Investigation of Low-intensity Focused Ultrasound Parameters
NCT: NCT05134233
09/12/2024

INSTRUCTIONS:

- *Use this "TEMPLATE PROTOCOL (HRP-503)" to prepare a study protocol outlining your research plan.*
- *Depending on the nature of your study, some major sections might not be applicable to your research. If so, simply mark as "N/A." For example, a simple survey might have many sections with "N/A." For subsections (e.g., 1.x or 8.x) you can mark as "N/A"if you are certain that the subsection is not applicable.*
- *Once the IRB/HRPP approves your submission, your latest approved version of the protocol will be stored in the IRB Protocol Management online system.*
- *If your research plan changes and you need to modify the protocol, please submit an amendment to Protocol Management with the requested modifications. Download your current protocol from Protocol Management and indicate the changes/revisions using the track changes feature in order to make review of the modifications easier to follow. If you are unable to use track changes, please create a new paragraph wherever you need to make a change, and indicate "Amendment: Date" before making a change to any section. Protocol management will store the older versions of your protocol if the IRB or HRPP staff need to compare them during the review.*

PROTOCOL TITLE:

Include the full protocol title.

Investigation of low-intensity focused ultrasound parameters

PROTOCOL NUMBER:

Include the number assigned in Protocol Management (verify this has been added before submitting protocol to HRPP).

21-882

PRINCIPAL INVESTIGATOR:

Full Name and Degrees: Wynn Legon PhD
Department: Fralin Biomedical Research Institute at VTC
Telephone Number: 540-526-2184
Email Address: wlegon@vt.edu

FUNDING:

Sponsor(s): N/A
Funded already or in the proposal phase?: Proposal phase
Is Virginia Tech the primary awardee or the coordinating center of this grant or contract? If not, list the primary institution: Yes

VERSION NUMBER/DATE:

Include the version number and date of this protocol. Versions should start at 1.0.

1.1

REVISION HISTORY:

Use this table to keep track of changes. Add more rows as needed.

Revision #	Version Date	Brief Summary of Changes (i.e., the different sections)	Consent Change?
1	01/31/2022	Section 7 & 8, added physiology measurements, added set of questionnaires. Section 15, timeline/compensation from questionnaire addition. Section 20, added BDI risk.	Y
2	07/28/2022	Adding respiratory and ECG physiology collection. Section 10, added that participant will be offered a copy of their MR images on CD.	Y
3	01/11/2023	Adding foreign participant payment details & FDA NSR determination	Y
4	05/16/2024	Adding menstruation questions	N

Table of Contents

1.0 Study Summary

Study Title	Investigation of low-intensity focused ultrasound parameters
Study Design	Systematic study of set parameters in a multiple visit study
Primary Objective	Understanding the interaction of varying ultrasound parameters
Secondary Objective(s)	Document the tolerability of the parameters
Study Population	Healthy volunteers aged 18-65
Sample Size	80
Research Intervention(s)/ Investigational Agent(s)	Low-intensity focused ultrasound, Transcranial magnetic stimulation, EMG will be used to record motor responses.
Study Duration for Individual Participants	7 total study sessions, ~2.5 hours per session, for a total of ~17.5 hours.
Acronyms and Definitions	LIFU = low-intensity focused ultrasound TMS = transcranial magnetic stimulation MRI = magnetic resonance imaging CT = computed tomography EMG = electromyography ECG = electrocardiogram

2.0 Objectives

2.1 Describe the purpose, specific aims, or objectives of this study:

An understanding of the interaction of ultrasound parameters on human brain function is critical to rationally design clinically useful therapeutic methods that result in predictable outcomes. Experiments in small and large animal are confounded by small cranial vault size, anesthesia and thinner and differently shaped skulls making translation to human uncertain. Human studies remove translation and scaling issues and can be directly related for clinical use. Here, we will systematically test ranges of ultrasound parameters (intensity, duration, duty cycle, pulse repetition frequency) in a fully parametric design delivered to the primary motor cortex (M1) in healthy human volunteers. This will allow for an evaluation of what parameters change effect (and by how much), and determine how they interact with other parameters. Studies will be conducted in a cortical brain area using different outcome measures to test robustness and reproducibility.

2.2 State the hypotheses to be tested:

We hypothesize that duty cycles between 10 and 30% will result in inhibition and duty cycles > 50% will result in excitation independent of intensity and duration. We further hypothesize that longer durations will result in inhibition whereas shorter duration will

results in excitation. Finally, we hypothesize that 1000 Hz is the optimal PRF for these effects.

3.0 Background

3.1 Summarize the relevant prior research on this topic and gaps in current knowledge within the field of study:

Ultrasound has a rich parameter space. In addition to the fundamental frequency, parameters include intensity (directly relates to pressure), duration, number of cycles (relates to pulse duration), duty cycle and pulse repetition frequency (PRF). There are multiple studies in small and one in large animals explicitly testing how different parameters affect neuromodulation. King et al. (in mice) found that the product of intensity and duration best predicted success rates, Kim et al. (in rats) found a strong inter-dependency on duration and duty-cycle such that 300 milliseconds of LIFU at 50% duty cycle was most effective, and Yoo et al. (in rabbits) found PRF interacted with duty cycle such that high duty cycles (50%) at low PRFs (10 Hz) resulted in excitation whereas higher PRFs (100 Hz) at low duty cycle (5%) resulted in inhibition. In large animal (sheep), Yoon et al. reported inhibition was best achieved with a 3 and 5% duty cycle at PRFs of 60 and 100 Hz respectively and that 0.5 milliseconds at 70% duty cycle to be most effective for excitation. There are now several studies in primate and human showing neuromodulatory effect, though parameters have not been explicitly been tested.

Additionally, we have conducted a retrospective study of symptoms and safety in TMS and LIFU, which reported no serious adverse events in any of the studies monitored (Sci Rep 2020 Legon).

3.2 Describe any relevant preliminary data:

Primary motor cortex. We have collected N = 6 healthy participants under a full-parametric design looking at 3 intensities (6 W/cm2, 12 W/cm2, 24 W/cm2 Isppa in the head), 6 duty cycles (1%, 5%, 10%, 30%, 50%, 70%), 3 durations (100 msec, 500 msec, 1000 msec) and 4 PRFs (10 Hz, 100 Hz, 500 Hz, 1000 Hz) delivered to the primary motor cortex using concurrent and concentric TMS/LIFU (Sci Rep 2018 Legon). Experiments were conducted at the University of Minnesota and University of Virginia, and reviewed by the corresponding university IRBs. Preliminary analysis revealed duty cycle to be a critical parameter (DC 30% = inhibition of ~10-15%; DC 50 – 70% = facilitation of ~15 – 25%). Interestingly, duty cycle does not look to interact with intensity but does so with duration. These effects were independent of PRF. The results have similarities to a theoretical model that takes into consideration how pressure affects cell membranes (eNeuro 2016 Plaskin).

3.3 Based on the existing literature, provide the scientific or scholarly rationale for and significance of your research and how will it add to existing knowledge:

Neurological and psychiatric diseases are on the rise and present an immense challenge for health services globally. Current treatment methods are limited in their efficacy, particularly for patients with advanced or drug-resistant disorders, leaving a critical need to develop effective new treatment pathways. Low-intensity ultrasound is a new method of brain modulation that can be focused anywhere in the brain with precise targeting to alter brain activity and potentially repair dysfunctional brain circuits. Before this promise can be fulfilled, the development of tools and methods to aid in precise and predictable energy delivery are critical. This project will develop new means for precise, predictable delivery and determine effective ultrasound parameters for robust and reproducible neuromodulation. This information will help to progress ultrasound as a future targeted therapeutic that could be useful for a range of psychiatric and neurological disorders.

4.0 Study Endpoints

*4.1 Describe the primary and secondary **study** endpoints. See links below for discussion of study endpoints and how they may differ from study objectives. These are most common in clinical trials but are sometimes applicable to other types of biomedical research, as well as social, behavioral, or educational research. See link below for a discussion.*

https://docs.google.com/document/d/1Wocz7K7a0hCQJPPO_khh5l1SQQjhGDDGHzcOPRHR5Tw/edit?usp=sharing

Primary endpoint: Waveform combinations that induce reliable excitation and inhibition

Secondary endpoint: Documentation of tolerability of waveforms

*4.2 Describe any primary or secondary **safety** endpoints. These should be included for all studies that are greater than minimal risk. (Minimal risk: The probability and magnitude of harm or discomfort anticipated in the research that are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests.)*:

Primary safety endpoint: Subjective report questionnaire on neurological/physiological feelings.

Participants can request study procedures be stopped at any point throughout the experiment.

Based upon previous literature from our lab in humans we expect the LIFU effect to last up to 30 minutes. There is no evidence as yet that the proposed administration lasts longer than 30 minutes. All participants will be involved in laboratory procedures during this interval and be queried via the attached Report of Symptoms Questionnaire for any signs or symptoms from the LIFU intervention pre and post intervention.

Post-stimulation Monitoring:
Participants will be monitored for at least 30 minutes after stimulation has ended. Participants will complete a Report of Symptoms questionnaire prior to leaving the facility. Responses will be monitored with each given session and the change of any symptom to 'severe' on the post-stimulation questionnaire compared to the pre-stimulation questionnaire will require PI evaluation.

5.0 Study Design and Statistical Analysis Plan

5.1 Describe the basic study design/approach (e.g., qualitative study using five focus groups of first year students to describe assimilation into the university community; randomized controlled trial of a behavioral change intervention to increase dietary intake of whole grains; pre- post-test evaluation of new pedagogical techniques to improve adult literacy):

A total of 80 participants will be enrolled in study procedures. We will examine the interaction of ultrasound parameters through repeat testing of set parameters of low-intensity focused ultrasound (LIFU) while administering transcranial magnetic stimulation (TMS) to generate motor evoked potentials (MEP) which will be detected by electromyography (EMG). This study will be collected through multiple study visits. Visit 1 includes anatomical scans which will include 3T MRI and CT scans. Additional study visits will consist of LIFU/TMS parameter testing. It is anticipated participants will complete 7 study sessions, depending on participant availability.

