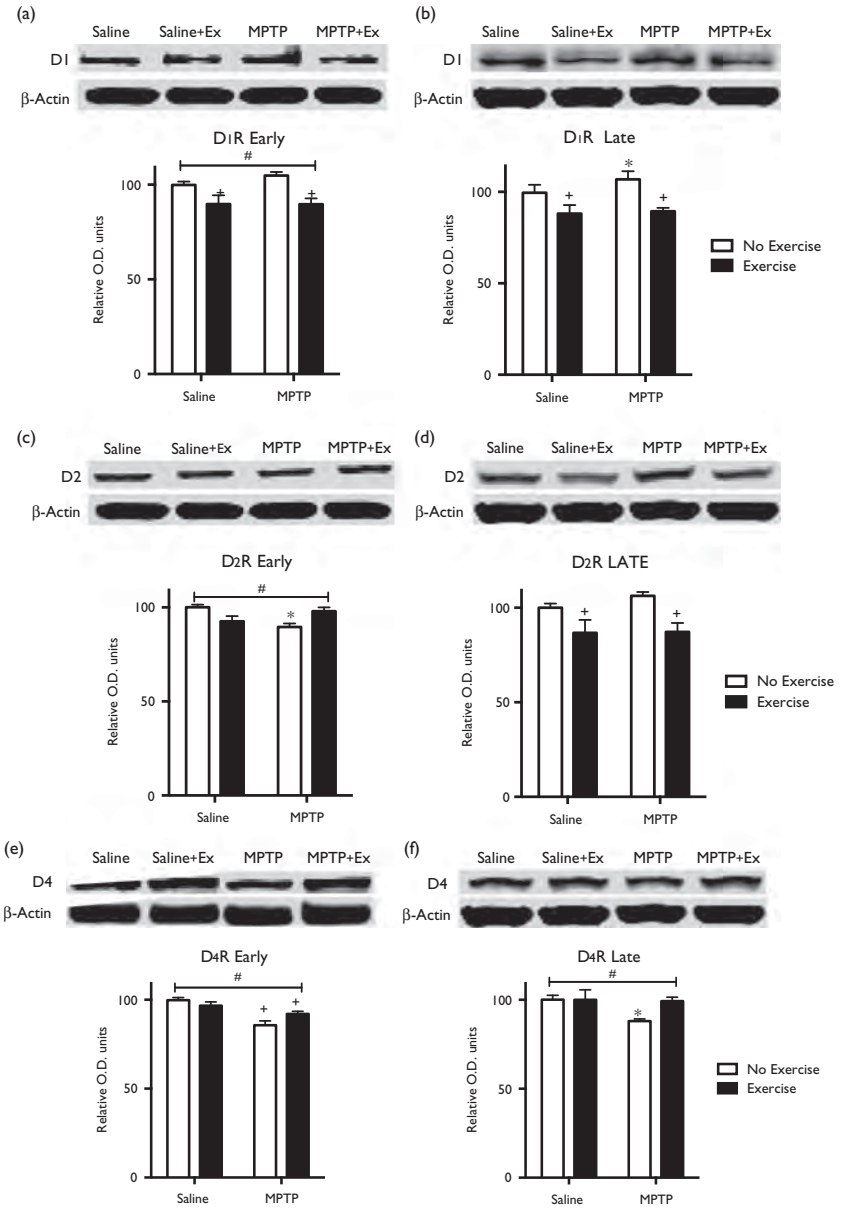
HPLC analysis of dopamine (DA) levels within the dorsal striatum (dStr). DStr samples were analyzed at 1 week ($N=4$/group) and 6 weeks ($N=7$/group). Data are expressed as ng of DA per mg of protein (ng/μg). Error bars represent SD. (a) 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) significantly decreased DA levels in the dStr compared with saline at 1 week. $^{+}$MPTP effect [$F(1,11)=300.9$, $P<0.0001$]. (b) MPTP significantly decreased DA levels in the dStr compared with saline at 6 weeks. $^{+}$MPTP effect [$F(1,24)=326.9$, $P<0.0001$]. (b, c). Western immunoblot analysis of tyrosine hydroxylase (TH) protein in the dStr. dStr samples were analyzed at 1 and 6 weeks ($N=4$ animals/group/time point). Data were normalized to the saline group, designated at 100 00 arbitrary optical density (OD) units. Error bars indicate SD. (c) The upper panel shows representative scans of western immunoblotting results collected at 1 week for TH and an antibody against β-actin in the lower bands to normalize for gel loading. MPTP significantly decreased TH levels in the dStr compared with saline mice at 1 week.*MPTP effect [$F(1,12)=1385$, $P<0.001$]. (d) The upper panel shows representative scans of western immunoblotting results collected at 6 weeks for TH and an antibody against β-actin in the lower bands to normalize for gel loading. MPTP significantly decreased TH levels in the dStr compared with saline mice at 6 weeks. $^{+}$MPTP effect [$F(1,12)=160.8$, $P<0.001$].

