Investigation of Low-intensity Focused Ultrasound Pressure
NCT: NCT06343883
Date: 5/13/2026

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 Pressure

PROTOCOL NUMBER:

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

24-353

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

1.0

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?

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1.0 Study Summary

Study Title	Investigation of low-intensity focused ultrasound (LIFU) pressure
Study Design	Multiple subject repeated measures
Primary Objective	Assess the relationship between LIFU pressure to Somatosensory Cortex and event-related MRI and EEG signals.
Secondary Objective(s)	Assess the safety and tolerability of LIFU to Somatosensory Cortex.
Study Population	Healthy human volunteers aged 18-65
Sample Size	36
Research Intervention(s)/ Investigational Agent(s)	Low-intensity focused ultrasound, continuous Electroencephalography, and functional Magnetic Resonance Imaging.
Study Duration for Individual Participants	Cohort 1 - 3 study visits, & a follow-up questionnaire; 4 hrs Cohort 2 - 4 study visits, & a follow-up questionnaire; 8 hrs Cohort 3 - 4 study visits, & a follow-up questionnaire; 8 hrs
Acronyms and Definitions	LIFU = low-intensity focused ultrasound SEP = Somatosensory Evoked Potential MRI = magnetic resonance imaging CT = computed tomography HR = heart rate BP = blood pressure

2.0 Objectives

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

AIM 1. An understanding of the effect of peak LIFU pressure on human brain function is critical to rationally design clinically useful therapeutic methods that result in predictable outcomes. Experiments in small and large animals 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. However, thus far, there has been no comprehensive assessment of the relationship between peak LIFU pressure and its effect on human brain function. Consequently, the field lacks consensus regarding the optimal pressure levels for effective application. Here, we will systematically test a range of LIFU pressures between 0 and 1.25 MPa delivered to the somatosensory cortex (S1) in healthy human volunteers and will evaluate the relationship between delivered peak pressure and both EEG evoked potential amplitudes and BOLD activity recorded via functional Magnetic Resonance Imaging to test robustness and reproducibility.

AIM 2. LIFU has a favorable safety profile at pressures below 1 MPa and FDA guidelines for the application of diagnostic ultrasound--a conservative estimate of the

bioeffects in the human brain--indicate that the delivery of 1.25 MPa using a single-element 500 kHz ultrasound transducer falls well below the established safety indices; however, there is limited safety and tolerability data in human subjects to confirm these recommendations. Here, we will deliver up to 1.25 MPa to the S1 in N=8 healthy individuals and hypothesize that LIFU will have a favorable safety profile and be well-tolerated as assessed by adverse event reports and structural brain magnetic resonance imaging examined by an attending neurologist.

2.2 State the hypotheses to be tested:

We hypothesize that LIFU at incremental pressures to the S1 reduces the amplitude of the somatosensory evoked EEG signal in a dose-dependent manner.

We hypothesize that LIFU at incremental pressures to the S1 reduces the somatosensory evoked fMRI BOLD signal in a dose-dependent manner.

We hypothesize that applications of LIFU to the S1 at a pressure of 1.25 MPa will have a favorable safety profile and be well-tolerated as assessed by adverse events and structural brain MRI examined by an attending neurologist.

3.0 Background

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

Low-intensity focused ultrasound (LIFU) is a new and promising non-surgical technique that uses mechanical energy to induce transient plasticity in the brain with high spatial resolution and adjustable focus. LIFU has been used safely and effectively for cortical neuromodulation in animal models from rodents up through non-human primates and has also been demonstrated to be an effective method of transient cortical and sub-cortical neuromodulation in humans. Considering specific outcome measures, LIFU has been shown to affect the amplitude of evoked potentials, the power, phase and frequency of the electroencephalogram (EEG); the blood oxygen level dependent (BOLD) magnetic resonance imaging signal, as well as certain behaviors.

LIFU operates within a diverse parameter space, including fundamental frequency, intensity (directly related to pressure), duration, cycles, duty cycle, and pulse repetition frequency. While small animal models have provided valuable insights into its neuromodulatory effects, investigations in primates and humans underscore the importance of explicit parameter testing. This becomes increasingly critical as LIFU transitions into clinical applications, where optimizing parameters is essential for achieving the most effective and durable therapeutic outcomes in clinical populations.

