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TITLE: Effect of SMARTfit training on motor, cognitive functions and brain connectivity in individuals with Parkinson's disease: a pilot study

PRINCIPAL INVESTIGATOR(S): Charles Liu, MD, PhD

CO-INVESTIGATOR(S): Beth Fisher, PhD, PT, FAPTA
Judy Pa, PhD

SPONSOR: None

PARTICIPANTS/LOCATIONS: Individuals with Parkinson's disease/ Division of Biokinesiology and Physical Therapy (CHP building) and Stevens Neuroimaging and Informatics Institute

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TABLE OF CONTENTS

1.0 BACKGROUND AND HYPOTHESES

Although Parkinson's disease (PD) has been mainly viewed as a movement disorder, the pathophysiology of declined motor function incorporates impairments of multiple systems (sensory, motor, and cognition). Specifically, it has been demonstrated that cognitive function may play an essential role in daily motor function for individuals with PD[1,2]. Cognitive dysfunction involving set-shifting and attentional control has been found to be associated with movement slowness in performing a finger sequence task and freezing of gait[3–8]. The above results are in line with the clinical observations that motor performance in individuals with PD is degraded when the cognitive load of motor tasks increases, such as talking on the phone while walking or maneuvering between people in a crowded mall. Furthermore, a recent rodent model indicates that cognitive dysfunction may occur prior to the onset of motor symptoms[8]. Human studies also show that 25-30% of individuals with PD exhibit cognitive impairments at the time of diagnosis [9,10]. The overall evidence suggests that cognitive dysfunction may contribute to degraded motor function.

Interestingly, emerging evidence demonstrate that aerobic exercise and resistance training can improve cognitive function in individuals with PD[11–15]. One study done by Ridgel et al. (2009) shows enhanced cognitive function after 30-min passive cyclying[12]. The findings indicate that there is a tight interplay between motor and cognitive function in individuals with PD. Therefore, targeting cognitive function and incorporating cognitive training into physical rehabilitation may be important as it may better help individuals with PD to transfer the learned skill into real-world daily activities. Although there is mounting evidence for the benefits of physical exercise in PD, few studies investigate whether combining cognitive training with physical exercise can provide additional benefits than physical exercise alone.

It has been suggested that exercise exerts its beneficial effects in PD, at least partially, through changes in the brain16. However, few studies have linked these improvements in motor and cognitive function with alterations of the brain and the exact nature of these alterations is not completely understood. Neuroimaging studies using functional magnetic resonance imaging (fMRI) have documented altered brain functional connectivity in PD[17,18]. Relative to non-disabled adults, people with PD showed reduced functional connectivity in mesolimbic-striatal and corticostriatal loops, with strengthened connectivity between cortical areas, such as pre-SMA and M1. Exercise has been shown to change altered brain functional connectivity, as demonstrated by inducing an increase in cortico-subcortical connectivity and a decrease in cortico-cortical connectivity after cycling training[19,20]. In addition, it remains to be answered whether physical exercise can also modulate the disease progress in PD. Blood-based α-synuclein and DJ-1[21–24], as well as quantitative electroencephalogram (qEEG)[25,26] have been demonstrated to be predictive biomarkers related to disease progress and cognition decline in individuals with PD. EEG is a routine test procedure commonly used in clinical neurology and a gross measure of neural electric activities from the scalp. Amplitude of a given activity reflects the degree of neuronal synchronization underneath the recording lead. The relative magnitude distribution among different frequency bands reflects the brain status whether in sleep, relaxation or vigilance. In addition to the epileptic spikes found in seizure patients, individuals with different mental disorders often have different patterns in their EEG profile. More recent studies in conjunction with other neural imaging measures have shown that EEG may predict the state energy metabolism in the brain as well as how the cognitive information is processed. The characteristics of EEG resonance were further found to be closely associated with how cognitive information is encoded in the brain[27]. Significant deviation of the alpha EEG (8-12 Hz) from the norm was found in various illnesses. Quantitative EEG is a non-invasive, information rich, and easy-to-use objective test. Recent studies have demonstrated that it is sensitive to detect minor neurological changes. Exploring the changes in biomarkers following physical training will provide more insights into potential mechanisms underlying training-induced benefits in PD. We expect that EEG changes will be seen along with physical and cognitive changes.

The SMARTfit (https://smartfitinc.com/) is a novel technology that provides an opportunity to combine physical training with cognitive training. SMARTfit technology focuses on providing a multi-sensory approach for exercise by simultaneously using visual and auditory stimuli/feedback while delivering physical and cognitive training games. SMARTfit intends to design physical training with game technology by using time and score to ignite engagement and enjoyment during physical training, as well as providing cognitive challenges. In consideration of the imbedded cognitive and motivational components in SMARTfit, it can be utilized as a model to investigate the benefits of combining cognitive challenges with physical training on individuals with PD. Thus, the purpose of this pilot study is to investigate the effectiveness of a SMARTfit training in individuals with PD. Our hypothesis is that SMARTfit training will promote motor and cognition improvements over conventional physical training by altering brain functional connectivity. To test the hypothesis, individuals with mild PD will receive both SMARTfit training and conventional physical training in a counterbalanced order with a washout

period. For each training program, participants will receive a 1-hour training session 3 times per week for 8 weeks. The changes in disease biomarkers before and after training will also be explored.

Parkinson's disease: Approximately 630,000 people in the US were diagnosed with Parkinson's disease (PD) in 2010 and it is estimated that PD prevalence will double by 2040[28]. PD progression leads to decreased independence and quality of life, as well as increased reliance on the healthcare system. The economic impact of PD, including treatment, social security payments, and lost income from inability to work, exceeded $14.4 billion in 2010[28]. There is no known cure for this degenerative disease that results in progressive deterioration of motor skills along with reduced sensory and cognitive function. The current treatment for PD is medication (levodopa, dopamine agonists) and surgical intervention (deep brain stimulation). These treatments only partially treat the symptoms and are less effective over time. Furthermore, they often have undesirable side effects. In the past 15 years, mounting evidence has shown that exercise can ameliorate PD symptoms. Even though exercise has been demonstrated to be beneficial for improving PD motor symptoms, significant gaps in this knowledge limit the ability to create specific exercise prescription to optimize neuroplastic changes in the PD brain[16].

