Study Title:
Cognitive Enhancement through Transcranial Laser Therapy (LLLT)

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Title:
Cognitive Enhancement through Transcranial Laser Therapy

1. **Principal Investigator:**
Name: Andreana Haley
EID: aph355
Department: Psychology

2. **Purpose:**

Low-level light/laser therapy (LLLT) has been proven effective in increasing cognitive function in several photobiomodulation models (Barrett and Gonzalez-Lima, 2013; Blanco et al., 2015). LLLT shows promise in its ability to minimize or prevent the loss of cognitive function associated with cardiovascular disease in high risk patients.

Cardiovascular diseases have been determined to be associated with diminished cognitive function. An ideal solution to this issue would be to restore proper cardiovascular status through improved lifestyles. However, an alternative intervention is increasing cerebral oxygen consumption by up-regulating mitochondrial respiration using LLLT. As shown by Haley et al., 2007, atherosclerosis of the carotid artery is a strong predictor of cognitive decline. The carotid artery intima media thickness (IMT) is also recognized as a risk factor for brain damage in asymptomatic patients (Nguyen-Thanh and Benzaquen, 2009).

We believe that the beneficial effects of LLLT can be used to combat the negative cognitive effects associated with vascular disease in high-risk patients. We hypothesize that, relative to their own baseline, participants who receive LLLT will exhibit improved cognitive performance (higher task accuracy, faster reaction times) in cognitive tests and have a greater Blood Oxygen Level Dependent (BOLD) fMRI response to a working memory task post-treatment. The therapeutic response to LLLT will likely be moderated by level of carotid atherosclerosis (measured by IMT) and genes involved in lipid transport (APOE).

3. **Procedures:**

Summary of general design and methodology.

We would classify this research study as a "non-significant risk device" study. Cognitive performance will be evaluated in working memory tasks in middle-aged and older adults across three groups: low intima media thickness (IMT) (1 STD below the mean), average IMT (mean), and high IMT (1 STD above the mean). Within each group there will be two possible interventions: the LLLT treatment or the placebo treatment. Cognitive tests will emphasize attention and executive functions, which are impaired in adults with cardiovascular risk factors (Cohen et al., 2009) and are enhanced by LLLT in young adults (Barrett and Gonzalez-Lima, 2013). Carotid atherosclerosis will be measured and documented via vascular ultrasound. BOLD-fMRI response to cognitive tasks will be measured pre- and post-treatment to determine physiological response to LLLT. 100 men and 100 post-menopausal women, aged 45 and older, will participate in this study after providing written informed consent, for 1 session of cardiovascular assessment, 1 session of cognitive testing and MRI scanning, followed by 6

weekly sessions of LLLT/placebo treatments, followed by post-treatment cognitive testing and MRI scanning.

Sequence of events:
Telephone Screening
Visit 1: Baseline cardiovascular assessment: 1 hour (Vascular Aging Lab, Bellmont Hall)
Visit 2: Baseline cognitive testing/MRI visit: 2-2.5 hours (Clinical Neuroscience Lab, Seay building; UT Imaging Research Center, Norman Hackerman Building)
The order of Visits 1 & 2 is interchangeable. The visit that comes first will include the informed consent procedure.
Visits 3-8: Treatment visits: 1 hour (Human Laser Lab, Seay building)
Visit 9: Post-treatment cognitive testing/MRI visit: 2-2.5 hours (Clinical Neuroscience Lab, Seay building; UT Imaging Research Center, Norman Hackerman Building)
Visit 10: One month post-treatment discontinuation cognitive testing visit: 1 hour (Clinical Neuroscience Lab, Seay building)

In an effort to reduce study burden for participants with Mild Cognitive Impairment, the visit to Bellmont Hall will be removed. Participants will come to the Seay building for all visits. They will undergo a shortened cardiovascular assessment at the Clinical Neuroscience Lab during visit 1.

