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MOVEMENT DISORDERS - ORIGINAL ARTICLE

Running wheel activity restores MPTP-induced functional deficits

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Abstract Wheel-running and treadmill running physical exercise have been shown to alleviate parkinsonism in both laboratory and clinical studies. MPTP was administered to C57/BL6 mice using two different procedures: (a) administration of a double-dose regime (MPTP 2 × 20 or 2 × 40 mg/kg, separated by a 24-h interval), vehicle (saline 5 ml/kg) or saline (vehicle 2 × 5 ml/kg), and (b) administration of a single-dose weekly regime (MPTP 1 × 40 mg/kg) or saline (vehicle 1 × 5 ml/kg) repeated over 4 consecutive weeks. For each procedure, two different physical exercise regimes were followed: (a) after the double-dose MPTP regime, mice were given daily 30-min periods of wheel-running exercise over 5 consecutive days/week or placed in a cage in close proximity to the running wheels for 3 weeks. (b) Mice were either given wheel-running activity on 4 consecutive days (30-min periods) or placed in a cage nearby for 14 weeks. Behavioral testing was as follows: (a) after 3 weeks of exercise/no exercise, mice were tested for spontaneous motor activity (60 min) and subthreshold L-Dopa (5 mg/kg)-induced activity. (b) Spontaneous motor activity was measured on the fifth day during each of the each of the first 5 weeks (Tests 1–5), about 1 h before injections (first 4 weeks), and continued on the 5th days of the 6th to the 14th weeks (Tests 6–14). Subthreshold L-Dopa (5 mg/kg)-induced activity was tested on the 6th, 8th, 10th, 12th and 14th weeks. (b) Mice from the single-dose MPTP weekly regime were killed during the 15th week and striatal regions taken for dopamine analysis, whereas frontal and parietal cortex and hippocampus were taken for analysis of brain-derived neurotrophic factor (BDNF). It was shown that in both experiments, i.e., the double-dose regime and single-dose weekly regime of MPTP administration, physical activity attenuated markedly the MPTP-induced akinesia/hypokinesia in both the spontaneous motor activity and restored motor activity completely in subthreshold L-Dopa tests. Running wheel activity attenuated markedly the loss of dopamine due to repeated administrations of MPTP. BDNF protein level in the parietal cortex was elevated by the MPTP insult and increased further by physical exercise. Physical running wheel exercise alleviated both the functional and biomarker expressions of MPTP-induced parkinsonism.

Keywords Exercise · Running wheel · MPTP · Motor activity · L-Dopa · Locomotion · Rearing · Motor activity · Restoration · Dopamine · BDNF · C57/BL6 mice

Introduction

Physical exercise, as defined by one recent account (cf. Morris and Schoo 2004), attenuates the neurodegenerative process in Parkinson's disease (PD): an observation that

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enjoys some degree of clinical support (Bilowit 1956; Hurwitz 1989; Palmer et al. 1986). Long-term exercise benefits brain functioning by increasing the blood and oxygen flow to the brain, mobilizing growth factors that promote neurogenesis and synaptic plasticity (Hunsberger et al. 2007), and facilitating performance, e.g., motor or cognitive, through release of neurotransmitters, such as DA, noradrenaline, serotonin and glutamate (Morishima et al. 2006; Waters et al. 2008). Basic and clinical research suggests that the "intensity" property of exercise (i.e., repetitiveness, velocity and complexity) may contribute to activity-dependent neuroplasticity and CNS alterations in response to physical activity, e.g., neurogenesis (Adkins et al. 2006) of damaged brains, including conditions such as PD with basal ganglia damage (Fisher et al. 2004; Nudo et al. 1996). In this regard, motor training was shown to facilitate self-repair following unilateral lesions of the striatum in adult rats (Döbrössy and Dunnett 2001, 2003), thereby suggesting that the adult brain is capable of significant neuronal plasticity (cf. Gomez-Pinilla et al. 2002). The possible influence of exercise on parkinsonism was demonstrated using 6-hydroxydopamine (6-OHDA) lesioning in the brain: unilateral administration of 6-hydroxydopamine (6-OHDA) in adult male rats induces an extreme motor asymmetry due to almost exclusive use of the favored ipsilateral limb with severe neglect of the contralateral limb. A plaster of paris cast, placed on the ipsilateral limb on the 7 days following lesioning, forces the animal to use the contralateral limb during the immediate post-lesioning period, and was found to abolish the motor asymmetry induced by unilateral lesion (Tillerson et al. 2001). It was shown too that both DA and DOPAC were increased markedly in the "casted" 6-OHDA-treated rats in comparison with the "non-casted" 6-OHDA-treated animals (Cohen et al. 2003). Furthermore, glial cell line-derived neurotrophic factor (GDNF), a potent survival factor for DA neurons, levels were enhanced markedly during the immediate 7-day post-lesion period when the ipsilateral limb was casted (ibid. see also Döbrössy and Dunnett 2006; Smith and Zigmond 2003).

One established method offering a mouse model of PD is the repeated (two or more times) administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to C57/BL6 mice typically. MPTP induces parkinsonism in human and nonhuman primates (Langston 1985), resulting in the loss of *substantia nigra* cells in the *pars compacta* of adult animals. It destroys selectively nigrostriatal neurons thereby inducing acute, sub-acute and long-term effects resembling certain features of PD, particularly the hypokinesic effect. Systemic administration of MPTP (2×40 mg/kg, s.c.) to C57 BL/6 mice caused L-Dopa reversible hypoactivity (Fredriksson et al. 1990; Sundström et al. 1990). A less rigorous dose treatment, e.g., 2×20, or 25 or 30 mg/kg, of MPTP has been found not to reduce motility in the C57 black mice, although DA concentrations may indicate up to 50–80% reductions (Heikkila et al. 1989; Sonsalla and Heikkila 1986). The parameters of MPTP treatment neurotoxicity are long-lasting (up to and beyond 52 weeks after treatment) with a good correlation between the functional deficits, particularly hypokinesia, the neurochemical concomitant, severe depletions of DA, and a dose- and time-dependent recovery of several parameters of motor behavior following treatment with the DA precursor, L-Dopa (Archer and Fredriksson 2003; Fredriksson and Archer 1994; Fredriksson et al. 1999; Sundström et al. 1990). Neonatal administration with iron (Fe^{2+}, at doses of 7.5 or 15 mg/kg) potentiated both the functional and neurochemical deficits induced by both a lower (2×20 mg/kg) and a higher (2×40 mg/kg) dose of MPTP (Fredriksson and Archer 2003; Fredriksson et al. 2001).

