EFFECTS OF EXERCISE THERAPY ON FUNCTIONAL CONNECTIVITY IN PARKINSON'S DISEASE

by

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Effects of Exercise Therapy on Functional Connectivity in Parkinson's Disease

<u>Abstract</u>

by

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Forced-rate lower-extremity exercise has recently emerged as a potential safe and lowcost therapy for Parkinson's Disease (PD). The efficacy is believed to be dependent on high rates of exercise. In this study, we use functional connectivity (FC) MRI to further elucidate the mechanism underlying this rate-dependent effect. PD subjects were randomized to complete 8 weeks of forced, voluntary, or no exercise. Changes in brain connectivity were then analyzed as a function of pedaling rate. Changes in task-related FC (trFC) from the most affected motor cortex (*M1) to the ipsilateral thalamus were significantly positively correlated to pedaling rate. Changes in trFC between *M1 and the supplementary motor area were significantly negatively correlated to pedaling rate. This relationship persisted after 4 weeks of follow up. This indicates that patients who pedal faster tend to show stronger increases in cortico-subcortical trFC, and stronger decreases in cortico-cortical trFC.

Background and Significance

Parkinson's Disease

PD is a progressive neurodegenerative disorder in which dopaminergic neurons of the nigrostriatal pathway are lost. The result is decreased stimulation of the motor cortex through the pathways of the basal ganglia, as has been documented through neurophysiological and fMRI studies [1], [2]. Hypoactivity of the nigrostriatal pathways and decreased input to the motor cortex manifests itself through bradykinesia, tremor, and rigidity, among other symptoms. Current treatments for PD aim to treat these symptoms and restore quality of life. Medical treatments for PD are mainly designed to replete or substitute for dopamine. Unfortunately, although these therapies often improve motor symptoms there are potential significant side effects such as on/off phenomena, dyskinesias, sedation, and psychiatric symptoms including compulsive behavior. Further, as the disease progresses, the therapeutic window for medical therapies narrows with increasing frequency of side effects. When advanced PD patients can no longer be effectively treated with medications without significant side effects they are considered for surgical therapies. Deep brain stimulation (DBS) is an effective therapy for late stage Parkinson's disease, but carries with it significant cost as well as an invasive procedure for placement of the device. The present study focuses on a potential new, low-cost, effective therapy for Parkinson's disease, forced exercise.

Exercise and PD

Exercise has recently emerged as a possible therapeutic modality for PD. It has the potential to serve as an inexpensive alternative or adjunct therapy without side effects.

Experiments in animal models of PD have shown that exercise can improve motor function [3], produce an increase in neurotrophic factors [4], and have a neuroprotective effect [4], [5]. Using a 6-OHDA model of PD, Tillerson et al [3] showed that running rats on a treadmill after lesioning can preserve striatal dopamine as compared to rats that were not exercised. Further, the exercised rats showed improvements in motor function, including limb use asymmetry and forelimb akinesia [3]. A later study by Poulton & Muir [6] found that although dopamine levels were preserved, there were no improvements in motor function seen in exercised rats. This may be related to the slightly increased levels of neurotoxin used in the Poulton study, but may also be partially related to the timing and rate of exercise implemented. Tillerson et al exercised the rats sooner after lesioning (2-4 hrs vs. 1 day), and at a faster rate (15 m/min vs. 13 m/min) [3], [6], indicating that the rate of exercise may be important for the degree of effect seen. Attempts to reproduce these results in human studies of exercise have had mixed results, with a recent meta-analysis concluding that there is "insufficient evidence to support or refute the effectiveness of exercise therapy for patients with Parkinson's disease." [7] The apparent inconsistency between human and animal studies is likely related to differences in the type of exercise performed. In animal studies, the subject is forced to exercise at a rate greater than its voluntary exercise rate, whereas in human studies, the subjects exercise at their voluntary rate. This is an inherent problem in PD, a disease characterized by bradykinesia, as patients are frequently not able to voluntarily achieve a rate high enough to be effective. To investigate this hypothesis recent studies compared the effects of voluntary exercise (VE) versus forced exercise (FE) on the symptoms of PD patients[8]. After completing 8 weeks of exercise, those patients randomized to FE

showed 35% improvement in clinical motor ratings, while patients randomized to VE did not show improvement [8]. The mechanism underlying these changes is not well understood. Functional magnetic resonance imaging (fMRI) and functional connectivity MRI have the potential to provide additional information regarding the mechanism of improvement.

Functional Connectivity fMRI (fcMRI)

fMRI traditionally uses changes in blood oxygen level-dependent (BOLD) signal to determine areas of neuronal activation during a certain task. This has the drawback that it is highly sensitive to task performance issues (and therefore problematic in PD). Further, it only provides information on brain regions having a systematically altered blood flow in response to manipulating the level of task difficulty (and therefore insensitive to regions required equally at different levels of task difficulty). BOLD images can also be used to determine functional connectivity, which is defined as temporal correlations in a neurophysiological measurement across different brain regions [9]. In 1995, Biswal et al. [10] analyzed BOLD signal in functionally connected areas of the *resting* brain. They showed that low frequency BOLD fluctuations (LFBF) in these areas were highly temporally correlated [10]. Methodology was developed to make this technique applicable across the whole brain and in clinical scanners [11], and temporally correlated spontaneous LFBFs are now held as representative of functional connectivity in the resting state [12]. Research is actively being conducted on how resting state functional connectivity (rsFC) is affected in various neuropsychiatric disorders [13]. For example, in a neuroimaging study on depression, increased fMRI activation was found in both

cortical and limbic regions, but rsFC between these regions was found to be decreased [14]. This is consistent with the hypothesis that cortical regulation of limbic structures is disrupted in depressed patients [14], and illustrates how fcMRI can provide additional and complimentary information to that gleaned from traditional fMRI. Thus, fcMRI can be applied to understand how fundamental neurological connections are altered in specific disease states. Further, it has the potential to determine the effectiveness of current and new treatments for these disorders. Both resting state and continuous-task fcMRI are used in this study to better understand alterations in network changes resulting in the therapeutic effects of forced exercise therapy in PD.

