Title: Revitalizing Cognition in Older Adults at Risk for Alzheimer's Disease using Near Infrared Photobiomodulation

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Protocol

1. Title: Revitalizing Cognition in Older Adults at Risk for Alzheimer's Disease using Near Infrared Photobiomodulation

2. Investigators

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3. Abstract

The proposed multi-site collaborative project brings together research teams at the University of Florida (UF) and University of Arizona (UA) to test a novel, relatively low cost, low risk, and potentially high impact therapeutic intervention in older adults who are at increased risk for Alzheimer's disease. The intervention involves transcranial and intranasal delivery of near infrared (NIR) light via light emitting diodes, aka *photobiomodulation*. Prior research in cellular and animal models suggest that red and infrared light are neuroprotective and thought to improve mitochondrial function by promoting increased production of intracellular ATP. Transgenic mouse models of Alzheimer's disease demonstrate reduced beta-amyloid and neurofibrillary tangles in response to transcranial NIR versus sham stimulation. Preliminary human studies have also shown promising behavioral findings in young adults and those with TBI, aphasia, and Alzheimer's disease. From our team, pilot phosphorous magnetic resonance spectroscopy (31P MRS) and cognitive data in older adults support this mechanism of action and provide compelling evidence for a Phase II clinical trial. To more fully determine whether this novel stimulation approach has potential for enhancing cognition in cognitively normal but "at risk" individuals for Alzheimer's disease, we plan to conduct a multi-site double blinded randomized sham-controlled Phase II clinical trial. Our overall hypothesis is that exposure to NIR stimulation will have beneficial effects on brain health via influence on mitochondrial function as measured by changes in 31P MRS-based markers of ATP, neural network changes in functional connectivity (rs-fMRI), and improved cognitive performance. To test this hypothesis, we plan to randomize 168 older adults with subjective cognitive complaints, and a first-degree family history of Alzheimer's disease to sham or real treatment groups and evaluate neuroimaging and cognitive outcome measures, before and after a 12-week intervention involving transcranial and intranasal NIR-PBM. The protocol will involve "lab" and "home" sessions, and a 3 month post-intervention follow-up. This trial will determine: 1) whether NIR stimulation, relative to sham, improves performance on memory and executive tasks sensitive to hippocampal and frontal brain function in older adults with increased risk for Alzheimer's disease; 2) whether NIR stimulation, relative to sham, enhances brain function and connectivity measured by changes in MRS phosphorous ATP and resting state functional connectivity; and 3) how differences in demographic, neuroimaging, and Alzheimer-related risk factors influence the brain response to NIR stimulation versus sham in older adults with increased risk for Alzheimer's disease. Results will provide key insights into whether this novel NIR intervention can enhance cognition in older adults with increased risk for Alzheimer's disease and will provide the necessary data for a future Phase III randomized clinical trial.

4. Background

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, delivery of near-infrared (NIR) stimulation.

By history, current nonsurgical device approaches to brain stimulation have primarily involved magnetic (e.g., transcranial magnetic stimulation [TMS]) or electrical stimulation (e.g., transcranial direct current stimulation [tDCS]), which alter synaptic firing rates and influence circuitry long after the stimulation is removed. Instead of directly modulating neural networks per se, NIR stimulation appears to facilitate a supportive milieu via optimizing mitochondrial function (Freitas et al., 2018; Karu et al., 2010; Gonzalez-Lima et al., 2014). As such, it is viewed as a nonspecific and more general mechanism that has the potential to induce benefits for several neurological disorders, including Alzheimer's disease, Parkinson's disease, and TBI (Johnstone et al., 2016: Hamblin, 2016). Its influence on neuronal connectivity is thought to be indirect rather than direct. Different terms have been used to reflect this form of stimulation, including low level *laser* therapy (LLLT), low level light therapy (LLLT), and most recently photobiomodulation, or PBM (Anders et al.2015). In the current proposal, we use **the terms NIR** and **"near infrared-Photobiomodulation (NIR-PBM)**".

Historically, the beneficial effect of NIR-PBM was discovered serendipitously in the 1960's when application of low level laser light was noted to stimulate wound healing and improve hair growth on the backs of shaven mice (Mester et al., 1967, 1972); for review, see Hamblin (2016), Rojas et al. (2012). The FDA originally approved NIR technology for humans in 2003 and currently it is approved for treatment of musculoskeletal conditions (e.g., wound healing, muscle injury, pain, arthritis). Current studies of NIR stimulation use lasers, light emitting diodes (LEDs), or **superluminous diodes** (SLD), an LED variant that produces stronger light; the latter approach (SLD) is planned for the current proposal. In humans, delivery is typically **transcranial** (over the scalp) and/or via diodes inserted into the nose (i.e., **intranasal**). Intranasal stimulation is thought to provide more direct access to ventral, limbic, and subcortical brain regions (Pitzschke, 2015). Positive outcomes have been found with both focal, high energy light (lasers) and more widely distributed lower intensity light with superluminous LEDs (Chung et al., 2012). The critical parameters leading to efficacy appear related to wavelength, power density, energy density, and total energy (Hamblin, 2016).

Mechanism of NIR Stimulation. Evidence from cellular and animal research suggests that application of light in red (630-700 nm) and NIR wavelengths (808-904nm) improves mitochondrial function by promoting increased production of intracellular ATP (Wang et al., 2016; Freitas & Hamblin, 2018). It does so by triggering photon absorption by *cytochrome oxidase (CCO*), the

terminal enzyme in the mitochondrial transport chain that plays a key role in neuronal oxygen utilization for energy metabolism (Wong-Riley et al., 2005). In turn, this leads to: 1) increased intracellular levels of ATP (Mochizuki-Oda et al., 202; Wong-Riley et al., 2005; Oron et al., 2007); 2) increased expression of genes supporting cell proliferation and mitochondrial energy metabolism; 3) increased blood oxygenation and blood flow (Chung et al., 2012; Tian et al., 2016); 4) decreased expression of genes for pro-inflammatory proteins such as interleukin-1, interleukin10, and cytokine receptors Chen et al., 2014; Whelan et al., 2008); and 5) up-regulation of anti-oxidant genes (Huang et al., 2009). Recently, Wang et al. (2016, 2017), using broadband near-infrared spectroscopy, provided the first evidence in humans that exposure to NIR stimulation upregulated oxidized CCO and increased oxygenated hemoglobin in the human brain. Others have described neuroimaging changes in functional connectivity in a stroke patient who underwent NIR-PBM.

Relevance to Alzheimer's Disease. The central view of Alzheimer's disease pathogenesis has focused on the amyloid-tau cascade hypothesis (Hardy et al., 1992, 2002), but with some additional views implicating alternative mechanisms, including prions (Jaunmukatane et al., 2015) and vasculopathy (de la Torre et al., 2004). Recently, there has been a resurgence of interest in mitochondrial dysfunction as a potentially important feature of AD pathogenesis (Swerdlow et al., 2004, 2014). If mitochondria become damaged or dysfunctional, potentially due to aging or other stressors, their efficacy and ATP yield becomes reduced. In turn, this can lead to increases in toxic reactive oxygen species (ROS), generating oxidative stress and subsequent neuronal death, as has been observed in AD (Wang et al., 2016; Hauptmann et al., 2009; Moreira et al., 2018; Cheng et al., 2018). Whereas mitochondrial dysfunction has been implicated in many neurodegenerative diseases, including AD, it is unclear whether it is a *cause, a mediator, or an effect of* other underlying pathology (Swerdlow, 2018). ***Regardless of whether mitochondrial dysfunction is a trigger or just an effect of Alzheimer's disease, reducing mitochondrial dysfunction as part of developing Alzheimer's disease pathology could be an important therapeutic target.*** Given the observed mechanism of action in improving mitochondrial respiration, NIR stimulation has great potential as a promising intervention for exerting disease-modifying effects.

NIR Stimulation as an Intervention: Animal and Human Studies. Recent animal and human studies provide initial support for benefits of NIR-PBM on brain and cognitive function. Across these studies, a key mitigating factor is ***penetrance of NIR signal through the skull***. When applied transcranially, red and NIR light penetrate the skull up to 1-3 cm, with approximately 2-3% reaching the cortex (Rojas et al., 2012; Nawashiro et al., 2012; Wan et al., 1981; Jagdeo et al., 2012; Haeussinger et al., 2011). Lapchak et al. (2015) compared NIR transmission through skulls of 4 different species, and observed an inverse relationship between skull thickness and penetrance. In humans, penetrance correlated with skull thickness, but not density. While transcranially applied NIR light can reach cortex, it may be less useful for directly targeting white matter or subcortical structures, which may be influenced indirectly via afferent and efferent connectivity.

Animal NIR Studies. Daily exposure to transcranial NIR light delivered via LED over 20 days was found to significantly reduce beta-amyloid load (APP/PSI line) and neurofibrillary tangles (K3 line) in transgenic Alzheimer's disease mice who received treatment but not sham (Purushothuman et al., 2014). In the K3 line, histochemical analyses not only showed reduction in neurofibrillary tangles, but also reduction in hyperphosphorylated tau and oxidative stress markers in cortex and hippocampus along with increased cytochrome c oxidase activity. DeTobaoda et al. (2011) reported similar pathologic findings in a transgenic APP mouse model following stimulation over a 3-month

period. Importantly, there was corresponding improvement in behavior/cognition in NIR-exposed mice on the **Morris Water Maze**, a widely used behavioral learning task. This finding ***directly links to our pilot data and to Aim 1 of our proposal,*** in which our primary cognitive outcome, ARENA, is a **direct human analogue to the Morris Water Maze** (Thomas et al., 2001; Laczo et al., 2010).