Sequence of events:
Telephone Screening
Visit 1: Abbreviated baseline cardiovascular assessment/cognitive testing: 1-2 hours (Clinical Neuroscience Lab, Seay Building)
Visit 2: Baseline MRI visit: 2 hours (Clinical Neuroscience Lab, Seay building; UT Imaging Research Center, Norman Hackerman Building)
Visits 3-8: Treatment visits: 1 hour (Human Laser Lab, Seay building)
Visit 9: Post-treatment cognitive testing/MRI visit: 2-2.5 hours (Clinical Neuroscience Lab, Seay building; UT Imaging Research Center, Norman Hackerman Building)
Visit 10: One month post-treatment discontinuation cognitive testing visit: 1 hour (Clinical Neuroscience Lab, Seay building)

Research Protocols:

Interested participants who are eligible after the telephone screening will receive a packet of information in the mail including the study consent forms, medical history questionnaires, and maps and parking information (please see attachments: Initial contact cover letter.doc; Consent form.doc; Mailing Packet.pdf). The questionnaires in the Mailing Packet include: Medical Screening Questionnaire, Food Diary, Edinburgh Handedness Questionnaire, Beck Depression Inventory, State Trait Anxiety Questionnaire, Cognitive Difficulties Scale, and 36-Item Short Form Survey Instrument (SF-36). Should they continue to express interest in the study, they will be scheduled for their first study visit (see details below) and instructed to bring the completed Mailing Packet to their first visit.

Cardiovascular assessment.

This visit will take place at the UT Cardiovascular Aging Lab located Bellmont Hall under the supervision of Dr. Hirofumi Tanaka (co-I). The assessments included in this visit were also included in the approved protocol #2011-07-0025. This study will be covered under consolidated IBC eProtocol IBC-2015-00143, "Cardiometabolic risk and brain function" (see attached file: IBC eProtocol Number.docx). Participants with MCI will undergo an abbreviated version of the assessment in the Clinical Neuroscience Lab under the supervision of Dr. Andreana Haley (PI). The lab has biosafety Level II certification and operates under consolidated IBC eProtocol IBC-2015-00143. Participants will be instructed to fast and refrain from caffeine and chewing gum for at least eight hours prior to their visit. During the visit, participants will undergo the following assessments:

1) Resting blood pressure will be measured non-invasively using arm and ankle cuffs while participants rest in a supine position.

2) Participant's waist and hip circumferences will be measured with a tape measure.

3) Body weight and height will be measured with a beam balance scale for the purpose of calculating body mass index (BMI)

4). Body composition will be measured non-invasively by dual energy X-ray absorptiometry (DEXA) using Lunar DPX by General Electric Medical Systems. This procedure requires the subject to lie down approximately five minutes on a padded table while a small arm that emits energy to measure tissue density passes overhead. The DEXA assessments are performed by appropriately trained operators for research purposes only, and not for medical diagnostic purposes. The DEXA unit is operated in compliance with the Texas Administrative Code and the Texas Department of Health Services under the medical supervision of Patrick List, M.D., UT University Health Services physician, and radiation safety oversight of the UT Office of Environmental Health and Safety. This measure will be removed from the protocol for participants with MCI.

5) Arterial Stiffness will be measured non-invasively using ultrasound. This measure will be removed from the protocol for participants with MCI.

6) A blood sample will be drawn (~ 15 mL) through venipuncture by a trained phlebotomist to measure cholesterol, triglycerides, blood glucose, insulin levels. Additionally, proteins related to inflammation and arterial stiffness will be measured due to their importance to vascular health and cognition. This measure will be removed from the protocol for participants with MCI. Instead, they will undergo a finger prick.

7) A saliva sample will be obtained using an OMNIgene saliva collection kit (DNA Genotek, Ontario, Canada) to test for the presence of genetic polymorphisms with relevance to lipid transport and atherosclerosis (ApoE e4). Due to the preliminary nature of the investigation, no information about the person's genetic status will be released to the participant. Participants who have consented to this portion of the study will be instructed to spit into a small tube until approximately 1 mL of saliva is obtained. Saliva samples awaiting genetic analyses will be stored under participants' study number according to proper storage temperatures in a biosafety level 2 area (SEA 4.111B) until they are transferred to the UT Austin DNA Sequencing Facility (DSF) for analysis.