Brain-derived neurotrophic factor (BDNF), a neurotrophin with widespread expression in the brain, is linked with neurogenesis, neuronal survival and neuroreparation in the brain and CNS (Cui 2006; Huang and Reichardt 2001; Numakawa et al. 2010). Physical exercise induces improvements in motor ability and enhances BDNF expression, thereby contributing to neuronal integrity (Macias et al. 2009). Physical exercise is associated also with elevations in hippocampal levels of BDNF (Neeper et al. 1996a; Oliff et al. 1998); both a 5-day (Cotman and Berchtold 2002) and a 7-day (Johnson et al. 2003) regime of running wheel exercise was sufficient to elevate hippocampal BDNF levels. Physical activity, for instance in the form of voluntary running, has been shown to enhance neuronal plasticity by amplifying the major signaling trophic factor, BDNF, which is implicated in the diversity of molecular pathways augmenting neurogenesis (Stranahan et al. 2009) covering a wide range of health-facilitatory measures (Post 2010). BDNF mediates a variety of essential morphological changes at neuronal levels including dendritic arborization (Imamura and Greer 2009; Liu et al. 2009; Mishra et al. 2008; Zhou et al. 2008), axonal and dendritic remodeling (Yacobian and Lo 2000), synaptogenesis (Liu et al. 2009; Menna et al. 2009; Tchantchou et al. 2009) and synaptic efficacy (Boulanger and Poo 1999; Matsuda et al. 2009; Sallert et al. 2009). Physical activity, through its capacity to enhance the expression of BDNF in the brain (Seifert et al. 2010) and particularly the hippocampus, (Neeper et al. 1996a, b; Vaynman et al. 2003), notably under conditions of neuropsychiatric adversity, has provided positive outcomes in both the elderly (Laske et al. 2010) and in patients afflicted with affective disorder (Sylvia et al. 2010).

The purpose of the present study was to investigate whether or not regular physical exercise may restore the functional motor aspect of Parkinsonism, as well as dopamine concentrations and BDNF expression, following either a short period or an extended period of physical exercise or no exercise. To accomplish this, daily wheel-running exercise, which is assumed to be comparable to treadmill exercise, with two different types of MPTP administrations, a two-dose regime, separated by 24 h, and progressive, incremental dose regime consisting of four doses, one/week over 4 weeks was designed. In the former case, a low-dose (2 × 20 mg/kg) and a high-dose (2 × 40 mg/kg) regime of MPTP was administrered and a 3-week, 5 days/week schedule of wheel running over 30-min intervals was maintained. In the latter case, mice were introduced to the wheel-running activity on the first 4 days of each week, starting from the first week, then tested for spontaneous motor activity (60 min) on the fifth day of each week, and afterward administered MPTP over 4 consecutive weeks. From the 5th to the 14th week, the groups were allowed either wheel-running exercise or not and tested for spontaneous motor activity on the fifth day of each week. Subthreshold L-Dopa-induced activity was assessed following the 1-h tests of spontaneous motor activity during the 6th, 8th, 10th, 12th and 14th weeks, using the same procedures as those applied previously (Archer and Fredriksson 2003; Fredriksson and Archer 1994; Fredriksson et al. 1999, Sundström et al. 1990).

Materials and methods

Animals

Male C57 Bl/6 mice (aged 90 days and weighing 16–18 g) were purchased from B&K, Sollentuna, Sweden, and were maintained, five to a cage, in plastic cages in a room at a temperature of 22 ± 1°C and a 12/12-h constant light/dark cycle (lights on between 0600 and 1800 hours). They were placed and maintained in groups of four to six animals in a room maintained for male mice only following arrival at the laboratory for about 2 weeks to acclimatize. Free access to food and water was maintained throughout, except for the day previous to the initiation to wheel-running exercise, which occurred at the end of the second week following arrival. Wheel-running exercise and activity chamber testing occurred only during the hours of light (0800–1500 hours), performed in a normally lighted room. In each experiment, half of the mice administered MPTP or vehicle were wheel-running exercised, whereas the other half were placed in an adjoining cage in the same room in which the running wheels were placed. Motor activity was tested in a test room, in which all 12 ADEA activity test chambers, each identical to the home cage, were placed, was well secluded and used only for this purpose. Each test chamber (i.e., motor activity test cage) was placed in a soundproof wooden box with 12 cm thick walls and front panels and a small double-glass window to allow observation; each box had dim lighting.

Experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) after approval from the local ethical committee (Uppsala University and Agricultural Research Council), and by the Swedish Committee for Ethical Experiments on Laboratory Animals (license S93/92 and S77/94, Stockholm, Sweden).

Drugs

MPTP (Research Biomedical Inc., MA, USA, 2 × 20 mg/kg or 2 × 40 mg/kg, s.c., with a 24-h interval between injections in each case) was dissolved in saline and administered s.c. in a volume of 2 ml/kg body weight. Saline was used as vehicle in each case.

Behavioral measurements and apparatus

Activity test chambers

An automated device, consisting of macrolon rodent test cages (40 × 25 × 15 cm), each placed within two series of infrared beams (at two different heights, one low and one high, 2 and 8 cm, respectively, above the surface of the sawdust, 1 cm deep), was used to measure spontaneous motor activity (RAT-O-MATIC, ADEA Elektronic AB, Uppsala, Sweden). The distance between the infrared beams was as follows: the low level beams were 73 mm apart lengthwise and 58 mm apart breadthwise in relation to the test chamber; the high level beams, placed only along each long side of the test chamber, were 28 mm apart. According to the procedures described previously (Archer et al. 1986), the following parameters were measured: locomotion was measured by the low grid of infrared beams. Counts were registered only when the mouse in the horizontal plane ambulated around the test cage. Rearing was registered throughout the time when at least one high level beam was interrupted, i.e., the number of counts registered was proportional to the amount of time spent rearing. Total activity was measured by a sensor (a pick-up similar to a gramophone needle, mounted on a lever with a counterweight) with which the test cage was constantly in contact. The sensor registered all types of vibration received from the test cage, such as those produced both by locomotion and rearing as well as shaking, tremors, scratching and grooming. All three behavioral parameters were measured over three consecutive 20-min periods. The motor activity test room, in which all 12 ADEA activity test chambers, each identical to the home cage were placed, was well secluded and used only for this purpose. Each test chamber (i.e., activity cage) was placed in a soundproof wooden box with 12 cm thick walls and front panels and day lighting. Motor activity parameters were tested on one occasion only, over three consecutive 20-min periods, at the age of 3–4 months.

Running wheel units

These were small rodent running exercise wheels, purchased from a pet store and considered suitable for small rodents. The wheels were adapted and modified for use by mice and placed altogether in a large soundproof room within the animal section of the laboratory. All 25 running wheels were placed equidistant from each other with adjacent wheels in two long rows, such that the sounds of the wheels turning by any one wheel could easily be heard by the occupants of all the other wheels. A photograph of the types of running wheel used, presenting a row of the activity running wheels applied in all the experiments as well as the 'holding' cages in which the non-exercise groups remained, has been depicted previously (Archer and Fredriksson 2010). In previous neuroteratology studies that observed wheel-running exercise following different types of perinatal treatments, it was observed that each wheel had to be isolated from each of the others since the noise emitted by one animal served to evoke wheel-running behavior in the other animals. However, for the purposes of the present experiments, it was considered to be an advantage if the mice in the exercise groups stimulated each other to perform physical exercise.