2.0 OBJECTIVES AND PURPOSE

The aims of this pilot study are:

1) to examine the changes in clinical and physical performance measures after SMARTfit training and conventional physical training. Outcome measures will include MDS-UPDRS III (motor) score and the modified Physical Performance Test (mPPT) before and after SMARTfit training and conventional physical training. We hypothesize that clinical and physical performance will improve after SMARTfit training more than after conventional physical training.
2) to examine the changes in cognitive function measures after SMARTfit training and conventional physical training. Outcome measures will include Trail-Making test (TMT) and Parkinson's Disease-Cognitive Rating Scale (PD-CRS) before and after SMARTfit training and conventional physical training. We hypothesize that cognitive function will improve after SMARTfit training more than after conventional physical training.
3) to examine the changes in brain connectivity after SMARTfit training and conventional physical training. Functional magnetic resonance imaging during rest and a cognitive task will be measured before and after SMARTfit training and conventional physical training. We hypothesize that cortico-subcortical connectivity will increase and cortico-cortical connectivity will decrease after SMARTfit training more than after conventional physical training.
4) to examine the changes in biomarkers after SMARTfit training and conventional physical training. Blood-based α-synuclein and DJ-1, as well as quantitative EEG will be measured before and after SMARTfit training and conventional physical training.

3.0 STUDY DESIGN

3.1 Methods

Sample size and participants

This pilot study aims to investigate the effects of 8-week SMARTfit training versus conventional physical training on motor function, cognition and brain functional connectivity in individuals with PD. The target sample size is a maximum of 12 participants with PD. Recruitment to this study at this time is based primarily on word of mouth from prior participants. We will also recruit through the use of flyers posted on campus and its immediate vicinity, and through a website. All eligible recruits will sign a written informed consent to participate in the experimental protocol approved by the Institutional Review board of the University of Southern California. All participants will be screened for inclusion and will be excluded from the study if they do not meet the inclusion criteria.

Prior to their arrival, participants will complete one brief (about 5 minutes) intake questionnaire that includes questions about their medical and psychiatric history and their current use of alcohol, nicotine and prescription or non-prescription drugs to screen their eligibility for MRI. The purpose of the interview is to obtain more detailed information about any factors that may influence either their eligibility to participate in the procedures or the results of the procedures themselves. Potential subjects will also be screened for contraindications for MRI experiment such as pregnancy, pacemakers and implants.

Procedures and tasks

This pilot study is a cross-over trial to estimate the effects of 8-week SMARTfit training versus conventional physical training on behavior, brain measures and biomarkers in individuals with Parkinson's disease. Participants will receive both 8-week SMARTfit training and 8-week conventional physical training with an 8-week washout period in between. The order of received training will be counterbalanced between participants. Each training regimen will consist of 24 training sessions over an 8-week period (3 days/week). Motor function, cognitive function, the Unified Parkinson's Disease Rating Scale (UPDRS) measures and functional connectivity of the brain will be assessed before and after each training program.

Ten individuals with mild Parkinson's disease, who are medically approved for both intense exercise and (MRI), will be recruited for the study if they are willing to consent. Each participant's personal physician will be contacted and involvement in the study will be discussed and approved by the physician. All participants will undergo informed consent as approved by the Internal Review Board of the University of Southern California. Following assessment by either the principal investigator or the study coordinator to assure that the participant meets rule-in and rule-out criteria to be in the study. All participants will be given standard instructions to ensure reliability for each evaluation. The following tests will be conducted on participants at the following time points: baseline, 8 weeks, 16 weeks and 24 weeks. The first baseline measures will be taken within the week prior to beginning the first training program. The first post-training evaluation (8 weeks) will be taken during the week after the first training has been completed (after 24 training sessions which occur over an 8-week period). A second baseline evaluation (16 weeks) will be taken after an 8-week washout period following the first training,and within the week prior to beginning the second training. The second post-training assessment (24 weeks) will be taken during the week after the second training has been completed. Data will be coded to protect the confidentiality of the participants and will be stored in a locked office and password-protected computer accounts. Data entry will also not disclose the identity of the participants.

Clinical assessments

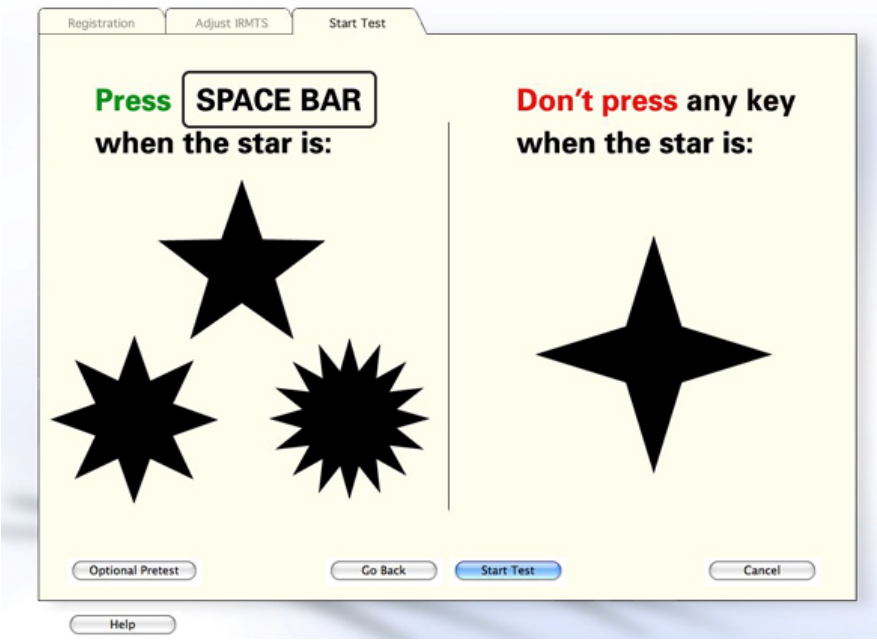
- MDS Unified Parkinson's disease Rating Scale (UPDRS): The UPDRS is an assessment of Parkinson's disease severity and progress. The UPDRS has four sections. The first section assesses mentation, mood, and behavioral changes. The second section assesses historical activities of daily living. The third section is the motor examination portion of the UPDRS and includes evaluations of tremor, rigidity, bradykinesia, gait, and postural instability. The fourth section evaluates complications of therapy including motor fluctuations and dyskinesias. A 5-point scale is used a well-defined criterion. The UPDRS will be administered by a blinded research investigator who has been trained using the Movement Disorders UPDRS training videos. The UPDRS has universal acceptance as a rating scale for individuals with PD and it has been shown to be reliable and valid. This measure will be used to determine symptom severity. MDC will be 2.5 points between baseline and post-training.

