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Safety of focused ultrasound neuromodulation in humans with temporal lobe epilepsy

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ABSTRACT

Objective: Transcranial Focused Ultrasound (tFUS) is a promising new potential neuromodulation tool. However, the safety of tFUS neuromodulation has not yet been assessed adequately. Patients with refractory temporal lobe epilepsy electing to undergo an anterior temporal lobe resection present a unique opportunity to evaluate the safety and efficacy of tFUS neuromodulation. Histological changes in tissue after tFUS can be examined after surgical resection, while further potential safety concerns can be assessed using neuropsychological testing.

Methods: Neuropsychological functions were assessed in eight patients before and after focused ultrasound sonication of the temporal lobe at intensities up to 5760 mW/cm^2. Using the BrainSonix Pulsar 1002, tFUS was delivered under MR guidance, using the Siemens Magnetom 3T Prisma scanner. Neuropsychological changes were assessed using various batteries. Histological changes were assessed using hematoxylin and eosin staining, among others.

Results: With respect to safety, the histological analysis did not reveal any detectable damage to the tissue, except for one subject for whom the histology findings were inconclusive. In addition, neuropsychological testing did not show any statistically significant changes in any test, except for a slight decrease in performance on one of the tests after tFUS.

Significance: This study supports the hypothesis that low-intensity Transcranial Focused Ultrasound (tFUS) used for neuromodulation of brain circuits at intensities up to 5760 mW/cm^2 may be safe for use in human research. However, due to methodological limitations in this study and inconclusive findings, more work is warranted to establish the safety. Future directions include greater number of sonications

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as well as longer exposure at higher intensity levels to further assess the safety of tFUS for modulation of neuronal circuits.

Key Points

- tFUS is a novel brain stimulation technique with not yet fully established safety guidelines.
- tFUS was administered to patients electing to undergo resective brain surgery. tFUS does not appear to cause damage to tissue.

Introduction

Noninvasive, controllable, and reversible modulation of regional brain activity is a major interest in current neuroscience because current neuromodulation technologies are *either* invasive, or limited in their spatial resolution. An ideal neuromodulation technology would be noninvasive, but also allow for precise targeting of both deep and superficial brain circuits.

The emerging technology of transcranial focused ultrasound (tFUS) offers the unique possibility of noninvasive, targeted neuromodulation. It is a promising new potential neuromodulation tool, because it can preferentially target and stimulate deep brain regions (e.g., thalamus), with high spatial specificity, whilst having minimal effect on other regions [1–4]. It allows for the noninvasive delivery of acoustic energy to a well-localized and circumscribed brain region of a few millimeters in diameter, depositing mechanical or thermal energies [5,6].

The administration of tFUS has been demonstrated to a have a variety of neuromodulatory effects. While there is ongoing debate as to whether these effects rely on primarily a thermal or non-thermal mechanism [7–9], the effects themselves have been documented widely. Several studies have demonstrated that, when used at low-intensities for neuromodulation, the mechanism of action is primarily non-thermal [10–12]. Further, the short acoustic wavelengths of high frequency ultrasound enable focusing the sonication to regions limited to several millimeters and, by increasing the surface area of the scalp over which the ultrasound is applied (either with larger spherical-section transducers, or using phased arrays), the focal spot can be placed in deep brain targets, such as thalamus or amygdala [5] while depositing minimal energy outside of the targeted region. tFUS differs from high-intensity focused ultrasound (HIFU) because the tFUS energies are an order of magnitude lower than HIFU. Whereas HIFU is administered continuously for ablation, tFUS is administered in short pulses, which reduces total energy deposition. Given these advantages, tFUS is a tool with great potential in both therapy and diagnostics [13].

Early investigations assessed safety with various ex vivo preparations. A 2008 study by Tyler and colleagues showed that repeated stimulation of brain slices every 8 min for 36 h did not result in changes to the cytoarchitecture or integrity [10]. Upon further examination, there was no evidence of damage to the integrity of the BBB, and there was not a difference in the frequency of apoptotic neurons [14]. Numerous additional investigations have used a variety of histologic assessments, including hematoxylin and eosin (H&E) staining, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), and vanadium acid fuchsin (VAF) staining with toluene blue counterstaining, and none of them showed evidence of damage [12,15–17].