In the realm of human LIFU experimentation, consensus on parameters remains elusive. However, recent studies have shown promising outcomes with pressures reaching or surpassing 1MPa in cranial applications. Riis et al. reported a compelling case where

delivering 1MPa to the subcallosal cingulate cortex of a patient with severe, treatment-resistant non-psychotic depression led to an 11-point drop in her Hamilton depression rating, sustained for at least 44 days post-intervention. Nakajima et al. found that administering 1MPa LIFU to the anterior putamen and the subthalamic nucleus—regions implicated in controlling response inhibition—yielded significant impairments in stopping performance. Similarly, Mahoney et al. achieved noteworthy results by applying 1.6 MPa LIFU to the nucleus accumbens in individuals with co-occurring substance use disorders (n = 4), resulting in a substantial reduction in drug cravings for at least 90 days post-treatment. The procedure and follow-up evaluations showed no safety concerns, with no unexpected adverse events related to the LIFU procedure or serious adverse events recorded. Behavioral assessments, including HAM-D and CSSRS, were normal during and after intervention. Brain MRIs conducted immediately post-sonication, at 24 hours, and at 1-week post intervention revealed no edema, hemorrhage, or significant structural changes. Based on these results, the adoption of LIFU protocols delivering pressures exceeding 1MPa holds significant promise from a clinical perspective. However, to date, there are no studies systematically examining the relationship between LIFU pressure and the extent of its effects, necessitating explicit testing.

To investigate this relationship, we will examine the impact of a range of LIFU pressures on two measures of neural activity: the amplitude of the N20 and P27 components of the somatosensory evoked potentials (SEPs) as well as pre/post changes in BOLD responses to median nerve stimulation. The SEP generated by electrically stimulating the median nerve during EEG recordings has been extensively studied and its components are labeled based on their polarity (negative - N or positive - P) and latencies in milliseconds. The N20 component of the median nerve-evoked SEP is known to represent sensory input from the dorsal column–medial lemniscal pathway which terminates in Brodmann area 3b, located in the anterior bank of the postcentral gyrus within the primary somatosensory cortex (S1). Incorporating BOLD responses to a median nerve stimulation alongside SEPs enhances our evaluation of the impact of ultrasound pressure. This integration is beneficial because there exists a linear correlation between BOLD signal intensity and evoked potential amplitude, allowing for a more comprehensive examination of our hypotheses.

Because a new parameter set is being applied, we have carefully evaluated the safety profile of the highest-pressure dose delivered during our study. To ensure safe application, we adhere to the existing guidelines defined for obstetric diagnostic ultrasound defined by the Food and Drug Administration (FDA) for both mechanical and thermal bioeffects. The FDA safety guidelines include derated limits of spatial peak pulse average (I_{SPPA}) of 190 W/cm2, a spatial peak temporal average (I_{SPTA}) of 720 mW/cm2, and a mechanical index (MI) of 1.9. Considering the thermal bioeffects, the allowable temperature rise due to ultrasound is limited to ≤ 2° Celsius at any time in soft tissue. It's important to note that these regulatory guidelines may be overly conservative estimates of bioeffects, potentially resulting in underdosing.

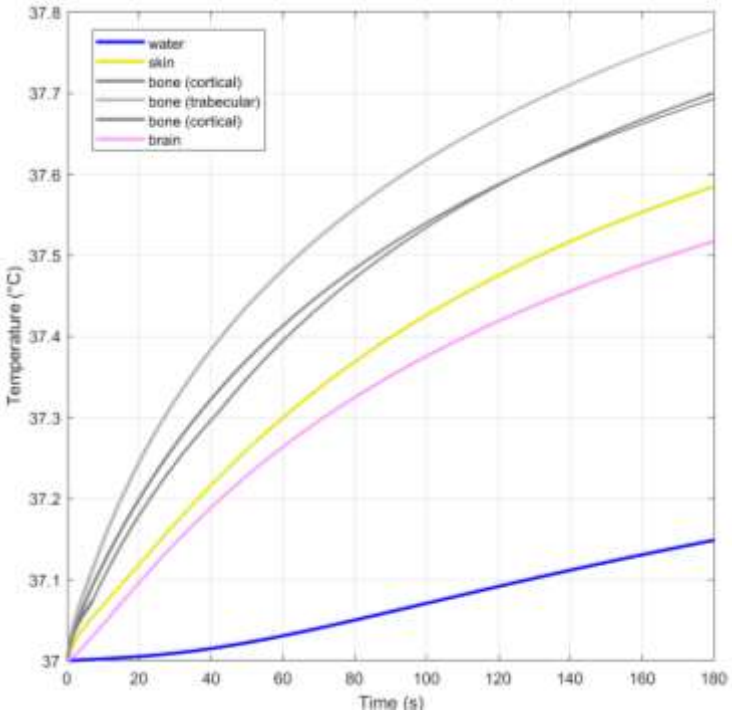
I_{SPPA} measures the energy delivered per unit area during each ultrasound pulse and is calculated using a formula $I_{SPPA} = \frac{P_{pk}^2}{2*Z}$ which incorporates the peak pressure and the