Human NIR Studies. At least 5 studies have been conducted in ***young healthy adults***. Most have used brief (8 minute) single doses of laser-delivered NIR light over frontal brain regions. Relative to sham, single doses of NIR stimulation (808nm) have resulted in improved attention and memory (Barrett et al., 2013), fewer errors and improved set-shifting on the Wisconsin Card Sort Test (Blanco et al., 2010, and better prefrontal 'rule based' learning (Blanco et al., 2017). Mood-related changes have also been described (Farfara et al., 2015; Disner et al., 2016; Henderson et al., 2017). In ***clinical populations***, beneficial effects of NIR-PBM have been observed in individuals with *chronic aphasia* due to focal stroke (Naeser, 2013; Gazova et al., 2012), with *TBI* who underwent a six-week intervention (Naeser et al., 2014, 2011), and in a small group of individuals with *major depression* (Henderson et al., 2017; Schiffer et al., 2009). Effect sizes on memory and executive tasks have ranged from modest to large. Importantly, none of these studies have been designed as RCT's, with most being single case studies or case series. The exception was a study of individuals with acute stroke, who were seen in an Emergency Department (Lampl et al., 2007; Zivin et al., 2009; Hacke et al., 2014). These studies, however, were limited by using a low sensitivity yes-no outcome and a single dose of NIR stimulation. Most importantly, the nature of stroke differs dramatically from the older adults we are targeting in the current study.

NIR Stimulation for Alzheimer's disease. To date, there have been ***no published RCTs on the efficacy of NIR-PBM stimulation in those at risk for Alzheimer's disease*****.** A study by Saltmarche et al. (2017) was limited to a **case series of 5 individuals with probable AD** receiving transcranial and intranasal NIR-PBM over 12 weeks of active treatment, followed by a 4-week withdrawal period. Immediate post-testing after 12-weeks of intervention revealed significantly higher scores on the Mini-Mental State Exam and Alzheimer's Disease Assessment Scale (ADAS-Cog), as well as improved functional behaviors. With discontinuation of NIR-PBM, all participants showed significant decline. In a second study, **independently living older adults** (N=12, ages 49-90 yrs) received weekly stimulation (1064 nm, laser, 8 minutes duration) for 5 weeks (Vargas et al., 2017). Findings were significant for pre-post intervention changes in reaction time, memory, and resting state EEG, and a more efficient fMRI response over the frontal brain region. Critically, **neither study with older adults included a sham control group.**

Pilot Data from UF. We recently completed a small well-controlled proof of concept pilot study to determine whether NIR-PBM might benefit cognition and neuroimaging. Our pilot was randomized, double-blinded, sham controlled and designed to derive effect sizes. It involved transcranial (116 MedX Console) and intranasal (Vielight Inc.) delivery of active or sham NIR light using methods described by Naeser et al. (2014) Participants completing this initial pilot study included 13 healthy community residing older adults in their mid-70's who were randomized to Active (N=8) or Sham groups (n=5). The intervention involved six 40-minute sessions, given 3 times a week over a 2-week period. The Active and Sham conditions were identical with the exception that no actual NIR light was delivered during Sham. For both conditions, six transcranial superluminous clusters were placed on the scalp*, following the 10-20 EEG system*, targeting bilateral frontal, temporal, parietal and central sites. Intranasal diodes were used to target inferior frontal and limbic regions. Cognitive/behavioral outcomes were obtained before and immediately after intervention.

The cognitive/behavioral outcomes were *measures* of memory, executive function, and emotion. Executive function was tested using components of the NIH Examiner (Kramer et al., 2014), a series of computer-based executive function tasks. Episodic memory was tested using a spatial learning task, ARENA (Thomas et al., 2001), that is associated with hippocampal function and MCI (Laczo et al., 2010, 2011, 2012; Hort et al., 2007, and is a virtual human analogue of the Morris Water Maze (Hort et al., 2007; Gazova et al., 2012). Emotion was tested using subtests from the Emotion module of the NIH Toolbox (Gershon et al., 2013). A subset of participants underwent brain imaging including 31P MRS to index mitochondrial function (i.e., ATP) and resting state fMRI to index changes in functional connectivity pre-post NRI stimulation. All outcome measures were administered by individuals who were blind to group assignment.

The focus of this small pilot was to determine **effect sizes** for our power calculations. We initially calculated pre-post change scores for the outcome measures and computed effect size differences using Cohen's *d*. As shown in **Fig. 1**, large effect sizes were observed for memory (ARENA), Negative Affect, and rs-fMRI measures. Medium effect sizes were found for the executive function measures (working memory, fluency), psychological well-being, and ATP changes. Comparable effect sizes resulted when data were analyzed using a repeated measure analysis of variance (ANOVA). ***Importantly***, the correlations between the emotion and memory/executive measures were not significant, suggesting that 'mood' was not driving the cognitive changes. There was no long-term follow-up for this pilot study.

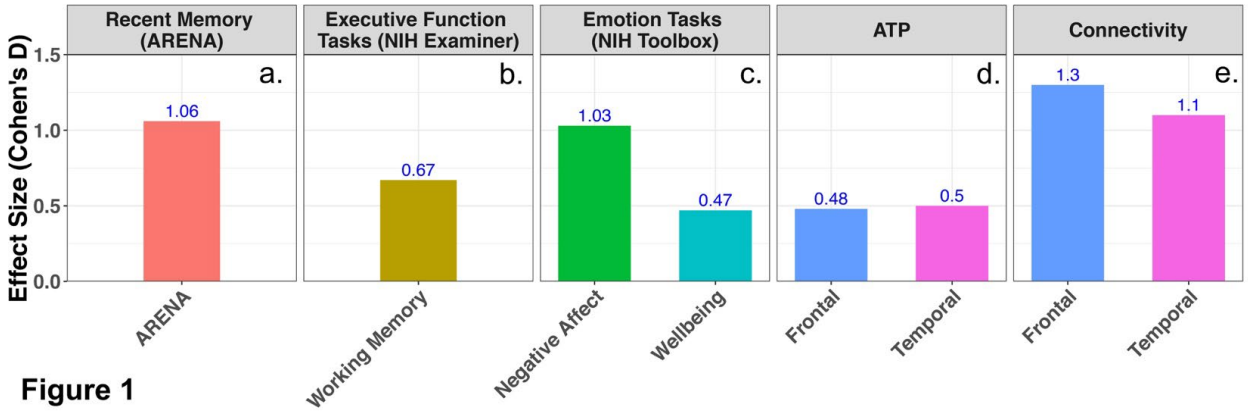
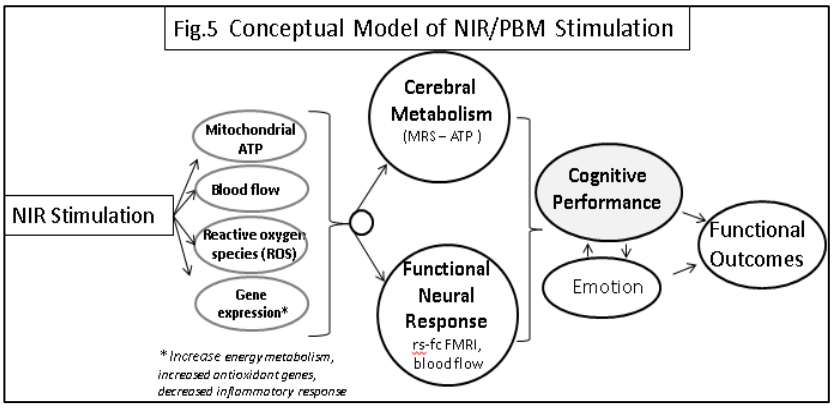


Figure 1

5. Summary

Our pilot findings provide compelling evidence to move forward with a stringently controlled Phase II clinical trial. The intervention, NIR-PBM, holds promise in light of its presumed mechanism of change (mitochondrial, blood flow, gene expression), the extensive research in animal models of neurodegeneration (e.g., Alzheimer's disease, suggesting NIR may be neuroprotective against brain 'insult', and a growing literature on mitochondrial function in relationship to AD). The conceptual

model for this intervention is shown in the figure to the right. It depicts the proposed influence of NIR stimulation at the cellular level, including increased mitochondrial function, improved blood oxygenation, and upregulation of gene expression. In turn, these cellular changes are presumed to affect cerebral metabolism and functional neural responses. In our proposed study, this will be assessed by changes in neuroimaging outcomes, namely 31P MRS indices of ATP and indices of resting state functional connectivity in frontal and temporal lobes. We anticipate improvement in cognitive functions mediated by neural networks involved in episodic memory (temporal) and executive (frontal) functions. Thus, an intervention that is potentially neuroprotective or delays onset/trajectory of Alzheimer's disease related changes could have critically important implications for quality of life and wellbeing of older adults.



Our ***overall hypothesis*** is that exposure to NIR stimulation will have positive effects on brain health via influence on mitochondrial function as measured by changes in MRS-based markers of ATP, neural network changes (rs-fMRI), and improved cognitive performance. To test this hypothesis, we plan to conduct a multi-site intervention at the University of Florida and University of Arizona. We will randomize 168 cognitively unimpaired but "at risk" older adults with subjective cognitive complaints and a family history of Alzheimer's disease to 12 weeks of sham or real treatment and evaluate 31P MRS, rs-fMRI functional connectivity, and cognitive outcomes before, immediately after intervention, and 3 months later.