5.2 Describe corresponding data analysis plan/approach (e.g., content analysis of focus group transcripts; descriptive analysis followed by linear regression modeling; nonparametric analysis of pre- and post-test measures):

MEP and SEP amplitude data will be analyzed separately using 4-way ANOVAs (intensity ×3, duty cycle ×6, duration ×3, PRF ×4) to determine significant main effects and interactions. Interactions will be decomposed using appropriate post-hoc examination (2/3-way ANOVA, t-tests, Tukey etc.). We will also employ stepwise regression. Iterative linear, quadratic and polynomial models will be tested for fit. Phase-plane diagrams will be produced for significant models.

6.0 Setting

6.1 Describe the sites or locations where your research team will conduct the research. Consider each of the items listed below:

- *Identify where your research team will identify and recruit potential subjects.*
- *Identify where the team will perform the research procedures.*
- *Describe the composition and involvement of any community advisory board(s).*
- *For research conducted in other locations, describe:*

- *Site-specific regulations or customs affecting the research at those locations.*
- *Local scientific and ethical review structure at those locations. Examples include work in other cultures or ethnic groups (within or outside of the U.S.) and work with churches. The HRPP will provide additional guidance for international research.*

Participants will be recruited from the general public in Roanoke, VA and surrounding areas. Participants will be recruited independently of other studies being conducted in the Legon lab.

All research procedures will be conducted at the Fralin Biomedical Research Institute at VTC in Roanoke, VA.

7.0 Study Intervention(s)/Investigational Agent(s)

7.1 Describe the study interventions (including behavioral interventions) and/or investigational agents (e.g., drugs or devices) to be used in this study. Consider each of the items listed below:

- *Drug/Device Handling: If the research involves drugs or devices, describe your plans to store, handle, and administer the drugs or devices so that they will be used only on subjects, and only by authorized investigators.*
- *Describe whether any of the following will be used: microwaves, X-rays, DEXA scans, general anesthesia, or sedation*
- *If control of the drugs or devices used in this protocol will be accomplished by following an established, approved organizational SOP (e.g., Research Pharmacy SOP for the Control of Investigational Drugs, etc.), please reference the SOP in this section.*

LIFU: The primary procedure involves the use of a Low-intensity focused ultrasound (LIFU) device. Ultrasound energy will be used. The device can only be activated by a computer controlled by the researchers. Study staff will be properly trained to operate this device.

TMS: The primary procedure involves the pairing of LIFU with TMS. A stimulating coil produces an electric current via electro-magnetic induction of a specific brain region to cause MEPs. Study staff will be properly trained to operate this device.

MRI: Participants will also undergo an MRI scan. Staff will review safety issues related to MRI with the subject and answer any questions. The MRI scanner used has a magnet strength of 3T, which has been approved for clinical use by the FDA. Participants will be able to communicate with study staff via an intercom. Staff operating the MRI have been trained to do so through both classroom and hands on education.

CT: Participants will undergo a head CT to obtain necessary images of their skull. CT scans will be a low-dose scan with expected radiation dose of about 2mSv. This dose gives a lifetime cancer risk of about 1 in 10,000. The lifetime natural incidence rate is about 1 in 2. Equipment will be operated by trained personnel only.

Stereotaxic Neuronavigation: Stereotaxic neuronavigation is the process of using a subject-specific anatomical MRI/CT images and co-registering them to their actual head and the LIFU transducer and TMS coil for real-time navigation of the treatment. The subject is fitted with a head-band that contains 3D infrared tracking bulbs. The LIFU transducer is also fitted with these tracking bulbs. Both the subject and treatment device are then tracked in real-time using an infrared camera. This procedure allows for precise targeting of specific brain regions using the subject-specific MRI and is wholly noninvasive. This procedure is ongoing throughout the experiment during the LIFU and TMS intervention.

7.2 List the name of all drugs (including any vitamins, supplements, herbs, or nicotine) to be used in the study. Indicate whether they have FDA approval, and list any limitations for their use:

N/A

7.3 List all devices, how they will be used, their purpose in the study, and if they will be used in a manner consistent with their approved uses. If they will be used in ways that are not yet FDA approved, indicate whether they need an IDE or a determination that they are exempt from the IDE Determination. If a determination of significant risk or non-significant risk is needed for any of the devices, include the researcher's recommendation for each of those devices:

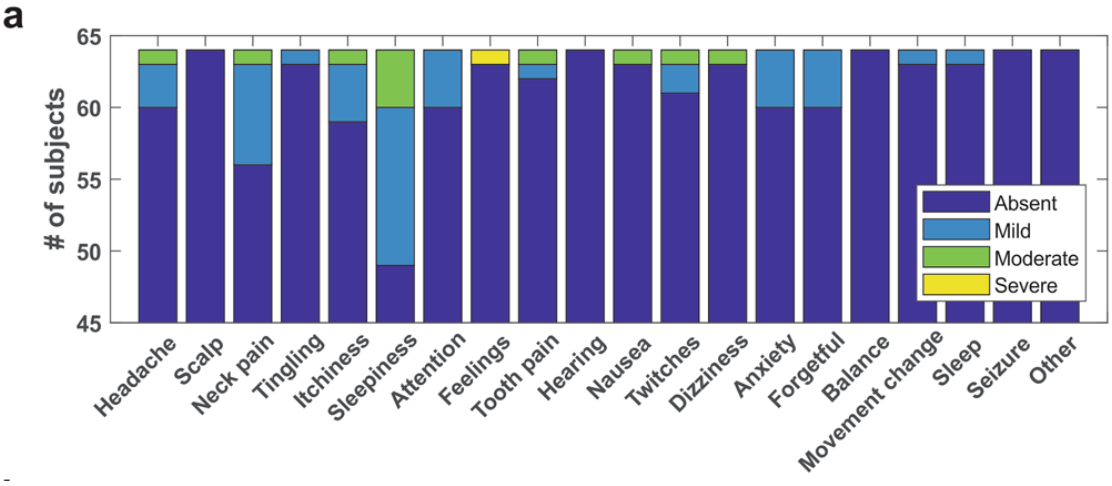
LIFU: In its current state, ultrasound for neuromodulation follows the safety guidelines of the Food and Drug Administration (FDA) for obstetric diagnostic ultrasound and adult cephalic applications. The device is not FDA approved for neuromodulation. . The device has been deemed a nonsignificant risk (NSR) by the FDA. These include derated limits of spatial peak pulse average (Isppa) of 190 W/cm2, a spatial peak temporal average of 720 mW/cm2 (94 mW/cm2 for adult cephalic) and a mechanical index (MI = peak negative pressure/√fc) of 1.9. MI is an indication of the ability to produce cavitation related bio-effects and can be used as an indication for potential micromechanical damage. We will use less than 50 W/cm2 (4x lower Isppa) for these studies.

In addition to settings listed above, the following parameters are to be tested in this study. 3 Intensity (I) settings (6, 12, and 24 W/cm2), 6 duty cycle (DC) settings ranging from 1-70%, 3 duration (Dur) settings ranging from 100ms-1s, and 4 pulse repetition frequency (PRF) settings ranging from 10-1000Hz. As this is a parametric design, we are testing 216 ultrasound settings. Though each setting will take less than 1 minute to test, testing is

expected to be divided between 6 study sessions (up to 36 per session) to reduce burden to the participant.

These levels of energy are adhered to in human ultrasonic neuromodulation studies though there are no specific guidelines for energy deposition for ultrasonic neuromodulation and indeed, explicit expository and dosimetry for diagnostic and therapeutic applications, despite their prevalence and long history, has proven challenging and is still largely lacking. Ultrasound for transient neuromodulation is different from the use of ultrasound for surgery where very high intensities are used to thermally ablate tissues or for blood-brain barrier (BBB) opening where high intensities are also used in combination with contrast agents to intentionally produce cavitation as a means of opening the BBB. To date, a total of N = 179 in 8 published studies of healthy neurologically intact participants (plus one 1 TBI patient) have undergone a variant of LIFU for neuromodulation with no published report of severe adverse events. We have published on minor adverse events from LIFU (Legon et al. 2020).

We recently compiled a qualitative report of side effects and symptoms from a subset of participants (N = 64) that have participated in one or more of our LIFU studies. We queried participants about their experience/tolerability with LIFU and any symptoms they experienced immediately after the intervention and also out to 22 months in a sub-cohort. There were no reports of any serious adverse events. The most common reported side effects were sleepiness, mild neck pain, headache and scalp tingling (Figure). These extinguished rapidly and no new symptoms were reported upon follow-up out to 1 month. We subsequently compared this side effect profile to other forms of non-invasive neuromodulation such as TMS, deep TMS and transcranial direct current stimulation (tDCS). We found the symptom profile to be similar to other forms of non-invasive neuromodulation. Over 300 healthy volunteers have undergone a variant of LIFU to different brain regions demonstrating that LIFU is safe and effective for transient non-invasive neuromodulation in humans.



-

Figure Preliminary self-report of symptoms in N = 64 healthy volunteer participants in LIFU studies in our lab (Legon et al. 2020). 'Feelings' refers to the question asking about experiencing any unusual feelings, attitudes or emotions. Please note the instance of severe 'feelings' in one individual was followed up by a neurologist and deemed unrelated to the intervention. Data excerpted from Legon et al. (2020)

As currently employed, human neuromodulation with LIFU typically involves coupling a single (or multiple) focused single-elements usually in the ~ 250 to 600 kHz range (for efficient energy transfer through skull) to the scalp to target a desired cortical brain region. Transducers for cortical targeting are generally small (~ 30 mm diameter with a ~ 30 mm focal length); produce a ~ 3-4 millimeter lateral and ~1 – 2 centimeter axial resolution, and can be placed anywhere on the head similar to other current methods of noninvasive brain stimulation.

Ultrasound is also capable of reaching brain targets deep to the cortical surface as the acoustic waves can be focused to any desired depth with certain limits. Transducers for deep brain modulation are typically larger (~ 70 mm diameter) to achieve this deeper focal length at reasonable axial resolutions. In addition to adjusting focal lengths, there are a number of parameters that can be manipulated when using ultrasound including the acoustic frequency, amplitude, duration, duty cycle, pulse repetition frequency etc. and the efficacy of some of these for successful neuromodulation has been addressed in small animal studies though the precise mechanism of acoustic energy for neuronal modulation is largely theoretical and the impact of parameter space in humans is not yet well-described. The bioeffects of ultrasound for neuromodulation in humans as described here is likely largely mechanical as opposed to thermal as the parameters used are of low intensity and duration and do not generate sufficient temperatures for thermal modulation. As described, our study does not meet any of the definitions of a significant risk device as outlined by the FDA or VT and therefore does not require an IDE.

