



Original Research

Transcranial ultrasound (TUS) effects on mental states: A pilot study

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ABSTRACT

Background/Objective: Transcranial ultrasound (TUS) can modulate brain function. To assess possible TUS modulation of mental states, we investigated effects on subjective reports of pain and mood of sub-thermal TUS versus placebo applied to frontal scalp and brain of chronic pain patient volunteers.

Methods: With IRB approval and informed consent, subjects with chronic pain completed two visual analog scales for pain (NRS) and mood (VAMS/Global Affect), and their vital signs were recorded 10 min prior to, and 10 min and 40 min following exposure to either subthermal TUS (8 MHz) or placebo (in a double blind crossover study) using the 12L-RS probe of a LOGIQe ultrasound imaging machine (General Electric, USA). A physician, also blinded for TUS versus placebo, applied the probe (with gel) to scalp over posterior frontal cortex, contralateral to maximal pain, for 15 seconds. A second investigator operated the ultrasound machine, randomizing TUS versus placebo. The process was then repeated, applying the opposite modality (TUS or placebo). **Results:** Subjective reports of Mood/Global Affect were improved 10 min ($P = 0.03$) and 40 min ($P = 0.04$) following TUS compared with placebo. NRS pain reports slightly improved following TUS ($P = 0.07$) at 40 min.

Conclusion: We found improvement in subjective mood 10 min and 40 min after TUS compared to placebo. TUS can have safe neurophysiological effects on brain function, and is a promising noninvasive therapy for modulating conscious and unconscious mental states and disorders. We suggest TUS acts via intra-neuronal microtubules, which apparently resonate in TUS megahertz range.

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Introduction

Therapeutic brain stimulation delivering electric and/or magnetic fields either via implanted electrodes or non-invasive transcranial approaches has been utilized and studied since the 1950s. Transcranial magnetic stimulation (TMS) was shown to safely modulate cortical function [1], and electrical techniques including transcranial direct current stimulation (tDCS) and transcranial alternating current stimulation (tACS) have shown promise for acute and chronic pain [2,3], and memory [4].

Another transcranial modality is ultrasound (TUS), cyclic mechanical vibrations with frequency greater than the upper limit

of human hearing ($\sim 20,000$ Hz, 'Hz'; 20 kHz, 'kHz'). Used medically since the 1920s, and shown since 1929 [5] to have effects on excitable tissue, ultrasound occurs in a range between 20 kHz and around 20 MHz (20,000,000 Hz, 20 'MHz'). Ultrasound penetrates bodily tissue including bone, and is widely used to image anatomical structures via pulse echo, e.g. fetuses in utero, and various structure in medical and surgical diagnosis, nerve blocks and catheter placements. Virtually every part of the body, including the brain, has been safely imaged with ultrasound [6].

At any particular frequency, the thermal energy or heat, as well as any physiological or pathological effects are determined by the intensity, duration and pulse width of the delivered ultrasound, along with specific properties of tissue through which it passes. With extreme levels, e.g. leading to heating and cavitation, ultrasound can damage cells and tissue. Indeed extremely high intensity, continuous (very long pulse width) focused ultrasound is used for ablation, e.g. to destroy specific thalamic brain regions for chronic intractable pain (heating to 53 °C) [7]. More moderate ultrasound

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thermal effects can selectively damage rapidly dividing cells, e.g. blocking excessive mitosis in malignant brain tumors [8]. At still lower thermal levels, ultrasound has historically been used for soft tissue injury ('diathermy' for muscle relaxation and vasodilatation).

At lower levels, e.g. sub-thermal exposure at 5.7 MHz and 280 mW per square centimeter (mW/cm^2), ultrasound can stimulate or inhibit excitable tissue by mechanical vibrations without detectable damage or heating [9–11]. For example low level ultrasound focused on specific brain regions of animals (both directly and transcranially) alters behavior, electrophysiology and synaptic plasticity [12–16]. Focused on motor cortex of mice, ultrasound stimulation evokes paw movements without detectable changes in structure or function [13]. Ultrasound can thus modulate neuronal activity, e.g. presumably including brain activities related to higher-level cognition and consciousness. In 2002, following neuroimaging experiments in psychiatric patients, Bystritsky et al. [17] proposed that ultrasound could be used for neuromodulation with therapeutic benefit for mental and neurological disorders [18].

