Memory or Mood Problems?

Clinical Trial of Transcranial Ultrasound ('TUS')

Patients with memory, mood or cognitive problems from dementia, Alzheimer's, head injury or other brain disorders are invited to enter a free clinical trial of painless, non-invasive brain ultrasound.

Physician or self-referral at
BUMC Department of Anesthesiology 520-235-0510
Information http://anesth.medicine.arizona.edu/

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The Department of Anesthesiology and Center for Consciousness Studies at BUMC, and the Department of Psychology at U of A are sponsoring an 'open label' trial of transcranial ultrasound ('TUS') for memory, mood and cognitive disorders in patients with dementia, Alzheimer's disease and traumatic brain injury. Administered from the fronto-temporal scalp ('temporal window'), brief, low intensity TUS is safe, painless and shown to enhance mood in hundreds of human volunteers at the University of Arizona. In the lab, ultrasound increases synaptic connections, and improves symptoms and pathology in mice with genetically-induced Alzheimer's. In this study, patients will perform brief cognitive tests before and after low intensity TUS, 2 minutes per day, several days per week for several weeks.

Investigators for the new study include Stuart Hameroff, MD (Anesthesiology, BUMC), Jay Sanguinetti & John JB Allen, PhD (Psychology, University of Arizona), and Michael Lemole, MD (Neurosurgery, BUMC)

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1) Introduction/Background

Memory and cognitive dysfunction in people with brain disorders, including Alzheimer's disease and traumatic brain injury, are enormous problems.

Various drugs, brain stimulation and training programs have been tried with minimal success. These include non-invasive transcranial direct current stimulation (tDCS, e.g. Fregni 2005), and transcranial magnetic stimulation (TMS). tDCS creates a weak electric current in the brain from electrodes placed on the scalp, and has had some success in improving verbal working memory. TMS imposes a magnetic field in the brain, and has shown promise for some cognitive symptoms of depression. But both tDCS and TMS are inconsistent, have poor spatial resolution and lack known neurophysiological mechanisms of action. A third technique is transcranial ultrasound (TUS). Ultrasound consists of

mechanical vibrations, usually in low megahertz (MHz), used for many years in medical imaging. But ultrasound has also been shown to stimulate excitable nerve and muscle tissue since Harvey's studies in 1929. More recently Tyler (2011), Bystritsky (2015), Yoo (2011) and others have shown electrophysiological and behavioral effects of brain ultrasound, and our group (Hameroff et al, 2012) was the first to show effects of TUS on mental states in human volunteers.

Figure 1 Transcranial Ultrasound ('TUS') from temporal window at 8 MHz with a GE Logiqe with imaging screen.

