# Low Intensity Focused Ultrasound of Medial Temporal Lobe Regions for the Improvement of Learning and Memory / Low Intensity Focused Ultrasound as a Non-Invasive Neural Prosthetic for the Improvement of Emotion Regulation

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## APPROACH

**Participants.** Participants will consist of 21 adults aged 35-90

**Subject Recruitment.** Participants will be recruited from the Human Connectome Project – Aging participant pool and through fliers placed around campus and greater Los Angeles.

**Eligibility.** Inclusion criteria: 1) age 35-90, 2) right handed, 3) English dominant language. Exclusion criteria: contraindications for MRI (e.g. metal implants, pregnancy), history of head injury sufficient to warrant medical attention, history of alcohol or substance abuse or dependence, history of major psychiatric illness requiring treatment, history of cancer or other neoplastic syndromes, history of major neurologic illness (e.g. epilepsy).

**Procedures.**

***Crossover Design.*** This study will be conducted using a crossover design in which each participant will complete two visits spaced two weeks apart. At one visit, they will receive LIFUP sonication to the right amygdala, and at the other, they will receive sonication to the left entorhinal cortex. The order of conditions will be randomized and counterbalanced across participants. Participants will be blinded to condition order, and all other testing will be conducted the same at both visits.

***Screening.*** Participants who have consented to be contacted regarding future research projects will be contacted by study staff who will conduct a telephone screening to ensure eligibility.

***fMRI and LIFUP administration.*** Prior to receiving LIFUP sonication, the LIFUP transducer will be aimed at the appropriate target region for the visit (either the amygdala or the entorhinal cortex) and gently strapped in place to their head. Participants will then return to the scanner where a T1 image will verify the position of the LIFUP transducer and allow for estimation of the spatial location of the sonication beam focus. If needed, adjustments to transducer placement will be made to ensure that the beam focus is correctly aimed; an additional T1 will be collected after adjustment. Once correct placement of the transducer has been confirmed, LIFUP will be administered in 10 sonications at 650kHz, 720mW/cm, duty cycle 5% duration 30s, with 30s spacing between sonications. LIFUP will be administered with a pulse repetition frequency of 10Hz for amygdala sonication and 100Hz for entorhinal cortex sonication. BOLD fMRI will be collected simultaneously during LIFUP administration.

***MRI Data Acquisition.*** We will use a 3-Tesla Siemens PRISMA scanner. We will collect T1, T2, hi-res hippocampal T2, and diffusion-weighted structural images for each participant. Additionally, we will collect arterial spin labeling and BOLD fMRI both before and after LIFUP, as well as BOLD fMRI during LIFUP administration. We will be using Human Connectome Project scan sequences to collect these images; detailed information about the protocols can be obtained here: http://www.humanconnectome.org/documentation/Q1/imaging-protocols.html.

***Behavioral Testing.*** Participants will be administered the behavioral tests described below twice at each of their in-person sessions; once before LIFUP and once after LIFUP.

***Verbal Free Recall Task.*** Verbal memory will be assessed using the Rey Auditory Verbal Learning Test (RAVLT) which involves repeated presentation of a list of 15 words. The order of RAVLT form administration will be randomized and counterbalanced across participants.

***Spatial Navigation Memory Task.*** Participants will be presented with a computerized spatial arena simulating a museum art gallery. They will be instructed to learn the locations of three objects placed throughout the gallery. After the Learning Phase, the subjects will then be asked to locate the objects' shown location sans stimuli immediately after learning the set of locations of each object. After each learning phase, participants will complete an unrelated Distractor Task

within the task but unrelated to the Mission. The navigation and distractor task paradigm will be repeated three times. During the fourth and final trial, retrieval of spatial information during this trial will be measured as the distance between the location that participants selected and the true location of the object. Shorter distances will indicate better spatial memory performance. This assessment has four forms, the order of which will be randomized and counterbalanced across participants.

***Brief Visuospatial Memory Test.*** In this test, the participant is presented with a 2x3 array of geometric figures for 10 seconds, then instructed to draw the figures from memory in the correct location on the page. The task consists of three learning trials, one long delayed recall trial, and one recognition trial. Learning performance will be calculated as the proportion of presented figures and figure locations successfully recalled during the initial presentations of the six-figure array. Long delayed recall memory performance will be calculated as the proportion of presented figures and figure locations successfully recalled freely after a 20-minute delay. Memory retention will be calculated as the proportion of presented figures successfully identified during a forced-choice recognition task. This assessment has 4 forms that will be used for repeat testing, the order of which will be randomized and counterbalanced across participants.

***State Trait Anxiety Inventory.*** This is a questionnaire used to measure anxiety-like symptoms. The first half of the questionnaire asks about the degree to which several listed statements align with how a participant feel at the current moment (state anxiety). The second half asks about the degree to which the listed statements align with how a participant typically feels (trait anxiety).

***International Affective Picture Stimuli Task.*** In this task, participants complete multiple trials in which a 15-second fixation cross is followed by a 2-second color-coded instruction for one of three trial types: WATCH (passively view a negative image), VIEW (passively view a neutral image), or REAPPRAISE (actively reappraise a negative image). The instruction image is followed by a 5 second presentation of a negatively or neutrally-valenced image. After each image presentation, participants rate their subjective level of current arousal and the degree of awfulness of the image (valence). The task consists of 24 VIEW trials, 24 WATCH trials, and 48 REAPPRAISE trials. Objective reactivity to each stimulus will be measured using galvanic skin response and heart rate. Emotional reactivity in this task will be quantified using the difference between negative and neutral stimuli (N-S). Emotional reappraisal will be quantified by taking the difference of the negative reappraisal stimuli and the negative stimuli (NR - N).

