Revitalize Cognition: Near Infrared Stimulation in Older Adults

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IRB Protocol

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1. Title:

Revitalize Cognition: A Proof of Concept Study with Transcranial Near Infrared Stimulation in Cognitively Normal Older Adults, those with Mild Cognitive Impairment or Parkinson Disease

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3. Abstract

Cognitive decline and transition to dementia, particularly Alzheimer's disease, is one of the most prominent public health concerns of the 21st century. Over the past decade, progress has been made in identifying subtypes of dementia, preclinical biomarkers and associated risk factors, along with strides in understanding the role of various suspected pathophysiologic mechanisms. Even so, there is a dearth of clinically meaningful treatment options at this point in time for individuals who are at increased risk for transitioning to dementia, particularly those with the amnestic variant of mild cognitive impairment (aMCI). While waiting for causative cures and preventive approaches, we are faced with the task of identifying modifying therapies that might alter the course or slow down the transition from aMCI to Alzheimer's dementia. Similar concerns face other neurodegenerative disorders (e.g., Parkinson disease) regarding transitions from mild cognitive impairment to dementia.

The proposed study hopes to contribute to this mission by testing the viability of a different type of intervention, one involving transcranial delivery of near-infrared (NIR) wavelengths (808-904nm). Near-infrared stimulation is safe, non-invasive and appears to improve mitochondrial function by promoting increased production of intracellular ATP and possibly improved blood flow. Perhaps most compelling are recent findings of reduced beta-amyloid and neurofibrillary tangles in transgenic Alzheimer's mouse models after exposure to real vs sham transcranial NIR stimulation. Similar observations have emerged in mouse models of

Parkinson disease. Preliminary human studies involving TBI, stroke, and young adult populations have also been promising in terms of positive effects of NIR on cognition.

The overall goal of the present study is to learn whether this unconventional NIR stimulation approach has potential for improving cognition in older adults. To do so, we will conduct a randomized sham controlled pilot trial. The intervention will involve six sessions, over a 2-week period in which real or sham stimulation is transcranially applied using a delivery system that has been FDA-approved as a nonsignificant risk since 2003. We hope to learn whether NIR stimulation, relative to sham, has positive effects on: a) novel tasks of memory that are sensitive to hippocampal function and amyloid burden; b) executive tasks from the NIH Examiner, and c) emotion measures, including those from the emotion module of the NIH Toolbox. An exploratory neuroimaging aim will examine pre-post intervention changes in resting state connectivity and MRS phosphorus ATP in a subset of participants who are eligible to undergo magnetic resonance imaging. A subgroup of participants with Parkinson disease will participate and will undergo similar procedures in addition to motor testing. If findings from this study are positive, this may set the stage for more finely tuned trials examining various dosage parameters. Transcranial NIR stimulation is an understudied intervention that could potentially represent a strategy for enhancing thinking and memory in older adults and those with mild cognitive impairment or pre-Alzheimer's disease.

4. Background

The proposed project represents a novel, relatively low cost, low risk, though potentially high impact approach for a cognitive/ mood intervention in older adults. It involves transcranial delivery of near infrared (NIR) light via light emitting diodes. Before describing the study design, we will briefly describe the background and rationale for this unconventional approach

By history, current nonsurgical device approaches to brain stimulation primarily involve magnetic (rTMS, TMS, deep TMS) or electrical stimulation approaches (transcranial direct current stimulation (tDCS) which alter synaptic firing rates and neuronal membrane potentials.

In contrast, a very different type of brain stimulation approach involves near infrared light (NIR). Application of light in red (630-700nm) and near-infrared wavelengths (808-904nm) appears to improve mitochondrial function by promoting increased production of intracellular ATP, which is important for cellular metabolism, respiration and oxygenation. It increases blood flow and upregulates the expression of neuroprotective genes. Specifically, it targets the cytochrome oxidase c integral membrane protein of the mitochondrial membrane (electron transport chain) and leads to: a) increased intracellular levels of ATP (Mochizuka-Oda et al., 20002, Wong-Riley et al., 2005, Oron et al., 2007), b) increased expression of genes supporting cell proliferation and mitochondrial energy metabolism (Zhang et al., 2003), and c) decreased genes for pro-inflammatory proteins such as interleukin-1, interleukin10, and cytokine receptors (Whelan et al., 2003). Additionally, near infrared light may increase blood flow (Chung et al., 2012; Nawashiro et al., 2012) and up-regulate antioxidant genes (Chen et al., 2009). Thus, rather than directly modulating neural networks per se, application of NIR appears to create a supportive environment for optimal neuronal functioning. Its influence on neural connectivity is thus indirect rather than direct.

Recent animal and human studies have provided tentative support for positive benefits of this NIR approach in terms of "brain and cognitive function". Animal studies have included reports of reduced beta-amyloid load and neurofibrillary tangles in transgenic mice (APP/PSI and

K3Tau) expected to develop Alzheimer's disease after exposure to real vs sham transcranial NIR stimulation (Purushothuman et al., 2014, 2015). Others, using mouse and macaque models of Parkinson disease, have found that leads with NIR- attached diodes implanted into the ventricles protected dopaminergic neurons in the substantia nigra from MPTP-induced degeneration and also preserved locomotor activity (Moro et al, 2013, 2014). Still others have reported that NIR light stimulation attenuates post-stroke deficits in rabbits (Lapchak et al., 2007, 2010) and increases cortical metabolism and memory retention in healthy rats (Rojas et al., 2012).

In human studies, near infrared stimulation has been applied via diodes directly to the scalp and is thought to penetrate 1-3 centimeters deep, with approximately 2-3% of the light reaching the cortex (Wan, 1981; Naeser et al., 2014). Stimulation is carried out using FDA-cleared devices that are deemed safe, painless and non-invasive. Studies of NIR stimulation typically use lasers, light emitting diodes (LEDs), or superluminous diodes (i.e., an LED variant that produces stronger light). The FDA originally approved the technology in 2003 as a nonsignificant risk device and the application of this technology in humans has been shown to be safe and effective (Rojas & Gonzalez-Lima, 2013).

In humans, positive effects with NIR stimulation have been observed in individuals with chronic aphasia due to focal stroke (Naeser et al., 2012, 2013), in individuals with traumatic brain injury who underwent a six week intervention study (Naeser et al., 2014), and in young healthy adults (Barrett et al., 2013). The latter study was 'sham controlled' and with young adults undergoing only one session of NIR stimulation, resulting in changes on tasks of executive functioning and processing speed. Patients with TBI showed improvement on tasks of executive function and recent memory, with relatively large effect sizes ranging from changes on the order of 1-2 standard deviations. At least one neuroimaging study has reported pre-post changes in resting state functional connectivity MRI following application of real vs. sham NIR.

Summary: While findings from both animal and human studies are promising, it remains unclear whether such a novel, unconventional approach might be viable for enhancing in older adults, including those at risk for developing Alzheimer's disease. More carefully controlled studies are clearly needed. Currently, we have available NIR instrumentation (MedX, FDA cleared as nonsignificant risk device) that was obtained from a McKnight Brain Institute shared instrumentation grant. The current proposal intends to use this system to pilot a randomized sham-controlled study in cognitively normal older adults, those with mild cognitive impairment (amnestic, nonamnestic), and nondemented individuals with Parkinson disease.

5. Specific Aims

1. **Aim 1**: ***To test the hypothesis that NIR stimulation will result in greater pre-post intervention improvement on tasks of executive function (co-primary outcome) and recent memory*** (co-primary outcome). Executive function will be tested using individual and composite scores from the NIH Examiner, a battery of executive function tests (Kramer et al., 2014) and an experimental Stroop task. The co-primary outcome includes a novel task of recent memory that is sensitive to hippocampal function and amyloid load, the Spatial Navigation Task which is also known as ARENA (Thomas et al., 2001; Laczo et al., 2011). Additional executive and memory measures will serve as secondary cognitive outcomes..

2. **Aim 2**: ***To test the hypothesis that NIR stimulation will result in greater pre-post intervention improvement, relative to sham, on measures of mood, negative affect and wellbeing*****.** This will be tested using indices from the Emotion module of the NIH Toolbox (Salsman et al., 2013) and traditional measures of depression, anxiety, and apathy.

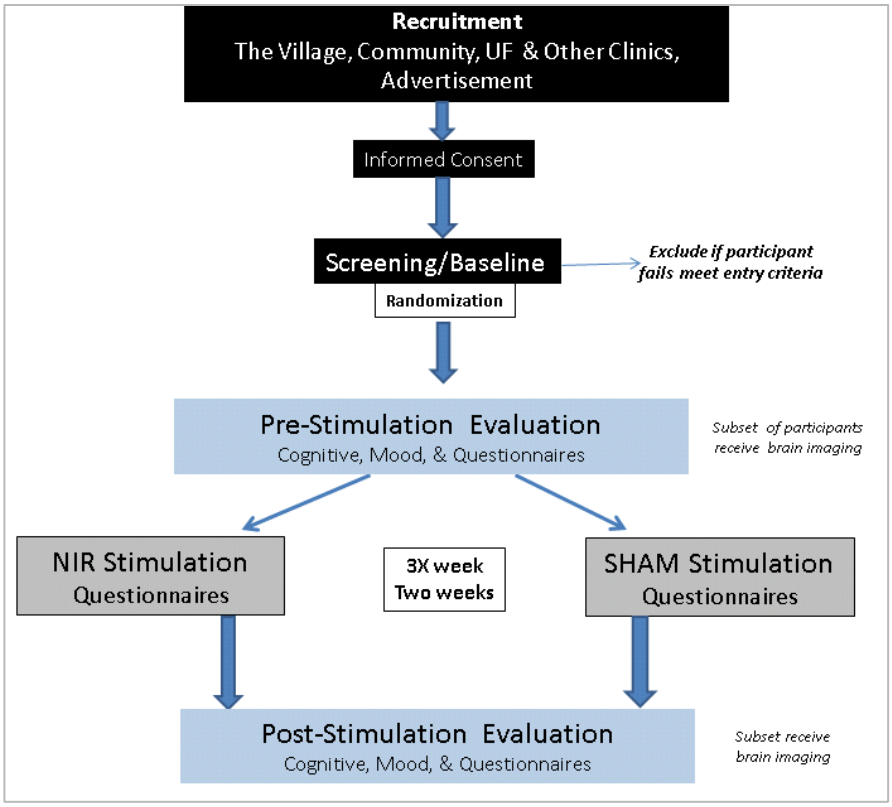
3. **Aim 3:** (exploratory): ***To learn whether there are intervention-related changes in resting state functional connectivity and/or MRS-based markers of ATP function***. This will be sampled in a subgroup of participants who undergo structural and functional MRI before and after intervention, either NIR treatment or sham conditions.