- Modified Physical Performance Test (mPPT): The mPPT is a 9-item test that assesses multiple dimensions of physical function (basic and complex activities of daily living) with different levels of difficulty. This measure correlates well with degree of disability, loss of independence, and early mortality. It is also significantly correlated to UPDRS III and Hoehn and Yahr scale in PD. It is a reliable measure with a high inter- and intra-rater reliability. Standard instructions will be given to ensure reliability. Participants will be asked to complete functional tasks (i.e. writing a sentence, simulated eating, lift a book and put it on a shelf, turning 360 degrees, 50-foot walking test, stair climbing etc.). Participants' performance will be videotaped and the score of each task will be rated based on the completion time.

- Self-Efficacy for Exercise (SEE) scale: The SEE is to capture an individual's confidence in their ability to continue exercising in the face of barriers to exercise. The SEE consists of 9 items describing potential barriers to participation in exercise (eg, "too busy with other activities," "did not enjoy exercise," "felt pain with exercise," "bored by the exercise"). For each item, participants circled a number from 0 ("not confident") to 10 ("very confident") that best described their belief that they could exercise 3 times a week for 20 minutes. Reliability, validity, and internal consistency have been established for the SEE.

- Quotient test: The Quotient test is an innovative device that objectively measures three domains of cognition: hyperactivity, inattention and impulsivity. This easy-to-administer tool uses advanced motion tracking technology to track micromovements while participants complete a computerized test that takes less than half an hour. Below is an example of the cognitive task involved, it consists of a simple go/no go task which is generally used to clinically assess frontal lobe function. After the test is completed, patterns of motion, accuracy of the responses, and fluctuations in attention state are quickly analyzed and scored using proprietary algorithms based on 19 or more parameters. The motion detection includes 6 movement metrics. The Attention Response Analysis

includes measures of accuracy, omission errors, commission errors, latency, variability, and response time variability. Data for each patient is compared to age and gender matched cohorts. Scores and test data provide both the existence of related deficits and the magnitude of those deficits. Quotient is cleared by the FDA with the intended use to provide objective measures of hyperactivity, impulsivity and inattention to aid in the clinical assessment.



- Trial-Making Test (TMT): The Trail Making Test is a neuropsychological test of visual attention and task switching. It consists of two parts in which the participant is instructed to connect a set of 25 dots as quickly as possible while still maintaining accuracy. The test can provide information about visual search speed, scanning, speed of processing, mental flexibility, as well as executive functioning.

- Parkinson's Disease-Cognitive Rating Scale (PD-CRS): The PDCRS is is a valid, reliable and useful neuropsychological battery designed to cover the full spectrum of cognitive defects associated with PD. The PD-CRS includes 10 'subcortical-type' items (attention, working memory, Stroop test, four verbal fluencies, immediate and delayed verbal memory, clock drawing), and two 'cortical-type' items (naming, copy of a clock). Total score ranges from 0 to 204, subcortical score from 0 to 174, and cortical score from 0 to 30, with higher scores indicating a better functioning.

Magnetic resonance imaging (MRI) protocol

All participants will be screened using the MRI screen questionnaire. The MRI scans will be performed at the Stevens Neuroimaging and Informatics Institute at the USC Health Sciences Campus. Participants will undergo MRI tests at 4 time points (baseline, 8 weeks, 16 weeks and 24 weeks).

MRI preparation and procedures

Participants will be instructed in a behavioral task that they will be asked to do as part of a functional imaging study. In some cases, they will go through sample trials of the task as part of a familiarization period. The participants will then be asked to lie on the bed of the MRI scanner and will be given headphones and ear-protectors. In some cases, a comfortable head restraining device (such as cloth tape or pads on the sides of the head) may be used to reduce excess head motion. The duration of the MRI procedure will be 1 hour. The participants will lie with their head motionless and either relax passively or perform the required behavioral task.

The MRI equipment makes a variety of noises during scanning from repetitive tapping to beeping noises. Anyone in the scanner room while the scanner is in operation must use hearing protection in the form of earplugs and/or headphones to avoid hearing injury from the acoustic noise generated by the scanner. The earplugs and/or headphones provided reduce the noise levels to a level comfortably below the FDA guidelines. The scanners are equipped with a squeeze bulb that allows the participant to set off an audible alarm to attract the operator's attention. The squeeze bulb will be made available to participants. After completion of the imaging, the participant will be removed from the instrument. We interview the participants informally for any adverse or unexpected

reactions that may have taken place during the study.