The mechanism by which non-thermal ultrasound modulates neuronal activity is unknown. Applied to peripheral neurons, sub-thermal ultrasound with shorter duration pulses tends to activate, and longer pulses to inhibit, action potential amplitude and velocity [19,20]. In mouse hippocampus, low intensity ultrasound modulates neuronal activity, presumably by influencing voltage gated sodium and calcium channels [13]. This is consistent with a recent hypothesis that non-thermal ultrasound-induced neuromodulation occurs via mechanical stretching of membrane lipid bilayers [21], and thus stimulating stretch-sensitive voltage-gated ion channels [22]. An alternative, or complementary view, that ultrasound modulates neuronal functions through resonant vibrations in intraneuronal microtubules, will be described in the Discussion.

Transcranial ultrasound (TUS) is a promising, safe technique to therapeutically modulate brain functions, potentially including mental states and disorders. To clinically evaluate TUS modulation of mental states in human volunteers, we studied subjects with chronic pain, a complex disorder involving peripheral nociception, overactive central nervous system responses, and major changes in mood, affect and quality of life [23]. Chronic pain is associated with alterations in brain structure, for example in 'default-mode' networks associated with background mental states [24–26]. Treatment of chronic pain with opiate drugs has many side effects including dependency, thus a myriad of alternate drugs and therapies have aimed at reducing chronic pain, including tACS and tDCS [2,3,27]. Chronic pain and its associated mood disorders were the mental states targeted in our TUS pilot study.

Methods

We set out to determine whether TUS can beneficially alter mental states, as determined by measures of pain and mood in subjects with chronic pain.

For TUS we used a General Electric (GE) LOGIQe ultrasound system with a 12L-RS probe, e.g. utilized in anesthesiology practice for imaging in nerve blocks and vascular catheter placement. The LOGIQe is approved for adult brain imaging, fetal imaging and newborn brain imaging through fontanelles [28,29]. To avoid heating or cavitation, we approached TUS conservatively, as described below. Ours is among the first TUS studies in humans aimed at modulation of mental states.

Transcranial ultrasound (TUS)

A General Electric LOGIQe ultrasound machine with 12L-RS probe in B mode was utilized [28]. The industry recommendation to avoid heating and cavitation is acoustic output below 720 mW per

square centimeter (720 mJ per second per square centimeter) [29]. Effects depend on tissue characteristics, described by three parameters monitored and displayed on the LOGIQe console: Thermal Index (TI), Mechanical Index (MI), and a relative Acoustic Output (AO) value. TI describes tissue heating, and is the ratio of total acoustic power to power needed to raise tissue temperature by 1°C . Depending on type of tissue involved, two TI parameters may be selected and monitored. The TI choices are either Soft tissue: TIs, Bone: Tlb, or Skull/Cranium: Tlc, though Tlc as an index of heating may be somewhat misleading due to ultrasound absorption near internal skull surface [30]. The LOGIQe then derives and displays MI and AO values during operation. MI relates to cavitation and is the peak pressure at the point of maximum pulse intensity integral, divided by the square root of the ultrasonic center frequency in megahertz. FDA guidelines give a maximum MI permitted of 1.9 and require an explanation if TI is greater than 6.0. We selected and monitored TIs and Tlc display options, and carefully monitored MI and AO values during exposure (see below).

The LOGIQe has other settings contributing to tissue effects of acoustic output. These include (with our chosen settings) power (100%), frequency (8 MHz), depth (3.5 cm), harmonics (on), cross-Xbeam (on), mode (B), and duration of exposure (15 s). The LOGIQe also has a 'Freeze' button which, when pressed, stops ultrasound emission from the probe, and which we used for placebo. TUS was applied as shown in Fig. 1 and described below. Full power at posterior frontal scalp with these settings gave acoustic output values of $\text{MI} = 0.7$, $\text{TIs} = 0.5$ and $\text{Tlc} = 0.2$, well below FDA guidelines of maximum MI of 1.9 and TI of 6.0. The global maximum acoustic output (AO) was $152\text{ mW}/\text{cm}^2$, about 20% of the recommended limit of 720 mW per centimeter, and about 30% of Tyler's stated safety limit of 500 mW/cm^2 [21]. Our parameters were thus well within safe levels.

Probe application site

The greatest clinical experience in ultrasound to the brain comes from transcranial Doppler ultrasound used to measure blood flow velocity in specific brain arteries. A variety of devices using both phased array and simple two-element probes spanning 1–10 MHz have been used [31].

