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# 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

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**Studies have suggested that there are beneficial effects of exercise in patients with Parkinson's disease, but the underlying molecular mechanisms responsible for these effects are poorly understood. Studies in rodent models provide a means to examine the effects of exercise on dopaminergic neurotransmission. Using intensive treadmill exercise, we determined changes in striatal dopamine in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned mouse. C57BL/6J mice were divided into four groups: (1) saline, (2) saline plus exercise, (3) MPTP, and (4) MPTP plus exercise. Exercise was started 5 d after MPTP lesioning and continued for 28 d. Treadmill running improved motor velocity in both exercise groups. All exercised animals also showed increased latency to fall (improved balance) using the accelerating rotarod compared with nonexercised mice. Using HPLC, we found no difference in striatal dopamine tissue levels between MPTP plus exercise compared with MPTP mice. There was an increase detected in saline plus exercise mice. Analyses using fast-scan cyclic voltammetry showed increased stimulus-evoked release and a decrease in decay of dopamine in the dorsal striatum of MPTP plus exercise mice only. Immunohistochemical staining analysis of striatal tyrosine hydroxylase and dopamine transporter proteins showed decreased expression in MPTP plus exercise mice compared with MPTP mice. There were no differences in mRNA transcript expression in midbrain dopaminergic neurons between these two groups. However, there was diminished transcript expression in saline plus exercise compared with saline mice. Our findings suggest that the benefits of treadmill exercise on motor performance may be accompanied by changes in dopaminergic neurotransmission that are different in the injured (MPTP-lesioned) compared with the noninjured (saline) nigrostriatal system.**

*Key words: in situ* **hybridization; neurochemistry; neuroplasticity; substantia nigra; Parkinson's disease; tyrosine hydroxylase**

## Introduction

Recent studies from several laboratories including ours have shown that exercise can have a beneficial effect in patients with Parkinson's disease (PD) and in rodent models of PD (Comella et al., 1994; Tillerson et al., 2001, 2003; Bezard et al., 2003; Fisher et al., 2004; Chen et al., 2005; Faherty et al., 2005). In both the 6-hydroxydopamine (6-OHDA) lesioned rat and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) lesioned mouse, exercise initiated either before or during neurotoxicant exposure has been shown to be neuroprotective, as demonstrated by the attenuation of striatal dopamine loss. Our previous studies have shown that high-intensity treadmill exercise, initiated 5 d after administration of MPTP, a period when neurotoxicant-induced cell death is completed, can also lead to improved motor performance in the MPTP lesioned mouse (Jackson-Lewis et al., 1995; Fisher et al., 2004). In addition, we found downregulation of the dopamine transporter (DAT), a protein important in regulating the uptake of dopamine, and upregulation of dopamine receptor D2, a receptor whose activation is important in eliciting motor behavior. Given the critical role of dopamine in motor learning and execution, and our interest in neuroplasticity of the injured basal ganglia, in this study we have examined the effects of exercise on other potential compensatory changes of the striatal dopaminergic system including total striatal dopamine levels and release. We further examined whether exercise-induced changes in proteins important in dopamine biosynthesis (tyrosine hydroxylase, TH) and uptake (DAT) may be accompanied by changes in their respective mRNA transcript expression within midbrain dopaminergic neurons and whether exercise leads to changes in the number of substantia nigra neurons.

For these studies, we used four groups of mice including (1) saline, (2) saline plus exercise, (3) MPTP, and (4) MPTP plus exercise. Intensive treadmill running was initiated 5 d after MPTP

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lesioning and continued for 28 d of running (5 d/week). Brain tissue was harvested from all four groups at the completion of exercise. Analysis included (1) measurement of striatal dopamine and its metabolites by HLPC and (2) striatal dopamine release using fast-scan cyclic voltammetry in slice cultures. Unbiased stereological counting of nigrostriatal dopaminergic neurons was used to determine any differences in cell numbers between groups. Our studies indicate that exercise may have a differential effect on the dopaminergic system in MPTP- versus saline-treated mice. In the MPTP-lesioned mice, exercise had an effect on dopamine release, which was region specific, but not in total striatal dopamine levels. In saline-treated mice, exercise increased total striatal dopamine levels without a significant effect on dopamine release. In addition, in our study, we determined that MPTP administration led to 50% cell loss, with no difference in substantia nigra cell numbers between MPTP and MPTP plus exercise mice. Downregulation of DAT and TH transcript expression was detected in saline plus exercise-treated mice only.

## Materials and Methods

*Animals.* Mice used for these studies were young adult (8–10 weeks old) male C57BL/6J mice supplied from The Jackson Laboratory (Bar Harbor, ME). There were four treatment groups including the following: (1) saline, (2) saline plus exercise, (3) MPTP, and (4) MPTP plus exercise. Animals were housed five to a cage and acclimated to a 12 h shift in light/dark cycle so that the exercise occurred during the animals normal wake period. All experiments were performed in accordance with the National Institutes of Health (NIH) *Guide for the Care and Use of Laboratory Animals* (NIH publication 80-23, revised 1996) and approved by the University of Southern California Institutional Animal Care and Use Committee.

*MPTP lesioning.* MPTP (Sigma, St. Louis, MO) 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% loss of nigrostriatal neurons, as determined by unbiased stereological techniques for both TH staining and Nissl substance and an 80–90% depletion of striatal dopamine levels (Jackson-Lewis et al., 1995; Jakowec et al., 2004). Nigrostriatal cell loss is complete by day 3 after MPTP administration as determined by counting remaining nigrostriatal TH immunoreactive cells and reduced silver staining for degenerating neurons (Jackson-Lewis et al., 1995; Jakowec et al., 2004).

*Treadmill exercise.* One week before the start of the treadmill exercise paradigm, sixty mice that could maintain a forward position on the 45 cm treadmill belt for 5 min at 5.0 m/min were randomly assigned to the four groups to ensure that all animals performed similarly on the treadmill task before MPTP lesioning. The treadmill used in these studies was a model EXER-6M treadmill manufactured by Columbus Instruments (Columbus, OH). A non-noxious stimulus (metal-beaded curtain) was used as a tactile incentive to prevent animals from drifting back on the treadmill. As a result, shock-plate incentive was not used and stress related to the activity was minimized. Exercise was initiated 5 d after saline or MPTP lesioning when cell death is complete. Mice from each of the two exercise groups (saline plus exercise and MPTP plus exercise) were run at the same time in the six-lane treadmill. Exercise duration was incrementally increased starting with 30 min on day 1 to reach the goal duration of two sessions of 30 min each (for a total of 60 min), 5 d/week (with a 5 min warm-up period), for a total of 28 d of exercise (corresponding to a final of 42 d after MPTP lesioning). Treadmill speed and exercise duration for each group was increased when all mice within each group maintained a forward position on the treadmill belt for 75% of the running period. To control for any nonexercise effects of treadmill running (handling, novel environment, noise, and vibration), nonexercised groups were placed on the top of the treadmill apparatus for a time period equivalent to exercise training (Fukai et al., 2000; Kojda et al., 2001).

*Motor behavior analysis with the rotarod.* An accelerating rotarod (five-lane accelerating rotarod; Ugo Basile, Comerio, Italy) was used to measure motor balance and coordination during the treadmill exercise period. The rotarod consisted of a rotating spindle (diameter, 3 cm) where mice were challenged for both speed and alternating rotational direction. Velocity of the rod was set to 30 rpm with changes in direction (forward-reverse) every 24 s. Each mouse was placed in a separate compartment on the rotating rod and the latency to fall was automatically recorded by magnetic trip plates. All groups of mice were tested once per week. The first week was recorded 3 d after starting the treadmill exercise. The test consisted of five consecutive trials each separated by 1 min, with a maximum cutoff latency of 200 s. Data were subsequently collected for each mouse once per week until completion of the exercise paradigm (corresponding to week 6).

*Tissue collection.* Brain tissue from all groups of mice was collected on the last day of exercise (day 28 of exercise corresponding to 42 d after MPTP lesioning). Striatal brain tissue was also collected from a subset of animals from each experimental group at 5 d of exercise (10 d postlesioning) to determine the degree of dopamine depletion at an earlier exercise and MPTP time point. Mice were killed by cervical dislocation for fresh tissues (for HPLC, fast-scan cyclic voltammetry, and *in situ* hybridization histochemistry) or by pentobarbital followed by transcardial perfusion with fixative (for immunohistochemistry and unbiased stereological counting). Striatal tissues for HPLC analysis were collected fresh en bloc corresponding to anatomical regions from bregma 1.20 to bregma 0.60, with borders dorsal to the anterior commissure, ventral to the corpus callosum, medial to the lateral ventricle, and 2.5 mm lateral from midline, and frozen until analysis. *In situ* hybridization histochemistry, fast-scan voltammetry, and immunohistochemistry were performed on coronal sections corresponding to bregma 1.30 to bregma 0.00. In addition, to evaluate the initial degree of MPTP-mediated striatal dopamine depletion, brain tissue for HPLC analysis was collected at 10 d post-MPTP lesioning from a subset of nonexercise mice from both the saline and MPTP groups.