DA-D_2R expression by 9.4% in MPTP + exercise mice (98.0 ± 2.1) compared with MPTP mice (89.6 ± 1.9, $P<0.0001$), but a significant decrease of 7.5% in saline + exercise mice (92.6 ± 2.8) compared with saline mice (100.1 ± 1.4, $P<0.0001$). Furthermore, PFC DA-D_2R expression was significantly reduced by 10.5% in MPTP mice ($P<0.0001$), but not in MPTP + exercise mice ($P=0.9951$), compared with saline mice. At 6 weeks, there was a significant effect of exercise on PFC DA-D_2R expression [$F(1,24)=94.57$, $P<0.0001$] (Fig. 3d). DA-D_2R expression was significantly reduced by 17.9% in MPTP + exercise mice (87.3 ± 4.7) compared with MPTP mice (106.3 ± 2.0, $P<0.001$) and by 13.3% in saline + exercise mice (86.8 ± 6.8) compared with saline mice (100.0 ± 2.2, $P<0.0001$). Furthermore, there was a trend toward increased DA-D_2R expression in MPTP mice compared with saline mice, but this effect did not reach statistical significance ($P=0.0585$). These data suggest that exercise had differential effects on PFC DA-D_2R expression at 1 and 6 weeks that appeared to be inversely related to exercise effects on PFC DA levels. Specifically, exercise prevented an MPTP-induced decline in DA-D_2Rs at 1 week when PFC DA levels were significantly decreased in MPTP-lesioned mice. Conversely, exercise decreased PFC DA-D_2R levels at 6 weeks when exercise normalized PFC DA levels in MPTP-lesioned mice.

DA-D_4 receptor

At 1 week, MPTP significantly reduced PFC DA-D_4R expression compared with the saline mice [$F(1,16)=121.2$, $P<0.0001$] (Fig. 3e). Specifically, DA-D_4R expression was

Fig. 3



Western immunoblot analysis of dopamine (DA) receptors protein in the PFC. PFC samples were analyzed from five to seven animals/group at 1 and 6 weeks. Data were normalized to the saline group designated at 100 00 arbitrary optical density (OD) units. Error bars indicate SD. (a) The upper panels are representative scans of western immunoblotting results of prefrontal cortex (PFC) tissue samples collected at 1 week (left) for DA-D_1R and an antibody against β-actin in the lower bands to normalize for gel loading. At 1 week, there was a significant interaction between 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and exercise, where PFC D_1R expression was significantly increased in MPTP mice, but significantly reduced in MPTP + exercise mice compared with saline mice. $^{\#}$Interaction [$F(1,23) = 4.88$, $P = 0.0375$]. There was also a significant effect of exercise, where exercise reduced D_1R levels in the PFC in both MPTP and saline mice compared with the respective nonexercised groups. $^{+}$Exercise effect [$F(1,23) = 110$, $P < 0.001$]. (b) At 6 weeks, exercise significantly reduced D_1R expression in the PFC compared with the nonexercised groups. $^{+}$Exercise effect [$F(1,24) = 91.57$, $P < 0.001$]. In nonexercised mice, MPTP significantly increased D_1R levels in the PFC compared with saline mice. *MPTP effect in nonexercised mice ($P = 0.011$). (c) Western immunoblot analysis of DA-D_2R protein preparations from the PFC shows that there was a significant interaction between MPTP and exercise on PFC DA-D_2R expression at 1 week. $^{\#}$Interaction [$F(1,23) = 97.68$, $P < 0.001$]. This interaction was because of a significant increase in DA-D_2R expression in MPTP + exercise mice compared with MPTP mice and a significant decrease in saline + exercise mice compared with saline mice. In the nonexercised groups, MPTP significantly reduced DA-D_2R expression compared with saline mice.*MPTP effect in nonexercised mice ($P < 0.001$). (d) There was a significant effect of exercise on PFC DA-D_2R expression at 6 weeks. $^{+}$Exercise effect [$F(1,24) = 94.57$, $P \leq 0.001$]. There was no significant difference between MPTP mice and saline mice in the nonexercised groups ($P = 0.059$). (e) Western immunoblot analysis of DA-D_4R protein from the PFC showed that MPTP significantly reduced DA-D_4R expression at 1 week compared with the saline groups. $^{+}$MPTP effect [$F(1,16) = 121.2$, $P < 0.001$]. There was also a significant interaction between MPTP and exercise on DA-D_4R expression because of a significant increase in DA-D_4R expression in MPTP + exercise mice compared with MPTP mice, with no statistical difference between saline mice and saline + exercise mice. $^{\#}$Interaction [$F(1,16) = 30.41$, $P < 0.001$]. (f) There was a significant interaction between MPTP and exercise on DA-D_4R expression in the PFC at 6 weeks. $^{\#}$Interaction [$F(1,23) = 21.51$, $P < 0.001$]. This interaction was primarily because of a significant decrease in DA-D_4R expression in MPTP mice, but not in MPTP + exercise mice compared with saline mice.*MPTP effect in nonexercised mice, $P < 0.001$. Furthermore, no statistical difference was observed between saline mice and saline + exercise mice.