However, not every study is consistent. A study of sheep brain showed micro-hemorrhaging after repetitive sonications of V1 with a 50% duty cycle [18]. Importantly, this study used a high spatial peak, pulse average intensity (I_{sppa}) of 6.6 W/cm^2. It's worth noting that edema, cell necrosis, or localized inflammatory processes were not detected with the H&E staining that was used. Indeed, reported adverse effects from tFUS are exceedingly rare [19]. While ultrasound at higher intensities may produce effects such as hemorrhage, apoptosis, or opening of the blood-brain barrier (BBB), it is difficult to establish the safety limits of the ultrasonic neuromodulation technique at which these effects occur, as published studies do not report parameters in a consistent manner.

The ability to suppress neuronal activity could be of great interest in a variety of neurological and psychiatric disorders. Suppressing epileptiform activity in patients would be an ideal application of the technique. In fact, tFUS has already been used in animal models of epilepsy. In 2011, Byoung-Kyong Min and others reported that focused ultrasound could suppress PTZ-induced acute epileptic EEG activity by targeting thalamus in rats [20]. They utilized a 0.5 ms pulse duration with a 5% duty cycle, 100 Hz PRF & 130 mW/cm^2 I_{spta}. Histology confirmed that the ultrasound did not induce any changes or damage to the sonicated brain regions. Several years later, Hakimova, et al., continued this line of research in a kainic acid chronic murine model of temporal lobe epilepsy, demonstrating that 30 s of tFUS (delivered as 1 ms pulses repeated at 500 Hz) could inhibit acute epileptic activity, prevent status epilepticus, and reduce the number of chronic seizures [21].

Recently, more literature has come out regarding the pentylenetetrazol model of epilepsy. Stimulating through cortex, hippocampus, and thalamus, Chen and colleagues were again able to suppress acute EEG spikes in rats with acute epilepsy using tFUS [22]. The safety of tFUS in human epilepsy, however, has yet to be demonstrated.

The objective of the current study was to assess the safety and feasibility of tFUS neuromodulation in the human brain. To determine if tFUS damages brain tissue, we utilized human participants with medication-resistant temporal-lobe epilepsy, who were already scheduled to undergo resective brain surgery for epilepsy treatment. This allowed us to apply tFUS to the temporal lobe prior to its scheduled removal and enabled the detailed histopathological evaluation of the tFUS-exposed tissue for damage. We also performed neuropsychological testing before and after tFUS exposure to assess whether the ultrasound resulted in measurable cognitive changes.

Materials & methods

All experimental procedures were approved by UCLA Institutional Review Board (IRB: 13–000670) and were regulated by an Investigational Device Exemption (IDE) G130290 from the US Food

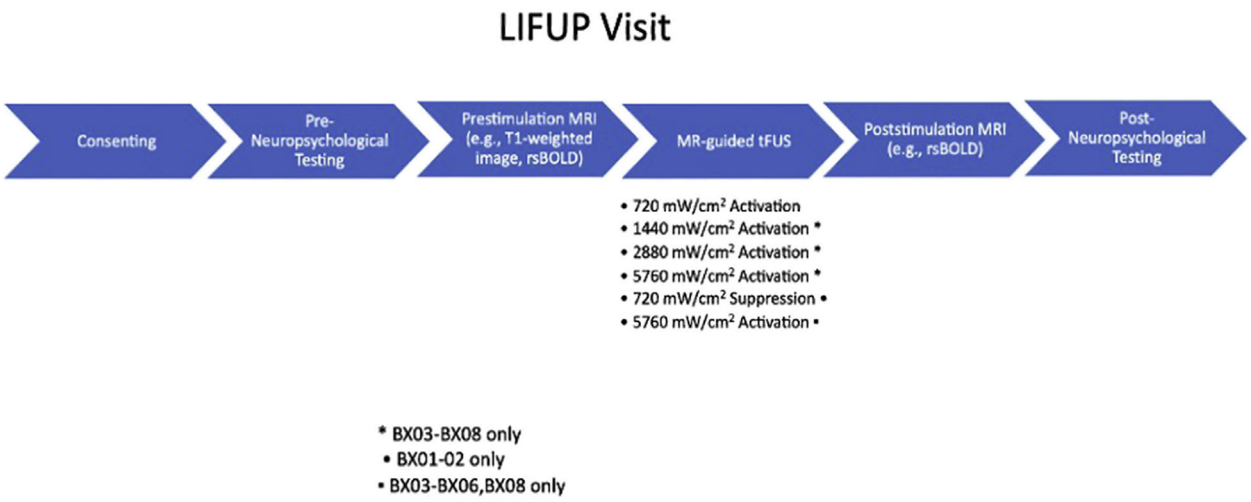

Fig. 1. Flowchart of study events.

and Drug Administration (FDA). A flowchart of study events (excluding surgery and histology) is in Fig. 1.