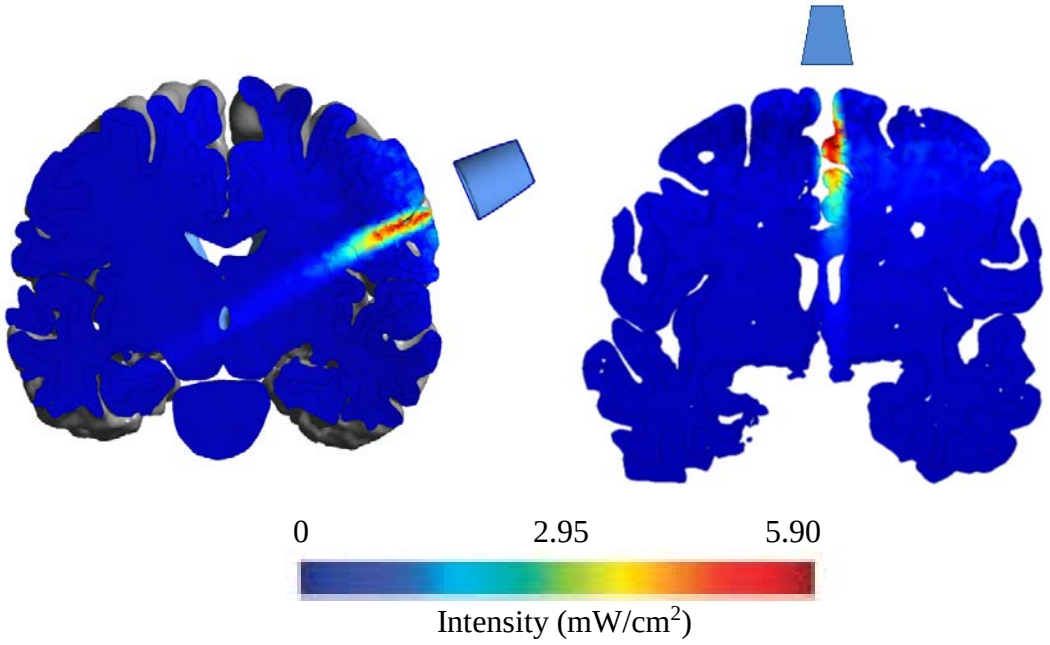
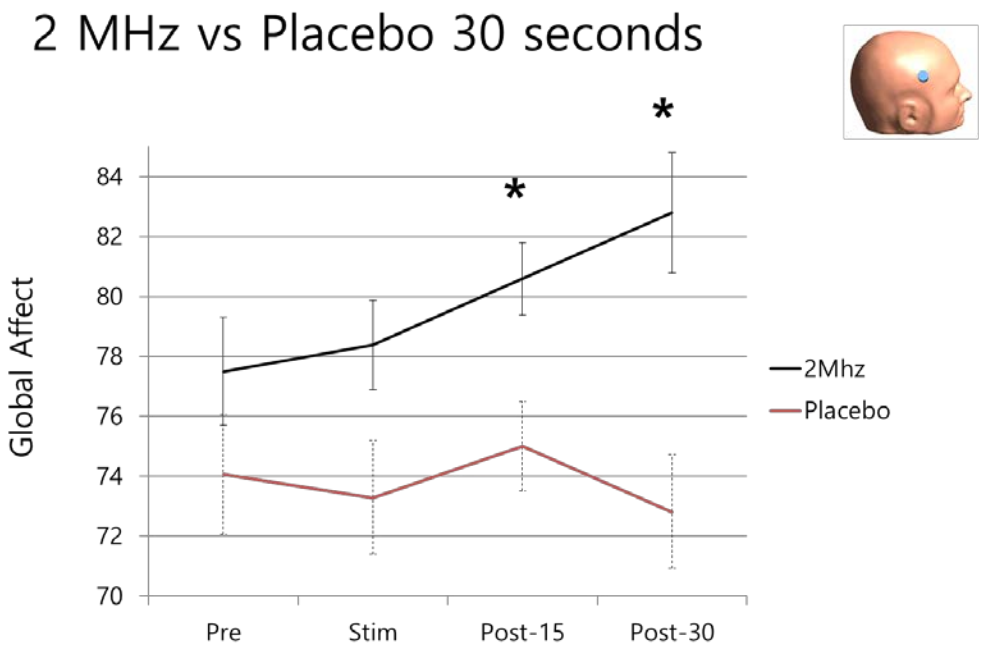


Figure 2. Simulation of TUS penetration delivered at the scalp at 150 mW/cm^2 at temporal window (left) and vertex (right).

Applied at the scalp and aimed at the brain at low intensities, and for reasonably brief durations, TUS is painless and safe. TUS is significantly attenuated by the skull, with lower frequencies (e.g. 2 MHz or less) penetrating more readily. Approximately 3 percent reaches the brain to echo back through the skull for surface images (Figures 1 and 2). Regarding safety, high intensity ultrasound can heat and cavitate tissue, and is used to cause therapeutic destructive lesions. Mid-range TUS intensitites can open the blood brain barrier. On the contrary, we use low intensity, sub-thermal TUS (~150 mW/cm^2) aimed at augmenting natural brain activities. The FDA has designated an ultrasound thermal threshold at 720 mW/cm^2. Sub-thermal TUS (~150 mW/cm^2) can safely and painlessly stimulate brain activity without long-term effects or damage (Dalecki, 2007). Simulation (Figure 2) suggests scalp levels of 150 mW/cm^2 results in peak brain levels of ~6 mW/cm^2. Moreover TUS can be pulsed (on-off cycles at arbitrary frequencies and patterns), and used in either focused or unfocused beams or scanning modes.