Design and treatment procedures

The rationale for Experiment I was to examine whether or not running wheel exercise following the standard MPTP treatment regime (2 × 40 mg/kg, s.c., separated by 24 h), applied in all our previous studies, would alleviate the functional and neurochemical deficits. On the other hand, the rationale for Experiment II was to examine whether or not running wheel exercise following a progressive incremental, repeated dose (1 × 40 mg/kg, once per week), presented over 14 weeks to optimize recovery, would alleviate the functional and neurochemical deficits under conditions whereby double the dosage of MPTP (80 vs. 160 mg/kg) was applied. In a previous study (Archer and Fredriksson 2010), an 8-week exercise period showed significant, but limited, functional and DA recovery. In Experiment I, mice were administered either MPTP (Table 1) or vehicle, and then either given access to wheel-running activity or not during the succeeding 3 weeks. During the week after, all the mice, MPTP/vehicle and exercised/non-exercised, were tested on a 60-min spontaneous motor activity test and a 180-min L-Dopa-induced activity test.

In Experiment II, mice were administered single weekly doses of MPTP (1 × 40 mg/kg, s.c.), after a test of spontaneous motor activity, which followed 4 consecutive days of wheel-running activity (see Table 2), over 4 consecutive weeks, with a similar procedure during the fifth week except that there was no administration of MPTP after the test of motor activity. After this, all the mice were left for 2 weeks without treatment or wheel-running exercise and then tested again on the spontaneous motor test followed by the L-Dopa-induced motor activity test. After this, all the mice were then maintained under conditions of wheel-running exercise or sedentary placement in plexiglas cages over the following 9 weeks, but tested at 2-week intervals where both spontaneous motor activity (Tests 1–14) and L-Dopa-induced activity (Tests 1–5, during weeks 6, 8, 10, 12 and 14 of the experiment) were assessed (Table 2). In the following week (week 15), MPTP and vehicle mice were killed and the frontal cortex, parietal cortex, hippocampus and striatal regions dissected out for neurochemical analysis of DA and

Table 1 The experimental design and treatment of mice administered either MPTP or vehicle, with or without 3 weeks of running wheel exercise as carried out in Experiment I

Group	Condition	Design and treatment used in Experiment I			
		Treatment	Exercise wheel	Behavior	
	First week Wheel (5 days/week)	Second week	3 weeks (5 days/week)	SMA (60 min)[a]	L-Dopa (180 min)[b]
1	Cage	Saline	Cage	×	×
2	Wheel	Saline	Wheel	×	×
3	Cage	MPTP 20 mg/kg × 2	Cage	×	×
4	Wheel	MPTP 20 mg/kg × 2	Wheel	×	×
5	Cage	MPTP 40 mg/kg × 2	Cage	×	×
6	Wheel	MPTP 40 mg/kg × 2	Wheel	×	×

[a] Spontaneous motor activity test (60 min)

[b] L-Dopa (5 mg/kg) test (180 min)

Table 2 The experimental design and treatment of mice administered either MPTP or vehicle, with or without 3 weeks of running wheel exercise as carried out in Experiment II

Time and test	Day	Vehicle	MPTP	MPTP + Exercise
Week 1–4	Monday	Cage	Cage	Exer
	Tuesday	Cage	Cage	Exer
	Wednesday	Cage	Cage	Exer
	Thursday	Cage	Cage	Exer
Test 1–4[a]	Friday	Test + sal	Test + MPTP[b]	Test + MPTP[b]
Week 5–8	Monday	Cage	Cage	Exer
	Tuesday	Cage	Cage	Exer
	Wednesday	Cage	Cage	Exer
	Thursday	Cage	Cage	Exer
Test 5–8[a]	Friday	Test + sal	Test	Test
Week 9–14	Monday	Cage	Cage	Exer
	Tuesday	Cage	Cage	Exer
	Wednesday	Cage	Cage	Exer
	Thursday	Cage	Cage	Exer
Test 9–14[a]	Friday	Test + sal	Test	Test

Spontaneous motor activity tests over 60-min intervals and subthreshold L-Dopa tests are indicated

L-Dopa tests 1–5 on weeks 6, 8, 10, 12 and 14, respectively, following the spontaneous motor activity tests. L-Dopa (5 mg/kg, s.c.) after 60-min habituation to test cages

[a] Spontaneous motor activity over 60 min

[b] MPTP (40 mg/kg) injected during the first 4 weeks

BDNF. According to this design, in only one vehicle group (non-exercised was included as we have shown previously (Archer and Fredriksson 2010)) wheel-running exercise produced no behavioral alterations in the vehicle-injected animals.

Neurochemical analysis

Dopamine

Mice were killed by cervical dislocation within 1–2 weeks of completion of behavioral testing. Determination of DA was performed using a high-performance liquid chromatograph with electrochemical detection (HPLC-EC), according to (Björk et al. 1991), as modified by (Liu et al. 1995). Striatal regions were rapidly dissected out and stored at −80°C until neurochemical analysis. DA concentration was measured as follows: the frozen tissue samples were weighed and homogenized in 1 ml of 0.1 M perchloric acid, and alpha-methyl-5-hydroxytryptophan was added as an internal standard. After centrifugation (12,000 rpm, i.e., 18,600*g*, 4°C, 10 min) and filtration, 20 µl of the supernatant was injected into the HPLC-EC to assay DA. The HPLC system consisted of a PM-48 pump (Bioanalytical Systems, BAS) with a CMA/240 autoinjector (injection volume 20 µl), a precolumn (15 × 3.2 mm, RP-18 Newguard, 7 µm), a column (100 × 4.6 mm, SPHERI-5, RP-18, 5 µm) and an amperometric detector (LC-4B, BAS, equipped with an Ag/AgCl reference electrode and an MF-2000 cell) operating at a potential of +0.85 V. The mobile phase, pH 2.69, consisted of K_2HPO_4 and citric acid buffer (pH 2.5), 10% methanol, sodium octyl sulfate, 40 mg/l, and EDTA. The flow rate was 1 ml/min, and the temperature of the mobile phase was 35°C.