Blood oxygen level dependent (BOLD) functional MRI (fMRI)*:* fMRI is one of the most widely used MRI techniques for studying human brain function. Since the seminal discovery of functional connectivity (FC) between distributed brain areas during the resting state, resting state fMRI (rs-fMRI) has become a major tool for characterizing the dynamic organization of large scale brain networks. With the recent development of simultaneous multi-slice (SMS) or multiband imaging, whole brain BOLD fMRI using gradient-echo echo-planar imaging (GE-EPI) can be performed with an isotropic spatial resolution of a few mm^3 and a temporal sampling rate of a few hundred milliseconds per volume, as demonstrated by the Human Connectome Project (HCP[29]). At ultrahigh magnetic fields, fMRI with a sub-millimeter spatial resolution has been applied for studying human brain function at the cortical layer or column level. Each test session consists of fMRI during a cognitive task and at resting state.

- Task-based fMRI: Participants will perform a word-color Stroop paradigm that has been previously adapted for use in fMRI experiments[30]. The task is composed of two conditions: congruent and incongruent, interspersed with periods of no stimuli (baseline). The visual stimuli consist of four words: red, blue, green and yellow. In the congruent condition, words appear in their matching colors, whereas in the incongruent condition, the colors do not match their content. Participants will be instructed to press the buttons as quickly as possible according to the color of the ink presented while disregarding the meaning of the word. Responses will be made using the index and middle finger of both the left and right hand using a response box that contained four buttons. The same number of stimuli is given to the left and right hand. The color of the four buttons is presented on the top of the computer screen. Stimuli will be presented at a rate of 1 sec for color word interleaved with 2 sec of a blank screen. Each task block contains 12 different words of which 1/3 are distracters (congruent in incongruent trials and vice versa), interleaved with a 10 sec baseline which consists of a red fixation point at the center of the screen. There are seven alternating blocks of the two conditions (four incongruent and three congruent) and eight baseline blocks. For all participants, adequate task performance will be assured by familiarization and practice of the task prior to entering the MRI scanner.
- Resting state fMRI: Participants will be instructed to keep their eyes open fixation on a projected image of a white cross on a black background, to remain motionless, and to not to think of anything in particular. The scanning session will last for 6 mins.

Quantitative electroencephalogram (qEEG) protocol

A 19-channel EEG acquisition system (Deymed Diagnostic, TruScan 32) will be used in recording. Participants will be instructed to sit quietly with eyes closed whilst wearing a skull cap. An electrode cap designed according to standard International 10-20 Montage system is placed over the head with proper skin preparation to ensure each electrode is well contacted (impedance < 5k). Ten minutes of rest data will be collected. Data will be amplified and digitized at 512 point/c and stored on a computer hard disk for off-line analysis.

Blood sample collection protocol

A venous blood sample of up to 40 ml will be obtained from participants. The amount drawn from participants will not exceed the lesser of 50ml or 3ml per kg of the participant's weight in an 8 week period.

Training program

All participants will receive the following trainings with a counter-balanced order. Training sessions will take place at the Division of Biokinesiology and Physical Therapy (CHP building).

(1) SMARTfit training
(2) Conventional physical training.

For both SMARTfit training and conventional physical training, participants receive three 1-hour training sessions per week for 8 weeks. During each week, participants will receive physical training focused on six tasks, which are the functional tasks individuals with PD commonly have difficulty with. The six tasks are paired into 3 pairs (see Table 1). Participants will focus on practicing one pair of tasks during each session.

Table 1. Functional task training

Pair	Task 1	Task 2

1	Sit-to-stand	Multi-plane locomotor tasks (obstacle course)
2	Gait	Reach & Grasp
3	Floor-to-stand; Stand-to-floor	Single limb standing

For each of the trained task, the five components (movement amplitude/speed; endurance; balance; vision; accuracy) can be manipulated to scale up and down based on each participant's capability. Each component has three difficulty levels. Please see Appendix 1 for the details of three difficulty levels for each component. This provides the training therapist a standardized guideline to adjust the task difficulty to fit each participant's priority and participants' progress over 8 weeks to make sure that each training session is at the optimal challenge point in order to maximize the effect of training[31]. For the SMARTfit training, there is an additional cognition component that can be manipulated using features provided by SMARTfit. After each training session, participants' rating of perceived exertion (RPE) will be obtained using a 1-10 Borg scale. Participants' RPE should be between 6 to 8. If the participant's RPE exceeds 8, then in the next training session the challenge level of trained task will be scaled down until they are at or below 8 on the Borg scale. At any point during the session, participants are allowed to terminate by telling the researcher that they would like to stop.

3.2 Dependent measures

Primary outcome measure

1. Modified Physocal Performance Test score (mPPT)

Secondary outcome measures

1. UPDRS score
2. Self-Efficacy for Exercise scale (SEES)
3. Trail-making test (TMT)
4. Parkinson's Disease-Cognitive Rating Scale (PDCRS)
5. Quotient system test
6. Functional connectivity
7. Quantative EEG measures
8. α-synuclein and DJ-1

3.3 Data processing

Statistical Parametric Mapping will be used to process the fMRI data. The fMRI analysis focuss on the whole brain. An inclusion criterion for fMRI image analysis is head motion < 2 mm in any direction. The functional images are subjected to geometric distortion (field map) correction and motion correction. The structural images will be co-registered to the mean unwarped and motion corrected functional image for each participant and functional and structural images will be normalized to Montreal Neurological Institute (MNI) space. Functional volumes will be smoothed with a Gaussian kernel of 8 mm3 (FWHM). Correlational analyses between the BOLD signal from an a priori selected seed and every other brain voxel during the entire acquisition condition will be run to provide seed-to-voxel connectivity estimations for each participant. Movement parameters will be entered as first-level covariates in the model. Before averaging individual voxel data, the waveform of each brain voxel will be filtered using a bandpass filter to reduce the effect of low-frequency drift and high-frequency noise. Several sources of spurious variance along with their temporal derivatives will then be removed from the data through linear regression: signal from movement, signal from ventricular regions, and signal from the white matter. Because further steps include between-sessions comparisons, temporal connectivity maps will be generated for each participant across the conditions. These images will be then included in a second-level analysis.