*HPLC analysis of dopamine and its metabolites.* Neurotransmitter concentrations were determined according to an adaptation of Irwin et al. (1992) from the method of Kilpatrick et al. (1986). Tissues for analysis were homogenized in 0.4 N perchloric acid and centrifuged at 12,000 × *g* to separate precipitated protein. The protein pellet was resuspended in 0.5 N NaOH and the total protein concentration determined using the Coomassie Plus protein assay system (Pierce, Rockford, IL) using a Biotek Model Elx800 microplate reader (Biotek Instruments Wincoski, VT) and KCjunior software. The concentrations of dopamine, 3,4-dihydroxyphenylacetic (DOPAC), and homovanillic acid (HVA) were assayed by HPLC with electrochemical detection. Samples were injected with an ESA (Chelmsford, MA) autosampler. Dopamine and its metabolites were separated by a 150 × 3.2 mm reverse phase 3-$\mu$m-diameter C-18 column (ESA) regulated at 28°C. The mobile phase MD-TM (ESA) consisted of acetylnitrile in phosphate buffer and an ion-pairing agent delivered at a rate of 0.6 ml/min. The electrochemical detector was an ESA model Coularray 5600A with a four-channel analytical cell with three set potentials at −100, 50, and 220 mV. The HPLC was integrated with a Dell GX-280 computer with analytical programs including ESA Coularray for Windows software and the statistics package InStat (GraphPad Software, San Diego, CA).

*Measurement of dopamine release by fast-scan cyclic voltammetry in brain slices.* Fast-scan cyclic voltammetry was used for the analysis of dopamine release from coronal, *in vitro* brain slices of the striatum (Patel and Rice, 2006). Brains were removed and placed in cooled (1–4°C), modified, and oxygenated artificial CSF (aCSF) containing the following (in mM): 124 NaCl, 1.3 $MgSO_4$, 3.0 KCl, 1.25 $NaH_2PO_4$, 26 $NaHCO_3$, 2.4 $CaCl_2$, 10.0 glucose, equilibrated with a 95% $O_2$/5% $CO_2$ mixture to obtain a pH value of 7.3–7.4. In the modified aCSF, some sodium was replaced with sucrose to reduce tissue excitability during brain slice cutting (sucrose 124 mM, NaCl 62 mM) to maintain the osmotic balance of normal aCSF. Hemicoronal striatal slices were cut from the rostral end of the tissue at a thickness of 400 $\mu$m with a Vibratome 1000 (Vibratome, St. Louis, MO). Slices were immediately placed in oxygenated aCSF and were slowly brought to room temperature (23°C). Slices remained in solution for 2 h before and throughout all recording sessions. Single slices were transferred to the recording chamber (Haas ramp style gas interface

chamber) and bathed continuously with the oxygenated aCSF solution maintained at a temperature of 32°C. Disc carbon fiber microelectrodes (CFMEs) were made from 7 mm unsized carbon fiber (Goodfellow Corporation, Devon, PA) by electrophoretic anodic deposition of paint (Schulte and Chow, 1996). Extracellular dopamine was monitored at the CFME every 100 ms by applying a triangular waveform (−0.4 to +1.0 V vs Ag/AgCl, 300 V/s). Currents were recorded with a modified VA-10× Voltammetric and Amperometric Amplifier (NPI Electronic, Tamm, Germany). Data acquisition was controlled by Clampex 7.0 software (Molecular Devices, Menlo Park, CA). Electrical stimulation was used to elicit dopamine efflux with a twisted, bipolar, nichrome electrode placed on the surface of the slice. Single pulses (0.1 ms, 200 $\mu$A) were generated with a Master-8 pulse generator (AMPI, Jerusalem, Israel). Constant current of 200 $\mu$A and 0.1 ms duration were obtained by using an A360R Constant Current Stimulus Isolator (World Precision Instruments, Sarasota, FL). Stimulus intervals between pulses were not <5 min. The CFMEs were inserted 75–100 $\mu$m into the slice at a position 100–200 $\mu$m from the stimulating electrode pair (Miles et al., 2002). Each slice was sampled for dopamine at five sites, which represented medial to lateral and dorsal to ventral dimensions. Three rostral slices were examined in each mouse and the values were averaged for each animal. Changes in extracellular dopamine were determined by monitoring the current over a 200 mV window at the peak oxidation potential for dopamine. Background-subtracted cyclic voltammograms were created by subtracting the current obtained before stimulation from the current obtained in the presence of dopamine. To convert oxidation current to dopamine concentration, electrodes were calibrated with dopamine standard solutions after experimental use. The mechanism of evoked dopamine release in our voltammetry experiments was tested in a set of brain slices by stimulating striatal tissue using the following sequence of solutions changes: control aCSF containing 2.4 mM $Ca^{2+}$ and 1.3 mM $Mg^{2+}$ followed by a reduced $Ca^{2+}$ aCSF solution containing 0.5 mM $Ca^{2+}$ and 3.2 mM $Mg^{2+}$, followed by washout with the control aCSF solution and then ending in a control aCSF solution containing 1 $\mu$M tetrototoxin (TTX).

The kinetics of the dopamine signal evoked by intrastriatal stimulation was studied by monitoring the cyclic voltammetry signal for 1 s before and 5 s after intrastriatal stimulation at a sampling rate of once every 100 ms (10 Hz). The decay of the dopamine signal was determined by normalizing postpeak dopamine measurements to the peak dopamine measured. The decay constant was then determined from a single exponential fit of the decay in dopamine signal according to the following equation: $y = Ae^{-kt}$, where $A$ is the peak dopamine signal at time 0 and the constant $-k$ is the decay rate for exponential decay of the dopamine signal. ANOVAs were performed between all groups for the decay rate constant ($-k$) (Mosharov and Sulzer, 2005).

*Immunohistochemical staining.* The relative expression of striatal TH immunoreactivity (TH-ir) and DAT immunoreactivity were determined in tissues sections using commercially available primary antibodies, including rabbit polyclonal anti-TH and mouse monoclonal anti-DAT (Millipore, Bedford, MA) using a validated approached to compare relative patterns of expression as described by Burke et al. (1990). Primary antibody binding was visualized using fluorescently labeled secondary antibodies with AlexaFluor 680 or AlexaFluor 800 (Licor, Lincoln, NE). To ensure that differences in staining intensity were because of differences in antigen expression, multiple sections from each of the different treatment groups were handled in identical staining conditions concurrently. Control experiments excluding either primary antibody or secondary antibody were also performed to verify staining specificity. For image analysis, three or four animals per treatment group and 10–12 sections per animal were captured at low magnification and digitized. The relative optical density (expressed as arbitrary units within the linear range of detection) of the dorsal lateral striatum was determined by subtracting the relative optical density of the corpus callosum as background.

*Unbiased stereological counting of dopaminergic neurons.* The number of nigrostriatal dopaminergic neurons in the substantia nigra pars compacta (SNpc) was determined using unbiased stereology with the computer-imaging program BioQuant Nova Prime (BioQuant Imaging, Nashville, TN) and an Olympus BX-50 microscope (Olympus Optical, Tokyo, Japan) equipped with a motorized stage and digital Retiga-cooled CCD camera (Q-Imaging, Burnaby, British Columbia, Canada). Brain tissue was prepared from three mice in each group. Tissue was sliced at 30 $\mu$m thickness and every sixth section collected and stained for TH-ir using a rabbit polyclonal antibody (Millipore) and counterstained for Nissl substance (Jakowec et al., 2004; Petzinger et al., 2005). The SNpc was delineated from the rest of the brain based on TH-ir. Section collection started rostral to the substantia nigra at bregma −2.50 mm before the closure of the third ventricle through to the prominence of the pontine nuclei at bregma −4.24 mm according to the stereotaxic atlas of the mouse brain (Paxinos and Franklin, 2001). Each stained ventral mesencephalon section was viewed at low magnification (10× objective) and the SNpc outlined and delineated from the ventral tegmental-immunoreactive neurons using the third nerve and cerebral peduncle as landmarks. Neurons were viewed at high magnification (80× objective) and counted if they displayed TH-ir and had a clearly defined nucleus, cytoplasm, and nucleolus. The total number of SNpc dopaminergic neurons was determined based on the method of Gundersen and Jensen (1987).

In situ *hybridization histochemistry.* Brains for *in situ* hybridization were quickly removed and frozen in isopentane on dry ice, and tissues were processed as described previously (Jakowec et al., 1995, 2004). Selected slides were dipped in NTB-2 (Kodak, Rochester, NY) photographic emulsion, developed in D-19 developer, and counter stained with cresyl violet. To minimize potential sources of variation between different experiments, slides that were to be compared were processed in the same experiment using identical hybridization buffers, probe concentration, probe preparation, wash regimen, and emulsion exposure. Images of midbrain cells were captured using an Olympus BX-51 microscope and the computerized image analysis program BioQuant (BioQuant Imaging). Emulsion grains were counted above individual neurons within the SNpc if they displayed a stained cytoplasm and evident nucleus.