TMS: Transcranial Magnetic Stimulation (TMS) is a neurophysiologic device that allows for non-invasive stimulation of the human brain (Rossi et al. 2009). We use the (Magstim 2002, Magstim Inc. Morrisville NC, USA). TMS is minimal risk as deemed by the FDA. Possible adverse risks are described in risk section below. During TMS, the stimulating coil is held over a subject's head and produces an electric current in the subject's brain via electro-magnetic induction. Single-pulse TMS is used to probe the state of the motor system. The TMS coil is placed over the motor cortex and a single-pulse (120% of individual maximum threshold) is conducted. This is repeated 10 – 20 times at an inter-stimulus-interval (ISI) of 5 – 10 seconds. The FDA approved TMS parameters for the treatment of major depression is 10Hz stimulation with 120% motor threshold; 4-second pulse trains; 26-second intertrain intervals, and each treatment session is last for 37.5 minutes, this is a total of 3,000 pulses. The proposed TMS parameters will stay below parameters for safety consideration.

LIFU and TMS have been paired in numerous studies in the past, resulting in no serious adverse events. Transcranial focused ultrasound neuromodulation of the human primary motor cortex (Sci Report 2018 Legon et al), Systematic examination of low-intensity ultrasound parameters on human motor cortex excitability and behavior (Elife 2020

Fomenko et al), and Time course of the effects of low-intensity transcranial ultrasound on the excitability of ipsilateral and contralateral human primary motor cortex (NeuroImage 2021 Xia et all).

MRI: Participants will receive an MRI scan. The Siemens 3T scanner has been approved by the FDA and will be used in a manner consistent with approval.

CT: Participants will receive a CT scan of their head. The Somatom Confidence RT Pro scanner has been approved by the FDA and will be used in a manner consistent with approval.

In addition to study interventional devices listed above, the following device is utilized to obtain subject data throughout the experiments.

EMG: Electromyography (EMG) involves attaching disposable surface electrodes to the skin overlying muscles of interest of the hand/ forearm in order to record muscular activity. Identification of the muscle is achieved by asking the participant to hold a light tonic contraction. The skin is prepared with a mild abrasive gel to remove surface debris and subsequently disinfected with rubbing alcohol. EMG electrodes are held in place with double-sided tape. We utilize the BrainSight device from Rogue Research.

BP: Blood pressure will be measured using the QardioArm blood pressure monitor. This is a noninvasive smart blood pressure sleeve that is placed around the arm at the bicep and is controlled through a phone app. The QardioArm is FDA approved for BP monitoring.

GSR and PPG: The galvanic skin response (GSR) sensor is used for real-time GSR biofeedback. The Shimmer GSR+ sensor monitors skin conductivity noninvasively between two reusable electrodes attached to two fingers of one hand. A stimulus causes the sweat glands to become more active, increasing moisture on the skin and allowing the current to flow more readily by changing the balance of positive and negative ions in the secreted fluid (increasing skin conductance). The Shimmer GSR+ unit also collects PPG data via an optical pulse sensor attached to a finger or earlobe.

ECG: Two ECG leads will be applied, to collect an electrocardiogram (ECG). This will noninvasively record electrical heart activity. This device will be operated by trained study staff.

Respiratory: A commercial respiration belt (SleepSense) will be wrapped around the chest to collect continuous respiration data. The belt uses Velcro closure and is noninvasive.

7.4 If the drug is investigational (has an IND) or the device has an IDE or a claim of abbreviated IDE (non-significant risk device), include the following information:

- *Identify the holder of the IND/IDE/abbreviated IDE.*

- *Explain procedures followed to comply with sponsor requirements for FDA regulated research for the following:*

	Applicable to:		
FDA Regulation	***IND Studies***	***IDE studies***	***Abbreviated IDE studies***
21 CFR 11	***X***	***X***	
21 CFR 54	***X***	***X***	
21 CFR 210	***X***		
21 CFR 211	***X***		
21 CFR 312	***X***		
21 CFR 812		***X***	***X***
21 CFR 820		***X***	

N/A

8.0 Procedures Involved

8.1 Describe and explain the study design:

This is a systematic study of set parameters in a multiple visit study.

Session 1: MRI and CT anatomical scans

Session 2-7: LIFU parameter testing with TMS and EMG while utilizing neuronavigation and physiological data collection such as PPG, BP, ECG, Respiration, and/or GSR.

8.2 Provide a description of:

- *All research procedures being performed*
- *If the study has more than one procedure, session, and/or subject population, describe each procedure, session, and/or study population separately. For complex studies, you are encouraged to include a figure or chart.*

Recruitment and Screening: Individuals indicating interest in participating in this research study will complete a screening and demographic form as part of the screening process (see Safety Screening Form and Demographic Form). This information will be reviewed to determine eligibility and to screen for any contraindications to MRI, CT, and TMS.

Consent: Participants will be asked to sign a consent form upon arrival for their first study visit. Participants will have as much time as they need to review the consent form and may ask questions of the PI or study team prior to participation.

Pregnancy Screening: Prior to any study procedures, female participants will provide a urine sample which will be screened for pregnancy. As indicated in the consent form, if

testing indicates the participant is pregnant, they will be notified and excluded from the study.

Participants will be healthy adults age 18-65 with no contraindications to MRI, CT, or TMS. We will enroll approximately 80 individuals.

This study includes low-intensity focused ultrasound (LIFU), transcranial magnetic stimulation (TMS), magnetic resonance imaging (MRI), and computed tomography (CT). Additionally, stereotaxic neuronavigation is utilized.

Study Procedures:

Session 1	Session 2-7
Imaging	36 Parameter Trials
MRI & CT	LIFU + TMS
2.5 hours	2.5 hours

*parameters per session will be chosen randomly within the set values

Session 1 - An MRI and CT exam will be performed and questionnaires will be administered.

Sessions 2-7 - Utilizing imaging collected on previous visit, subjects will complete a testing session. These sessions will involve LIFU and TMS stimulation. Additionally EMG, PPG, BP, ECG, Respiration, and GSR will be collected. Questionnaires will be administered at each session.

Follow up - An electronic questionnaire of Report of Symptoms will be sent about 1 week following final study participation. If requested, this questionnaire can be completed verbally on the phone with study staff.

MRI session: Participants will receive an MRI scan to obtain a high-resolution image of their head to be used for proper setup of LIFU parameter sessions. During the MRI session, the experimenter and participant are able to communicate with one another at all times, ensuring the comfort of the subject. In addition, participants are provided with an emergency ‘squeeze bulb’ which can terminate the scanning procedure at any time.

CT session: Participants will receive a CT scan to obtain detailed imaging of the skull. CTs are collected as this is the only current option to properly image the skull. This is necessary for our computer models of acoustic wave propagation to ensure accurate intracranial intensities for each individual.

Stereotaxic neuronavigation: Stereotaxic neuronavigation allows us to track the positioning of the ultrasound transducer on the subject's head in real time using the MR/CT images for guidance.

LIFU: Low-intensity focused ultrasound (LIFU) is a method of brain neuromodulation using ultrasonic acoustic energy. An ultrasound transducer is placed on the participant's head and is tracked using the neuronavigation system. Brief ultrasound pulses are delivered to the desired brain area. A small amount of ultrasound gel will be placed on the face of the single-element focused ultrasound transducer. The transducer will then be fitted on the scalp over the desired brain area and held in place with a mechanical arm. Brief pulses (0.1-1.0 seconds; duty cycle 1 – 70%; pulse repetition frequency 10 – 1000 Hz) of low-intensity (< 50 W/cm2 Isppa), sub-thermal ultrasound (0.3 - 0.5 MHz) will be delivered in order to determine the area of activation, as well as the patterns of brain activity generated. Subjects may experience a mild buzzing sensation on their scalp depending upon stimulation parameters used. Participants will be familiarized with the sensations before formal testing. The ultrasound intensity used will be below the intensities recommended for the safe use of ultrasound by the FDA (190 W/cm2 Isppa) and will be brief in duration at these low-intensities as not to generate any heating. While no discomfort is anticipated, participants will be informed if they experience any warming sensation on their scalp or discomfort to notify the investigator and the procedure will be immediately terminated. Participants may be given ear plugs to wear during the procedure and/or a white noise machine may be utilized in the testing space. This is to provide ear protection and noise cancellation to reduce participant focus on LIFU device function. We have significant experience using LIFU including our published reports of human neuromodulation experiments.

TMS: transcranial magnetic stimulation (TMS) is a noninvasive method of stimulating the brain using magnetically induced electrical currents. The stimulating coil is placed over a subject's motor cortex and a single pulse is conducted. This is repeated 10-20 times at an inter-stimulus interval of 5-10 seconds per trial. A threshold setting will be determined for each participant before each study session to obtain an average motor evoked potential of approximately 0.75mV, measured with EMG recordings. This will be the setting used throughout the testing session.

EMG: Electromyography (EMG) involves attaching disposable surface electrodes to the skin overlying muscles of interest of the hand and forearm in order to record muscular activity. Identification of the muscle is achieved by asking the participant to hold a light tonic contraction. The skin may be prepared with a mild abrasive gel to remove dead skin and surface debris and subsequently disinfected with rubbing alcohol. EMG electrodes are held in place with double sided tape. This signal is used to detect LIFU effects and TMS amplitude.

PPG, GSR, ECG, BP, Respinration: Photoplethysmograph (PPG) and galvanic skin response (GSR) will be collected using a lightweight wearable Shimmer3 GSR+ unit and blood pressure will be collected using a wireless blood pressure monitor (QardioArm). A commercial respiration belt (SleepSense) will be wrapped around the chest to collect continuous respiration data. Electrocardiogram (ECG) will be collected using two leads. This data is collected to assess for any effects of ultrasound but more importantly as a monitor of the state of the participants' autonomic nervous system as a potential contributor/confound that may affect responses.