EEG will be recorded using Deymed acquisition system and then converted to *.EDF format for offline analysis. Raw data will be carefully inspected and edited to reject all movement artifacts. Total of 4-min artifact free epochs will be included in the quantitative analyses. All data will be analyzed by a fast Fourier transform routine using 1,024 point FFT window to yield a power density spectrum for each channel. According to clinical EEG convention, power density will be calculated at the following frequencies: delta (<4Hz), theta (4-8Hz), alpha (8-12Hz), and beta (12-30), respectively. In addition to fast Fourier transform, we will calculate cross-channel correlation in time domain, coherence in frequency domain and power density mappings. A contour map will also be generated for each frequency band using the nearest neighbor algorithm. NBRL NeuroRef, which applies LORETA technology to convert EEG voltage signals into current density, will be applied to map out the distribution in 3-D space by overlapping with a morphed MRI image. Individual's data will be further analyzed and compared with an FDA approved normative EEG database licensed from NYU to determine degrees of deviation of respected variables from the norm. Multivariate discriminant function analysis will be used to determine degrees of deviation of individual's data from norm by linear composite of variances from the means of

database. Z-scores will be calculated for the following measures: (1) absolute power of all frequencies in all channels, (2) relative power of all frequencies in all channels, (3) mean frequency of alpha rhythm in all channels, (4) 19 X 19 coherence matrix and mappings for each frequency, and (5) 3-D current density mappings for interested frequency using sLORETA technology. Results of analyses will be exported to a spreadsheet for further statistical analysis.

Blood samples labeled with participant ID will be frozen and shipped to the Laboratory of Molecular Psychiatry at University of California, Irvine for data processing and analysis. Alpha-synuclein and DJ-1 levels will be measured using an established protocol in previous research[32].

4.0 DRUG/DEVICE INFORMATION

The following devices will be utilized in this pilot study:

1. MRI: The MRI scanning procedure requires that the participants be confined in a small, partially enclosed space. The sound of the MRI scanner can be loud, but will be reduced by special ear plugs. The tasks may be boring or difficult. In addition, the magnetism of the machine attracts certain metals; therefore, people with these metals in them (specifically pacemakers, infusion pumps, aneurysm clips, metal prostheses, joints, rods, or plates) will be excluded from the study. The "metal" in dental fillings is less susceptible to magnetism and is therefore allowed. There are no known adverse effects resulting from exposure to the MRI scan. There are no other known risks when the scanner is operated within its nominal range as discussed above. The FDA Center for Devices and Radiological Health, released the document "Criteria for Significant Risk Investigations of Magnetic Resonance Diagnostic Devices" on July 15, 2003. This describes their current evaluation of risk related to MRI. Notably, both software and hardware features of the instrument prevent this software from executing commands outside of a well-defined range defined as safe by the FDA.
2. Quantitative EEG: TruScan 32 EEG Acquisition System (Deymed Diagnostics, Payette, ID), including EEG cap, EEG amplifier and EEG adapter, will be used in the study.
3. Quotient system: Quotient® (formerly known as MMAT, the McLean Motion and Attention Test) is a non-invasive device providing objective measures of an individual's ability to inhibit motor activity, sustain attention and suppress impulsive response. Quotient uses motion tracking technology to track an individual's micromovements while he or she completes a 15-20 minute computerized test. After the test is completed, patterns of motion, accuracy of the responses, and fluctuations in attention state will be analyzed and scored using proprietary algorithms. Quotient is cleared by the FDA with the intended use to provide objective measures of hyperactivity, impulsivity and inattention to aid in the clinical assessment of ADHD.
4. SMARTfit™ Mini 9 On-Frame: The SMARTfit™ Mini 9 On-Frame is a vertical frame-mounted 9 target 46″ x 46″ Panel with CPU controller, timeclock, score display, sound system and 4 tracks of themable voice/tones/music. The SMARTfit™ Mini 9 On-Frame can capture the accuracy and movement time, which serve as performance feedback to participants.

5.0 SELECTION AND WITHDRAWAL OF SUBJECTS

5.1 Inclusion Criteria:

1) 50-85 years of age
2) Diagnosis of idiopathic Parkinson's disease using the UK Brain Bank criteria (as determined by the study movement disorders neurologist) with Hoehn and Yahr stage 1-2
3) No contraindications to exercise including untreated cardiovascular disease or stroke
4) Medically stable and optimized on their medications
5) Able to ambulate independently with or without device
6) No other neurologic, neuromuscular, or orthopedic disease
7) No serious cognitive deficits and able to participate in the informed consent process
8) With medical clearance from primary care physician to participate in the physical therapy intervention
9) No contraindications for MRI

5.2 Exclusion Criteria

Participants will be excluded from participation if they have:

1) Severe cardiac disease (New York Heart Association classification II-IV)
2) Systolic blood pressure reduction of greater than 20 mmHg with standing
3) A history of poorly controlled or brittle diabetes
4) A history of lower limb amputation

5) Been prescribed any new dopamine replacement medications or new mood stabilizer medications.
6) Presence of a lower limb non-healing ulcer
7) Montreal cognitive assessment score of less than 21
8) The presence of any medical condition which the investigator believes might present an unacceptable health risk to the subject should they participate in the study
9) Electrically, magnetically, or mechanically activated implant (such as cardiac pacemakers or intracerebral vascular clip)
10) Metal in any part of the body including metal injury to the eye
11) History of brain lesions (such as stroke), seizures, or unexplained spells of loss of consciousness
12) Pregnant or breast-feeding
13) With other neurologic, neuromuscular, or orthopedic disease that would interfere with ability to participate in exercise training
14) Currently participating in other studies

Cardiac Tolerance

Cardiac conditions that would cause exclusion from the study include (but are not limited to) unstable angina within last 3 months, myocardial infarction within last 3 months, serious cardiac dysrhythmia, right main coronary artery disease, aortic stenosis, currently decompensated heart failure of pulmonary edema, severe system hypertension (SBP > 180 or DBP > 110), fixed-rate artificial pacemaker, hypertrophic cardiomyopathy, pulmonary embolus or infarction within the last 6 months. Each participant's cardiac status will be verified with his/her physician.