6. Specific Aims

Aim 1: To evaluate whether NIR-PBM stimulation can improve cognitive function in older adults with increased risk for Alzheimer's disease. Specifically, we plan to test whether 12 weeks of NIR stimulation, relative to sham, improves performance on memory (primary outcome) and executive function tasks sensitive to hippocampal and frontal brain function in older adults with increased risk for Alzheimer's disease. We hypothesize that NIR stimulation will result in greater improvement on tasks of H1.1) episodic memory and H1.2) executive function relative to sham. The primary outcome measure will be a spatial navigation task (ARENA), a human analogue to the Morris Water Maze[17], that is sensitive to hippocampal function. A secondary cognitive outcome measure will be the executive function working memory composite score from the NIH Examiner battery known to be sensitive to frontal brain function.

Aim 2: To evaluate whether NIR-PBM intervention enhances brain function and connectivity in those at increased risk for Alzheimer's disease. We plan to test whether 12 weeks of NIR stimulation leads to enhanced markers of 31-P MRS ATP and rs-fMRI functional connectivity in older adults with increased risk for Alzheimer's disease. We hypothesize that: H2.1) NIR stimulation will produce pre-post increases in frontal and medial temporal brain markers of MRS ATP function and

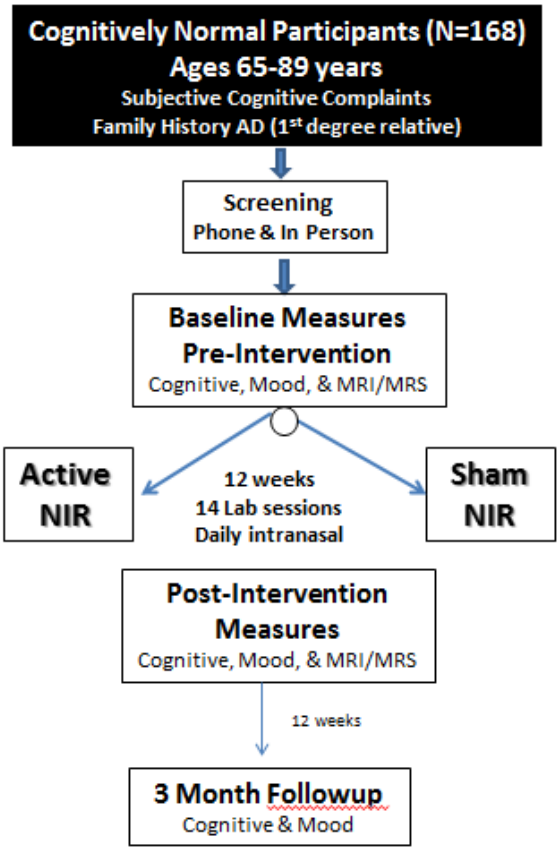
H2.2) pre-post increases in functional connectivity in frontal and medial temporal lobe mediated resting state brain networks.

Exploratory Aim: To examine how baseline demographic, mood, vascular risk, genetic, and neuroimaging factors influence individual differences in cognitive outcome for NIR-PBM intervention. We plan to evaluate how baseline MRI white matter abnormalities, apolipoprotein E (APOE) ε4 status, and metabolic/vascular risk factors of hypertension and diabetes influence cognitive outcomes after 12 weeks of NIR stimulation. Additional factors include demographic (age, education, gender), mood status, and general processing efficiency. For this exploratory aim, we hypothesize that: HE.1) greater white matter lesion load on FLAIR MRI, HE.2) metabolic-vascular risk factors of hypertension and diabetes, and HE.3) APOE ε4 status will each predict poorer NIR-PBM outcomes in memory and executive function.

7. Research Plan

7.1 Design Overview.

This is a blinded sham-controlled parallel group multi-site clinical trial where nondemented older adults will be randomized to active or sham treatment groups. The intervention phase will extend over 12 weeks and includes transcranial and intranasal stimulation that has "lab" and "home" components. The lab component includes weekly transcranial + intranasal NIR-PBM sessions, whereas the 'at home' protocol involves daily intranasal stimulation. The devices used to deliver "real" and "sham" stimulation are identical in all respects to that of our pilot study. The use of a sham design controls for the effects of placebo, activation, and practice.



Our participants will include cognitively unimpaired individuals with **"subjective" cognitive complaints** and a **family history of Alzheimer's disease** (first degree relatives). We have selected these criteria based on a growing literature that: a) subjective cognitive decline (SCD) is linked to increased AD biomarker abnormalities and increased risk for progressing to MCI and dementia (Saykin et al., 2005; Jessen et al., 2014; Kielb et al., 2017; Kryscio et al., 2014; Colijn et al., 2015; Lista et al., 2015; Contreras et al., 2017; Risacher et al., 2015; Swinford et al., 2018).; and b) SCD combined with a family history of Alzheimer's disease may further heighten AD risk, with findings of resting state patterns that mirror early AD (Verfaellie et al., 2018). We expect that these additive risk factors result in greater vulnerability for cognitive decline, representing an 'early' preclinical AD group who would benefit from intervention

Participants will be tested at 3 primary time points: 1) pre-intervention; 2) immediately post-intervention; and 3) three months later. At each time point, we will obtain cognitive (memory,

executive function) and emotion measures. Metabolic, functional and structural neuroimaging measures will be obtained at the pre and post-intervention time points. APOE status and health histories will be ascertained. *Our outcomes* center on our specific aims. Aim 1 tests improvement in cognition before and after the 12-week NIR intervention, whereas Aim 2 assesses neuroimaging changes pre- and post-intervention. **Aim 3** focuses on predictors and moderators of the intervention response**. The primary outcome** for Aim 1, as well as the **overall trial primary outcome**, is ARENA, a spatial memory task that is a human analogue to the Morris Water Maze. **Aim 2 neuroimaging outcome** involves changes in 31-P MRS indicators of ATP function and resting state connectivity. Additional secondary and exploratory outcomes are associated with each aim.

7.2 Participants

Participants will include 168 older adults with **subjective cognitive complaints** and a first degree family history of Alzheimer's disease. Study participants will be randomized to Active or Sham groups using a 1:1 randomization scheme, stratified by age, education, and gender. We hope to recruit equal numbers of men and women. Half the participants will be enrolled at the University of Florida and half at the University of Arizona. Because each participant will have an informant, this means we will need 336 individuals to sign consen formst (i.e., 168 X 2 [participant plus informant]). Due to estimated potential screen failures (up to 350) and drop outs (i.e., 100), we plan to consent up to 768 individuals who meet inclusion-exclusion criteria. One half the participants will be enrolled at the University of Florida and ½ at the University of Arizona).

Inclusion Criteria

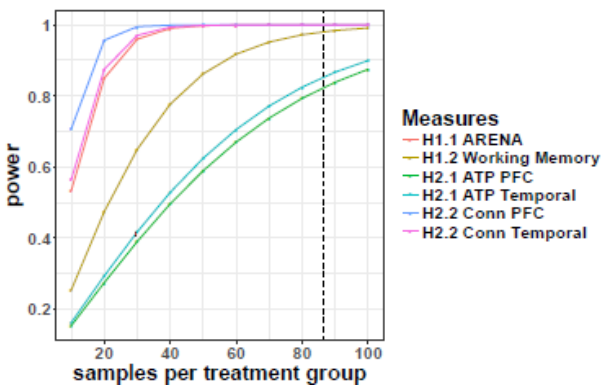
- Age 65-89 years, at least 8th grade education, community dwelling
- Subjective report of cognitive complaints with scores $\geq$16 on the **Cognitive Change Index** (CCI-20), a validated scale of subjective cognitive decline (Saykin et al., 2013).
- No evidence of dementia or mild cognitive impairment based on cognitive screening (e.g., Montreal Cognitive Assessment (MoCA) score is within normal limits for age, education and sex.)
- No psychometric evidence of cognitive impairment based on performance on the Neuropsychological Battery from the National Alzheimer's Coordinating Center Unified Data Set (NACC-UDS), version 3 (Weintraub et al., 2018)
- Reading at $\geq$ 8th grade level based on the reading subtest of the Wechsler Test of Adult Reading (WTAR).
- Global Clinic Dementia Rating (CDR) score must be 0 (Hughes et al.)
- Family history of dementia/probable Alzheimer's disease in first degree relative (parents, children, siblings)
- Willingness to be randomized to Sham or Active Intervention
- Can devote 12 weeks to the intervention with additional time for pre and post testing
- Normal functional behavior in terms of daily activities, based on the Functional Activities Questionnaire from the NACC-UDS
- Able to perform cognitive and emotion measures on a computer
- Availability of an informant (family member, colleague, friend) to provide information about activities of daily living and cognitive complaints (see Molienueva et al., 2017).

Exclusion Criteria

- Sensory loss (vision, hearing) or motor deficits that would preclude participation in the experimental tasks or neuropsychological assessment
- English as a second language or Non-English speaking
- Inability to undergo brain imaging due to claustrophobia or implants such as pacemakers, heart valves, brain aneurysm clips, orthodontics, non-removable body jewelry, or shrapnel containing ferromagnetic metal
- Previous major strokes or other known significant brain abnormalities or diseases affecting the brain and/or cognition (e.g., Parkinson disease, multiple sclerosis, seizure disorder, brain surgery, moderate TBI, REM Behavior Sleep Disorder, untreated sleep apnea, etc.)
- Unstable and uncontrolled medical conditions (metastatic cancer, HIV, moderate-severe kidney disease, uncontrolled diabetes, uncontrolled hypertension, severe cardiac disease, etc.). No current cancer diagnosis
- 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. Scores > 20 on the Beck Depression Inventory-II. We are not excluding individuals who are taking antidepressant or anti-anxiety medications. However, use of these medications will be recorded and data analyzed in post-hoc analyses
- Use of antipsychotics, sedatives, or other medications with significant anticholinergic properties (due to potential influence on memory)
- Use of prescribed 'memory enhancing' medications such as Aricept or Namenda
- Use of photo-sensitive medications such as steroids or retin-A within 15 days of the study intervention.
- Previous participation in a cognitive training study within the last 6 months or current involvement in another study involving cognitive, physical or other intervention at the time of participation.