Using low intensity, sub-thermal TUS levels, we published the first study of TUS on human mood using 8 MHz TUS in a double blind protocol (Hameroff et al, 2012; Sanguinetti et al, 2014). 15 seconds of 8 MHz TUS at temporal window resulted in ~ 1 hour mood enhancement, measured by visual analog mood scale ratings, in a double blind crossover study. We have continued to show that brief (e.g. 30 seconds) low intensity TUS targeting the right frontal cortex enhanced mood (Sanguinetti et al, 2015). Other studies (e.g. Legon et al, 2014) have shown TUS enhances cognitive function in humans, and in a recent study on mice with genetically-induced Alzheimer's disease, TUS to temporal cortex restored memory deficits and reduced amyloid plaques (Leinenga and Götz 2015).

TUS and Mood



Sanguinetti et al., under review

Using 8 MHz TUS at 150 mW/cm2 in a double blind protocol, our group published the first study of TUS on human mood (Hameroff et al, 2012, c.f. Sanguinetti et al, 2014a; 2014b). We have continued to show that brief (e.g. 30 seconds) low intensity TUS from the scalp at the 'temporal window' aimed at right prefrontal cortex (PFC) results in approximately an hour of enhanced mood, as measured by subjective scales in randomized double blind trials (Sanguinetti et al, 2015). Other studies (e.g. Legon et al, 2014) have shown TUS enhances cognitive function in humans, and in a recent study in mice with genetically-induced Alzheimer's disease, TUS applied to temporal cortex restored memory deficits (Leinenga and Götz 2015).

With IRB approval, we have completed 3 previous clinical TUS studies on several hundred healthy human subjects without problems. The current study proposes to test effects of brief, sub-thermal TUS for memory, mood and cognition in patients with memory and/or cognitive dysfunction following brain injury (including concussion), and those who have, or may have, Alzheimer's disease or other dementia. In this experiment, patients with memory dysfunction possibly due to dementia including Alzheimer's disease and brain injury (including concussion) will be given ultrasound for cognitive enhancement and mood improvements.

Studies in cell culture show brief, low intensity ultrasound accelerates neuronal growth and synaptic formation (ref). But the mechanism by which low intensity TUS affects cognitive function and mental states is unknown. Suggestions include stimulation of mechano-sensitive membrane receptors, and enhanced vibrations of cytoskeletal microtubules, known to have megahertz resonance dynamics. 2 MHz stimulation causes optimized polymerization of microtubules, which are disrupted in Alzheimer's disease and brain injury.