*Statistical analysis of data.* Statistical analysis was performed using SPSS version 14.0 for Windows (SPSS, Chicago, IL) or InStat software (GraphPad Software). Differences in behavioral tests between groups were analyzed using repeated-measures ANOVA with the between-subjects factors being lesion (saline or MPTP) and intervention (exercise or no exercise) and the within subject factor being time. For HPLC analysis, immunocytochemistry staining, and grain counting for *in situ* hybridization histochemistry, a two-way ANOVA was performed to compare the different groups and examine for significant interactions. *Post hoc* contrasts with Bonferroni correction were performed to determine the locus of any significant differences. The data from the studies of dopamine release using fast-scan cyclic voltammetry were analyzed using repeated-measures ANOVA with the between-subjects factors being lesion (saline or MPTP) and intervention (exercise or no exercise) and the within-subjects factor being electrode location (positions 1–5). *Post hoc* testing was performed in those cases where warranted. Within-subject effects were performed using the Huynh-Feldt correction for sphericity. For all analyses, a significance level of $p < 0.05$ was used.

## Results

The time course of improvement in running velocity of both the saline plus exercise and MPTP plus exercise groups over the 28 d of treadmill running is shown in Figure 1. Saline plus exercise mice at day 1 started at 13.3 m/min and the MPTP plus exercise mice at day 1 started at 7.6 m/min. In the first week of treadmill running, the saline group increased velocity to 14 ± 1.4 m/min that further increased to 22.6 ± 0.3 m/min by the final week. The MPTP plus exercise group had a running velocity that increased to 9.2 ± 1.1 m/min during the first week that further increased to 20.5 ± 0.7 m/min in the last week. As shown in our previous study, *post hoc* analysis demonstrated a significant difference in velocity at day 1 between the saline plus exercise and MPTP plus exercise groups and this difference was not significant at the completion of the treadmill running regimen (Fisher et al., 2004). The

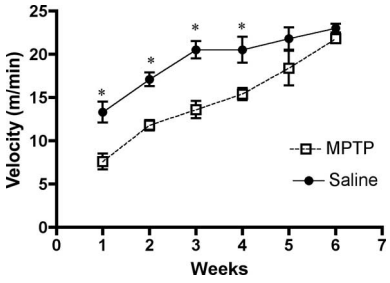


**Figure 1.** Analysis of motor behavior on motorized treadmill. Both saline and MPTP-lesioned mice were run on the motorized treadmill for 28 d (5 d per week). The running velocity of the mice in each group ($n = 12$) were determined three times per week and compared. The graph demonstrates that (1) MPTP-lesioned mice had a running velocity less than the saline group, where the asterisk indicates significant difference ( $p < 0.05$), and (2) both saline- and MPTP-lesioned groups improved in running velocity, and by the final 2 weeks, the difference between the two groups was not significant ( *post hoc* analysis; $p < 0.05$; *t* test). Error bars indicate SEM.

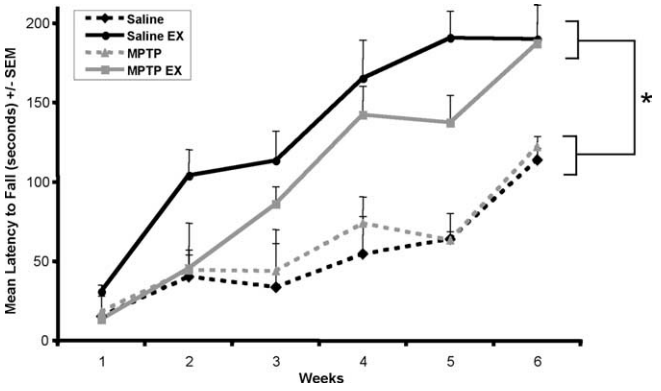


**Figure 2.** Analysis of behavior on rotarod mice from all groups were tested once a week for latency to fall from an accelerating rotarod (mean and SEM in seconds per week). There was a significant effect of exercise (treadmill running) on the mean latency to fall (in seconds) from the accelerating rotarod, where the asterisk represents significant differnce between the exercise and no exercise groups ($F_{(3,44)} = 9.587$; $p < 0.0001$). Both MPTP plus exercise and saline plus exercise mice performed better on the rotarod compared with the nonexercised groups. There was no significant effect of MPTP on mean latency to fall ($F_{(3,44)} = 0.851$; $p = 0.504$) and no significant interaction between exercise and MPTP on mean latency to fall ($F_{(3,44)} = 0.965$; $p = 0.435$).

running velocity of the MPTP nonexercise mice at the final day of treadmill exercise was similar to the velocity of the MPTP plus exercise group at day 1 of the exercise regimen (data not shown) (Fisher et al., 2004).

As a second measure of motor performance, mice from all four groups were tested for their latency to fall from the accelerating rotarod (Fig. 2). Because of the high degree of challenge of this task, mice from all groups initially performed poorly. We observed overtime, however, that there was a significant effect of exercise (treadmill running) on the mean latency to fall (in seconds) from the accelerating rotarod ($F_{(3,44)} = 9.587$; $p < 0.0001$). Both MPTP plus exercise and saline plus exercise mice performed better on the rotarod compared with the nonexercised groups. There was no significant effect of MPTP on mean latency to fall ($F_{(3,44)} = 0.851$; $p = 0.504$) and no significant interaction between exercise and MPTP on mean latency to fall ($F_{(3,44)} = 0.965$; $p = 0.435$).

HPLC analysis was used to determine levels of striatal dopamine, its metabolites HVA and DOPAC, and the metabolites turnover ratio, defined as follows: [(DOPAC + HVA)/dopamine]. These data are shown in Table 1 and Figure 3. First, in the 5 d exercise group, there was a significant effect of MPTP on dopamine levels ($F_{(3,16)} = 39.52$; $p < 0.0001$) and dopamine turnover ($F_{(3,16)} = 88.30$; $p < 0.0001$). MPTP induced dopamine depletion in the MPTP plus no exercise group (48.0 ± 8.4 ng dopamine/mg protein) compared with saline plus no exercise group (269.5 ± 24.9 ng dopamine/ng protein), which corresponded to 82% depletion. The MPTP induced dopamine depletion in the MPTP plus exercise group (4.9 ± 0.2 ng dopamine/mg protein) compared with saline plus exercise group (285.1 ± 30.3 ng dopamine/ng protein), which corresponded to 98% depletion. There were no significant differences in striatal dopamine levels between exercise and no exercise animals in either the MPTP- or saline-treated animals. However, there was a significant decrease in turnover ratio in the MPTP plus exercise compared with the MPTP plus no exercise groups only ($F_{(3,16)} = 8.30$; $p < 0.02$)

HPLC analysis of striatal dopamine at the completion of the 28 d of treadmill running showed that there was a significant effect of MPTP on the degree of dopamine depletion ($F_{(3,16)} = 229.3$; $p < 0.0001$). MPTP induced dopamine depletion in the MPTP plus no exercise group (77.9 ± 12.0 ng dopamine/mg protein) compared with saline plus no exercise group (246.9 ± 19.8 ng dopamine/ng protein), which corresponded to 68% depletion. The MPTP induced dopamine depletion in the MPTP plus exercise group (69.8 ± 11.7 ng dopamine/mg protein) compared with saline plus exercise group (315.2 ± 9.0 ng dopamine/ng protein), which corresponded to 78% depletion. In addition, there was a significant interaction between lesioned state and exercise on striatal dopamine levels ($F_{(3,16)} = 7.78$; $p = 0.015$). This interaction was because of a significant effect of exercise on the saline-treated group, in which saline plus exercise mice had a higher level of striatal dopamine compared with saline mice. There was no significant difference in striatal dopamine levels comparing MPTP plus exercise with MPTP plus no exercise mice. There were no significant effects of MPTP or exercise, or interaction between these two factors on turnover ratio, with ratios of 0.36 for MPTP, 0.34 for MPTP plus exercise, 0.26 for saline, and 0.34 for the saline plus exercise group.