Resting State Functional Connectivity in PD

Until recently, there has been little study of functional connectivity in PD patients. The literature is relatively young, with studies having only emerged in the last 3 years [15]. A study by Wu et al. in 2009 [16], in which they used a graph-theoretic measure of global connectivity, found that rsFC in the supplementary motor area, left dorsolateral prefrontal cortex, and left putamen were significantly decreased in PD patients off medications, compared to normal controls. Global connectivity to the left cerebellum, left primary motor cortex, and L parietal cortex was increased relative to controls, and these changes were correlated to clinical ratings of motor symptoms. When on medication, these differences became nonsignificant, indicating that medication aids in normalizing the functional FC in these regions, and may help improve motor planning and symptoms [16]. The graph-theoretic, global measure of FC used in this study produces some difficulty in predicting changes in typical region-to-region FC, but we hypothesize that

regions which change in opposite directions in global connectivity would decrease in their direct connection strength. More recently, another study comparing 31 PD patients with 44 healthy controls has found that PD patients have significantly increased FC between the subthalamic nucleus (STN) and cortical motor regions, when compared to controls [17]. This is a significant result, as the STN is a commonly targeted region in DBS for PD, and previous studies have revealed an overactive "hyperdirect" pathway between these regions. In this work, we show that some of these FC deficits can be improved after 8 weeks of exercise.

Significance and Objectives

For years it has been anecdotally observed that movement – exercising and even dancing – seems to relieve PD symptoms, but the mechanism behind why it does so has been an enigma. Recently, studies have begun to uncover this mechanism by showing that forced exercise, rather than voluntary exercise, produces consistent symptomatic improvement [8], indicating that the therapeutic effect is related to exercise rate. In order to gain insight into the neural mechanism of this effect, we undertook a randomized, controlled trial in which subjects were randomized to complete 8 weeks of forced, voluntary, or no exercise. Changes in brain connectivity as a function of pedaling rate were then analyzed to determine the effect of exercise therapy on functional connectivity in PD. Based on previously published FC changes in PD, we hypothesized that, after exercise, FC between cortical motor areas would increase, and that FC between cortico-subcortical motor areas would also increase, except for the globus pallidus internus and STN, in which a decrease was expected. Further, these changes were expected to be related to pedaling rate, and to persist 4 weeks after the end of therapy.

Research Design and Methods

The current study is an extension of a larger single-center, parallel-group, randomized controlled study evaluating the effect of forced exercise on motor and non-motor symptoms in PD, and changes in task oriented fMRI activation. The study reported here is designed to analyze functional connectivity in patients with PD as measured by fcMRI, to determine how FC is changed after either forced or voluntary exercise, and to thereby investigate the mechanism by which exercise may relieve symptoms in PD.

Experimental overview:

Patients were consented and enrolled, and baseline studies were conducted including clinical evaluation, MRI imaging, and cardiovascular testing (all described below), in a protocol approved by the Cleveland Clinic Institutional Review Board. Subjects were randomized to one of three groups: forced exercise, voluntary exercise, or no-exercise (control). The study duration was 16 weeks: an eight-week period in which the randomly assigned exercise intervention occurred, and an eight-week follow up period without intervention. Clinical evaluation will occur every 4 weeks and MRI imaging will be done at baseline, after the 8 weeks of therapy (EOT), and four weeks thereafter (EOT+4).

Study sample:

A total of 42 patients with mild-to-moderate idiopathic PD were enrolled in the MRI portion of the study. <u>Primary inclusion criteria included</u>: clinical diagnosis of idiopathic PD, age between 30-65 years, not currently engaged in formal exercise intervention or

clinical study, and Hoehn and Yahr stage II-III [18] when off PD medication. <u>Primary</u> <u>exclusion criteria included</u>: existing cardiopulmonary disease or stroke, presence of dementia, and any medical or musculoskeletal contraindications to exercise. Potential study candidates were pre-screened with the American Heart Association exercise preparticipation questionnaire [19], to exclude those with major signs or history of cardiovascular or pulmonary disease. <u>MRI-related exclusion criteria included</u>: severe claustrophobia, health concerns with exposure to the magnetic fields, or MRIincompatible metal implant.

Exercise Intervention:

Prior to randomization, patients underwent cardiopulmonary exercise fitness testing on a cycle ergometer, with EKG and blood pressure monitoring, in order to ensure adequate exercise tolerance and determine their baseline voluntary exercise rate. Patients randomized to VE or FE underwent three exercise sessions per week, separated by at least one day, for eight weeks. The aerobic exercise intensity was matched for both groups, as the target heart rate (THR) for patients in both groups was in the range of 60-80% of the age-predicted maximum (220 minus patient's age) [20]. Each session comprised a 10-minute warm up and cool down period, separated by a 40 minute main exercise set, during which patients were instructed to keep their heart rate in their THR.

To deliver forced exercise, a specialized stationary cycle was developed that includes a motor driven pedal system that can augment patients' voluntary exercise rate, and which monitors heart rate, power produced by the subject, power contribution of the motor, cadence(pedaling rate), exercise time, and ambient temperature, among other variables. The motor is controlled by a feedback mechanism responsive to pedaling rate, patient work, and heart rate.

Subjects in the VE group operated the cycle without motor assistance, and voluntarily determined their resistance level and cadence. Subjects in the FE group received motor assistance to pedal at a rate 35% greater than their voluntary rate, as determined by the preliminary fitness testing. Both groups were instructed to maintain their heart rate in the THR. Patients randomized to the control group maintained their baseline level of activity.

Motor Assessment:

Patients underwent clinical assessment of motor symptoms using the UPDRS-III motor assessment [21] on five occasions: at baseline, half way through and at the end of the eight-week intervention, and after four and eight weeks of follow up. The rater was blinded to the treatment group of the subject. Additional tests included the Trail Making Test, and a bimanual dexterity test similar to that in [8].

MRI Data acquisition:

Patients underwent scanning on three occasions: at baseline, at the end of the eight-week intervention, and after 4 weeks of follow up. Although subjects were on PD medication for the duration of the study, scans were conducted while the patients were off medication for 12 hours. Subjects were scanned using a 12 channel receive-only head coil in a Siemens Trio 3T scanner (Siemens Medical Solutions, Erlangen, Germany), with the use of a bite bar to reduce head motion. During each session, scans included:

Scan 1: Anatomic 3D whole-brain T1 study: T1-weighted inversion recovery turboflash (MPRAGE). 120 axial slices; thickness=1.2 mm; field of view (FOV)=256 mm×256 mm; inversion time (TI)/echo time (TE)/repetition time (TR)/flip angle (FA)=1,900 ms/1.71 ms/900 ms/8°.

Scan 2: fMRI motor activation study. 160 repetitions of 31-4 mm thick axial slices acquired using a pulse sequence based on the prospective motion-controlled, gradient-recalled echo, echoplanar acquisition [22]; TE/TR/FA=29 ms/2,800 ms/80°; matrix= 128×128 ; FOV=256 mm×256 mm; bandwidth=250 kHz. Patients were instructed to perform the complex finger tapping pattern described below.

