Published in final edited form as:

Neurobiol Dis. 2015 May ; 77: 71–87. doi:10.1016/j.nbd.2015.02.020.

Recruitment of the prefrontal cortex and cerebellum in Parkinsonian rats following skilled aerobic exercise

Zhuo Wang^a, Yumei Guo^a, Kalisa G. Myers^a, Ryan Heintz^a, and Daniel P. Holschneider^{a,b,*}

^aDepartment of Psychiatry and Behavioral Sciences, University of Southern California, Los Angeles, California, U.S.A.

^bDepartments of Biomedical Engineering, Neurology, Cell and Neurobiology, University of Southern California, Los Angeles, California, U.S.A.

Abstract

Exercise modality and complexity play a key role in determining neurorehabilitative outcome in Parkinson's disease (PD). Exercise training (ET) that incorporates both motor skill training and aerobic exercise has been proposed to synergistically improve cognitive and automatic components of motor control in PD patients. Here we introduced such a skilled aerobic ET paradigm in a rat model of dopaminergic deafferentation. Rats with bilateral, intra-striatal 6-hydroxydopamine lesions were exposed to forced ET for 4 weeks, either on a simple running wheel (non-skilled aerobic exercise, NSAE) or on a complex wheel with irregularly spaced rungs (skilled aerobic exercise, SAE). Cerebral perfusion was mapped during horizontal treadmill walking or at rest using [¹⁴C]-iodoantipyrine 1 week after the completion of ET. Regional cerebral blood flow (rCBF) was quantified by autoradiography and analyzed in 3-dimensionally reconstructed brains by statistical parametric mapping. SAE compared to NSAE resulted in equal or greater recovery in motor deficits, as well as greater increases in rCBF during walking in the prelimbic area of the prefrontal cortex, broad areas of the somatosensory cortex, and the cerebellum. NSAE compared to SAE animals showed greater activation in the dorsal caudate-putamen and dorsal hippocampus. Seed correlation analysis revealed enhanced functional connectivity in SAE compared to NSAE animals between the prelimbic cortex and motor areas, as well as altered functional connectivity between midline cerebellum and sensorimotor regions. Our study provides the first evidence for functional brain reorganization following skilled aerobic exercise in Parkinsonian rats, and suggests that SAE compared to NSAE results in enhancement of prefrontal cortex- and cerebellum-mediated control of motor function.

© 2015 Published by Elsevier Inc.

*Corresponding author: Daniel P. Holschneider, M.D., 1975 Zonal Ave., KAM 400, MC 9037, Department of Psychiatry and Behavioral Sciences, University of Southern California, Los Angeles, CA 90089-9037, U.S.A., Tel: +1 (323) 442-1536, Fax: +1 (323) 442-1587, holschne@usc.edu.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Disclosure statement

The authors have no conflicts of interest.

Keywords

rehabilitation; exercise; motor skill learning; motor adaptation; functional brain mapping; functional connectivity; prefrontal cortex; cerebellum; Parkinson's Disease; motor function; rats

Introduction

There is now extensive evidence that exercise training (ET) has neurorehabilitative benefits for motor symptoms in Parkinson's disease (PD) (Goodwin et al., 2008; Petzinger et al., 2013; van der Kolk and King, 2013). ET has been proposed to elicit regional brain neuroplasticity, leading either to the restoration of function of the impaired circuit or to the recruitment of compensatory circuits (Yang et al., 2007; Petzinger et al., 2013). Clinical studies have examined a wide range of ET modalities, including aerobic exercise (treadmill walking, cycling), resistance training, balance training, and multifaceted ET paradigms (Tai Chi, dancing, agility exercise) (Scandalis et al., 2001; Hirsch et al., 2003; Hackney et al., 2007; Herman et al., 2007; Li et al., 2007; King and Horak, 2009). Their findings suggest that different ET modalities may preferentially improve different aspects of PD motor symptoms. A key clinical challenge is to understand how different ET parameters determine rehabilitative outcomes, so that rehabilitative regimens can be optimized for patients (Nieuwboer et al., 2009; Salgado et al., 2013).

The implication of motor skill learning in ET-based rehabilitation in PD has received increasing attention (Nieuwboer et al., 2009). Two types of motor skill learning have been extensively studied in healthy subjects, motor sequence learning and motor adaptation (Dayan and Cohen, 2011). Functional neuroimaging evidence suggests that both the basal ganglia-thalamocortical and the cerebellar-thalamocortical systems are crucial during the acquisition of these tasks. Whereas the basal ganglia circuit plays a more important role in the consolidation and retention of sequence learning, the cerebellar circuit is more critical in the consolidation and retention of motor adaptation (Doyon et al., 2009). Neuroimaging studies suggest that motor sequence learning in PD patients compared to normal elderly subjects requires additional recruitment of neural resources and different neural networks, including the cerebellum and dorsolateral prefrontal cortex (Mentis et al., 2003; Wu and Hallett, 2005).

Behavioral studies have shown that despite their slower learning-rates, PD subjects retain more or less intact motor learning (Nieuwboer et al., 2009). However, it is well known that individuals with PD demonstrate a context-dependency in their acquisition of new motor skills (Onla-or and Winstein, 2008). Such 'motor set inflexibility' or 'task-switching deficits' has made it difficult for individuals with PD to translate learning acquired in a rehabilitation session to a real-world situation where responses must be adapted to the environmental context. It has been suggested that motor rehabilitation programs for PD patients should include a relatively high cognitive demand, such that by forcing patients to practice task-switching over a sufficient number of practice trials, they might be able to overcome their context-dependency (Onla-or and Winstein, 2008). Recently, Petzinger et al. (2013) proposed that exercise incorporating goal-based motor skill training and aerobic

exercise may synergistically improve both cognitive and automatic components of motor control in subjects with mild to moderate PD. Earlier work by Ploughman et al. in a rat stroke model suggests that running exercise facilitates learning of a subsequent skilled forelimb task, which may synergistically improve outcomes (Ploughman et al., 2007).

Research in animal models of dopamine deficiency has demonstrated ET-induced behavioral recovery (Tillerson et al., 2003), as well as ET-induced neuroplasticity (Fisher et al., 2004; Petzinger et al., 2007) and functional brain reorganization (Wang et al., 2013b). However, such studies have primarily utilized relatively simple ET paradigms using the horizontal treadmill (Fisher et al., 2004; Sung et al., 2012; Toy et al., 2014), rotarod (Holschneider et al., 2007), and running wheel (O'Dell et al., 2007; Wang et al., 2013b). Although these ET paradigms involve a certain level of motor skill learning, particularly in lesioned and functionally impaired animals, they are primarily considered aerobic tasks. Recently, we characterized in rats with intra-striatal 6-hydroxydopamine (6-OHDA) lesion, the behavioral improvements and functional brain reorganization induced by ET using simple running wheels (non-skilled aerobic exercise, NSAE) (Wang et al., 2013b). In the current study, we adapted a complex running wheel with irregularly spaced rungs as a tool for skilled aerobic exercise (SAE). This allowed us to assess in the 6-OHDA rat model whether SAE compared to NSAE resulted in greater motor recovery and different functional brain reorganization.

Materials and Methods

Animals

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institutional Animal Care and Use Committee of the University of Southern California. Sprague-Dawley (3-month old at the time of stereotaxic surgery) rats were purchased from Harlan Laboratories (Indianapolis, IN, USA) and housed in pairs on a 12-hr light/12-hr dark cycle with free access to water and rodent chow. Animals were randomized into two groups: Lesion/SAE/Walk ($n = 9$) and Lesion/SAE/Rest ($n = 8$), with 'Walk' and 'Rest' referring to the behavioral state of the animal at the time of blood flow mapping. Comparison was made to six other groups we reported on previously (Wang et al., 2013b): Lesion/NSAE/Walk ($n = 11$), Lesion/NSAE/Rest ($n = 12$), Lesion/No-ET/Walk ($n = 9$), Lesion/No-ET/Rest ($n = 10$), Sham/No-ET/Walk ($n = 10$), Sham/No-ET/Rest ($n = 9$), with a focus on the comparison between the SAE and NSAE groups. All experiments were performed by the same group of researchers in the same place, with the same equipment and conditions. We chose not to repeat the previously reported experiments in conformation to the reduction and refinement directives of animal welfare. Nevertheless, we acknowledge that not running all experiments in parallel is a caveat.

Overview of the experimental protocol

The protocol has been described in detail previously (Wang et al., 2013b). As shown in Fig. 1, the animals were trained in motor tests, and their baseline motor performance was measured prior to the stereotaxic surgery. Motor performance was measured once a week thereafter. Animals were allowed two weeks of recovery for the lesion to mature. Starting in

Week 3, rats were subjected to forced exercise training, either in a simple running wheel for NSAE, or in a complex running wheel with irregularly spaced rungs for SAE, or sham training (No-ET) for 4 weeks. At the beginning of Week 7, animals were intravenously cannulated and allowed to recover for 4 days. Cerebral blood flow (CBF) mapping experiments were performed at the end of Week 7 while the animals were either walking on a horizontal treadmill (Walk) or resting on a stopped treadmill (Rest). Whole brain sectioning was performed, followed by autoradiography and tyrosine hydroxylase (TH) staining for the quantification of dopaminergic lesion. For discussion of behavioral and immunohistochemical data, the 'Walk' and 'Rest' animals were merged into 4 large groups, Lesion/SAE ($n = 17$), Lesion/NSAE ($n = 23$), Lesion/No-ET ($n = 19$), and Sham/No-ET ($n = 19$). In the current study, rats were trained on the running wheel, with motor function tested on different motor tasks such as the rotarod and beam crossing. This avoided a potential confound of learning effects on the motor outcome measures in favor of the exercised groups. For the same reason, functional brain mapping was performed during walking on a horizontal treadmill – a new motor task easy enough for all animals to perform.

Animal model and stereotaxic surgical procedure

The 6-OHDA basal ganglia injury model is a widely accepted acute model of dopaminergic deafferentation, associated with motor deficits of PD (Cenci et al., 2002). To prevent any noradrenergic effects of the toxin, animals received desipramine (25 mg/kg in 2mL/kg bodyweight in saline, i.p., Sigma-Aldrich Co., St. Louis, MO, USA) before the start of surgery. They were then placed under isoflurane anesthesia (1.5% in 30% oxygen and 70% nitrous oxide) in a stereotaxic apparatus (David KOPF Instruments, Tujunga, CA, USA) and received injection of 6-OHDA (Sigma-Aldrich Co.) at four injection sites targeting the dorsal striatum (dorsal caudate-putamen, dCPu) bilaterally: anterior-posterior (AP) + 0.6, medial-lateral (ML) $\pm$ 2.7, dorsal-ventral (DV) – 5.1 mm, and AP – 0.4, ML $\pm$ 3.5, DV – 5.5 mm, relative to the bregma. Injection of 10 μ g of 6-OHDA dissolved in 2 μ L of 1% L-ascorbic acid/saline was made at each site through a 10 μ L Hamilton microsyringe (Hamilton Company, Reno, NV, USA) fitted with a 26 gauge, blunted needle, at 0.4 μ L/min controlled by a Micro4 microsyringe pump controller (World Precision Instruments, Sarasota, FL, USA). Toxin was made up fresh on the day of surgery from the same batch of 6-OHDA. Sham-lesioned rats received 4 injections of an equal volume of vehicle. After injection, the needle was left in place for 5 min before being slowly retracted at $\sim$ 1 mm/min.

Cannulation surgery

At the beginning of Week 7, animals were anesthetized (isoflurane 2% in 30% oxygen and 70% nitrous oxide). The right external jugular vein was cannulated with a 5 French silastic catheter (Dow Corning Corp., Midland, MI, USA), advanced into the superior vena cava. The port at the distal end of the catheter was tunneled subcutaneously and externalized dorsally in the region rostral to the scapula. All animals were allowed to recover for 4 days. Postoperatively, the catheter was flushed daily to ensure patency (0.3 mL of sterile 0.9% saline, followed by 0.1 mL saline containing 40 unit/mL heparin).