BDNF (ELISA)

The methods and procedures described by Viberg et al. (2008) were maintained. Frontal cortex, parietal cortex and hippocampus tissue from the mice in each group were sonicated in 20 volumes (w/v) of ice-cold lysis buffer (137 mM NaCl; 20 mM Tris–HCl, pH 8.0; 1 mM phenylmethyl-sulfonyl fluoride; 10 µg/ml aprotinin; 1 µg/ml leupeptin). The homogenate was centrifuged for 20 min at 20,000×*g* at 4°C, and the supernatant was acidified (pH < 3) with HCl and neutralized back to pH 7.6 with NaOH. The Promega Emax TM ImmunoAssay System was used to determine the amount of BDNF in the samples according to the technical bulletin supplied by the distributor. Briefly, BDNF from each sample was captured with a monoclonal antibody (mAb) against BDNF; captured BDNF was then bound to a second specific polyclonal antibody (pAb) against BDNF. After washing, the amounts of specifically bound pAb were detected by using

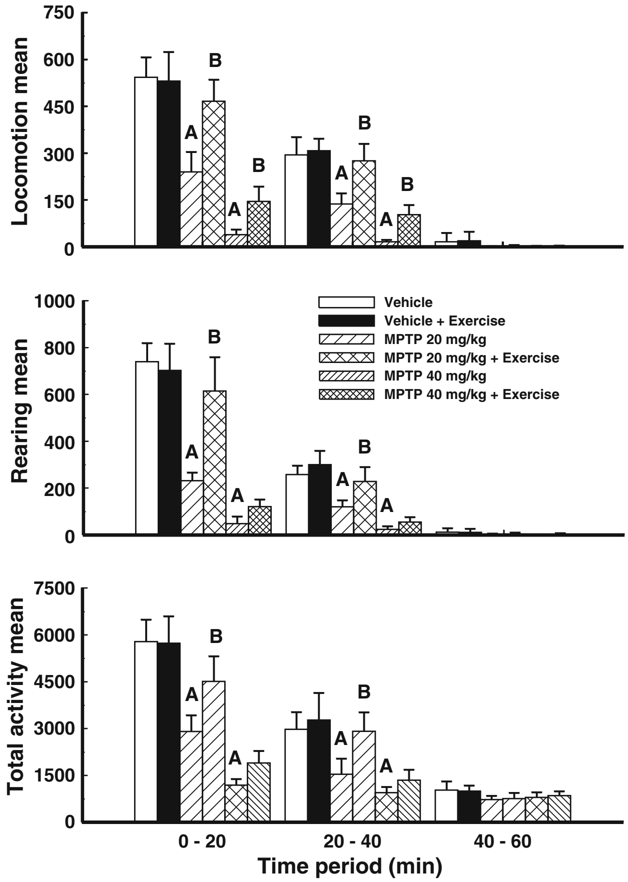

Fig. 1 Mean locomotion, rearing and total activity counts (±SD) over consecutive 20-min intervals by mice administered MPTP (either 2 × 20 or 2 × 40 mg/kg, s.c., with a 24-h interval between injections) or vehicle (2 × 2 ml/kg, s.c.) and then given access to running wheel exercise or not over 3 weeks (5 days/week) before testing for spontaneous motor activity

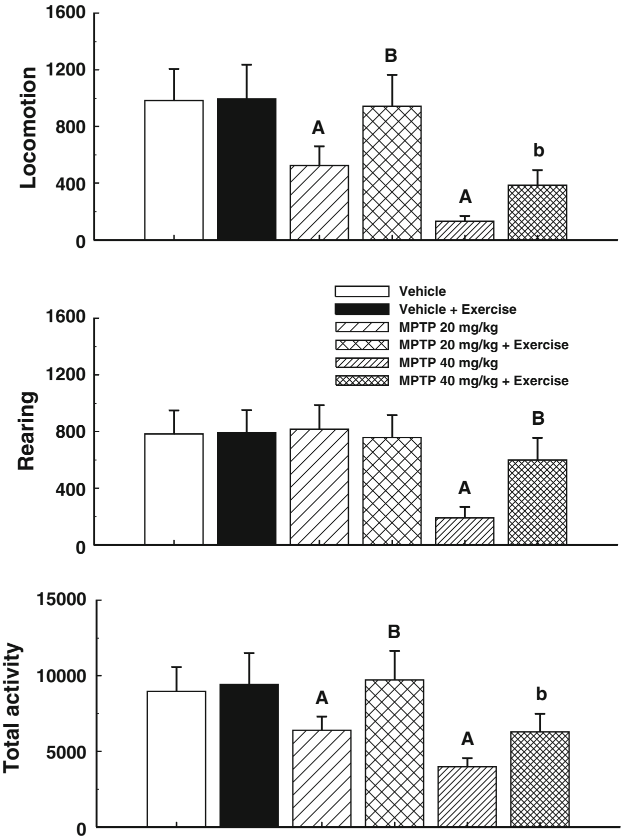

Fig. 2 Mean locomotion, rearing and total activity counts (±SD) over a single 180-min test interval following a subthreshold dose of L-Dopa (5 mg/kg, s.c.) by mice administered MPTP (either 2 × 20 or 2 × 40 mg/kg, s.c., with a 24-h interval between injections) or vehicle (2 × 2 ml/kg, s.c.) and then given access to running wheel exercise or not over 3 weeks (5 days/week) before testing for L-Dopa-induced activity

a specific anti-IgY antibody conjugated to horseradish peroxidase (HRP) as a tertiary reactant. Unbound conjugate was removed through washing and after an incubation period with a chromogenic substrate, the color change was measured in a micro-plate reader at 450 nm. The amount of BDNF was proportional to the color change generated and compared with a standard curve. The cross-reactivity to other neurotrophic factors was less than 3% and the purity of the anti-BDNF antibodies was greater than 95%.

Statistical analysis

The locomotion, rearing and total activity data over three consecutive 20-min periods in the activity test chambers from the spontaneous motor activity data were submitted to a split-plot ANOVA design (Kirk 1995). Brain (striatal) regional levels of dopamine, locomotion, rearing and total activity over the full 60-min period following administration of apomorphine each were submitted to a one-way ANOVA based on a completely randomized design (Kirk 1995). Pairwise testing between the different treatment groups was performed with the Tukey HSD test (Kirk 1995). The 1% level of significance was maintained throughout unless otherwise stated (Fig. 1).

Results

Experiment I

The low (2 × 20 mg/kg) and high (2 × 40 mg/kg) doses of MPTP each caused low and high levels of hypokinesia reflected by the locomotor, rearing and total activity counts, respectively. The regime of running wheel exercise, daily over 30-min consecutive 5-day periods, each week over 3 weeks, in each case attenuated significantly these hypokinesic effects. Thus, split-plot ANOVA indicated significant treatment × time period interaction effects for locomotion, rearing and total activity counts. Locomotion: $F(10, 108) = 55.62$, $P < 0.0001$; rearing: $F(10, 108) = 77.10$,

$P < 0.0001$; and total activity: $F(10, 108) = 35.49$, $P < 0.0001$. Figure 2 presents the mean and SD values for locomotion, rearing and total activity.