Cognitive testing/MRI visit.

This visit will take place at the UT Department of Psychology and the UT Imaging Research Center in the Norman Hackerman Building. The assessments included in this visit were also included in the approved protocol #2011-07-0025. Upon arrival, participants will be screened for a second time on paper or electronically to ensure that they are eligible to safely participate in an MRI study (please see attachment: IRC MRI Screening Form.pdf).

Prior to being scanned, participants will complete questionnaires and a brief battery of neuropsychological paper-and-pencil tests assessing attention, memory, visual-spatial functioning, and language at the UT Department of Psychology (please see attachment: Cognitive test packet.pdf, which includes the Mini-Mental Status Examination, Dementia Rating Scale, Clinical Dementia Rating Scale, Wechsler Test of Adult Reading, California Verbal Learning Test, Brief Visuospatial Memory Test, Trail Making Test A & B, WAIS Digit Span, Controlled Oral Word Fluency Test, Stroop Test, California Verbal Learning Test alternate, and Boston Naming Test). The cognitive testing during the last visit will also include the 36-Item Short Form Survey Instrument (SF-36), which was included as part of the mailing packet. They may also practice the experimental tasks to be performed in the scanner on a laptop or desktop computer. Participants with MCI will complete cognitive testing on visit 1 and MRI scanning on visit 2 to reduce study burden. They will be offered a snack after the abbreviated cardiovascular assessment is completed on visit 1, before cognitive testing begins.

Neuroimaging will be conducted at the UT Imaging Research Center in the Norman Hackerman Building. Participants will be asked to change into medical scrubs and to store their personal items in a locker. Upon entering the magnet room, participants will be given earplugs to wear (specially chosen to limit the sound intensity produced by the MRI scanner). They will be instructed to lie supine on a table. Participants will place their head inside a standard head coil. In order to acquire MR images, it is necessary that the subject's head does not move appreciably; they will therefore be outfitted with pads and wraps. We will adjust the head stabilizers until both stability and participant comfort are attained. At this point, any cables connecting the coil array to the scanner will be inspected and adjusted to guarantee the absence of any loops or direct contact with the subject. The table will then be slid into the scanner so that the participant's head and upper body are inside the scanner tube.

Once in the scanner, participants will always be given a "squeeze ball" as a top-priority communication device. They will be instructed that squeezing the ball indicates that they wish to stop the scan and/or need immediate assistance. This sounds an alarm in the control room where the researchers sit while running the MRI scanner. The scanner will be stopped if the alarm sounds during a scan. Standard communication and instruction is performed via a built-in intercom system. Participants will also be given a response pad for use during the cognitive task.

A typical scan session will take approximately 40 minutes. We will begin by taking a few anatomical MR images of the participant's brain (6-10 minutes). We will then collect a series of functional MR images (12 minutes) while the subject views visual displays and performs the cognitive and control tasks. Acquisition of magnetic resonance spectroscopy (MRS) and resting state functional MR data will take an additional 20 minutes. Participants will not be required to do anything during those acquisitions. Participants will be encouraged to terminate the session if they become uncomfortable in any way.

Treatment visits: Low-level light therapy.

LLLT or placebo will be administered once a week for 6 weeks, following the same stimulation procedure as in our previously-approved IRB protocols (2011-05-0022, 2015-09-0018) and as reported in Barrett and Gonzalez-Lima (2013). Administration of LLLT consists of applying light of a specific wavelength (1064 nanometers) using a laser diode, the HD laser (Cell Gen Therapeutics, LLC). This apparatus is FDA-cleared for temporary relief of muscle and joint pain, muscle spasm, stiffness associated with arthritis, improving circulation, and relaxation of muscle tissue. The HD laser has been evaluated and the standard operating procedure reviewed by the University of Texas at Austin Laser Safety Program for the purpose of occupational safety. The individuals who will operate the HD laser have completed the OH 304 Laser Safety class through UT's Environmental Health and Safety office. Both participants and experimenters wear protective eyewear, though the administrators of the LLLT are careful not to shine the light in or near the eyes.