Scan 3: fcMRI continuous-task connectivity study. Whole-brain LFBF fcMRI study. 137 repetitions of 31-4 mm thick axial slices; TE/TR=29 ms/3,000 ms; matrix=128×128; FOV=256 mm×256 mm; receive bandwidth=250 kHz. The subject performed a biofeedback force-tracking motor task with the most affected hand (MAH) during the entire scan, as described below.

Scan 4: Resting Connectivity scan (rest): Whole-brain LFBF fcMRI study. Same protocol as scan 3, but the subject was instructed to rest with eyes closed and refrain from any voluntary motion.

The subjects performed the following tasks during scanning:

Complex bilateral finger tapping task. The task was performed with a pair of fiber optic data gloves (Fifth Dimension Technologies, Irvine, CA). Data were collected and synchronized with MRI scanning using an acquisition system designed and built in-house [23]. Subjects performed a bilateral complex finger tapping task beginning with a 60 second rest period, followed by four "on/off" cycles, each of which comprised 45 seconds of tapping and 45 seconds of rest (hereinafter "block paradigm"). This task was performed during scan 2 above.

Continuous fingertip force tracking task. This task was performed using a customized pinch grip force system designed in house [24]. Data were acquired at 128 Hz. Subjects applied a pinch grip (thumb and index finger only) to the device with their MAH to generate force readings. Immediately prior to each scanning session, each subject's maximum voluntary contraction force (MVC) was measured as an average of three trials of five seconds each, separated by at least one minute. Each subject's target force was set at 5% MVC. During the task, subjects were given real-time feedback of their applied force relative to the target and instructed to match the target force for the entirety of the scan. Subjects were trained and practice trials were conducted prior to scanning to ensure comprehension and minimize practice effect.

Task performance analysis

Complex bilateral finger tapping task. Finger tapping was monitored to ensure adequate task performance for motor activation and connectivity seeding (see below). The

performance metric was designed to ensure the subject was tapping during the 'on' blocks and resting during the 'off' blocks:

Performance = [(% time tapping during 'on' blocks) + (% time resting during rest blocks)]/2

Performance was calculated in this manner, as opposed to an overall % correct activity, so that the active and inactive periods receive equal weight (the paradigm includes more rest time than tapping time). Minimum performance was set to 90%. Studies not meeting this criterion were manually inspected to determine the reason for poor performance. Those with minor and identifiable deviations were analyzed with a modified block paradigm according to their tapping pattern, and performance was re-calculated. Using such a modified paradigm to generate fMRI activation maps can be problematic, as a trend function is also used in the calculation, and the modification can introduce a nonzero projection of the paradigm on the trend. The method was deemed acceptable in this case as the number of studies affected was small, the activation maps were visually inspected to ensure typical motor activation, and the fMRI analysis involved calculating an average map from several activation maps. The average activation map was then used only for seed localization, as described below. Studies with poor performance and major deviations were excluded from use in further analysis.

Continuous fingertip force tracking task. Force tracking was monitored to establish minimum performance criteria, and to analyze the frequency power spectrum. The force

tracking performance metric was designed to determine whether the patient was indeed performing the task and did not discontinue. It was calculated as the percentage of scan time during which the patient maintained force above a minimum, which was set to 75% of his/her target. Minimum performance was set to 75% of scan time, and studies in which this was not achieved were manually examined to determine the reason. Statistical distributions of change in task performance from baseline were calculated used to ensure there was no relation to the intervention.

The force tracking frequency spectrum was analyzed to ensure there was minimal force fluctuation below 0.08 Hz, the region of the spectrum in which intrinsic LFBFs lie [10], [11]. If subjects had been manipulating the force tracker at these frequencies, BOLD fluctuations in this part of the spectrum would be a direct result of task performance and represent activation rather than intrinsic fluctuations arising from functional connectivity. A fast Fourier transform (FFT) of the force tracker data was performed and the system was found to have characteristic 1/f noise ("pink noise"). The noise was modeled according to the following equations:

$$noise = C \cdot \frac{1}{f^{\alpha}}$$
$$\log(noise) = \log C - \alpha \log f$$

Where alpha is in the vicinity of 1 (white noise or Brownian noise occur for $\alpha=0$ or 2, respectively). Parameters *C* and α were estimated via linear transformation by plotting on a log-log scale: C was fit to the intercept and α was estimated as the slope in the most linear region that excluded our region of interest, between 0.1 and 10 Hz. No restrictions

were placed on α during estimation but its distribution was verified after fitting. The true power was then calculated as the power above the noise floor (power –noise). The low frequency power percent (LFP) was calculated as the true power below 0.08 Hz divided by that in the entire spectrum (up to 64 Hz). As a control case LFP for a block paradigm (as opposed to a constant target) was also calculated. The distribution of LFPs was examined and statistical distributions of change in LFP from baseline were calculated used to ensure there was no relation to the intervention.

As a further confirmation that task performance frequency did not directly cause LFBFs, this process was repeated after first convolving the force tracker data with the expected hemodynamic response function (HRF) of the BOLD response ##. The data was then re-sampled at a temporal resolution in accord with the TR of the fcMRI scan, in order for the Nyquist frequency to match that of the imaging data. This resembles the actual fcMRI experiment: the patient performs the task, there is a blood flow response, and we measure it using fcMRI at a certain sampling frequency (determined by the TR). Further, it allows us to determine whether the effects of task performance at a higher frequency are aliased into our pass band. In this case the frequency spectrum is limited (due to the TR of 3.0 seconds and resultant Nyquist frequency of ~ 0.17 Hz), which does not allow for estimation of noise as above. Thus, the absolute low frequency power <0.08 Hz was calculated as the area in the region of the spectrum 0 < f < 0.08 Hz. A block paradigm case was again used as a control.

Image Post Processing

The fMRI and fcMRI data were postprocessed in a manner similar to [23], including the following steps:

- 1. Retrospective motion correction using the AFNI 3dvolreg routine [25]. This step aligns all volumes in a 4D data set with the initial volume, and produces a 6 degree of freedom (DOF) set of motion parameters for each volume that is used in later analysis.
- Physiologic (cardiac and respiratory) noise source estimation and regression using PESTICA [26], [27].
- Second-order motion correction to correct for the effects of voxel-level motion (see Motion Analysis section for further detail).
- 4. Spatial filtering with a 4-mm full width at half maximum (FWHM) Gaussian filter. Such spatial filtering is generally not needed at 3T as it unnecessarily decreases spatial resolution. If it is done, a Hamming filter should be used as it is a matched filter designed to increase the signal-to-noise ratio [28]. The design of this study was such that each subject's seed voxel for FC analysis was propagated to all of their time points via coregistration, which can result in a partial voluming of the voxel and a resultant shift in the seed by a maximum of 1 voxel. Thus a 4-mm FWHM Gaussian blur was chosen to allow spatial coherence between study time points without significantly degrading the spatial resolution of the data.
- (fcMRI data only) Temporal filtering to remove all fluctuations above 0.08 Hz
 [10], [11].