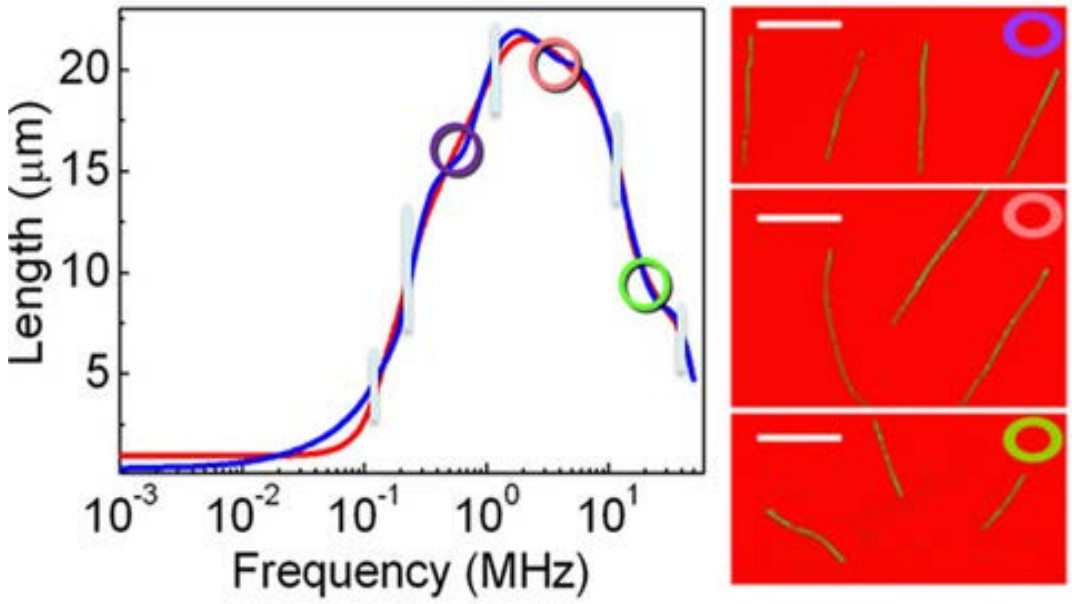


Figure 3. The mechanism of TUS is unknown, but self-assembly of microtubules, major component of neuronal cytoskeleton, is optimized at 2 MHz stimulation (Sahu et al, 2014). TUS may resonate microtubules.

TUS is painless and safe when properly administered, improves mood in humans and Alzheimer's symptoms and pathology in mice. It is potentially beneficial in brain disorders, and appears to cause no harm. The time is right. As world leaders in clinical TUS, we propose to test effects of brief, sub-thermal TUS on cognitive tests for memory and mood in patients with dementia and brain injury. Although our previous studies have been double blinded, we choose here an open label approach. We recognize a possible placebo response, but also see a potential stress response, e.g. for potentially confused subjects. So we'll start with an open label 'pilot study' and consider a subsequent double blind study.

2) Study Protocol

Subjects with memory and cognitive dysfunction can be referred from physicians throughout Banner-University Medical Center and elsewhere. Referred subjects with a history of dementia, Alzheimer's and/or brain trauma will be pre-screened by phone.

‘Alzheimer’s’ will be considered to include patients in any stage of Alzheimer’s previously diagnosed by medical professionals at BUMC or elsewhere. Subjects with mild-to-moderate brain injury including concussion will be solicited from Neurosurgery (e.g. Dr Lemole), Neurology (e.g. Dr Hishaw), Sports Medicine and elsewhere. Overall, approximately 60 dementia and 60 head injury patients are sought.

Those with a recent history of major medical conditions (< 6 months) will be excluded. Major medical conditions include: heart attack, stroke, trauma, respiratory distress (pneumonia or other serious lung conditions), organ failure, or cancer. Patients with migraines will also be excluded.

The study will take place in the Center for Consciousness Studies (CCS) offices, rooms 105, 105a and 105c, in the Faculty Office Building (FOB) 1609 Warren St (directly across from the BUMC Emergency Department). FOB allows easy ground floor access and parking. Room 105 is a common area, and 105a and 105c are private rooms. The actual TUS will be conducted privately in 105a and 105c during which participants will interact only with researchers.

Upon arrival and entry to the study, subjects or their caretakers will be asked to complete intake information, written consent will be obtained, vital signs taken, and the pre-test cognitive test battery given (see below).

Patients will receive two minutes of low intensity TUS to the right and two minutes to the left temporal window twice a week for four weeks. A full battery of tests will be administered at the beginning and end of the four weeks, and a minor test battery after each exposure. Results will be assessed through pre-and post-TUS test scores, and comments from caretakers and patients.

In this study we will use a GE Logiq E imaging ultrasound (Figure 1) in broad beam scanning mode at 2 MHz, aimed roughly at pre-frontal and medial temporal cortex using sphenoid ridge as a landmark. These areas are considered key to memory in Alzheimer’s disease, and executive function in brain injury.

The significance of TUS benefits would be profound, as (1) TUS is painless, safe, inexpensive, and could be widely available, and (2) memory and cognitive dysfunction in dementia and brain injury currently have no effective methods to improve cognition.

Personnel and Resources Although we consider the possibility of medical emergencies unlikely, anesthesiology and emergency medicine personnel and services are immediately available. On-call anesthesiology physicians are aware of the study and available for consultation and intervention if necessary.

The study will be conducted by Jay Sanguinetti PhD (post-doc in Psychology), Chris Chan (U of A undergrad), Stuart Hameroff MD, Alian Aquino MD, James Nelson MD, Mike Lucas MD (Anesthesiology), and Abi Behar-Montefiore and Ethan Yang (Center for Consciousness Studies). The researchers will NOT have access to patient medical records.