Skilled and non-skilled aerobic exercise training

Liebetanz et al. (2007) used complex running wheels with irregularly spaced rungs in a voluntary setting for the detection of motor deficits in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mice, which displayed a reduced maximum speed and running distance compared to control mice. Motor deficits in PD subjects have been examined with a similar test that requires subjects to step on a treadmill over randomly approaching obstacles on either side (Michel et al., 2009). Here, we adapted the complex running wheel as a training tool in a forced, skilled ET paradigm (SAE) that incorporated both motor skill learning and aerobic exercise. In the SAE paradigm, running wheels (wheel diameter 34.4 cm) with irregularly spaced rungs (rung diameter 0.3 cm) were used as depicted in Fig. 2A. A pseudo random pattern of rung spacing was achieved by repeating a pattern OOOOXOX, with O indicating a rung and X indicating a missing rung (regular inter-rung spacing 1.3 cm). For non-skilled aerobic exercise (NSAE), we used simple running wheels with regularly placed rungs and an inner plastic ‘floor’ covering the metal rungs (Fig. 2B). Pilot experiments showed that the modification made it easy for lesioned rats to learn, and therefore minimized the “motor skill” factor.

Starting in Week 3, animals assigned to the ET groups were trained in running wheels (Lafayette Instrument, Lafayette, IN, USA) for 20 min/day, 5 consecutive days/week with no training over the weekends. No-ET animals were handled and left in a stationary running wheel for 30 min/day. On the day of training, the animal was placed in a running wheel for 5 min, followed by four 5-min running sessions, with 2-min inter-session intervals. In Week 3, animals were habituated to the wheels and trained with a starting speed of 2 m/min. For the NSAE animals, speed was ramped up with either 1 or 2 m/min increments from session to session within a day, and each day’s starting speed was progressively increased depending on how fast the animals learned. Starting in Week 4, we applied an *adaptively challenging paradigm* for ET training (Fig. 2C). The purpose was to train the animal progressively at the highest speed without causing significant stress. Defecation and urination were monitored as signs of acute stress responses. A failure was defined as the animal falling behind the speed of the turning wheel, allowing its body to reach a near vertical position at the back of the wheel before getting tossed over (with both hindpaws airborne). The following rules were applied in adjusting the speed. If the animal had no more than 1 failure and showed no sign of stress over 2 consecutive sessions, the speed was increased by 1 m/min. If the animal had as many as 6 failures in a single session, the session was ended and the speed was decreased by 1 m/min in the next session. It is important to note that the paradigm was too conservative for the initial learning phase of the NSAE rats, and therefore was not applied to these animals in Week 3. In Week 7, for two days prior to the CBF experiments, rats were exercised for two 5-min sessions at half of their training speed at the end of Week 6.

For SAE animals, a simple wheel was used on the 1st day to familiarize the animals to the wheel walking task, and a complex wheel was used for the rest of training. The adaptively challenging paradigm for speed adjustment was applied to the SAE animals starting in Week 3. SAE and NSAE were titrated to a level of near maximal effort. Therefore, the ET protocols were matched for relative intensity, but not absolute intensity (running speed). In practice, the slower wheel speed of SAE animals is not challenging enough to engage

animals trained in the simple wheel. At nonchallenging speed, NSAE animals tend to exhibit behaviors in simple wheels that disrupt the ET, such as turning around, walking backward, and sitting.

Assessment of motor deficits

Accelerating rotarod: Rats were familiarized with the rotarod (Columbus Instruments, Columbus, OH, USA, spindle diameter 7.3 cm) starting 1 week before the stereotaxic surgery. For 2 consecutive days, rats were run on the rotarod at 10 rpm (2.29 m/min) for 3 min each day. For the next 3 days, rats were run using an acceleration paradigm (initial speed: 5 rpm = 1.15 m/min, acceleration rate: 6 rpm/min = 1.38 m/min², 2 trials/day, 30-min intertrial interval) until they fell onto a padded surface or reached the 5 min cutoff time (maximum speed: 35 rpm = 8.02 m/min).

Rearing: No prior training was required for this test. The rearing test was always the first motor test of the day. The room was illuminated with lights from an adjacent room through a half opened door. The animal was habituated to the room for 15 min. It was then put in an arena for 5 min. The number of rearings was counted manually from video recordings. A rearing was defined as when the animal lifted both forepaws off the ground. To maintain the level of novelty, arenas of different shapes and wall decorations were used. Olfactory cues were minimized by wiping the arena with 1% ammonia solution between animals.

Beam crossing: Rats were familiarized with the paradigm 1 week prior to the stereotaxic surgery. A beam was placed horizontally and fixed with tape between a small platform (6 × 8 cm) and the edge of a bench, 80 cm apart. The beam was 50 cm from the floor. A Plexiglas black box (L: 30, W: 15, H: 15 cm) was placed on the edge of the bench, with its opening facing the small platform. The room light was turned off and a lamp illuminated the small platform from 50 cm above. For a trial of beam crossing, the animal was put on the small platform with its head facing the black box. The time for the animal to cross the 80 cm distance was recorded. The animal was left in the box for 20 s at the end of each trial before being returned to its homecage. On the first day of training, the animal was first put into the black box and allowed to explore for 20 s. It was then trained for 3 trials using a 2.5 × 2.5 cm square beam, followed by 3 trials using a 1.3 × 1.3 cm square beam, with 3-min intertrial intervals. The animal was guided by the experimenter during the first few trials if necessary. Over the next 5 days of training, as well as in future weekly testing, the animal was tested for 3 trials/session using the 1.3 × 1.3 cm square beam, followed by 3 trials using a round beam (diameter = 1.3 cm) with 3-min intertrial intervals each day. In a few trials, when lesioned rats failed to complete the round beam crossing test by falling off the beam, the time to cross was recorded as 300% of the baseline.

Adjusting footstep: For 3 days before baseline testing, rats were habituated to the handling by the experimenter for 3 min/day, including the grip as described below. Baseline data were measured for the next two days. The experimenter held the rat with one hand fixing the hindlimbs with a towel, and the other fixing the forelimb not tested. The hind part of rat was slightly raised above the table surface with the tested paw touching the surface and bearing some bodyweight. The rat was then moved slowly sideways (0.9 m in 5 s), first in the

forehand and then in the backhand direction. The number of adjusting footsteps was counted for both forepaws in the backhand and forehand direction of movement (Olsson et al., 1995). The test was repeated twice each day with a 5-min intertrial interval.

Brain Mapping

All animals were habituated to a horizontal treadmill for 4 days: 2 days at the end of Week 6 and 2 days prior to cerebral perfusion experiments in Week 7. They were individually placed on a stationary, horizontal treadmill (L: 50 cm, W: 7 cm) for 10 min followed by 3 min of walking at 8 m/min. Front and side panels (H: 30 cm) guided the animal's motion forward. On the day of the perfusion experiment, the animal was allowed to rest in the treadmill for 10 min. A piece of silastic tubing was filled with radiotracer [^{14}C]-iodoantipyrine (125 $\mu\text{Ci/kg}$ in 300 μL of 0.9% saline, American Radiolabelled Chemicals, St. Louis, MO, USA). The radiotracer-filled tubing was then connected to the animal's cannula on one end, and to a syringe filled with euthanasia agent (pentobarbital 50 mg/mL, 3M potassium chloride) on the other. The animal was allowed to rest for another 5 min. For animals assigned to the 'Walk' condition for CBF, the treadmill was then turned on and set at 8 m/min, while it remained off for animals assigned to the 'Rest' condition for CBF. After 2 min, the radiotracer was infused at 2.25 mL/min by a motorized pump, followed immediately by 0.7 mL of the euthanasia solution, which resulted in cardiac arrest within ~ 10 s, a precipitous fall of arterial blood pressure, termination of brain perfusion, and death. This approach uniquely allowed a 3-dimensional (3-D) assessment of functional activation in the awake, unrestrained animal, with a temporal resolution of $\sim 5\text{--}10$ s and an in-plane spatial resolution of $100\text{ }\mu\text{m}^2$ (Holschneider et al., 2002). Time of day and duration of testing, persons performing the testing, lighting, ambient sound levels, room temperature were kept constant between sessions. Olfactory cues were minimized by wiping the treadmill with a 1% ammonia solution.

Autoradiography

Brains were removed, flash frozen at $\sim -55^\circ\text{C}$ in methylbutane over dry ice and serially sectioned for autoradiography (57 coronal 20- μm slices, 300- μm interslice distance beginning at ~ 4.5 mm anterior to bregma). Autoradiographic images of brain slices were digitized on an 8-bit gray scale using our prior methods (Nguyen et al., 2004). CBF related tissue radioactivity was measured by the classic [^{14}C]-iodoantipyrine method (Sakurada et al., 1978). In this method, there is a strict linear proportionality between tissue radioactivity and CBF when the data is captured within a brief interval (~ 10 s) after the tracer injection (Jones et al., 1991).

Image Analysis

A 3-D reconstruction of each animal's brain was conducted using 57 serial coronal sections (starting at $\sim$ bregma + 4.5 mm) with a voxel size of $40\text{ }\mu\text{m} \times 300\text{ }\mu\text{m} \times 40\text{ }\mu\text{m}$ (Nguyen et al., 2004). Adjacent sections were aligned manually in Photoshop (version 9.0, Adobe Systems Inc., San Jose, CA, USA) and using TurboReg, an automated pixel-based registration algorithm implemented in ImageJ (version 1.35, <http://rsbweb.nih.gov/ij/>). This algorithm registered each section sequentially to the previous section using a nonwarping geometric model that included rotations and translations (rigid-body transformation) and

nearest-neighbor interpolation. One “artifact free” brain was selected as reference. All brains were spatially normalized to the reference brain by statistical parametric mapping (SPM, version 5, Wellcome Centre for Neuroimaging, University College London, London, UK). Spatial normalization consisted of applying a 12-parameter affine transformation followed by a nonlinear spatial normalization using 3-D discrete cosine transforms. All normalized brains were then averaged to create a final rat brain template. Each original brain was then spatially normalized to the template. Normalized brains were smoothed with a Gaussian kernel ($\text{FWHM} = 3 \times \text{voxel dimension}$). Voxels for each brain failing to reach a specified threshold in optical density (70% of the mean voxel value) were masked out to eliminate the background and ventricular spaces without masking gray or white matter. To account for any global differences in the absolute amount of radiotracer delivered to the brain, adjustments were made by the SPM software in each animal by scaling the voxel intensities so that the mean intensity for each brain was the same (proportional scaling). Prior work has demonstrated that 6-OHDA striatal lesions in rats result in no significant difference in absolute cerebral blood flow (Lindvall et al., 1981). To identify brain regions showing significant changes in rCBF as the main effect of Skill and Walk, as well as Skill $\times$ Walk interaction, we ran factorial analyses among the 4 ET groups (Lesion/SAE/Walk, Lesion/SAE/Rest, Lesion/NSAE/Walk, Lesion/NSAE/Rest). Threshold for significance was set at $P < 0.05$ at the voxel level and an extent threshold of 100 contiguous voxels. This combination reflected a balanced approach to control both Type I and Type II errors. The minimum cluster criterion was applied to avoid basing our results on significance at a single or small number of suprathreshold voxels. Brain regions were identified according to a rat brain atlas (Paxinos and Watson, 2007) and data interpretation was focused on gray matter. The directionality of rCBF changes was analyzed with nonbiased, voxel-by-voxel Student's *t*-tests contrasting Walk and Rest, as well as SAE and NSAE. Threshold for significance was set at $P < 0.05$ at the voxel level and an extent threshold of 100 contiguous voxels.