Questionnaires: Questionnaires will be collected either electronically using a VT approved platform such as Redcap, or on paper. The following questionnaires will generally be completed during the imaging and consent visit but may be completed at following visits if time does not allow during the first visit: Beck Depression Inventory-II (BDI), Beck Anxiety Inventory (BAI), State Trait Anxiety Inventory (STAI), International Physical Activity Questionnaire (IPAQ), Medical Outcomes Survey Short Form-8 (SF-8), Sleep Scale from the Medical Outcomes Study, Patient Health Questionnaire-2 (PHQ-2), Generalized Anxiety Disorder 2-item (GAD-2), Tobacco, Alcohol, Prescription medications, and other Substance Tool (TAPS), and Perceived Stress Scale (PSS). Questionnaires may be used as a means of documenting potential confounds such as anxiety, fear, depression, etc. Questionnaires are estimated to take 30-45 minutes to complete. Additionally, the STAI (state only), Experience Questionnaire, Daily Questionnaire, and Report of Symptoms questionnaires will be administered at all stimulation sessions (sessions 2-7). The Daily Questionnaire exists to help quantify any variance between participant sessions. This includes items such as amount of sleep, hormone changes, and caffeine intake. Lastly, subjects will be contacted up to one month following the final session to complete the participant Report of Symptoms questionnaire. All questionnaires are attached to this application.

8.3 Describe:

- *Procedures or safeguards intended to reduce the probability and magnitude of risks. (For example: Reducing the risk of injury in a virtual reality study either by having the subjects sit during the study or by providing an obstacle-free space for walking.)*
- *Be sure to describe all drugs and devices used in the research, when they will be administered or used, and their purpose.*
- *Methods used to collect data about subjects. Please upload all data collection forms to Protocol Management. Some common examples are:*
  - *Screening questionnaires*
  - *Survey(s), including online surveys*
  - *Demographic questionnaire(s)*
  - *Interview guide(s), e.g., questions or pool of questions for semi-structured interviews*
  - *Focus group guide(s)*
  - *Other documents used to collect data*

Every effort will be made to ensure the subject's comfort and to reduce any risk. Subjects will be thoroughly screened to ensure that MR and CT contraindications are not present. All females will be screened for pregnancy. If, at any time, subjects experience discomfort, dizziness, or claustrophobia they will be able to discontinue immediately.

During MRI scanning subjects will have access to an emergency squeeze bulb they can indicate at any time. Staff operating the MRI undergo Advanced MR/Operator Training. This is required for persons wishing to conduct research on the Human Neuroimaging Lab (HNL) MRIs. Training consists of presentations, observation, and hands-on practice. Topics include safety and emergency procedures, subject screening and preparation, scanner set-up and operation, and troubleshooting. Each person must also complete at least 4 hours of observation (to include 2 different studies) and at least 16 hours of supervised scanning (to include 4 different studies). Certification is granted upon completion of the training and passing of a competency exam.

During CT scanning subjects will be in communication with the trained technician. A trained and certified radiology technician will operate the CT.

During LIFU stimulation sessions participants will be in constant communication with the study staff. Staff operating the LIFU device, TMS device, and EMG/PPG/BP/GSR/ECG/Respiratory recording device will undergo training under the direction of the PI. Additionally, participants will be reminded they can terminate the session at any time. Screening for side effects will take place pre and post all stimulation sessions.

Methods used to collect data:
We will collect questionnaires and screening information (see attached forms and questionnaires) using an electronic Virginia Tech approved system such as Redcap or Qualtrics, or via paper form.

The MRI and CT will be used to collect brain images, and EMG will be used to collect muscle contraction signals. Standard devices will be used to collect PPG, BP, ECG, Respiration, and GSR data. All data will be stored on FBRI servers with the subject coded identifier.

8.4 What data will you collect during the study and how you will obtain them? Please include descriptions of electronic data collection, database matching, and app-based data collection:

Images will be obtained of the subjects' head/brain from MRI and CT scans. EMG signals will be collected using standard EMG equipment. Photoplethysmograph (PPG), blood pressure, heart rate, respiration, and galvanic skin response data will be collected using standard equipment. Electronic data such as questionnaires or screening forms may be collected through secure web-based survey tools, such as RedCap, provided by Virginia Tech. The Daily Questionnaire exists to help quantify any variance between participant sessions. This includes items such as amount of sleep, hormone changes, and caffeine intake. Additionally, questionnaire or screening data may be recorded on paper, entered into a digital database and then stored securely. All data will be stored on secure FBRI servers.

8.5 Who will transcribe or code audio and/or video recordings?:

N/A

8.6 Include a description of any deception to be used in the study. Include justification for the use of deception (why the deception is necessary), describe the debriefing process, and describe how the study meets <u>all</u> the following criteria for alteration of consent (deception is considered an alteration of informed consent):

- *The research involves no more than minimal risk to the subjects*
- *The alteration will not adversely affect the rights and welfare of the subjects*
- *The research could not practicably be carried out without the alteration/deception*
- *(Optional but encouraged in most cases) Subjects will be provided with additional pertinent information after participation (i.e., debriefing for studies involving deception)*

N/A

8.7 If the study involves long-term follow-up (once all research related procedures are complete), describe what data will be collected during the follow up period and when it will occur:

N/A

9.0 Data and Specimen Long Term Storage and Use

9.1 If you will store data or specimens for future use, describe where you will store the data or specimens, how long they will be stored, and how and by whom the data or specimens will be accessed:

All data collected from subjects will be assigned a coded designation. MRI, CT, EMG, and behavioral data will be stored on secure FBRI servers indefinitely. All of this data will have no identifying information. Only the code will link it to the PII of the subject. The de-identified data will be made available to authorized study staff, and may be stored elsewhere such as Dropbox, iCloud, or a local drive on a desktop or laptop. Further, after publication of results, de-identified data may be made available to the public in accordance with funding and publication requirements, or otherwise.

9.2 For specimens, list the data to be stored or associated with each specimen:

N/A

9.3 Describe the procedures to release data or specimens outside of the research team, including the process to request a release, approvals required for release, who can obtain data or specimens, and what data will be provided with specimens:

Prior to publication of results, de-identified data will be made available to study staff as listed on the approved IRB. After publication, de-identified data may be made public as required by funding sources, journals, or otherwise.

9.4 Describe the identifiers to be included with stored data or specimens, as well as any key or code that could be used to make them identifiable. Describe where the code will be stored, who will have access to it, and when it will be destroyed:

Data will be coded using a randomly generated identifier. The study key linking name to identifier is stored digitally under password access, only accessible by authorized members of the research team.

Participant Social Security Number (or Taxpayer ID) and street address are collected in order to compensate participants, via W9 form or W-8BEN. This information is entered into the ClinCard system, which is a HIPAA compliant payment system approved by Virginia Tech. After entry the form is stored by central administration at Virginia Tech to be used if it is required. Study staff do not store documents with SSN or address long term. International participants will also be asked to provide a copy of passport, visa documents, any work authorizations, as well as the Foreign National Data Form.

Documents with other identifiers, such as name, phone number, email, or elements of dates are stored separately from de-identified study data, either via encryption or in a locked file cabinet, accessible only by study staff.

9.5 Please select the identifiers you will obtain (whether directly from participants or from another source), including but not limited to:

☒	*Name*
☒	*Geographical subdivisions smaller than a state, including street address, city, county, precinct, zip code, and equivalent geocodes (note, the initial three digits of a zip code are not considered identifiable)*
☒	*Elements of dates (except year) for dates directly related to an individual, including birth date, admission date, discharge date, date of death, and single year of age over 89 and all elements of dates (including year) indicative of such age (note, such ages and elements may be aggregated into a single category of age 90+)*

☒	*Phone numbers*
☐	*Fax numbers*
☒	*Electronic mail addresses (e-mail)*
☒	*Social Security numbers*
☐	*Medical record numbers*
☐	*Health plan beneficiary numbers*
☐	*Account numbers*
☐	*Certificate/license numbers*
☐	*Vehicle identifiers and serial numbers, including license plate numbers*
☐	*Device identifiers and serial numbers*
☐	*Web Universal Resource Locators (URLs)*
☐	*Internet protocol (IP) address numbers*
☐	*Biometric identifiers, including finger and voice prints (audio recording)*
☐	*Full face photographic images and any comparable images (including video recording)*
☐	*Student record number or identification number*
☐	*User name for online or computer accounts*
☒	*Any other unique identifying number, characteristic, or code (note this does not mean the unique code assigned by the investigator to code the data)***:** Passport ID

10.0 Sharing of Results with Subjects

10.1 Describe whether you will share results (study results or individual subject results, such as results of investigational diagnostic tests, genetic tests, or incidental findings) with subjects or others (e.g., the subject's primary care physician). If so, describe how you will share the results and include this information as part of the consent document. Upload materials you will use to explain the results to subjects:

Study results will not be shared with participants directly as they are de-identified prior to analysis and/or publication. Participants will be offered a CD with raw (non-analyzed) MRI structural images, if desired. It is explained in the consent that these images are not reviewed by a physician and participants must go through their medical provider for any neurologic evaluations.

11.0 Study Timelines

11.1 Describe:

- *The duration of an individual subject's participation in the study (for example, 1 hour, 2-4 weeks, 3-5 years).*
- *The amount of time expected to enroll all study subjects (weeks, months, years, etc.)*
- *The amount of time expected for the investigators to complete this study including primary data analyses.*

Participants will be asked to participate in 7 study sessions lasting from 2-2.5 hours each. Participants will complete scanning tasks (MRI and CT), parameter tasks (LIFU/TMS), and/or questionnaires in any given session. It is anticipated that there will be a minimum of 1 week between a first and second study session to allow for image processing. Total study participation is expected to last 2-4 weeks per subject. Timeline for study data collection and primary analysis is two years.

12.0 Inclusion and Exclusion Criteria

12.1 Describe how you will screen individuals for eligibility. When will screening occur and what procedures will you use? Upload any screening scripts or surveys to Protocol Management:

Subjects will be screened with electronic screening forms prior to scheduling a study visit to determine study, MRI, and CT eligibility. Study screening forms are attached. Once consented, female subjects will be screened for pregnancy by study staff.

12.2 Describe the eligibility criteria that define who will be included and who will be excluded from enrollment for each procedure of your study. Include any geographic criteria (e.g., Virginia Tech undergraduate students, a national sample of adults with engineering degrees, minors aged 8-12 in the New River Valley, university faculty in Virginia and Paris, France):

Inclusion criteria: Healthy volunteers, age 18-65. Both females and males of all ethnicities, who understand and speak English.