4. **Aim 4:** (Parkinson specific): **To learn whether there are intervention-related changes in motor skills.** This will be tested using measures of fine motor speed and dexterity (Grooved Pegboard), gait and balance, and clinical measures of Parkinson disease severity.

6. Research Plan

6.1 Design Overview

This is a pilot study of the efficacy of NIR stimulation for enhancing cognition and mood in older adults including those who are cognitively normal, those with mild cognitive impairment (amnestic and nonamnestic), and individuals with Parkinson disease. The overall hypothesis, drawn from previous literature, is that exposure to NIR stimulation will have positive effects on brain health and will result in better cognitive and mood performance. We propose to conduct a blinded parallel group sham controlled pilot trial, with half the participants in each group randomized to the real NIR treatment and half to the sham treatment condition. The two conditions are identical in all respects except that no near-infrared light stimulation will occur during the sham condition. The participants will consist of older adults recruited from the community, including The Village, and from UF and other clinics. The study will take place at the UF-VITAL lab located on The Village campus or in the Cognitive Neuroscience Laboratory at the Fixel Center for Neurologic Disease. The study design is shown below:



6.2 Participants

Participants will include individuals, age 62 years and older, who are independently living and willing to undergo a two-week intervention program. Our study groups will consist of the following: 70 cognitively normal individuals (N= 35/group), 20 individuals with amnestic MCI (N=10/group), 20 individuals with nonamnestic MCI (N=10/group), and 40 nondemented individuals with Parkinson disease (N=20/group). Based on our previous intervention studies with older adults, we anticipate a 20% attrition rate and up to 50 screen failures. To account for screening failures and study attrition, we anticipate consenting 206 individuals.

Informed Consent: Informed consent will be obtained according to university and federal guidelines. The informed consent process may begin with the initial telephone screening call, when a prospective participant is contacted or calls to find out more information about the study (See Telephone Script). After that first phone contact, the participant will be sent a copy of the Informed Consent via hardmail or via email if they are interested, and/or scheduled for an appointment in the VITAL laboratory or at the Fixel Center for Neurologic Diseases to discuss the study in more detail and in person.

Other potential participants, particularly those seen during the clinics of the PI or co-investigators, will be directly asked about their interest in this study and will be provided a copy of the informed consent to review.

During the first study visit, the project will be explained in more detail to the potential participant by the study coordinator or approved staff and the Informed Consent will be reviewed. Participants will be informed that this is a randomized trial and that there is 50% likelihood they may receive the sham intervention. The participant will be given the opportunity to ask questions. No study interventions will begin until the Informed Consent

(ICF) paperwork is signed. Participant will be given a signed copy of the ICF paperwork, and a copy will be kept on file with the Principal Investigator.

6.3 Recruitment

Participants will be recruited from the Village and the community through advertisements, brochures, and flyers (See Appendix A). Targeted locations might include bulletin boards at community grocery stores, physician's offices, medical centers, retirement homes, etc. Occasionally, the project coordinator and investigators will make informational presentations about age-related changes in various community venues (e.g. health fairs, socials). As part of these presentations, we will encourage interested individuals to contact the VITAL lab to learn more ongoing research. Staff members of The Village who obtain signed permission from residents will forward resident names and contact information to the Study Coordinator.

Additional recruitment may occur through the clinics of the PI and co-investigators or other clinics at the Fixel Center (via flyers). These clinics focus on older adults including those with Parkinson disease, and potential participants will be directly asked about their interest in this study by their clinicians. Additionally, flyers will be distributed to other physicians at the Fixel Center.

Finally, recruitment may occur via IRB approved clinical and research databases. Thus, recruitment of normal older adults may take place via two IRB-approved research databases that are maintained by one of the co-investigators, Dr. Marsiske. These include the Claude D. Pepper Recruitment Registry (IRB #201601352, previously 415-2007) and the Participant Registry for Aging Research (IRB #201601448, previously 131-2002).

Recruitment of individuals with mild cognitive impairment or Parkinson disease may take place via INFORM (IRB #201501166, previously #416-2002), a clinical research database that is maintained by the Center for Movement Disorders and Neurorestoration (CMDNR). The INFORM database includes patients with both movement and cognitive disorders, including those who are seen in Dr. Weisbrod's and Maraganore's Cognitive Disorders and Dementia clinics at the Fixel Center.

6.4 Inclusion/Exclusion criteria

6.4a. General Inclusion Criteria for all Participants

- Age 62 years or above
- Able to provide informed consent and perform cognitive and mood measures on a computer
- Willingness to be randomized to Sham or Real intervention
- Can devote 2 weeks to the intervention, and additional time for pre and post testing
- 8^{th} grade education and ability to read on 8^{th} grade level based on scores on the Wechsler Test of Adult Reading (WTAR) or the Wide Range Achievement Test-IV (WRAT-IV); or a reading test at 14 pt. text
- On stable doses of major medications; Since some older adults with memory complaints may be prescribed acetylcholinererase inhibitors or related medications by their primary care physicians (i.e., donepezil, rivastigmine, galantaominhe, memantime, or other potential memory-enhancing agent(s), we will not exclude them as long as they have been on stable medications for at least two months and plan to continue this medication during study participation.

- Willingness to allow a study partner (spouse, family member, friend) to answer questions about their cognitive, mood, and other behaviors. This does not apply to individuals with Parkinson disease.

6.4b. *General Exclusion Criteria for All Participants*

- Sensory loss (vision, hearing) or motor deficits that would preclude participation in the experimental cognitive tasks or neuropsychological assessment
- Unstable and uncontrolled medical conditions (metabolic encephalopathy, HIV, moderate to severe kidney or liver disease)
- Previous major strokes or other known significant brain abnormalities or diseases affecting cognition (i.e., multiple sclerosis, seizure disorder, brain surgery, moderate TBI, etc.). No history of brain surgery. Exceptions are a diagnosis of Parkinson's disease for the PD subgroup.
- Evidence of potential dementia based on cognitive screening (e.g., scores < 5^{th} %ile on the Montreal Cognitive Assessement (MoCA) or the Dementia Rating Scale-2 (DRS-2) based on appropriate age, education and sex norms.
- Current or past history of major psychiatric disturbance including schizophrenia, or active psychosis, bipolar disorder, current major depressive episode, current alcohol or substance abuse or history thereof within the past six months. This will be assessed using the *Mental Health Screen* v.3 (Carroll & McGinley), a modification of the Structured Clinical Interview for DSM-IV psychiatric disorders. We are not excluding individuals who are taking antidepressants or anti-anxiety medications, however, use of antidepressants and anxiolytics will be recorded and data will be analyzed in post-hoc analyses
- Use of antipsychotics, sedatives, or other medications with significant anticholinergic properties (due to potential influence on memory)
- Use of photo-sensitive medications such as steroids or retin-A within 15 days of the study intervention
- Diagnosis of active cancer
- Previous participation in a cognitive training study within the last 6 months

6.4c. Additional criteria for participants in the cognitively normal, MCI, and Parkinson groups:

Cognitively Normal Group

- No evidence of objective cognitive decline based on scores on neuropsychological screening measures involving delayed memory (HVLT-R or WMS Story Recall), confrontation naming (Boston Naming Test), and executive function (Trails B, Category Fluency, Judgement of Line Orientation). Scores on these measures must be no lower than -1 standard deviation below normative values.
- Lives independently with no impairment in social or occupational function
- No impairment in social and/or occupational function
- Does not meet DSM-V criterial for Major or Minor Neurocognitive Disorder

Amnestic MCI Groups – Amnestic and Nonamnestic

- Subjective memory or cognitive complaints that are confirmed by a study partner
- Global Clinical Dementia Rating (CDR) Scale of 0.5 with CDR sum of boxes no greater than 2.0 (Morris, 1993)

- For amnestic MCI, objective memory impairment based on HVLT-R delayed word list recall or delayed paragraph recall (WMS) of -1.5 standard deviation or below expected levels for age, education, ethnic/culture. Scores on other neuropsychological screening or baseline measures can be no more than 1.5 SD below normative values. Note that an amnestic component is necessary but non-amnestic component is not required.
- For nonamnestic MCI, scores on memory tasks (e.g., delayed recall of stories from WMS) must be within normal limits (e.g., at least -1.0 SD or better than normative values). Scores in the executive domain should be -1.5 SD or below normative values..
- No impairment in social and/or occupational function
- Does not meet DSM-V criteria for Major Neurocognitive Disorder

Parkinson Disease Group

- Participants must have a diagnosis of idiopathic Parkinsons disease by a movement disorders specialist based on UK Brain Bank criteria (Hughes et al., 1992a, 1992b).
- No previous history of brain surgery (DBS, pallidotomy, thalamotomy, or fetal cell implants).
- No evidence of dementia based on scores on a cognitive screener (e.g., Dementia Rating Scale-2 (DRS-2) and score on delayed paragraph recall cannot be lower than 1.0 standard deviation of normative values
- May have difficulties with activities of daily living, but this is due to physical symptoms of Parkinson disease and not because of cognitive problems
- Unwillingness to undergo baseline and followup visits when they are 'off' standard dopamine medication
- Inability to undergo a brain scan

Additional exclusion criteria apply to those individuals who participate in the ***optional brain imaging*** study. The brain scan is optional for all participants except those with Parkinson disease. In that case, inability to undergo a brain scan, before and after intervention, is an exclusion. Exclusion criteria for a brain scan include

- Presence of claustrophobia
- Implants such as pacemakers, heart valves, brain aneurysm clips, orthodontics, non-removable body jewelry, or shrapnel containing ferromagnetic metal.