Pairwise testing using Turkey's HSD test revealed differences between the different MPTP treatment groups and the vehicle groups, as follows:

Locomotion:

First 20-min period: Veh, Veh + Exer, MPTP20 + Exer > MPTP20 > MPTP40 + Exer > MPTP40.
Second 20-min period: Veh, Veh + Exer, MPTP20 + Exer > MPTP20, MPTP40 + Exer > MPTP40.

Rearing and total activity:

First 20-min period: Veh, Veh + Exer, MPTP20 + Exer > MPTP20 > MPTP40 + Exer, MPTP40.
Second 20-min period: Veh, Veh + Exer, MPTP20 + Exer > MPTP20 > MPTP40 + Exer, MPTP40.

In vehicle-treated mice, there was a distinctive decrease in motor activity in all spontaneous behavioral variables over the consecutive 20-min periods, a normal profile of spontaneous motor behavior (cf. Archer et al. 1986). Mice administered vehicle in combination with exercise (Veh-Exer) did not differ from the vehicle group.

In the subthreshold L-Dopa-induced (5 mg/kg) motor activity test over 180 min, motor activity was restored partially by daily exercise at the 40 mg/kg dose and completely restored at the 20 mg/kg dose of MPTP (locomotion and total activity).

The subthreshold dose of L-Dopa (5 mg/kg) did not alter the hypokinesic effects of MPTP by itself. However, the 3-week exercise regime restored partially yet significantly motor activity following acute L-Dopa in the case of the 40 mg/kg dose of MPTP, and completely for the 20 mg/kg (but not for rearing wherein no functional deficit was observed). Thus, one-way ANOVA indicated significant between-groups effects for: locomotion $F(5, 54) = 43.22$, $P < 0.0001$; rearing $F(5, 54) = 25.84$, $P < 0.0001$ and total activity $F(5, 54) = 23.86$, $P < 0.0001$. Figure 3 presents the locomotion, rearing and total activity counts of the low- and high-dose MPTP groups given exercise or not.

Pairwise testing using Tukey's HSD test revealed differences between the different MPTP treatment groups and the vehicle groups, as follows:

Locomotion:

Veh, Veh + Exer, MPTP20 + Exer > MPTP20 > MPTP40 + Exer > MPTP40.

Rearing:

Veh, Veh + Exer, MPTP20 + Exer, MPTP20 > MPTP40 + Exer > MPTP40.

Total activity:

Veh, Veh + Exer, MPTP20 + Exer > MPTP20, MPTP40 + Exer > MPTP40.

Neurochemical analysis.

Experiment II

Dopamine

MPTP (2 × 40 mg/kg) induced a marked loss of striatal DA. The running wheel exercise regime alleviated this loss of DA significantly. Figure 3 presents the levels of striatal DA from vehicle, vehicle+exercise, MPTP, and MPTP+exercise groups.

A single weekly dose of MPTP (40 mg/kg) induced a progressive akinesia, which reached an asymptotic level after the second administration of the neurotoxin. The regime of wheel-running exercise applied in the experiment (30 min/day 4 days each week) blocked all expression of hypokinesia in the MPTP-treated mice until after the fifth administration of the neurotoxin. Thus, split-plot ANOVA performed on the locomotion, rearing and total activity counts indicated significant groups × test days interactions for locomotion: $F(26, 336) = 23.93$, $P < 0.0001$; rearing: $F(26, 336) = 16.85$, $P < 0.0001$; and total activity: $F(26, 336) = 15.23$, $P < 0.0001$. Figure 4 presents mean and SD values for mean locomotion, rearing and total activity counts for MPTP-treated and vehicle mice that were exercised or non-exercised over 14 weeks of testing spontaneous motor activity.

Pairwise testing using Tukey's HSD test revealed that the MPTP group showed significantly less activity than the MPTP–exercise group during Tests 1–14, which in turn showed significantly less activity than the vehicle and

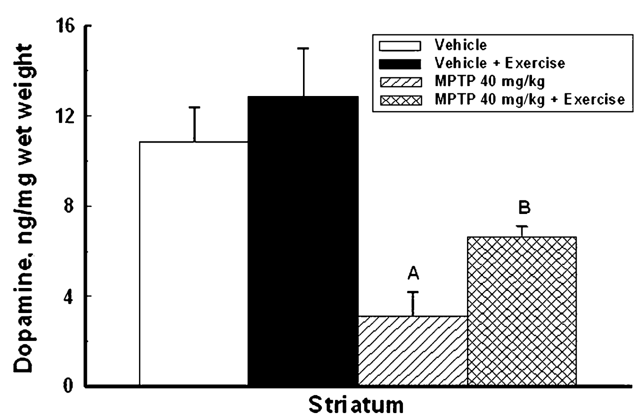

Fig. 3 Mean striatal dopamine concentrations of mice administered MPTP (2 × 40 mg/kg, s.c., with a 24-h interval between injections) or vehicle (2 × 2 ml/kg, s.c.) and then given access to running wheel exercise, or not, over 3 weeks (5 days/week) before behavioral testing

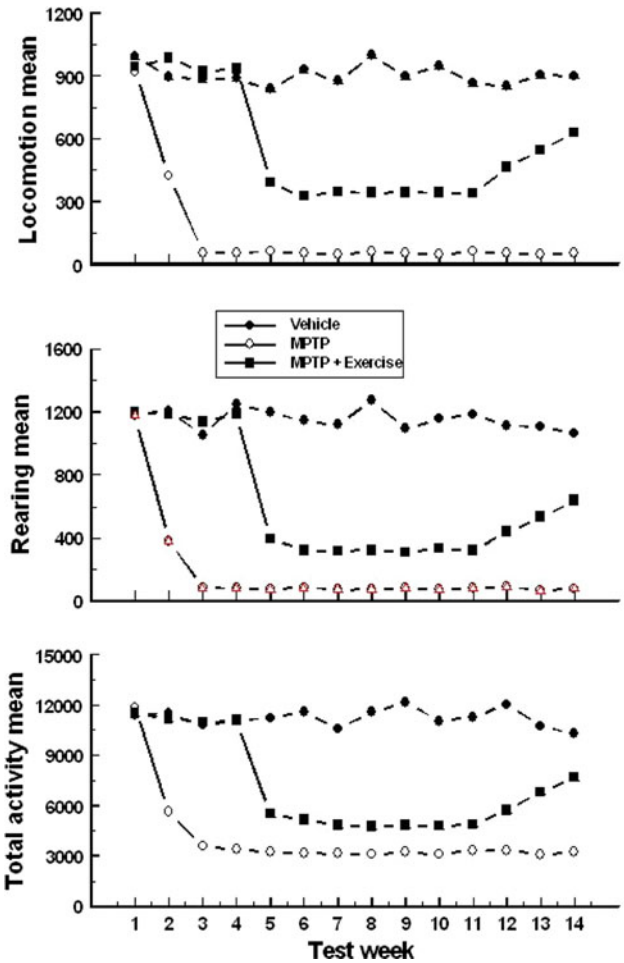

Fig. 4 Mean locomotion, rearing and total activity counts (±SD) in consecutive once weekly (Fridays) 60-min tests of spontaneous motor behavior over 14 weeks. During the first 4 weeks, MPTP (40 mg/kg, s.c.) or vehicle (2 × 2 ml/kg, s.c.) was administered, once weekly (Fridays). The two MPTP groups were either given access to running wheel exercise or not over 14 weeks