Pertinent medical information will be obtained upon entry to the study, and from reports by referring physician. Informed Consent forms will be provided and signed for entry. Patients and caretakers will be given an opportunity to ask questions or address any concerns while reading over the consent forms. Caretakers may also participate in this process if the patient is unable to consent for themselves. In addition, caretakers will be presented with consent forms for their part in the study. Where applicable, caretakers will be referred synonymously with the patient as a subject. Following informed consent and/or completion of the procedure, subjects will have another opportunity at the end of each session to ask questions and/or voice concerns.

If there are any updates to procedures during the duration of the experiment, patients will be notified of said updates by a telephone call.

Protocol details

Patients will participate in two sessions per week for four weeks with the procedure below following given consent. Data involving mood change and cognitive performance will be collected during each day. Medical grade tools (Welch Allyn PROPAQ CS monitor, UMC 1047005) donated by the Department of Anesthesiology will be used to measure blood pressure, heart rate, oxygen saturation by pulse oximetry and body temperature. Quality of life assessments scales (see appendix 5) will be taken from the caretaker on the first and last day of the study.

Dosage of ultrasound treatment will be held at intensity well below 720 mW/sq^2, well below FDA safety threshold. Ultrasound will be administered for 2 minutes targeting the right and another 2 minutes targeting the left temporal regions of the brain, via the "temporal window," or the skull area covering the temporal cortex. The 10-20 International EEG System will be used to target the temporal cortex.

All procedures are done for research purposes. No standard care is provided to patients.

Week 1

On the first day, patients will fill out an intake form and medical intake form (see appendix 7a and 7b). Patients will be given the ADAS-Cog test while the caretakers complete the ADCS-ADL life satisfaction scale (**1.6**), which will take about an hour. They will also be given the cognitive tasks outlined in section **1.5** below. Patients will then receive ultrasound stimulation on the right and left temporal windows for two minutes at intensity well below the FDA limit.

On day 2 (week 1), they will be given the cognitive tasks outlined in **1.5** below. Patients will then receive ultrasound stimulation on the right and left temporal windows for two minutes.

Week 2

On days 3-4 (week 2), they will be given the cognitive tasks outlined in **1.5** below. Patients will then receive ultrasound stimulation on the right and left temporal windows for two minutes on each day.

Week 3
On days 5-6 (week 3), they will be given the cognitive tasks outlined in **1.5** below. Patients will then receive ultrasound stimulation or sham stimulation(?) on the right and left temporal lobes for two minutes.

Week 4
On days 7-8 (week 4), they will be given the cognitive tasks outlined in **1.5** and the ADAS-Cog (patients) and ADCS-ADL (caretakers**) outlined in 1.6** below. Patients will then receive ultrasound stimulation or sham stimulation(?) on the right and left temporal windows for two minutes. Caretakers will repeat the ADCS-ADL at this session(repetitive?).

Cognitive and Mood Tasks

On each day, participants will perform the following simple cognitive tasks on paper or on computer:

- **Digit span (forward and backward) task (10-15 min):** Digit span is used to assess working memory. Participants are briefly shown an increasing amount of digits and prompted to sequentially repeat the digits back to the researcher. Successful recollections will result in increasing lengths of digits until the participant is no longer successful. This can be done by repeating from the beginning to the end or then end to the beginning of the digits shown.

- **Trail making A and B test (5-15 min):** Trail making tests the domain of executive function. In Part A, participants are given a form with scattered encircled numbers (1-25). The objective of the test is to connect the numbers in increasing order. The time taken to complete the test is measured. Part B introduces letters, alongside numbers, in the test. Participants will then connect 1 to A to 2 to B to 3 to C and so on in order to complete the test.

- **Word list learning (10-20 min):** Word list learning is a straightforward task that tests episodic memory by asking participants to recall a list of words. The list is presented three times to the participant, each time asking the participant to repeat the words back following each word shown. After several minutes have passed the participant is asked to repeat as many words back. Recall is again tested several minutes after the first recall session. The number of correct recalls will be measured. Each day's testing will have different word lists to account for learning effects.