**Note that we are highly experienced in working with older adults who are identified as experiencing current major depression or other psychological disturbances or who perform below cutoff on standard cognitive screening measures. The PI [Bowers] is a boarded clinical neuropsychologist who has been working with this clinical population for over 30 years. The clinical Co-I's (Maraganore, Wagle-Shukla, Weisbrod, Hess) are experienced neurology clinicians, and the doctoral students on this protocol are clinical psychologists in training with experience in diagnosis and treatment of mood and cognitive disorders. Discussions will be held with individuals of concern and recommendations will be made to appropriate health care providers with permission of the participant and in line with standards of care.

6.5 Screening Interview and Measures

Participants will be screened for inclusion/exclusion criteria. This will involve review of background and demographic information, medical and psychiatric history, current medications, and assessment of functional activities, along with screening of current cognitive

status using the MoCA or DRS-2, the HVLT-R or WMS-III Logical Memory, and other cognitive measures. Screening will be administered by the project coordinator.

Table 1: Screening & Baseline Measures

Dementia Screen	Montreal Cognitive Assessment (MoCA: Nasreddine et al., 2005) or Dementia Rating Scale (DRS); these are widely used cognitive screening measures for dementia; See text for cutoff scores for Cognitively Normal and MCI groups
Objective Memory	Hopkins Verbal Learning Test-Revised and the WMS Logical Memory Stories: delayed recall scores on these measures are used to assign participants to Cognitively Normal (CN), amnestic and nonamnestic MCI groups, or to exclude Parkinson patients; (see text)
Other Cognitive measures	Executive Function - Trailmaking Test (Optional: Wisconsin Card Sort) (optional for PD) Visuospatial – Judgement of Line Orientation (JOLO) (optional for PD) Language - Boston Naming Test, Category Fluency (optional for PD) Attention & Processing Speed - Digit Span, Digit Symbol (optional for PD)
Medical	Background medical history- rule out medical conditions (i.e., neurodegenerative, TBI, etc.),
	Charlson Comorbidity: Assesses for variety of comorbid medical conditions, computes risk factor score
	List of Medications: stable medications for 3 months; rule out use of anticholinergic medications; rule out use of photo-sensitive medications within 15 days of intervention
Psychiatric	Mental Health Screen V.3 (Carroll & McGinley) - this is modification of Structured Clinical Interview for DSM-IV psychiatric disorders, both current and historical (SCID-IV, First, Spitzer, Gibbon, & Williams); Goal is to rule out major depressive disorder, schizophrenia, psychosis, current substance abuse, and other Axis 1 disorders
Reading Literacy	Wechsler Test of Adult Reading (WTAR) or the reading subtest from the Wide Range Achievement Test –IV (WRAT-IV); participant reads aloud single words in order to estimate reading level; All must read on at least an 8th grade level
Vision	Vision & Color Vision Screening;. Basic visual acuity will be measured using a Snellen chart and color vision will be tested using Ishihara color plates.

Participants will require a **Study Partner** (e.g., spouse, family member, friend) to provide independent information about their cognitive and behavioral status. The study partner will complete structured questionnaires about the participant. Both the participant and the study partner must provide independent consent for this to take place. The requirement for a study partner is 'optional' for those individuals with Parkinson disease.

It is possible that some participants, particularly those in the MCI or Parkinson groups who are recruited through the UF Cognitive Disorders and Dementia clinics or CMNDR, may have already had some of the screening measures, cognitive tasks, and rating scales associated with the current study. If so, we will request permission to use these, rather than repeat them, if they have been completed within 2 months. We will also request permission to access relevant medical and neuroimaging records.

If a participant does not meet inclusion/exclusion criteria, they will be thanked for their participation and given information as to the reason for their screen failure. There are several reasons why a participant might fail inclusion/exclusion criteria. Some that are of particular concern are poor test performance on the dementia screening measures (MoCA or DRS), presence of current major depression, substance abuse, or psychosis (i.e., hallucinations, delusions). If any of these scenarios emerge, we will discuss with the participant and, if appropriate, we will offer a referral for additional clinical evaluation through their primary physician or a specialty health care provider. Should individuals report suicidal ideation, the PI or co-investigators will meet with the participant to assess risk and refer for further evaluation

or treatment if necessary. With participant permission, we will provide results of our screening to their health care provider and/or them or a family member. As mentioned above, the project team is experienced in diagnosis and treatment of mood/cognitive disorders.

6.6 Pre and Post-Intervention Measures

Participants who meet study criteria and who remain interested in participating in the 2 week intervention will be randomized to treatment or sham groups and undergo a baseline/ pre-intervention testing. The pre-intervention measures will be administered by a trained psychometrist or research assistant who will be blinded to group assignment (sham, real NIR stimulation).

The pre-intervention testing will take approximately 3 – 3.5 hours, and will consist of measures of executive function, recent memory, and mood. These same measures will be given after the two-week intervention has completed.

Individuals with Parkinson disease will complete the pre and post intervention visits when they are "off" their typical dopaminergic medications. This is identical to what Parkinson patients are asked to do as part of standard clinical care when seen at the UF Movement Disorders Clinic. To do so, the Parkinson patients will refrain from taking their parkinsonian medications overnight, with no dopa medication after 10:00 PM the night before. The theoretical rationale for evaluating patients when off dopamine medication is to obtain an index of the true status of their disease state.. The remainder of the protocol (i.e., screening, intervention) will be completed when participants are on their normal medications. When tested 'off medication', patients may experience increased slowness, tremors and stability. Because of this, they will be closely monitored and provided a wheel chair. If they become too uncomfortable they can take their medication and will be withdrawn from the study.

In previous IRB- approved studies, we have tested Parkinson patients off medication while they completed an array of cognitive, EEG, and other tasks. We have found that the tolerability was quite good and no participants dropped out of the study for the medication issue. In the current study, we are implementing testing 'off dopa' medications at the recommendation of the Parkinson Foundation.

6.6.1 Executive Measures [NIH Examiner, Stroop]

The executive measures include the NIH Examiner, a computerized battery consisting of 9 individuals subtasks described below, and a measures of cognitive inhibition (Stroop task). During post-intervention, an executive measure given as part of screening (Trail Making Test) will be repeated.

6.6.1a Description of the NIH Examiner: The National Institutes of Health Executive abilities: Methods for Neurobehavioral Evaluation and Research (Kramer et al., 2012). The NIH Examiner is a computer based battery that involves nine tasks of frontal-executive function and takes around 45 minutes to complete. Funding for development of the NIH Examiner was provided by NINDS in 2005 to develop sensitive, domain-specific tasks of fronto-executive function. The NIH Examiner consists of 9 individual tasks that can be combined into three domain specific scores: *Cognitive Control, Working Memory, and Fluency.* An overall *Executive Composite* score can also be computed (Kramer et al., 2014). In the current study, the **dependent variable for the primary aim will be the "executive composite**"

score calculated from the tasks within the NIH Examiner. Described below are each of the 9 tasks of the battery.