Functional connectivity analysis

We applied seed-ROI (region of interest) correlation analysis to assess functional connectivity of the prelimbic cortex, as well as of the cerebellar vermis (Wang et al., 2013a). Structural ROIs were hand drawn in MRIcro (version 1.40, <http://cnl.web.arizona.edu/mricro.htm>) over the template brain according the rat brain atlas: one in the right hemisphere of the PrL and one in the gray matter layer of the cerebellar lobule 2 near the midline. The structural ROIs were then intersected with clusters defining regional functional activation showing the main effect of Skill in the SPM factorial analysis to create functional seed ROIs. Mean optical density of the seed ROIs were extracted for each animal using the MarsBaR toolbox for SPM (version 0.42, <http://marsbar.sourceforge.net/>). Correlation analysis was performed in SPM for each group using the seed values as a covariate. Threshold for significance was set at $P < 0.05$ at the voxel level and an extent threshold of 100 contiguous voxels. Regions showing significant correlations (positive or negative) in rCBF with the seed ROI are considered functionally connected with the seed.

Correlation analysis of regional cerebral blood flow and motor function

We applied correlation analysis to assess relationship between the functional activation of the prelimbic cortex during walking and the animal's motor function following ET. Within-

group correlation was calculated for the SAE/Walk and the NSAE/Walk group. To remove nonlinearities in the raw scores of motor tests, animals in each group were ranked for each test such that the animal with the worst performance was ranked #1. Correlation analysis was then performed in SPM for each group and each motor test using the ranking number as a covariate. Threshold for significance was set at $P < 0.05$ at the voxel level and an extent threshold of 100 contiguous voxels.

Tyrosine hydroxylase staining of dopaminergic neurons

Staining was done in batches of 6–8 rats as the experiments progressed. Sections from a subgroup of animals of each treatment group were fixed for 10 min at RT with 2% PFA, rinsed with PBS, and quenched with 0.3% H_2O_2 +0.3% NGS in PBS for 30 min at RT. After rinsing in PBS, slides were blocked with 4% NGS/x1 PBS and incubated overnight at 40C in primary antibody solution (1:2000 anti-tyrosine hydroxylase, clone LNC1, Millipore, Billerica, MA in 2% NGS/x1 PBS). Sections were washed the following day in PBS and incubated in secondary antibody solution (biotinylated anti-mouse IgG, Vectastain Elite ABC kit) 1:2500 in 2% NGS/x1 PBS, 30 min at RT. Staining was developed with DAB (10mg DAB+10 μ l 30% H_2O_2 in 10mL PBS), until optimal contrast on sections was achieved. Sections were then dehydrated, mounted, and dried overnight. The striatum, substantia nigra compacta (SNc), and cerebral hemisphere were defined bilaterally in the digitized, thresholded images of each rat by manual tracing using ImagePro Plus 4.0 (Media Cybernetics, Rockville, MD, USC) (striatum: 6 coronal sections anterior and 3 sections posterior to bregma, AP: + 1.56 mm $\rightarrow$ - 1.14 mm; substantia nigra: 5 coronal sections at AP: - 5.20 mm $\rightarrow$ - 6.40 mm; 300 μ m interslice distance). Lesion of the striatum were evaluated as reduction in the optical density (OD) of the striatum. OD values were normalized in each animal by values obtained in the corpus callosum using the formula $1000 * \log_{10}(\text{OD}_{\text{corpus callosum}} / \text{OD}_{\text{striatum}})$. Lesion of the SNc was similarly evaluated as changes in normalized optical density. We have chosen to use densitometry rather than stereology to simplify the evaluation of lesion in the SNc. Comparable results in the SNc using both methods have been shown in intrastriatal 6-OHDA-lesioned rats (Xavier et al., 2005).

Statistical analysis

Motor tests data were normalized to each animal's baseline value and presented as percentages (mean $\pm$ SEM). Week 6 motor test data and postmortem TH staining data were analyzed using one-way ANOVA, followed by Fisher's Least Significant Difference (LSD) *post hoc* test in SPSS (version 13.0, IBM, Armonk, NY, USA). $P < 0.05$ was considered statistically significant.

Results

Skilled exercise resulted in partial recovery in tyrosine hydroxylase staining in the striatum

The SAE paradigm using complex running wheels was highly demanding, requiring rapid adjustment of footsteps throughout the ET period. In contrast, the NSAE paradigm using simple running wheels was predominantly an aerobic task, which once acquired became largely automatic. The final speed achieved by the NSAE group in our adaptively

challenging ET paradigm was significantly greater than that of the SAE group (5.4 ± 0.5 vs. 4.2 ± 0.2 m/min at the end of Week 6, $P < 0.05$, Student's *t*-test).

Bilateral, intrastratial injection of 6-OHDA significantly reduced the optical density of TH staining bilaterally in the striatum and SNc (Fig. 3A, 3C, Lesion/No-ET vs. Sham/No-ET). NSAE further significantly reduced TH staining in the striatum and SNc (Fig. 3, Lesion/NSAE vs. Lesion/No-ET). Skilled compared to non-skilled exercise increased TH staining significantly in both the striatum and SNc. There was no significant difference in TH optical density between the Lesion/SAE and Sham/No-ET, and between the Lesion/SAE and Lesion/No-ET groups.

Compared to non-skilled exercise, skilled exercise resulted in equal or greater improvement in motor deficits

Motor test results at the end of Week 6 are summarized in Fig. 4. There were statistically significant differences between groups ($P < 0.001$) in all motor tests determined by one-way ANOVA. LSD *post hoc* tests revealed significant differences between pairs of groups. Lesion-induced motor deficits were apparent in all motor tests (Lesion/No-ET vs. Sham/No-ET). Compared to Lesion/No-ET, both NSAE and SAE significantly improved motor function in all tests. Both NSAE and SAE recovered the motor performance to sham group level in rearing, with SAE additionally recovering performance in square and round beam crossing, and 3 of the adjusting footstep tests. Furthermore, SAE compared to NSAE, resulted in significantly greater improvement in the round beam crossing and adjusting footstep tests (left forehand, left backhand, and right backhand).

Effects of skilled exercise on functional brain activation (Figs. 5, 6, Tables 1, 2)

Factorial analysis was performed in SPM in a 2×2 design with Skill (SAE or NSAE) and Walk (Walk or Rest) as the factors. The direction of changes was further analyzed in SPM *t*-tests contrasting two groups at a time. The results are presented with an emphasis on motor related regions. A complete list of results may be found in Tables 1 and 2.

Main effect of Walk—Fig. 5 displays brain regions showing main effect of Walk in rCBF and those showing Walk-induced changes in rCBF (Walk vs. Rest) in SAE and NSAE animals. Main effect of Walk was seen broadly in the prefrontal, motor, sensory cortices, basal ganglia, cerebellum, thalamus, limbic and paralimbic areas, as well as the brainstem.

The SAE and NSAE animals showed similar general pattern of Walk-induced rCBF changes with important differences. In both SAE and NSAE animals, Walk compared to Rest resulted in increased rCBF in the primary and secondary motor cortex (M1, M2, posterior aspect) and the cerebellar vermis (Cb), with decreased rCBF noted in the substantia nigra (SN), globus pallidus (GP), thalamus (ventrolateral, VL; central medial, CM) and superior colliculus (SC). Also noted was increased rCBF in the primary somatosensory (barrel field, S1BF; dysgranular zone, S1DZ; forelimb, S1FL; shoulder, S1Sh; trunk, S1Tr), secondary somatosensory (S2, posterior aspect), parietal (medial association, MPtA; lateral association, LPtA; dorsal posterior area, PtPD; rostral posterior area, PrPR), primary and secondary visual (V1, V2) cortices. Decreased rCBF was noted in the jaw and upper lip areas of the S1

(S1J, S1ULp), insular cortex (Ins), and the anterior aspect of S1FL, S1DZ, and S2. An important difference between SAE and NSAE was that activation in the dorsal caudate-putamen (dCPu, including dorsolateral and dorsomedial areas) was noted in the NSAE (and in Sham/No-ET animals, data not shown), but not the SAE animals.

In both SAE and NSAE animals, Walk induced increased rCBF in limbic and paralimbic brain regions including, the dorsal hippocampus (dHPC), lateral septum (LS), as well as in the prelimbic (PrL), infralimbic (IL), cingulate (area 1, Cg1; area 2, Cg2), entorhinal (dorsolateral, DLEnt; dorsal intermedial, DIEnt; ventral intermedial, VIEnt; medial, MEnt; central, CEnt), and retrosplenial (RS) cortices. Decreased rCBF was noted in the amygdala, ventral CPu (vCPu), periaqueductal gray (lateral, LPAG; dorsolateral, DLPAG; dorsomedial, DMPAG), and raphe (rostral linear n., RLi; dorsal part of dorsal n., DRD; median n., MnR). Whereas NSAE compared to SAE animals showed greater activation in the dHPC and greater deactivation in the brainstem, SAE animals showed greater deactivation in the amygdala.

Main effect of Skill and Skill $\times$ Walk interaction—Fig. 6 displays brain regions showing the main effect of Skill and the Skill $\times$ Walk interaction in rCBF, as well as those showing Skill-related differences in rCBF (SAE vs. NSAE) during Walk and Rest. Main Skill effect was seen in the prelimbic cortex (during Walk and Rest, SAE $>$ NSAE in rCBF), primary somatosensory cortex S1Tr, S1BF, S1ULp (during Walk and Rest, SAE $>$ NSAE), medial CPu (during Rest, SAE $<$ NSAE), and insular cortex (during Walk, SAE $<$ NSAE). Main Skill effect and Skill $\times$ Walk interaction were noted in the dorsal CPu (during Walk, SAE $<$ NSAE), globus pallidus (during Walk, SAE $<$ NSAE), dorsal hippocampus (during Walk, SAE $<$ NSAE; during Rest, SAE $>$ NSAE), and cerebellar vermis (during Walk, SAE $>$ NSAE). Significant Skill $\times$ Walk interaction was also noted in the inferior colliculus (IC) and auditory cortex (during Walk, SAE $>$ NSAE; during Rest, SAE $<$ NSAE).

Skilled exercise induced changes in functional connectivity of the prelimbic cortex and cerebellum

Seed correlation analysis revealed functional connectivity of the right PrL in the Walk state (Fig. 7). SAE compared to NSAE animals showed more widespread positive functional correlation with the ipsilateral motor cortex (M1 and M2), bilateral primary somatosensory cortex (S1FL, S1J, S1DZ), ipsilateral dorsal CPu, and bilateral ventral CPu, as well as more negative functional correlation with the cerebellar vermis.

Seed correlation analysis also revealed alterations in functional connectivity of the cerebellar vermis during treadmill walking (Fig. 8). In the SAE animals, the cerebellar seed showed significant positive correlation with the posterior M1/M2, primary somatosensory cortex (S1BF, S1HL, S1Sh) and thalamus (ventral anterior/ventrolateral, VA/VL), as well as negative correlation with the PrL, Cg2, S1ULp, Ins, S1ULp, and GP. In the NSAE animals, the cerebellar seed was positively correlated with the S1BF, S2, and Pir, and negatively correlated with anterior M1, dCPu, vCPu, Ins, GP, and Ce.

Correlation between functional activation of the prelimbic cortex during walking and motor function

In the SAE animals, performance in the round beam crossing, square beam crossing, and rearing (but not the other motor tests, data not shown) was positively correlated with rCBF in the prelimbic cortex during treadmill walking. Significant correlation was bilateral, and present broadly across the anterior-to-posterior extent of the PrL (Fig. 9, top panel). In the NSAE animals, the correlation of rCBF of the PrL and the motor outcome measures was present in a more limited fashion, being significant for only a subregion of the left PrL for the square beam crossing and rearing tests (Fig. 9, lower panel).

In both the SAE and NSAE animals, performance in the rotarod and rearing, but not the other motor tests, was *negatively* correlated with rCBF in areas of the cerebellum (data not shown).

Discussion

Petzinger et al. (2013) recently proposed that incorporating intensive and challenging goal-based practice in combination with aerobic training may synergistically enhance both cognitive (volitional) and automatic (unconscious) motor control in mild to moderate PD patients. Studies to date on the effects of exercise in rodent models of dopamine deficiency have used relatively simple and primarily aerobic exercise paradigms. In the current study, we applied a complex running wheel for skilled aerobic exercise training (SAE). In contrast, nonskilled aerobic exercise (NSAE) in simple running wheels remained primarily an aerobic challenge. Using the 6-OHDA rat model of PD, we compared the effects of SAE and NSAE on the nigrostriatal dopaminergic system, motor function, and functional brain reorganization. To our best knowledge, this is the first neuroimaging study to delineate differences in the neural substrates of neurorehabilitation following skilled and non-skilled aerobic exercise in a rodent model of PD.