*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. Dr. Bowers, at the UF site, is a boarded clinical neuropsychologist who has been working with this clinical population for over 30 years. Two Co-I's (Dekosky, Weisbrod) are experienced neurology clinicians. All are experienced with diagnosis and treatment of mood and cognitive disorders. Similar clinical expertise exists at the University of Arizona. 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. Specifics regarding how we address suicidality is described on page 19 of this protocol.

Sample Size, Attrition, Power, and Intent to Treat. Our planned sample size includes 168 cognitively normal individuals (N=84 Active/N=84 Sham intervention). This sample size was determined directly from the effect sizes of our pilot data. Importantly, the study was powered to assess hypotheses proposed in Specific Aims 1 and 2, with 80% power. The vertical dash line in the figure to right shows the projected point at which 80% power will be achieved at p=0.05, two



tailed, relative to a sample size of the two treatment groups derived from the effect sizes of our pilot data. Based on our previous intervention studies with older adults, we anticipate a 30% attrition rate and up to 82 screen failures.

To account for screen failures and study attrition, we anticipate consenting up to 300 individuals. * Intent to treat analysis will be used to reduce selective attrition bias; participants who are unwilling/unable to complete the intervention will be encouraged to attend the post intervention testing. *(With informants, the number of involved individuals is doubled.)

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 may be sent a copy of the Informed Consent via regular mail or via email if they are interested, and/or scheduled for an appointment at the McKnight Brain Institute or the Fixel Center for Neurologic Diseases to discuss the study in more detail and in person.

Other potential participants, particularly those seen during UF clinic visits with Drs. Weisbrod or Bowers, will be directly asked about their interest in this study and will be provided a copy of the informed consent to review in person or via mail or email.

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.

7.3 Recruitment

We have considerable experience in conducting study recruitment with community living older adults for both research and clinical studies including an array of interventions (i.e., exercise, cognitive training, mindfulness, tDCS) with cognitive and neuroimaging outcomes. These studies have been funded by NIH, the McKnight Brain Research Foundation, the State of Florida, the State of Arizona, and private foundations. We plan to implement strategies we have successfully used in our other studies with older adults.

To recruit participants from the community we will use the following strategies: 1) Place IRB approved advertisements in newspapers, magazines, church bulletins, and newsletters; 2) Place IRB approved brochures and fliers in areas frequented by adults in the target age range (e.g., doctor's offices, senior centers, retirement communities); 3) Mail IRB-approved recruitment post-cards to households with age-eligible residents and to individuals who might have age-eligible parents; 4) Contact physicians, physical therapists, and other health care professionals about this study; 5) Participate in health fairs; 6) Make presentations at senior centers, community centers, retirement communities, and organizations that provide services or advocate for older adults (e.g., local area agencies on aging, AARP offices).

At UF, additional recruitment may occur through Dr. Bowers or Dr. Weisbrod's UF clinics or other UF clinics focused on older adults (via flyers). These clinics focus on older adults, and potential participants will be directly asked about their interest in learning about this study. If so, they will be provided IRB approved recruitment materials in person, with a subsequent follow-up phone call and/or mailing/emailing of a consent form for them to review.

Another recruitment strategy at UF will involve use of the Integrated Data Repository (IDR). Patients who meet criteria for this study may be identified via the IDR and those who are participants in Consent2Share may be sent a recruitment letter along with IRB approved flyers. Additionally, flyers will be distributed to other physicians and psychologists at the Fixel Center, UF neuropsychology, and practices at the University of Florida where patients can be asked about their potential interest in learning more about this study.

Finally, recruitment may occur via IRB approved clinical and research databases. Thus, recruitment of normal older adults at UF may take place via the Cognitive Aging and Memory/Clinical Translational Research Program Consent to Contact Participant Registry (IRB#201500568).

7.4. 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 and the Neuropsychological Battery from the National Alzheimer's Coordinating Center Unified Data Set (NACC/UDS), version 3. This battery (Weintraub et al., 2018) consists of tests that are sensitive to cognitive aging and AD, including measures of attention, episodic memory (verbal, visual), language (naming, fluency), executive function and processing speed. Specific tests are depicted in the Table below.

Dementia Screen	Montreal Cognitive Assessment (MoCA) - Widely used screening measure for dementia; total of 30 possible points
	Clinical Dementia Rating Scale - Scale for staging dementia based on information from informant; Participants must be rated "0"
Subjective Cognitive Decline	Cognitive Change Index (CCI) - Well validated 20 item questionnaire that assesses self-perception of cognitive decline using 5 point likert scale; must score $\geq$ 16 to be classified as having subjective cognitive decline. Domains that are covered include multi-tasking, learning new things, recalling old memories, thinking quickly, etc. Includes participant and informant versions
Cognitive Screen	Neuropsychological Battery (NACC/UDS, version 3) - Participants must score within normal limits on this battery which includes measures of: a) attention span (Number Span); b) episodic memory (Craft Story, Benson design) ; c) confrontation naming (Multilingual Naming Test); d) verbal fluency (Category & Letter); and e) executive function (Trailmaking Test)
Reading Literacy	Wechsler Test of Adult Reading (WTAR) - This single word reading measure requires participants to read aloud and provides estimate of reading grade level and premorbid IQ. It also enables us to assess ability to read 14 font words.
Medical	Background medical history- Goal is to rule out medical conditions (i.e., neurodegenerative, TBI, etc.),

	List of Medications - Stable medications for 3 months; rule out use of anticholinergic medications (including computation of Magellan Risk score); rule out use of photo-sensitive medications and steroids within 15 days of intervention
	Charlson Comorbidity - Assesses for variety of comorbid medical conditions, computes risk factor score, derived from background medical history
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
Daily Activities	Functional Activities Questionnaire - this 10 item scale measures independent activities of daily living and is part of the NACC/Unified Data Set
Vision	Vision & Color Vision Screening – this involves reading words printed in 14 point font; color vision will be tested using Ishihara color plates and a color discrimination task.

Informant. Information about the participant's overall cognitive and functional status will be obtained from a spouse, family member, friend, or another person who knows the participant. The "informant" will answer questions regarding cognitive changes they have noticed over the past 5 years, along with changes in the participant's ability to perform daily activities. Specific informant versions of the following measures will be administered, as described in the table above: Clinical Dementia Rating Scale (CDR), Functional Activities Questionnaire, (FAQ) and Cognitive Complaints Index (CCI-informant version). We will collect information pertaining to the informant's age, education, gender, nature and duration of the relationship, and frequency of interactions. This information along with the information from questionnaires will be linked to the participant. This information may be collected in person, via telephone and/or via mail survey. Once collected, we will remove specific identifiers (i.e., name, contact information) from the collected information.

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 cognitive screening measures (e.g., MoCA), presence of current major depression, substance abuse, or psychosis (e.g., 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. Members of the project team are highly experienced in diagnosis and treatment of mood/cognitive disorders. A further discussion of 'at risk' procedures regarding neuropsychiatric symptoms is on page 19.

7.5. Pre and Post-Intervention Measures

Participants who meet study criteria and who remain interested in participating in the 12 week intervention will undergo a baseline/ pre-intervention testing and be randomized to treatment or sham groups. The pre-intervention measures will be administered by research assistants or psychometrists who are blinded to group assignment (sham, real NIR stimulation).

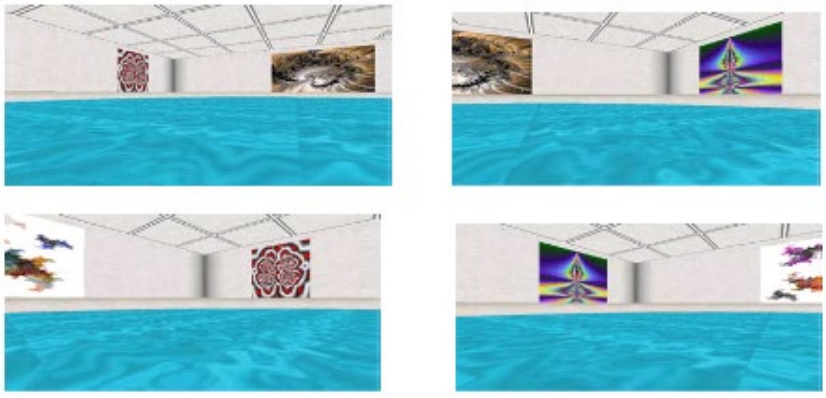
Pre-intervention/baseline testing involves cognitive and mood measures, questionnaires, blood draw, and neuroimaging. The cognitive measures include tasks of executive function, recent memory, and processing speed. The mood measures include the emotion module from the NIH Toolbox and paper-pencil questionnaires. Participants will undergo brain imaging (1.5 hrs). These same measures will be given after the 12 week intervention has been completed and again at 3 months post-intervention. The exception is neuroimaging and a blood draw which are not given at the 3 month follow-up.

7.5.1 **Cognitive Outcome Measures (Aim 1)**

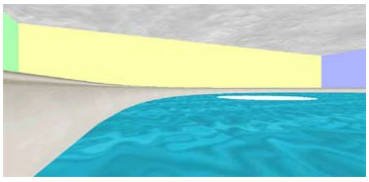
The cognitive outcome measures are described in more detail below and include the **primary outcome (ARENA)** and various experimental and clinical exploratory measures. An additional subset of cognitive measures is optional and depends on available time. These optional measures will allow comparison with an ongoing study that is IRB-approved.