1. *Dot Counting (working memory*): Participants look at a mixed array of green circles, blue circles, and blue squares. They are asked to count all of the blue circles and remember the final total, before counting the blue circles on new displays. The number of new displays increases from two to seven over a total of six experimental trials. The dependent variable is total correct score over trials 1-6, which contributes to the Executive Composite and the Working Memory factor scores.
2. *N-back (working memory*): Participants view a series of white squares that appear in different locations on a black screen. In the 1-back trials, participants are required to remember the location of squares and compare the location of each new square to the previous square. In the 2- back trails, participants are required to compare each new square location to the location of the square two trials before. The 2-back will only be administered if sufficient performance is reached on the 1-back (computerized adaptive testing). The 1-back consists of 30 trials total while the 2-back consists of 90 trials. Discriminability (d-prime) is calculated as the difference between the z-transforms of the hit rate and the false positive rate. The dependent variables are the 1-back and 2-back d-prime scores and contribute to the Working Memory factor score and the Executive Composite.
3. *Flanker (inhibition)*: In this task, participants view a row of five arrows presented at the center of the screen and then indicate the direction of the center arrow using the arrow keyboard keys. In some sets of trials, all arrows point in the same direction (congruent); in other sets of trials, the center arrow points in the opposite direction of the rest of the arrows (incongruent). Reaction times are typically longer on the incongruent trials. A total of 48 trials are administered. Total accuracy score and reaction time scores on the incongruent trials are calculated and added together to create the total flanker score. The dependent variable is the total flanker score, which contributes to the Executive Composite and the Cognitive Control factor score.
4. *Continuous Performance Test (inhibition):* Participants are asked to press a button when they see a five-pointed start, and not respond when they see any other shape. A total of 100 trials are presented, 80% consisting of the five-pointed star. The dependent variable is the total number of false alarm errors made on the stimuli. This variable contributes to Executive Composite and Cognitive Control factor scores.
5. *Anti-saccades (inhibition):* In this task, participants are asked to watch a moving dot on the computer screen and move their eyes upon presentation of a laterally presented stimulus. Some trials involve moving eyes in the direction of the presented stimulus (prosaccade, 10 trials), and some involve moving eyes in the opposite direction (anti-saccade, 40 trials). The dependent variable is the total number of correct anti-saccade trials, which contributes to the Executive Composite and Cognitive Control factor scores.
6. *Set Shifting (set-shifting*): Participants are required to match a stimulus on the top of the screen to one of two stimuli located in the lower corners of the screen. Stimuli are matched either based on color or shape depending on the cue. There are three tasks: Task A involves matching stimuli based on shape (homogenous; 20 trials), Task B involves matching stimuli based on color (homogenous; 20 trials), and Task C alternates between matching stimuli based on shape and color (heterogeneous; 64 trials; 32 shape, 32 color). Both reaction time score and accuracy scores are calculated across all blocks. These two scores are summed to create the dependent variable, a total set shifting score. This total set shifting score is used to calculate the Executive Composite and the Cognitive Control factor scores.
7. *Phonemic Fluency (fluency*): In this task, participants are asked to generate as many words that begin with a particular letter of the alphabet. The task is timed for one minute and participants are given two different letters (F and L) for which they must generate words. The dependent variables are the total number of correct words generated on F and L trials, which contribute to the Executive Composite and Fluency Factor scores.
8. *Category Fluency (fluency*): This task is similar to “phonemic fluency”. However, participants are

asked to generate as many words possible that belong to a particular category. This task is timed for one minute and participants are given two categories (animals and vegetables) for which they must name items. The dependent variables are the total number of correct words generated on animal and vegetable trials, which contribute to the Executive Composite and Fluency Factor scores.

9. *Unstructured Task* (planning): In this task, participants are presented with three booklets, each containing five pages of puzzles. Puzzles are cognitively simple but may take anywhere from 4 to 60 seconds to complete, and are associated with a specific point value. Participants are given six minutes to complete puzzles and are required to use judgment and planning skills in order to earn as many points as possible. The dependent variable is the total number of points earned during the 6 minutes. The score on this task is not factored in to the Executive Composite or the factor scores.

6.6.1b. Description of the Stroop (Golden version). This version of the Stroop is a standard task given as part of the typical neuropsychological exam and is pre-approved by the IRB. It involves 3 parts: Word Reading, Color Naming, and Color-Word Interference. During Word Reading, a participant reads the words (red, blue, green) typed in black ink on a white page, vertically. Final trial score is total number of words read in 45". During Color Naming, the participant rapidly names the color patches (red, green, blue) that are arrayed on a card. Final trial score is total number of patches accurately identified in 45". During the Color-Word Interference condition, a word (i.e., Red) is printed in color ink that differs in meaning from the meaning of the written word. The participant's task is to name the ink color of the word, and inhibit reading the word. Final trial score is total number of items identified over 45". The ***dependent variable*** is the cognitive inhibition score, which is based on the Color-Word interference score, taking into account performance on the 2 baseline trials.

6.6.2 Recent Memory Tasks [AVLT, Arena Task, MST]

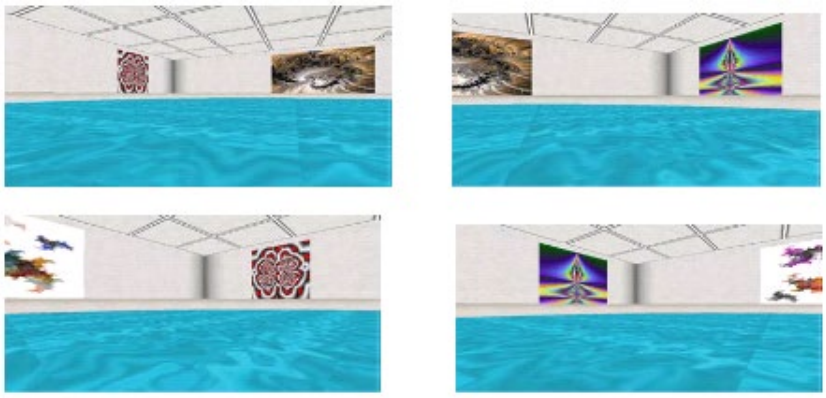
The recent memory measures include a verbal word list learning task (AVLT), a spatial navigation and learning measure (ARENA), and an object recognition memory task (MST). During post-intervention, the memory measure given as part of screening (Logical Memory Stories) will be repeated.

6.6.2a. ***Description of the Auditory Verbal Learning Test (AVLT)****:* The AVTL is a word list learning task that assesses learning, proactive interference, along with retention and recognition over 30 minute delays (Rey, 1964; Schmidt, 1996). It is widely used in clinical settings and is similar to other word list learning tasks, except that the individual items are not semantically related to each other. Thus, participants must impose their own unique strategies in order to optimize learning. The AVLT consists of two 15 item lists, with List A given over 5 trials, followed by one presentation of List B, with subsequent immediate recall of List B, then List A. Later 30 minute recall and recognition of List A are obtained. The primary dependent variables for this task are overall learning, the degree of proactive interference, and delayed recall after 30 minutes.

6.6.2b. Description of Computerized Arena Task (Laurance et al., 2012): This is a task of spatial memory and navigation that has been linked to hippocampal function and is a human analogue to the Morris water maze used in animal studies. At UF, this task has been used in other IRB-approved studies with older adults (the VITAL study), those with Essential tremor, those with mild cognitive impairment and Alzheimer's disease.

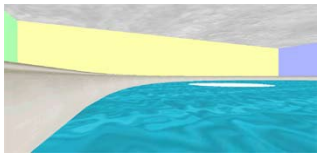
The task is given on a computer and participants are trained in the use of a joystick or direction pad before beginning the task. Participants must navigate in a virtual, 3-D room displayed on a computer screen. They must start from different positions to find an invisible target on the

floor as quickly and efficiently as possible. Shown below are sample walls of the virtual room. The overall goal is to find a 'hidden target' on the floor of the arena.



The participant uses either a direction pad or joystick to navigate this "arena". Navigation is constrained to a circular "arena" designed to look like a pool of water (the blue on the pictures above). The arena is bordered by a low wall and is in its entirety located in a perfectly square room, with each of the four walls of the room differentiated from the others by 1-2 unique, fractal-based patterns

Practice and Visible Target Trials. The task begins by providing a participant with a 5-10 minute orientation session, in which they practice using the joystick or direction pad and then navigate to visible targets within the environment. In the figure to right the white elliptical circle represents the target. Performance on up to 5 visible-target navigation trials ends the practice session and the recorded path length and time to acquire the target will serve as a sensorimotor baseline against which invisible target trials will be evaluated.



Hidden target condition. After subjects have learned the task, they will participate in 8 invisible-target acquisition trials. In these trials the target is not visible from afar and is discoverable only when the joystick or direct pad icon is hovered over the target. A trial begins with a fixed tour of a new room, where each wall is shown for approximately 2 seconds during two sweeps of the room. Participants are instructed that the hidden target will be in the same place on each trial and that their task is to find it as soon as possible. Participants are typically given 120 seconds to find the target. During the first 2 trials, the examiner takes over after 120 seconds and assists the participant finding the target. No assistance is given after the initial two trials. If the target has not been located after 120 seconds in the later trials, the trial ends and a 10-second inter-trial interval (ITI) ensues, and the next trial begins. If the subject acquires the target on any trial, it becomes visible and a pleasant auditory signal additionally alerts them to target acquisition. They are allowed to stay on the acquired target for 15 seconds and are encouraged to rotate-in-place to survey the environment. After this, the 10-second ITI ensues and the next trial begins at a different compass point. A final spatial memory probe occurs following the 8th trial. On this final trial, the invisible target is removed unbeknownst to the participant. This means that target acquisition is not possible, but search behavior is evaluated with regard to the percent of the 120-second trial that is spent in the quadrant containing the target. On each acquisition trial, path length, time to acquire the target, and target acquisition (yes-no) are recorded. On the probe trial, path length and percent of time spent in each quadrant of the Arena is recorded. A composite score, consisting of mean z-scores on path length, time to acquire target, and percent of time spent in the proximal quadrant on the probe trial, will also be calculated. **The dependent variable will be this navigational performance composite.**

6.6.2c. Description of the Mnemonic Similarity Test (MST; Starke et al., 2013, 2015, 2017): This task is sensitive to hippocampal dysfunction and the effects of normal aging. It relies on process of pattern separation, the process of grouping similar inputs into distinct memory representations. The MST measures recognition memory performance for objects using traditional targets, novel foils, and other items ("lures") that are perceptually and semantically related to the targets. During the learning/encoding phase, participants are shown a series of designs/objects. This is followed by a recognition task in which the items are true replications (hits), novel items (foils), or are similar but not identical to the hits (i.e., lures). The task is completed on a computer and takes about 15 minutes

6.6.3. Processing Speed

Processing speed, a potential contributor or mediator of both memory and executive function, will be assessed using reaction time subtests from the **California Computerized Assessment Package (**CALCAP-RT; Miller, 2013).

The **CAPCAP** is a comprehensive tool for assessing reaction time, speed of information processing, rapid visual scanning, form discrimination, brief memory and divided attention. We plan on administering the **abbreviated version**, which consists of 4 subtests that take approximately 8-10 minutes. All tasks are given on a computer. The 4 subtests are described below.