Fig. 1A shows four commonly used sites for transcranial Doppler (TCD) ultrasound. These are 1) trans-orbital, to visualize ophthalmic artery and internal carotid siphon through orbital fissure and optic foramen, 2) sub-mandibular, for distal internal carotid artery through angle of mandible, 3) sub-occipital, for vertebral and basilar arteries through foramen magnum and 4) trans-temporal, for circle of Willis through 'trans-temporal window'. The trans-temporal window passes through the thin squamous part of the temporal bone, but may vary in location and/or be absent [32,33]. Site 5 in Fig. 1A was used in the present study. The ultrasound 12L-RS transducer probe (aligned longitudinally) was placed at the scalp, slightly anterior and superior to the 'temporal window' (Fig. 1B). At a frequency of 8 MHz and depth setting of 3.5 cm, placement at this site demonstrates scalp, skull and the posterior aspect of the right frontal cerebral cortex (Fig. 1C). This confirms TUS penetrated the skull to reach the brain, echoing back to the probe to be imaged.

Study design

The study was a double blind crossover, with subjects receiving either TUS or placebo/sham, and then the alternative (Groups 1 and 2, Fig. 2). The LOGIQe console has a "Freeze" button which, when pressed, stops the ultrasound emission through the machine and stays on. Freeze button "ON" was used for placebo, and Freeze

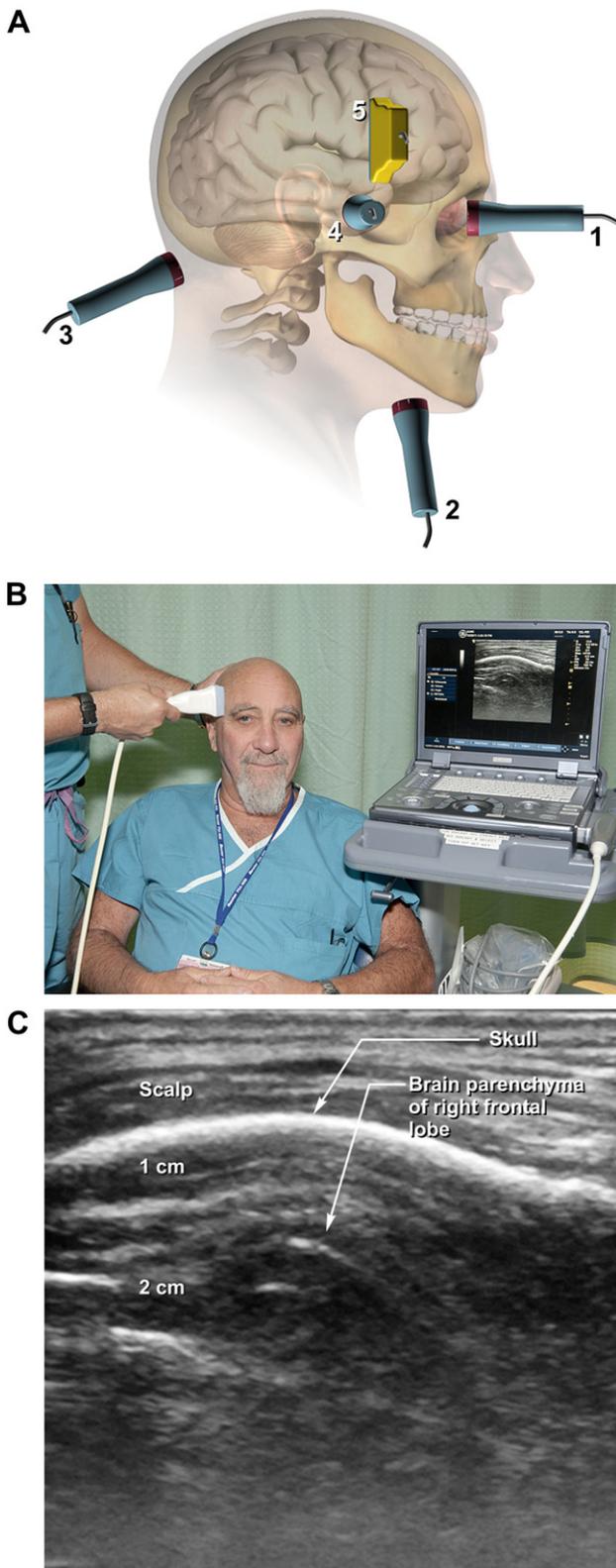


Figure 1. (A) Sites for transcranial ultrasound (TUS). 1–4 are used in Doppler blood flow studies. 1: trans-orbital, 2: sub-mandibular, 3: sub-occipital, 4: temporal window. 5 is the site used in present study, overlying frontal-temporal cortex. (B) Transcranial ultrasound as used in present study (at site 5 in Fig 1A). A 12L-RS probe from a GE LOGIQe ultrasound imaging machine is applied with gel on the scalp overlying frontal-temporal cortex. The image is shown on the monitor of the LOGIQe device. (C) Longitudinal gray scale image from Fig. 1B, typical of images from study participants. Scalp, skull and posterior aspect of right frontal lobe of cerebral cortex are visible, confirming TUS in our study penetrated the skull to reach the brain.