The diameter of the circular surface receiving the LLLT is 4 centimeters. The laser power output used is 3.4 Watts. The irradiance (or power density) used, 250 milliwatts/centimeter^2, as well as the cumulative fluence (or energy density) used, 60 Joules / centimeter^2, are the same parameters that showed psychologically beneficial effects in Schiffer et al. (2009) and in Barrett and Gonzalez-Lima (2013), and the same parameters approved in our previous approved IRB protocol (2011-05-0022). From ANSI Z136.1-2007, Table 7, the Maximum Permissible Exposure (MPE) to skin at the emission wavelength is 1.0 W/ cm^2. (The MPE standard for this wavelength is unchanged in Table 7b of the 2014 revision of the ANSI Z136.1 Safe Use of Lasers.) At the power level described, the energy emitted by the HD laser at this setting is one quarter of the skin MPE (0.250 W/cm^2), exposure to it is not deemed harmful to tissue, and it causes no detectable physical damage and negligible heat. In fact, similar settings are used clinically by Cell Gen Therapeutics for the treatment of disorders such as lower back pain, sciatica, and migraine headaches.

The LLLT treatment will occur in Seay 3.304, a lockable room in the Gonzalez-Lima lab that has been approved by the University of Texas at Austin Laser Safety Program for housing of the HD laser apparatus. The experimenter will lock both himself and the participant inside the room, put a sign on the outer door indicating that the apparatus is in use, and make sure that protective eyewear (900 nm - 1000 nm: 5+, 1000 nm - 2400 nm: 7+; 2900 nm - 10600 nm: 7+) is worn by both individuals, following the approved standard operating procedure for the Gonzalez-Lima lab's use of the apparatus. The laser's power output is automatically calibrated by an internal mechanism, every time a power level is set by the user; however, in addition to this calibration, the power density in mW/cm^2 (and thus the energy density dose in J/cm^2) will be confirmed independently using a Newport model 1916-C power meter attached to a Newport model 918D-SL photodiode detector, prior to the onset of treatment.

The laser will be directed at the right side of the forehead, targeting the middle frontal gyrus of the cerebral cortex, the area of the brain with the strongest relationship between carotid IMT and working memory activation in our prior study (Haley et al., 2007). The treatment duration is eight minutes. The control group will undergo the same procedure as the treatment group, but will receive brief (5-second) stimulation to the intended site on the forehead, followed by 55 seconds of no stimulation, for each 1-minute cycle. Only a fraction of the transmitted light will penetrate the skin, skull and dura to the brain, and the vascularity of the scalp efficiently removes heat and prevents any significant heat accumulation.

During the treatment visits, LLLT-related cognitive enhancement will be assessed using two tasks: the psychomotor vigilance task (PVT), a test of sustained attention; and the delayed match-to-sample memory task (DMS), a test of visual working memory. These executive/cognitive processes are mediated by the frontal cortical regions that the LLLT treatments target. These tasks are the same as those approved in our previously-approved IRB protocols (2011-05-0022, 2015-09-0018).

The first test will be a pre-treatment baseline evaluation. (This will be preceded by a brief 1-minute-long introduction to both tasks; this data will not be recorded.) Then, after the first treatment with LLLT or placebo, participants will repeat the PVT and DMS tasks. Participants will again repeat the PVT and DMS tasks after the sixth (final) treatment is completed. We have previously demonstrated an improvement in performance on both the PVT and DMS tasks after LLLT treatment (Barrett and Gonzalez-Lima, 2013).

4a. Location.