acoustic impedance of the biological tissue. I_{SPTA} is an alternative safety measure considering both the intensity and duration of the treatment and is calculated with the formula $I_{SPTA} = I_{SPPA} * DC$, ensuring that the overall energy delivery does not produce thermal bioeffects. The mechanical index ($MI = \frac{P_{pk}}{\sqrt{f}}$) is an important safety measure that indicates a system's potential to induce cavitation-related effects as a function of peak rarefaction pressure and transducer fundamental frequency, where bubbles form in the liquid due to pressure changes. Keeping MI within this limit helps prevent tissue disruption and adverse effects while allowing for effective neuromodulation. In vivo, it appears that substantially higher rarefactional pressures are necessary to induce inertial cavitation. For example, Gateau et al. 2011 demonstrated that an MI an order of magnitude greater than the FDA limit was necessary to detect cavitation in a large animal brain. Despite these findings, an arbitrary regulatory threshold of MI 1.9 continues to be used to standardize the use of diagnostic ultrasound. Importantly, while this threshold has been instrumental in demonstrating the mechanical safety of diagnostic ultrasound below this level, there is no evidence suggesting immediate mechanical or cavitation damage upon exceeding MI 1.9. Below is a table displaying the recommended parameters compared to the upper limit proposed to be used in our study.

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	I_{SPPA} W/cm2	I_{SPTA} mW/cm2	***MI***
FDA Guidelines	190	720	1.90
Proposed Study	48.3	483	1.76

To assess the thermal effects of our updated parameters, we employ computational models to gauge temperature increases in various tissues: skin, cortical skull bone, trabecular skull bone, and brain tissue. The accompanying figure illustrates the temperature elevation in each tissue. Notably, the trabecular bone experiences a maximum increase of less than 0.8°C, comfortably below the FDA's 2°C threshold for permissible temperature rise.



Although the safety metrics for our parameter set reside below the well-validated FDA recommendations for diagnostic ultrasound, we have chosen to further evaluate the safety profile of our highest-pressure dosage in cohort 1 of this study before proceeding on to the efficacy arms of the study in cohorts 2 and 3. To do this, we will deliver the highest-pressure condition of 1.25 MPa to the S1 in 8 subjects and have

chosen this cohort size based on existing TMS safety testing and feasibility literature (Levkovitz et al. 2007).

3.2 Describe any relevant preliminary data:

While the above human studies have successfully applied 1MPa and 1.6MPa of pressure respectively, there are no published studies to date that specifically aim to assess effects of pressures in measurable outcomes.

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 the relationship between LIFU pressure and effect outcome to yield more 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: Change in MN-SEP amplitudes and BOLD signal in S1.
Secondary endpoint: Documentation of tolerability of up to 1.25 MPa LIFU to S1.

*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/psychological feelings.
Secondary safety endpoint: Physiological recordings and structural MRI evaluated by an attending neurologist.

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. Furthermore, participants in cohort 1 will undergo a structural MRI scan 24-72 hours after the highest-pressure session to confirm that there were no adverse events.

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 8 participants will be enrolled study procedures for cohort 1. We will examine the safety profile of LIFU at 1.25 MPa delivered to the S1 assessed by adverse event reports and structural brain magnetic resonance imaging examined by an attending neurologist. Visit 1 includes the informed consent session and anatomical scans which includes 3T anatomical MRI and CT scans. Visit 2 consists of LIFU sonication at a peak pressure of 1.25 MPa delivered to the S1 preceded by and followed up with a battery of questionnaires (Daily Q, Experience Q, Report of Symptoms). Visit 3 takes place no less than 48 hours after visit 2 and includes a secondary 3T anatomical MRI scan and a Report of Symptoms questionnaire.