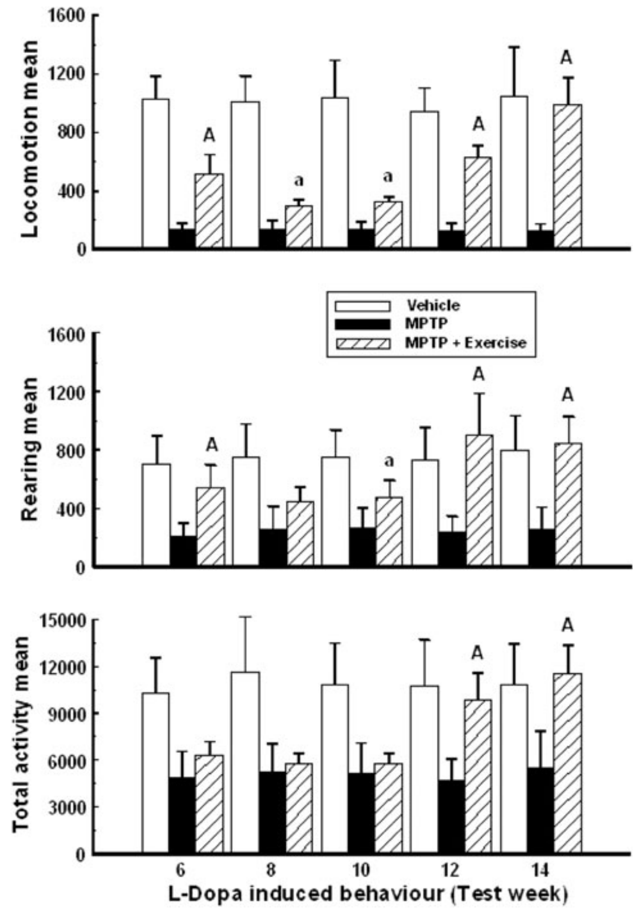

Fig. 5 Mean locomotion, rearing and total activity counts (±SD) in consecutive once weekly (Fridays) 60-min tests of subthreshold L-Dopa (5 mg/kg, s.c.)-induced motor activity during Tests 1–5 over the 6th, 8th, 10th, 12th and 14th weeks of exercise or no exercise. The two MPTP groups were either given access to running wheel exercise or not over 14 weeks

vehicle–exercise group during Tests 5–14. However, the MPTP–exercise group showed significantly more activity during Tests 12–14 than the previous seven tests.

Subthreshold administration of L-Dopa (5 mg/kg) failed to induce increases in the motor activity of MPTP-treated mice that had not received exercise, but did induce activity in the MPTP-treated mice that had received physical activity in all three tests. The levels of motor activity decreased from Test 1 (6th week) to Test 2 (8th week) and then increased successively from Test 3 (10th week) to Test 4 (12th week) to Test 5 (14th week), after which activity levels did not differ from the vehicle controls. It appears that maintenance of wheel running over 9 weeks after the final MPTP dose induced complete recovery. Thus, split-plot ANOVA performed on the locomotion, rearing and total activity counts indicated significant groups × test days interactions for locomotion: $F(8, 120) = 10.85$; rearing: $F(8, 120) = 4.80$; total activity: $F(8, 120) = 5.27$. Figure 5 presents mean and SD values for mean locomotion, rearing and total activity counts for MPTP-treated and vehicle mice, administered acute subthreshold L-Dopa, and that were exercised or non-exercised over the 6th, 8th, 10th, 12th and 14th weeks of testing.

Pairwise testing using Tukey's HSD test revealed that the MPTP group showed significantly less L-Dopa-induced activity than the MPTP–exercise group during Tests 1–5, which in turn was less active than the vehicle group during Tests 1–4 (6th, 8th, 10th and 12th weeks), but not Test 5 (14th week).

Neurochemical analysis

Dopamine

Wheel-running exercise over 14 weeks, maintained 9 weeks after the final MPTP administration, attenuated the

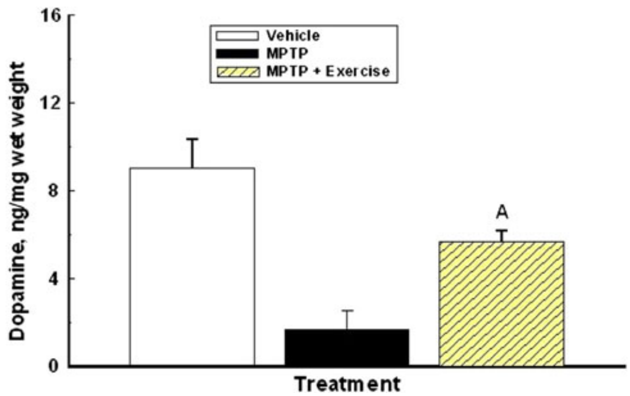

Fig. 6 Mean striatal dopamine concentrations of mice administered MPTP (4 × 40 mg/kg, s.c., with a 24-h interval between injections) or vehicle (4 × 2 ml/kg, s.c.) and concurrently given access to running wheel exercise, or not, over 14 weeks (5 days/week)

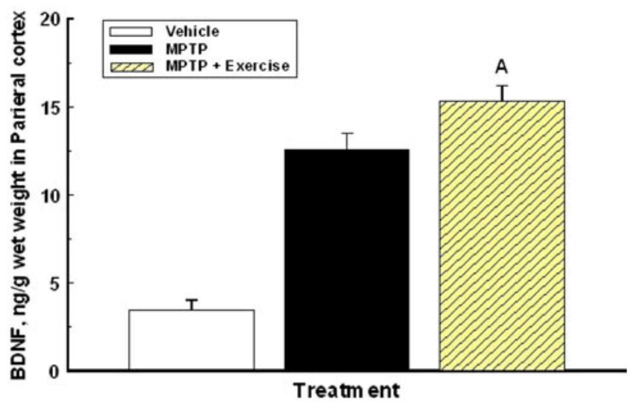

Fig. 7 Mean concentrations of BDNF in the parietal cortex of mice administered MPTP (4 × 40 mg/kg, s.c., with a 24-h interval between injections) or vehicle (4 × 2 ml/kg, s.c.) and concurrently given access to running wheel exercise, or not, over 14 weeks (5 days/week)

MPTP-induced loss of DA markedly; thus, the MPTP + Exer group showed higher dopamine levels than the MPTP–no exercise group. One-way ANOVA indicated that there was a significant between-groups effect: $F(2, 18) = 103.01$. Thus, Tukey HSD-testing indicated significantly more striatal DA in the MPTP–exercise mice (64% of vehicle control value) than in the MPTP–no exercise mice (17% of vehicle control value). Figure 6 presents the dopamine concentrations by groups of mice administered MPTP (1 × 40 mg/kg, administered once a week progressively over 4 consecutive weeks) and either given wheel-running exercise (30 min/day 4 days each week) or placed in a cage near the running wheels in Experiment II or administered vehicle (saline, 5 ml/kg) without wheel-running exercise.