**Data Analysis Plan.**

**Hypothesis 1a.** LIFUP of the amygdala will decrease BOLD activation in the amygdala and functionally associated regions (e.g. DLPFC), as well as functional connectivity between the amygdala and connected regions. It will also increase perfusion in the amygdala. These changes will not be observed in response to LIFUP of the entorhinal cortex.

**Hypothesis 1b.** LIFUP of the entorhinal cortex will increase BOLD activation in the entorhinal cortex and functionally associated regions (e.g. DLPFC), as well as functional connectivity between the entorhinal cortex and connected regions. It will also increase perfusion in the entorhinal cortex. These changes will not be observed in response to LIFUP of the amygdala.

**MRI Analysis.** Analyses will be performed using FSL Version 5.0 (www.fmrib.ox.ac.uk/fsl). Stimulation-related functional data processing will include motion correction to the mean image, spatial smoothing (Gaussian Kernel FWHM = 8mm), high-pass temporal filtering ($t > 0.01$ Hz) and regression-based removal of outliers. Processing of rs-fMRI will include correcting for

motion artifacts (using framewise displacement calculations, DVARS and within-subject independent component analysis), scrubbing, slice timing, unwarping and spatial smoothing (Gaussian Kernel FWHM = 8mm). Functional data will be linearly registered to stereotaxic MNI space and co-registered with anatomic data for each participant. Processing of diffusion imaging will include correction for motion, eddy distortion and susceptibility induced distortion and then fitting diffusion tensors to the data, Subjects with greater than 3mm mean displacement will be excluded from analyses. Structural data (T1 and DTI) will be linearly registered to the standard MNI152 T1 2mm brain.

**Stimulation-related fMRI Analysis.** BOLD fMRI will be collected continuously during the LIFUP paradigms. A block-design model will be used to statistically compare the BOLD signal during LIFUP stimulation blocks to the BOLD signal during no-stimulation blocks. To examine effect of stimulation to target ROI activity, we will extract average BOLD response rates in both the targeted and control (non-targeted) region for each of the blocks and use mixed-effect models to compare activity during LIFUP conditions (on / off) within each ROI. To examine LIFUP-related network connectivity, we will use a seed-based approach and examine whole brain connectivity with the LIFUP target of interest and compare between stimulation (on-off) conditions using psychophysiological interaction modeling (PPI). To assess changes in connectivity in relationship to LIFUP, we will use dynamic connectivity to evaluate changes in ROI across each LIFUP condition. Finally, dynamic causal modeling will allow us to assess and compare how changes in activation in the LIFUP target may causally affect activation in other regions (e.g. increased amygdala activation leads to increased DLPFC activity).

**RS-fMRI.** We will compare the functional connectivity of the brain at rest before and after stimulation. Independent component analyses (ICA) will be used to statistically extract functional networks from each participant's rs-fMRI. Dual-regression analysis will then compare resting state networks between baseline and post-LIFUP to determine the effect of LIFUP on resting state functional connectivity. Age, sex and education will be included as additional regressors. Dual regression will also assess the association between these LIFUP-related longitudinal resting state network changes and emotional reactivity and memory performance.

**Arterial Spin Labeling.** Pulsed Arterial Spin Labeling (PASL) scans produce a perfusion image with voxel values representing local perfusion rates. For each subject, will be collected both before and after LIFUP. These PASL images will then be linearly registered to each subject's T1 structural image. Perfusion images will be processed by using the BASIL (Chappell et al., 2008) toolbox, including partial volume correction, then transferred to MNI space using non-linear registration in FSL. Then, a comparison of pre-vs-post tFUS sonication perfusion in the amygdala and entorhinal cortex will be conducted individually for each subject by subtracting the registered pre- sonication perfusion map from the post-sonication perfusion map. A $2 \times 2$ repeated measures analysis of variance (ANOVA), corrected for multiple comparisons using False Discovery Rate (FDR), will then be used to compare the longitudinal perfusion changes between amygdala sonication and ErC sonication.

**Hypothesis 2.** LIFUP of the amygdala will result in reduced subjective emotional reactivity to emotionally-salient stimuli compared to those presented before LIFUP; this reduction will not be observed with entorhinal cortex LIFUP.

**Behavioral Analysis.** Subjects, who will be unaware of the exact timing of the sonication onset, will be asked to report any unusual feelings, sensations or distress. To determine the effect of LIFUP on emotional reactivity, analyses of variance (ANOVA) will compare subjective valence

and arousal ratings between pre-LIFUP and post-LIFUP administrations of the IAPS task. Additionally, state and trait anxiety scores will be compared pre and post-LIFUP to determine LIFUP-related changes in anxiety levels.

**Physiological Analysis.** To determine the effect of LIFUP on psychophysiological reactivity to emotionally-salient stimuli, interbeat intervals (IBIs) will be calculated for each stimulus presentation using Autonomic Nervous System Laboratory (ANSLab). These IBIs in response to stimuli will be analyzed using multilevel modeling with a repeated measures within-subjects design, with target (amygdala / entorhinal cortex), timepoint (pre / post LIFUP), and instructions (view, watch, reappraise) included as model factors.

**Hypothesis 3.** LIFUP of the entorhinal cortex will result in better performance on memory tasks compared to both amygdala LIFUP and pre-LIFUP conditions.

**Behavioral Analysis.** To determine the effect of LIFUP stimulation on memory performance, performance on memory assessments before LIFUP, after entorhinal cortex LIFUP, and after amygdala LIFUP will be compared using ANOVA.