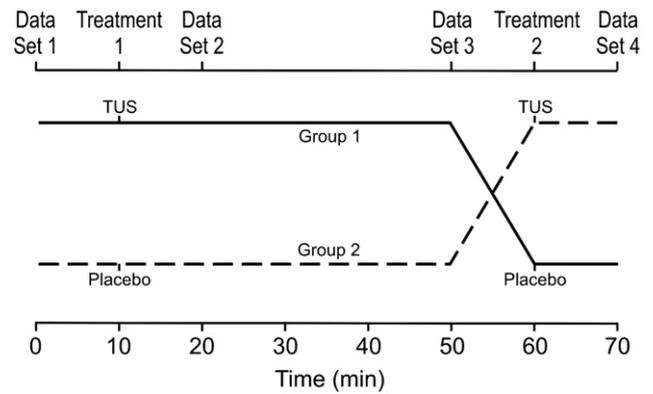


Figure 2. Study design. A double blind crossover was utilized, in which subjects served as their own control. Subjects were randomized into two groups (Group 1 and Group 2). After intake information and one set of data were obtained, Group 1 subjects received TUS, and Group 2 subjects received placebo. After 10 min, and again another 30 min later (40 min post-exposure), subjects crossed-over and Group 1 subjects received placebo, and Group 2 subjects received TUS. Data sets 40 min post first exposure served as baseline 10 min before second exposures. After 10 min, another set of data was obtained. Because of time constraints in the clinic, data sets 40 min post second exposure were not obtained for any subjects.

button “OFF” used for TUS treatment. Subjects received either TUS or placebo, and then the alternative. Subjects were assigned by coin flip to either Group 1 (TUS, then placebo), or Group 2 (placebo, then TUS).

Patient selection

With approval from the Institutional Review Board of the University of Arizona Medical Center, and that of University of Arizona Medical Center-South Campus, subject volunteers were recruited from among patients in the Chronic Pain Clinic at University of Arizona Medical Center-South Campus. Inclusion criteria were subjects with chronic pain who had not experienced changes in pain or treatment regime in the past month. Most subjects suffered from post-surgical back pain, and most were taking opioid pain medication (Tables 1 and 2). Subjects with severe neurological, cardiopulmonary or psychiatric problems, minors and pregnant females were excluded.

Subjects were told: 1) therapeutic use of TUS was experimental, 2) ultrasound at levels to be used in the study had been safely applied to virtually all regions of the body, 3) brain ultrasound was commonly used in newborn babies, and in studying blood flow in adults, 4) the particular ultrasound device and intensities to be used in the study were FDA approved for brain imaging without time constraints [21]. Informed, written consent was obtained. Intake data included age, chronic pain origin, medical problems, medications and recent treatment.

Thirty one outpatients in the Chronic Pain Clinic at University of Arizona Medical Center South Campus were approached for this study, met criteria, and were enrolled. Of those approached, none were excluded. Subject demographics are described in Results.

Probe application/Exposure

The ultrasound probe (12L-RS, General Electric, USA) with Aquasonic 100 ultrasound transmission gel (Parker Laboratories, USA) was applied by a physician at the scalp, contralateral to most severe pain and perpendicular to the skin. The LOGIQe ultrasound machine was controlled in accordance with randomization (via the Freeze button) by another researcher, with the LOGIQe console

Table 1
Demographics Group 1 (Ultrasound, then placebo).

| Gender | Age | Pain source | Side | Handedness | Pain medications | Probe application |
|--------|-----|---------------------|------|------------|-------------------------|-------------------|
| M | 46 | Lower back | C | R | Opioid, Muscle relaxant | R |
| F | 39 | Neck | C | L | Opioid | R |
| F | 53 | Shoulder, Back | L | L | Opioid, Benzodiazepine | R |
| F | 63 | Shoulder, Arm, Back | R | R | Opioid, Benzodiazepine | L |
| M | 31 | Rib, Chest | L | R | None | R |
| M | 70 | L face, R leg | L&R | R | Benzodiazepine | L |
| F | 51 | Neck, Shoulder | R | R | Opioid | L |
| F | 54 | Whole body | L | R | Opioid | R |
| F | 37 | Lower back | C | R | Opioid | L |
| F | 62 | Lower back | C | L | Opioid | R |
| M | 83 | Lower back | C | R | Opioid | L |
| M | 51 | Lower back | C | R | Muscle relaxant | L |
| M | 67 | Lower back | L | R | Anti-inflammatory | R |
| F | 29 | Back | C | R | Opioid, Muscle relaxant | L |

unseen by study subject or physician applying the probe (the light accompanying the Freeze button was covered). In the case of midline or non-lateralized pain, the non-dominant hemisphere was used (Tables 1 and 2).