Participants

Overall, eight patients were referred to the study from the UCLA Seizure Disorders Center after being scheduled for an anterior-mesial temporal lobe resection as treatment for medication-resistant temporal lobe epilepsy.

The FDA initially limited inclusion to only those participants who were undergoing resection of the non-dominant hemisphere, but later expanded the criteria to include both dominant and non-dominant temporal lobes. The criteria for inclusion at study launch were: age 18–60 years, a diagnosis of temporal lobe epilepsy that has been resistant to at least 3 appropriate and FDA-approved anti-seizure medications, adherence to anti-seizure medication treatment, maintenance of a seizure diary, a seizure frequency of at least 3 seizures/month and an epilepsy surgery evaluation that identified unilateral hippocampal dysfunction and seizure onsets. The diagnostic evaluation included the intracarotid amobarbital procedure (IAP) and neuropsychological testing. As this is an exploratory study, the study protocol was amended multiple times to allow for flexibility in enrollment and to enroll a wide variety of subjects to better assess safety in different patients.

Participants who had a cognitive or psychiatric disorder that limited their ability to give informed consent were excluded. We excluded participants if their recent history included status epilepticus or seizures due to alcohol or illicit drugs. In addition, participants with implants or other metal components not compatible with MRI were excluded due to elevated risk. Lastly, we also excluded pregnant participants. Participant demographic information is listed in Table 1.

tFUS device

We utilized various models of the BX Pulsar device (BrainSonix Inc., Sherman Oaks, CA). The BX Pulsar is designed to deliver tFUS energy to the human brain, and to be acceptable for simultaneous use within a 3 T MRI (MR Conditional [23]). The BX Pulsar transducer uses a spherically focused piezo element with a 6.1 cm aperture diameter and a fundamental resonance of 650 kHz. Acoustic intensity measurements in a water tank [24] showed that the transducer has a focal maximum pressure at a depth of 6.2 cm and a focal volume (the region in which the pressure is more than one half the maximum pressure) that is a prolate spheroid of approximately 4 mm × 4 mm × 28 mm in water.

For the first two participants, the transducer was placed within a water-filled holder with a membrane that allowed for deformation to fit the participant's head surface. A second-generation holder

Table 1
Participant demographics.

Pt.	Age/ Gender	Side of Resection	Years Since Diagnosis	Medication Taken During Study	Seizure Type	Seizure frequency (per month)
BX01	44/F	Right	24	Levetiracetam, lamotrigine, phenobarbital	Focal seizure with impaired awareness	4
BX02	22/F	Right	10	Levetiracetam, lacosamide, phenobarbital	Focal seizures that progress to bilateral tonic-clonic	3–4
BX03	41/M	Left	4	Levetiracetam, lacosamide	Focal seizure with impaired awareness	8
BX04	24/F	Left	7	Levetiracetam, oxcarbazepine	Focal seizure with impaired awareness	1
BX05	27/M	Left	15	Levetiracetam, lamotrigine, eslicarbazepine,	Focal seizure with impaired awareness	6
BX06	37/F	Left	3	Lacosamide, brivaracetam	Focal seizure with impaired awareness	8
BX07	25/M	Right	2	Eslicarbazepine, clobazam	Focal seizure with impaired awareness	4
BX08	65/F	Left	58	Lacosamide, brivaracetam, perampanel	Focal seizure with impaired awareness	5

was used for the remainder of the study. The latter incorporated a 1-cm thick ultrasound conducting gel-pad (Aquaflex, Parker Labs, Fairfield, NJ) between the transducer surface and the participant's head. The gel-pad provides the same contour benefit as the water-filled holder. We applied ultrasound gel (Aquasonic, Parker Labs, Fairfield, NJ) to both sides of the gel pad prior to putting the device onto the head. This acoustic coupling is necessary to avoid impedance mismatches in the ultrasonic path and to ensure correct beam propagation and focus. For all participants, the transducer was placed approximately over the temporal window (the thinning of the skull just posterior to the temple) and attached to the head with an adjustable strap.

MRI and tFUS procedures

We administered tFUS, with MRI guidance, to the temporal region on the side scheduled for resective surgery, within the anterior temporal lobe. The tFUS suggestion took place at least one day prior to the resection surgery. The ultrasound was focused on the region within the temporal lobe to be resected. Functional MRI of the brain was obtained throughout the tFUS session.