All study activities will occur at the University of Texas at Austin. The cardiovascular assessments will occur in the Cardiovascular Aging Laboratory of Dr. Hirofumi Tanaka, a research-dedicated facility in Bellmont Hall. Cognitive testing will occur in the Clinical Neuroscience Laboratory of Dr. Andreana Haley, Seay building. The fMRI testing will occur in the Imaging Research Center (IRC) located in the Norman Hackerman Building. The laser treatment will be done in the dedicated room in the Gonzalez-Lima laboratory in Seay 3.304. This room has already been evaluated and posted by the UT Austin Laser Safety Program for the use of the HD laser apparatus.

4b. Resources.

This research will be supported by NIH grant R21AG050898 and a grant by the Darrell K Royal research fund for Alzheimer's disease.

4c. Study Timeline.

We anticipate that this study will take three years to gather data and one year to analyze and disseminate the results.

5. Measures:

The PVT and DMS are implemented by a program called PEBL, the Psychology Experiment Building Language, an open-source programming language. For the PVT, this file includes dependent variables for each trial, such as reaction time in milliseconds, as well as a code number indicating whether the trial was a success (response in less than 30 seconds), a lapse (no response in 30 seconds), or a false alarm (responded with a button press prior to the onset of the cue). Participants will be identified by a code number (their randomly-assigned subject number) typed into the program, prior to the start of each block of trials. For the DMS, the dependent variables include reaction time in milliseconds, and success/failure on the choice part of the task; participants are indexed by their randomly-assigned number. For the intima media thickness (IMT) measurements, ten measurements will be averaged to give a final value; this data will also be encoded using the participant's randomly-assigned subject number. The fMRI data consist of a Blood Oxygen Level Dependent (BOLD) contrast imaging signal which

shows brain metabolic activity; these measures will also be recorded under each participant's randomly-assigned subject number. Additional measures used for sample characterization (also indexed with each participant's randomly-assigned code number) include responses to the questionnaires seen in the uploaded attachment Cognitive Test Packet.pdf (which includes Medical Screening Questionnaire, Food Diary, Edinburgh Handedness Questionnaire, Beck Depression Inventory, State Trait Anxiety Questionnaire, Cognitive Difficulties Scale, and 36-Item Short Form Survey Instrument (SF-36)).

6. Participants:

6a. Target population and subject numbers.

Men (n=100) and post-menopausal women (n=100), aged >45, of any ethnic background from the surrounding community will be considered for the study. Equal numbers of men and women are expected to enroll. Racial and ethnic composition is expected to reflect that of the state of Texas. Participants will not be excluded on the basis of gender, race, ethnicity, or socioeconomic status.

6b. Inclusion/exclusion.

The primary inclusion criterion is age: subjects must be 45 years old or older. However, there are a number of exclusionary criteria. (Please see attachment: Exclusionary criteria screening checklist.docx.) Female participants will be limited to post-menopausal women. The criteria will be such that participants will be excluded that have been diagnosed with a psychotic disorder, have a history of violent behavior, are currently pregnant, or have ever been institutionalized or imprisoned. Participants with subjective complaints regarding modest decline cognitive decline from a previous level of performance will be included as long as the cognitive deficits do not interfere with their capacity for independence in everyday activities (Clinical Dementia Rating Score=0 or 0.5). Participants with CDR=0.5, who were randomly assigned to the placebo-treated group, will be given the chance to return for 6 weeks of active therapy. Thus, they will have the option to receive the potential benefits from LLLT. These visits will be offered as a courtesy and participants will not receive additional monetary compensation for them.

For the MRI, participants will be excluded if they have a history of neurological disease (e.g., large vessel stroke, seizure disorder, Parkinson's disease, clinically significant traumatic brain injury, multiple sclerosis, or brain infection/meningitis), major psychiatric illness (e.g. schizophrenia, bipolar disorder), MRI contraindications, including BMI > 40, or show evidence of large vessel stroke or clinically significant white matter disease confirmed by a licensed neuroradiologist. Participants with MCI with MRI contraindications will be enrolled in the study, but will not complete MRI scanning. Excluded participants will be encouraged to see their primary care physician for further care. Uninsured participants will be provided with low cost medical care resources in the Austin area organizations.