Subjects sat in a comfortable chair with constant lighting and ambient sound. A study physician then held the 12L-RS probe with gel at the subject's posterior frontal scalp (Fig. 2) for 15 s. With the Freeze button off, the imaging screen showed scalp, skull and brain parenchyma (e.g. Fig. 1C). Both subject and physician applying the probe were blinded as to whether the ultrasound machine was in Freeze mode or not (i.e. placebo vs TUS). Following exposure, subjects were asked if they detected any sound, heat or sensation from the probe, and all said no. (In a separate test, an acoustic spectrum analyzer showed a faint 12.8 kHz signal from the probe when Freeze button was off, and ultrasound emitted. But this was inaudible to us, and to subjects in ambient sound.)

Data collection

After intake demographic information was obtained, the following data were recorded 10 min before exposure, and 10 min and 40 min after exposure: Vital signs (Heart rate, systolic and diastolic blood pressure, oxygen saturation), NRS: Numerical rating scale for pain [34], and VAMS (Visual Analog Mood Scale) [35].

Post-exposure 1 after 40 min also served as Pre-exposure 2 (10 min prior to the second exposure). Unfortunately, we were unable to include 40 min Post-exposure 2 data because our time in the clinic was limited. This is an admitted weakness in our study.

Table 2
Demographics Group 2 (Placebo, then ultrasound).

| Gender | Age | Pain source | Side | Handedness | Pain medications | Probe application |
|--------|-----|------------------|------|------------|-------------------------|-------------------|
| F | 65 | Back | C | L | Opioid | R |
| F | 67 | Cervical spine | C | R | Opioid | R |
| F | 66 | Cervical spine | R | R | Opioid, Antidepressant | L |
| F | 50 | Cervical spine | C | R | Opioid | L |
| F | 39 | Back, Arms, Legs | C | R | None | L |
| M | 48 | Lower back | R | R | Opioid | L |
| F | 35 | Lower back | L | R | Opioid, Anticonvulsant | R |
| M | 83 | R thigh, L hip | R&L | A | None | L |
| F | 50 | Neck, Arms | C | R | Opioid, Anticonvulsant | L |
| M | 66 | Lower back, Neck | R&C | R | Opioid | L |
| M | 67 | Both feet | R&L | R | None | R |
| F | 36 | Neck | L | R | Opioid, Muscle relaxant | R |
| M | 44 | Face, Head | R | L | Opioid, Antidepressant | R |
| F | 30 | Lower back | C | R | Opioid, Muscle relaxant | L |
| F | 41 | Lower back | L | R | Opioid, Muscle relaxant | R |
| F | 43 | Lower back | R | R | Opioid | L |
| M | 72 | Lower back | C | R | Opioid | L |

NRS (Numerical Rating Scale) is a visual analog 0–10 scale. Subjects were instructed to circle the number that best corresponds to their pain at the moment: 0 = no pain, 1–3 = mild pain, 4–6 = moderate pain, and 7–10 = severe pain. VAMS (Visual Analog Mood Scale) includes 8 categories on a 0–100 scale. Categories include Happy, Calm, Sad, Tense, Alert, Sleepy, Effort and Weary. From VAMS, two global parameters, Global Affect (GA) and Global Vigor (GV) are derived: $GA = 10 \times [(happy) + (calm) + 20 - (sad) - (tense)]/4$; $GV = 10 \times [(alert) + 30 - (sleepy) - (effort) - (weary)]/4$.

Results

Demographics

Table 1 shows pertinent information about Group 1 (ultrasound, then placebo), and Table 2 for Group 2 (placebo, then ultrasound). Pain Source refers to anatomical location of greatest pain, Side refers to laterality of pain (L: left, R: right, C: center), Handedness refers to subject's dominant hand (A: ambidextrous), and Pain medications indicate the types of medications taken by each subject for pain. Probe Application refers to the side of the head where the probe was placed.