Targeting of the anterior temporal lobe entailed an iterative process. We first placed the transducer in a position over the temporal bone. A brief 3D T1-weighted MR image (FLASH, TR = 3.15 ms, TE = 1.37 ms, flip angle = 8 degrees, 1.6 mm^3 isotropic voxels) series was acquired to determine if the transducer was positioned accurately over the desired region of the anteromesial temporal lobe. If not, the transducer was repositioned; another 3D T1-weighted MR image was then acquired. We repeated this process as needed until we achieved proper placement [1]. Proper placement was assessed by drawing a line orthogonal to the face of the transducer along the main axis of the ultrasound beam to the depth of the ultrasound transducer. If this point was within the desired focal area, then the placement was accurate. We delivered tFUS concurrently with T2*-weighted BOLD imaging (TR = 700 ms, TE = 33 ms, 2.5 mm^3 isotropic voxels, MB = 6).

The tFUS stimulus was administered under 2 different pulsing paradigms that were classified as “activation” and “suppression” based on previous preclinical research in animals. The fundamental frequency was 650 kHz in both instances. Activation tFUS involved brief pulse trains with a 50% duty cycle [16]. Suppression tFUS involved 30-s pulse trains with a 5% duty cycle [20]. The epilepsy study by Min et al., used a 5% duty cycle to inhibit activity. For this reason, we first delivered stimulation at 50% duty cycle, and then later attempted to replicate the Min findings by stimulating with a 5% duty cycle.

As part of the first-in-human safety testing, initial delivery of tFUS was limited to a derated Spatial Peak, Time Average intensity ($I_{SPTA.3}$) of 720 mW/cm^2. This limit value comes from the FDA Guidance document for Track 3 diagnostic ultrasound devices [25], and assumes a uniform tissue attenuation or derating of 0.3dB/cm-MHz While this derating approach is not predictive of the therapeutic transcranial situation, it provides an upper bound on *in-situ* exposure. The pulse paradigms are shown in the table below.

The specific intensities that each subject received are shown in Table 2 below. One participant (BX07) did not receive the suppression paradigm due to time constraints during the stimulation session.

Neuropsychological assessments

Before and after the tFUS/MRI procedure participants underwent neuropsychological testing to determine if they had any significant decrease in dominant/non-dominant hemisphere functioning as a result. The original test battery contained several redundant or duplicative measures, so it was modified as the study progressed. The most used assessments were the Rey Auditory Verbal Learning Test (RAVLT), which evaluates verbal learning and memory, as well as either the Rey-Osterrieth Complex Figure Test (ROCFT), the Brief Visuospatial Memory Test-Revised (BVMT-R), or the Taylor Complex Figure Test (TCFT), all of which evaluate visuospatial learning and memory. It has been hypothesized that the RAVLT accesses predominantly dominant hemisphere functions [26], whereas the other tests access predominantly non-dominant hemisphere functions [27]. Different forms of each measure were used pre-test to post-test. Pre-test was administered immediately before treatment and post-test was administered immediately after treatment. While each measure offers a delayed recall score only the immediate recall scores were used. Raw scores were converted to standard scores and then to percentile ranks. The process of aggregating tests within a domain into a composite score is a common principle in neuropsychological studies [28,29]. After testing for normality, we ran the Wilcoxon Signed Rank Test, a non-parametric test to test the null hypothesis of no effect of tFUS.

Histology

After the scheduled surgical resection of the anterior-mesial temporal lobe [30], the removed tissue was fixed in 10% formaldehyde, embedded in paraffin, and sectioned at 10 μm. Apoptosis assays were performed using the *In situ* Apoptosis Detection kit per manufacturer's instructions (Abcam). Briefly, sections were deparaffinized and subsequently treated with terminal deoxynucleotidyl transferase dUTP Nick End Labeling (TUNEL) and counterstained with methyl green. As a positive control, non-sonicated tissue was treated with DNase I to generate free 3′-OH ends for TUNEL labeling.

To visualize potential damage due to sonication, adjacent paraffin sections were stained with either hematoxylin and eosin (H&E), or vanadium acid fuchsin (VAF) with toluidine blue counterstain. H&E staining is customarily used for examination of tissue integrity, whereas VAF-toluidine blue staining is performed to detect the presence of acidophilic neurons which are indicative of acute neuronal injury and subsequent apoptosis or necrosis. VAF staining also allows for the visualization of extravasation and blood vessel disruption [31]. The samples were imaged at the UCLA Translational Pathology Core Laboratory (TPCL) using Applied Imaging Leica Aperio Versa.