The animal model

The 6-OHDA lesioning model in the rat is a well-characterized rodent model for dopamine deficiency (Cenci et al., 2002). As we reported previously (Wang et al., 2013b), bilateral intrastratial injection of 6-OHDA caused decreases in TH optical density in the striatum and substantia nigra pars compacta, consistent with retrograde cell death from the injured fibers terminating in the dorsal striatum (Cadet et al., 1991; Blandini et al., 2007). Significant deficits were observed in all behavioral tests compared to sham animals (Fig. 4). The lesion is considered “moderate” according to the classification by Schwarting and Huston (1996). The modest TH loss in the dorsal caudate-putamen and substantia nigra in our study is in line with that observed in PD patients shortly after diagnosis, as reported by Kordower et al. (2013). Notwithstanding the limits of the 6-OHDA model for PD in general, our protocol produced a rodent model of dopaminergic deafferentation in which motor deficits were apparent, yet the animals remained functional enough to carry out vigorous, daily exercise training. This research is therefore relevant to using exercise training for neurorehabilitation in subjects with mild to moderate PD. Exercise training was initiated 2 weeks after 6-OHDA administration when lesion maturation was well underway (Sauer and Oertel). This allowed

us to examine primarily the neurorestorative effects of exercise, rather than its neuroprotective effects on attenuating 6-OHDA toxicity (Tillerson et al., 2003; Yoon et al., 2007; Real et al., 2013). While most studies of 6-OHDA examined unilateral lesions, we chose bilateral lesion to minimize compensation by the contralateral side in a unilateral setting (Yang et al., 2007).

Effect of exercise on tyrosine hydroxylase staining

Review of the literature has emphasized that the extent of TH changes following neurotoxic brain injury and exercise is dependent on a number of factors, including prelesion motor training, exercise type, intensity and duration. Exercise-related increases (Yoon et al., 2007; Foley and Fleshner, 2008), decreases (Hattori et al., 1994; Petzinger et al., 2007; Mabandla et al., 2009), as well as no change (O'Dell et al., 2007) have been reported, and there may be a variable time course of TH changes following ET (Hattori et al., 1994) and possibly a stress effect (Howells et al., 2005). In our study, the relatively low level of effect on the measures of TH in lesioned, non-exercised rats may have been due to neuroprotection from motor enrichment as a result of the 1-week motor tests training prior to stereotaxic surgery. Pretraining prior to lesioning has been previously reported to significantly attenuate loss of TH (Cohen et al., 2003; Faherty et al., 2005; Real et al., 2013). An additional factor, as noted above, was that exercise was delayed for 2 weeks until lesion maturation was largely complete. This may have attenuated the impact of NSAE in 'rescuing' TH, as some have shown when exercise is initiated within days of lesioning (Tillerson et al., 2003; Yoon et al., 2007; Real et al., 2013). The differential loss of TH in lesioned rats receiving NSAE compared to those receiving SAE suggests the possibility that the higher running speed in the NSAE group may have played a role in the magnitude of TH decrease. Indeed, treadmill training at high intensity has been reported to induce decreases in striatal TH staining in naïve rats (Hattori et al., 1994), and in MPTP-lesioned mice while improving motor function (Petzinger et al., 2007).

Effects of skilled aerobic exercise training on lesion-induced motor deficits

As we reported previously (Wang et al., 2013b), 6-OHDA lesion caused significant deficits in all of the motor tests, while NSAE resulted in significant improvement in all motor tests. SAE, despite being performed at a lower speed than NSAE led to equal or greater motor improvement compared to NSAE (Fig. 4). SAE compared to NSAE, resulted in significantly greater improvement in the round beam crossing and adjusting footstep tests. The better performance in beam crossing and adjusting footstep tests likely reflects improved balance and agility as a result of motor skill learning in the complex wheel. Our behavioral and neuroimaging findings (described below) were based on motor tasks different from the exercise paradigm, and therefore reflected a transfer of motor skill learned. A central question of motor learning is if performance improvements can generalize beyond the training task and context, and what factors contribute to such a generalized learning outcome (Green and Bavelier, 2008). Recent findings from behavioral studies suggest that subjects may be able to acquire general, transferable knowledge about skill learning processes (Seidler, 2004; Bhatt and Pai, 2009; Wang et al., 2011; Schaefer et al., 2013). However, the ability to interpolate new motor solutions based on prior learning experience is believed to be limited largely to tasks that have relatively similar requirements, and may share some of

the building blocks at different levels of the motor hierarchy (“motor primitives”) (Thoroughman and Shadmehr, 2000; Flash and Hochner, 2005). At the level of brain circuits, such generalization may be the result of learning-induced neuroplasticity in overlapping motor circuits. SAE in the lesioned rat led to equal or greater motor improvement compared to NSAE. Differences were significant for round beam crossing and adjusting footstep tests, which likely involved greater coordination of fine motor skills than the rearing and accelerating rotarod tests. This suggests that SAE compared to NSAE may provide the animal with a modestly enhanced ability to generalize a trained motor task, with largest effects noted for task requiring skilled limb placement. Similar findings have been made previously by Ploughman et al. in a rodent stroke model in which forelimb reach training improved recovery on a different skilled reaching task, but did not transfer to gross motor skills such as postural support and overall gait (Ploughman et al., 2007).

Regarding the adjusting steps, variability in data cannot be ruled out to account for the discrepancy between right forehand and left forehand. Given the bilateral nature of 6-OHDA lesion and the exercise paradigms, unilateral changes were not expected. A possible explanation is that rats may show limb preferences during motor tasks, which may result in laterality in their motor recovery. For example, it has been shown in monkeys that laterality can affect recovery of hand motor function after motor cortical injury (Darling et al., 2013).

Functional brain activation in response to treadmill walking

Treadmill walking elicited a similar overall pattern of functional brain activation in SAE and NSAE animals (Fig. 5, t-tests), a pattern also noted in 6-OHDA rats without ET and in sham rats (see Wang et al., 2013b). Treadmill walking was associated with activation in the motor and somatosensory cortices, cerebellum (vermis) and hippocampal formation (dorsal hippocampus, subiculum, entorhinal cortex). Deactivation was noted in the basal ganglia (substantia nigra, globus pallidus, caudate-putamen) and thalamus. These responses in motor areas are consistent with those reported previously (Holschneider et al., 2003; Nguyen et al., 2004). This general consistency in brain responses during similar motor tasks across multiple independent datasets provides validation for the reproducibility of the perfusion-based functional brain mapping method.

Functional brain reorganization following skilled aerobic exercise training

Prefrontal cortex and cognitive motor control—SAE compared to NSAE animals showed greater rCBF in the prelimbic cortex during both the Walk and the Rest state (Fig. 6). Seed correlation analysis in the Walk state further revealed that the PrL had broader functional connectivity in the SAE animals with the primary and secondary motor cortices, primary somatosensory cortex, dorsal and ventral caudate-putamen, and cerebellum. In addition, in the SAE animals, performance in the round beam crossing, square beam crossing, and rearing was significantly, bilaterally and positively correlated with rCBF in the PrL during treadmill walking. In the NSAE animals, on the other hand, the correlation was more limited, being significant only for the square beam crossing and rearing tasks and largely restricted to the left hemisphere.

The key roles of the PrL in executive and cognitive functions have been the subject of extensive research (Miller and Cohen, 2001). The PrL is a multifunctional region implicated in motor control, attentional set shifting (Newman and McGaughy, 2011), risk-based decision making (St Onge and Floresco, 2010), and spatial working memory (Ragozzino et al., 2002), among other functions. Excitotoxic lesions of the PrL and neighboring infralimbic cortices disrupt motor preparatory processes in the rat (Risterucci et al., 2003). The SAE paradigm likely required greater efforts in motor preparatory processing, motor control and set shifting than that required for the NSAE, and therefore involved the PrL more functionally. This made the PrL and its associated motor pathways a central target for experience-dependent neuroplasticity as a result of SAE.

The PrL in rodents is generally considered a part of the medial prefrontal cortex, with features of dorsolateral prefrontal cortex (DLPFC) (Uylings et al., 2003), the pregenual anterior cingulate cortex (Vogt et al., 2013) and dorsomedial prefrontal cortex (Balleine and O'Doherty, 2010) of primates. In humans, the dorsomedial prefrontal cortex has been implicated in top-down control of motor cortex for inhibition of inappropriate motor responses (Narayanan et al., 2006; Narayanan and Laubach, 2006), with FC increasing between the frontal lobes and basal ganglia during set shifting (Nagano-Saito et al., 2008). Prefrontal areas are also critically involved in the early stage of motor learning, referred to as fast motor skill learning (Doyon and Benali, 2005; Dayan and Cohen, 2011) or cognitive stage of learning (Nieuwboer et al., 2009). In humans, the DLPFC has been implicated in this learning stage, showing decreased activation and functional coupling as learning progresses and performance improves (Floyer-Lea and Matthews, 2005; Sun et al., 2007). Changes in prefrontal and frontal areas in response to long-term exercise training have also been reported in human subjects (Harada et al., 2004; Kemppainen et al., 2005; Voss et al., 2010). Our findings of PrL hyperactivation and increased FC with other motor areas are consistent with experience-dependent enhancement of the prefrontal area in terms of cognitive motor control.

Early stages of PD are characterized by deficits in executive function, including internal control of attention, set shifting, planning, inhibitory control, dual task performance, and decision-making tasks (Dirnberger and Jahanshahi, 2013). Decreases in the regional CBF in the DLPFC and DLPFC-associated cognitive impairment have been shown to correlate with decline in executive function and clinical progression of PD in later stages (Kikuchi et al., 2001; Zgaljardic et al., 2006). Therefore, an ET paradigm that engages the prefrontal cortex and thereby induces experience-dependent neuroplasticity in the area may enhance cognitive motor control and result in improved motor function.

Motor cortex—Results of our factorial analysis showed a significant skill effect in M1 and M2 (Fig. 6). These results are consistent with earlier work in rats demonstrating that complex motor training, as opposed to simple motor activity, results in increased synaptogenesis and increased levels of the brain-derived neurotrophic factor receptor TrkB (Kleim et al., 1996; Klintsova et al., 2004). In human subjects, Pascual-Leone et al. using transcranial stimulation mapping have demonstrated that training on complex finger tasks compared to simple finger tasks produces significantly greater physiological changes (increased cortical excitability) in motor cortex (Pascual-Leone et al., 1995).

Striatum, cerebellum, and automatic motor control—Motor-learning based changes in the brain depend both on the stage of learning, as well as the type of motor task. While the striatum and the cerebellum are both involved in initial consolidation, these structures take on a specific role during later automatization, with the striatum being responsible for learning predictable motor sequences and the cerebellum being dominant in motor adaptation tasks (Doyon et al., 2009). Much of the work underlying these observations stems from human subjects performing finger and hand tasks during functional magnetic resonance imaging (fMRI). However, little is known regarding whether such observations generalize to a situation where motor sequence learning or motor adaptation are incorporated as part of aerobic exercise. Exercise in the complex running wheel was highly demanding for attention and rapid adjustment of footsteps throughout the training period. As such, the task had a significant component of constant motor adaptation. In contrast, exercise in the simple running wheel was predominantly an aerobic task that required the acquisition of sequential movements into a well-articulated behavior (motor sequence learning), and which once acquired became largely automatic. Our results in lesioned rats were consistent with a greater engagement of the cerebellum following SAE, and a greater engagement of the striatum following NSAE, as discussed below.