General results

Subjects reported no subjective experience during exposure with either TUS or placebo. Scalp, skull and brain parenchyma were visible on the LOGIQe imaging screen during TUS, demonstrating penetration of TUS to the brain, and echoing back to the probe

(Fig. 1A). One subject experienced exacerbation of a headache following TUS which quickly subsided without sequelae. He was followed, and his data are included in our results. There were no other untoward effects during or after treatment, or in follow-up phone interviews up to 4 months after the study.

Data analysis

We measured values for each subject at 4 times in 7 categories: 1) Numerical Rating Scale (NRS), a subjective measure of pain, 2) Global Affect, a rating of mood, 3) Global Vigor, a measure of energy, or motivation. We also measured vital signs: 4) systolic and 5) diastolic blood pressure, 6) heart rate, and 7) blood oxygen saturation as measured by finger probe.

Values were obtained: 1) at baseline (10 min before first exposure), 2) 10 min after first exposure, and 3) 40 min after first exposure. This 3) 40 min post first exposure value also served as baseline 10 min prior to second exposure. Finally, values were obtained 4) 10 min post second exposure (but unfortunately not 40 min post-exposure because of clinic time constraints, an admitted weakness in our study). Thus post-40 min analysis included only 17 placebo and 14 TUS data points, whereas the post-10 min analysis included all 31 patients.

Table 3 shows average values (plus or minus standard error of the mean, SEM) in each of the 7 categories at the different measurement times. The differences in values for NRS, GA and GV, produced by either placebo or TUS 10 min and 40 min post-exposure were each compared (TUS versus placebo) using Student's paired *t*-tests. Vital signs data (systolic and diastolic blood pressure, heart rate, oxygen saturation) were similarly compared using unpaired Student's tests (because not all data points could be matched, i.e. these values are not expected to be constant). Because NRS, GA and GV (the latter 2 derived from VAMS scores) were anticipated to improve as a result of TUS treatment (NRS decreasing, and GA and GV increasing), they were analyzed using one-tailed *P*-values. Changes in vital signs were not anticipated as a result of TUS, so these were analyzed using two-tailed *P*-values.

In Table 3, columns 5 and 8 show statistical significance for these comparisons in terms of *P* values, with *P* = 0.05 (95 percent confidence) a general standard. All values had *P* values above 1.0, and are reported as NS (not significant) with the following exceptions: NRS (Numerical Rating Scale for pain) showed slight reduction for TUS compared to placebo at 40 min post-exposure, but with only 90% confidence interval (*P* = 0.07).

We also explored the possibility that TUS may produce changes in mood, as measured by Global Affect (GA) derived from the VAMS test. We found that TUS elicited significant improvement in Global Affect 10 min (*P* = 0.03) and 40 min (*P* = 0.04) post-exposure compared with placebo.

In vital signs measurements we found reduced values 10 min post-exposure for placebo compared with TUS for systolic (*P* = 0.03) and diastolic (*P* = 0.06) blood pressure. We also found increased oxygen saturation 10 min post-exposure for TUS

compared with placebo (*P* = 0.05). There were no significant changes from baseline in vital signs 40 min after TUS.

In summary, we found significant improvement in Global Affect, a measure of mood, 10 min and 40 min after TUS compared to placebo, and a slight improvement in pain score (NRS) 40 min after TUS compared to placebo. We found reduction in systolic and diastolic blood pressure for placebo versus TUS 10 min post-exposure. We also found increase in blood oxygen saturation for TUS compared with placebo after 10 min. There were no changes in any vital signs 40 min post-exposure for either TUS or placebo.

Discussion

A new genre of non-invasive therapeutic brain modalities aimed at mental and neurological dysfunctions includes transcranial electrical (alternating and direct current), magnetic and ultrasound stimulation (tACS, tDCS, TMS, TUS). Their potential to safely modulate brain processes offers a wealth of therapeutic opportunities. As clinicians (anesthesiologists, radiologist) who routinely use ultrasound imaging for nerve blocks, vascular access and diagnosis, and are familiar with ultrasound technology and potential risks, we find transcranial ultrasound, TUS, particularly interesting, and chose to study its effects on mental states, namely pain and mood in chronic pain patients. Our study design and measured parameters were imperfect, and chronic pain and depression are difficult to quantify. We were limited in clinic time, and unable to perform extensive psychological testing. We emphasized safety in what may be considered a pilot study for TUS in a clinical setting.

Ultrasound effects depend on intensity, frequency and other factors including tissue properties. High intensity ultrasound can cause heating and cavitation which can damage or destroy tissue. Mid-range intensity can cause mild, beneficial heating (diathermy) in soft tissue injury. Our intent was brief, low intensity, non-thermal ultrasound, perhaps enhancing naturally-occurring vibrations in brain proteins involved in mechanisms supporting conscious mental states.