	Sonication Duration (s)	Number of Sonications	Pulse Width (ms)	Pulse Repetition Frequency (Hz)	Duty Cycle
Activation	0.5	8	2	250	50%
Suppression	30	2	0.5	100	5%

Table 2
Ultrasound exposure values.

Suppression 5% Duty Factor - BX03-BX06, BX08							
$I_{SPTA.0}$ mW/cm^2	$I_{SPTA.3}$ mW/cm^2	$P_{r.0}$ MPa	$P_{r.3}$ MPa	$I_{SPPA.0}$ W/cm^2	$I_{SPPA.3}$ W/cm^2	MI	TIC
7607	5760	1.985	1.727	152.1	115.2	2.14	2.88

Activation 50% Duty Factor - BX03-BX08							
$I_{SPTA.0}$ mW/cm^2	$I_{SPTA.3}$ mW/cm^2	$P_{r.0}$ MPa	$P_{r.3}$ MPa	$I_{SPPA.0}$ W/cm^2	$I_{SPPA.}$ W/cm^{2}3	MI	TIC
951	720	0.23	0.19	1.90	1.44	0.24	0.36
1902	1440	0.33	0.27	3.80	2.88	0.34	0.72
3803	2880	0.46	0.39	7.61	5.76	0.48	1.44
7607	5760	0.65	0.55	15.21	11.52	0.68	2.88

Suppression 5% Duty Factor - BX01, BX02							
$I_{SPTA.0}$ mW/cm^2	$I_{SPTA.3}$ mW/cm^2	$P_{r.0}$ MPa	$P_{r.3}$ MPa	$I_{SPPA.0}$ W/cm^2	$I_{SPPA.3}$ W/cm^2	MI	TIC
951	720	0.702	0.611	19.0	14.4	0.75	0.36

Activation 50% Duty Factor - BX01, BX02							
$I_{SPTA.0}$ mW/cm^2	$I_{SPTA.3}$ mW/cm^2	$P_{r.0}$ MPa	$P_{r.3}$ MPa	$I_{SPPA.0}$ W/cm^2	$I_{SPPA.3}$ W/cm^2	MI	TIC
330	250	0.14	0.11	0.66	0.50	0.14	0.12
660	500	0.19	0.16	1.32	1.00	0.20	0.25
951	720	0.23	0.19	1.90	1.44	0.24	0.36

Results

The estimated tFUS focus is shown overlaid on a T1-weighed MRI in Fig. 2, along with an image of the transducer. Apoptosis was not detected in tissue blocks resected from patients undergoing tFUS sonication (Fig. 3). There was a consistent lack of TUNEL labelling in the non-sonicated tissue (Fig. 3A), the white matter (Fig. 3B) and grey matter (Fig. 3C) regions in the sonicated block. Positive control TUNEL labeling in the nucleus is shown in Fig. 3D. Examination of the H&E (not shown) and VAF stained (Fig. 3E and zoomed in, Fig. 3F) samples did not show signs of tissue damage-such as necrosis, vascular damage, acidophilic/ischemic neurons, or extravasation. These changes are visualized by loss of vascular and/or cellular integrity, which is not seen here.

For all subjects, the “active” or “sonicated’ region was drawn on the MR-image and provided to the surgeon who performed the resection. The control region for histology came from the ipsilateral anterior pole of temporal lobe. An example of these two regions is shown in Fig. 4.

We note that in one participant (BX08), acidophilic neurons and extravasation in the sonicated tissue was evident, but the significance of this finding is not known. These changes were also noted in the non-sonicated areas from this patient's resection material processed by both the UCLA Neuropathology Service and the Neurosurgery UCLA Rare Epilepsies & Brain Disease Tissue Bank using TUNEL, H&E, and VAF staining. The non-sonicated tissue was damaged during resection surgery. It is therefore unclear if the damage seen in the sonicated region is caused by tFUS or by the resection. Therefore, these findings in subject 8 were inconclusive of tFUS related effect, and as such, are not shown.