7.5.1a. Primary Outcome – ARENA: 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. We selected **ARENA** (Thomas et al., 2001) as our **primary cognitive outcome** for the following reasons: a) the large effect size noted in our pilot NIR study; b) its sensitivity to hippocampal neuronal loss and early amnestic MCI (Laczo et al., 2010, 2011, 2012; Hort et al., 2007; Gazova et al.m 2012); and c) observations that the animal version (Morris Water Maze) benefitted from NIR stimulation in a transgenic mouse model (DeTaboada et al., 2011).

The task is given on a computer and participants are trained in the use of a joystick before beginning the task. Participants must navigate in a virtual, 3-D room displayed on a computer screen and find an invisible target on the floor as quickly and efficiently as possible. Shown below are sample walls of the virtual room. The participant uses a joystick to navigate this "arena", which is designed to look like a pool of water (the blue on the pictures). 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 with a 5-10 minute orientation session, in which they practice using the joystick and then navigate to visible targets on the floor. 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. The recorded path length and time to acquire the target serves as a baseline against which invisible target trials will be evaluated.



Hidden target condition. Practice trials are followed by 8 invisible-target learning trials. In these trials the target is not visible from afar and is discoverable only when the joystick hovers 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 during the 9th and final 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 are recorded. The **primary outcome variable** is 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 (Trial 9). Additional variables are also computed.

7.5.1.b Secondary Cognitive Outcome – Working Memory Composite from the NIH Examiner. Our secondary cognitive outcome involves a validated index of executive function, Working Memory, from the NIH Examiner Battery (Kramer et al., 2012). The NIH Examiner is a computerized battery consisting of 9 individual subtests from which 4 composite indices are derived: Overall Executive Composite, Working Memory, Fluency, and Cognitive Control. We selected Working Memory as our secondary cognitive outcome because of its sensitivity to NIR intervention in our pilot study and because working memory is a component of executive function (Miyake et al., 2000). The Examiner's *Working Memory Composite* is based on two working memory tasks, one spatial (***N-Back***) and one verbal *(**Dot Counting***), described below. The ***Working Memory composite*** consists of accuracy data drawn from these two tasks.

- *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.

- *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.

7.5.1.c Exploratory Cognitive Measures. Our exploratory cognitive outcomes include experimental and clinical measures of memory and executive function, described below.

- ***Mnemonic Similarity Test*** (MST, Stark et al., 2013, 2015, 2017). The MST involves *object-based pattern separation*, the process of grouping similar inputs into distinct memory representations; it contrasts with the spatial demands of our primary outcome measure, ARENA. The MST is sensitive to hippocampal dysfunction (Yassa et al., 2011; Kirwan et al., 2012) and the effects of normal aging (Stark et al, 2015) and has been validated in older adults, those with MCI and Alzheimer's disease Stark et al., 2013). The task itself measures recognition memory 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. A *Lure Discrimination Index* is calculated as the difference between the rate of "Similar" responses given to the lure items minus "Similar" responses given to the foils. *Recognition* for repeat items is calculated as the difference between the rate of "Old" responses given to repeat items minus "Old" responses given to foils.
- ***Auditory Verbal Learning Test*** (AVLT). This word list learning tasl (Schmidt, 1996) is widely used in clinical settings and assesses verbal learning for semantically unrelated words, proactive interference, and delayed recall/retention over 30-minute delays. The task consists of a 15-item word list, given over 5 learning trials, followed by a second list (to assess proactive interference) with subsequent 30' delayed recall/recognition of the initial target list. Primary measures are overall learning, the degree of proactive interference, and delayed 30' recall.
- ***NIH Examiner.*** The NIH Examiner is a computerized battery consisting of 9 individual subtests. The two working memory subtests represent the secondary cognitive outcome and are described in the preceding section. The tasks below reflect other NIH Examiner subtests that are part of our exploratory outcomes. **Total time to complete all the NIH Examiner subtests is approximately 35-40 minutes**.
    - ***Verbal Fluency*** *(*NIH Examiner). This task is widely used clinically and is administered in 60 second trials, consisting of two letter fluency and two semantic fluency trials. Total number of correct items, repetitions, and intrusions are computed.
    - ***Flanker Task:*** This task measures cognitive and motor inhibition across 48 reaction time (RT) trials. Participants view a row of arrows and indicate the direction that the center arrow points. On some trials, all the arrows point in the same direction. On others, the center arrow points in a different direction (Incongruent). Total accuracy score and RT scores on the incongruent trials are calculated and added together to create the total flanker score.

- ***Continuous Performance Task***: This task measures inhibition. Participants press a button when they see a five-pointed star, and do 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.
- ***Anti-Saccade Task*** (NIH): Participants are asked to watch a moving dot on the computer screen and move their eyes. On some trials, participants move their eyes in the direction of the stimulus (prosaccade, 10 trials), On others, participants are asked to move their eyes in the opposite direction (anti-saccade, 50 trials). The dependent variable is the total number of correct anti-saccade trials. This score contributes to the Executive Composite and Cognitive Control factor score
- ***Set Shifting*** In this task participants are cued to match stimuli on the basis of color or shape across 104 trials. Both RT and accuracy scored are computed across blocks, resulting in an overall set-shifting score
- ***Unstructured Task***: Participants are presented booklets that contain five pages of puzzles and are given six minutes to complete. Each puzzle is associated with a particular number of points. Some simple puzzles have a lot of points, and some hard ones have few points. Puzzles are simple but may take anywhere from 4 to 60 seconds to complete.The goal is to get as many points as possible. Participants are asked to use judgement and planning to get as many points as possible.

- ***Stroop Color Word Task.*** This measure assesses cognitive inhibition (Golden, 1978). Naeser et al. (2011) reported improvement on this measure in a case series of TBI patients who underwent NIR stimulation. The task requires participants to inhibit "reading' words (green, red, blue) that are printed in conflicting colors and instead name the ink color. The dependent variable is a computed interference score, adjusting for baseline reading and coloring naming times.

Trailmaking Test: This well validated task involves speeded visual search land set-shifting in a 'connect the dot' format. Performance is compared to baseline trial that does not have a set-alternation condition. This task is already given as part of the Neuropsychological screening battery. It will be additionally given post-intervention and at t 3-month follow-up.

Logical Memory Stories (WMS). This is among the mostly highly used measures of episodic memory in clinical settings. It involves immediate and 25-30' delayed recall of two novel stories.

Brief Visuospatial Memory Test (BVMT-R): This is a task of visual memory that involves learning and retention of various visual designs and figures. Both learning and delayed memory drawings are obtained.

7.5.2 Imaging Outcome Measures (Secondary) (Aim 2)

All participants will undergo a standard neuroimaging protocol before and after the NIR intervention. We plan to focus on cerebral mitochondrial function (**31P MRS ATP)** and resting state functional connectivity **(rs-fMRI).** The first imaging outcome is change in ATP, one of the key mechanisms associated with NIR stimulation. We plan to use a single voxel method for MRS to achieve optimal sensitivity and will measure from two ROIs (temporal, frontal), corresponding to our primary (memory) and secondary (executive) cognitive outcomes from Aim 1. The second imaging outcome involves changes in rs-fMRI connectivity which will enable us to document system level network brain changes.

Neuroimaging Protocol. Imaging will be performed on the AMRIS 3T Siemens Prisma MRI systems at UF and a 3T Siemens Skyra at UA. Inside the brain scanner, participants will lie on a padded table with foam pads used to hold the participants head in place. 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 may take up to 1.5 hours.

The protocol will be optimized for standardized acquisition for structural (e.g., T1, T2, etc.), functional (resting state fMRI), and magnetic resonance spectroscopy (phosphorous MRS). We will apply the ADNI-3 scanning sequences using a standard Siemens head coil and 31P MRS sequences on a dual-tuned whole head phosphorous coil (RapidMRI). Specifics are detailed below.

MRI. Whole brain axial gradient-echo MPRAGE T1-weighted images will be acquired for 31P and FMRI localization (~6 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 assessment of possible structural improvements related to intervention (~5 minutes duration). A T2* sequence to quantify presence of cerebral microbleeds–a marker of tissue damage. A 3D PASL sequence will enable quantification of changes in cerebral perfusion related to CT. A single shell diffusion-weighted imaging sequence will be acquired together with a reverse phase encode scan for distortion correction, enabling quantification of diffusion tensor analytic approaches. These measures will provide insight into white matter integrity. A high resolution hippocampal subfield structural sequence, combined with the T1/MPRAGE scan, will enable quantification of subfield structures potentially sensitive to CT. We will perform an fcMRI resting state block with eyes open and visual fixation using echoplanar BOLD imaging. Processing and analyses will use Statistical Parametric Mapping (SPM12), the CONN toolbox and in house software. We will present one ten-minute block of rs-fcMRI during MRI sessions to test stimulation related change in functional networks.

MRI Sequences			
Run	**Sequence**	**Domain Assessed**	**Min(s)**
1	T1/MPRAGE	Structure	6:20
2	3D FLAIR	White matter hyperintensities	5:30
3	Hippocampal	Hippocampal subfield measurement	4:20
4	T2* GRE	Cerebral microbleed assessment	4:10
5	ASL	Cerebral perfusion	4:00
6	Diffusion	White matter integrity	7:30
7	EPI-BOLD	Resting State	10:00
-	Coil Change	Change 32-channel coil for 31P coil	10:00
8	31P MRS	Frontal ATP and 31P metabolites	10:00
9	31P MRS	Left temporal ATP and 31P metabolites	10:00
		Total Time	71:50

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 (~9 minutes duration). Analyses: We will evaluate concentrations for phosphorous metabolites (e.g., alpha, beta, and gamma ATP, inorganic-phosphate (Pi), phosphocreatine (PCr), etc.