Motion Analysis

Parkinson's Disease is a condition that produces significant motor impairment including tremor and rigidity. As such, PD patients comprise a unique population with regard to motion concerns for fMRI studies. Indeed, Helmich et al [29] found that PD patients move more during scanning than controls, and used second order motion correction (SOMC) to partially correct for these effects. Rather than either ignoring these issues or excluding PD patients from the benefits of functional neuroimaging studies, methods should be developed to identify and reduce artifact related to this, as was done here. We use three methods to address motion in this study: SOMC, peak-to-peak displacement exclusions, and statistical exclusions of FC maps (described in the FC analysis section).

Second order motion correction. The motion parameters generated from the retrospective motion correction (Post processing step 1) were used to trigonometrically calculate the x, y, z, and total displacement at each voxel [30], [31]. These displacement and certain parameterizations of them are known to correlate with signal change caused by motion and are regressed from the signal to perform SOMC [31]. The number of regressors used was held to 15 such that the degrees of freedom in the data would not be excessively reduced, and such that the contributions of the regressors could be accurately calculated (> 8 time points per regressor). The 15 motion estimators that were used included each of the displacements, their squares, lagged values (by 1 volume), and first derivatives, except for the first derivative of the x displacement, as these were found to be optimal in a separate analysis. The method was applied to all fMRI and fcMRI scans. Activation

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maps generated from fMRI data were visually assessed with and without the use of second order motion correction.

Peak to peak displacement exclusions. Motion parameters were also used to calculate the head displacement in each frame and subtracted to find the peak-to-peak displacement in the fcMRI data. Statistical distributions were analyzed to assess for group differences. Any study with a peak to peak displacement above 1.2 mm at any time during the scan was excluded from further analysis. This threshold is similar to or stricter than that reported in many studies, but is more lenient than that typically used by our group [23]. It was used due to the additional implementation of exclusions based on visual evaluation and statistical characterization of FC maps.

Image Analysis

fMRI data analysis. The fMRI data were analyzed by computing a least squares fit of the block paradigm described earlier to the timeseries data at each voxel [32]. A modified block paradigm was used for certain studies as described in the task performance section. This resulted in a whole-brain Student's t map, which was thresholded to determine regions of significant involvement in the complex bilateral finger tapping task. For each subject, fMRI data from all study time points (Baseline, EOT, EOT+4) were coregistered, and this coregistration was applied to the activation maps. The maps were then averaged to create a single average activation map for each subject, which was used for seed localization in the FC analysis.

fcMRI data analysis. FC analysis was done using the seed-based correlation method with the seed defined by activation, similar to the methods used in [23], [33]. The FC analysis involved:

- 1. Regions of interest (ROIs) were manually drawn for the left and right hand areas of the primary motor cortex (M1) based on both anatomical location and activation strength. These were drawn for each individual activation map and combined across each subject's multiple study time points via coregistration and a logical OR operator. The final ROIs were used in combination with the average activation map for seed selection.
- 2. Seed voxels were selected as the single voxel with the largest average activation within each ROI, after the ROIs and average activation map were coregistered to the subject's baseline fcMRI data.
- Seed voxels were then propagated to the later fcMRI scans via coregistration. This was performed so that the seed would represent the same region in every follow up study of that subject.
- 4. A reference timeseries was produced by calculating the arithmetic average of the timeseries' of the seed voxel and the 8 surrounding in-plane voxels in the fcMRI dataset.
- 5. The reference timeseries was correlated with the timeseries at every voxel in the brain, and the correlation coefficient converted to a Student's t statistic [34]
- 6. The Student's t map is z-score corrected by fitting a normal distribution to the FWHM of the distribution of Students t scores for all voxels as described in [11].

The result is a whole-brain map of z-scores representing functional connectivity to the seed region (FC map).

FC map quality assessment.

A rigorous quality assessment of the FC maps was undertaken to ensure that these maps represented motor network connectivity and did not contain artifacts significant enough to produce erroneous results in second level statistical analysis. All maps were visually inspected based on these criteria. None of the FC maps were excluded on this subjective basis, though some were found to be less ideal than others. Thus a statistical characterization of FC maps was performed to objectively exclude the least ideal maps.

The z-score correction described above is an empirical method to correct for global effects that bias the t-score distribution. The mean (μ_f) and standard deviation (σ_f) that are derived from the fit are used to center and normalize the distribution and thereby produce a z-score, which is useful for comparison across subjects [11]. Here, μ_f can be taken to represent the average magnitude of the biasing effects, where as σ_f is partially related to the variability in those effects. Thus, FC maps in which the t distribution has large μ_f or σ_f values may potentially have artifacts or otherwise be of poor quality. It is important to note that these parameters are not the sample mean and sample standard deviation of the t-score distribution, but rather parameters calculated from the normal fit to the FWHM of the t-score distribution. The fit is restricted to the FWHM because this is the Gaussian component, and the tails contain higher order correlations [11], which are generally of interest, though can also be produced by artifactual sources. Standardized moments are

useful to describe such statistical distributions, where the nth standardized moment is given by:

$$n^{th}moment = \frac{E[(x-\mu)^{n}]}{\left(E[(x-\mu)^{2}]\right)^{n/2}} = \frac{E[(x-\mu)^{n}]}{\sigma^{n}}$$

where μ is the population mean and σ is the population standard deviation. We can see from the numerator that an odd standardized moment will be signed and thus describe asymmetry in the distribution, and further that higher moments will be weighted more heavily at further distances from the mean. Thus the first standard moment is zero, and the third is the skewness, a common measure of asymmetry. The fifth standard moment provides a characterization of asymmetry that is sensitive further from the mean (i.e. in the tail regions) and thus provides the information desired. The sample fifth standardized moment (γ) was calculated for N voxels in the brain as

$$\gamma = \frac{\frac{1}{N} \sum_{i=1}^{N} (x_i - \overline{x})^5}{\left(\frac{1}{N} \sum_{i=1}^{N} (x_i - \overline{x})^2\right)^{5/2}}$$

Statistical distributions of μ_f , σ_f and γ were examined to determine if any relationship existed with visual assessment of the maps.