Blood Pressure/Heart Rate at rest and with exercise[33]

Resting: Participants in the study must have a resting SBP<180 and DBP<110. Heart rate must be <100 at rest and systolic blood pressure must not drop > 20 with standing.

Exercise: Participants in the study must have a SBP<200 and DBP<110 when measured after 2 minutes rest from exercise.

Participants whose heart rate or blood pressure is greater than the values stated above will be excluded from the study.

5.3 Withdrawal Criteria

Participants will have the option to withdraw from this study at any time. Withdrawal from this study will not affect their participation in any other study within the USC Neurorestoration center. The investigators may also choose to terminate a participant from this study, if he or she suffers a severe adverse event, does not follow study requirements, or investigators feel that continued participation will put the participant at a greater risk than indicated.

6.0 STRATIFICATION/DESCRIPTIVE FACTORS/RANDOMIZATION SCHEME

Participants will be assigned to receive the SMARTfit training or conventional physical training first with stratified randomization. Specifically, Hoehn and Yahr stage I and II will be used as the two strata. Randomization will be performed within each Hoehn and Yahr stage (I and II) using sealed envelopes with a 1:1 allocation ratio between the two training orders. The number of participants with Hoehn and Yahr stage I and II will be evenly assigned to the two training orders to ensure equivalent disease severity.

7.0 STUDY AGENT ADMINISTRATION OR INTERVENTION AND TOXICITY MANAGEMENT PLAN

For training protocol, see section 3.1 at page 7. Training will be discontinued if participants continue experiencing a RPE above 8 or show signs of cardiac intolerance (shortness of breath, leg cramps, agina, lightheadness, confusion, nausea, blood pressure above guidelines lists in Section 5.2). Individuals whose symptoms resolve after training is discontinued will be invited to participate in the next training session (48 hours later). If these symptoms occur a second time, individuals will be removed from the study and will be encouraged to see a physician as soon as possible.

8.0 ASSESSMENT OF EFFICACY AND SAFETY

8.1 Side effects/Toxicities to be monitored.

The risks to participants associated with this study include the time needed to complete the physical therapy sessions and the potential for muscle soreness, strains, or sprains that is a natural part of participating in a training program.

The overall risk classification is greater than minimal risk but less than significant risks.

Potential Risks of Exercise

There are minimal risks associated with participation in exercise and physical training. These risks include muscle strains and/or sprains and muscle soreness, in addition to cardiovascular events such as heart attack and stroke. Physical training in this proposed study is considered safe and effective for improving motor performance in PD. To minimize these risks, all sessions will be monitored by research personnel that are CPR/First Aid certified. Additionally, the participant to researcher (1:1) ratio will be low so that research personnel can provide adequate attention to participants during training.

Potential Risks of EEG

The use of EEG is largely low risk although placement of disks may cause slight scalp irritation in that the discs must be placed directly on the scalp. To prevent more than minimal discomfort we will be in constant communication with the participants on how the placement of the EEG cap feels and will modify placement based on reported comfort level. If the cap becomes too uncomfortable for the participant to bear then the cap will be removed and the participant is free to discontinue participation in the study.

The gel used to put the EEG discs on the head is sometimes sticky and the discs may scratch the outer layer of scalp skin. Investigators will take care to try to minimize scratching the scalp skin during application.

Potential Risks of blood sample collection

There are minimal risks associated with blood sample collection. There is a small risk of bruising and fainting, and a rare risk of infection.

Summary of MRI risks

The FDA considers MRI devices to be of non-significant risk if they meet the following criteria (we summarize only the relevant portions of this document):

a. Field strength less than 8 Tesla
b. RF power deposition (measured as the specific absorption rate or SAR) to the patient is limited by law, and by the instrument, to less than 3 W/kg in the head. Notably, human imaging with SAR of double that has been demonstrated to be safe in normal subjects[34].
c. Any time rate of change of gradient fields (dB/dt) sufficient to produce severe discomfort or painful nerve stimulation.
d. A-weighted root mean square (rms) sound pressure level greater than 99 dBA with hearing protection in place.

Under no circumstances will our instrument be operated outside of these non-significant ranges on human subjects. The small risk of metal objects being dislodged will be reduced by the screening process previously described. Headphones and ear protectors will be used to reduce any discomfort that might be associated with the scanner noise. The use of headphones and video systems will minimize the potential discomfort of claustrophobia.

Protection against MRI risk

The 3T Prisma whole-body MRI systems installed at the Center of Image Acquisition (CIA), Stevens Neuroimaging and Informatics Institute located at HSC, which offers state-of-the-art imaging platforms to characterize TMS induced physiological, metabolic and functional changes of human brain.

The participants will be screened for appropriateness for study participation using the inclusion and exclusion criteria. In addition, participants will be introduced to exercise activities and monitored by a trained Physical Therapist (PT) at each visit. The PT will keep documentation records for each treatment session that will monitor 1) the subjects cardiovascular exercise tolerance through vital sign assessment and 2) any signs of musculoskeletal adverse response. The PT will be instructed to contact the PI immediately if an adverse response that requires medical intervention occurs. Participants will practice

the activities with the PT in the clinical setting and will receive feedback to ensure proper form and safety during each session. In addition, subjects will be notified that they can call the PI at any time if any adverse effects occur.