significantly reduced by 14.1% in MPTP mice (85.7 ± 2.4) and by 7.8% in MPTP + exercise mice (92.0 ± 1.5) compared with saline mice (99.8 ± 1.5). There was also a significant interaction between MPTP and exercise on PFC DA-D_4R expression [$F(1,16) = 30.41$, $P < 0.0001$]. This interaction was largely because of differences between the MPTP and the MPTP + exercise groups. Specifically, exercise significantly increased PFC DA-D_4R expression by 7.4% in MPTP + exercise mice compared with MPTP mice ($P = 0.004$). However, there was no statistical difference between saline + exercise mice (96.7 ± 2.1) and saline mice ($P = 0.0886$). These data indicate that exercise mitigates the MPTP-induced decrease in PFC DA-D_4Rs at 1 week. At 6 weeks, PFC DA-D_4R expression remained significantly reduced in MPTP mice, but was restored in MPTP + exercise mice (Fig. 3f). Specifically, there was a significant interaction between MPTP and exercise on PFC DA-D_4R expression [$F(1,23) = 21.51$, $P < 0.001$], where PFC DA-D_4R expression was significantly reduced by 12.1% in MPTP mice (88.0 ± 1.3, $P < 0.0001$), but not in MPTP + exercise mice (99.3 ± 2.2, $P = 0.967$) compared with saline mice (100.1 ± 2.5). Furthermore, there was no significant difference in DA-D_4R expression in saline + exercise mice (100.0 ± 5.7) compared with saline mice ($P = 0.0886$). However, there was a significant increase in D_4R expression by 12.9% in MPTP + exercise mice compared with MPTP mice ($P < 0.0001$). These data indicate that DA depletion significantly reduces and exercise normalizes PFC DA-D_4R expression in MPTP mice.

Discussion

Our previous work showed that treadmill exercise could reverse motor deficits and improve striatal DA neurotransmission in the MPTP-lesioned mouse model [9,10]. The primary aim of the current study was to investigate the impact of intensive treadmill exercise on DA neurotransmission, specifically within the PFC, focusing on DA receptor expression. We found that MPTP lesioning led to the depletion of total DA levels and TH protein expression in the PFC, which was reversed by exercise. Also, MPTP lesioning increased DA-D_1R expression and exercise decreased both DA-D_1R and DA-D_2R expression in MPTP-lesioned and saline mice. This differential exercise effect on DA receptors appears to be regionally specific depending on the anatomical sites as we previously reported that exercise increased DA-D_2R expression without changes in DA-D_1R expression in the striatum of MPTP-lesioned mice [10]. In this study, we also found that MPTP lesioning reduced DA-D_4R expression and exercise reversed this effect, reverting DA-D_4R levels to that observed in saline mice.