BDNF

Four weekly administrations of MPTP (40 mg/kg), in the absence of any wheel running, were shown to have increased parietal BDNF concentration 9 weeks later. Wheel-running exercise over 14 weeks, maintained 9 weeks after the final MPTP administration, elevated BDNF concentrations in the MPTP–exercise group compared with the MPTP–no exercise group. One-way ANOVA indicated that there was a significant between-groups effect: $F(2, 13) = 269.84$. Thus, Tukey HSD-testing indicated significantly more parietal cortex BDNF in the MPTP–exercise mice (4.53-fold increase over vehicle control values) than in the MPTP–no exercise mice (3.57-fold increase over vehicle control value). Figure 7 presents the parietal BDNF concentrations by groups of mice administered MPTP (1 × 40 mg/kg, administered once a week progressively over 4 consecutive weeks) and either given wheel-running exercise (30 min/day 4 days each week) or placed in a cage near the running wheels in Experiment II or administered vehicle (saline, 5 ml/kg) without wheel-running exercise.

Discussion

The present study examined the propensity for physical exercise (daily wheel-running activity) to restore, although partially, the functional, severe or less severe hypokinesic deficits induced by (a) MPTP administration at a lower or a higher dose (2 × 20 or 2 × 40 mg/kg), and (b) MPTP administration at higher dose (40 mg/kg) once each week over 5 weeks. It was shown previously that there were no differences at all between vehicle-treated groups given access to either 3, 6 (unpublished data) or 8 weeks of running wheel exercise or non-exercised (Archer and Fredriksson 2010). The results may be summarized as follows:

1. The hypokinesic effects of MPTP at both the 20 and the 40 mg/kg doses upon spontaneous motor activity at 20-min intervals were restored almost completely (20 mg/kg) or partially (40 mg/kg) by daily exercise.
2. The effects of subthreshold L-Dopa upon MPTP-induced motor activity deficits were restored almost completely (20 mg/kg) or partially (40 mg/kg) by daily exercise, over the 180-min test interval.
3. For both spontaneous and subthreshold L-Dopa-induced activity, the functional deficits were more markedly severe in the 40 mg/kg dose of MPTP than in the 20 mg/kg dose, as observed previously (Archer and Fredriksson 2003, 2006, 2007; Fredriksson and Archer 2003, 2007; Fredriksson et al. 2001).

4. After 14 weeks of exercise, the complete loss of spontaneous motor activity induced in the MPTP–non-exercised group was restored substantially by the regular exercise schedule. Subthreshold L-Dopa testing showed a complete recovery by the final test.
5. In both experiments, there was a marked increase in striatal DA following the respective exercise regime, although DA restoration was seen in Experiment II.
6. In Experiment II, repeated MPTP, without exercise, induced a marked increase in parietal BDNF; this result confirms that brain injury induces increased levels of BDNF (Hughes et al. 1999; Takahashi et al. 1999). The exercise regime (MPTP–exercise group) further elevated levels of BDNF in the parietal cortex.

In Experiment I, the test of L-Dopa-induced activity showed that the functional deficit was much more severe in mice treated with the 2 × 40 mg/kg dose of MPTP; the restorative effect of exercise was partial, though significant. In this experiment, the activity deficits accruing to the 2 × 20 mg/kg dose of MPTP did not include rearing behavior (see Fig. 2, middle panel); nevertheless, locomotor and total activity following the subthreshold dose of L-Dopa were restored completely by the 3-week period of exercise with the test interval over 180-min. In Experiment II, the exercise regime again provided a partial restorative affect upon spontaneous motor activity, although the dose of MPTP was twice as high as in Experiment I, with functional restoration nearing completion; for the subthreshold L-Dopa test, after 14 weeks of exercise, the restoration was complete. These observations agree plausibly with the findings of Muhlack et al. (2007) regarding the effects of exercise on levodopa efficacy in PD patients: they found that, although levodopa plasma absorption did not differ between exercise and non-exercise conditions, the motor response was significantly improved 120 and 150 min after levodopa intake on the day with exercise than on the day with rest. The authors concluded that moderate exercise increased the clinical efficacy of levodopa in PD patients (Muhlack et al. 2007).

Applying a similar procedure to that used in Experiment II, except that access to wheel-running exercise was given only during the first 5 weeks, it was found that, although exercise delayed markedly the functional deficits in both the spontaneous motor activity and L-Dopa (5 mg/kg) tests, the motor performance of these animals deteriorated throughout in the absence of exercise (Archer and Fredriksson 2010). Dopamine analyses in that study indicated that, after 4 × 40 mg/kg MPTP accompanied by 5 weeks of exercise, the MPTP–no exercise group showed 11% of vehicle control (non-exercised) and the MPTP–exercise group 24% of vehicle control. In the present Experiment II, both spontaneous motor activity and L-Dopa activity improved with the exercise schedule until it was terminated at 14 weeks; the dopamine analyses indicate that the MPTP–no exercise group showed 17% of vehicle control (non-exercised) and the MPTP–exercise group 64% of vehicle control. Thus, the real benefits of extended exercise appear to be clear-cut.