Dopamine release induced by intrastriatal stimulation was determined using fast-scan cyclic voltammetry at completion of the 28 d of treadmill running. Coronal slices at the level of midstriatum were sampled with electrodes placed at five sites, as illustrated in Figure 4. For measurements in the contralateral striatum, a mirror-image placement was used. Measurements were made using four to six mice from each group and from four slices per mouse. For each animal, a single mean value was determined at each electrode location by averaging all data collected. Stimulation-induced dopamine release varied with the location of the electrode ($F_{(3,20)} = 9.211$; $p < 0.001$) in both saline and MPTP-treated mice. MPTP lesioning dramatically decreased stimulation-induced dopamine release compared with saline-treated mice ($F_{(3,20)} = 26.450$; $p < 0.001$). Exercise had a significant effect in the MPTP-lesioned mouse that was dependent on electrode location ($F_{(3,20)} = 2.875$; $p = 0.039$). Additional analysis using *post hoc t* tests demonstrated that there was significant dopamine release at electrode positions three ( $p = 0.036$) and four ( $p = 0.023$) but not electrode positions one ( $p = 0.723$), two ( $p = 0.252$), and five ( $p = 0.141$) in MPTP plus exercise mice compared with MPTP plus no exercise mice. Exercise did not have an effect in the unlesioned animals (no overall effect, $F_{(3,20)} = 0.084$, $p = 0.781$; no interaction between exercise and striatal location, $F_{(3,20)} = 2.586$, $p = 0.088$), but it did have an effect on lesioned animals, depending on the location in the stri-

**Table 1. HPLC analysis of striatal dopamine and its metabolites**

| | | | Dopamine | DOPAC | HVA | Turnover |
|---|---|---|---|---|---|---|
| Exercise day 5 | No exercise | Saline | 269.5 ± 24.9 | 33.4 ± 4.5 | 33.1 ± 3.2 | 0.3 ± 0.08 |
| | | MPTP | 48.0 ± 8.4* | 11.7 ± 1.9* | 87.2 ± 9.6 | 2.1 ± 0.28* |
| | Exercise | Saline | 285.1 ± 30.3 | 36.2 ± 1.8 | 26.3 ± 2.2 | 0.2 ± 0.06 |
| | | MPTP | 4.9 ± 0.2* | 1.4 ± 0.1* | 4.7 ± 0.1# | 1.2 ± 0.14*,# |
| Exercise day 28 | No exercise | Saline | 246.9 ± 19.8 | 36.5 ± 4.5 | 27.4 ± 1.7 | 0.3 ± 0.01 |
| | | MPTP | 77.9 ± 12.0* | 14.7 ± 2.4* | 13.5 ± 1.9* | 0.4 ± 0.01 |
| | Exercise | Saline | 315.2 ± 9.0+ | 39.3 ± 2.8 | 25.9 ± 1.3 | 0.2 ± 0.01 |
| | | MPTP | 69.8 ± 11.7* | 11.3 ± 1.5* | 13.5 ± 1.2* | 0.4 ± 0.05 |

The concentration of dopamine, DOPAC, and HVA and turnover ratio were analyzed in each experimental group ($n = 6$ per group) at 5 d of exercise (corresponding to 10 d postlesioning) and also at 28 d of exercise (corresponding to 42 d postlesioning). The turnover ratio is defined as [(DOPAC + HVA)/dopamine]. At day 5 of exercise, there was a significant effect of MPTP on dopamine and DOPAC levels and turnover ratio, compared with saline-treated animals (the asterisk represents significance at $p < 0.0001$). There was no significant effect of exercise on dopamine levels in either MPTP or saline-treated groups. Exercise at day 5 caused a significant decrease in turnover ratio and HVA levels in the MPTP plus exercise group compared with the MPTP plus no exercise group only (# represents significance at $p < 0.02$). At day 28 of exercise, there was a significant effect of MPTP on dopamine, DOPAC, and HVA levels, compared with saline-treated animals (the asterisk represents significance at $p < 0.0001$). There was a significant increase in straital dopamine levels in the saline plus exercise animals compared with the saline plus no exercise animals (+ represents significance at $p = 0.015$), but there was no significant effect of exercise on dopamine levels in the MPTP-treated groups.

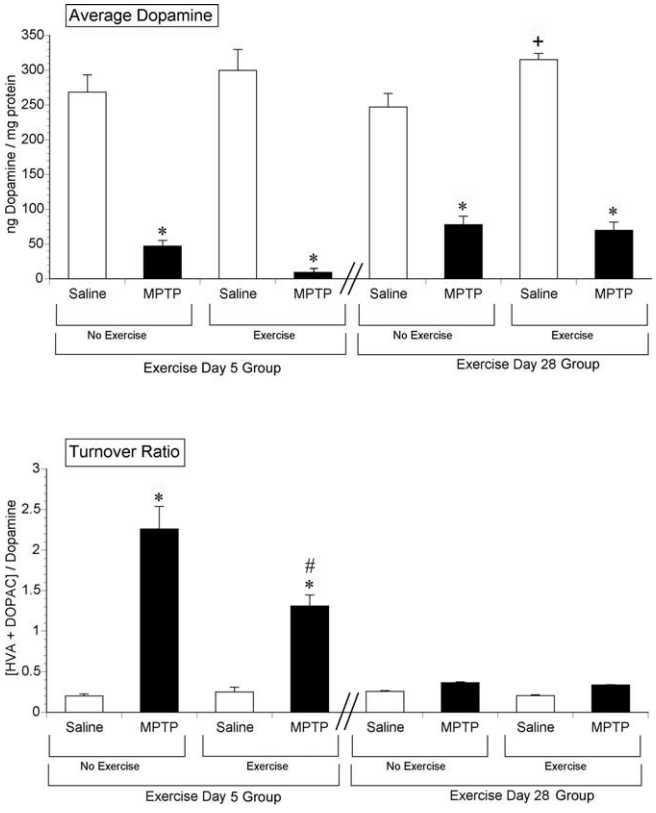


**Figure 3.** HPLC analysis of striatal dopamine levels and dopamine turnover. This figure shows data from Table 1 for striatal dopamine levels and turnover ratio at both 5 d of exercise (corresponding to 10 d postlesioning), and also at 28 d of exercise (corresponding to 42 d postlesioning). At day 5 of exercise, there was 82–98% depletion of dopamine in all MPTP-treated animals (exercise and no exercise) compared with their respective saline groups (asterisk represents significance at $p < 0.0001$). There were no significant differences in striatal dopamine levels between exercise and no exercise animals in either the MPTP- or saline-treated animals. Turnover ratio was significantly elevated in all MPTP- compared with saline-treated animals (asterisk represents significance at $p < 0.0001$). There was a significant decrease in turnover ratio in the MPTP plus exercise compared with the MPTP plus no exercise animals (hash mark represents significance at $p < 0.02$). At day 28 of exercise, there was >68 –78% depletion of striatal dopamine levels in all MPTP-treated animals (exercise and no exercise) compared with their respective saline groups (asterisk represents significance at $p < 0.0001$). There was a significant increase in striatal dopamine levels in saline plus exercise compared with saline plus no exercise groups only (cross represents significance at $p = 0.015$). Exercise had no effect on turnover ratio. Error bars indicate SEM.

atum (there was an interaction of exercise with location). The mechanism of dopamine release induced by our intrastriatal stimulation was investigated by sequentially testing the evoked dopamine response in control aCSF (containing 2.4 mM $Ca^{2+}$ and 1.3 mM $Mg^{2+}$), reduced $Ca^{2+}$ aCSF (containing 0.5 mM $Ca^{2+}$ and 3.2 mM $Mg^{2+}$), and washout with normal aCSF followed by addition of aCSF containing 1 $\mu$M TTX. The reduced $Ca^{2+}$ aCSF decreased the evoked dopamine response to 25.85 ± 5.44% ($n = 3$) of control, which returned to the control amplitude with washout by normal aCSF. Subsequent addition of TTX completely blocked the evoked dopamine release ($n = 3$).

The decay rate of the dopamine signal evoked by intrastriatal stimulation was also compared for site 4 between each of the groups (see Materials and Methods for determining decay rate constant, $-k$). Site 4 was chosen, because this site showed the greatest exercise-induced difference for peak dopamine release in the MPTP-lesioned group. Exercise had an effect on the decay constant for the evoked dopamine signal ($-k$). *Post hoc* analysis revealed a significant slowing in dopamine decay rate in the MPTP plus exercise group ($n = 11$) compared with the MPTP alone group ($n = 7$; $p < 0.05$; $t$ test). A similar trend was seen between saline plus exercise group ($n = 11$) and the saline only group ($n = 11$; $p = 0.13$).

As shown previously, there was a significant reduction in striatal DAT and TH protein expression in MPTP plus exercise mice compared with MPTP mice ( $p < 0.05$) (Fisher et al., 2004). MPTP-treated mice were also shown to have a significant decrease in TH and DAT protein expression compared with saline-treated mice ( $p < 0.001$). No significant differences in TH and DAT protein were observed between saline plus exercise and saline mice. These data are shown in Figure 5. To determine whether changes in protein level were accompanied by changes in the relative expression of either TH or DAT mRNA transcript in midbrain dopaminergic neurons, *in situ* hybridization histochemistry in conjunction with grain counting of emulsion-dipped sections was used. Mice from all four groups were analyzed after the 28 d of exercise and these data are shown in Figure 6. MPTP lesioning caused a significant decrease in the relative expression of TH and DAT transcript expression in midbrain dopaminergic neurons ($F_{(3,12)} = 26.1$, $p < 0.001$ and $F_{(3,12)} = 37.29$, $p < 0.001$, respectively). Exercise caused a significant decrease in TH and DAT mRNA transcript expression in saline-treated but not in MPTP-treated mice. Specifically, although exercise had no overall effect on TH transcript expression ($F_{(3,12)} = 2.26$; $p = 0.133$), there was a significant interaction between exercise and MPTP lesioning ($F_{(3,12)} = 51.7$; $p < 0.001$). This interaction was because of a 28% reduction in TH mRNA expression in the saline plus exercise group compared with saline plus no exercise group. Exercise also caused a significant decrease in DAT transcript expression, ($F_{(3,12)} = 73.2$; $p < 0.001$), and there was a significant interaction between exercise and MPTP lesioning ($F_{(3,12)} = 33.7$; $p < 0.001$). This interaction was again because of a 43% reduction in DAT mRNA expression in the saline plus exercise group compared with the saline plus no exercise group.