- Simple Reaction Time. Subjects are asked to press a key as soon as they see anything at all on the screen. This procedure provides a basal measure of reaction time.
- Choice Reaction Time for Single Digits. Subjects are asked to press a key as soon as they see a specific number such as '7', otherwise they are to do nothing. This procedure adds a simple element of memory to the task.
- Serial Pattern Matching 1 (Sequential Reaction Time 1). Subjects are asked to press a key only when they see two of the same number in sequence, for example, if they see the number '3' followed by a second occurrence of the number '3'. This procedure adds a more complex element of memory since the subject must keep in mind the last number that was seen.
- Serial Pattern Matching 2 (Sequential Reaction Time 2). Subjects are asked to press a key only when they see two numbers in sequence (increasing order). For example, if they see the number '3' followed by the number '4', the number '6' followed by '7' and so on.

6.6.4 Mood Measures [Emotion module of NIH Toolbox, STAI, POMS, BDI-II, AS]

6.6.4a. Description of NIH Toolbox Emotion Module (Salsman et al., 2013). The NIH Toolbox for the Assessment of Neurological and Behavioral Function (www.nihtoolbox.org) is a standardized set of Web-based measures developed through a contract initiated by the NIH Blueprint for Neuroscience Research (Gershon et al., 2013). It contains four modules: Motor, Sensation, Cognition, and Emotion.

This study will use the Emotion module. It is a 12 to 22 minute computer administered, self-report measure of emotional health and psychological function. It surveys positive and negative emotions such as joy, sorrow, fear, etc.. Questions consist of likert-type items that are rated by a participant. The module consists of 4 scales: Psychological Well Being, Negative Affect, Social Relationships, and Stress& Self Efficacy. Each is described below. The dependent variable is the T score from each measure. The first two scales are the co-primary outcomes and the latter are the secondary outcomes:

1. *Psychological wellbeing* (co-primary outcome). Subscales making up this domain include: general life satisfaction, meaning and purpose, and positive affect. Participants rate statements such as "I

am satisfied with my life", "I value my activities", and "there is not enough purpose in my life". There are a total of 76 items on the Psychological Wellbeing scale.

2. *Negative affect* (co-primary outcome; subscales making up this domain include anger, fear, and sadness). Participants rate statements such as "I feel worthless" and "I felt envious of others". A total of 89 items are given on the Negative Affect scale.
3. *Social Relationships* (exploratory outcome, subscales making up this domain include social support, companions, social distress, and positive social development), Participants rate statements such as "people in my life act as if they don't care about me" and "people don't listen when I ask for help". A total of 26 items will be given on the Social Relationships scale.
4. *Stress and Self-efficacy* (exploratory outcome; subscales making up this domain include: perceived stress and self-efficacy). Participants rate statements and questions such as "I can handle whatever comes my way" and "how often in the past month did you feel nervous and 'stressed'". A total of 20 items will be given on the Stress and Self-efficacy domain.

6.6.4b. Description of State Trait Anxiety Inventory (STAI**;** Spielberger,1983a; 1983b; 1989). The STAI is a 40-item questionnaire that is used to assess current (state) and general (trait) levels of anxiety. Higher scores represent higher levels of anxiety, or feelings of worry, tensions, and stress. Items on the questionnaire include ratings of statements such as "I feel calm", "I am jittery", and "I feel nervous". The STAI has been used extensively in clinical and research settings and displays a test-retest reliability of 0.31 to 0.86 and internal consistency alpha coefficients of 0.86 to 0.95 (Spielberger et al., 1983a). Further, this measure shows high convergent validity with other measures of anxiety (0.72 to 0.86; Spielberger et al. 1989).

6.6.4c. Description of Profile of Mood States, short form (POMS, Curran et al., 1995). This is a 30 item self- report questionnaire of mood states such as "discouraged", "grouchy", "vigorous", and "efficient". The short form version of the POMS is easy to administer, and yields information about transient current mood states. The measure is grouped into subscales including tension, depression, anger, fatigue, and vigor. Test-retest reliability ranges from 0.65 to 0.74, internal consistency is 0.93, and the test also features high degrees of construct validity (Curran et al., 1995).

6.6.4d. Description of Beck Depression Inventory-II (BDI-II; Beck et al., 1996). This is a 21 item self-report questionnaire of symptoms of depression such as sadness, self-criticalness, loss of interest, or somatic changes such as insomnia or increased sleep, increased or decreased appetite, and low energy. This questionnaire allows one to characterize levels of depression as mild, moderate, or severe, depending on the total score. The BDI-II has strong convergent validity, internal consistency (α=.91) and test-retest reliability (r=.93) and has been used extensively in clinical and psychological research.

6.6.4f. Description of Apathy Scale (AS, Marin et al., 1991). This is a 14 item self-report questionnaire that assesses for symptoms of motivation, drive, and initiative. Participants answer questions on a 5 point likert scale ranging from 0 to 4. The AS has been subgrouped into components reflecting cognitive, affective, and behavioral components of apathy. Test-retest reliability is strong, and the AS has strong convergent validity with real world indicators of behavior (Ferencz et al., 2012).

6.6.5 Other Measures

6.6.5a. Placebo Control Questionnaire (PCQ): This is a 4-item questionnaire that is given after the end of the 2-week intervention. It asks questions regarding which group (real, sham) the participant believes they were assigned.

6.6.5b. Adverse Events Log (AE). This is a standard open ended questionnaire regarding symptoms or adverse events that the participant has experienced since beginning the intervention. This will be assessed at the end of each intervention session and will prompt for symptoms the participant may have experienced during the session and/or since the previous intervention session. It queries for severity, how long the symptoms last, and whether (if known) the symptom might be related to the intervention. This will also be assessed during the followup session.

6.6.5c. Fitzpatrick Skin-Type Questionnaire (Fitzpatrick, 1986): This is a 10 item self-report form where participants indicate hair, eye, and skin color, as well as rate burning and tanning to sun exposure. This information is being obtained because skin and hair pigmentation may impact the penetrance of infrared light through the skin and scalp. This scale is often used in dermatology and will be used to as a covariate in statistical analyses.

6.6.5d. Cognitive Change Index (CCI) On this 20 item questionnaire, participants rate changes in their own thinking and memory. They use a 5 point Likert scale that goes from 1 (no problem/normal ability) to 5 (much worse/severe problem). Domains that are covered include multi-tasking, learning new things, recalling old memories, thinking quickly, retrieving names, etc. This questionnaire will be given before and after the intervention to assess perceived changes.

6.6.5e. Expectancy Questionnaire: This measure is given prior to the intervention and asks participants to rate their expectations about changes they anticipate will change as a result of the intervention.

6.6.5f. Followup Questionnaire: This measure is given after the intervention. It asks participants to rate the extent that they experienced changes in particular cognitive and functional domains

6.6.5g. Pittsburgh Sleep Quality Index: This is well-known and well-validated sleep scale for detecting insomnia and sleep quality. It will be given before and after the intervention.

6.6.5h. Fatigue Severity Scale (FSS): This is standard self-report questionnaire that measures degree of fatigue that an individual experiences. It will be given before and after the intervention.

6.6.5i. Pain Rating Scale. This is analogue rating scale where participants rates the current severity of any ongoing pain.

6.6.6 Motor Measures (UPDRS, Grooved Pegboard, Walking-Balance & GaitRite)
Individuals with Parkinson disease will receive additional motor measures before and after NIR intervention. These include the *Unified Parkinson Disease Rating Scale* for rating disease severity, a task of manual fine motor speed and dexterity (*Grooved Pegboard Task*) and walking speed and balance that is measured using a Gait Rite mat which has digital sensors that records speed and foot placement and other motor measures.

6.7 Near-infrared (NIR) Stimulation Protocol

Overview: Participants in both the real and sham conditions will participate in a total of six intervention sessions, 3 sessions a week, over a two-week period. Each session will include 40

minutes of “stimulation” time, followed by completion of a brief adverse events questionnaire and a brief mood questionnaire (POMS). Total session time in the lab may take up to 1.5 hours. During each session, the participant will be seated comfortably in a chair. Clusters of LED’s will be positioned across various regions of the scalp via a flexible head cap. Onset of the NIR light stimulation will be controlled by a MedX Console unit. During the 40 minutes of NIR stimulation or sham stimulation, the participants will view nature documentaries (BBC Life documentary series; Gunton, 2009) that are presented during the period of stimulation. This is being done in order to standardize cognitive engagement and control for differences in cognitive activity during stimulation.

In addition to stimulation in the laboratory, participants may be asked to do daily intranasal stimulation in their home. They will be provided Vielight intranasal devices (described below) and asked to stimulate themselves for 25 minute sessions on the days they do not come to the lab (i.e., 2 days during week 1 and 2 days during week 2).

6.7.1 Equipment: MedX Rehab Console System.

Near infrared (NIR) stimulation will be delivered to the cranium via two MedX 1116 Rehab Console Systems (MedX Health, LLC, Mississauga, ON, Canada). See Figure 2. The MedX system was FDA-cleared in 2003 as a Class II medical device (K032231, 21 CFR 890.5500) and has been freely available on the market since that time. It is not viewed as causing harm or injury, and falls under the category of an infrared lamp with indicated use for “increase in local blood circulation” as well as muscle relaxation and relief of muscle and joint aches, pains, and stiffness. It has been used in transcranial studies of cognition and mood in humans since 2009, without any known adverse side effects (See review by Rojas & Gonzalez-Lima, 2013).