Exclusion criteria:
The exclusion criteria below are regularly implemented in MRI, CT, and TMS experiments for the safety of the subjects and for data quality assurance.
1. Claustrophobia (scanning environment may be uncomfortable).
2. Contraindications to MRI: including pacemaker, aneurysm clips, neurostimulators, cochlear implants, metal in eyes, steel worker, or other implants.
3. Contraindications to CT: pregnancy.
4. Active medical disorder or treatment with potential CNS effects (e.g. Alzheimer's)
5. History of neurologic disorder. (e.g. Parkinson's, Epilepsy, or Essential Tremor)
6. History of head injury resulting in loss of consciousness for >10 minutes.
7. History of alcohol or drug dependence (through self-report).
8. Failure to provide a Social Security Number or Tax ID Number. This is required for tax purposes or payment cannot be processed.

Healthy individuals will include any persons who are not excluded based on the above listed criteria.

The minimum age for inclusion is 18 years. This is based upon literature citing that the safety of non-invasive brain stimulation in children/adolescents is unknown (Krishnan et al. 2015). There is evidence that the structural (Caviness et al. 1996) anatomy and importantly the mechanical properties of the child/adolescent brain is different from the adult (Chatelin et al. 2012). This is critical when using ultrasound as the effect of ultrasound is directly linked to the mechanical properties of tissue (Tyler 2012). Thus, studies in children/adolescents may not relate similarly to adult and thus the findings may not translate to the general population.

The maximum age for inclusion is 65 years. Non-invasive brain stimulation looks to be safe in the elderly (Krishnan et al. 2015) and may have benefit (Legon et al. 2017). However, structural anatomy in the elderly is different including enlarged ventricles and CSF space (Ambarki et al. 2010; Sullivan et al. 2002). This may increase pressure from ultrasound in the brain and/or produce cavitation and/or unwanted standing waves – all of which are potential safety hazards. Functional anatomy of the elderly brain also shows differences from the adult brain (Hafkemeiger et al. 2013; Madden et al. 1999) that may affect outcomes measures of this study and not relate to the general population. The skull of the elderly is also different in that it may contain greater calcification. This is a potential safety hazard as it can heat abnormally from ultrasound.

Chatelin, S., Vappou, J., Roth, S., Raul, J. S., & Willinger, R. (2012). Towards child versus adult brain mechanical properties. Journal of the mechanical behavior of biomedical materials, 6, 166-173.
Krishnan, C., Santos, L., Peterson, M. D., & Ehinger, M. (2015). Safety of noninvasive brain stimulation in children and adolescents. Brain stimulation, 8(1), 76-87.
Legon, W., Punzell, S., Dowlati, E., Adams, S. E., Stiles, A. B., & Moran, R. J. (2016). Altered prefrontal excitation/inhibition balance and prefrontal output: markers of aging in human memory networks. Cerebral Cortex, 26(11), 4315-4326.
Ambarki, K., Israelsson, H., Wåhlin, A., Birgander, R., Eklund, A., & Malm, J. (2010). Brain ventricular size in healthy elderly: comparison between Evans index and volume measurement. Neurosurgery, 67(1), 94-99.
Sullivan, E. V., Pfefferbaum, A., Adalsteinsson, E., Swan, G. E., & Carmelli, D. (2002). Differential rates of regional brain change in callosal and ventricular size: a 4-year longitudinal MRI study of elderly men. Cerebral Cortex, 12(4), 438-445.
Hafkemeijer, A., Altmann-Schneider, I., Oleksik, A. M., van de Wiel, L., Middelkoop, H. A., van Buchem, M. A., ... & Rombouts, S. A. (2013). Increased functional connectivity and brain atrophy in elderly with subjective memory complaints. Brain connectivity, 3(4), 353-362.
Madden, D. J., Turkington, T. G., Provenzale, J. M., Denny, L. L., Hawk, T. C., Gottlob, L. R., & Coleman, R. E. (1999). Adult age differences in the functional neuroanatomy of verbal recognition memory. Human brain mapping, 7(2), 115-135.

12.3 Indicate specifically whether you will include or exclude each of the following special populations: (You may not include members of these populations as subjects in your research unless you indicate them in the description of your subject population.)

- *Minors, as defined by state law where the study is performed (infants, children, teenagers)*
- *Pregnant women (can be included in minimal risk studies by mentioning in section 13.1)*
- *Prisoners (including all incarcerated individuals)*
- *Adults not capable to consent on their own behalf*

Pregnant women, minors, prisoners, and adults unable to consent on their own behalf will be excluded.

13.0 Vulnerable Populations

13.1 If the research involves individuals who are vulnerable to coercion or undue influence, please describe additional safeguards you will include to protect their rights and welfare. Consider the applicable items listed below:

- *If the research involves Virginia Tech students, indicate whether these are students of any of the investigators. If so, describe whether the activities will take place during class time as part of the curriculum and the steps you will take to reduce the possibility that students feel obliged to participate in order to improve their course grade. The HRPP can provide further guidance as needed. Describe whether you will request access to student records (e.g., SAT, GPA, GRE scores).*
- *If the research involves employees of Virginia Tech or the research sponsor, describe steps you will take to ensure that the employees are freely participating and describe how their data will be protected from inspection by their supervisors.*
- *If the research involves Virginia Tech NCAA athletes, you must obtain approval from the athletic department.*
- *For research involving Montgomery County Public Schools, you must obtain county approval (after obtaining contingent Virginia Tech approval). Other locales have different requirements; please check on these and describe here. Approval is typically granted by the superintendent, principal, and classroom teacher (in that order). Approval by an individual teacher is insufficient. School approval, in the form of a letter or a memorandum should be uploaded as a supporting document.*
- *If the research involves pregnant women, review "CHECKLIST: Pregnant Women (HRP-412)" to ensure that you have provided sufficient information in this protocol.*
- *If the research involves prisoners, review "CHECKLIST: Prisoners (HRP-415)" to ensure that you have provided sufficient information in this protocol.*
- *If the research involves persons who have not attained the legal age for consent to treatments or procedures involved in the research*

(minors), review the "CHECKLIST: Minors (HRP-416)" to ensure that you have provided sufficient information in this protocol.

- *If the research involves cognitively impaired adults, review "CHECKLIST: Cognitively Impaired Adults (HRP-417)" to ensure that you have provided sufficient information in this protocol.*

All subjects will be informed of the purpose of the study, the potential value to society, the lack of value to the subject personally, and all potential risks to the subject. It will be explained to the subject that they are under no obligation to participate, and that they may discontinue at any time during the study, without penalty. Employees will be told that their employment status will in no way be affected by their decision to participate in the study. Students will be advised that their decision to participate will not affect their student status, grades, coursework, or extra-curricular activities.

14.0 Number of Subjects

14.1 Indicate the total number of subjects to be enrolled and how this number was determined (e.g., sample size calculation [show], number of available subjects in a finite pool, number of tests funding award would allow):

We anticipate enrolling 80 subjects in this study. This population size should allow for adequate statistical significance and analysis.

It is anticipated that 70 percent of participants will complete the study sessions. To account for this we anticipate enrolling 80 participants to yield approximately 56 participants.

Based upon preliminary data (N = 5) and only two variables (duty cycle and duration) we found maximal changes of motor evoked potential amplitudes of 46.35% ± 12.33 and -25.54% ± 15.56 depending upon variable selection. Minimal changes with change of variables was 5.99 ± 7.87. We propose an N = 56 and while N is not sufficient to detect a statistically significantly difference of ~ 6% an N = 56 is sufficient with a power of 0.8 and a p = 0.05 to detect a ~15% that we suggest is the minimal significant change that is required for potentially clinically significant change.

14.2 If this is a multi-site study, indicate the number of subjects to be enrolled at this site and the total to be enrolled from all sites:

N/A

14.3 If applicable, indicate the number of potential subjects you expect to screen for enrollment, and the number of subjects you will need to complete the research procedures:

It is anticipated that 70 percent of participants will complete the study sessions. To account for this we anticipate enrolling 80 participants to yield approximately 56 participants.

14.4 If the study has more than one procedure, indicate the total number of subjects to undergo each procedure separately:

It is anticipated that all participants will complete all study procedures, given subject interest.

15.0 Recruitment Methods

15.1 Describe when, where, and how you will recruit potential subjects:

Subjects aged 18-65 years old will be recruited from the general population.

Interested individuals will be invited to complete an online set of screening and demographic forms using an approved VT platform such as Redcap or Qualtrics. See attached screening forms.

Subjects that meet eligibility criteria for both the study, and MRI, CT, and TMS safety, will be invited to participate and enrolled in the study.

Subjects will be recruited through flyers in the community, word of mouth, and electronic communication. For example, invitation to participate in research studies are posted on the FBRI at VTC webpage, Facebook, and Twitter. Only IRB approved images and wording will be utilized.

Communication with subjects will occur both through phone and electronic means such as email.

15.2 Describe the source of subjects (for example, clinic patients with specific conditions, students in the library, community members at a gathering, or members of a local gym):

Subjects will be recruited through the Fralin Biomedical Research Institute at VTC, as well as the general public.

15.3 Describe the methods that you will use to identify potential subjects:

Interested study volunteers will be invited to complete an electronic screening form (attached), which will be stored on a secure server. Study personnel will review

screening forms to locate eligible subjects which will then be contacted by email or phone to invite them to participate in the research.

15.4 Describe materials that you will be use to recruit subjects. Attach copies of these documents with this protocol in Protocol Management and be sure to include the IRB protocol number on each document.

- *For flyers, attach the final copy of printed flyers.*
- *For Virginia Tech News, Facebook postings and ads, newspaper ads, websites, MTurk/SONA/online survey systems, etc., attach the final wording and graphics to be used.*
- *For email recruitments, please include the subject line.*
- *For advertisements meant for audio broadcast, please submit the wording of the advertisement prior to taping (to avoid having to re-record with approved language) and submit the final recorded version for IRB review before use.*
- *Describe any compensation to subjects. Separate compensation into appropriate categories, such as: reimbursement for expenses, time and effort, and additional incentives for study participation. For each category, specify the amount (including any pro-rated amount), schedule, and method of payment.*

See attached recruitment document, flyers, and images.

Compensation: Subjects will be compensated for their time in the research study. Subjects will be compensated $20 for the first hour, and $10 for each additional half hour of study procedures after the initial hour of participation. This compensation applies for each study session, including all study procedures. In the event of a screen fail, standard compensation procedures still apply. There is an electronic questionnaire that can be completed remotely, which once confirmed is an additional $10 in compensation. Subjects will be informed of this payment scheme prior to participation. Subjects are able to withdraw at any time, without penalty.