- **Paired associates learning task (10-25 min):** The paired associate learning task also tests for episodic memory, associative memory. Participants are shown Two lists of words and tasked to learn the words. They are then instructed to learn randomly-paired words from the earlier task and later recall these arbitrary pairings. This is done in a manner similar to word recall.

• **Word recognition task (10-20 min)**: Similar to the free recall task, word recognition tests episodic memory. Participants are shown a list of 12 words and asked to repeat back to the researcher. Unlike the free recall task, participants will only be given one instance to learn this list. After 5 minutes participants are asked to distinguish words from a list of 24 words, 12 of which are from the initial list and 12 are new words. The number of correct responses will be measured.

• **Object knowledge task (5-15 min)**: The object knowledge task is used to test for mainly semantic memory. Participants will be shown 12 objects, four of which have frequent exposure, four with medium exposure, and four with low exposure, and asked to name the object. This is repeated for naming each finger on the hand. They can be given clues to each object's function, as specified by the ADAS-Cog manual. Participants will not be allowed to touch the objects.

• **Category fluency task (5-15 min)**: Category fluency tests for semantic memory in participants. They are given a minute to list as many words from a given category. Examples of such categories are animals, vehicles, and household items. A recording of this task will be done for future scoring purposes.

• **4 Mountains task (10-20 min)**: The 4 Mountains task is given to participants as a measure of spatial memory. A computer-generated landscape with four distinct mountains is shown to participants. Following a 2 second exposure to a blank screen, four panels are shown. One of the panels shows the initial mountain configuration, modified by reorientation and/or non-spatial features (vegetation, lighting, environment, etc.) while the other three panels will show similarly modified images of different mountains. A training phase will be given prior to actual administration. This can also be done with images printed on paper and given accordingly.

• **Block design task (5-15 min)**: Used in the Wechsler Adult Intelligence Test, the block design task measures spatial capabilities. Participants are given 16 cubes that are half red and white such that a line divides the colors at four vertices and two faces of the cube are completely red, completely white, and white and red. Participants are then given a 4x4 design and asked to replicate the design with all cubes. The amount of time taken to replicate the design is used to gauge performance.

• **Visual Analogue Mood Scale**

A simple self-report scale. On a single sheet of paper, patients rate their current mood on six dimensions: Alertness, Sadness, Tenseness, Happiness, Weariness, Calmness, and Sleepiness. They will mark their answer along a 100mm line, from "Very Little" to "Very Much."

1.6 ADAS-Cog (60-75 min; appendix 8): The Alzheimer's Disease Assessment Scale- Cognition is a standardized cognitive battery used commonly in NIH-funded trials for Alzheimer's Disease. The domains that are tested by word recall, commands, constructional praxis, naming, ideational praxis, orientation, and word recognition. The majority of these tasks are administered in less than 5-10 minutes.

ADCS-ADL (15-20 min): The Alzheimer's Disease Cooperative Study - Activities of Daily Living measures quality of life from the caretaker's perspective. Like the ADAS-Cog, the ADL is also a standardized assessment commonly used in AD trials. The ADL consists of less than forty questions that can be administered briefly.

Since they will be aware of the study hypotheses before the experiment begins, there will be no need for a debriefing.

After one month, and again after three months, caretakers will be given a follow-up phone call and asked the questions from the ADCS-ADL.

Cost to subjects

Subjects will incur no cost during this study except their time.

Risks to subjects

Both devices (GE Logiq E, U+) are *non-significant* risk devices with parameters well under FDA limits for transcranial ultrasound devices (see appendix 9 for U+, and appendix 10 and 11 for manuals). The outputs for U+ were tested with a hydrophone by Russ Witte, PhD, who runs the Experimental Ultrasound and Neural Imaging Laboratory in Radiology.

The FDA limits diagnostic ultrasound to 720 mW/cm^2 for adults. Above these doses, ultrasound can potentially damage tissue. Ultrasound stimulation has also been shown to be safe as studies have found effects using intensities as low as 24 W/cm^2 and 150 mW/cm^2, both of which are well below the 700 mW/cm^2 intensity FDA allows for safe non-thermal ultrasound (i.e., the intensity used in imaging ultrasound). Thermal ultrasound refers to intensities that could damage tissue or skin.