Previously we had shown that 6-OHDA rats without ET compared to sham rats showed hypoactivation of the dCPu during treadmill walking, as well as at rest. In addition, 6-OHDA lesions resulted in a loss of FC of this region with the motor network. Four weeks of NSAE partially restored the functional activation of the dCPu during walking (Wang et al., 2013b), as well as resting-state functional connectivity of the dCPu with the motor network (Wang et al., 2014). Neuroplasticity in the striatum following treadmill (low-skill aerobic) exercise has been documented in naïve rodents (Ferreira et al., 2010; Real et al., 2010; Garcia et al., 2012). In the dorsolateral striatum of MPTP mice, treadmill exercise induces increases in dopaminergic D2 receptor binding (Vuckovic et al., 2010) and a reversal of dendritic spine loss (Toy et al., 2014). A recent PET study showed that long-term treadmill exercise partly restores the level of dopamine D2 receptor binding in the striatum in early PD patients (Fisher et al., 2013). Such neuroplasticity in the dopaminergic signaling pathway may underlie the restoration of functional activation, as well as the reintegration of the dorsal striatum into the resting-state motor network we observed in 6-OHDA rats following NSAE (Wang et al., 2013b; Wang et al., 2014).

Animals that received SAE, in contrast to the NSAE animals, showed less activation of the dCPu, as well as greater deactivation (decreases in rCBF) of the globus pallidus, one of the major output sites of the basal ganglia. However, activation in the cerebellar vermis was greater, consistent with a greater recruitment of the cerebellar system in compensation of the basal ganglia dysfunction. The idea that the cerebellum is a supervised learning system dates back to the hypotheses of Marr (Marr, 1969) and Albus (Albus, 1971). PD-related hyperactivation of the cerebellum has been reported in neuroimaging studies in PD patients (Mentis et al., 2003; Werheid et al., 2003; Wu and Hallett, 2005) and in the 6-OHDA animal model (Yang et al., 2007). Our results on the differential effects of SAE and NSAE on the cerebellum are consistent with prior studies in naïve rats demonstrating that acrobatic exercise compared to mere motor activity elicits preferential morphological and functional

changes in the cerebellum (Black et al., 1990; Isaacs et al., 1992; Anderson et al., 1994; Kleim et al., 1997; Klintsova et al., 2004; Garcia et al., 2012). In particular, it has been suggested that the vermis and intermediate lobes play an important role in the control of the actual execution of movement by correcting for deviations from an intended movement through feedback comparison with information coming from the spinal cord (Shumway-Cook and Woollacott, 2001; Barlow, 2002). Seed analysis revealed that SAE compared to NAE animals showed greater positive functional correlation of the cerebellar midline area (lobule 2) with posterior M1/M2, primary somatosensory cortex and the ventral anterior/ventrolateral thalamus (Fig. 8). Our results are consistent with the known functional connection from the cerebellum to motor cortex (Townsend et al., 2006; Lu et al., 2012). That the SAE animals compared to NAE animals showed greater cerebellar activation but no activation of the dorsal striatum suggests that SAE preferentially enhanced the cerebellar-thalamocortical compensatory circuit, while NAE preferentially restored the function of the basal ganglia-thalamocortical circuit.

Hippocampus: A final point of interest was that functional brain activation in the dorsal hippocampus of NAE animals differed from that of SAE animals, with a significant Skill $\times$ Walk interaction (Fig. 6) and greater activation for the ‘walk versus rest’ comparison (Fig. 5). Our finding is consistent with a report in mice that running wheel exercise (non-skilled aerobic), but not acrobatic training (motor learning), improved hippocampus-dependent spatial memory as well as increased synaptophysin levels in the hippocampus (Lambert et al., 2005).

Limitations: Intensity is one of several critical parameters in locomotor training. In our study, ET protocols were matched for relative intensity based on an animal’s ability to maintain its position on the running wheel without slipping or signs of stress, but not for absolute intensity (running speed). This criterion attempted to model the clinical situation in which patients with different levels of disability are optimally challenged based on their physical ability. Additional work is needed to confirm if the skill effects reported in our study would be maintained if animals had been matched not by performance criteria but by an absolute measure of treadmill speed. Our data indicated that SAE, even when performed at a lower speed than NAE resulted in a modestly enhanced ability to transfer the trained motor task to other tasks. Additional motor testing is needed to discern the full extent of the transferability of SAE training.

We decided not to include sham animals with SAE and NAE in the present study for two main reasons. Sham rats, being less impaired than lesioned rats, are able to run at substantially higher speeds, both on the simple as well as on the complex wheels. Hence, controlling for both effort (intensity) and distance across groups presents a practical challenge. Second, lesioned rats likely recruit different, compensatory motor circuit during wheel running as compared to sham rats. Therefore, comparison of sham and lesioned rats may not be straightforward. Although not optimal, the current design is comparable to clinical studies in which the effects of exercise are assessed only in PD subjects, but not in healthy control subjects. Nevertheless, the effect of exercise in normal animals is of great

interest (Holschneider et al., 2007) and would need to be explored in a separate study that ideally would evaluate a dose response effect of training.

Our findings in TH staining confirmed 6-OHDA-induced loss of dopaminergic neurons. It would be important to confirm the current findings in absence of pre-lesion motor training, and under more severe dopamine deficiency. Since modulation of the dopaminergic system by exercise likely involves changes in dopamine transporters, receptors, release, and synthesis, a more multifaceted evaluation of dopaminergic function may help to elucidate the underlying mechanisms.

Conclusions

Skilled aerobic exercise training using complex running wheels was efficacious in alleviating lesion-induced motor deficits in 6-OHDA rats. In addition, it resulted in distinct functional brain reorganization compared to NSAE. SAE, despite being performed at a lower speed, led to equal or greater motor improvement compared to NSAE. SAE compared to NSAE showed greater functional activation of the prelimbic area of prefrontal cortex and the cerebellum, but less activation of the dorsal striatum. At the same time, SAE compared to NSAE animals showed greater functional connectivity of the PrL with motor areas. Our results suggest that skilled aerobic exercise results in enhanced compensatory motor control by the prefrontal cortex and the cerebellar-thalamocortical circuit. This improved understanding of the neural substrates of exercise-induced neurorehabilitation in Parkinsonian rats can critically inform future exercise regimens aimed at optimizing cognitive motor control for the treatment of PD.

Acknowledgements

This research was supported by a United States National Institute of Child Health & Human Development (NICHD) grant 1R01HD060630.

References

- Albus JS. A theory of cerebellar function. *Math Biosci.* 1971; 10:25–61.
- Anderson BJ, Li X, Alcantara AA, Isaacs KR, Black JE, Greenough WT. Glial hypertrophy is associated with synaptogenesis following motor-skill learning, but not with angiogenesis following exercise. *Glia.* 1994; 11:73–80. [PubMed: 7520887]
- Balleine BW, O'Doherty JP. Human and rodent homologies in action control: corticostriatal determinants of goal-directed and habitual action. *Neuropsychopharmacology.* 2010; 35:48–69. [PubMed: 19776734]
- Barlow, JS. *The cerebellum and adaptive control.* Cambridge, UK: Cambridge University Press; 2002.
- Bhatt T, Pai YC. Generalization of gait adaptation for fall prevention: from moveable platform to slippery floor. *J Neurophysiol.* 2009; 101:948–957. [PubMed: 19073804]
- Black JE, Isaacs KR, Anderson BJ, Alcantara AA, Greenough WT. Learning causes synaptogenesis, whereas motor activity causes angiogenesis, in cerebellar cortex of adult rats. *Proc Natl Acad Sci U S A.* 1990; 87:5568–5572. [PubMed: 1695380]
- Blandini F, Levandis G, Bazzini E, Nappi G, Armentero MT. Time-course of nigrostriatal damage, basal ganglia metabolic changes and behavioural alterations following intrastratial injection of 6-hydroxydopamine in the rat: new clues from an old model. *Eur J Neurosci.* 2007; 25:397–405. [PubMed: 17284180]

- Cadet JL, Last R, Kostic V, Przedborski S, Jackson-Lewis V. Long-term behavioral and biochemical effects of 6-hydroxydopamine injections in rat caudate-putamen. *Brain Res Bull.* 1991; 26:707–713. [PubMed: 1933391]
- Cenci MA, Whishaw IQ, Schallert T. Animal models of neurological deficits: how relevant is the rat? *Nat Rev Neurosci.* 2002; 3:574–579. [PubMed: 12094213]
- Cohen AD, Tillerson JL, Smith AD, Schallert T, Zigmond MJ. Neuroprotective effects of prior limb use in 6-hydroxydopamine-treated rats: possible role of GDNF. *J Neurochem.* 2003; 85:299–305. [PubMed: 12675906]
- Darling WG, Helle N, Pizzimenti MA, Rotella DL, Hynes SM, Ge J, Stilwell-Morecraft KS, Morecraft RJ. Laterality affects spontaneous recovery of contralateral hand motor function following motor cortex injury in rhesus monkeys. *Experimental brain research.* 2013; 228:9–24. [PubMed: 23652723]
- Dayan E, Cohen LG. Neuroplasticity subserving motor skill learning. *Neuron.* 2011; 72:443–454. [PubMed: 22078504]
- Dirnberger G, Jahanshahi M. Executive dysfunction in Parkinson's disease: a review. *Journal of neuropsychology.* 2013; 7:193–224. [PubMed: 24007368]
- Doyon J, Benali H. Reorganization and plasticity in the adult brain during learning of motor skills. *Curr Opin Neurobiol.* 2005; 15:161–167. [PubMed: 15831397]
- Doyon J, Bellec P, Amsel R, Penhune V, Monchi O, Carrier J, Lehericy S, Benali H. Contributions of the basal ganglia and functionally related brain structures to motor learning. *Behav Brain Res.* 2009; 199:61–75. [PubMed: 19061920]
- Faherty CJ, Raviie Shepherd K, Herasimtschuk A, Smeyne RJ. Environmental enrichment in adulthood eliminates neuronal death in experimental Parkinsonism. *Brain Res Mol Brain Res.* 2005; 134:170–179. [PubMed: 15790541]
- Ferreira AF, Real CC, Rodrigues AC, Alves AS, Britto LR. Moderate exercise changes synaptic and cytoskeletal proteins in motor regions of the rat brain. *Brain Res.* 2010; 1361:31–42. [PubMed: 20858463]
- Fisher BE, Petzinger GM, Nixon K, Hogg E, Bremmer S, Meshul CK, Jakowec MW. Exercise-induced behavioral recovery and neuroplasticity in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned mouse basal ganglia. *J Neurosci Res.* 2004; 77:378–390. [PubMed: 15248294]
- Fisher BE, Li Q, Nacca A, Salem GJ, Song J, Yip J, Hui JS, Jakowec MW, Petzinger GM. Treadmill exercise elevates striatal dopamine D2 receptor binding potential in patients with early Parkinson's disease. *Neuroreport.* 2013; 24:509–514. [PubMed: 23636255]
- Flash T, Hochner B. Motor primitives in vertebrates and invertebrates. *Curr Opin Neurobiol.* 2005; 15:660–666. [PubMed: 16275056]
- Floyer-Lea A, Matthews PM. Distinguishable brain activation networks for short- and long-term motor skill learning. *J Neurophysiol.* 2005; 94:512–518. [PubMed: 15716371]
- Foley TE, Fleshner M. Neuroplasticity of dopamine circuits after exercise: implications for central fatigue. *NeuroMolecular Medicine.* 2008; 10:67–80. [PubMed: 18274707]
- Garcia PC, Real CC, Ferreira AFB, Alouche SR, Britto LRG, Pires RS. Different protocols of physical exercise produce different effects on synaptic and structural proteins in motor areas of the rat brain. *Brain Research.* 2012; 1456:36–48. [PubMed: 22521043]
- Goodwin VA, Richards SH, Taylor RS, Taylor AH, Campbell JL. The effectiveness of exercise interventions for people with Parkinson's disease: a systematic review and meta-analysis. *Mov Disord.* 2008; 23:631–640. [PubMed: 18181210]
- Green CS, Bavelier D. Exercising your brain: a review of human brain plasticity and training-induced learning. *Psychology and aging.* 2008; 23:692–701. [PubMed: 19140641]
- Hackney ME, Kantorovich S, Levin R, Earhart GM. Effects of tango on functional mobility in Parkinson's disease: a preliminary study. *J Neurol Phys Ther.* 2007; 31:173–179. [PubMed: 18172414]
- Harada T, Okagawa S, Kubota K. Jogging improved performance of a behavioral branching task: implications for prefrontal activation. *Neurosci Res.* 2004; 49:325–337. [PubMed: 15196781]