A total of 36 participants will be enrolled in study procedures for cohorts 2 and 3. We will examine the effect of LIFU stimulation to the cortex on EEG and fMRI signals. This study will be collected through multiple study visits. Visit 1 includes anatomical scans which will include 3T anatomical MRI and CT scans as well as questionnaires. Additional study visits will consist of either EEG or fMRI scans, electrical median nerve stimulation, and LIFU at one of three pressures between 0 and 1.25MPa. It is anticipated that participants may complete 3-6 additional study visits, depending on participant availability and interest.

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):

SEP EEG data analysis
A separate one-way analysis of variance (ANOVA) will be conducted with the main factor PRESSURE for each of the SEP potentials (N20, P27, N33, P50, N70, P100 and N140). Mauchly's test for sphericity will be performed and Greenhouse-Geisser correction applied accordingly. Suitable post-hoc comparisons will be used to investigate main effects. Comparisons will be adjusted for error, and nonparametric data will be analyzed using nonparametric tests (e.g., Kruskal–Wallis). Significance will be set at $p<0.05$.

BOLD fMRI data analysis
The fMRI data will be processed in Analysis of Functional NeuroImages (AFNI). The data will be 3D motion correction, linear and quadratic trends removed, a Gaussian filter with full width half maximum of 3 mm applied, slice timing correction, and distortion correction applied. A general linear model analysis will be utilized to generate a statistical parametric map with a reference function generated by convolving the hemodynamic response function with the task function. This process is performed for all subjects' fMRI data to isolate the individual representations of the S1 using a $t = 5$ ($p = 1e{-}6$ uncorrected). To measure volume changes, a region of interest (ROI) will be drawn around the S1. Activated voxels ($t = 5$; $p = 1e{-}6$) in this ROI will be used to calculate the activation volume in S1 due to LIFU. To test for differences between different LIFU pressures, the total number of voxels that met this threshold within this ROI will be subjected to a paired student's t test.

For percent signal change analysis, we will concentrate on a brain volume at the measured focal volume of the ultrasound beam (see Fig. 3). These coordinates will be found for each subject and an ROI of 125 mm3 ($5 \times 5 \times 5$ mm) will be drawn to encompass partial volume of the ultrasound pressure field. Based upon free water field ultrasound beam measurements, the FWHM volume of the beam is ~ 230 mm3. Percent signal change between LIFU and sham conditions will be compared with a paired t test. To further investigate the spatial selectivity of the LIFU effect, a $5 \times 5 \times 5$ mm ROI will be also placed at the region of strongest activations in each participant to examine if LIFU has effects on these representations despite not being directly targeted for stimulation. Group paired t-tests will be performed separately for the S1 at different pressures

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.

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.

MRI: Participants will undergo MRI scans. 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.

SEP: The somatosensory evoked potential (SEP) will be generated using an electrical stimulation. This will be done with a commercial device placed on the participants arm. The device will generate brief stimulation (1-5mA for 100ms) of the muscle which may feel twitchy or mildly uncomfortable. This is not expected to be painful, however the participant can stop the procedure at any time.

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 for real-time navigation of the stimulation. The subject is fitted with a device that contains 3D infrared tracking bulbs, such as a headband or glasses. The LIFU transducer is also fitted with these tracking bulbs. Both the subject and treatment device are then tracked using an infrared camera. This procedure allows for precise targeting of specific brain regions using the subject-specific MRI and is wholly noninvasive.

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. 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 60 W/cm2 (4x lower Isppa) for these studies.

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).