DA-D_1R and DA-D_2R are the most abundantly expressed DA receptors in the brain [13] and exert opposing effects on PFC function. The importance of DA in regulating PFC cognition is shown by an 'inverted-U'-shaped relationship, where high or low levels of DA outside a critical narrow concentration range result in the disruption of EF [14]. Furthermore, studies in DA-depleted animal models, including MPTP-lesioned and 6-hydroxy-dopamine (6-OHDA)-lesioned rodents and nonhuman primates, suggest that the loss of DA impairs working memory function and behavioral flexibility [15,16]. DA-D_1Rs are preferentially activated under low concentrations of extracellular DA, resulting in increased pyramidal cell excitability leading to increased corticostriatal long-term potentiation [17]. Conversely, DA-D_2Rs are preferentially activated by high concentrations of extracellular DA, leading to decreased pyramidal cell excitability and increased corticostriatal long-term depression). For example, higher long-term potentiation (and/or reduced long-term depression) expression has been observed at corticostriatal synapses after DA depletion with MPTP or 6-OHDA [11,18]. As DA-D_1Rs are preferentially activated under conditions of low DA concentrations, the exercise-induced decrease in PFC DA-D_1R, but not DA-D_2R expression at 1 week of exercise, may be part of a compensatory response to restore the physiological balance between DA receptors in MPTP mice. In contrast, at 6 weeks (when exercise restored PFC DA levels in MPTP mice), exercise significantly reduced DA-D_1R and DA-D_2R expression in MPTP mice. This exercise-induced reduction was also observed in saline mice. Thus, exercise may help restore PFC function by dynamically regulating DA-D_1R and DA-D_2R expressions in response to PFC DA levels.

The DA-D_4R is highly expressed and is the predominant DA-D_2-like receptor in the PFC in contrast to its low level of expression within the striatum [19]. Studies suggest that DA-D_4Rs couple to hyperpolarizing inwardly rectifying potassium channels, modulate kinase activity, and regulate glutamate receptor trafficking, all potential mechanisms that can influence PFC activity [20]. For example, DA-D_4R-deficient mice show cortical hyperexcitability, reduced exploration of novel stimuli, and enhanced reactivity to unconditioned, but not conditioned fear [21]. Taken together, in PD and models of DA depletion, the reduced DA-D_4R signaling in the PFC is associated with increased cortical excitability and increased extracellular glutamate likely because of the inability of DA to exert its normal inhibitory influence over these neurons [22,23]. These changes in excitability may reflect the close relationship between this receptor and NMDA and AMPA glutamate receptors within the PFC [24]. In this study, exercise restored DA-D_4R expression in MPTP mice, suggesting that exercise may help restore the inhibitory influence of DA in the PFC by restoring DA-D_4R expression.

Running on a motorized treadmill is predominantly an aerobic form of exercise with some degree of cognitive engagement as animals must be cognizant of maintaining a forward position on the treadmill as well as the fact that running duration and speed increases with progressive

sessions [9]. In the MPTP-lesioned mouse, this form of exercise may have a predominant impact on motor circuitry within the basal ganglia, which is supported by changes in DA and glutamate neurotransmission localized to the dorsolateral striatum [9–11,25]. However, skill-based exercise interventions may better recruit higher order regions in the brain required for adaptive cognitive features such as EF localized to the PFC, and ultimately more effectively show the cognitive benefits affected by different forms of exercise. For example, Wang *et al*. [26] found that skilled-exercise training results in the enhancement of PFC-mediated and cerebellum-mediated control of motor function compared with nonskilled exercise training in 6-OHDA-lesioned rats, indicating that skilled forms of training may better target the PFC and its related neural circuitry. Future studies will determine whether skill-based exercise paradigms that require higher levels of cognitive engagement during the exercise intervention, either alone or in conjunction with aerobic training (i.e. treadmill running), are more effective at improving deficits in EF observed in MPTP mice.

Conclusion

We observed modest, yet statistically significant changes in DA receptor expression in the PFC of DA-depleted mice subjected to intensive treadmill exercise. Compared with the striatum, DA levels in the PFC are modest and therefore small changes may have a major impact on cognitive behaviors [14]. Different types of exercise in patients with PD that are cognitively engaging and have significant components of motor learning may be necessary for fine-tuning the type of exercise to maximize the therapeutic benefits of exercise [27]. In other words, it may not matter what form of exercise patients with PD engage in, but rather the degree of aerobic and cognitive engagement may be critical to enhance learning and thus promote neuroplasticity and manifest improvements in motor and cognitive behaviors [27].

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Conflicts of interest

There are no conflicts of interest.

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