- Hattori S, Naoi M, Nishino H. Striatal dopamine turnover during treadmill running in the rat: relation to the speed of running. *Brain Res Bull.* 1994; 35:41–49. [PubMed: 7953756]
- Herman T, Giladi N, Gruendlinger L, Hausdorff JM. Six weeks of intensive treadmill training improves gait and quality of life in patients with Parkinson's disease: a pilot study. *Arch Phys Med Rehabil.* 2007; 88:1154–1158. [PubMed: 17826461]
- Hirsch MA, Toole T, Maitland CG, Rider RA. The effects of balance training and high-intensity resistance training on persons with idiopathic Parkinson's disease. *Arch Phys Med Rehabil.* 2003; 84:1109–1117. [PubMed: 12917847]
- Holschneider DP, Yang J, Guo Y, Maarek JM. Reorganization of functional brain maps after exercise training: Importance of cerebellar-thalamic-cortical pathway. *Brain Res.* 2007; 1184:96–107. [PubMed: 17964551]
- Holschneider DP, Maarek JM, Harimoto J, Yang J, Scremin OU. An implantable bolus infusion pump for use in freely moving, nontethered rats. *Am J Physiol Heart Circ Physiol.* 2002; 283:H1713–H1719. [PubMed: 12234827]
- Holschneider DP, Maarek JM, Yang J, Harimoto J, Scremin OU. Functional brain mapping in freely moving rats during treadmill walking. *Journal of Cerebral Blood Flow & Metabolism.* 2003; 23:925–932. [PubMed: 12902836]
- Howells FM, Russell VA, Mabandla MV, Kellaway LA. Stress reduces the neuroprotective effect of exercise in a rat model for Parkinson's disease. *Behav Brain Res.* 2005; 165:210–220. [PubMed: 16159673]
- Isaacs KR, Anderson BJ, Alcantara AA, Black JE, Greenough WT. Exercise and the brain: angiogenesis in the adult rat cerebellum after vigorous physical activity and motor skill learning. *J Cereb Blood Flow Metab.* 1992; 12:110–119. [PubMed: 1370068]
- Jones SC, Korfali E, Marshall SA. Cerebral blood flow with the indicator fractionation of [¹⁴C]iodoantipyrine: effect of PaCO₂ on cerebral venous appearance time. *J Cereb Blood Flow Metab.* 1991; 11:236–241. [PubMed: 1900067]
- Kemppainen J, Aalto S, Fujimoto T, Kalliokoski KK, Langsjo J, Oikonen V, Rinne J, Nuutila P, Knuuti J. High intensity exercise decreases global brain glucose uptake in humans. *J Physiol.* 2005; 568:323–332. [PubMed: 16037089]
- Kikuchi A, Takeda A, Kimpara T, Nakagawa M, Kawashima R, Sugiura M, Kinomura S, Fukuda H, Chida K, Okita N, Takase S, Itoyama Y. Hypoperfusion in the supplementary motor area, dorsolateral prefrontal cortex and insular cortex in Parkinson's disease. *J Neurol Sci.* 2001; 193:29–36. [PubMed: 11718747]
- King LA, Horak FB. Delaying mobility disability in people with Parkinson disease using a sensorimotor agility exercise program. *Phys Ther.* 2009; 89:384–393. [PubMed: 19228832]
- Kleim JA, Lussnig E, Schwarz ER, Comery TA, Greenough WT. Synaptogenesis and Fos expression in the motor cortex of the adult rat after motor skill learning. *J Neurosci.* 1996; 16:4529–4535. [PubMed: 8699262]
- Kleim JA, Swain RA, Czerlanis CM, Kelly JL, Pipitone MA, Greenough WT. Learning-dependent dendritic hypertrophy of cerebellar stellate cells: plasticity of local circuit neurons. *Neurobiol Learn Mem.* 1997; 67:29–33. [PubMed: 9013498]
- Klintsova AY, Dickson E, Yoshida R, Greenough WT. Altered expression of BDNF and its high-affinity receptor TrkB in response to complex motor learning and moderate exercise. *Brain Res.* 2004; 1028:92–104. [PubMed: 15518646]
- Lambert TJ, Fernandez SM, Frick KM. Different types of environmental enrichment have discrepant effects on spatial memory and synaptophysin levels in female mice. *Neurobiol Learn Mem.* 2005; 83:206–216. [PubMed: 15820856]
- Li F, Harmer P, Fisher KJ, Xu J, Fitzgerald K, Vongjaturapat N. Tai Chi-based exercise for older adults with Parkinson's disease: a pilot-program evaluation. *Journal of aging and physical activity.* 2007; 15:139–151. [PubMed: 17556781]
- Lindvall O, Ingvar M, Stenevi U. Effects of methamphetamine on blood flow in the caudate-putamen after lesions of the nigrostriatal dopaminergic bundle in the rat. *Brain Res.* 1981; 211:211–216. [PubMed: 7225839]

- Lu MK, Tsai CH, Ziemann U. Cerebellum to motor cortex paired associative stimulation induces bidirectional STDP-like plasticity in human motor cortex. *Front Hum Neurosci.* 2012; 6:260. [PubMed: 23049508]
- Mabandla MV, Kellaway LA, Daniels WM, Russell VA. Effect of exercise on dopamine neuron survival in prenatally stressed rats. *Metab Brain Dis.* 2009; 24:525–539. [PubMed: 19844780]
- Marr D. A theory of cerebellar cortex. *J Physiol.* 1969; 202:437–470. [PubMed: 5784296]
- Mentis MJ, Dhawan V, Nakamura T, Ghilardi MF, Feigin A, Edwards C, Ghez C, Eidelberg D. Enhancement of brain activation during trial-and-error sequence learning in early PD. *Neurology.* 2003; 60:612–619. [PubMed: 12601101]
- Michel J, Benninger D, Dietz V, van Hedel HJ. Obstacle stepping in patients with Parkinson's disease. Complexity does influence performance. *J Neurol.* 2009; 256:457–463. [PubMed: 19266148]
- Miller EK, Cohen JD. An integrative theory of prefrontal cortex function. *Annu Rev Neurosci.* 2001; 24:167–202. [PubMed: 11283309]
- Nagano-Saito A, Leyton M, Monchi O, Goldberg YK, He Y, Dagher A. Dopamine depletion impairs frontostriatal functional connectivity during a set-shifting task. *J Neurosci.* 2008; 28:3697–3706. [PubMed: 18385328]
- Narayanan NS, Laubach M. Top-down control of motor cortex ensembles by dorsomedial prefrontal cortex. *Neuron.* 2006; 52:921–931. [PubMed: 17145511]
- Narayanan NS, Horst NK, Laubach M. Reversible inactivations of rat medial prefrontal cortex impair the ability to wait for a stimulus. *Neuroscience.* 2006; 139:865–876. [PubMed: 16500029]
- Newman LA, McGaughy J. Adolescent rats show cognitive rigidity in a test of attentional set shifting. *Dev Psychobiol.* 2011; 53:391–401. [PubMed: 21365641]
- Nguyen PT, Holschneider DP, Maarek JM, Yang J, Mandelkern MA. Statistical parametric mapping applied to an autoradiographic study of cerebral activation during treadmill walking in rats. *Neuroimage.* 2004; 23:252–259. [PubMed: 15325372]
- Nieuwboer A, Rochester L, Muncks L, Swinnen SP. Motor learning in Parkinson's disease: limitations and potential for rehabilitation. *Parkinsonism Relat Disord.* 2009; 15(Suppl 3):S53–S58. [PubMed: 20083008]
- O'Dell SJ, Gross NB, Fricks AN, Casiano BD, Nguyen TB, Marshall JF. Running wheel exercise enhances recovery from nigrostriatal dopamine injury without inducing neuroprotection. *Neuroscience.* 2007; 144:1141–1151. [PubMed: 17157992]
- Olsson M, Nikkhah G, Bentlage C, Bjorklund A. Forelimb akinesia in the rat Parkinson model: differential effects of dopamine agonists and nigral transplants as assessed by a new stepping test. *J Neurosci.* 1995; 15:3863–3875. [PubMed: 7751951]
- Onla-or S, Winstein CJ. Determining the optimal challenge point for motor skill learning in adults with moderately severe Parkinson's disease. *Neurorehabil Neural Repair.* 2008; 22:385–395. [PubMed: 18326891]
- Pascual-Leone A, Nguyet D, Cohen LG, Brasil-Neto JP, Cammarota A, Hallett M. Modulation of muscle responses evoked by transcranial magnetic stimulation during the acquisition of new fine motor skills. *J Neurophysiol.* 1995; 74:1037–1045. [PubMed: 7500130]
- Paxinos, G.; Watson, C. *The Rat Brain in Stereotactic Coordinates.* 6th Edition. New York: Elsevier Academic Press; 2007.
- Petzinger GM, Fisher BE, McEwen S, Beeler JA, Walsh JP, Jakowec MW. Exercise-enhanced neuroplasticity targeting motor and cognitive circuitry in Parkinson's disease. *Lancet Neurol.* 2013; 12:716–726. [PubMed: 23769598]
- Petzinger GM, Walsh JP, Akopian G, Hogg E, Abernathy A, Arevalo P, Turnquist P, Vuckovic M, Fisher BE, Togasaki DM, Jakowec MW. Effects of treadmill exercise on dopaminergic transmission in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned mouse model of basal ganglia injury. *J Neurosci.* 2007; 27:5291–5300. [PubMed: 17507552]
- Ploughman M, Attwood Z, White N, Dore JJ, Corbett D. Endurance exercise facilitates relearning of forelimb motor skill after focal ischemia. *Eur J Neurosci.* 2007; 25:3453–3460. [PubMed: 17553014]
- Ragozzino ME, Detrick S, Kesner RP. The effects of prelimbic and infralimbic lesions on working memory for visual objects in rats. *Neurobiol Learn Mem.* 2002; 77:29–43. [PubMed: 11749084]