6c. Benefits.

There is no guarantee of direct benefits; however, our previous work (Barrett and Gonzalez-Lima 2013, Hwang, Castelli and Gonzalez-Lima 2016) successfully demonstrated the beneficial effects of LLLT on sustained attention and working memory tasks, as well as

executive function (Blanco, Maddox and Gonzalez-Lima 2015) and alleviating symptoms of depression (Disner, Beevers and Gonzalez-Lima 2016), providing evidence for LLLT as a non-invasive, easily administered, and clinically useful tool to augment brain activity. These results suggest that LLLT could be a way to increase a subject's ability to sustain attention for longer periods of time, and even provide a non-invasive, non-pharmacological treatment for disorders such as attention deficit disorder, which may be useful for subjects that are treatment-unresponsive to drugs like Ritalin, or who simply do not want to be treated pharmacologically for such a disorder. Similarly, the beneficial effect on mood indicates that LLLT could provide a non-invasive, non-pharmacological, easily administered and clinically useful way to treat mood disorders. Participants can also elect to receive feedback from their cognitive and cardiovascular testing (see attachments: Cognitive feedback letter template.doc; Cardio feedback letter template.doc).

6d. Risks.

As an NIH-NIA funded study, this study is registered as a clinical trial (NCT02851173) and subject to oversight by an NIA-approved external Data Safety and Monitoring Board. The three members of the DSMB are: Lawrence Sweet, Ph.D. UGA (Chair); Noah Philip, M.D., Brown (Safety Officer), and L. Stephen Miller, Ph.D. UGA (member). A copy of the Data and Safety Monitoring Plan will be uploaded separately (see attachment: DSMP.doc). In this DSMP is a complete description of the Risks (section 1.1), Adverse Event and Serious Adverse Event Collection and Reporting (section 1.2), and Protection against Study Risks (section 1.3).

Possible Discovery of Findings Related to MRI. The MRI scans (which possibly could result in claustrophobia as a possible risk) that will be performed are NOT necessarily equivalent to those used to diagnose medical problems. Many potentially serious problems may be undetectable on these scans and ultrasounds. A lack of findings should not be used to avoid physician visits. Participants who have are having physical symptoms that they find concerning will be encouraged to see their primary physician, who will determine the examinations required to arrive at a proper medical diagnosis.

The MRI scans will produce anatomical images that may be reviewed by a certified radiologist. In the unlikely event that the images show clinically significant findings, the investigator will contact the participant and inform him or her of the findings. The initial contact will be by phone and followed up with a letter. Two weeks after the initial contact, the participant will be called again to see if he or she needs any further assistance. The participant will be told that the decision as to whether to proceed with further examination or treatment lies solely with the participant and his or her physician. The participant will be informed that the investigators, the consulting radiologist, and The University of Texas are not responsible for any medical examination or treatment that the participant may undertake based upon our findings. Because the images obtained in this study do not comprise a proper clinical MRI study, these images will not be made available to the participant or his or her physician.

The Imaging Research Center has an approved protocol in place regarding the possible discovery of findings, and that protocol will be followed. The specific steps to be carried out to inform the participant of potentially problematic findings are as follows:

1-Images with suspected incidental findings will be flagged and reported to the IRC. They will be referred to Dr. Neal Rutledge, a licensed neuroradiologist and medical director of the IRC, for interpretation.

2-Should contact with the participant is deemed appropriate by Dr. Rutledge, the participant will be contacted and a phone conference arranged during which Dr. Rutledge can explain the incidental findings to them and offer advice about follow-up care.

3-The phone call will be followed up with a letter summarizing the findings and recommendations.

6e. Recruitment.