Second level statistical analysis

Spatial normalization into Talairach space. In order to perform group level analyses, FC maps were registered into a common space. The fcMRI datasets were coregistered with the anatomical images acquired in scan 1 using the align_epi_anat utility [35]. Anatomical images were normalized into the standard Talairach-Tournoux space using the AFNI auto-Talairach tool in combination with a modified template developed inhouse to correctly align the AC-PC line [36]. These transformations were then applied to the FC maps to normalize them into Talairach space which involved resampling to 1x1x1 mm resolution. FC maps were also spatially smoothed with a 6-mm FWHM Gaussian filter in preparation for group-level analysis.

Effect of exercise on functional connectivity. To determine the change in FC from baseline, difference maps were created by subtracting the baseline FC map from the EOT or EOT+4 map (i.e. $\Delta FC_{EOT} = FC_{EOT} - FC_{Baseline}$). These could only be calculated if neither FC map had been excluded. For the constant task connectivity data, since patients performed the task with the most affected hand (MAH), R M1 or mirrored L M1 FC maps were used for those with L or R MAH, respectively. Thus these maps represent FC to the most affected M1 and appear as connectivity to the right motor cortex. The ΔFC maps for subjects in the exercise groups were concatenated into a 4D dataset (3D x subject) and voxel-wise correlations were then performed with cadence to determine regions in which ΔFC is related to the subject's average pedaling rate. The correlation measure used was a Student's t statistic. Correction for multiple comparisons across voxels was performed using a family wise error (FWE) correction, computed via a 10

000 iteration Monte Carlo simulation, to determine significant cluster sizes. The significance level was set at p<0.05, corrected.

<u>Results</u>

Subject demographics

Subject demographics are presented in Table 1. All three groups were comparable with respect to all these factors, except the exercise exposure (cadence). The FE group did pedal at a faster average rate than the VE group, however this rate was only 19% greater than that of the VE group. This corresponds to 55% of the designated increase in pedaling rate in the FE group (35% above voluntary rates). Although this was designed for a cross-over setting, it was also the increase at which a measureable clinical effect was seen in preliminary studies [8]. Considering that the therapeutic efficacy is believed to be related to the pedaling rate, the expected effect size with a 19% increase would be much smaller than that previously seen. As this study was not powered to detect such small group differences, the analysis scheme was changed to a multivariate regression model in which the cadence was correlated to the outcome, as specified in the Methods above.

| | Control group | VE group | FE group |
|---|-----------------|--------------------|-----------------|
| | (n=10) | (n=16) | (n=16) |
| Mean age (SD) | 60.9 (9.6) | 57.8 (7.5) | 56.8 (9.0) |
| # Female, <i>n</i> (%) | 3 (30%) | 5 (31%) | 7 (44%) |
| # Dominant hand = \mathbf{R} , n (%) | 9 (90%) | 14 (88%) | 14 (88%) |
| # Most affected hand = \mathbf{R} , n (%) | 4 (40%) | 9 (56%) | 6 (38%) |
| Median # months PD (IQR) | 53.5 (7 – 57) | 45.5 (16.5 - 83.5) | 40.5 (19 – 63) |
| Median LEDD (IQR) | 491 (200 – 780) | 600 (363 – 774) | 364 (113 - 649) |
| Mean baseline UPDRS III motor (SD) | 20.1 (7.3) | 22.5 (11.3) | 23.6 (9.5) |
| Mean cadence (SD) | N/A | 72.33 (12.78) | 86.15 (6.53) |

| Table 1 | Characterist | ics of stud | y subjects |
|---------|----------------------------------|-------------|------------|
|---------|----------------------------------|-------------|------------|

SD=standard deviation; IQR = interquartile range; LEDD = Levodopa equivalent daily dose. UPDRS = Unified Parkinson's Disease Rating Scale.

Accordingly, demographic variables were checked for codependence and then for interaction with the exercise exposure (cadence). Codependence was found only between LEDD and PD duration, thus only PD duration was included in the test for interaction with cadence. None of the variables were strongly related to cadence. The only variables which trended towards correlation with cadence were MAH and Age (0.05), with age being the stronger predictor. As age is a plausible confounder and there is an a priori reason for cadence to be affected by age, it was adjusted for in the final correlation analysis. It was the only adjuster used, as additional use of marginally related regressors needlessly reduces the degrees of freedom. However, the analysis was repeated with the additional inclusion of MAH as a regressor to ensure results were not significantly different.





Figure 1. Complex bilateral finger tapping task performance: histogram of task performance across all subjects and time points.

Complex bilateral finger tapping task. Glove data was not available for two of the 118 fMRI activation studies. These two studies were analyzed in the standard manner, and

activation maps were confirmed to represent motor activity. For this reason, and due to the high fidelity of task performance among all subjects (as described below, shown in Figure 1), these two studies were included in the analysis. Of the remaining 116 studies, 96 met the minimum performance criteria and fMRI activation maps were later generated for these using the standard block paradigm reference function. Manual examination of the studies that did not meet criteria revealed that 3 of 20 had small errors and high noise levels, but their performance was >85%. Thus these were deemed acceptable and analyzed in the standard manner. An additional 14 of 20 had minor deviations from the standard block paradigm as described in Table 2. Activation maps for these studies were generated with modified block paradigms and visually confirmed to represent typical motor activation. Tapping performance was also recalculated with these modified block paradigms. Three of the 20 studies not meeting criteria had unacceptably poor performance and were not included in further analysis. A histogram of final tapping performance measures is shown in Figure 1.

| Case | Deviation description | Shared | # of |
|------|--|------------------------|---------|
| | | variance | studies |
| | | with trend function | |
| 0 | No deviation (standard block paradigm used) | 0% | 99 |
| 1 | Did not tap during 1st 'on' cycle | 6% | 4 |
| 2 | Started tapping at beginning of scan and tapped through initial rest | 18% | 6 |
| | period | | |
| 3 | Same as #2, and also stopped tapping at midpoint of 4 th 'on' cycle | 29% | 1 |
| 4 | Continued tapping after 4 th 'on' cycle until end of study | 18% | 1 |
| 5 | Switched the 'on' and 'off' portions of each cycle | 18% | 2 |

Table 2. Studies with minor deviations in tapping from standard block paradigm

Continuous fingertip force tracking task: performance. Force tracker data was available for 99 of the 116 continuous task fcMRI studies. Performance was >75% in 94 of 99 studies. On manual inspection of the 5 studies that did not meet criteria, it was found that

these studies had low force targets and high noise relative to these targets. For these studies, the minimum force was lowered by an amount proportional to the amplitude of the noise relative to the target. The new minimums were between 60-70% of the subject's target, as opposed to the original 75%. Four of these five now met the minimum performance criteria (Figure 2, left panel), and one study was excluded from further analysis. Change in performance from baseline to EOT and baseline to EOT+4 was calculated for each subject, and no relationship was found with cadence (p=0.20 and 0.48, correlation, respectively).