Incidental MRI Findings: One remote possibility is the occurrence of incidental findings on the MRI. Should this occur, the study site MD will discuss this finding with the participant, provide them with a CD containing their MRI (with software for viewing the images) and encourage the participant to follow-up with their primary care physician (PCP). With the participant's permission, the study MD may discuss the findings with their PCP.

7.5.3. Potential Moderators and Mediators of Cognitive and Neuroimaging Outcomes (Aim 3)

We plan to examine various factors that might influence how well participants respond to NIR-PBM stimulation. These include demographic factors (i.e., age, education, sex); b) cardiovascular and

other health comorbidities; c) neuroimaging variables (i.e., white matter hyperintensity volume, hippocampal volume, cerebral perfusion, etc.), d) APOE-4 carrier status, f) general cognitive efficiency/processing speed; e) physical (e.g., walking speed), and f) mood, and other factors.

7.5.3a ***APOE and Biomarker Status:*** We will obtain blood samples at two time points, at study entry and during the initial followup evaluation. Blood draws will be taken by trained Phlebotomists. Blood samples will be de-identified and stored at the McKnight Brain Institute biorepository maintained by the Center for Aging and Memory (CAM) until processed. We plan to use the blood samples to obtain APOE□4 genotyping at baseline and to examine changes in biomarkers (e.g., serum tau, serum amyloid-beta, inflammatory biomarkers, etc.) at baseline relative to post-intervention. The reason doing so is that APOE-e4 status has been linked to increased risk for Alzheimer's disease in older adults. We want to know whether the presence of e4 alleles is differentially associated with treatment response. Similarly, we want to know whether blood based biomarkers of Alzheimer's disease or other health-related factors drawn from the blood might be sensitive to our intervention (NIR-photobiomodulation).

Banking of medical and contact information for future research: As part of this study, participants will be asked about their interest having left over blood stored to be used in future research protocols. For those participants who are interested, they will sign a separate banking protocol consent form. Banking is not required to participate in this study.

7.5.3b ***Mood Measures:*** We plan to use the Emotion module from the NIH Toolbox (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.

The Emotion Module 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.

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.

Other Mood Measures. In addition to the Toolbox, more traditional clinical measures of mood and motivation may also be given. These are described below.

Anxiety	State-Trait Anxiety Inventory (STAI, Speilberger, 1983a,b; 1989). Widely used 40 item questionnaire for assessing current (state) and general (trait) levels of anxiety. Strong psychometric characteristics. Higher scores represent higher levels of anxiety, or feelings of worry, tensions, and stress.
Depression	Beck Depression Inventory -II (BDI-II, Beck et al.,1996). Widely used 21 item self-report questionnaire of symptoms of depression. Allows one to characterize mild, moderate, and severe symptoms, depending on score. Strong psychometric characteristics, even in older adults
Apathy	Starkstein Apathy Scale. abnormal performance on this task of visual object naming is used to exclude individuals with potential brain based cognitive decline
Emotion/Mood	Profile of Mood States, short form (POMS, Curran et al., 1995). – This is 30 item self-report questionnaire regarding occurrence of transient mood states such as "discouraged", "vigorous", and "grouchy". The measure is grouped into subscales. Psychometric characteristics are strong. Including test-retest reliability, internal consistency, and construct validity. The POMS will be at study entry , weekly during the intervention, and during post-intervention and followup sessions..

Mood Concerns: Participants are screened for depression and other psychological disturbances on 4 occasions: a) during Screening using a semi-structured DMS-IV type interview and the BDI-II; and b) during baseline, post-intervention, and at the 3 month followup session. During these latter 3 timepoints, experimental cognitive measures are given along with self-report mood measures (BDI-II, STAI, AS, POMS.) that gauge the symptom severity of depression, anxiety, and apathy. The reason to give these mood measures is due to the known influence of mood on cognitive status. Thus, we want to track whether cognitive changes might be due to mood changes.

Above and beyond the theoretic reason for examining mood, a critical symptom of concern involves suicidality. This symptom is captured by one of the items of the BDI and also during the Mental Health Screen given during screening. If a participant endorses suicidality, the PI or another qualified clinician (e.g., Weisbrod, DeKosky, Lopez) will be **contacted immediately**. We will follow standard criteria for determining suicidal risk including the following: clarifying the distinction between passive and active suicidal ideation, determining whether there is a method, plan, or intent for execution, determining whether there are barriers for executing intent (i.e., strong faith, not wanting to hurt family), and determining whether there is a history of previous attempt(s), including aborted or interrupted attempts. For acute crisis, the investigator will accompany the participant to the Emergency Department of UF Health and call ahead to alert the Psychiatrist on Call or will contact the local sheriff officials to arrange transport to the ED. Otherwise, we will discuss counseling and psychological care options vis a vis referral. This is contained in the body of the consent form and also in the protocol.

Our team is highly experience in working with older adults who are depressed and/or have other psychological disturbances. The PI (Dr. Bowers) is a board certified clinical psychologist and neuropsychologist who has been working with this population for over 35 years. She is '*certified*" in the use of the Columbia Suicide Rating Scale (C-SSRS). The doctoral students on her team are masters level psychologists with experience in working with depressed patients and are highly sensitive to "risks' of this sort. The clinical CO-I's are also experienced clinicians with longstanding expertise win working with older adults who have increased risk for depression and psychological

comorbidities.

In our experience, active suicidality is rare in the clinical research studies we have conducted to date. In fact, the PI has never encountered this scenario among research participants in over 35 years. Even so, we are keenly sensitive to mood changes in our patients.

7.5.3c 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.

7.5.4 Other Measures

7.5.4a. Expectations Questionnaire. This measure asks questions about the participant's expectations regarding effects of intervention on potential cognitive, mood, and functional changes.

7.5.4b. 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.

7.5.4c. 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 follow-up session.

7.5.4d. Placebo Control Questionnaire (PCQ): This questionnaire is given after the end of the intervention. It asks questions regarding which group (real, sham) the participant believes they were assigned.

7.5.4e Followup Questionnaire. This questionnaire is given after the intervention. It asks questions regarding the participant's subjective experience of change due to the intervention and the degree to which the participant found the intervention enjoyable, time consuming etc.

7.5.4.f. **Fatigue, Sleep and Pain** Questionnaires. Additional surveys will be completed including the Pittsburgh *Sleep Quality index*, *the Fatigue Severity Scale*, and a *Pain Rating Scale*.

7.6. Near-infrared (NIR) Intervention Protocol

We plan to use two **MEDx Rehab Console systems** (Medx Health, LLC, Mississauga, On, Canada) for transcranial delivery of NIR light and the 810 **Vielight system** for delivery of **intranasal stimulation**. We are currently using these systems in other IRB approved protocols without any concerns or adverse effects.

The MedX system was FDA-cleared in 2003 as a Class II medical device (K032231, 23 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).

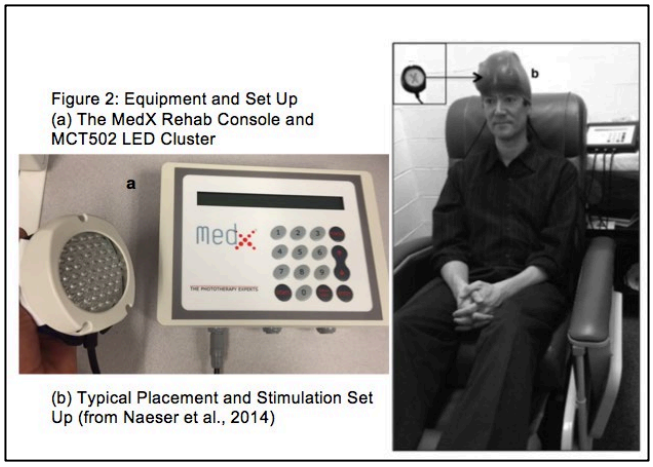


Figure 2: Equipment and Set Up
(a) The MedX Rehab Console and MCT502 LED Cluster
(b) Typical Placement and Stimulation Set Up (from Naeser et al., 2014)

Each Medx console consists of a control unit and 3 superluminous diodes). Each superluminous LED cluster (3MedX MCT502) consists of 52 near infrared diodes and 9 visible red diodes (See Figure). 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. Note that the commercially sold MedX unit typicall includes just 2 LED clusters and 1 laser attachment. For the current study and other studies we conduct using MedX, we have exchanged the laser attachment for an LED attachment. Thus, no laser is used in this study, just 3 LED's.

The Vielight 810 Infrared Intranasal Device (Vielight, Inc., Toronto, ON, Canada) will be used to deliver NIR light intranasally. It is considered safe and painless and does not require FDA clearance. 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. Each intransal unit is automatically set by Vielight to deliver stimulation for 25 minutes and then go off.

7.6.1 The **intervention protocol** will span 12 weeks and includes:

a. 16 stimulation sessions in the laboratory using the MedX Rehab Console System and Vielight intranasal unit. Each session in the lab will last approximately 1.5 hours and will involve 40 minutes of NIR stimulation. 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 40 minutes. Placement of LED clusters on the scalp follows the recommendations of Naeser (personal communication, 2014, 2015, and 2016).

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 net 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 for 25 minutes (one dose) and subsequently removed.

Throughout the session, participants are seated comfortably in a chair in front of a Video monitor. During the 40 minutes of stimulation, participants will view nature documentaries (BBC Life documentary series, Gunton, 2009, One Planet-Seven Worlds series). This is being done in order to standardize cognitive engagement and control for differences in cognitive activity during stimulation.