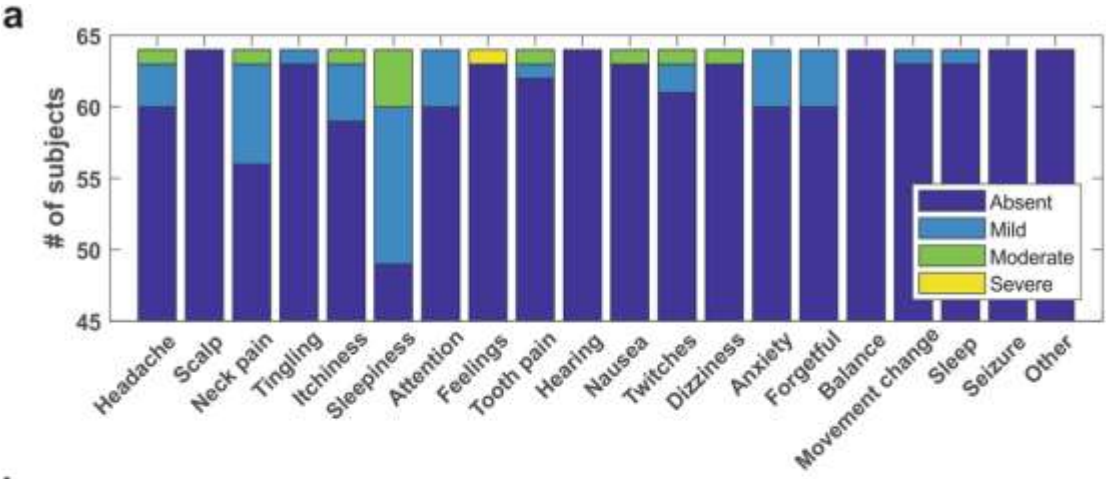


Figure. Preliminary self-report of symptoms in N = 64 healthy volunteer participants in LIFU studies in our lab. 'Feelings' refers to the question asking about experiencing any unusual feelings, attitudes or emotions. Please note the instance of severe 'feelings' in one individual was followed up by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

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. Pressures delivered in the seven LIFU studies varied across three transducers. The pressures resulted in mechanical index values (MI) of 0.78-0.9. 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.

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.

MRI: Participants will receive MRI scans. 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.

SEP: Participants will receive brief electrical shocks to their arm using a standard GRASS SD9 electrical stimulator. The max voltage of the device is 100V, with a max frequency of 200Hz. The unit has a built in isolation, providing an additional layer of safety.

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 multi-cohort sham-controlled repeated measures design collected in multiple study sessions.
Cohort 1 -
Session 1 - MRI and CT scans
Session 2 - LIFU (highest pressure only)
Session 3 - 24-72 hr follow-up MRI
Follow-up Questionnaire done virtually
Cohort 2 -
Session 1 - MRI and CT scans (if needed)
Sessions 2-4 - SEP + EEG + LIFU (Pressure 1, 2, or 3)
Follow-up Questionnaire done virtually
Cohort 3 -
Session 1 - MRI and CT scans (if needed)
Sessions 2-4 - SEP + fMRI + LIFU (Pressure 1, 2, or 3)
Follow-up Questionnaire done virtually

Cohort 2 and 3 will only be done once Cohort 1 has completed collection. Cohorts 2 and 3 will be updated pending the outcome of cohort 1. This is an added safety check.

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 and CT.

Consent: Participants will be asked to sign a consent form upon arrival for the 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. Verbal consent will be obtained at all other study visits.

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, 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 or CT. We will enroll approximately 36 individuals.

This study includes low-intensity focused ultrasound (LIFU), functional magnetic resonance imaging (fMRI), computed tomography (CT), and a Somatosensory Evoked Potential (SEP) task. Additionally, stereotaxic neuronavigation is utilized and physiological recordings are taken.

Three pressures of LIFU will be applied throughout the three cohorts. These will be 'low', 'medium', and 'high'. Participants will not know which they are receiving (except cohort 1). The high cohort will not exceed 1.25 MPa.

Cohort 1

Session 1	Session 2	Session 3 (24-72 hours later)
Imaging	Stimulation	Imaging
MRI & CT	LIFU	fMRI
2 hours	1 hour	1 hour

Session 1 - An MRI and CT exam will be performed (if needed). If participant has MRI and CT scans on file with the lab this visit may be omitted.

Session 2 - LIFU will be applied to the S1 using the 'high' setting.

Session 3 - 24-72 hours later a follow-up MRI will be performed. This may be a combination of structural and functional scans.

Follow-up Questionnaire: 1 week after LIFU application a Report of Symptoms questionnaire will be administered through an email.

Cohort 2 and 3 will not be performed until Cohort 1 is complete. The outcome of cohort 1 will be determined prior to enrolling cohort 2 and 3. This cohort is an additional safety check and meant to mitigate risk in the additional cohorts.