Kurz et al. (2007) injected male C57/BL mice with ten doses of MPTP (25 mg/kg) and probenecid (250 mg/kg) over 5 weeks with control mice receiving probenecid alone. From 15 weeks after the final MPTP injection onwards, MPTP and control mice were videotaped on the sagittal plane, using a digital camera, as they ran on a motorized treadmill at a speed of 10 m/min. They found that MPTP mice showed a significantly more variable stride length and less certain gait pattern than the control mice. However, they made no attempt to compare the effects of motorized treadmill exercise and non-exercise upon subsequent measures of motor function. Petzinger et al. (2007) administered a series of four i.p. injections of MPTP, or saline, at 2-h intervals for a total of 80 mg/kg and treadmill running on an accelerating rotarod was initiated for half the MPTP and saline mice 5 days later. All the exercised mice, with MPTP and saline administration, showed increased latencies to fall off the treadmill (i.e., indicating improved balance) compared with the non-exercised mice. There was no difference in striatal DA levels between MPTP–exercised and DA–non-exercised mice. Nevertheless, examination of the striatal DA, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) of the MPTP-treated mice on Exercise day 5 (post-lesion day 10) and Exercise day 28 (post-lesion day 42) indicates a marked increase in all three variables for the exercised mice compared with the exercised mice (ibid.), suggesting some small degree of DA neuron plasticity (see also Fisher et al. 2004). In contrast, the forced non-use of limbs in unilateral 6-OHDA-lesioned rats exacerbated the denervation effects (Tillerson et al. 2002). Thus, several reviews and/or meta-analyses report that physical exercise is beneficial for physical functioning, strength, balance and gait speed, as well as quality of life in individuals with PD (Goodwin et al. 2008; Pohl et al. 2003; Toole et al. 2005). Laboratory studies using animal models confirm these notions: O'Dell et al. (2007) found that unilaterally lesioned 6-OHDA-infused rats that received exercise showed improved motor behavior outcomes relative to their sedentary lesioned controls, particularly during post-operative days 17–24. It will be noted that in the present study motor activity tests occurred between post-treatment days 20–21, and that the exercised mice performed wheel running during 15 of those days. However, there were no differences between exercised or sedentary 6-OHDA-lesioned rats with regard to loss of striatal DA transporters and tyrosine-hydroxylase-positive nigral cells (O'Dell et al. 2007).

It is possible that other mechanisms, separate from, or parallel to, DA nigrostriatal integrity may be involved, such as levels of stress at the time of testing in a novel chamber environment: e.g., prenatally stressed rats display enhanced anxiety-like behavior in the open-field test (McFadyen-Leussis et al. 2004), as do rats exposed to mild prenatal stress (Mabandla et al. 2008). Exercise was shown to reduce anxiety-like behavior that was associated with exploratory behavior in the open field (Shallert 2006). In contrast, it has been found that exercise induced anxiogenic, rather than anxiolytic, effects in the open field (Fuss et al. 2010). Nevertheless, exposure to stress has been shown to dissipate the neuroprotective effects offered through exercise. Using the unilateral 6-OHDA model of PD with acute apomorphine administration, Howells et al. (2006) found that the DA agonist, apomorphine, caused "stressed exercisers" and "non-exercisers" to rotate vigorously away from the side of the lesion (contralaterally); this behavior is used as a model of parkinsonism in the laboratory, whereas the "exercisers" rotated significantly less than "stressed exercisers" and "non-exercisers". In addition to indicating that voluntary exercise is neuroprotective, the study shows too that mild stressors cancel the neuroprotection afforded by voluntary exercise. Further, Mabandla et al. (2009), again applying the unilateral 6-OHDA lesion technique, demonstrated that voluntary exercise induced a neuroprotective effect that was expressed as improved motor control and reduced forelimb asymmetry in the exploration of a novel environment and tyrosine-hydroxylase cells in the substantia nigra pars compacta, as well as attenuating DA cell loss in the 6-OHDA-lesioned rats. Prenatal stress, on the other hand, enhanced the toxic effects of 6-OHDA. These authors argued that this dimunition of exercise-mediated neuroprotection was due to reductions in the compensatory adaptations to exercise. Certainly, two notions associated with the role of neurotrophins appear clear-cut: (a) stress reduces BDNF, and (b) exercise increases BDNF (cf. Post 2010; Soya et al. 2007; Vaynman et al. 2004). For example, Marais et al. (2009) have showed that exercise increased BDNF levels in the striatum and decreased depressive-like behavior in chronically stressed rats, whereas maternal separation stress reduced hippocampal neurotrophin levels (see also Greisbach et al. 2008; Johnson et al. 2003). Li et al. (2009) showed that there was lower BDNF expression in the CA3 and dentate gyrus regions of the hippocampus following stress in both young-stressed and aged-stressed rats, and that the aged-stress group showed lower BDNF expression compared to the young-stressed group at every testing time point (see also Marais et al. 2008). The notion that exercise-induced elevations in BDNF may be of significance for the treatment of aging disorders is not novel, since memantine, a medium-affinity uncompetitive *N*-methyl-D-aspartate receptor antagonist applied clinically as a neuroprotective agent to treat AD and PDs, increased BDNF mRNA levels markedly in the limbic cortex at clinically relevant doses (Marvanová et al. 2001). The present findings that (a) MPTP treatment induced a marked increase in parietal BDNF, and (b) exercise over 14 weeks further increased levels of BDNF in the parietal cortex, appear to lend credence for the involvement of BDNF in the exercise-induced recovery of function and DA-innervation following repeated doses of MPTP. The lack of exercise-induced changes in hippocampal BDNF suggests that the level of running wheel exercise per day and week under present conditions was insufficient. Nevertheless, it is not unreasonable to indicate that exercise regimes inducing hippocampal BDNF alterations are linked to improvements in cognitive performances. In contrast, in the present circumstances involving the MPTP-induced denervation of DA, the exercise regime, sufficient to produce marked improvement in both motor function and striatal DA concentration, induced significant increases in parietal cortex DA. It appears that parietal cortex BDNF may exert an important mediatory role, hitherto unobserved, upon functional and biomarker recovery in experimental parkinsonism. The manifest benefits of physical exercise on neurodegenerative states are dependent on a variety of parameters that determine prognosis, intervention and outcome, not least pertaining to the particular disorder under consideration (Archer 2010; Archer et al. 2010a, b).

Certain aspects of exercise ought to be considered: Fox et al. (2006) have presented five key principles of exercise that enhance neuroplasticity in association with PD, namely: (a) intense activity maximizes synaptic plasticity; (b) complex activities promote greater structural adaptation; (c) 'rewarding' activities increase DA levels thereby promoting learning/relearning (e.g., motor); (d) dopaminergic neurons are highly responsive to exercise, on the one hand, and inactivity, on the other ("use it or lose it"); (e) early introduction of exercise retards disease progression. In conclusion, the present experiments offer further demonstrations of the extent to which regular 30-min periods of exercise suffice to attenuate the effects of DA-denervation on motor activity deficits. Currently, it would appear that these results may confirm (f) the responsiveness of DA neurons to exercise; (g) the necessity of introducing the exercise regime as early as possible; and finally (h) that neuroreparative processes, provoked by insult, are further mobilized through physical activity. It is pertinent that the MPTP insult produced a remarkable increase in parietal cortex (measured at least 10 weeks after the final MPTP administration), which suggests a noteworthy self-recovery attempt, albeit inadequate. Physical exercise induced a further increase in parietal BDNF; this increment implies, tentatively, a mediatory role of the neurotrophin in functional motor and striatal DA recovery. Further studies will determine the optimal conditions of physical exercise necessary to ensure more effective BDNF mobilization and complete restoration.

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