As a measure of the integrity of the midbrain nigrostriatal dopaminergic neurons, we determined the number of TH-ir neu-

rons in the SNpc using unbiased stereological counting in mice from the saline, saline plus exercise, MPTP, and MPTP plus exercise groups ($n = 3$ per group) (Fig. 7). MPTP lesioning caused a significant decrease in the number of TH-ir SNpc neurons ($F_{(3,8)} = 106.9$; $p < 0.001$). However, exercise caused no significant effect on the number of TH-ir SNpc neurons ($F_{(3,8)} = 0.022$; $p = 0.885$), and there was no significant interaction between exercise and MPTP on the number of TH-positive neurons.

## Discussion

As reported previously, intensive treadmill exercise leads to improvement of motor performance in MPTP-lesioned (Tillerson et al., 2003; Fisher et al., 2004) and saline-treated mice (Fisher et al., 2004). In the present study, we also demonstrate that intensive treadmill exercise leads to increased latency to fall (improved balance) on the accelerating rotarod. This finding suggests that intensive treadmill exercise may, through adaptive changes of the basal ganglia and motor circuitry, lead to improvement in related motor tasks (balance) in both MPTP and saline-treated animals. Our findings indicate, however, that the beneficial effects of exercise are accompanied by differential effects on the dopaminergic system that may be dependent on the presence or absence of nigrostriatal injury (lesioned versus unlesioned). Specifically, although intense exercise improved motor performance in both groups, using HPLC analysis, we observed that this functional benefit was accompanied by a significant increase in total striatal dopamine after 28 d of exercise in saline plus exercise compared with saline plus no exercise mice only. There were no significant differences in total striatal dopamine levels in MPTP mice, examined after either 5 or 28 d of treadmill running, compared with their corresponding no exercise groups. In addition, exercise had no effect on the time course of dopamine return in MPTP-lesioned mice. Specifically, we observed a partial return of striatal dopamine in all MPTP-lesioned mice at day 28 compared with day 5, and this effect was observed to the same degree in exercise and no exercise mice, and in accordance with previous reports (Ricaurte et al., 1986; Bezard et al., 2000; Jakowec et al., 2004). Although HPLC analysis of total tissue catecholamines is an excellent measure of the total dopamine pool, including synaptic, extra-synaptic, vesicular, and cytoplasmic, it may not be an accurate estimate of the amount of dopamine released with activity (Garris et al., 1997; Yavich and MacDonald, 2000; Dentresangle et al., 2001).

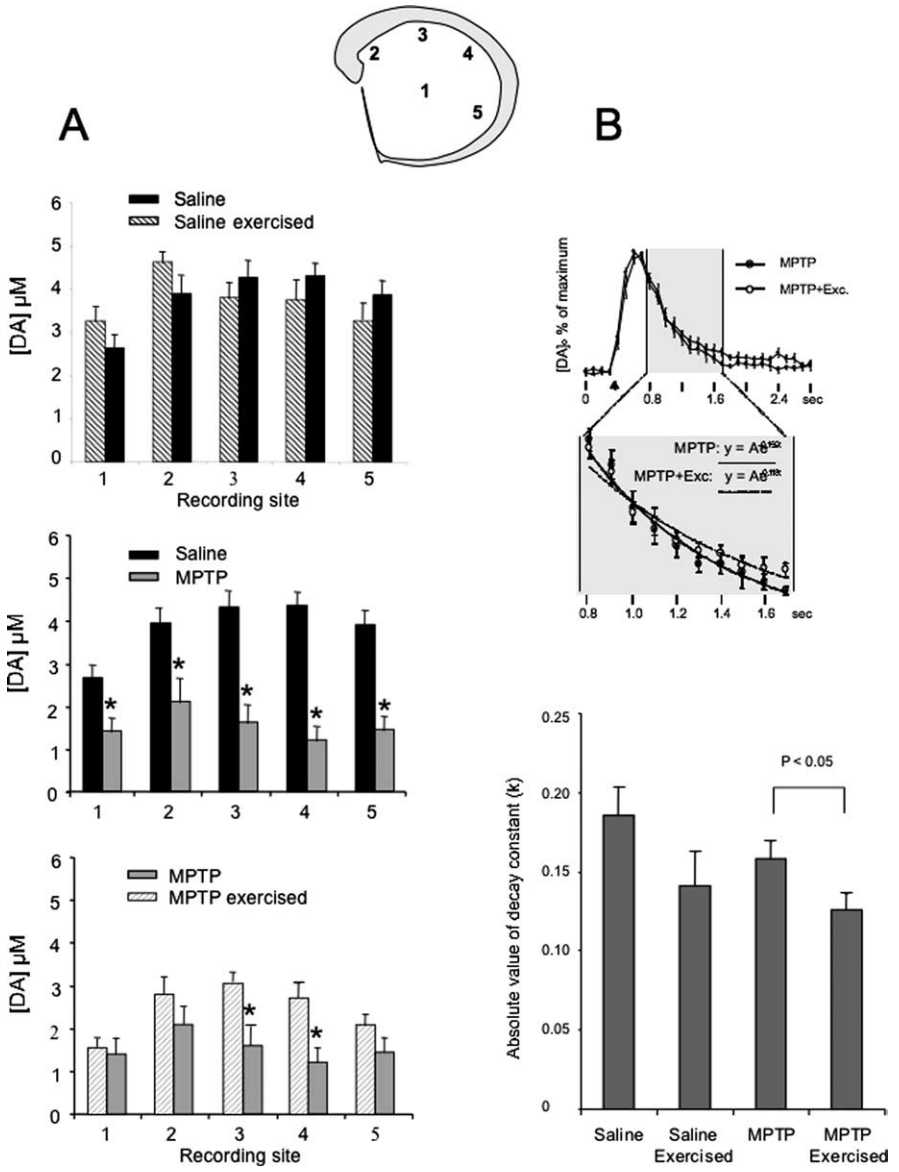


**Figure 4.** Analysis of dopamine release using fast-scan cyclic voltammetry. The amount of dopamine release was determined at five regions within the striatum (at bregma level ~1.00 to bregma 0.80) including (1) mid-striatum, (2) dorsomedial, (3) dorsal, (4) dorsolateral, and (5) ventrolateral in representative mice from all four groups (see brain slice insert). ***A***, Comparison of peak dopamine released by a single intrastriatal stimulus (200 A, 0.1 ms). Top, Data comparing saline and saline plus exercise mice. There was no significant difference in dopamine release in any region. Middle, Data comparing saline and MPTP groups. There was a significant decrease in dopamine release in MPTP mice and the asterisk represents significant difference compared with saline ( $p < 0.001$). Bottom, Data comparing MPTP and MPTP plus exercise mice. There was a significant increase in dopamine release in the MPTP plus exercise mice at dorsal sites 3 and 4 compared with MPTP mice, and the asterisk represents significant difference ( $p < 0.05$). ***B***, Comparison of dopamine signal (peak and decay) evoked by intrastriatal stimulation. Top, A plot of average time to peak and decay in the dopamine signal at electrode position 4 for MPTP plus no exercise ($n = 7$, filled circles) and MPTP plus exercise ($n = 11$, open circles). The intrastriatal stimulus was delivered at the time indicated by the filled triangle (between 0 and 0.8 s). Data points are normalized to the peak-evoked dopamine signal. The middle panel illustrates the decay phase of the graph (shaded area), which was fit with a single exponential function. Best fit for the average data from MPTP plus no exercise mice (solid line) and MPTP plus exercise mice are shown (dotted line). The bottom panel illustrates the averages of the decay constant ($k$) obtained by the exponential fit of the decay phase for each recording and are shown for each group (mean ± SEM). MPTP plus exercise mice had a significantly lower decay constant compared with MPTP plus no exercise mice ( $p < 0.05$; $t$ test). A similar trend was seen in the saline plus exercise compared with the saline plus no exercise animals ( $p = 0.13$; $t$ test). Exc, Exercise.