Participants will be recruited using newspaper/craigslist advertisements, and flyers posted around the greater Austin area and at various organizations (please see attachments: Newspaper advertisement text.doc; Flyer.ppt) To facilitate recruitment of participants with mild cognitive impairment, we have added recruitment materials that specifically target this population (see uploaded documents: Newspaper advertisement text MCI.docx; Flyer_MCI.ppt). Participants will contact the lab and complete a brief screening form over the phone or online via Research Electronic Data Capture (REDCap) to determine if they meet the inclusion criteria and would like to participate (please see attachments: Recruitment telephone script.docx; Exclusionary criteria screening checklist.docx; see below for information on REDCap).

Additionally, participants from previous IRB-approved studies conducted by our lab, whose consent forms did not preclude future contact, will be contacted about enrollment in the current study. Participants who do not wish any future contact will be placed on a no-contact list. If participants have not responded to the invitation to contact the lab after three messages, they will be moved to the no-contact list. Participants from previous studies who are interested in participating in the current study will complete a brief screening form over the phone or online to ensure continued eligibility (please see attachments: Recruitment telephone script.docx; Exclusionary criteria screening checklist.docx).

Screening forms collected online, and contact information for participants who have agreed to be recontacted for future studies, will be managed using REDCap. REDCap is a secure web application designed to support data capture for research studies, providing user-friendly web-based case report forms, real-time data entry validation (e.g. for data types and range checks), audit trails and a de-identified data export mechanism to common statistical packages (SPSS, SAS, Stata, R/S-Plus). REDCap also provides a powerful tool for building and managing online surveys. The research team can create and design surveys in a web browser and engage potential respondents using a variety of notification methods. The system was developed by a multi-institutional consortium which includes University of Texas at Austin and was initiated at Vanderbilt University. Network transmissions (data entry, survey submission, web browsing, etc.) in REDCap are protected via Secure Sockets Layer (SSL) encryption. REDCap data collection projects rely on a thorough study-specific data dictionary defined in an iterative self-documenting process by all members of the research team with planning assistance from the PRC. The iterative development and testing process results in a well-planned data collection strategy for individual studies. REDCap provides a secure, web-based application that is flexible enough to be used for a variety of types of research, provide an intuitive interface for users to enter data and have real time validation rules at the time of entry.

6f. Obtaining Informed Consent.

While the consent form will be mailed as part of the Mailing Packet to interested participants, informed consent will be obtained while the PI or research assistants are present, when the participants come to their first appointed experiment time. One of the researchers will guide the participants through the consent form with an oral explanation, and then the participant will be given the opportunity to read through the consent form. When this is completed, the researcher will ask if there are any questions or concerns. The consent form will be signed and confidentially kept in a locked file cabinet. Participants chosen to be scanned with MRI will fill out a separate consent form.

Participants will be asked to provide consent to be contacted in the future regarding opportunities to participate in any follow-up components to the present study, pending continued grant funding. Participants who do not wish future contact are able to check an "opt-out" box that will preclude them from future contact.

7. Privacy and Confidentiality:

The data will not be shared by other researchers for research purposes not detailed in this study. Information collected during the initial experiment screening will contain no identifying information until the participant signs the informed consent. No record will be kept of screens of persons determined not eligible for the study, or who decide not to participate. The participant will maintain control throughout the study over the content and amount of information he/she chooses to share with the experimenter, and has the right to withdraw from the study at any time without any penalty. Individuals will participate in the experiment one at a time, to prevent anyone's choice to participate from becoming public. The performances on the reaction time tasks or the results of the questionnaires of any participant will never be revealed to another participant. Participants can skip any question on any of the questionnaires. The consent and debriefing sessions will also be conducted by a trained investigator. Privacy and confidentiality of participants will always be maintained.

Efforts will be made to ensure participant privacy within the constraints of the study design. The study visits will be scheduled in accordance to the participant's availability as long as resources are available. These visits will be conducted only in the presence of trained research assistants. Participants will also be contacted by phone or email, depending upon their preference, to schedule appointments and for appointment reminders. Permission to leave messages will be asked at the time of screening.