Timing of lab stimulation sessions*:* Following Saltmarche et al., (2017), the "in lab" stimulation sessions will occur three times a week during Weeks 1 and 2 of the intervention, and once a week during the remaining weeks of intervention (Weeks 3-12).

b) ***Daily home use of an intranasal NIR stimulator*** (Vielight 810 intranasal unit). Following the procedures of Saltmarche et al., 2017), participants will stimulate each nostril daily for 25 minutes in their home setting. During Weeks 1-2, they will do this 2 days a week on days they do not come to the lab). During Weeks 3-12, they will self –stimulate 4 days a week on days they do not come to the lab. During this stimulation period, they will be asked to watch television or videos, listen to music, or some other engaging activity, but refrain from talking with others. They will NOT self-stimulate on those days when they come to the laboratory, because the laboratory sessions include both transcranial and intranasal stimulation. Participants will keep a journal for recording the day/date and time of each of their stimulation sessions and will bring both their journal and intranasal unit to lab visits. At the end of the intervention, participants will return 810 Intranasal units.

Each participant will receive their own personal intranasal lead for use with the 810 Vielight intranasal device. Thus, the leads that are inserted into the nose will not be shared among participants. Participants will be provided sanitary wipes to clean the intranasal leads after each use. Should a participant become sick with a cold/rhinitis, they can continue use of the intranasal device if they are comfortable doing so. Otherwise, they will just record reason for not stimulating and will be asked to let the PI or project coordinators know. Depending on the situation, it might be possible to shift the intranasal stimulation to a different day. That will be decided on an individual basis.

The parameters for stimulation such as energy density and length of application were adapted from parameters used in an ongoing study in our lab and that of Saltmarche et al. (2017). The LEDs do not pose risk to vision and thus participants are not required to wear goggles.

In the unlikely event a participant should become incapacitated (i.e., TBI, severe illness, etc.), then standard IRB procedures will be implemented. An adverse events report will be submitted to the IRB, the study safety officer will be contacted, as will the study's DSMB. The participant will be withdrawn from the study.

7.6.2. NIR Procedures

All participants will attend 16 NIR stimulation sessions in the laboratory. During each session, the participant will be seated comfortably in a chair. The LED clusters will be positioned on the scalp and the intranasal units will be positioned in the nostrils. Approximately 40 minutes of stimulation will occur, broken into two 20 minutes segments. Intranasal stimulation will occur for 25-50 minutes.

During the actual stimulation period, ***participants will view nature documentaries*** (BBC Life Nature documentary series; Gunton, 2009; One Planet, Seven Worlds series, Keeling, 2019) 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. Episodes of the BBC Life Nature Documentary will be presented in the same order for each participant, with a different episode played at each stimulation session. The episodes include the following:

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.
7. "Fish": This episode features the countless number of fish that fill the seas and almost all other waters on the blue planet.
8. "Reptiles & Amphibians": This episode features reptilians, which survive from the age of the Saurians. Examples include lizards like the giant Komodo dragon, the feared crocodilian

hunters, color-changing chameleons and snake species fitted for most (warm) ecosystems. Featured amphibians include numerous specialized (tree) frogs and toads. Both groups are cold-blooded, hence vulnerable while warming up

9. "Insects": This episode feature insects, which outnumber all other species on earth. Their immense variety effect adaptation to an extreme range of ecological conditions. Nearly 60,000 fly species cover about the globe.
10. "Hunters and the Hunted": This episode features the struggle of life which is often based on eat and/or be eaten. Therefore, evolutionary success is based on struggle for skills to survive as prey or hunter. Mammals are particularly successful worldwide because the add to anatomical adaptation an intelligence allowing quick and greatly diverse strategies to find preys, shelter, fight (back)
11-16. "One Planet-Seven Worlds": This BBC series consists of one hour episodes, each involving the wildlife and unique landscapes/geography of each continent, emphasizing how the development of each continent shaped the diversity of animal life.

Questionnaires: Following the NIR stimulation, participants will complete 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.

7.6.3. SHAM NIR Condition and Blinding

Participants in the sham group will undergo identical informed consent, screening, pre-post testing, and NIR intervention sessions. The only difference is that "sham" MedX and Vielight devices will be used. These are identical in all respects to real devices except that they do not deliver NIR. The research assistant administering the lab stimulation session will be aware of the participant's treatment status, but will have no role in pre- or post-intervention assessments. The research assistant involved in testing and neuroimaging acquisition will be blinded to the treatment status of the participant.

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

Table 2: Overview of Procedures and Protocol

Measures	Pre-screen	Screening	Base-line	Intervention	Follow-up	3-month
Telephone Screening	X					
Inclusion / Exclusion	X	X				
Informed Consent		X				
Contact Information		X				
Demographic Info		X				
Medical & Psychiatric History		X				
Medications/Magellan		X				
MoCA		X				
NACC Neuropsych Battery		X				
Mental Health Screen V.3		x				

Charleson		X				
WTAR		X				
Vision Screen		X				
Functional Activity Questionnaire (FAQ)		X			X	X
Clinical Dementia Rating		X				
Cognitive Change Index (CCI) – participant & informant		X			X	X
Blood Draw			X		X	
MRI Screener	X	X	X		X	
MRI			X		X	
Skin type Questionnaire			X			
Expectation Questionnaire			X			
ARENA			X		X	X
NIH Examiner (Working Memory and other Examiner subtests)			X		X	X
MST			X		X	X
Rey AVLT			X		X	X
Verbal Fluency			X		X	X
Stroop			X		X	X
Trail Making Test		X			X	X
Logical Memory Stories			X		X	X
Brief Visuospatial Memory Test-Rev			X		X	X
Emotion Module - NIH Toolbox			X		X	X
BDI-II			X		X	X
STAI			X		X	X
Apathy Scale			X		X	X
Pittsburgh Sleep Inventory			X		X	X
Pain Scale			X		X	X
Fatigue Severity Scale			X		X	X
10 Meter Walk Test			X		X	X
POMS			X	X	X	X
Cal-Cap			X		X	X
Adverse Events Log			X	X	X	X
Daily Journal (participant)				X		

NIR Sensation Stimulation Questionnaire				X		
Placebo Control Quest					X	X
Followup Questionnaire					X	X
3-mo Followup Medical					X	X

MoCA=Montreal Cognitive Assessment; NACC=National Alzheimer's Coordinating Center; Charlson = Charleson Comorbidity Index; WTAR=Wechsler Test of Adult Reading; Skin type Questionnaire =Fitzpatrick Skin-Type Questionnaire; MST=Mneumonic Similarity Test; Rey AVLT = Rey Auditory Verbal Learning Test;; CALCAP = California Computerized Assessment Package; BDI-II=Beck Depression Inventory, 2nd edition; STAI=State Trait Anxiety Inventory; POMS=Profile of Mood States, Short Form; Daily journal = completed by participant for recording time of daily intranasal treatment.

7.7 Videotaping

A random subset of approximately 10% of the participants taking part in the intervention will be asked to have a baseline session and an intervention session video and audio taped. These videos or photos will be used for "quality" control. We want to insure that our research team is conducting the testing and the intervention in a standard manner. These videos or photos will be reviewed by experts at the University of Florida to provide feedback to the research team. Agreeing to be video and audiotaped is not a requirement for study participation.

7.8 Compensation

Participants will be compensated up to $500 for completing the study. The distribution of payment is as follows:

Completion of Baseline, MRI, and Blood Draw	$50
Completion of Intervention, post-MRI and follow-up, return of intranasal 810	$350
Completion of 3 month Follow-up	$100

8. Outcome and Statistical Approaches

8.1. Outcome Measures

Change in performance on cognitive and imaging parameters (functional connectivity, MRS ATP markers) before and after intervention, relative to the sham control group. Intervention changes must exceed the effects of practice based on the sham control group.

As shown, the protocol as a whole has <u>one primary outcome</u> (ARENA, Aim 1) and <u>three secondary outcomes</u> (*Working Memory*-Aim 1, *31-P MRS ATP*- Aim 2, rs-fMRI - Aim 2).

STUDY MEASURES AND OUTCOMES				
Measures	**ASSESSMENT SCHEDULE**			
	Baseline	During Inter-vention	Post Intervention	3-Month Followup
Outcome Measures				
Primary Outcome				
ARENA: Aim 1	X		X	X
Secondary Outcomes				
Working Memory: Aim 1	X		X	X
31-P MRS ATP: Aim 2	X		X	
rs-fMRI: Aim 2	X		X	
Exploratory Outcomes				
Verbal Fluency	X		X	X
Mneumonic Similarity (memory)	X		X	X
Auditory Verbal Learning Test	X		X	X
Stroop Color Word	X		X	X
Other Imaging	X		X	
Other Cognitive	X		X	x
Measures of Potential Mediators, Moderators , and Covariates				
Genotype for APOE-e4 status	X			
AD Biomarkers & other	X		X	
Vascular comorbidities	X			
Fitzpatrick Skin Type Questionna	X			
Processing Speed (CalCap-RT)	X		X	X
Mood (NIH Toolbox Emotion)				
Negative Affect	X		X	X
Subjective Wellbeing	X		X	X
Other Imaging variables				
Other Measures				
Daily Diary Home Log		Continuously During Intervention		
Profile of Mood States	X	X	X	X
Adverse Event	X	X	X	X
Fidelity				
Session Completion	X	X	X	X
Expectation Questionnaire	X			
Placebo Control & F/U Questionnaire			X	X

Aim 1 examines memory and executive function.

- Changes in pre-post scores on ARENA, a human Morris Water Maze analog sensitive to hippocampal function (Primary outcome)
- Changes in ore-post scores on Working Memory subtests from NIH Examiner (Secondary outcome).
- Changes in pre-post treatment scores on Verbal Fluency (NIH Examiner), the Mnemonic Similarity Task, Rey Auditory Verbal Learning Test, Stroop, and traditional neuropsychological measures of executive function (Exploratory outcomes).