Study payments will be loaded onto an electronic Clincard.^

Expected compensation is as follows:

Session 1 - MRI and CT scan, 2.5 hours, $50
Session 2-7 - LIFU/TMS/EMG, 2.5 hours per visit, $50 per visit
Follow up - Online questionnaire, <15 minutes, $10

Total - $360

^ International subjects will be paid via Hokiemart and processing may take a few business days. Amount of compensation will not change.

16.0 Withdrawal of Subjects

16.1 Describe circumstances under which you anticipate subjects could be withdrawn from the research without their consent:

As is standard with MRI and TMS research, if subjects experience unusual sensations, they will be withdrawn. In some cases, due to the magnetic field (as described in risks below), subjects may experience peripheral nerve stimulation, e.g. tingling or twitching. They will be withdrawn from the study if this occurs. Researchers reserve the ability to withdraw a subject at any point in the study.

16.2 If applicable, describe any procedures for orderly termination (e.g., discontinuation of a study drug or debriefing after a behavioral intervention):

N/A

16.3 Describe procedures that you will follow when subjects withdraw from the research, including partial withdrawal from procedures with continued data collection (e.g., participant declines to continue with regular blood draws, but continues with periodic behavioral questionnaires):

Subjects will be compensated for their time, as described above, regardless of completion of all study tasks.

17.0 Risks to Subjects

17.1 List the reasonably foreseeable risks, discomforts, hazards, or inconveniences to the subjects related the subjects' participation in the research. Include for the IRB's consideration a description of the probability, magnitude, duration, and reversibility of the risks. Consider physical, psychological, social, legal, privacy, and economic risks. Do not indicate "No risk" or "N/A." Instead, for studies with very low risk (e.g., anonymous online questionnaire on a mundane topic) indicate "The investigators are not aware of any risks from participation in this study." or "No more than risks than are found in everyday life." The example consent form presents a tabular method for risk information, which you can also use here. Common risk types include:

- *Physical (e.g., potential for pain, discomfort, infection)*
- *Psychological (e.g., potential for stress, discomfort, and/or embarrassment)*
- *Social (e.g., potential for discrimination or stigmatization and disruption of personal and family relationships)*
- *Legal (e.g., potential for disclosure of illegal activity, negligence)*

- *Privacy (e.g., potential for personal information being accessed, used, or disclosed without the subjects' knowledge or consent, breach of confidentiality/security)*
- *Economic (e.g., potential for individuals to lose access to economic services, employment, insurability)*

MRI Data Collection: Movement or heating of metallic implants is a potential risk; Subjects will be screened to exclude people with metallic implants, fragments, or pacemakers. Some individuals experience claustrophobic reactions in the scanner. Subjects will be informed of this prior to the study. Any subject experiencing claustrophobia will be removed from the scanner immediately. The Siemens 3 Tesla (T) scanner has been approved by the FDA. However, there may be additional risks associated with scanning at 3 T compared to the conventional clinical scanners in the 1.5-2.0 T range. These include:

1. Effect of the static field. There is no conclusive evidence for irreversible or hazardous bioeffects to acute, short-term exposures of humans up to 2.0 T (Shellock and Kanal, 1996). Studies have indicated some side effects at 4.0 T, namely unusual sensations including nausea, vertigo, and metallic taste (Schenck, 1992). However, there is no evidence that this is either irreversible or harmful. If subjects experience unusual sensations, they will be withdrawn.

2. Effect of the gradient field. MRI operates by rapidly changing small additional fields, called gradients. This will induce small electrical currents in any conductor, and thus could theoretically induce mild peripheral nerve stimulation. However, this is not substantially different at higher magnetic fields since the gradients are separate from the main magnet. There is no evidence that the effect of the gradients is any different at 3 T than at 1.5 T. However, if subjects experience peripheral nerve stimulation, e.g. tingling or twitching, they will be withdrawn.

3. Effect of the Radio Frequency (RF) electromagnetic field. The 3.0T magnetic field strength requires higher RF frequency pulses to excite the protons in the subject's brain. The limits of RF energy that humans can be safely exposed to are outlined by the FDA and the IEEE International Committee on Electromagnetic Safety: a) The exposure to RF energy below the level of concern is a Specific Absorption Rage (SAR) of 4 W/kg or less averaged over the body, and 10.0 W/kg or less spatial peak in any 10 g of tissue, and 3.2 W/kg or less average over the head; or b) The exposure to RF energy that is sufficient to result in a core temperature increase of 1 degree C and localized heating to no greater extent than 38 degrees C in the head, 39 degrees C in the trunk, and 40 degrees C in the extremities, except for patients with impaired systemic blood flow and/or perspiration (Institute of Electrical and Electronics Engineers, 2006) The scanners used have a large monitor indicating the RF power level which can be limited to a specific maximum; We will adhere to the approved limits for the head.

4. Acoustic Noise Levels. Rapid changes in the currents in the gradient coils of the MRI scanner produce significant levels of acoustic noise. The levels of noise approximately

range between 65 and 95 dB, but could have higher peaks. Communications with subjects takes place with the standard pneumatic headphones provided by the scanner manufacturer. These headphones provide some degree of noise reduction. Foam ear plugs with a Noise Reduction Rating of 31 dB will be provided.

While in the scanner, participants lie still for approximately 15 minutes so that we may take images of their brain. They will be asked to relax and remain still, thus no physical harm can reasonably be expected from this procedure, other than perhaps mild stiffness from remaining still.

All individuals who will be operating the scanners are required to pass an fMRI safety training course. However, it should be noted that over the past several years and over 3000 imaging sessions, we have had no scanner-related emergencies.

CT Data Collection: Participants will undergo a head CT to obtain necessary images of their skull. CT scans will be a low-dose scan with expected radiation dose of about 2mSv. This does gives a lifetime cancer risk of about 1 in 10,000. The lifetime natural incidence rate is about 1 in 2. Equipment will be operated by trained personnel only.

To reduce both time burden and ionizing radiation exposure, if previous MRI or CT scans have been taken with the Legon lab, they can be used in this study and the first study visit procedures may be omitted.

Low-Intensity Focused Ultrasound: Brief pulses (0.1-1.0 seconds; duty cycle 1 – 70%; pulse repetition frequency 10 – 1000 Hz) of low-intensity (< 50 W/cm2 Isppa), sub-thermal ultrasound (0.3 - 0.5 MHz) will be delivered in order to determine the area of activation, as well as the patterns of brain activity generated. Subjects may experience a mild buzzing sensation on their scalp depending upon stimulation parameters used. Participants will be familiarized with the sensations before formal testing. The ultrasound intensity used will be below the intensities recommended for the safe use of ultrasound by the FDA (190 W/cm2 Isppa) and will be brief in duration at these low-intensities as not to generate any heating. While no discomfort is anticipated, participants will be informed if they experience any warming sensation on their scalp or discomfort to notify the investigator and the procedure will be immediately terminated.

Comparison between the LIFU parameters used in proposed study to those for obstetric diagnostic imaging: The FDA guidelines for obstetric diagnostic images include derated limits of spatial peak pulse average (Isppa) of 190 W/cm2, a spatial peak temporal average of 720 mW/cm2 and a mechanical index (MI = peak negative pressure/√fc) of 1.9. This study will use less than 50 W/cm2 (4x lower Isppa) for these studies. We have significant experience using LIFU including our published reports of human neuromodulation experiments.

Transcranial Magnetic Stimulation: Single-pulse TMS is used to probe the state of the motor system. The FDA approved TMS parameters for the treatment of major depression is 10Hz stimulation with 120% motor threshold; 4-second pulse trains; 26-second

intertrain intervals, and each treatment session is last for 37.5 minutes, this is a total of 3,000 pulses. The proposed TMS parameters will stay below parameters for safety consideration. The device creates a clicking noise which can result in tinnitus. Hearing protection will be provided to participants to reduce this risk. Participants should not feel any discomfort, but will feel a small twitching movement in their hand. This may lead to residual soreness. Some people experience headaches or dizziness, which may be due to excessive muscle tension of the face and neck. Breaks will be taken where possible to reduce this tension. TMS may cause lightheadedness or fainting. We will encourage participants to eat a full meal and hydrate before study visits. There is a remote possibility that TMS can induce a convulsion even in the absence of neurological disease, epilepsy or other risk factors for seizures.

Electromyography: EMG may involve skin prep with a mild abrasive gel to remove dead skin and surface debris and subsequently disinfection with rubbing alcohol. This may cause mild skin irritation.

Due to the investigative nature of this study there may be other risks that are currently unknown.

Personally Identifiable Information (PII) Collection: There is the risk that personally identifiable information (associated with the research materials) could be compromised. Study staff will take recommended and industry approved precautions to ensure confidentiality and security of the data.

17.2 Indicate the measures you will use to minimize risks and monitor subjects for safety. (e.g., asking a subject at regular intervals to rate how they are feeling from 1 to 10, or to slowly crouch in order to check their balance.)

Participants will be screened for safety and any MRI, CT, TMS or ultrasound contraindications, including pregnancy testing. MRI and CT operators will utilize a microphone/headphone system to communicate after positioned in the scanner, while the scanner is not operating. A squeeze bulb is provided during MRI in order for the participant to indicate that they would like to stop or are uncomfortable. Study staff will be present throughout all LIFU/TMS procedures and they will be halted if the subject experiences any unexpected sensations. Breaks will be offered periodically to reduce participant discomfort. Monitoring for side effects will take place pre and post all stimulation sessions.

17.3 If applicable, indicate which procedures might have risks to the subjects that are currently unforeseeable. This will be rare, and usually applicable when testing a new drug or device or a new use of an existing drug or device:

N/A

17.4 If applicable, indicate which procedures might have risks to an embryo or fetus should the subject be or become pregnant:

CT should not be performed in pregnant women. Ionizing radiation can cause developmental problems in fetuses, particularly in the first trimester. Risks of using TMS with pregnancy women are currently unknown. Pregnant women will be excluded from this study. At the first study visit, study staff will screen for pregnancy in all females.

17.5 If applicable, describe risks to others who are not subjects (e.g., collection of sensitive health data that might affect sexual partners if disclosed, mandatory reporting of abuse, DNA testing that might affect family members or relationships):

N/A

18.0 Potential Benefits to Subjects

18.1 Describe the potential benefits that individual subjects might experience from participating in the research. Include the probability, magnitude, and duration of the potential benefits, as this will be useful to the IRB's risk:benefit analysis. Do not include benefits to society or others. Do not list monetary or non-monetary compensation for participation, as this is not a benefit These should be included in section 2 or 3 of this document:

There are no anticipated direct benefits for participants.