For TUS we used a GE LOGIQe ultrasound imaging machine at 8 MHz for 15 s exposure to the posterior frontal scalp (and frontal cortex) at about 20 percent of FDA-recommended limits. Subjects could not detect ultrasound output (nor could clinicians applying the probe).

In a double blind crossover study comparing TUS versus placebo, we found significant improvement in Global Affect, a measure of mood, both 10 min (*P* = 0.03) and 40 min (*P* = 0.04) after TUS compared to placebo. We also found slight improvement in pain ratings (NRS) 40 min after TUS exposure compared to placebo (*P* = 0.07).

We found reductions in blood pressure 10 min post-placebo exposure, perhaps indicating relaxation or normal variation. This reduction was not seen 10 min following TUS, suggesting TUS might lead to brain-induced sympathomimetic effects. Similarly, increased blood oxygenation, also observed 10 min post-TUS, may indicate brain respiratory stimulation by TUS.

Table 3

Average \pm SEM at baseline, 10 min post treatment, and 40 min post treatment for placebo and transcranial ultrasound (TUS).

| | Baseline | | 10 min post | | 5. <i>P</i> | 40 min post | | 8. <i>P</i> |
|---------------------------------|-----------------|-----------------|-----------------|----------------|-------------|-----------------|-----------------|-------------|
| | 1. Placebo | 2. TUS | 3. Placebo | 4. TUS | | 6. Placebo | 7. TUS | |
| NRS/Pain Score [scale 1–10] | 6.5 \pm 0.5 | 6.5 \pm 0.5 | 6.4 \pm 0.5 | 6.2 \pm 0.6 | NS | 6.9 \pm 0.6 | 6.2 \pm 0.8 | 0.07 |
| Global affect [scale 1–100] | 58.4 \pm 4.1 | 54.6 \pm 4.5 | 58.3 \pm 4.2 | 59.3 \pm 4.3 | 0.03 | 56.8 \pm 6.1 | 58.6 \pm 5.5 | 0.04 |
| Global vigor [scale 1–100] | 49.4 \pm 3.1 | 47.5 \pm 3.4 | 48.9 \pm 3.2 | 44.8 \pm 3.4 | NS | 45.4 \pm 4.5 | 49.6 \pm 4.2 | NS |
| Systolic blood pressure | 140.9 \pm 4.4 | 137.4 \pm 4.1 | 134.5 \pm 4.1 | 138.4 \pm 4 | 0.03 | 135.1 \pm 5.1 | 141.14 \pm 6 | NS |
| Diastolic blood pressure | 84.8 \pm 1.8 | 84.5 \pm 2.0 | 79.1 \pm 1.9 | 84.0 \pm 1.9 | 0.06 | 82.9 \pm 1.6 | 84.1 \pm 3.4 | NS |
| Heart rate [BPM] | 79.2 \pm 2.7 | 78.6 \pm 2.4 | 77.0 \pm 2.5 | 78.5 \pm 2.3 | NS | 76.6 \pm 2.8 | 76.7 \pm 4.5 | NS |
| O ₂ Blood saturation | 96.3 \pm 0.5 | 93.0 \pm 2.3 | 96.7 \pm 0.5 | 96.6 \pm 0.5 | 0.05 | 97.0 \pm 0.6 | 96.70 \pm 0.9 | NS |

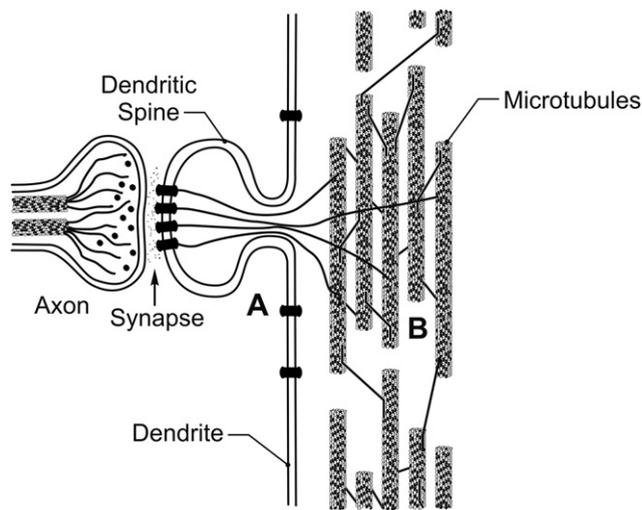


Figure 3. Schematic of synapse and neurons with two possible explanations for ultrasound effects. Left: Axon terminal releasing neurotransmitters into synapse and onto receptors in membrane of dendritic spine. Actin filaments (as well as soluble second messengers, not shown) connect to cytoskeletal microtubules in main dendrite. Two microtubules are seen in the axon (left); dendritic microtubules (right) are arranged in local networks, interconnected by microtubule-associated proteins (MAPs). Tyler [21] proposed ultrasound affected viscoelastic properties of neuronal membranes and extracellular fluid, altering membrane conductance (zones marked by A). We suggest ultrasound acts via microtubules (B) which have resonant frequencies in TUS megahertz range [40–43]. Artwork by Dave Cantrell.