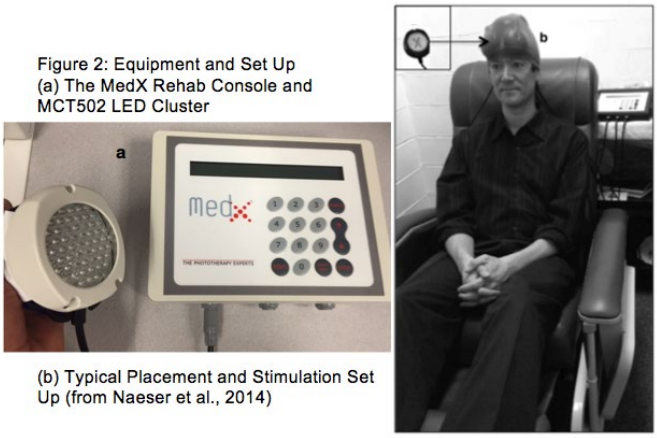


Figure 2: Equipment and Set Up
(a) The MedX Rehab Console and MCT502 LED Cluster
(b) Typical Placement and Stimulation Set Up (from Naeser et al., 2014)

Each MedX console consists of a control unit and 3 superluminous diodes - a cluster of LED’s (3 MedX MCT502). Each superlumious LED cluster consists of 52 near infrared diodes and 9 visible red diodes (see Figure 2). The energy delivered by the device is 1 Joule/cm^2 in 45 seconds at treatment wavelength of 870 nm. The LED cluster has an irradiance of 22.2 mW/cm^2 and treats an area of 22.48 cm^2.

In 2013, we were awarded monies from a shared instrumentation grant from the McKnight Brain Institute for purchase of two MedX console systems (each approximately $5000). Since then we have acquired additional units, including two units that are ‘sham’.

Vielight 810 Infrared Light will be delivered intranasally using a Vielight 810 Infrared system (Vielight, Inc., Toronto, ON, Canada). The Vielight 810 Infrared System uses light that is considered safe and painless, and does not require FDA clearance (considered a low-risk device). The Vielight 810 consists of an infrared diode that delivers light at a wavelength of 810 nm, with an irradiance of 7.6 mW/cm^2 and a pulse frequency of 10 Hz.

6.7.2 Duration of Treatment, Dosage Parameters, and Placement of LED Clusters

The intervention protocol will span 2 weeks and will include laboratory (six sessions) that involves transcranial plus intranasal stimulation and an optional four 'at home' sessions involving intranasal stimulation (. For Parkinson patients, we may add up to four at home sessions with intranasal stimulation (25 minutes/session). Both the duration of treatment (6 sessions over two weeks) and the dosage of stimulation were originally adapted from prior investigations in TBI patients (Naeser et al., 2014), young adults (Barrett & Gonzalez-Lima, 2013), and individuals with Alzheimer's disease (Saltmarche et al., 2017), showing that NIR stimulation was safe, well tolerated and had no side effects. In our pilot work using the laboratory based protocol with older adults, we found positive effect sizes with this dosing for memory and executive function, along with increased ATP and neural connectivity. In a parallel IRB study with older adults we have increased the duration of treatment to 8 weeks, based on recent findings by Saltemarche et al. (2017) with Alzheimer's patients. However, we continue to keep the current protocol brief as it is designed to determine potential effect sizes and feasibility.

Placement of Clusters and Dosage. During each laboratory session, actual stimulation via the LED clusters will occur for a total of 40 minutes. Six LED clusters will be applied in 2 distinct configurations. There will be 20 minutes of stimulation at each of these configurations. Each configuration will target 8 sites (6 cranial + 2 intranasal), for a total of 16 sites over the course of the 40 minute session. Placement of LED clusters on the scalp follows the recommendations of Naeser (personal communication, 2014, 2015, and 2016). See Figure 3 for site placement.

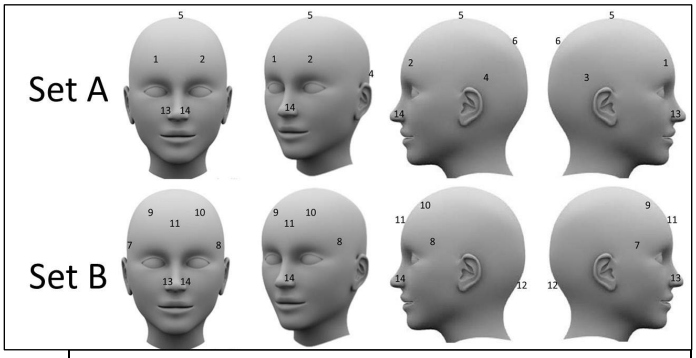


Figure 3: Placement of LED Clusters in 2 Configurations

The power density used will be 500 mW with a cumulative fluence (energy density) of 336 J/cm^2 (26 J/cm^2 applied at 12 cranial sites + 12 J/cm^2 at 2 nasal sites). It is estimated that approximately 6 J/cm^2 will reach the cortex with each daily treatment. At this energy level, the MedX MCT502 accessory does not cause tissue damage or physical damage and emits negligible heat. There is no nominal ocular hazard distance (NOHD) for the LED accessory, as LEDs do not pose a serious risk to vision.

Parameters for stimulation such as energy density and length of application were adapted from

parameters used in Naeser et al., 2014 as well as personal correspondence in September 2015. In 2013, Naeser and colleagues demonstrated that an energy density of 13.3 J/cm^2 resulted in changes in resting state- connectivity, whereas an energy density of 2 J/cm^2 did not. Current studies conducted by the Naeser group involve an energy density of 26 J/cm^2.

6.7.3 Stimulation Session Procedure

In Lab Sessions: All participants will attend six NIR stimulation sessions. During each session, the participant will be seated comfortably in a chair. Clusters of LED's will be positioned across various regions of the scalp via a flexible nylon head cap that is appropriate to the participants head size. Onset of the NIR light stimulation will be controlled by the MEDX Console. Twenty minutes of stimulation will occur during each Set, for a total of 40 minutes NIR light application, with LEDs repositioned to a new position for each set. During the session, the 810 nm intranasal Vielight will be placed in each nostril and turned on once per session for 25 minutes (one dose) and subsequently removed.

During the actual stimulation period, participants will view nature documentaries (BBC Life documentary series; Gunton, 2009) that are shown on a monitor during the duration of stimulation. This is being done in order to standardize cognitive engagement and control for differences in cognitive activity during stimulation. This will also be done to reduce conversation between the examiners and the participant during the period of stimulation, which could differ between participants. Six episodes of the BBC Documentary will be presented in the same order for each participant, with a different episode played at each stimulation session. A brief description of each episode is provided below:

1. "Challenges of Life": This episode is the series opener and features stories of animal behavior such as feeding, hunting, and courting from around the world. Animals featured include killer whales, poison-dart frogs, and penguins.
2. "Mammals": This episode features the life of mammals from around the globe. Stories feature animals such as reindeer, polar bears, African elephants, and humpback whales.
3. "Birds": This episode features stories of birds from across the globe. Nesting, parenting, and migratory behaviors are described from animals such as humming birds, pelicans, grouses, and bowerbirds.
4. "Creatures of the Deep": This episode features an exploration of life in the deep ocean. Deep-sea creatures such as jellyfish, spider crabs, sea urchins, octopus, and coral reefs are described.
5. "Plants": This episode utilized time-lapse photography and videography to explore the behavior of plants. Featured plants include Venus flytraps, sundews, milkweeds, dragons blood trees and mangrove trees.
6. "Primates": This episode features stories of primates and the attributes that have made them a highly successful group of mammals. Animals featured include lemurs, gibbons, orangutans, and macaques.

Other episodes include "Reptiles and Amphibians", "Fish", "Insects", and "Hunters and Hunted". These videos may be played if there is reason to need an extra video.

At Home Intranasal Sessions (optional). Each participant will be loaned an intranasal device, that is identical to the one used during the laboratory session. Participants will be asked to use the intransal device for 25 minutes each day, except for days they visit the laboratory. The participants are trained to use the device during the initial lab visit and shown that the device

automatically cuts off after 25 minutes. Participants will be asked to keep a log of usage and to return the device at the completion of the study.

Equipment Sterilization

Following each session, the 6 MedX LED clusters and 2 Vielight intranasal leads will be sterilized with disinfectant wipes (PDI Super Sani-Cloth Germicidal Disposable Wipes) before storage. The manufacturer's recommendations are to clean equipment with sterilizing swabs in between sessions. In addition, a new flexible net caps will be used for each participant.

Questionnaires: Following the 40-minute stimulation period all participants will fill out the POMS short form and will be queried for adverse events (using the Adverse Events log). Completion of these questionnaires will take place at the end of each session.

6.7.4 SHAM NIR Condition and Blinding

Participants in the sham control group will undergo identical procedures as the intervention group - screening, baseline testing, and LED cluster placement procedures. We have sham devices that look similar in all respects to the active devices, both transcranial and infranasal. The sham and active devices are coded and only one of the Investigators knows the code. The research assistant administering the stimulation sessions will not be aware of the participant's treatment status, and will have no role in post-intervention assessments. The research assistant administering post-intervention testing will be blinded to the treatment status of the participant.

6.8. Optional Research MRI: Exploratory Aim

A subset of participants, from the Real and Sham Intervention groups will participate in collection of neuroimaging data, obtained before and after the intervention. This is an exploratory aim and designed to obtain pilot data. This portion of the study will be coordinated by Dr. Woods, an expert in multimodal imaging with older adults. We plan to obtain:

- structural MRI
- resting state fMRI. This will allow us to examine intervention changes in functional connectivity.
- Blood flow. This will allow us to examine changes in brain perfusion
- spectroscopy data using a newly acquired MRI/S head coil. The latter will allow us to assess for region-specific changes in phosphorous MRS-based markers of ATP function, one of the presumed mechanisms of NIR stimulation.