18.2 If applicable, specify that there are no anticipated direct benefits for participants:

There are no anticipated direct benefits for participants.

19.0 Data Management and Confidentiality

19.1 Describe procedures that you will use for quality control to ensure validity of collected data:

All research staff conducting study procedures are trained to do so, through Human Subjects Protections, MRI safety training, and device specific operation trainings. All CT scans will be performed by a trained radiology technician.

19.2 Describe any existing data or biospecimens you will obtain as part of this study. Include:

- *Variables or samples to be obtained*

- *Source of the data or specimens*
- *Your authorization to access or receive the data or biospecimens*
- *Whether the data or biospecimens are publicly available*
- *Whether the data or specimens you receive will contain identifiers*

N/A

19.3 Describe the steps that you will take to handle and secure study data during data collection, storage, use, and transmission. Include information about training of study staff, authorization of access, password protection, encryption, physical controls, certificates of confidentiality, separation of identifiers and data, etc.:

Each subject will be assigned a coded designation. All study data to be used in analysis will be de-identified using this coded designation, and contains no biographical data that can be linked to individuals. The key for study codes is stored in a secure server accessible only by limited authorized personnel. Any screening forms collected on paper will be stored in a locked filing cabinet within the Legon lab space. All electronic data collected will be stored on secure servers.

MRI, CT, EMG, and behavioral data will be stored on secure FBRI servers.

Participant screening and questionnaire data will be collected and stored on the secure platform Redcap.

The MRI, CT, EMG, and behavioral data files will have no identifiable information stored within them. Only the participant coded designation, whose key is only accessible by authorized study personnel, links these data to the participant. These de-identified data will be available to authorized personnel for data analysis, and may be made available to the public as required by funding source and journal used for study publication.

19.4 For multi-site studies, describe how data or specimens will be handled and secured for each site (e.g., central or disseminated data storage, data coordinating center):

N/A

19.5 Describe the plan for data disposition following the conclusion of the study (e.g., long term maintenance of data, data destruction methods).

- *What information will be included in the long term storage of data or specimens?*
- *How long will the data or specimens be stored?*
- *Where and how data or specimens will be stored?*
- *Who will have access to the data or specimens during long term storage?*
- *Who is responsible for receipt or transmission of the data or specimens?*
- *How will data or specimens be shared or transported?*
- *When and how will personal identifiers be destroyed?*

Data may be stored and archived indefinitely for future analysis. MRI, CT, EMG, and behavioral data is de-identified, coded, and contains no specific biographical information which can be related back to the individual. The study key is stored on a secure server which cannot be accessed unless specifically authorized. Questionnaire and screening data will be collected and stored on the secure platform Redcap.

20.0 Provisions to Protect the Privacy Interests of Subjects

20.1 Describe the steps that you will take to protect subjects' privacy interests. "Privacy interest" refers to a person's desire to place limits on with whom they interact or to whom they provide personal information (e.g., collecting the minimal amount of private information required to complete the study, protecting the data once it is obtained):

Only information required for study purposes will be collected. This includes subject screening, participation, and payment information. Once obtained these data are stored securely, accessible only by authorized personnel. Documents with subject name, such as the consent form, will be stored in a separate locked cabinet from files containing subject data with coded identifiers. The study key is only accessible by authorized staff.

20.2 Describe steps that you will take to make subjects feel at ease with the research situation in terms of the questions being asked and the procedures being performed. "At ease" does not refer to physical discomfort, but the sense of intrusiveness a subject might experience in response to questions, examinations, and procedures (e.g., use of a same gender investigator to place sensors on the torso, a private changing area if clothing must be changed, sensitivity when discussing pregnancy testing with subjects, making it clear on surveys that participants can discontinue at any time, not asking questions about private or sensitive issues unless necessary for the research):

Research staff will check in with participants throughout study sessions to ensure any concerns are addressed immediately. Study procedures do not involve any questionnaires

or tasks that are anticipated to be emotional in nature. During the consenting process, staff will ensure participants understand that they can discontinue at any time.

20.3 Describe how you plan to access existing sources of information about the subjects (e.g., medical records, grades) and how you will protect participant privacy through the data security plan:

N/A

20.4 Describe any required reporting that might occur as a result of your research questions, study populations, and data collection methods. Examples for Virginia and Virginia Tech include:

- ***Any*** *suspicions (e.g., circumstantial, disclosed) of child abuse (physical, emotional, sexual) and neglect*
- *Sexual discrimination and/or sexual violence that involves a student*
- *Disclosure or signs of intention to harm oneself (i.e., suicidal ideation and/or plan)*
- *Disclosure or signs of desire to harm others (i.e., homicidal ideation and/or plan)*
- *Suspected abuse, neglect or exploitation of vulnerable adults (e.g., individuals with a disability, elderly persons)*

The Beck Depression Inventory is included in our assessments. We will provide information with suicide hotline numbers as indicated by participants' responses.

The data we are collecting is for research purposes only, not for clinical diagnosis. As such, the data collected, including that from the Beck Depression Inventory (BDI), will not be reviewed by a physician, and will not be analyzed for some time after the participants visit. However, to ensure participants safety, the study personnel administering the BDI will check a participant answer to item 9 on the BDI (Suicidal Thoughts or Wishes). If the participant answers "2 I would like to kill myself." or "3 I would kill myself if I had the chance." the study staff administering the questionnaire will put them in touch with the appropriate resources. We have also added language in the consent form informing the participant that if they feel distressed that we will put them in touch with the appropriate mental health resources. Further, we state that upon request we will provide them with a number for a mental health hotline: (1-800-950-6264) – The National Alliance on Mental Illness Help Line.

21.0 Provisions to Monitor the Data to Ensure the Safety of Subjects

Safety monitoring is required when research involves greater than minimal risk and is sometimes appropriate for other studies.

21.1 Describe:

- *The plan to periodically evaluate the data collected regarding both harms and benefits to determine whether subjects remain safe (e.g., periodic reporting to the IRB, establishing a data monitoring committee, reporting data monitoring committee findings to the IRB and the sponsor).*
- *What data you will review, including safety data, unexpected events, and data that show the ability to produce the intended results.*
- *How the safety information will be collected (e.g., with case report forms, at study visits, by telephone calls with subjects).*
- *The frequency of data collection, including when safety data collection starts.*
- *Who will review the safety data and with what frequency.*
- *The statistical tests for analyzing the safety data to determine whether harm is occurring.*
- *Any conditions that will trigger an immediate suspension of the research (e.g., a serious adverse event).*

The PI will be responsible for continuous data and safety monitoring of all participants enrolled in this study. All report of symptoms questionnaires will be reviewed as the study progresses. Any unexpected events will be reviewed by the study PI. In the event of a serious adverse event, all data collection will be stopped until safety can be reviewed by the PI and IRB.

22.0 Compensation for Research Related Injury

22.1 If the research involves more than minimal risk to subjects, describe the available compensation in the event of research-related injury, if any:

N/A

22.2 Provide a copy of contract language, if any, relevant to compensation for research-related injury. At Virginia Tech, this is most common for sponsored research:

N/A

23.0 Economic Burden to Subjects

23.1 Describe any costs that subjects might be responsible for because of participation in the research, including any uncompensated costs for items such as transportation, missed work, and childcare:

Participation in the research study will take approximately 2-2.5 hours per session. Participants will be compensated for their time as described above.

24.0 Consent Process

24.1 Indicate the process by which you will obtain consent for study participation. Please upload all consent, parental permission, and assent forms, documents, and scripts referenced in this section to Protocol Management.

Describe the following:

- *Where the consent process will take place (e.g., clinic waiting area, classroom, online)*
- *The time interval between sharing the consent information with the prospective subject and obtaining consent. For lab, interview, and focus group studies, the Virginia Tech IRB prefers that subjects have at least 24 hours to review the consent form and study information before the appointment where consent will be obtained. For simple online survey studies, you can typically present the consent information immediately before subjects begin participation.*
- *If applicable, processes to ensure ongoing consent or assent (e.g., for multiple sessions; for research in which a minor will turn 18 during the study; for longitudinal research with minors who will later be asked to provide or affirm their assent).*
- *Please review "SOP: Informed Consent Process for Research (HRP-090)" for recommended procedure. Describe your process, being sure to include:*
  - *The name and role of all study personnel who will be trained and certified by the PI to conduct the consent process*
  - *The time that will be devoted to the consent discussion*
  - *Steps that you will take to minimize the possibility of coercion or undue influence*
  - *Steps that you will take to gauge or ensure the subjects' understanding*

Subjects who express interest in the study through recruitment efforts may be contacted by follow-up phone or email. If verbally screening, participants will provide verbal permission for study staff to collect screening information to determine study eligibility. If, after screening, participants are eligible for further participation, subjects will be asked to provide informed consent.

Consent will be obtained on-site prior to the first study visit in a quiet area. Once written consent is obtained, participants do not need to undergo the consenting process again for additional study visits. As this study involves seven study visits, this is designed to be considerate of participants' time.

Participants will be given as much time as they request to review the consent documents and ask questions. Consent documents for in person visits will be emailed to the participant at least 24 hours prior to their first visit. In order to determine understanding,

staff will discuss each section with the participant, verifying their understanding through conversation throughout.
Staff will confirm they have had all questions answered prior to obtaining the participants signature.

Participants will consent to the follow up questionnaire during the initial consent. A reminder of all relevant consent sections will be provided prior to questionnaire completion - see attached document. Report of Symptoms questionnaire will be sent to participants following in-person study participation. If preferred, this questionnaire can be completed verbally via phone with study staff, in which case the consent process will involve a verbal consent reminder as documented by study staff initials. In this instance, study staff will review the reminder consent document with participants prior to completing the verbal questionnaire.

Non-English Speaking Subjects

- *Indicate what language(s) other than English are understood by prospective subjects or representatives.*
- *If non-English speakers will be recruited, describe the process you will use to ensure that the oral and/or written consent information provided will be in a language that they understand.*
- *If you translate consent forms and study materials, please provide a certified translation of the form as well as the certification document.*
- *Indicate the spoken language that study personnel obtaining consent will use. Describe how you will assess fluency of personnel obtaining consent to ensure that the translation is accurate.*

Subjects must be able to understand and read English, as that is the language in which the screening documents and study instructions will be presented. They are not required to be native English speakers in order to participate in the research.