Both ultrasound devices can *only* administer doses well below the approved FDA guidelines for safe human application. Therefore, it will not be possible to administer a harmful dose of the ultrasound to participants using this device.

See the 2008 FDA guidelines on ultrasound:

http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM070911.pdf

Both ultrasound devices emit pulsed ultrasound. The ultrasound transducer will administer a single pulse lasting for only a few seconds, repeated over two minutes. Thus the transducer will be placed on the participant's head for two minutes at a time, but the stimulation will be not continuous throughout this time. This means the actual

"dose" will be minimal. We have chosen the smallest dose we expect an effect in order to minimize risk to the participants as much as possible.

Ultrasound has been used in countless settings on human tissue for over 50 years and has an excellent safety record over; it has been used by the PI hundreds of times in clinical settings. We will stay far below the FDA recommended dosages and will report any unforeseen adverse outcomes immediately to the IRB. All stimulation will be 2 MHz or below at intensity well below 720 mW/cm^2 which is deemed safe under the FDA guidelines and verified by published literature as a safe dose.

The ultrasound gel used on the transducer is hypoallergenic, but nonetheless could cause a very mild skin reaction in some subjects. This is very unlikely, but in the case that a researcher notices a mild reaction, or a subject complains of irritation, the study will be stopped immediately.

Potential benefits to subjects and/or society

The possible implications are wide-reaching if TUS improves memory and cognition in patients with memory and cognitive deficits in Alzheimer's, age-related decline in memory function, and brain trauma. Safe, painless and inexpensive, TUS offers potential benefit for a wide range of mental and cognitive brain disorders.

a. **Protection of subject privacy:**

   The entire study will take place in a private room in the Center for Consciousness Studies FOB 105. The door to the room will be shut and experimenters will ensure subject privacy. All contact via telephone or e-mail will be to schedule participants, and no identifiable or personal information will be asked in email. Therefore, should participants share an e-mail or phone line, no private information will be gleaned from our communications.

b. **Protection of data confidentiality:** Consent forms will be stored in Babcock 1114 until the end of the study. Per psychology department guidelines, at the end of the study all consent forms will be moved to the Psychology main office, room 312. Each participant will be given a unique participant identifying number that will not be recorded on his or her consent form. This unique participant number (and not any identifying information) will only be recorded on all experimental data collected. Therefore, all of the data in Babcock 1114 will be de-identified. The date of the participant's intake will be recorded on the consent form. This date will also be recorded in an Excel sheet of de-identified participant data. Consent forms will be stored in Psychology for six years should data ever need to be re-identified, as requested by the IRB. De-identified data will be secured indefinitely in electronic form on Dr. Allen's secure server. Paper copies, with no identifying information, will be shredded once entered onto the server.

Subject compensation

Patients will receive no payment for their time.

Withdrawal of subjects

Subjects will be reminded they can withdraw themselves from the study at any point if they feel uncomfortable with any part of the study design. Incomplete data will be analyzed.

Future use and long-term storage of data or specimens

As mentioned in *Protection of Data Confidentiality*, de-identified experimental data will be stored in a secure server belonging to Dr. John Allen. This data includes mood change, cognitive performance, quality of life, and consent forms of each subject. Physical copies will be shredded per regulation after the data is secure on Dr. Allen's server. All forms of data will not have any identifying information and will not be sold or shared with any pharmaceutical companies.

This is an 'open label', open enrollment study: All patients with memory problems attributable to dementia (including Alzheimer's) and brain injury (including concussion) – will be recruited for this study.

The caretakers of dementia patients will be recruited alongside their patient. Our rationale for an open label study is the following. For subjects with memory and cognitive impairment, visits will be both stressful and excitatory. Although the excitatory aspect of the visit could induce a placebo effect, visits are also stressful and we want to minimize untoward effects. If we see improvement we will then consider a double blind study.

An Institutional Review Board responsible for human subjects research at The University of Arizona reviewed this research project and found it to be acceptable, according to applicable state and federal regulations and University policies designed to protect the rights and welfare of participants in research.

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