In contrast to total tissue HPLC analysis, using fast-scan cyclic voltammetry in striatal slices of mice exercised for 28 d, we observed an exercise-dependent increase in stimulus-evoked dopamine release in MPTP plus exercise compared with MPTP plus

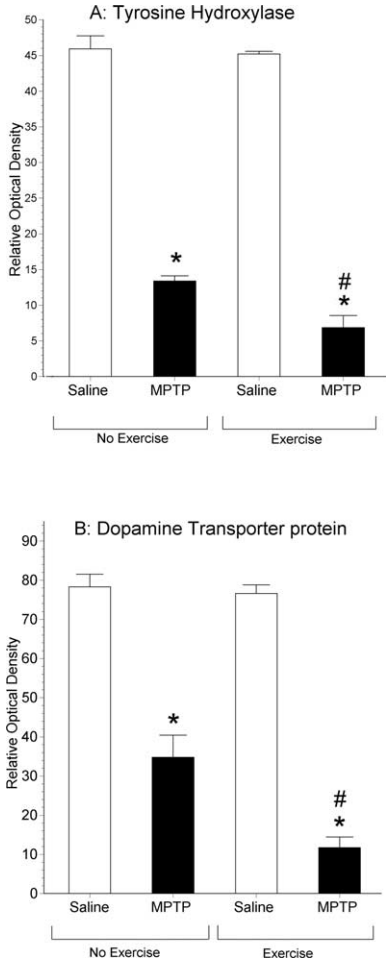


**Figure 5.** Analysis of striatal TH and DAT protein. ***A***, ***B***, The relative expression of striatal TH (***A***) and DAT (***B***) was determined using immunohistochemistry and computer-assisted image analysis after 28 d of exercise. Sections at the level of the midstriatum ($\geq$6–8 sections from 4 different mice within each group) were immunostained with an antibody against TH or DAT. Sections were scanned and the relative optical density mean plus SEM for each group was determined. MPTP causes a significant decline in the level of expression of striatal TH and DAT protein compared with saline-treated mice, and the asterisk represents significance at $p < 0.001$. There is a significant interaction between exercise and MPTP, with a significant decrease in the MPTP plus exercise compared with the MPTP plus no exercise mice, and the hash mark represents significance at $p < 0.05$.

no exercise mice. Our results show that the stimulus-evoked dopamine release is vesicular, because it was decreased by reduced extracellular $Ca^{2+}$ and it was completely blocked by blockage of $Na^{+}$ channels by TTX as reported previously (Cragg and Greenfield, 1997; Chen and Rice, 2001). This exercise-dependent effect was not seen in the saline plus exercise compared with saline plus no exercise mice. Interestingly, the exercise effect on stimulus-evoked dopamine release in the MPTP plus exercise mouse was most pronounced within the dorsolateral striatum. A possible explanation for this exercise and regional specific effect may be because of use-dependent forms of synaptic plasticity. The high degree of engagement required from the dorsolateral striatum for forelimb and hindlimb movement during treadmill exercise is supported by studies demonstrating a selective increase in blood flow and metabolic activity within the dorsolateral striatum during treadmill exercise (Cospito and Kultas-Ilinsky, 1981; Ebrahimi et al., 1992; Brown and Sharp, 1995; Holschneider et al., 2003; Nguyen et al., 2004).

Fast-scan cyclic voltammetry is a technique that allows for precise anatomical targeting and examination of evoked dopamine release at a number of distinct sites within the same brain slice during a single recording session, thus providing the ability to detect regional differences in dopamine release. Although an advantage of *in vivo* recording is the retention of intact afferent pathways, stimulation of the medial forebrain bundle (MFB) may be variable across these afferent fibers and lead to differences in dopamine release even within a hundred micrometers between striatal recording sites (Day et al., 1989). Brain slices may not retain an intact nigrostriatal pathway but it does avoid variation in evoked release because the entire recording field is excited and provides a reasonable tool to investigate the way this neurotransmitter system responds (Patel and Rice, 2006). In a set of control experiments, we verified that the release of dopamine was in fact mediated through an action potential by carrying out the fast-scan cyclic voltammetry studies in the presence of the sodium channel blocker TTX.

In agreement with our study, compensatory changes in dopamine release have been reported after injury of the dopaminergic system using 6-OHDA lesioning and in patients with PD during motor exercise (Zhang et al., 1988; Garris et al., 1997; Ouchi et al., 2001). In addition, a recent study from O'Dell et al. (2006) using 6-OHDA striatal lesioning in the rat, supports our findings that improvement in motor performance may not necessarily be accompanied by changes in total striatal dopamine levels after exercise. Specifically, this group reported exercise-enhanced return of motor behavior without the return of striatal dopamine levels to the prelesioning baseline (O'Dell et al., 2006). Our studies and those reported by O'Dell et al. (2006) are in contrast to others that show partial return of striatal dopamine in neurotoxicant-lesioned rodent models undergoing exercise (Tillerson et al., 2003). One possible explanation for this discrepancy may be because of differences in the MPTP-lesioning regimen and exercise paradigm. In our study, we used an MPTP-lesioning schedule that resulted in a moderate degree of nigrostriatal dopaminergic cell loss and initiated exercise 5 d after MPTP lesioning, when cell death is complete. Conversely, studies demonstrating a partial dopamine return have used milder lesioning regimens and have initiated treadmill running immediately (within 24 h) after lesioning, when the potential for exercise-induced downregulation of the DAT may influence both the amount of neurotoxin uptake and the degree of lesioning (Gainetdinov et al., 1997). Unbiased stereological counting of midbrain dopaminergic neurons in our study indicates that exercise-induced downregulation of DAT appears independent of the number of surviving substantia nigral neurons.

Using immunohistochemical analysis, we observed that 28 d of intense exercise leads to the downregulation of both DAT (a protein responsible for uptake and clearance of dopamine from the extracellular space) and TH (an enzyme responsible for the rate-limiting step in dopamine biosynthesis) (Jackson-Lewis et al., 1995; Fisher et al., 2004) protein. This finding is supported by our previous work demonstrating suppression of both TH and DAT protein in MPTP plus exercise mice using both Western immunoblotting and immunohistochemical staining, two complementary techniques to examine protein expression levels and anatomical patterns of distribution (Burke et al., 1990; Jakowec et al., 2004). To verify that this decrease of striatal DAT and TH protein was not because of exercise-induced nigrostriatal cell death, the number of midbrain dopaminergic neurons based on TH immunoreactivity were determined using unbiased stereological counting techniques. Comparison of the exercise and no exercise groups showed no significant differences in the total