Continuous fingertip force tracking task: low frequency power percent. Force vs. time plots are shown for a constant force tracking task and a block paradigm alternating task in Figure 3A and B, respectively. Figure 3C and D show log-log plots of the FFT in each case, with the linear fit for the noise super imposed. All α values were in the range $0.8 \le \alpha \le 1.25$. Normal scale plots of the frequency spectrum, with the low frequency region of interest highlighted, are shown in Figure 3E and F. In the block paradigm task there is a considerable amount of power above the noise floor in the region below 0.08 Hz, where as there is minimal power in this region in the constant force tracking task. The LFP histogram in Figure 2 (right panel) confirms this is true for all studies, with the majority of LFPs below 5% and a maximum of 15.4%. For comparison, the LFP in the block paradigm case was 44.7%. Change in LFP from baseline to EOT and baseline to EOT+4 was calculated for each subject, and no correlation was found with cadence (p=0.46 and 0.28, respectively).



Figure 2. Continuous fingertip force tracking task performance: Histograms of the task performance (left) and the low frequency power percent (right), for all subjects and time points.

The analysis after convolution with the canonical HRF and resampling at a temporal resolution of 3.0 seconds is shown in Figure 4. Figures 4A and B show the continuous and block paradigm tasks after convolution, respectively, which now represent the expected hemodynamic response to the force tracking task. Their respective FFTs are shown in Figures 4C and 4D, showing that there is nearly no power below 0.08 Hz for the continuous task, where as there is considerable power in this region for the block paradigm task. Figure 5 shows a histogram of the area in the region 0 < f < 0.08 Hz for all studies. Most studies had an area ≤ 100 , and one study had an area > 250. This study was excluded from further analysis. For comparison, the block paradigm task had an area of 835.



Figure 3. Example of the low frequency power percent (LFP) analysis. The left panel is an example of a typical constant force tracking task, whereas the right panel shows a block design task. The top row shows the task being performed, the middle row shows the linearization and fit of the 1/f noise model, and the bottom row depicts the power above the noise floor in the frequency range of interest. There is considerably less power <0.08 Hz in the constant force tracking task.



Figure 4. Low frequency power in the expected hemodynamic response to force tracking. The left panel is an example of a typical constant force tracking task, whereas the right panel shows a block design task. The top row shows the task being performed, after convolution with a canonical hemodynamic response function and down-sampling to the temporal resolution of fcMRI. The bottom row depicts the absolute power in the frequency spectrum. There is considerably less power <0.08 Hz in the constant force tracking task. (Note that there is a 10x magnitude difference in the vertical axes of panels C and D.)

The initial approach shows that subjects performed the task at frequencies above 0.08 Hz, and the second approach shows that this task performance would not produce low frequency BOLD fluctuations in the fcMRI data in most subjects. Thus, the LFBFs seen during the continuous-task fcMRI scan were due to intrinsic fluctuations and not directly caused by task manipulation.



Figure 5. Histogram of the low frequency power in the expected hemodynamic response to the force-tracking task for all subjects and time points.

Motion Analysis

Second order motion correction

For the fMRI data collected in scan 2, 56% of scans were identified as having motionrelated artifact before SOMC was implemented (Figure 6, Left). SOMC resulted in a reduction in motion-related artifact in the data (Figure 6, Right), and all activation maps were considered improved, though many were not necessarily ideal. However, via visual inspection of maps after SOMC, it was determined that all maps had appropriate motor activation and were useable for activation-based seed localization.



Figure 6. Examples of Activation maps prior to SOMC (Left) and after SOMC (Right). Motion artifacts are generally decreased in the majority of cases.

Peak-to-peak displacement exclusions

As described in the methods, a peak-to-peak displacement threshold of 1.2 mm was used to exclude fcMRI datasets from further analysis. This criterion resulted in the exclusion of 8 continuous-task fcMRI studies and 7 resting state fcMRI studies. The maximum peak-to-peak displacement in each of the remaining studies is displayed in histogram format in Figure 7. Further, the average level of peak-to-peak motion in each study was not related to cadence, and neither were changes in each subject's motion level from baseline to EOT or EOT+4, for both the continuous-task and resting state fcMRI scans.



Figure 7. Histogram of the largest peak-to-peak head displacement among all fcMRI studies not excluded for motion (constant task and resting state pooled).

Functional Connectivity Analysis

Functional connectivity map quality assessment.

Maps of FC to the R and L M1 regions were produced for each of the 116 constant task fcMRI studies and 118 resting state fcMRI studies, resulting in a total of 468 FC maps. These were visually inspected and none were found to be poor enough to exclude solely on this basis. The maps were rated on a 7 point rating scale (0-6) and 51% were found to be ideal (score 0-2), 23% were found to be good (score 3), and 26% were found to be non-ideal (score 4-6).



Figure 8. Example of a constant-task functional connectivity map (left) and its uncorrected t-score distribution (right). The FC map shows two axial slices of the motor cortex and the FC to the right hand area.

An example of a constant-task FC map of connectivity to R M1 is shown in Figure 8, along with the distribution of its t-scores (prior to z-score correction). The quality of the normal fit is good above the FWHM, but departure from normality is seen in the tail regions. This is an asymmetric distribution with a heavy positive tail (γ =4.1), and these higher order positive correlations are expected due to intrinsic FC (displayed in the connectivity map). Negative correlations are expected as well, but it is rare for one to expect asymmetry with a heavy negative tail as a result of intrinsic effects, though these often occur due to artifact. This was confirmed in the statistical characterization of the FC maps.

The fitted mean, fitted standard deviation, and 5th standardized moment (μ_f , σ_f and γ , respectively) were plotted against map rating to determine if any relationship existed between quality of the map and the statistical descriptions (Figure 9). The

parameters μ_f and σ_f did not have a direct relationship with map quality, though large σ_f was seen in poor maps. There was a direct relationship between γ and map quality with poorer maps tending to have a more negative γ . However, there was no γ threshold identified that could reliably identify a large number of poor maps. Due to these observations it was postulated that a 2-dimensional statistical descriptor space between σ_f and γ may allow a better separation, as poorer maps would exist in the high- σ_f , low γ subspace.