Cohort 2

Session 1	Sessions 2-4
Imaging	Stimulation
MRI & CT	LIFU + EEG + SEP
2 hours	2 hour

Session 1 - An MRI and CT exam will be performed (if needed).

Sessions 2-4 - Subjects will receive stimulations to generate SEPs before and after LIFU application. Subjects will receive one of the three LIFU pressures (high, medium, or low). EEG recordings will be taken throughout the study visit. The order of LIFU application will be randomized.

Follow-up Questionnaire: 1 week after LIFU application a Report of Symptoms questionnaire will be administered through an email.

Cohort 3

Session 1	Sessions 2-4
Imaging	Stimulation
MRI & CT	LIFU + fMRI + SEP
2 hours	2 hour

Session 1 - An MRI and CT exam will be performed (if needed).

Sessions 2-4 - Subjects will receive stimulations to generate SEPs before and after LIFU application. Subjects will receive one of the three LIFU pressures (high, medium, or low). fMRI recordings will be taken throughout the study visit. The order of LIFU application will be randomized.

Follow-up Questionnaire: 1 week after LIFU application a Report of Symptoms questionnaire will be administered through an email.

While subjects can choose to enroll in all of the cohorts, they may not be a part of two cohorts at the same time. I.e. they must complete all study visits in a given cohort before participating in an additional cohort.

During these sessions, HR and, BP data will be collected. Questionnaires will be completed at each study session.

MRI sessions: Participants will receive a structural MRI scan to obtain a high-resolution image of their head to be used for proper setup for LIFU stimulation. Functional MRI scanning will be performed while the participant is at rest and receiving SEP stims. The participant will be familiarized with the SEP sensation prior to lying in the scanner. During the MRI sessions, the experimenter and participant are able to communicate with

one another, ensuring the comfort of the subject. In addition, participants are provided with an emergency ‘squeeze bulb’ which can end the scan 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 MRI/CT images for guidance.

SEP: Somatosensory evoked potentials (SEPs) are generated in this study using an electrical stimulator placed on the participants arm. These electrical stimulations will be of both low voltage and amplitude. There is no risk for electrical shock to the participant. The stimulations will cause a muscle twitch and participants may find them irritating. They are not meant to be painful. If a high enough voltage (again, well below a safe level) is needed to create a muscle twitch it can be uncomfortable or cause pain. Participants are asked to report their sensations and if the stimulation is too painful they will be discontinued.

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 (0.2 to 2 seconds; duty cycle 0.5 – 5%; pulse repetition frequency 1 – 100 Hz) of low-intensity (< 60 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. LIFU application may occur inside of the scanner, time locked with the QST stimuli as described above, or outside of the scanner, immediately preceding the QST stimuli protocol inside of the fMRI. LIFU stimulations will be approximately 1 second each. These LIFU parameters align with previous studies conducted by Dr. Legon in his past research studies, including the 2020 Sci Reports publication analyzing LIFU safety and adverse events. 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. We have significant experience using LIFU including our published reports of

human neuromodulation experiments.

HR and BP: Physiological measurements will be collected using commercially available devices, such as the Biopac or Siemens systems. Blood pressure will be collected using a wireless blood pressure monitor (QardioArm) before and after study tasks. 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 be completed at each study visit: Experience Questionnaire, Daily Questionnaire, and Report of Symptoms questionnaires. Lastly, subjects will be contacted one week following the final LIFU session per cohort 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 (Safety_Screening_Form & Demographic_Form) 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 and SEP devices will undergo hands on training under the direction of the PI. All staff will complete a pilot run with PI approval prior to running participant sessions. Additionally, participants will be reminded they can terminate the study 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 (Daily Q, Experience Q, Report of Symptoms) and screening (Demographic Form, and Screening Form) information using an electronic Virginia Tech approved system such as Redcap or via paper form.

The MRI and CT will be used to collect brain images. Standard devices will be used to collect HR and BP 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. Heart rate and blood pressure 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. Additionally, questionnaire or screening data may be recorded on paper, entered into a digital database and then stored securely in a locked filing cabinet. 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, 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 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.

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)**:*** Click here to explain.

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 study sessions lasting from 1-2 hours. Study participation will vary by cohort. Cohort 1 - 3 visits, 4 hours. Cohort 2 - 4 visits, 8 hours. Cohort 3 - 4 visits, 8 hours. Participants will complete imaging scans (MRI and CT), LIFU stimulation, SEP and/or behavioral 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 EEG 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.