Mood improvement suggests TUS somehow affects brain activities underlying mental states, i.e. conscious experience. But the specific brain activities which underlie conscious experience, and the mechanism by which TUS may affect them, are both unknown.

The most prominent view of non-thermal TUS modulation of neuronal function proposes involvement of viscoelastic properties of neuronal membranes and surrounding fluid environments, altering action potentials and membrane conductance via effects on voltage gated channels and stretch-sensitive receptors [21]. We suggest that in addition to these neuronal membrane effects, TUS acts directly on solid-state intra-neuronal microtubules, major components of the cytoskeleton.

Microtubules (Fig. 3) organize neuronal interiors, form and regulate synapses, act as tracks for motor proteins which deliver synaptic precursors, and disintegrate in Alzheimer's disease [36]. Their lattice polymer structure has been theoretically linked to information processing, memory and consciousness [37–40]. Stimulating brain microtubule activities in a controllable manner could be extremely useful, and expected to have a somewhat delayed effect (e.g. 40 min) via motor protein delivery of synaptic components which are synthesized in cell body some distance away. But why would microtubules be sensitive to TUS?

Beginning in 2001, experiments began to show coherent excitations from living cells in the low megahertz range, with microtubule vibrations the most likely source [40,42]. A recent study shows specific resonant frequencies in single microtubules from 12 kHz to 30 MHz [43], precisely in the range for ultrasound (8 MHz used in our study is one particular microtubule resonant frequency).

TUS acting to resonate microtubules could be a double-edge sword, even at sub-thermal intensities. Excessive, prolonged vibrations could rattle and disrupt the cylindrical lattice polymers, e.g. inhibiting excessive mitosis in cancer cells [8]. But low to moderate intensity ultrasound could enhance microtubule activities, including synaptic plasticity, and be helpful in memory, dementias including Alzheimer's disease, and traumatic and

hypoxic brain injuries. TUS may perhaps also be used to optimize the quality of conscious experience, and thus be important not only in medicine, but as an induced form of meditation, relaxation, and beneficially altered conscious experience.

Our study is among the first TUS clinical trials aimed at mental states, and utilized only a tiny fraction of the TUS parameter space. Other parameters to be explored include TUS location, frequency, intensity, depth, duration, modulation by pulsation [8], mixture with other frequencies, intervals between treatments, and whether multiple TUS probes are used, e.g. aimed to intersect and potentiate in specific deep brain regions.

In this study we demonstrate clinical safety and potential utility of TUS. Among transcranial therapies, TUS may be most physiological, versatile and useful for neurological, psychological and psychiatric therapy, as well as cognitive and conscious enhancement.

Conclusion

Transcranial ultrasound (TUS) appears to be a safe, non-invasive technology for modulating brain activities with relevance to cognition, consciousness and mental states and disorders. In this double blind study we compared sub-thermal levels of 8 MHz TUS (versus placebo) applied at the scalp over fronto-temporal cortex in subjects with chronic pain. Compared to placebo, TUS resulted in statistically-significant improvement in Global Affect, a measure of mood, 10 min and 40 min post-exposure ($P = 0.03$ and $P = 0.04$, respectively), and slightly reduced pain levels 40 min post-exposure ($P = 0.07$). Our findings suggest TUS can beneficially affect mental state.

The mechanism for TUS effects on mental states is unknown, proposed to involve effects on neuronal membranes [21]. We suggest TUS also acts on intra-neuronal microtubules which have physiological resonances in low megahertz frequencies, including our 8 MHz exposure [41–43]. As microtubules are intimately involved in synaptic plasticity, and theoretically implicated in learning, memory and conscious experience [37–40], TUS may be useful in a variety of mental and neurological disorders including depression, traumatic and hypoxic brain injury, stroke, learning, Alzheimer's disease, psychiatric disorders, and altering states of consciousness. TUS is a promising non-invasive, inexpensive therapeutic tool for modulation of conscious and unconscious mental states and disorders.

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