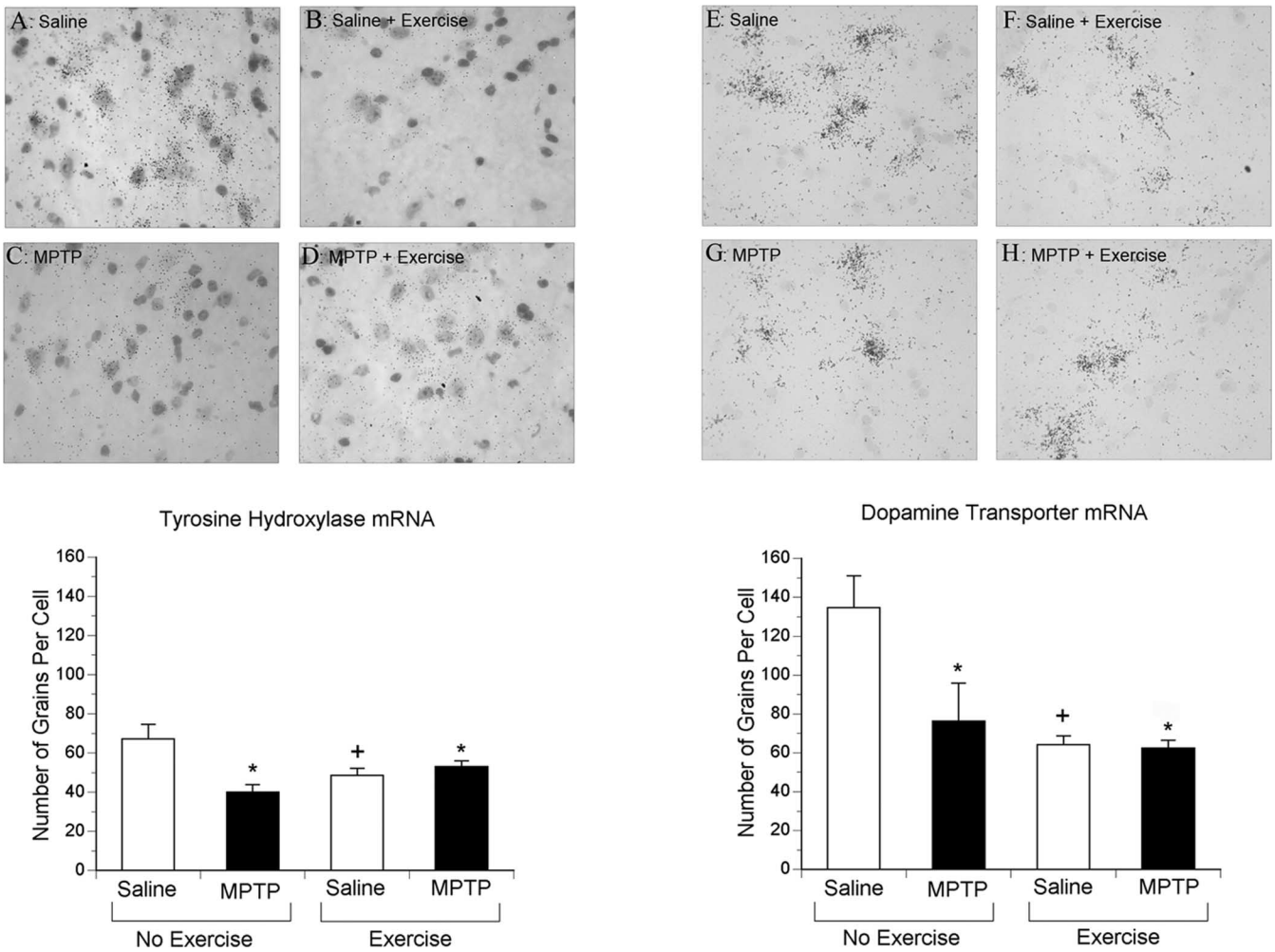


**Figure 6.** Analysis of TH and DAT mRNA in midbrain nigrostriatal neurons. The relative expression of TH and DAT mRNA in midbrain dopaminergic neurons were determined using *in situ* hybridization histochemistry followed by grain counting of emulsion-dipped sections. Representative sections showing neurons for the determination of TH mRNA expression are shown in panels ***A*** (saline), ***B*** (saline plus exercise), ***C*** (MPTP), and ***D*** (MPTP plus exercise). The respective analysis of the data for either TH or DAT is shown in the graphs below each set of images. At least 120 neurons with grains were counted for each treatment group. MPTP lesioning caused a significant decrease in both TH and DAT transcript compared with the saline-treated groups, and the asterisk represents significance at $p < 0.001$. Statistical differences were seen between the saline and saline plus exercise groups for both the TH and DAT transcript, and the cross represents significance at $p < 0.001$. There were no significant differences in transcript expression between the MPTP and MPTP plus exercise groups. Representative sections showing neurons for determination of DAT mRNA expression are shown in ***E*** (saline), ***F*** (saline plus exercise), ***G*** (MPTP), and ***H*** (MPTP plus exercise). Error bars indicate SEM.

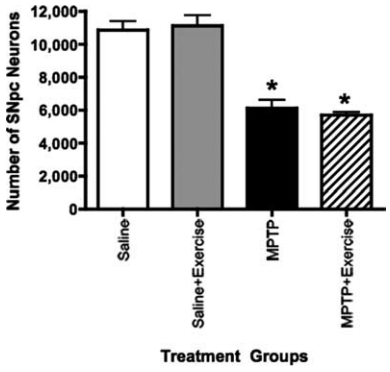


**Figure 7.** Determination of SNpc dopaminergic cell number. Unbiased stereological counting of TH-ir neurons was performed in mice ($n = 3$ per group) from all four groups at the end of the 28 d running regimen. MPTP lesioning resulted in an ~50% decline in SNpc dopaminergic neurons. There was no statistically significant difference between the saline and the saline plus exercise mice as well as no difference between the MPTP and MPTP plus exercise groups, indicating that there was no effect of exercise on the number of SNpc dopaminergic neurons. The asterisk represents significant difference from the saline group ( $p < 0.0001$).

number of midbrain dopaminergic neurons between either MPTP and MPTP plus exercise or saline and saline plus exercise mice, which indicated no additional cell death induced by exercise. This finding suggests that both DAT and TH proteins are downregulated by exercise in surviving midbrain dopaminergic neurons after MPTP lesioning. Downregulation of DAT may lead to increased synaptic availability and represent a compensatory effect of the dopaminergic system because of exercise in the injured state. Indeed, using fast-scan cyclic voltammetry analysis of the decay rate of the dopamine signal evoked by a single stimulus in striatal brain slices, we observed a slower dopamine decay rate in the MPTP plus exercise compared with the MPTP plus no exercise mice, possibly related to the decline in DAT. The increase in dopamine release seen in exercised mice could also reflect more diffusion of dopamine from synapses distant from the recording site when DAT is reduced (Cragg and Rice, 2004). The exercise-induced increase in evoked dopamine release in the dorsolateral striatum together with a DAT-related lengthening of the dopamine signal may underlie one mechanism by which intense exercise facilitates normal motor circuitry because corticostriatal

synaptic plasticity, both LTD and LTP, is modulated by dopamine. In the dorsolateral striatum, the increased release and decreased decay of dopamine could play an important role in maintaining normal LTD (Calabresi et al., 1992; Smith et al., 2001; Akopian and Walsh, 2007).

The expression of DAT and TH proteins, as well as the level of striatal dopamine, is closely linked. For example, DAT knock-out mice have been shown to express reduced striatal TH protein (Jaber et al., 1999). Although the precise link between DAT and TH is unclear, it is speculated that the increased synaptic bioavailability of dopamine, through the downregulation of DAT, may lead to increased activation of the dopamine autoreceptor, leading to the downregulation of TH (Fauchey et al., 2000). In support of our findings, studies with neuroimaging have shown rapid downregulation of DAT in patients with PD undergoing motor activity (walking) (Ouchi et al., 2001). A similar downregulation of DAT with activity can be induced in young mice exposed to environmental enrichment and this can in fact prove neuroprotective against MPTP lesioning (Bezard et al., 2003).

In our study, we used *in situ* hybridization histochemistry to examine the level of expression of mRNA transcripts of TH and DAT in surviving nigrostriatal dopaminergic neurons to determine whether alterations in transcription could account for exercise-induced suppression of striatal protein. We found a reduction in expression of TH and DAT mRNA in saline plus exercise compared with saline mice but not in MPTP plus exercise compared with MPTP mice. The effect of exercise on mRNA transcript expression may be dependent on the presence of a previous injury to the nigrostriatal dopaminergic neurons. MPTP can lead to reduction in TH mRNA expression in surviving midbrain neurons, and this may mask potential effects of exercise (Jakowec et al. 2004). Because exercise-induced changes in striatal protein cannot be accounted for by changes in transcript expression in surviving nigrostriatal dopaminergic neurons in MPTP mice, exercise may act through alternative mechanisms that influence TH and DAT rates of translation, axonal translocation, or turnover.

In conclusion, our results indicate that intensive treadmill exercise leads to improvement of motor performance in both MPTP and saline mice and this behavioral improvement is also observed in a related motor task. The beneficial effects of exercise may be because of alterations in dopaminergic neurotransmission, which may be different between the normal and injured basal ganglia. Exercise leads to compensatory changes in the MPTP-lesioned mouse resulting in increased synaptic dopamine availability through increased release, reduced uptake, and decrease in decay. In saline mice, exercise effects may be through elevated dopamine levels because of increased biosynthesis through increased TH activity. Currently, the molecular mechanism linking exercise and dopaminergic neurotransmission are unknown. However, reports in the literature suggest a role for neurotrophic factors, such as brain-derived neurotrophic factor, fibroblast growth factor, or glial-derived neurotrophic factor, which through activation of down-stream pathways, such as protein kinases, may influence synaptic plasticity and terminal neurotransmission (Gomez-Pinilla et al., 1997, 2002; Cohen et al., 2003). The glutamatergic corticostriatal pathway may be another candidate system involved in exercise-related alterations in dopamine release, because it is known to be an important modulator of dopamine release in the striatum, and our previous work has shown alterations in the density of striatal glutamate immunolabeling in the MPTP plus exercise mice compared with sedentary MPTP-lesioned mice (Molteni et al., 2002; Fisher et al., 2004; Dietrich et al., 2005). Overall, these results indicate that the recovery of motor behavior can in fact occur through novel compensatory mechanisms within the basal ganglia and that the mechanisms of this recovery may be different in the lesioned compared with the nonlesioned basal ganglia. These findings may be important in treating neurodegenerative disorders of the basal ganglia, including Parkinson's disease, in which the enhancement of neuroplasticity through exercise may lead to altered dopaminergic availability (release and uptake), which in turn may play a more critical role in maintaining normal synaptic connections than the restoration of absolute dopamine levels.

## References


Akopian G, Walsh JP (2007) Reliable long-lasting depression interacts with variable short-term facilitation to determine corticostriatal paired-pulse plasticity. J Physiol (Lond) 580:225–240.

Bezard E, Dovero S, Imbert C, Boraud T, Gross CE (2000) Spontaneous long-term compensatory dopaminergic sprouting in MPTP-treated mice. Synapse 38:363–368.

Bezard E, Dovero S, Belin D, Duconger S, Jackson-Lewis V, Przedborski S, Piazza PV, Gross CE, Jaber M (2003) Enriched environment confers resistance to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and cocaine: involvement of dopamine transporter and trophic factors. J Neurosci 23:10999–11007.

Brown LL, Sharp FR (1995) Metabolic mapping of rat striatum: somatotopic organization of sensorimotor activity. Brain Res 686:207–222.

Burke RE, Cadet JL, Kent JD, Karanas AL, Jackson-Lewis V (1990) An assessment of the validity of densitometric measures of striatal tyrosine hydroxylase-positive fibers: relationship to apomorphine-induced rotations in 6-hydroxydopamine lesioned rats. J Neurosci Methods 35:63–73.

Calabresi P, Maj R, Pisani A, Mercuri NB, Bernardi G (1992) Long-term synaptic depression in the striatum: physiological and pharmacological characterization. J Neurosci 12:424–4233.

Chen BT, Rice ME (2001) Novel $Ca^{2+}$ dependence and time course of somatodendritic dopamine release: substantia nigra versus striatum. J Neurosci 21:7841–7847.

Chen H, Zhang SM, Schwarzschild MA, Hernan MA, Ascherio A (2005) Physical activity and the risk of Parkinson disease. Neurology 64:664–669.