Thus, such a scatter plot was created (Figure 10) with the objective of separating outlying non-ideal maps (score ≥ 4) from the bulk. A linear separator of the simple form

$$\gamma = a\sigma_f + b$$

was optimized to separate out the largest number of poor maps with the highest fidelity (% of those separated out that are non-ideal), via parameter sweep over *a* and *b*. The constant-task FC maps were used as a training dataset for the optimization, and the resultant separator was applied to the resting-state FC maps. Optimal performance was confirmed for the resting state maps. The optimized separator was found to have a = 0.9, b = -2.4. For the constant-task FC maps, 22 of 232 maps were separated out, 21/22 (95%) of these were rated as non-ideal and were excluded from second-level analysis based on this objective confirmation. It should be noted that the 22nd map received a score of 2 (ideal), underscoring the need for visual inspection of FC maps. For the resting-state FCmaps, 14 of 236 maps were separated out, and 13/14 (93%) were rated as non-ideal, while 1/14 was rated 3 (good). These 14 maps were also excluded from further analysis.

Constant-task FC maps

Resting state FC maps



Figure 9. Relationship between FC map quality and fitted mean μ_f (top row), fitted standard deviation σ_f (middle row), and the 5th standardized moment of the distribution , γ (bottom row). Quality ratings of maps are plotted on each horizontal axis. Data are plotted in red, and the mean and 95% confidence intervals are overlaid in blue.



Figure 10. Two-dimensional statistical descriptor space between the fitted standard deviation (SD), σ_f , and the 5th standardized moment of the distribution, γ . The optimized linear separator is overlaid.

Effect of exercise on functional connectivity

Correlation Student's t maps were generated as described and thresholded/clustered to identify regions in which Δ FC was significantly related to cadence after adjusting for age. Positive and negative correlations are displayed as orange and blue regions in the correlation maps. Stronger correlation is indicated by a larger cluster or brighter color. The final numbers of subjects included in each correlation analysis (after exclusions) are listed in the figure captions. FC from the constant-task data is referred to as task-related FC (trFC), whereas that measured in the resting state data is the resting state FC (rsFC). Figure 11 shows regions in which the change in trFC from baseline to EOT (Δ trFC_{EOT}) is significantly related to cadence.



Figure 11. Correlation map of $\Delta trFC_{EOT}$ with cadence. Regions in which changes in task-related FC to the most affected M1 (at EOT) are correlated with cadence. Most affected M1 is displayed on the right side. N=20. Clusters are significant to p<0.05, FWE corrected.

Thus, the task related FC between the most affected M1 (*M1) and the ipsilateral thalamus tends to increase in those who cycle faster, where as that from *M1 to the medial prefrontal cortex (PFC) and supplementary motor area (SMA) tends to decrease. The thalamic effect is strongest in the posterolateral region of the thalamus ventrally, but also extends to the medial region dorsally. At EOT+4, after 4 weeks of follow up, this effect is preserved, and a positive correlation is also seen in the ipsilateral anterior putamen (Figure 12). A trend towards this was also seen at EOT, but did not meet

significance. A similar trend towards correlation was seen in the contralateral putamen and contralateral anterior thalamus



Figure 12. Correlation map of $\Delta trFC_{EOT+4}$ with cadence. Regions in which changes in task-related FC to the most affected M1 (at EOT+4) are correlated with cadence. Most affected M1 is displayed on the right side. N=18. Clusters are significant to p<0.05, FWE corrected.

At EOT+4, a positive correlation is also seen in the contralateral motor cortex, while negative correlations are seen in the supplementary motor area (SMA) and ipsilateral parietal cortex (Figure 12).



Figure 13. Correlation map of Δ rsFC with cadence. Regions in which changes in resting state FC to Right (top row) or Left (bottom row) M1 are correlated with cadence. Changes at EOT and EOT+4 are depicted as labeled. N=23, 21, 20, 21 for RM1 EOT, RM1 EOT+4, LM1 EOT, LM1 EOT+4, respectively. Clusters are significant to p<0.05, FWE corrected.

In the resting state data, $\Delta rsFC_{EOT}$ between R M1 and several subcortical areas is strongly negatively correlated to cadence, as seen in Figure 13 (top left). Those who pedal faster tend to decrease their connectivity between R M1 and the midbrain area, which may represent the bilateral subthalamic nucleus (STN) or part of the substantia nigra. This effect is also seen at EOT+4 (Figure 13, top right). Negative correlations were also seen bilaterally in the globus pallidus area, but did not reach significance. However, several artifactual regions also appear in the correlation maps for rsFC to R M1, including the regions in the ventricles and in the white matter. This indicates that the false-positive rate may be greater than that expected. Few correlations are seen with change in rsFC to the L M1 area (Figure 13, bottom row), and regions are not consistent from EOT to EOT+4.

Discussion

The primary aim of this study was to investigate the neural network changes that occur with exercise in PD using functional connectivity MRI. The characterization of functional connectivity in PD is a relatively young endeavor, with most studies having been published in the last 3 years. The reality that PD patients tend to move more during scanning than controls has only been slowly realized [29], and poses a unique challenge in studying this population using this technique. Although less motion generally occurs in fcMRI studies than fMRI studies due to the constant condition or state the subject is in, analysis may be more sensitive to motion as it is entirely data driven. Indeed, among the few studies that have been published, conflicting results are present [29], [37], [38]. While this is not entirely unexpected, and is likely to be partially related to true intrinsic variability in the population and disease, it may also be partially caused by motion-related artifacts. Appropriately addressing this issue will help create a consistent picture of the connectivity alterations in PD and aid in the development and assessment of new therapies.