- Real CC, Ferreira AF, Hernandez MS, Britto LR, Pires RS. Exercise-induced plasticity of AMPA-type glutamate receptor subunits in the rat brain. *Brain Res.* 2010; 1363:63–71. [PubMed: 20869354]
- Real CC, Ferreira AF, Chaves-Kirsten GP, Torrao AS, Pires RS, Britto LR. BDNF receptor blockade hinders the beneficial effects of exercise in a rat model of Parkinson's disease. *Neuroscience.* 2013; 237:118–129. [PubMed: 23396085]
- Risterucci C, Terramorsi D, Nieoullon A, Amalric M. Excitotoxic lesions of the prelimbic-infralimbic areas of the rodent prefrontal cortex disrupt motor preparatory processes. *Eur J Neurosci.* 2003; 17:1498–1508. [PubMed: 12713653]
- Sakurada O, Kennedy C, Jehle J, Brown JD, Carbin GL, Sokoloff L. Measurement of local cerebral blood flow with iodo [^{14}C] antipyrine. *Am J Physiol.* 1978; 234:H59–H66. [PubMed: 623275]
- Salgado S, Williams N, Kotian R, Salgado M. An evidence-based exercise regimen for patients with mild to moderate Parkinson's disease. *Brain Sci.* 2013; 3:87–100. [PubMed: 24961308]
- Sauer H, Oertel WH. Progressive degeneration of nigrostriatal dopamine neurons following intrastriatal terminal lesions with 6-hydroxydopamine: a combined retrograde tracing and immunocytochemical study in the rat. *Neuroscience.* 1994; 59:401–415. [PubMed: 7516500]
- Scandalis TA, Bosak A, Berliner JC, Helman LL, Wells MR. Resistance training and gait function in patients with Parkinson's disease. *Am J Phys Med Rehabil.* 2001; 80:38–43. quiz 44–36. [PubMed: 11138953]
- Schaefer SY, Patterson CB, Lang CE. Transfer of training between distinct motor tasks after stroke: implications for task-specific approaches to upper-extremity neurorehabilitation. *Neurorehabil Neural Repair.* 2013; 27:602–612. [PubMed: 23549521]
- Seidler RD. Multiple motor learning experiences enhance motor adaptability. *J Cogn Neurosci.* 2004; 16:65–73. [PubMed: 15006037]
- Shumway-Cook, A.; Woollacott, MH. *Motor control: Theory and practical applications.* 2nd Edition. Baltimore, MD: Lippincott Williams & Wilkins; 2001.
- St Onge JR, Floresco SB. Prefrontal cortical contribution to risk-based decision making. *Cereb Cortex.* 2010; 20:1816–1828. [PubMed: 19892787]
- Sun FT, Miller LM, Rao AA, D'Esposito M. Functional connectivity of cortical networks involved in bimanual motor sequence learning. *Cereb Cortex.* 2007; 17:1227–1234. [PubMed: 16855008]
- Sung YH, Kim SC, Hong HP, Park CY, Shin MS, Kim CJ, Seo JH, Kim DY, Kim DJ, Cho HJ. Treadmill exercise ameliorates dopaminergic neuronal loss through suppressing microglial activation in Parkinson's disease mice. *Life Sci.* 2012; 91:1309–1316. [PubMed: 23069581]
- Thoroughman KA, Shadmehr R. Learning of action through adaptive combination of motor primitives. *Nature.* 2000; 407:742–747. [PubMed: 11048720]
- Tillerson JL, Caudle WM, Reveron ME, Miller GW. Exercise induces behavioral recovery and attenuates neurochemical deficits in rodent models of Parkinson's disease. *Neuroscience.* 2003; 119:899–911. [PubMed: 12809709]
- Townsend BR, Paninski L, Lemon RN. Linear encoding of muscle activity in primary motor cortex and cerebellum. *J Neurophysiol.* 2006; 96:2578–2592. [PubMed: 16790591]
- Toy WA, Petzinger GM, Leyshon BJ, Akopian GK, Walsh JP, Hoffman MV, Vuckovic MG, Jakowec MW. Treadmill exercise reverses dendritic spine loss in direct and indirect striatal medium spiny neurons in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of Parkinson's disease. *Neurobiol Dis.* 2014; 63:201–209. [PubMed: 24316165]
- Uylings HB, Groenewegen HJ, Kolb B. Do rats have a prefrontal cortex? *Behav Brain Res.* 2003; 146:3–17. [PubMed: 14643455]
- van der Kolk NM, King LA. Effects of exercise on mobility in people with Parkinson's disease. *Mov Disord.* 2013; 28:1587–1596. [PubMed: 24132847]
- Vogt BA, Hof PR, Zilles K, Vogt LJ, Herold C, Palomero-Gallagher N. Cingulate area 32 homologies in mouse, rat, macaque and human: cytoarchitecture and receptor architecture. *J Comp Neurol.* 2013; 521:4189–4204. [PubMed: 23840027]
- Voss MW, Prakash RS, Erickson KI, Basak C, Chaddock L, Kim JS, Alves H, Heo S, Szabo AN, White SM, Wojcicki TR, Mailey EL, Gothe N, Olson EA, McAuley E, Kramer AF. Plasticity of brain networks in a randomized intervention trial of exercise training in older adults. *Front Aging Neurosci.* 2010; 2. [PubMed: 20552042]

- Vuckovic MG, Li Q, Fisher B, Nacca A, Leahy RM, Walsh JP, Mukherjee J, Williams C, Jakowec MW, Petzinger GM. Exercise elevates dopamine D2 receptor in a mouse model of Parkinson's disease: in vivo imaging with [(1)(8)F]fallypride. *Mov Disord*. 2010; 25:2777–2784. [PubMed: 20960487]
- Wang TY, Bhatt T, Yang F, Pai YC. Generalization of motor adaptation to repeated-slip perturbation across tasks. *Neuroscience*. 2011; 180:85–95. [PubMed: 21352898]
- Wang Z, Ocampo MA, Pang RD, Bota M, Bradesi S, Mayer EA, Holschneider DP. Alterations in prefrontal-limbic functional activation and connectivity in chronic stress-induced visceral hyperalgesia. *PLoS ONE*. 2013a; 8:e59138. [PubMed: 23527114]
- Wang Z, Myers KG, Guo Y, Ocampo MA, Pang RD, Jakowec MW, Holschneider DP. Functional reorganization of motor and limbic circuits after exercise training in a rat model of bilateral parkinsonism. *PLoS ONE*. 2013b; 8:e80058. [PubMed: 24278239]
- Wang Z, Guo Y, Myers KG, Heintz R, Peng YH, Maarek JM, Holschneider DP. Exercise alters resting-state functional connectivity of motor circuits in parkinsonian rats. *Neurobiol Aging* in press. 2014
- Werheid K, Zysset S, Muller A, Reuter M, von Cramon DY. Rule learning in a serial reaction time task: an fMRI study on patients with early Parkinson's disease. *Brain Res Cogn Brain Res*. 2003; 16:273–284. [PubMed: 12668237]
- Wu T, Hallett M. A functional MRI study of automatic movements in patients with Parkinson's disease. *Brain*. 2005; 128:2250–2259. [PubMed: 15958505]
- Xavier LL, Viola GG, Ferraz AC, Da Cunha C, Deonizio JM, Netto CA, Achaval M. A simple and fast densitometric method for the analysis of tyrosine hydroxylase immunoreactivity in the substantia nigra pars compacta and in the ventral tegmental area. *Brain Res Brain Res Protoc*. 2005; 16:58–64. [PubMed: 16310404]
- Yang J, Sadler TR, Givrad TK, Maarek JM, Holschneider DP. Changes in brain functional activation during resting and locomotor states after unilateral nigrostriatal damage in rats. *Neuroimage*. 2007; 36:755–773. [PubMed: 17481921]
- Yoon MC, Shin MS, Kim TS, Kim BK, Ko IG, Sung YH, Kim SE, Lee HH, Kim YP, Kim CJ. Treadmill exercise suppresses nigrostriatal dopaminergic neuronal loss in 6-hydroxydopamine-induced Parkinson's rats. *Neurosci Lett*. 2007; 423:12–17. [PubMed: 17644250]
- Zgaljardic DJ, Borod JC, Foldi NS, Mattis PJ, Gordon MF, Feigin A, Eidelberg D. An examination of executive dysfunction associated with frontostriatal circuitry in Parkinson's disease. *J Clin Exp Neuropsychol*. 2006; 28:1127–1144. [PubMed: 16840240]

HIGHLIGHTS

- Skilled vs nonskilled aerobic exercise (SAE vs NSAE) differentially affect the brain
- We show skill-based functional brain reorganization in a Parkinsonian rat model
- SAE compared to NSAE results in equal or greater recovery in motor deficits
- SAE recruits prefrontal cortex and cerebellum, while NSAE recruits the striatum
- Exercise complexity may play a key role in neurorehabilitative outcomes

Figure 1. Overview of experiments

(A) Timeline, (B) Representative slices showing tyrosine hydroxylase immunostaining in the caudate putamen and substantia nigra in a sham and a lesioned rat. White arrowheads point to areas showing lesion-induced loss in tyrosine hydroxylase staining. CBF: cerebral blood flow, CPu: caudate putamen, ET: exercise training, SNc: substantia nigra pars compacta.

A. Complex wheel for skilled aerobic exercise B. Simple wheel for non-skilled aerobic exercise

C

Figure 2. Non-skilled and skilled aerobic exercise

(A) Non-skilled aerobic exercise is implemented using simple running wheels, with regularly spaced rungs and an inserted plastic “floor”. (B) Complex running wheels with irregularly spaced rungs are used for skilled aerobic exercise. (C) Training speed during 4 weeks of forced exercise.

A. Striatum

B. Substantia nigra pars compacta

Figure 3. 6-OHDA induced lesion in the striatum and substantia nigra

Optical density of tyrosine hydroxylase staining is shown for the striatum (A) and substantia nigra pars compacta (B). Optical density has arbitrary units and should not be interpreted as a percentage. #: significantly different from the Sham/No-ET group; *: significantly different from the Lesion/No-ET group; @: significantly different from the Lesion/NSAE group ($P < 0.05$, LCD *post hoc* tests). ET: exercise training; SAE: skilled aerobic exercise; NSAE: non-skilled aerobic exercise.

Figure 4. Striatal lesioning-induced motor deficits and exercise-induced recovery in motor function

#: significantly different from the Sham/No-ET group; *: significantly different from the Lesion/No-ET group; @: significantly different from the Lesion/NSAE group ($P < 0.05$, LCD *post hoc* tests). ET: exercise training.; SAE: skilled aerobic exercise; NSAE: non-skilled aerobic exercise.

Figure 5. Brain regions showing significant effect of Walk

Color coded overlays over a selection of representative coronal slices of the template brain show statistically significant main effect of 'Walk' on regional cerebral blood flow (rCBF) (left panel, factorial analysis), significant changes in rCBF comparing Walk and Rest in animals receiving skilled aerobic exercise (SAE) (middle panel, Student's *t* test, red: increase in rCBF, blue: decrease in rCBF), and significant changes in rCBF comparing Walk and Rest in the non-skilled aerobic exercise (NSAE) group (right panel, Student's *t* test). Abbreviations: AM (anteromedial thalamic n.), APir (amygdalopiriform transition area), Au

(auditory cx.), BL (basolateral amygdaloid n.), BM (basomedial amygdaloid n.), Cb (cerebellar vermis), Ce (central amygdaloid n.), CEnt (central entorhinal cx.), Cg2 (cingulate cx. area 2), CM (central medial thalamic n.), dCPu (dorsal caudate-putamen), dHPC (dorsal hippocampus), DLEnt (dorsolateral entorhinal cx.), DLPAG/DMPAG (dorsolateral/dorsomedial periaqueductal gray), DRD (dorsal raphe n., dorsal part), GP (globus pallidas), IC (inferior colliculus), IL (infralimbic cx.), Ins (insular cx.), IP (interpeduncular n.), La (lateral amygdaloid n.), LPAG (lateral periaqueductal gray), LPtA (lateral parietal association cx.), LS (lateral septum), M1/M2 (primary/secondary motor cx.), mCPu (medial caudate-putamen), Me (medial amygdaloid n.), MPtA (medial parietal association cx.), Pir (piriform cx.), PLH (peduncular part of lateral hypothalamus), Post (postsubiculum), Pr5 (principal sensory 5), PRh (perirhinal cx.), PrL (prelimbic cx.), PrS (presubiculum), PT (pretectal n.), Re (reunions thalamic n.), Rh (rhomboid thalamic n.), RS (retrosplenial cx.), S1BF / S1DZ / S1J / S1ULp (primary somatosensory cx., barrel field / dysgranular / jaw / upper lip area), S2 (secondary somatosensory cx.), SC (superior colliculus), Sim (simple lobule), SN (substantia nigra), SO (superior olive), STh (subthalamic n.), Sub (submedialis thalamic n.), TeA (temporal association cx.), Tu (olfactory tubercle), V1/V2 (primary/secondary visual cx.), vCPu (ventral caudate-putamen), VMH (ventromedial hypothalamic n.), VP (ventral pallidas), VPL/VPM (ventral posterolateral/ventral posteromedial thalamic n.), VPPC (ventral posterior thalamic n., parvocellular). The right side of images corresponds to the left side of the animal.