The purpose of the neuropsychological testing was to determine whether the participants had any significant decrease in cognitive capacity between pretest and posttest as a result of undergoing the tFUS procedure, as assessed by the RAVLT and the BVMT-R (including, for some participants, either the ROCFT or the TCFT). A total of 8 participants were tested. They were divided into three groups *post* hoc based on whether the subject received inhibitory stimulation and what the maximum stimulation intensity was. Participants in Group A (BX01/02; n = 2) were administered both excitatory and inhibitory stimulation paradigms at a maximum intensity of 720 mW/cm^2. Participants in Group B (BX03/04/05/08; n = 4) were administered both excitatory and inhibitory stimulation paradigms at a maximum intensity of 5760 mW/cm^2. The participant in Group C (BX07; n = 1) was only administered an excitatory stimulation paradigm at a maximum intensity of 5760 mW/cm^2. Neither Group A nor Group C had enough participants to perform statistical analysis, so we did not include their neuropsychological results in statistical analysis. Anecdotally, one of the participants in Group A (BX-02) performed better on the RAVLT; and both participants in Group A (BX-01 and BX- 02) performed better on the BVMT-R.

As the data for Group B did not meet tests of normality, we used the Wilcoxon Signed Rank Test, a non-parametric test to test the null hypothesis that tFUS did not produce behavioral changes for comparing two dependent samples. Our two-tailed null hypothesis was that the median of differences between pretest and posttest scores was zero at $\alpha = 0.05$. On the RAVLT for Group B, there was a significant but only slight decline in mean scores pretest-to-posttest (pretest mean = 0.562 versus posttest mean −0.496). There was not a significant difference between pretest and posttest scores or Group B on those tests that evaluated visuospatial memory. These results are displayed at Table 3.

Discussion

In this study, we examined the safety of using transcranial tFUS designed to modulate temporal lobe brain activity in participants with temporal lobe epilepsy, who were scheduled previously for an anterior temporal lobe resection. The primary safety outcome was the determination of possible histologic damage from tFUS. There was no evidence of significant histopathologic damage in 7 of the 8 participants on light microscopy, while one participant had inconclusive results. These findings are in line with a recent safety review of both humans and animals, which did not find any adverse

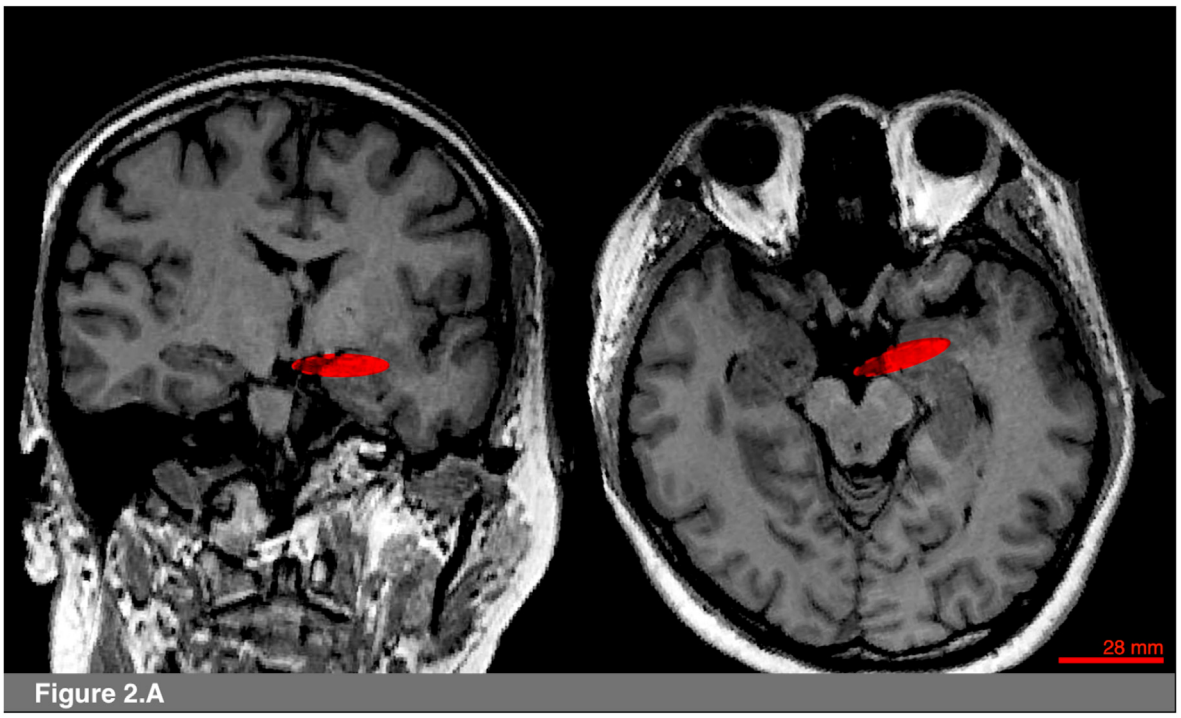

Fig. 2. Transducer and Targeting
Fig. 2. (**A**) Example T1-weighted MRI image with LIFUP focus overlaid in red. This represents the -6dB region where the intensity is greater than half the maximum. It is a cigar shape that is approximately 4 mm across and 28 mm long. (**B**) BrainSonix Transducer is shown with a gel pad. The air bubbles seen between the gel and transducer should be removed prior to application on a patient. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