Here we present a novel method for objectively assessing the quality of functional connectivity maps, based on the fitted standard deviation and 5^{th} standardized moment of the uncorrected t-score distribution. It is not designed to be a highly sensitive test to identify all poor or corrupted maps; visual assessment is still recommended. Rather, it is a specific test with a high positive predictive value that will provide objective criteria to exclude a map that is subjectively questionable. The general method should be widely applicable although the specific relationship will vary based on scan specifications, the type of study, and the network of interest. For example, while a motor network is

expected to have many strongly positive correlations, other networks such as the default mode network (DMN), along with its anti-correlated task-positive network, may display a more balanced distribution. Nevertheless, we expect the general pattern to hold, as it is uncommon to have an a priori expectation of an imbalance in the negative direction, as such an imbalance is often related to artifacts. For example, in Fox et al's [39] treatment of anticorrelations and the global signal, distributions of uncorrected correlations are seen to have heavy lower tails prior to global signal regression. The global effects they describe include physiologic (cardiac and respiratory) noise, among other sources, and after correction, the distributions generally have some positive asymmetry (Figure 2 in [39]). Such large negative asymmetries are not seen prior to z-score correction in the data presented here as physiologic noise is removed earlier in the processing stream. However, the example is instructive, as it indicates those FC maps with highly negative γ may have residual physiologic noise or other artifacts. With regards to the specific methodology, an argument can be made for using a nonlinear separator, for example, one that initially has a similar linear trajectory but then increases, approaching a vertical asymptote representing some maximum acceptable σ_{f} . This would allow for the exclusion of maps above this maximum σ_f , regardless of γ . This approach was not taken as there was no data in that region of the descriptor space to fit to. An advantage of using a linear separator, however, is its extendibility. If additional statistical descriptors (or other predictors) are identified, the space can be expanded into an N-dimensional cube, in which a (N-1)dimensional hyperplane can provide a similar linear separator with parameters that are simple to optimize. This method was implemented here to allow analysis of fcMRI data from PD patients while ensuring maps were of good quality.

Returning to the primary aim of the study, we found that PD patients who pedaled faster during their 8 weeks of exercise tended to have increases in cortico-subcortical connectivity *during task performance*. The *active* motor cortex in these patients showed a stronger connection to the ipsilateral thalamus (and possibly the putamen, though not statistically significant) after 8 weeks of exercise therapy, and a decrease in connectivity to the SMA. Both of these effects persisted 4 weeks after the end of the exercise period. These results are largely in accord with our hypotheses: we expected increases in cortico-subcortical subcortical connectivity (except to the STN or GPi) to be related to pedaling rate and to persist at EOT+4. We did not expect a decrease in connectivity between cortical motor regions (M1 and SMA), and the discrepant result may be as a result of determining general hypotheses based on resting state FC studies, as no studies have yet reported on task-related FC in PD. Further potential reasons for this result are discussed below.

The region of the thalamus in which the high correlation is seen is a large posterior area, ranging from posterolateral at the ventral aspect to more medial dorsally. The regions indicated likely include the pulvinar nucleus and the posterior aspect of the ventrolateral nucleus. The ventrolateral nucleus serves as a thalamic relay nucleus for the motor circuit, receiving input from the basal ganglia and cerebellum, and directing output to motor cortical regions [40]. The pulvinar nucleus is an association nucleus of the thalamus which connects largely to the parietal, occipital, and temporal association cortices [40]. This is consistent with the fact that improved connectivity to the pulvinar region was seen *during* the performance of a visuomotor feedback task, in which the subject must integrate visual sensory information to modulate his/her motor output. Thus, in patients who pedal faster, there tends to be improvement in thalamo-cortical task-

related connectivity, specifically in regions of the thalamus important for motor control and sensory integration.

Such task-related FC is not as commonly studied as resting-state FC, though several groups have published measures of effective connectivity during a task [41–45]. Effective connectivity (EC) differs from FC in that it is measured during a traditional fMRI scan (i.e. with alternating task blocks), rather than in what we refer to as an fcMRI scan, in which the subject is in a constant state throughout the scan. Further, EC measures the degree to which the activity of one region contributes to another [42]. A study of particular interest is that by Palmer et al. [44] as the force tracking device and task used was similar to that of this study, though it was in a block design. They report that effective connectivity was more robust to changes in task frequency than traditional activation measures, and report changes in EC of PD patients relative to controls [44]. EC during the task in PD patients was decreased in the striato-thalamo-cortical pathway, and increased in the cerebello-thalamo-cortical pathway, relative to normal controls [44]. Wu et al. [42] reported similar findings of EC during a self-initiated finger tapping task. They found that task-related EC between the putamen and cortical motor areas was negatively correlated to UPDRS and decreased compared to normal controls, whereas EC between M1 and the pre-SMA, premotor cortex, parietal cortex, and cerebellum was positively related to UPDRS and increased relative to controls [42]. The latter increases in EC are proposed to be compensatory mechanisms for the former decreases [42], [44]. These findings are consistent with our observation of increases in subcortical-cortical trFC, and decreases in trFC from M1 to SMA, in patients who pedal faster. This implies that a plausible mechanism for the therapeutic efficacy of exercise in PD is that it improves

striato-thalamo-cortical connectivity during the performance of a task, and results in less need for cortical compensation.

In the resting state analysis, those who pedaled faster tended to show decreases in connectivity from R M1 to the midbrain region, which may represent the STN or substantia nigra, and a trend towards this same pattern in the globus pallidus (not statistically significant). However, artifacts were also seen in the results and thus additional analysis is necessary to determine whether this relationship is accurate and determine the reason for these artifacts. Despite this, the effect is plausible, as the STN and internal globus pallidus are traditionally believed to be hyperactive in PD, and are common targets for deep brain stimulation, an effective therapy. STN overactivity in PD patients is believed to be a result of the hyperdirect pathway, and has been observed by Baudrexel at al [17] who found increased FC between the STN and cortical motor areas in PD patients relative to controls. Thus, exercise may additionally exert its effects by attenuating this overactivity, however further study is necessary to confirm this idea.

This study was limited by relatively low sample size. The original study was powered for the primary clinical aims, and only a subset of those participating were also enrolled in the MRI portion of the study. This may be responsible for the regions that did not meet statistical significance at one time point, but did at another. The issue was exacerbated by a second limitation, the degree of motion in the studies, as several studies had to be excluded due to motion. However this resulted in the identification of motion as an important problem in neuroimaging research in PD, and the development of methods to address it. A third limitation was the lack of an on-medication scanning session. Previous work in our laboratory has shown that the effects of PD medication and exercise on FC are related [46], and thus an on-medication scan would be useful in an analysis of exercise and FC. Thus, future work will involve larger sample sizes, an on-medication scanning session, and will reduce the complexity of the scanning protocol to lower the degree of motion.

This work provides important insight into the mechanism of the therapeutic effect of exercise in Parkinson's Disease. The effect is driven by strengthening of striatothalamo-cortical connectivity during task performance, and may also be related to decreases in resting connectivity between RM1 and STN. These directions of connectivity changes are correlated to pedaling rate during exercise, and thus more likely in those who pedal faster. Further, this work advances the field of neuroimaging in Parkinson's Disease by calling attention to the fact that PD subjects are more likely to move during scanning, and developing methods to address this.

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