Ongoing monitoring of risks and benefits will occur by a monthly site visit by the PI who will review all documentation. The PI will determine the balance between risks and benefits and will notify the IRB if a change in the intervention protocol in order to minimize risk is required. In the case of an actual adverse event, the PI will complete the "IRB Report of an Internal Adverse Event" form and report the event to the IRB within 5 days of the event.

8.2 Dosage change based on toxicity.

Not applicable. No modification of medication will be included.

8.3 Adverse Event Reporting

All adverse events will be reported to the IRB on the USC Health Sciences Campus. Any unexpected, severe, or fatal adverse events will be reported to the IRB within 24 hours. Minor adverse events will be summarized and included in the annual continuation review and final report.

8.4 Data Monitoring Committee (if applicable)

Not applicable.

9.0 CLINICAL AND LABORATORY EVALUATIONS AND STUDY CALENDAR

Evaluation	1st Pre-training assessment	1st Post-training assessment	2-month washout period	2nd Pre-training assessment	2nd Post-training assessment
Demographics form	X				
Montreal Cognitive Assessment (MoCA)	X				
Activities-specific Balance Confidence Scale (ABC)	X				
History of Falls Questionnaire	X				
Physical Activity Questionnaire	X				
Geriatric Depression Scale (GDS)	X				
Modified Physical Performance test (mPPT)	X	X		X	X
Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS)	X	X		X	X
Self-Efficacy for Exercise Scale (SEES)	X	X		X	X
Trail-making test (TMT)	X	X		X	X
Parkinson's Disease-Cognitive Rating Scale (PD-CRS)	X	X		X	X
Quotient system test	X	X		X	X
Functional MRI	X	X		X	X
Quantatitive EEG	X	X		X	X
α-synuclein and DJ-1	X	X		X	X

10.0 CRITERIA FOR EVALUATION AND ENDPOINT DEFINITIONS

All participants will be evaluated on the dependent variables listed in section 3.2. Additionally, basic information (such as past medical history and course of Parkinson's disease) will be collected via paper documents. The endpoint will occur once we have enrolled 12 participants.

11.0 SPECIAL INSTRUCTIONS:

Not applicable.

12.0 DATA COLLECTION AND MONITORING

All data in paper documents, such as clinical assessments and questionnaires, will be stored in locked cabinets in the USC Neurorestoration center. Trail-making test will be digitally recorded using PEBL test battery and saved in Excel[35]. Digitally recorded data, including some of the clinical assessment, MRI data and EEG data will be saved and secured in password-protected computer accounts in a computer located in the USC Neurorestoration center. All digital MRI data will also be archived onto secure, biometrically access-controlled integrated data archive (IDA) servers in a locked room within Stevens Neuroimaging and Informatics Institute.

The EEG and MRI data will be stored digitally. Prior to any transfer of information among investigators, or into any summary publication, all identifying information about the participants will be removed. Files will be identified by study and by an identification number assigned to the participant at the beginning of the study. The coding between participant name and ID will be maintained digitally in the records of the laboratory and, as needed, by the individual investigators in their private lab files and password-protected computers. For all scientific uses, the data will be displayed without identifying information.

The data will be recorded digitally on computer using only a coded designation. Specifically, EEG will be recorded Deymed acquisition system in *.dat data format. MR imaging in DICOM file format will be recorded using vender-provided data acquisition platform for 3T Siemens Magnetom Prisma (Siemens Medical Solutions USA, Inc., Malvern, PA; https://usa.healthcare.siemens.com/magnetic-resonance-imaging/3t-mri-scanner/magnetom-prisma). No one will have access to the codes or data except the study personnel. Participant data will not be released to anyone without written consent of the participant. When the study is completed, the data will be kept secure in the USC Neurorestoration center for seven years.

Blood samples labeled with participant ID will be frozen and shipped to the Laboratory of Molecular Psychiatry at University of California, Irvine (UCI) for data processing and analysis. The Laboratory of Molecular Psychiatry is responsible for receiving the de-identified blood sample from this pilot study, processing the samples, and analyzing the data. As such, the Laboratory of Molecular Psychiatry is not required to have IRB approval from UCI for the processing of the samples. The Laboratory of Molecular Psychiatry has no access to the link between the de-identified sample code and any identifying information.

13.0 STATISTICAL CONSIDERATIONS

The overall objective of this pilot study is to to estimate the effects of SMARTfit training versus conventional physical training on changes in motor and cognitive function, brain functional connectivity as well as disease biomarker measures. Three-way repeated measures ANOVA will be used to test the effect of training, training order and disease severity (Hoehn and Yahr stage) on the changes in motor and cognitive function, brain functional connectivity as well as disease biomarkers follow the SMARTfit training and conventional physical training. Baseline value of outcome measures before each training will be used as covariates to account for the potential impact of carryover benefit. The significance level is set at $p < .05$.

The projected sample size for this pilot study is 10 based on the recommendations for pilot studies[36,37]. Our expected goal is to have 10 participants to complete the training. Considering the potential drop-out rate, totally 12 participants will be recruited. The preliminary data will be used to estimate the effect size and calculate required sample size for future studies to achieve sufficient power.

14.0 REGISTRATION GUIDELINE

Individuals with Parkinson's disease interested in enrolling in the study will be directed to call (323) 442-1006 for more information. Once eligibility is confirmed and informed consent is signed, participants will be assigned to one of the two training orders (SMARTfit- conventional; conventional- SMARTfit). At the time of registration, two copies of a signed and dated Informed Consent form with Bill of Rights will be available (an original for patient's medical chart; one copy for the participant; and the other for the PI's file).

15.0 BIOHAZARD CONTAINMENT

Not applicable.

16.0 ETHICAL AND REGULATORY CONSIDERATIONS

All institutional and Federal regulations concerning the Informed Consent form will be fulfilled. The study will be conducted in adherence to ICH Good Clinical Practice.