Waiver or Alteration of Consent Process (consent will not be obtained, required information will not be disclosed, or the research involves deception)

- *Review the "CHECKLIST: Waiver or Alteration of Consent Process (HRP-410)" to ensure you have provided sufficient information for the IRB to make these determinations (i.e., that it meets the criteria for a waiver or alteration of the consent process).*

N/A

Subjects who are not yet adults (minors: infants, children, teenagers)

- *Describe the criteria that you will use to determine legal age for consent to treatments or procedures involved in the research under the applicable law of the jurisdiction in which the research will be conducted (e.g., in Virginia, individuals under the age of 18 years).*
  - *For research conducted in Virginia, review "SOP: Legally Authorized Representatives, Minors, and Guardians (HRP-013)" to determine which individuals in the state meet the definition of "minor."*
  - *For research conducted outside of the state, please describe the legal requirements for the definition of "minor."*
- *Describe the process for obtaining parental permission.*
  - *Permission from one parent is acceptable for studies that involve no greater than minimal risk OR involve greater than minimal risk but present the prospect of direct benefit to the minor subject.*
  - *Permission from both parents is required in all other cases (unless one parent is deceased, unknown, incompetent, or not reasonably available, or when only one parent has legal responsibility for the care and custody of the minor).*
- *Describe whether you will obtain permission from individuals other than parents or Legally Authorized Representatives, and if so, who will be allowed to provide permission. Describe the process you will use to determine these individuals' authority to consent to the minor's general medical care.*
- *Indicate whether you will obtain assent from all, some, or none of the minors. If you will obtain assent from some minors, indicate which minors will be required to assent. Consider chronological age and intellectual capacity when determining who will be required to provide assent (e.g., infants are unable to assent. However, teenagers are likely able to read and sign an assent form).*
- *When assent of minors is obtained, describe whether and how you will document it. Will minors sign an assent form or give verbal assent?*
- *Attach parental permission and minor assent forms or scripts in Protocol Management.*

N/A

Adults Unable to Consent

- *Describe the process you will use to determine whether an individual adult is capable of consent.*
- *List the individuals from whom you will obtain permission in order of priority (e.g., durable power of attorney for health care, court appointed guardian for health care decisions, spouse, and non-minor child).*

  - *For research conducted in the Virginia, review "SOP: Legally Authorized Representatives, Minors, and Guardians (HRP-013)" to determine which individuals in the state meet the definition of "legally authorized representative."*
  - *For research conducted outside of Virginia, please describe the legal requirements for obtaining permission from a legally authorized representative in the state where the research will occur.*
- *Describe the process for assent of the subjects.*
  - *Indicate whether you will require assent from all, some, or none of the subjects. If some, indicate which subjects will be required to assent and which will not.*
  - *If you will not obtain assent from some or all subjects, please provide justification for not obtaining assent.*
  - *Describe whether and how you will document assent.*

N/A

25.0 Process to Document Consent in Writing

25.1 Consult "SOP: Written Documentation of Consent (HRP-091)" for recommended procedures, and describe whether and how consent of the subject will be documented in writing:

Consent will be documented through participant printed name and signature on the informed consent form. For the follow-up questionnaire, electronic reminder of consent will be provided prior to questionnaire completion, or verbal reminder of consent will be indicated by study staff initials. All consent forms submitted.

25.2 If the research presents no more than minimal risk of harm to subjects and involves no procedures for which written documentation of consent is normally required outside of the research context, you can request that the IRB waive the requirement to obtain written documentation of consent (e.g., consent to participate is indicated by pressing a button for an online questionnaire – after the consent information is presented and before the questionnaire begins):

For study screening data collection, a waiver of consent is requested. Participants will be informed their data is being collected to determine study eligibility. Procedures will be explained to the participant by trained and authorized study staff and then the participant will be given as much time as they request to review the form and ask any questions prior to written consent. Follow up questionnaire reminder of consent will be provided either electronically or verbally per participant preference prior to questionnaire. If consent is reviewed verbally, reminder of consent will be documented with study staff initial. The consent forms are included in this application.

25.3 If you will document consent in writing, attach a consent document with places for signatures. If you will obtain consent, but not document consent in writing, please attach the consent script or text. Review "CHECKLIST: Waiver of Written Documentation of Consent (HRP-411)" to ensure that you have provided sufficient information. You should use "TEMPLATE CONSENT DOCUMENT (HRP-502)"to create the consent document or script:

Written and Electronic/verbal reminder of consent forms attached.

26.0 Resources Available

26.1 Describe the resources available to conduct the research. For example, as appropriate:

- *Describe the PI's availability to supervise the research.*
- *Justify the feasibility of recruiting the required number of suitable subjects within the agreed recruitment period. For example, how many potential subjects do you have access to? What percentage of those potential subjects do you need to recruit?*
- *Describe the time that you will devote to conducting and completing the research.*
- *Describe your facilities.*
- *Describe the availability of medical or psychological resources that subjects might need as a result of an anticipated or unanticipated consequence of participation in the research.*
- *Describe your process to ensure that all persons assisting with the research are adequately informed about the protocol, the research procedures, and their duties and functions (e.g., training plans, detailed study notebooks).*

The PI is a research-dedicated Faculty member at the Fralin Biomedical Research Institute at VTC.

PI Legon's lab is part of the Fralin Biomedical Research Institute at Virginia Tech Carilion (FBRI at VTC) at Virginia Tech, located in Roanoke, Virginia. The Research Institute was founded in 2010 as part of a commitment to biomedical sciences by Virginia Tech. Primary institute faculty including Legon have a primary faculty appointment within a traditional department at Virginia Tech, but are funded through and housed at the FBRI. Primary faculty comprise a broad interdisciplinary group working in a variety of areas such as neural computation, psychiatric and developmental disorders, genetics, substance abuse and molecular virology and biology. The FBRI also provides to the faculty an administrative staff of individuals with expertise in grants management, personnel management, supply ordering/tracking, web systems management, desktop support, laboratory animal care, IRB support, software development and facilities and operations support and planning as well as general administrative support.

The Legon lab is actively recruiting postdoctoral as well as graduate students. It is anticipated that undergraduate students will be involved in the lab as well to gain valuable research experience. As these individuals are incorporated into our research studies they will be added to the appropriate protocols. It is the intention of the lab to diligently recruit and run participants, while still providing sufficient time for PI oversight and ongoing data analysis.

Computing Resources:
The FBRI has dedicated data centers in the primary Riverside 2 facility as well as the Biomedical Research Addition (BRA / Riverside 4) opened in 2020. Both are secured with dual-factor biometric access and provide redundant and backup power through enterprise-class uninterruptible power supplies (UPS) and facilities-based generators. [Updated 4.22.2020]
Research Technology and Computing Resources
Dell / Intel HPC Cluster -SLURM (Dirac):
20 compute nodes, 960 cores, 3,840 GB RAM, 40Gb Interconnect
Dell / Intel HPC Cluster - SLURM (Hawking):
4 compute nodes totaling 192 cores, 768 GB RAM, 40Gb Interconnect
2 GPU nodes totaling 4 NVIDIA Tesla V100 16G Passive GPUs, 56 cores, 768 GB RAM, 40Gb Interconnect
10+ dedicated Linux servers (48 core AMD Opteron-based, 192GB RAM) are available for image and data analysis
Virtualized infrastructure using VMware vSphere with built-in redundancy between data centers serving Linux and Windows virtual machines available for general and scientific computing and image and data analysis
40 and 100Gbit/s storage connectivity for research data
10,40 and 100 Gbit/s Internal Local Area Network between file servers and cluster
10 Gbit/s Wide Area Network for access to Virginia Tech main campus / Internet
1.9 Petabytes of NAS centralized disk storage
1.2 Petabytes of off-site storage for disaster recovery
400 Terabytes of encrypted storage for scientific workloads
Nightly backups, snapshots, and off-site data retention
2.4Ghz / 5Ghz secure wireless network
1 Gbit/s commodity Ethernet network
Data Analysis Tools: MATLAB, SPM, AFNI, FSL, MRIcro, xjView, R, SAS, Graphpad Prism, SPSS
Productivity Tools: Adobe Suite, Microsoft Office Suite, vi, vim, emacs, etc.

MRI Scanning Resources (FBRI):
Virginia Tech has three research-dedicated Siemens 3T MR scanners (2 Siemens Magnetom TIM Trios, and 1 PRISMA-FIT) available. Each scanner bay is equipped with the following stimulation and response interfaces:
behavioral response: two-hand, eight-button optical response pads with USB, serial, and TTL output (Current Designs, Inc.) video stimulation: rear-projection video display (Hitachi CP-SX635) corrective lenses for use with video stimulation: MR-compatible frames with insertable polycarbonate lenses (prescriptions range from -8.00 to +8.00)

(Solo Bambini) stimulus delivery: dedicated computers for experiment presentation (Dell Optiplex 980) audio delivery: MRI compatible headphones.

CT Scanning Resources (FBRI):
Virginia Tech has a Siemens Somatom Confidence RT Pro CT Scanner available.

Mental Health Resources: In the unlikely and unexpected event that a participant requires or requests mental health resources, the research staff will offer a national mental health hotline number (National Alliance on Mental Illness helpline: 1-800-950-6264). Those staff and volunteers are trained to transfer calls to crisis management, if needed or work to answer questions about education programs, treatment options, symptoms of mental health conditions and more (additional information may be found here: https://www.nami.org/find-support/nami-helpline). In the event of a mental health or other emergency, the local hospital system, Carilion Clinic is located approximately 1 mile from the FBRI and is accessible by the public.

Training: Study staff are trained in Human Research Protections through approved programs, such as CITI. Additionally, study procedures, such as participant task instructions, staff instructions to introduce and/or present and start computer tasks, and visit checklists are developed for each study in order to maintain consistency across participants and ensure all procedures are completed and documented. All staff consenting and administering research tasks review both the approved protocol and consent form prior to study startup and participant recruitment. If there are amendments or changes, staff review the new documents. Study coordinators review the consent process and procedures on an ongoing basis.

27.0 Multi-Site Research

Contact the HRPP for multi-site research (involving multiple institutions) and the details required for this section will be provided. Otherwise, indicate N/A.

N/A