After giving informed consent and undergoing the appropriate prescreening measures, eligible participants will receive pre-intervention and post-intervention brain scans at the McKnight Brain Institute on the AMRIS 3T Philips scanner utilizing a dual tuned 31P-1H MRS coil. Structural MRI scans are also acquired as part of this scanning protocol. Inside the brain scanner, participants will like on a padded table with foam pads used to hold the participants head in place. During this portion of the study, participants will be asked to lie as still as possible. There will be no specific task or behavior that the participant must engage in. We anticipate that this protocol will take 1 hour. Specifics are shown below.

Neuroimaging Methods. We will conduct neuroimaging on a 3.0 Tesla Philips research dedicated scanners with a research agreement supporting all scanner sequences. We will use a Rapid MR International dual tuned 31P-1H MRI/S head coil for data acquisition.

MRI protocol. The scanning sequences will take 1.5 hour to acquire: 1) Structural MRI (MPRAGE), 2) Phosphorous MR Spectroscopy (31P-MRS) and 3) FMRI (EPI-BOLD).

Structural MRI. Whole brain axial gradient-echo MPRAGE T1-weighted images will be acquired for 31P and FMRI localization (TE/TR 3.57/2730ms, flip angle = 7 degrees, slice thickness=1mm, Gap=0 (contiguous slices), FOV=25.6cm, 256×256 matrix size & 2.0×2.0mm resolution; 5 minutes duration). 2D/3D FLAIR imaging will be acquired, time allowing within the 1.5 hour scan, to assess white matter hyper-intensity load (~6 minutes duration). High resolution hippocampal imaging will be performed, time allowing within the 1.5 hour scan, to allow localization of functional connectivity analysis seeds in the hippocampus and frontal regions and assessment of possible structural improvements related to intervention (~5 minutes duration). 3D PASL sequence (TE/TR=10.5/4885ms, PLDelay=2000ms, 1.9x1.9x4mm resolution, FOV = 240 x 240 x 160mm) will enable quantification of changes in cerebral perfusion related to intervention. Data will be analyzed using AFNI to compute regional and whole brain estimates of resting cerebral perfusion pre and post intervention.

Phosphorous MRS. A 31P-MRS pulse-acquired sequence will be acquired from two 6-cm^3 voxels centered in prefrontal cortex and temporal cortex to assess region-specific change in phosphorous MRS-based markers of ATP function and brain health. Parameters: TE 0.1ms; TR 4500ms; Spectral resolution 2.4 Hz/point; Spectral bandwidth 3000 Hz; 7 minutes duration). Analyses: Spectra will be analyzed using Tarquin to give concentrations for the following phosphorous metabolites: nucleoside di- and tri-phosphate (gamma, alpha, beta-NTP), phosphoethanolamine (PEtn), phosphocholine (PCho), glycerophosphoethanolamine (GPEtn), glycerophosphocholine (GPCho), 2,3 diphospho-glyceride (DPG), inorganic-phosphate (Pi), membrane-bound phospholipid (MP), and phosphocreatine (PCr). The signal amplitudes of brain tissue and CSF will be derived (corrected for T2 decay), with brain water signal used to correct for partial CSF volume. MRS Quality Assurance: The concentration measures for the metabolites will be accepted with Cramer-Rao lower bounds (%SD) of less than 20%, a reliable estimate for a particular metabolite for group comparisons. Scans resulting in spectra that do not meet quality criteria will be repeated. Line width and signal-to-noise resonance ratios are plotted over time (brain, phantom) to monitor spectral quality; inter- and intra-subject variability assessed by correlation and Bland and Altman plots.

fMRI. We will perform an fMRI resting state block using echoplanar BOLD imaging (EPI) methods, with a TR of 2500ms, TE 16ms, field of view = 192^2mm, matrix = 64^2 and voxel size of $3.5mm^3$. FMRI processing and analyses will use Statistical Parametric Mapping 12 (SPM12). We will present one ten minute block of resting state FMRI before and after intervention to provide insight into stimulation related change in functional networks. We will construct time series datasets implementing preprocessing methods to minimize physiological and motion artifact, with each volume assigned a condition based on task sequence. Whole-brain voxel-wise multiple regression analysis will verify activation patterns in resting state networks. Appropriate covariates, such as movement, for each participant's brain voxels will be included using the variation in BOLD signal over time. The REST toolbox and local specialized software will be used to obtain total interdependence (TI) values to assess functional connectivity between nodes in the resting state network, with particular focus on change in hippocampal connectivity. Primary dependent measures

will TI between each resting state ROI's per participant (FWE threshold p<.05). TI values will compare intervention-related changes in functional connectivity.

Shown below is an overview of the temporal overview of the procedures.

Table 2: Overview of Procedures and Protocol

Measures	Pre-sceen	Screening	Base-line	Pre-MRI*	Intervention	Follow-up	Post-MRI*
Telephone Screening	X	X					
Inclusion / Exclusion		X					
Informed Consent		X					
Demographic Info		X					
Medical History		X					
MoCA or DRS-2		X					
HVLT-R or WMS-III LM		X				X	
Mental Health Screen V.3		X					
Charleson		X					
Medications		X					
WTAR or WRAT-IV		X					
Vision Screen		X					
Digit Span (WIT)		X					
TrailMaking Test			X			X	
Wisconsin Card Sort			x			x	
Digit Symbol (WIT)			X			X	
Category Fluency		X					
JLO		X					
Functional Activity Questionnaire (FAQ)		X					
Cognitive Function Inventory (CFI)		X				x	
Cognitive Function Inventory –*Study Partner (not PD)*		X					
Clinical Dementia Rating Scale -*Study Partner (MCI group only)*		X					
Demographic Info - *Study Partner (Not PD)*		X					
MRI Screener**		X*		X*			X*
Cognitive Change Index (CCI)			X			X	

Skintype Questionnaire			X				
Arena Task			X			X	
AVTL			X			X	
MST			X			X	
NIH Examiner			X			X	
Stroop			X			X	
CalCap (processing speed)			X			X	
UPDRS (PD only)			X			X	
Grooved Pegboard (PD only)			X			X	
Walking-Balance (PD only)			X			X	
BDI-II			X			X	
STAI			X			X	
Apathy Scale			X			X	
NIH Toolbox Emotion			X			X	
POMS			X		X	X	
Fatigue Severity Scale			X			X	
Pittsburgh Sleep Quality Index			X			X	
Pain Rating Scale			X			X	
Adverse Events Log					X	X	
Expectation Quest Placebo Control Quest			X			X	

*MRI, only a subset of participants will be asked to undergo pre and post-MRI; these will be screened for eligibility using a standard MRI screener**. MoCA=Montreal Cognitive Assessment; HVLT-R=Hopkins Verbal Learning Test – Revised; WMS-III LM = Wechsler Memory Scale, Logical Memory subtest; Charlson = Charleson Comorbidity Index; WTAR=Wechsler Test of Adult Reading; JLO = Judgement of Line Orientation; Skin type Questionnaire =Fitzpatrick Skin-Type Questionnaire; AVT=Auditory Verbal Learning Test; MST = Mneumonic Similarity Test; CalCap = California Computerized Assessment Package; UPDRS = Unified Parkinson Disease Rating Scale; BDI-II=Beck Depression Inventory, 2nd edition; STAI=State Trait Anxiety Inventory; POMS=Profile of Mood States, Short Form; PCQ=Placebo Control Questionnaire; ; Daily journal = completed by participant for recording time of daily intranasal treatment.

- Note that individuals with Parkinson disease will receive the Unified Parkinson Disease Rating Scale, Grooved Pegboard Test, and Gait Rite analysis before and after intervention. Other participants will not.

7 Outcome and Statistical Approaches

7.1. Outcome Measures

Aim 1 examines executive function and memory using sensitive cognitive measures.

- Co-Primary Executive Outcome: Changes in pre-post treatment scores on: a) executive composite and domain scores derived from the NIH Examiner and b) the Stroop interference score.

- Co-Primary Memory Outcomes: Changes in pre-post treatment scores on memory measures including a) delayed recall scores and composite from the Arena and b) delayed recall score from the AVLT. Secondary outcomes include other executive and memory measures as well as potential mediator and moderator variables such as processing speed.

Aim 2 examines well-being and mood variables.

- Co-Primary outcome: Changes in pre-post treatment scores on the Negative Affect and Psychological Wellbeing scales from the NIH Toolbox emotion module.
- Secondary outcomes: Changes in pre-post treatment scores on traditional mood measures including the 1) State-Trait Anxiety Inventory (STAI; Spielberg, 1989), the 2) Profile of Mood States (POMS; Curan et al., 1995), 3) Beck Depression Inventory-II (BDI-II; Beck et al., 1996), and 4) Apathy Scale.

Aim 3 is an exploratory neuroimaging aim that will examine changes in resting state functional connectivity as a function of group status (NIR stimulation vs Control). We are particularly interested in potential changes in MRS spectroscopy, particularly those elements associated with phosphorous ATP

Aim 4 pertains to motor variables that are being examined in patients with Parkinson disease

- Outcomes include: Changes in pre-post intervention scores on the Grooved Pegboard Task, scores on the UPDRS rating scale, and gait and balance measures on the GAIT-Rite scale.