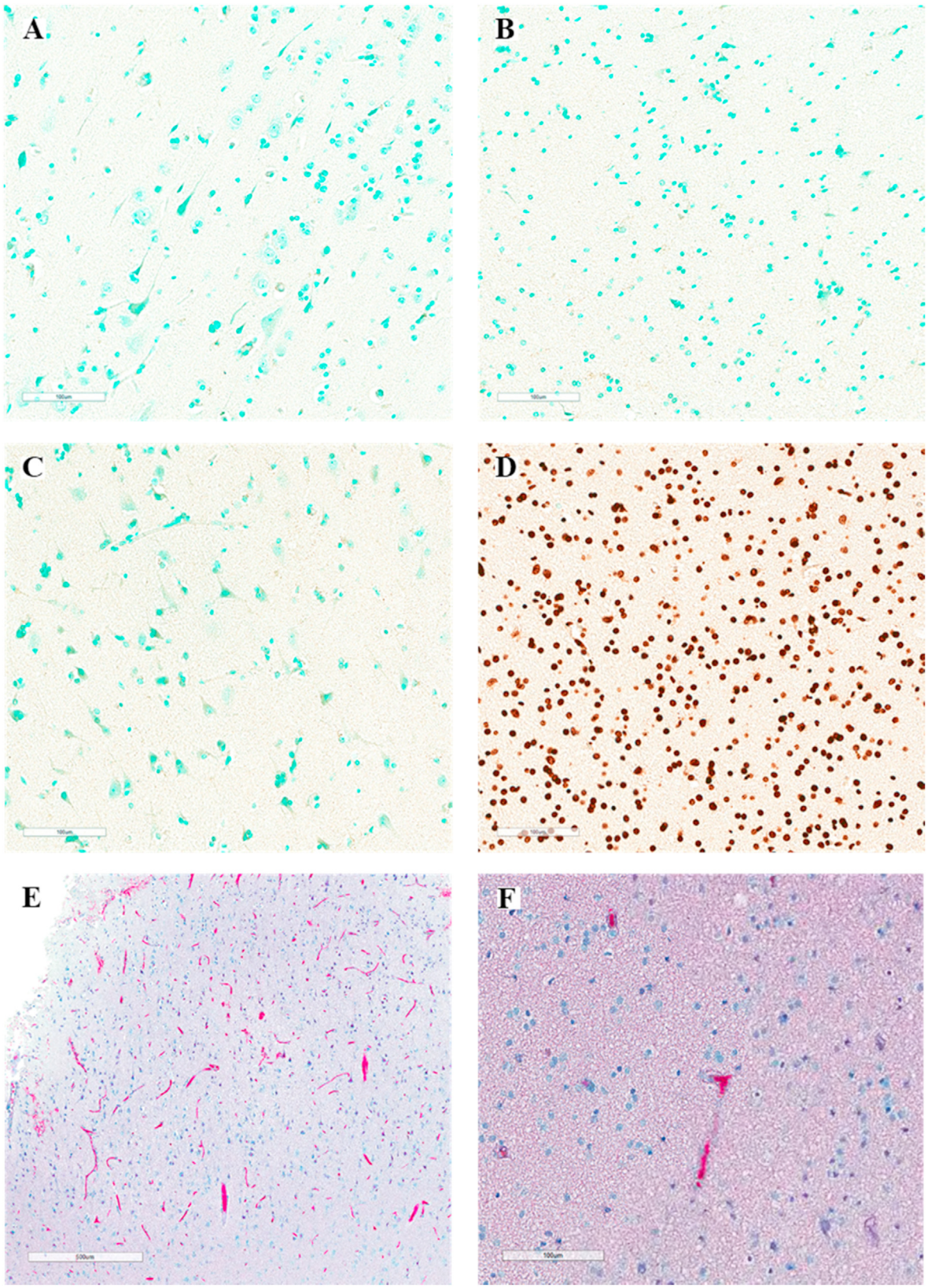

Fig. 3. Histopathology Findings
Fig. 3. Absence of TUNEL labeling in LIFUP tissue resected from adult epilepsy patients one week post-sonication. Representative photomicrographs of TUNEL labeling counter-stained with methyl green: (**A**) non-sonicated region, (**B**) white matter region within the sonicated field, (**C**) grey matter area within the sonicated field, (**D**) non-sonicated region treated with DNase I to provide positive TUNEL labeling. Scale bar = 100 μm. Tissue stained with vanadium acid fuchsin (VAF)/toluidine blue showed no evidence of vascular or cellular damage (**E** and **F**). Scale bars for **E** and **F** are 500 μm, and 100 μm, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