One important limit or exception to maintaining confidentiality comes in regards to the investigators identifying persons who pose a risk of harm to themselves or others. Under circumstances where any participant is identified as being at imminent risk of harming themselves, confidentially will be breeched to ensure the participant's safety. In such cases, a report of the risk will be made to the Psychiatric Crisis Services Department of the Austin Travis County Integral Care Center. If a subject indicates current suicidal ideation, we will contact the Behavioral Concerns Advice Line.

Regarding confidentiality of the research data, documents with participants' identifying information will be placed in a locked file cabinet located at the Department of Psychology. All data is represented using the participant's randomly-assigned subject number, not their name. Network transmissions (data entry, survey submission, web browsing, etc.) in REDCap are

protected via Secure Sockets Layer (SSL) encryption. Images collected during the MRI scan and ultrasound sessions will be stored on a Department of Psychology server under study and scan number. Personal information linking a participant with data will be maintained in a separate secure document, which is destroyed at the end of the experiment. Department of Psychology servers are backed up on a daily basis. Access to the scans and databases will be password protected and only available to relevant researchers designated by the PI. The consent forms will be kept for five years and then destroyed. All hard copies will be shredded, and digital copies will be deleted.

Saliva samples awaiting genetic analyses will be stored under participants' study number according to proper storage temperatures in a biosafety level 2 area (SEA 4.111B) until they are transferred to the UT Austin DNA Sequencing Facility (DSF) for analysis.

Anonymized data may be shared with other researchers, upon request, as per NIH guidelines.

8. Compensation:

Participants will receive $200 upon study completion. Should participants wish to discontinue the study early, they will be compensated $17 per study visit for each completed visit. Subjects will be paid at the end of their last visit, with a check, and they must sign the receipt.

REFERENCES:

Barrett DW, Gonzalez-Lima F (2013). Transcranial infrared laser stimulation produces beneficial cognitive and emotional effects in humans. Neuroscience 230: 13-23.

Blanco NJ, Maddox WT, Gonzalez-Lima F (2015). Improving executive function using transcranial infrared laser stimulation. Journal of Neuropsychology, May 28.

Cohen RA, Poppas A, Forman DE, et al. (2009). Vascular and cognitive functions associated with cardiovascular disease in the elderly. J Clin Exp Neuropsychol 31:96–110.

Disner SG, Beevers CG, Gonzalez-Lima F (2016) Transcranial laser stimulation as neuroenhancement for attention bias modification in adults with elevated depression symptoms. Brain Stimulation 9(5): 780-7.

Haley AP, Sweet LH, Gunstad J, Forman DE, Poppas A, Paul RH, Tate DF, Cohen RA (2007). Verbal working memory and atherosclerosis in patients with cardiovascular disease. An fMRI study. Journal of Neuroimaging 17(3): 227-233.

Huang YY, Chen ACH, Carroll JD, Hamblin MR (2009). Biphasic dose response in low level light therapy. Dose-Response 7: 358-383.

Hwang J, Castelli DM, Gonzalez-Lima F (2016) Cognitive enhancement by transcranial laser stimulation and acute aerobic exercise. Lasers in Medical Science 31(6): 1151-60.

Leal Junior ECP, Lopes-Martins RAB, Frigo L, De Marchi T, Rossi RP, de Godoi V, et al. (2010). Effects of low-level laser therapy (LLLT) in the development of exercise-induced skeletal muscle fatigue

and changes in biochemical markers related to postexercise recovery. Journal of Orthopedic and Sports Physical Medicine 40, 524-532.

Nguyen-Thanh HT, Benzaquen BS (2009). Screening for subclinical coronary artery disease measuring carotid intima media thickness. Am J Cardiol. 104(10):1383-8.

Schiffer F, Johnston AL, Ravichandran C, Polcari A, Teicher MH, Webb RH, Hamblin MR (2009). Psychological benefits 2 and 4 weeks after a single treatment with near infrared light to the forehead: a pilot study of 10 patients with major depression and anxiety. Behavioral and Brain Functions 5: 46-59.