7.2. Randomization and Statistical Methods

7.2.a. Randomization. Participants in the Cognitively Normal, the MCI, and Parkinson groups will be independently assigned to Sham and Treatment groups on a 1:1 ratio. For the Cognitively Normal group, we anticipate an N of 35 in each treatment group. For each MCI group, we anticipate an N of 10 per treatment group. For the PD group, we anticipate an N of 20 per treatment group. Randomization will be stratified according to age and MoCA/MMSE scores so as to prevent different average ages or cognitive status scores between the treatment groups. Treatment assignment will be pseudorandomized in order to have comparable distributions of age and cognitive screening scores across Sham and Treatment interventions. Procedures will be followed so that group assignments are not available to individuals administering and scoring baseline/pre-intervention and post-intervention cognitive/mood data.

7.2.b. Missingness and Intent to Treat.

All data will be checked for missingness, out-of-range values, and distributional form (i.e., normality, homogeneity of variance). Decisions regarding use of parametric vs. nonparametric statistics will be based on the results of those analyses. Standard summary statistics will be provided for pre- and post-stimulation. Intent to treat analysis will be used to reduce selective attrition bias; participants who are unwilling/unable to continue with intervention will be encouraged to attend the post intervention testing, if possible. Thus, statistical analyses will be performed on two data sets: 1) on all subjects who were successfully screened, randomized and able to participate in at least 1 week of the assigned intervention, regardless of study

completion (intention-to-treat analysis); and 2) on subjects who were successfully screened, randomized and able to complete at least 80% of the treatment assignment.

7.3. Statistical Analyses

Data for each group (Cognitively Normal, MCI, Parkinson) will be analyzed separately. However the same analytic approach will be taken for each, as described below. Some analyses will be truncated for the MCI groups due to the smaller sample size.

Aim 1: Aim1 will test the hypothesis that individuals in the active stimulation group will show improved cognitive function compared to the sham group. Separate mixed model ANOVAs (2 [Group: Active Stimulation, Sham stimulation] x 2 [Occasion: Pre-testing, Post-testing]) will be conducted using the executive and Memory outcome variables. When significant, appropriate post-hoc analyses will be conducted. Additional analytic techniques will involve 'change score' analyses, as well as analyses examining predictors of changes (i.e., age, education, gender).

Aim 2: Aim 2 will test the hypothesis that individuals in the active stimulation group will show improved emotion/psychological function compared to the sham group. Separate mixed model ANOVAs (2 [Group: Active Stimulation, Sham stimulation] x 2 [Occasion: Pre-testing, Post-testing]) will be conducted using the mood outcome variables (mood modules NIH Toolbox, STAI, POMS, BDI-II). When significant, appropriate post-hoc analyses will be conducted. Additional analytic techniques will involve 'change score' analyses, as well as analyses examining predictors of changes (i.e., age, education, gender).

Aim 3: This neuroimaging aim is exploratory and will test the hypothesis NIR intervention will produce pre-post increases in frontal and temporal brain markers of MRS ATP function and pre-post increases in connectivity in frontal and medial temporal lobe mediated resting state brain networks (resting state fMRI). Although this aim will require extensive data reduction, the overall data analytic plan is similar to that of Aims 1 and 2.

Aim 4: This is aim is specific to Parkinson disease and will test the hypothesis that active NIR stimulation will result in greater pre-post intervention improvement in motor parameters (UPDRS, fine motor speed, gait, balance) than sham. Separate mixed model ANOVA's will be conducted similar to those described in Aims 1 and 2.

Finally, we plan to examine whether various 'factors' might potentially be moderators or mediators to intervention success. WE plan to examine age, education, gender, health comorbidities, processing speed and mood.

8. Methodological Limitations

Only two previous human studies have conducted sham controlled trials. Thus, there is a potential impact of placebo in producing positive effects on cognition. The current study attempts to control for this. Another issue pertains to the penetrance of near infrared light through the skull. Most transcranial NIR light applied to the human cranium penetrates 1 cm into the tissue, reaching cortical tissue but not white matter or subcortical structures. As a result the dosage reaching particular brain structures depends on a variety of factors such as cranium thickness, degree of cortical atrophy, penetrance, source of light, and duration of treatment/energy density of light.

9. Study Time Line Schedule of Activities

The Schedule of Participant Activities for this study is as follows:

- **Call #1**- Telephone Prescreening to review the study details, and inclusion/exclusions.
- **Informed Consent Process and Screening Visit**- Informed Consent Process begins with the participant per UF, state and federal guidelines. Once the participant has signed the informed consent, complete medical history, inclusion/exclusion criteria, concomitant medications, and cognitive and mood questionnaires will be administered (listed in section C). If participant formally meets study criteria, then he or she is invited to participate in the intervention phase of the student consisting of baseline, NIR stimulation, and post-baseline testing.
- **Baseline Visit -** This assessment involves a series of computer based measures and other questionnaires. This visit may take approximately 3-3.5 hours. If participants undergo MRI, this will take an additional 1.5 hours.
- **NIR Invention (six in lab sessions over two weeks** - After completing baseline testing, participants will undergo a series of six interventions in the lab over a period of two weeks. The six treatment visits will be scheduled at the baseline visit to ensure availability of the participant. The treatment will be applied over the head and is detailed in section C. During each treatment visit, participants will fill out mood questionnaires such the POMS. .
- **Post-Treatment Visit**- Following the final day of stimulation, the participant will be scheduled for a post-testing assessment of cognitive and emotional function identical to the baseline visit. Participants will also fill out other questionnaires such as the adverse events and placebo control questionnaires.

10. Data Safety Monitoring Plan

The PI, Co-Is, or project coordinator will meet with each participant to review the adverse events questionnaires and assess for negative effects on the safety of participants. This questionnaire will be reviewed after every intervention session and at the follow up evaluation. As this intervention involves an infrared lamp that has been FDA approved as a non-significant risk, we do not anticipate adverse events or side effects. However, should a participant experience any adverse event of sufficient concern at any point during the intervention or at follow up, we will report to the IRB as directed. The PI and/or project coordinator will send via email a monthly report to the investigative team indicating the nature of any adverse events. If there are no reported adverse events, then an email summary will not be sent that month. The PI will take primary responsibility of the Data Safety and Monitoring Plan.

11. Data Management Plan

Data will be kept in a password protected encrypted database which can only be accessed by the investigator and her staff. All surveys and assessments will be under the direction of the Principal and 3.nvestigators who will supervise Research Assistants. Recruitment, screening, and project implementation will be coordinated by the co-investigator and/or research assistants. The existing data management group, supported by the IT department of the College of Public Health and Health Professions, will manage the database, monitor subject recruitment and distribute monthly enrollment reports, and produce reports summarizing the status of data acquisition, the baseline characteristics and the blinded primary variables.

The password protected encrypted database mentioned above is the storage place for all participants who call and wish to be pre-screened for the study. In many instances, the

prospective participant no longer wants to enroll in the study, once they learn of the time commitment and details involved in participating. At this point, they often ask if they could be contacted for other studies in the future. We will keep a column noting verbal consent to call in the future for short term, easier studies to participate in. If participants do not wish to be contacted in the future for other studies, that will be noted as well. This information will only be used by Dr. Dawn Bowers' laboratory for future research.

12. Possible Discomforts and Risks

The risks are minimal for individuals who participate in this study. Light is applied using light emitting diodes, which do not present as a serious ocular hazard. Near infrared light is non-thermal, painless, and was designated as a non-significant risk by the FDA in 2003. It is estimated that approximately 6 J/cm^2 is expected to reach the cortex with each daily treatment. At this energy level, the MedX MCT502 and Vielight 810 accessories do not cause tissue damage or physical damage.. As part of normal operation, the LED clusters may warm up during the application of light. The temperature of the MedX Clusters does not exceed 45° C (113 ° F) during operation.

There are four types of potential risks for participants in this study.

First, the completion of various screening and cognitive probes could potentially become boring or tiresome. To minimize fatigue associated with completion of screening and baseline tasks, participants will be given frequent breaks and the opportunity to rest.

Second, there is potential risk of loss of confidentiality. This will be minimized through assigning all data collection instruments a unique code without individual identifying information. All HIPAA regulations pertaining to protection of participants and eliminating identification will be followed.

Third, a subset of participants will undergo MRI. This is perhaps poses the greatest discomfort. Because the MRI scanner has a strong magnet, individuals with metal implanted in their body cannot participate. The scanner produces a loud hammering noise, which has produced hearing loss in a very small number of participants. All participants are provided with ear protection while in the scanner. There is not much room in the scanner and it can be uncomfortable to remain still for the scan. Individuals with known claustrophobia will be excluded from participating.

Fourth, it is also possible that incidental findings may emerge on the MRI. In this situation, the participant will be informed and recommendation/referrals will be made with the participant's permission. This could include provision of information to the participant's primary care physician and/or referral to another health care professional for followup.

For individuals with Parkinson disease, there is an additional potential discomfort. The PD participants may experience some increase in their symptoms (i.e., increased slowness, tremor, rigidity) when they are tested during the "off medication" state. This is similar to what occurs during their routine clinical evaluation at the Movement Disorders Clinic, when they are asked to come to their clinic appointment after being off their Parkinson medication overnight. For our research study, care will be taken to ensure that the Parkinson patients are transported by wheelchair when necessary and that they are accompanied by a member of the study team. They will be told that if they become too uncomfortable, then they should go ahead and take their medication.

It is possible that there are unknown risks for participating in this study. Individuals will be asked about side effects at each visit and encouraged to share information about subjective changes.

13. Possible Benefits

Cognitive and mood benefits cannot be guaranteed. However, it is possible that some participants may experience cognitive and mood benefits that may include improved memory, executive function, and mood. It is possible that individuals may experience some improvements solely due to expectations.

14. Conflict of Interest

No conflict of interest exists for the investigators of this protocol.

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