While participants with alcohol or drug abuse or addiction are excluded, participants who use substances and do not consider themselves as having an addiction are not excluded. Therefore, participants are asked to report any substance use on the day of each study visit. Anyone who has consumed alcohol or any recreational drugs on the day of a study visit will be rescheduled. This would both affect study data quality and presents a lack of ability to consent to study procedures sufficiently.

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 25 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 36 participants to yield approximately 25 participants.

Our sample yield size of 25 is based upon previous data that showed an attenuation of various MN-SEP potentials under the ultrasound condition as compared to the sham condition showing significant differences in the N20-P27 potential (sham, 1.22 ± 0.14 μV, s.e.m.; tFUS, 0.73 ± 0.15 μV; P = 0.014; Fig. 3) the P27-N33 potential (sham, −3.30 ± 0.65 μV; tFUS, −2.85 ± 0.46 μV; P = 0.017) and the late potential (sham, −0.66 ± 0.15 μV; tFUS, −1.42 ± 0.23 μV; P = 0.010) in N = 10 participants. Using these preliminary numbers at a desired p = 0.05 and power 0.9, an N = 20 is required. Given that these results were generated through an N = 10 study, the selected sample size is slightly larger since variability is likely to increase.

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 36 participants to yield approximately 25 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 at least one cohort. They have the option to participate in all three cohorts, however they cannot enroll in more than one cohort at a time.

15.0 Recruitment Methods

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

Subjects will be recruited from the general population within the age range of 18-65 years old. Subjects may participate in multiple Legon Lab studies.

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

Subjects that meet eligibility criteria for both the study, and MRI and CT 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:

Cohort 1
Session 1 - MRI/CT scan, 2 hours, $40
Session 2-3 - LIFU/fMRI, 1 hour per visit, $20 per visit
Follow up - Online questionnaire, <15 minutes, $10

Total - $90

Cohort 2

Session 1 - MR/CT scan, 2 hours, $40
Session 2-4 - SEP + EEG + LIFU, 2 hours per visit, $40 per visit
Follow up - Online questionnaire, <15 minutes, $10

Total - $170

Cohort 3
Session 1 - MR/CT scan, 2 hours, $40
Session 2-4 - SEP + fMRI + LIFU, 2 hours per visit, $40 per visit
Follow up - Online questionnaire, <15 minutes, $10

Total - $170

Max possible total - $430

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 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. If the voltage needed to generate an SEP is painful and the participant finds the sensation unbearable they will be withdrawn. 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 up to 60 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.

Computed Tomography 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 (0.2 to 2 seconds; duty cycle 0.5 – 5%; pulse repetition frequency 1 – 100 Hz) of low-intensity (< 60 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 60 W/cm2 (4x lower Isppa) for these studies. We have significant experience using LIFU including our published reports of human neuromodulation experiments.

Somatosensory evoked potential (SEPs) studies are generally considered safe procedures, albeit they may entail a minor discomfort, and potential risks could arise depending on individual medical conditions, thus warranting a subject-by-subject evaluation. During the procedure, electrodes are strategically placed on the scalp and on the median nerve located at the wrist. These electrodes administer painless electrical shocks between 0 and 6 times a second, eliciting somatosensory responses. The electrical activity captured by scalp electrodes is then recorded, amplified, and charted to facilitate interpretation by the investigator. While no discomfort is anticipated, participants will be informed if they experience any warming on the wrist electrode or discomfort to notify the investigator and the procedure will be immediately terminated.

Questionnaires: Participants will be asked to answer a wide array of questionnaires about their physical health, mental health, past and current drug use. Due to the sensitive and illegal nature of some of these questions there is a risk to the participant. While this information will not be freely shared with law enforcement there is a risk this data could be accessed.

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, 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/SEP procedures and they will be halted if the subject experiences any unexpected sensations. 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:

Pregnancy is a contraindication for CT. Participants will not participate if they are pregnant or trying to become pregnant. Study staff will screen for pregnancy in all females prior to any study visit with a CT scan.

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, physiological, 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, physiological, 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, physiological, 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)*

N/A

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 recorded physiological data and 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 1-2 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. Please see attached verbal screening script to be read prior to collection of screening information.
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. As this study involves four 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 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