Aim 2 examines neuroimaging changes

- Changes in frontal and temporal lobe MRS, including markers of ATP function, and structural imaging variables (Secondary outcome).
- Changes in frontal and medial temporal lobe mediated patterns of resting state functional connectivity (Secondary outcome).

Aim 3 examines potential mediators and moderators of treatment response, including demographic and other variables.

8.2. Randomization and Statistical Methods

8.2a. Randomization

Participants will be independently assigned to Sham and Active groups on a 1:1 ratio. Randomization will be stratified according to age and MoCA scores so as to prevent different average ages or cognitive status scores between the treatment groups (Active, Sham). 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.

8.2b. 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.

8.3. Statistical Analyses

For hypotheses associated with Aims 1 (cognitive) and 2 (imaging), we plan to conduct linear mixed effects models and other potential analyses. For Aim 3, we are interested in predictors of treatment responses and will explore a variety of potential mediators and/or moderators including APOE-4 carrier status, vascular comorbidities (including white matter burden), mood/emotion, processing speed, and demographic variables. To examine confounders, we will directly adjust these variables as covariates in a linear mixed model. Additional analyses will be conducted as appropriate.

8.4. Methodological Limitations

Few 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). The screening evaluation will take up to 1.5 hours. If participant formally meets study criteria, then he or she is invited to participate in the intervention phase of the study consisting of baseline, NIR stimulation, and post-baseline testing.

- **Baseline Assessment -** This assessment involves a series of computer based measures and other questionnaires, along with a brain scan and blood draw. This cognitive and mood assessment may take up to 5 hours and may be broken up across days, if necessary. The brain imaging will take between 60-80 minutes and the blood draw approximately 30 minutes.

- **NIR Intervention (16 sessions over twelve weeks)**- After completing baseline testing, participants will undergo 12 weeks of intervention. This includes 16 'in lab' sessions and daily at home intranasal sessions. The laboratory sessions will be scheduled at the baseline visit to ensure availability of the participant. The transcranial treatment will be applied over the head and is detailed in section 7.6. During each treatment visit, participants will fill out mood questionnaires such the POMS. During this 12 week period, participants will also complete daily journal indicating the time and location of their at home intranasal stimulation.

- **Post-Treatment Visit**- Following the final day of stimulation, the participant will be scheduled for a post-testing assessment of cognition, emotion, and neuroimaging, identical to the baseline visit. Participants will also fill out other questionnaires such as the adverse events and placebo control questionnaires and will be scheduled for a final blood draw.

- **3 Month Follow-up Visit** - Participants will receive final assessment of cognitive and emotion measures. They will not undergo MRI or a blood draw.

10. IRB Plan

The proposed study involves parallel data collection at two sites, the University of Florida and the University of Arizona. We plan to use a single IRB plan in compliance with NIH policy. Both the University of Florida and the University of Arizona are members of the SMART IRB, a master common reciprocal Institutional Review Board Authorization Agreement, which outlines standard operating procedures and guidelines for establishing reliant review of multi-site research.

We plan to establish the University of Florida as the IRB of record (Central IRB), whereas the University of Arizona will be the Relying IRB. As such, the UF IRB will provide ethical review for both UF and UA, whereas UA will retain responsibility for local institutional reviews (i.e., training, conflict of interest, HPPA privacy, biosafety, etc.). Additionally, UF will be the privacy board, and the relying IRB will be required to inform UF if there are any state HIPAA regulations in addition to Federal. Dr. Bowers (MPI), as the contact PI, will be responsible for the communication and overall conduct of the study and regulatory compliance and will submit regulatory IRB submissions on behalf of UF and the UA site. Dr. Alexander, MPI at the University of Arizona, will provide Dr. Bowers necessary information according to UF-IRB policies and procedures so that UF (Central IRB) can conduct an IRB review.

11. Data Safety Monitoring Plan

This project is very low risk to participants. The research team will follow procedures for data safety and monitoring per IRB guidelines. The research team will closely monitor participants for any potential adverse events. During the intervention phases, participants will complete adverse event questionnaires to assess for negative effects on safety of participants. These will be reviewed after every intervention session and at the follow-up evaluations. As this intervention involves an infrared lamp that is considered a non-significant risk, we do not anticipate adverse events or side effects. However, should a participant experience an adverse event of sufficient concern at any point during the intervention or at follow-up, we will report to the IRB as directed and to the Safety monitors at each site (i.e., Dr. Steve DeKosky at the University of Florida and Dr. Hishaw at the University of Arizona) who will complete Event Notification and Evaluation forms. The MPI's and Safety Officers will meet monthly via phone or other media to discuss any adverse events, should they occur. If there are no reported adverse events, then an email notification will be sent that month in lieu of a meeting.

We plan to have a **Data Safety Monitoring Board (DSMB)** who will consist of experts in cognitive interventions, clinical trials and neuroimaging. The committee will meet every six months via telephone, Skype, Zoom, or other format. Initially, the DSMB will advise NIH staff and MPI's on design and protocol changes, the analysis plan, and planned publications. The DSMB will monitor site performance through semi-annual reports from the research team.

12. Data Management Plan

Data will be kept in a password protected encrypted database which can only be accessed by the investigators and staff. All surveys and assessments will be under the direction of the Investigators who will supervise Research Assistants. Recruitment, screening, and project implementation will be coordinated by the Investigators and/or research staff. The 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 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.

Data Sharing with Collaborating Site: With permission of participants, de-identified data will be shared between the University of Florida and the collaborating site, the University of Arizona. De-identified questionnaire, cognitive, mood, and other data will be stored via the secured infrastructure of the University of Florida REdCap System. De-identified raw neuroimaging data will be transferred using Secure File Transfer Protocol (SFTP) onto UF Dropbox servers.

13. 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 is designated as a non-significant risk. 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 system and/or the

Vielight intranasal system do not cause tissue damage or physical damage. Both the Medx and Vielight systems are commercially available to the public.

There are five 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. A separate file linking the participant to their ID code will be stored separately in a password protected server/database. All HIPAA regulations pertaining to protection of participants and eliminating identification will be followed.

Third is discomfort associated with undergoing an MRI. 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 follow-up.

Fifth, there may be minor discomfort associated with having a blood draw taken. This is similar to what occurs when an individual has a blood draw as part of a standard clinical workup.

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.

14. **Guidelines and Risks in Relation to the COVID-19 Crisis**

Our study requires visits by older adults who may be at increased health risks related to COVID-19. We are addressing these risks in several ways by following guidelines provided by the State of Florida, the County, University of Florida, and the CDC. We will also follow guidelines put forth by the AMRIS facility for completion of MRI scans.

General Approach:
The research staff will check their own temperature daily upon entry into the laboratory. All will wear masks, practice social distancing, and wash hands or use sanitizer multiple times throughout the day. The only time a mask is not needed is when the individual works alone in an isolated office space. If an individual has a fever, or feels ill, they will leave the building immediately and return home. They will make arrangements to participate in the UF Health Test and Trace Program and will not return to the laboratory until cleared to do so.

The laboratory will be cleaned at least daily. Moreover, door-knobs, light switches, and touched surfaces (table tops, chairs, keyboards, mice, etc.) will be disinfected regularly, including before and after each participant is tested.

Study-Related Interactions with Participants

Upon entry into the Fixel Institute where our laboratory is located, all participants have their temperature screened by Fixel staff. Again, if a participant shows evidence of a temperature they will leave the building, return home, and are encouraged to seek guidance from their PCP.

Gloves and masks will be worn when a study coordinator interacts with a study patient. Participants will be expected to bring and wear a mask when attending a lab visit. If they do not bring one, participants will be given a mask to use and keep.

The offices that will be used for cognitive testing allow at least 6 feet separation between the participant and study coordinator. For the intervention, we are required to apply six light emitting diodes (LED's) on the scalp of the participant. As is standard practice, the LED's are cleaned before and after each use with sanitizer. During application of the diodes to the head (for NIR stimulation), the study coordinator will wear gloves. Again, as is standard practice, each participant will have their own unique 'net' for holding the diodes in place. The diodes will be sanitized with alcohol or other appropriate disinfectants after each intervention session. Finally, each participant has their own 'intranasal' stimulator that is used only by them throughout the study, both at home and during lab visits. These are cleaned using sanitizers.

If other lab personnel are in the lab on the day the patient is there, their office doors will be closed during the patient visit. We will use a staggered schedule for participant visits to avoid overlapping entry and exit into and out of the testing suite.

After Visit Procedures

After the study visit, the laboratory will be cleaned. This includes disinfecting door-knobs, light switches, and touched surfaces (table tops, door handles, keyboards, mice, pens, etc.) The diodes used for the intervention will be sanitized using recommended disinfectants.

Optional Telehealth Screening: We will offer participants the option to undergo the screening evaluation via telehealth. This option will depend on whether they have a computer or IPAD that can be safely and securely connected to the internet. If so, we will send the participant and their informant two copies each of the consent form. We will set up a time to review the consent forms via telephone. If participant continues to be interested, they will return signed copies of the consent forms in a pre-addressed stamped envelope. Once we receive the signed consent forms, we will set up the telehealth screening. This will be done via a UF zoom platform that is HPPA compliant, and identical in format to what we use in our clinical visits with patients at UF Health. With one exception, the majority of the relevant screening measures can be administered via telehealth. The exception is the Trailmaking Test, which will be given during the baseline visit instead of the screening visit.

15. 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.

16. Conflict of Interest

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

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