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Appendix 1

Pair 1: Sit-to-Stand						
Level	**Amplitude/speed**	**Endurance**	**Balance**	**Vision**	**Accuracy**	**Cognition**
1	Perform task big enough that able to complete task in one attempt – 18 inch height chair	Complete 10 repetitions	Both feet on the ground and allowed to use upper extremity support	No manipulation	Not using back of legs on the chair and standing up to full upright posture; no 'toes up'	Simple reaction time task
2	Perform task big enough that able to complete task in one attempt – 16 inch height chair	Complete 20 repetitions	Both feet on the ground with goal of no upper extremity support	Sunglasses or no body glasses		Choice reaction time task
3	Perform task big enough that able to complete task in one attempt – 14 inch height chair	Complete 40+ repetitions	No upper extremity support with pelvis or feet on an unstable surface (i.e., dynadisk; airex)	Eyes closed	Maintain end upright posture – no wobbling, no toes up end position	Working memory loading (e.g., counting backwards, adding numbers)

Pair 1: Multi-plane Locomotor Tasks: Turns, Stepping over, around and between obstacles, steps or stairs, (see obstacle course layout for this activity)							
Level	**Amplitude/speed**	**Endurance**	**Balance**		**Vision**	**Accuracy**	**Cognition**
1	Largest step at comfortable speed	15 minutes with unlimited # of rest breaks	PT UE support	Stable surface but obstructed path with obstacles to walk around and over (short objects)	No manipulation	Not knocking over any obstacles	Simple reaction time task
2	Maintain largest step length and increase speed	15 minutes with 1 -2 rest breaks allowed	Cane	Stable with obstacles to walk around and over (tall objects) and Unstable surfaces (thick floor mat)	Sunglasses or no-body glasses	Predictable start and stops and 90° turns	Choice reaction time task
3	Maintain largest step length and as fast as possible safely	15 minutes with no rest breaks	No Support	Stable with obstacles to walk around and over (tall objects and Unstable surfaces (step on airex pads or river stones)	Scanning environment with head turns	Unpredictable start and stops and turns of 90° (2-steps), 180° (max of 4 steps), or 360° (max of 8 steps)	Working memory loading (e.g., counting backwards, adding numbers)

Pair 2: Gait						
Level	**Amplitude/speed**	**Endurance**	**Balance**	**Vision**	**Accuracy**	**Cognition**
1	Largest step at comfortable speed	15 minutes with greatest number of rest breaks	Forward, side-ways and backward walking with PT in front giving UE support	No manipulation	No accuracy manipulation	Simple reaction time task
2	Maintain largest step length and increase speed	15 minutes with 1 -2 rest breaks allowed	Forward, side-ways and backward walking with a cane	Sunglasses or no-body glasses	PT directed stop and start	Choice reaction time task
3	Maintain largest step length and as fast as possible safely	15 minutes with no rest breaks	Forward, side-ways and backward walking with no UE support	Sunglasses or no-body glasses canning environment with head turns	Stop and start and regular turns (left, right, 180° or reversal from forward to back or side stepping direction	Working memory loading (e.g., counting backwards, adding numbers)
Pair 2: Reach & Grasp						
Level	**Amplitude/speed**	**Endurance**	**Balance**	**Vision**	**Accuracy**	**Cognition**
1	Sitting – maximum excursion (DISTANCE) of the reach with variations in both HEIGHT: Shoulder height, up & down AND DIRECTION: forward, lateral, and across	10 reaches each arm	Blocked direction and blocked height	No manipulation	Large object (water bottle) - palmer grasp	Simple reaction time task
2	Standing – Maximum excursion (DISTANCE) of the reach with variations in both HEIGHT: Shoulder height, up & down AND DIRECTION: forward, lateral, and across	20 reaches each arm	Blocked height variable direction	Sunglasses or no-body glasses	Grasp highlighter	Choice reaction time task
3	Standing: step and reach; Maximum excursion (DISTANCE) of the reach with variations in both HEIGHT: Shoulder height, up & down AND DIRECTION: forward, lateral, and across	40 reaches each arm	Variable height and direction	Look at object and then close eyes for reach and grasp	Grasp paper clip with pincer grasp	Working memory loading (e.g., counting backwards, adding numbers)

Pair 3: Floor-to-stand; Stand-to-floor						
Level	**Amplitude/speed**	**Endurance**	**Balance**	**Vision**	**Accuracy**	**Cognition**
1	With chair in front – down to knees and back up	5 repetitions	Using chair for upper extremity support	No manipulation	Best posture possible but OK if trunk is flexed; minimal sway or instability through the transition and in end positions	Simple reaction time task
2	Down onto hands and knees and back up	10 repetitions	Not using chair	Sunglasses or no-body glasses	Maintenance of extension in trunk; minimal sway or instability through the transition and in end positions	Choice reaction time task
3	All the way down into prone and back up	20 repetitions	Using least number of limbs possible (arms & legs)	Eyes closed	Maintenance of extension in trunk; minimal sway or instability through the transition and in end positions	Working memory loading (e.g., counting backwards, adding numbers)
Pair 3: Single limb standing						
Level	**Amplitude/speed**	**Endurance**	**Balance**	**Vision**	**Accuracy**	**Cognition**
1	Lift one foot up slightly to hip/knee flexion 15°, and to hip abduction 10°	5 repetitions	hand contact on an object as support	No manipulation	Maintain for 5 s per repetition	Simple reaction time task
2	Lift one foot up to hip/knee flexion 30°, and to hip abduction 20°	10 repetitions	Hands on hip	Sunglasses or no-body glasses	Maintain for 10 s per repetition	Choice reaction time task
3	Lift one foot up to hip/knee flexion 45°, and to hip abduction 30°	20 repetitions	With bilateral shoulder abduction/flexion 90°	Eyes closed	Maintain for 20 s per repetition	Working memory loading (e.g., counting backwards, adding numbers)