Figure 6. Brain regions showing significant effect of Skill

Color coded overlays over a selection of representative coronal slices of the template brain show statistically significant main effect of ‘Skill’ on regional cerebral blood flow (rCBF) (factorial analysis), significant ‘Skill $\times$ Walk’ interaction (factorial analysis), significant changes in rCBF comparing skilled aerobic exercise (SAE) and non-skilled ET (NSAE) during the Walk state (Student’s *t* test, red: increase in rCBF, blue: decrease in rCBF), and significant changes in rCBF comparing SAE and NSAE during the Rest state (Student’s *t* test). Abbreviations: Acb (accumbens n.), Cb (cerebellar vermis), CEnt (central entorhinal

cx.), Cg1 (cingulate cx. area 1), dCPu (dorsal caudate-putamen), dHPC (dorsal hippocampus), DMTg (dorsomedial tegmental area), GP (globus pallidas), IC (inferior colliculus), Ins (insular ctx.), LS (lateral septum), M1/M2 (primary/secondary motor cx.), mCPu (medial caudate-putamen), Me (medial amygdaloid n.), Pir (piriform cx.), PnO (pontine reticular n., oral part), PrL (prelimbic cx.), RS (retrosplenial cx.), Rt (reticular thalamic n.), S1BF / S1FL / S1Tr / S1ULp (primary somatosensory cx., barrel field / forelimb / trunk / upper lip area), S2 (secondary somatosensory cx.), VMH (ventromedial hypothalamic n.), VO (ventral orbital cx.), VP (ventral pallidas). The right side of images corresponds to the left side of the animal.

Figure 7. Functional connectivity of the prelimbic cortex

Comparison of the functional connectivity of the prelimbic cortex (PrL) in the skilled aerobic exercise (SAE) and non-skilled aerobic exercise (NSAE) animals during treadmill walking. Color coded overlays over a selection of representative coronal slices of the template brain show regions significantly correlated (red: positive correlation, blue: negative correlation) with the right prelimbic cortex. Abbreviations: Acb (accumbens n.), Cb (cerebellar vermis), Cg1/Cg2 (cingulate cx. area 1/2), CPu (caudate-putamen), DP (dorsal peduncular cx.), Ins (insular cx.), LO (lateral orbital cx.), LS (lateral septum), M1/M2 (primary/secondary motor cx.), Pir (piriform cx.), S1DZ / S1FL / S1J (primary somatosensory cx., dysgranular / forelimb / jaw area), VO/LO (ventral/lateral orbital cx.). The right side of images corresponds to the left side of the animal.

Figure 8. Functional connectivity of the cerebellum

Comparison of the functional connectivity of the cerebellum vermis (Cb) in the skilled aerobic exercise (SAE) and non-skilled aerobic exercise (NSAE) animals during treadmill walking. Color coded overlays over a selection of representative coronal slices of the template brain show regions significantly correlated (red: positive correlation, blue: negative correlation) with the cerebellum vermis seed. Abbreviations: Ce (central amygdaloid n.), Cg2 (cingulate cx. area 2), dCPu and vCPu (dorsal and ventral caudate-putamen), GP (globus pallidus), Ins (insular cx.), M1/M2 (primary/secondary motor cx.), Pir (piriform cx.), PrL (prelimbic cx.), RS (retrosplenial cx.), S1BF / S1HL / S1J / S1Sh / S1ULp (primary somatosensory cx., barrel field / hindlimb / jaw / shoulder / upper lip area), S2 (secondary somatosensory cx.), VA/VL (ventral anterior / ventrolateral thalamic n.). The right side of images corresponds to the left side of the animal.

Figure 9. Correlation between the regional cerebral blood flow in the prelimbic cortex during treadmill walking and motor function

Color coded overlays over a representative coronal slice of the template brain show regions significantly correlated (red: positive correlation, blue: negative correlation) with performance in 3 motor tests in the skilled aerobic exercise (SAE, upper panel) and non-skilled aerobic exercise (NSAE, lower panel) animals. Abbreviations: PrL (prelimbic cx.).

Table 1

Summary of statistical parametric mapping results in the cortex.

Cortical region	F-test		T-test			
	Skill	Interaction	Walk vs. Rest		SAE	NSAE
			Walk	Rest		
Auditory (Au)	**	**	++	-	++	-
Cingulate, area 1 (Cg1)	**		-	-	++	++
area 2 (Cg2)	*	**		+	++	++
Ectorhinal (Ect)	**		-	-	++ ^a , - ^p	-
Entorhinal, dorsolateral (DLEnt)	*	*	-	-	++	++
dorsal (DLEnt) & ventral intermed (VLEnt)	**		-		++	++
medial (MEnt)	**	**	-		++	++
central (CEnt)	**	*	++		++	
Frontal, area 3 (Fr3)	**				-	-
Infralimbic (IL)	**				++	++
Insular (Ins)	*	**	-		-	-
Motor, primary (M1)	**	**	- ^a , ++ ^p		- ^a , ++ ^p	- ^a , ++ ^p
secondary (M2)	**	**	+ ^a , - ^p		++	++
Orbital, ventral (VO)			+	+	+	
Parietal, association, medial (MPtA)	**	**	+	++	++	++
association, lateral (LPtA)	*	**	+	+	++	++
post area, dorsal (PPD) & rostral (PPR)	*		++	+	++	++

Cortical region	F-test				T-test			
	Skill	Interaction	Walk vs. Rest		Walk	Rest	SAE	NSAE
			Walk	Rest				
Perirhinal (PRh)	*	**	—	—	—	—	++	++
Piriform (Pir)		**	—	—	—	—	—	—
Prelimbic (PrL)	**	**	++	+	++	++	++	++
Retrosplenial (RS)	*	**	++	+	++	++	++	++
Somatosensory, primary								
barrel field (S1BF)	**	**	++	++	++	++	++	++
dysgranular zone (SIDZ)	**	**	++	++	++	++	— ^a , ++ ^p	— ^a , ++ ^p
forelimb (S1FL)	**	**	— ^a , ++ ^p	++	++	++	— ^a , ++ ^p	— ^a , ++ ^p
hindlimb (S1HL)	**		++	++	++	++	++	++
jaw (S1J)		**			—	—	—	—
shoulder (S1Sh)	**		++	++	++	++	++	++
trunk (S1Tr)	**	**	++	++	++	++	++	++
upper lip (S1ULp)		**			—	—	—	—
Somatosensory, secondary (S2)	*	** *	++		— ^a , ++ ^p	— ^a , ++ ^p	— ^a , ++ ^p	— ^a , ++ ^p
Temporal association (TeA)	*	**	—	—	—	—	—	—
Visual, primary (V1) and secondary (V2)	*	**	++	+	++	++	++	++

Functional brain activation was analyzed using statistical parametric mapping for four groups of animals (Lesion/SAE/Walk, $n = 9$; Lesion/SAE/Rest, $n = 8$; Lesion/NSAE/Walk, $n = 11$; Lesion/NSAE/Rest, $n = 12$). Results are summarized for the right and left hemisphere ($P < 0.05$ at the voxel level with an extent threshold of 100 contiguous voxels). ^a: anterior; P: posterior; +/—: increase / decrease in cerebral blood flow; *: significant main effect. NSAE / SAE: non-skilled / skilled aerobic exercise.

Table 2

Summary of statistical parametric mapping results in the subcortex.

	F-test		T-test			
	SAE vs. NSAE					
	Walk vs. Rest					
Walk						
	Skill	Interaction	Walk	Rest	SAE	NSAE
MOTOR RELATED						
Cerebellum, vermis (Cb)	*	*	*	+	-	+
interposed n. (Int), lateral n. (Lat)						-
simple lobule (Sim)	**	**	++			-
Caudate putamen, anterior (aCPu)	*	*			-	+-
dorsal (dCPu)	*	*	-	++		++
lateral (lCPu)	**				-	-
medial (mCPu)	*	*		-		
ventral (vCPu)	*	**	-		-	-
Globus pallidus (GP)	**	**	-	-	-	-
Motor trigeminal n. (5)						-
Oculomotor n. (3N)	**	**				-
Parasubthalamic n. (PSTh)	**				-	-
Red n., parvicellular part (RPC)	*					
magnocellular part (RMC)	*					
Substantia nigra (SN)	**				-	-

F-test		T-test				
		SAE vs. NSAE		Walk vs. Rest		
Walk						
	Skill	Interaction	Walk	Rest	SAE	NSAE
Superior colliculus (SC)	**	**	**	—	—	—
Superior olive (SO)	**	**	++	++	++	
Zona incerta (ZI)	*		++	++		
THALAMUS						
anteromedial n. (AM)	**				—	—
anterodorsal (AD), anteroventral n. (AV)	*	**		—		—
central medial n. (CM)	*		—		—	—
dorsal lateral geniculate n. (DLG)	**					
lateral posterior n. (LP)					—	
laterodorsal (LD)	**				—	
medial geniculate n. (MG)	**					—
posterior n. (Po)			+			
reticular n. (Rt)	*	*		—		—
reunions n. (Re), rhomboid n. (Rh)	*				—	—
submedius n. (Sub)	**					
ventral anterior n. (VA)					++	
ventral geniculate n. (VG)	**		—			
ventral posterolateral n. (VPL)	**			—	++	+

	F-test		T-test			
			SAE vs. NSAE		Walk vs. Rest	
	Skill	Interaction	Walk	Rest	SAE	NSAE
<i>Walk</i>						
<i>ventral posteromedial n. (VPM)</i>			+		+	
ventral posterior n., parvicellular (VPPC)	**				-	
<i>ventrolateral n. (VL)</i>	*	*		-	-	
<i>ventromedial n. (VM)</i>	**				-	
LIMBIC AND RELATED						
Accumbens n. (Acb)	**	**	-	-	-	
Amygdala, anterior cortical n. (ACo)	**				-	
amygdalopiriform transition (APir)	**					
basolateral n. (BL)	*	**	-	+	-	
basomedial n. (BM)	**	*	-		-	
central n. (Ce)	**		-		-	
lateral n. (La)	**	*	-		-	
medial n. (Me)	**	**	+	+	-	
sublenticular extended amygdala (EA)	*	**	-		-	
Fimbria (fi)	*	*	-	-	+	+
Hippocampus, dorsal (dHPC)	**	**	-	+	+	+
ventral (vHPC)						+
Hypothalamus, ventromedial n. (VMH)	**			+	-	-

F-test		T-test				
Walk						
	Skill	Interaction	Walk	Rest	SAE vs. NSAE	Walk vs. Rest
	*	**	+	+	-	-
		**			-	-
		**				-
	*	*	-	-	-	-
	*			-	-	-
	*	**		-		
		**			-	-
	*	*			-	-
		*			-	-
		*	+	-	+	-
		*	*	+		-
	**	**	-	-	++	++
	**	**		-	++	++
	**	*	-	+	-	++
	**	**	-			++
	**	**				
	**	*	++	-	++	++
	**	**				
	**	*				
	**	*	++	-	++	++
	**	*				
	**	*				
	**	*	++	-	++	++
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				
	**	*				

F-test		T-test		
Walk				
Skill	Interaction	Walk	Rest	SAE vs. NSAE
Ventral pallidum (VP)	**	+	+	+
OTHERS				
Anterior olfactory n., posterior (AOP)	**	+	+	+
Inferior colliculus (IC)	**	+	+	+
Interpeduncular n. (IP)	*	*	-	-
Olfactory tubercle (Tu)	**		+	+
Pontine n. (Pn)			+	
Pontine reticular n., oral part (PnO)	**	+	+	+
caudal (PnC)	**		+	+
Precuneiform n. (PrCnF)	*			+
Pretectal n. (PT)	**	+	+	+
Principal sensory 5 (Pr5)	**			+
Reticular formation, mesencephalic (mRt)	**		+	+
isthmus (isRt)	**			

Functional brain activation was analyzed using statistical parametric mapping for four groups of animals (Lesion/SAE/Walk, $n = 9$; Lesion/SAE/rest, $n = 8$; Lesion/NSAE/Walk, $n = 11$; Lesion/NSAE/Rest, $n = 12$). Results are summarized for the right and left hemisphere ($P < 0.05$ at the voxel level with an extent threshold of 100 contiguous voxels). a. anterior; P. posterior; +/-: increase / decrease in cerebral blood flow; *: significant main effect. NSAE / SAE: non-skilled / skilled aerobic exercise.