Cohen AD, Tillerson JL, Smith AD, Schallert T, Zigmond MJ (2003) Neuroprotective effects of prior limb use in 6-hydroxydopamine-treated rats: possible role of GDNF. J Neurochem 85:299–305.

Comella CL, Stebbins GT, Brown-Toms N, Goetz CG (1994) Physical therapy and Parkinson's disease: a controlled clinical trial. Neurology 44:376–378.

Cospito JA, Kultas-Ilinsky K (1981) Synaptic organization of motor corticostriatal projections in the rat. Exp Neurol 72:257–266.

Cragg SJ, Greenfield SA (1997) Differential autoreceptor control of somatodendritic and axon terminal dopamine release in substantia nigra, ventral tegmental area, and striatum. J Neurosci 17:5738–5746.

Cragg SJ, Rice ME (2004) Dancing past the DAT at a DA synapse. Trends Neurosci 27:270–277.

Day BL, Dressler D, Maertens de Noordhout A, Marsden CD, Nakashima K, Rothwell JC, Thompson PD (1989) Electric and magnetic stimulation of human motor cortex: surface EMG and single motor unit responses. J Physiol (Lond) 412:449–473.

Dentresangle C, Le Cavorsin M, Savasta M, Leviel V (2001) Increased extracellular DA and normal evoked DA release in the rat striatum after a partial lesion of the substantia nigra. Brain Res 893:178–185.

Dietrich MO, Mantese CE, Porciuncula LO, Ghisleni G, Vinade L, Souza DO, Portela LV (2005) Exercise affects glutamate receptors in postsynaptic densities from cortical mice brain. Brain Res 1065:20–25.

Ebrahimi A, Pochet R, Roger M (1992) Topographical organization of the projections from physiologically identified areas of the motor cortex to the striatum in the rat. Neurosci Res 14:39–60.

Faherty CJ, Raviie Shepherd K, Herasimtschuk A, Smeyne RJ (2005) Environmental enrichment in adulthood eliminates neuronal death in experimental Parkinsonism. Brain Res Mol Brain Res 134:170–179.

Fauchey V, Jaber M, Caron MG, Bloch B, Le Moine C (2000) Differential regulation of the dopamine D1, D2 and D3 receptor gene expression and

changes in the phenotype of the striatal neurons in mice lacking the dopamine transporter. Eur J Neurosci 12:19–26.

Fisher BE, Petzinger GM, Nixon K, Hogg E, Bremmer S, Meshul CK, Jakowec MW (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.

Fukai T, Siegfried MR, Ushio-Fukai M, Cheng Y, Kojda G, Harrison DG (2000) Regulation of the vascular extracellular superoxide dismutase by nitric oxide and exercise training. J Clin Invest 105:1631–1639.

Gainetdinov RR, Fumagalli F, Jones SR, Caron MG (1997) Dopamine transporter is required for in vivo MPTP neurotoxicity: evidence from mice lacking the transporter. J Neurochem 69:1322–1325.

Garris PA, Walker QD, Wightman RM (1997) Dopamine release and uptake rates both decrease in the partially denervated striatum in proportion to the loss of dopamine terminals. Brain Res 753:225–234.

Gomez-Pinilla F, Dao L, So V (1997) Physical exercise induces FGF-2 and its mRNA in the hippocampus. Brain Res 764:1–8.

Gomez-Pinilla F, Ying Z, Roy RR, Molteni R, Edgerton VR (2002) Voluntary exercise induces a BDNF-mediated mechanism that promotes neuroplasticity. J Neurophysiol 88:2187–2195.

Gundersen HJ, Jensen EB (1987) The efficiency of systematic sampling in stereology and its prediction. J Microsc 147:229–263.

Holschneider DP, Maarek JM, Yang J, Harimoto J, Scremin OU (2003) Functional brain mapping in freely moving rats during treadmill walking. J Cereb Blood Flow Metab 23:925–932.

Irwin I, Finnegan KT, DeLanney LE, Di Monte D, Langston JW (1992) The relationship between aging, monoamine oxidase, striatal dopamine and the effects of MPTP in C57BL/6 mice: a critical reassessment. Brain Res 572:224–231.

Jaber M, Dumartin B, Sagne C, Haycock JW, Roubert C, Giros B, Bloch B, Caron MG (1999) Differential regulation of tyrosine hydroxylase in the basal ganglia of mice lacking the dopamine transporter. Eur J Neurosci 11:3499–3511.

Jackson-Lewis V, Jakowec M, Burke RE, Przedborski S (1995) Time course and morphology of dopaminergic neuronal death caused by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Neurodegeneration 4:257–269.

Jakowec MW, Yen L, Kalb RG (1995) In situ hybridization analysis of AMPA receptor subunit gene expression in the developing rat spinal cord. Neuroscience 67:909–920.

Jakowec MW, Nixon K, Hogg L, McNeill T, Petzinger GM (2004) Tyrosine hydroxylase and dopamine transporter expression following 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurodegeneration in the mouse nigrostriatal pathway. J Neurosci Res 76:539–550.

Kilpatrick IC, Jones MW, Phillipson OT (1986) A semiautomated analysis method for catecholamines, indoleamines, and some prominent metabolites in microdissected regions of the nervous system: an isocratic HPLC technique employing coulometric detection and minimal sample preparation. J Neurochem 46:1865–1876.

Kojda G, Cheng YC, Burchfield J, Harrison DG (2001) Dysfunctional regulation of endothelial nitric oxide synthase (eNOS) expression in response to exercise in mice lacking one eNOS gene. Circulation 103:2839–2844.

Miles PR, Mundorf ML, Wightman RM (2002) Release and uptake of catecholamines in the bed nucleus of the stria terminalis measured in the mouse brain slice. Synapse 44:188–197.

Molteni R, Ying Z, Gomez-Pinilla F (2002) Differential effects of acute and chronic exercise on plasticity-related genes in the rat hippocampus revealed by microarray. Eur J Neurosci 16:1107–1116.

Mosharov EV, Sulzer D (2005) Analysis of exocytotic events recorded by amperometry. Nat Methods 2:651–658.

Nguyen PT, Holschneider DP, Maarek JM-I, Yang J, Mandelkern MA (2004) Statistical parametric mapping applied to an autoradiographic study of cerebral activation during treadmill walking in rats. NeuroImage 23:252–259.

O'Dell SJ, Gross NB, Fricks AN, Casiano BD, Nguyen TB, Marshall JF (2006) Running wheel exercise enhances recovery from nigrostriatal dopamine injury without inducing neuroprotection. Neuroscience 144:1141–1151.

Ouchi Y, Kanno T, Okada H, Yoshikawa E, Futatsubashi M, Nobezawa S, Torizuka T, Tanaka K (2001) Changes in dopamine availability in the nigrostriatal and mesocortical dopaminergic systems by gait in Parkinson's disease. Brain 124:784–792.

Patel J, Rice ME (2006) Monitoring dopamine release in brain slices. In: Encyclopedia of sensors (Grimes CA, Dickey EC, Pishko MV, eds), pp 313–334. Stevenson Ranch, CA: American Scientific.

Paxinos G, Franklin KBJ (2001) The mouse brain in stereotaxic coordinates, Ed 2. New York: Academic.

Petzinger GM, Fisher BE, Hogg E, Abernathy A, Arevalo P, Nixon K, Jakowec MW (2005) Behavioral recovery in the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-lesioned squirrel monkey (*Saimiri sciureus*): analysis of striatal dopamine and the expression of tyrosine hydroxylase and dopamine transporter proteins. J Neurosci Res 83:332–347.

Ricaurte GA, Langston JW, Delanney LE, Irwin I, Peroutka SJ, Forno LS (1986) Fate of nigrostriatal neurons in young mature mice given 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: a neurochemical and morphological reassessment. Brain Res 376:117–124.

Schulte A, Chow RH (1996) A simple method for insulating carbon-fiber microelectrodes using anodic electrophoretic deposition of paint. Anal Chem 68:3054–3058.

Smith R, Musleh W, Akopian W, Buckwalter G, Walsh JP (2001) Regional differences in the expression of corticostriatal synaptic plasticity. Neuroscience 106:95–101.

Tillerson JL, Cohen AD, Philhower J, Miller GW, Zigmond MJ, Schallert T (2001) Forced limb-use effects on the behavioral and neurochemical effects of 6-hydroxydopamine. J Neurosci 21:4427–4435.

Tillerson JL, Caudle WM, Reveron ME, Miller GW (2003) Exercise induces behavioral recovery and attenuates neurochemical deficits in rodent models of Parkinson's disease. Neuroscience 119:899–911.

Yavich L, MacDonald E (2000) Dopamine release from pharmacologically distinct storage pools in rat striatum following stimulation at frequency of neuronal bursting. Brain Res 870:73–79.

Zhang WQ, Tilson HA, Nanry KP, Hudson PM, Hong JS, Stachowiak MK (1988) Increased dopamine release from striata of rats after unilateral nigrostriatal bundle damage. Brain Res 461:335–342.