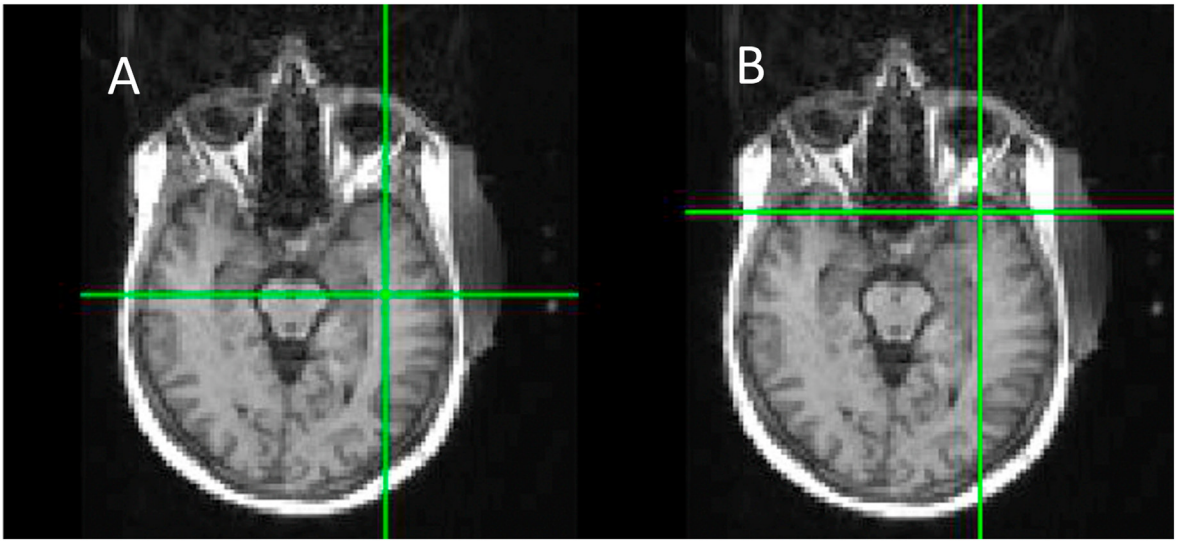

Fig. 4. This figure shows an example of the sonication (A) and control (B) regions. The histological findings from these regions were compared to each other.

effects in animal or human work that was done at or near the fundamental frequency that we used (650 kHz) [19]. A recent study by Gaur et al. revealed a similar finding [32]. In their work using focused ultrasound in sheeps, they found extravascular blood cells in the blocks that were analyzed after in-vivo ultrasound. Extravascularization of blood was seen in both the active and sham groups, suggesting a cause other than ultrasound application, and the authors themselves state that "the absence of this expected tissue reactivity within our sheep cohort confirm that meningeal and extravascular red blood cells seen across both hemispheres and experimental groups were artifact due to post-mortem tissue extraction." Nevertheless, additional exploration of safety in humans is essential.

The neuropsychological testing was exploratory in this first-in-human study. The number of participants was small because of safety concerns, so results may not be fully reliable.

Factors such as medication may have also played a role in the variability of our results. For example, all participants differed in their anti-seizure medication regimens. Some participants experienced claustrophobia and were administered a 2 mg tablet of diazepam to take prior to the MR-guided tFUS administration, and this could affect the post-tFUS neuropsychological testing.

Nevertheless, the neuropsychological testing revealed a significant decrease in participant's verbal memory. As there was no sham procedure, we cannot know definitively if this resulted from the tFUS itself causing functional suppression, or some other aspect of the procedures. While we did use alternative forms of the RAVLT, these were not designed to be used on the same day, and therefore may have been contaminated. Furthermore, participants were visibly fatigued from all the procedures, which likely affected their performance on the post-test. However, there is still the distinct possibility that the decrease was due to disruption of verbal memory centers of the brain. This should be further explored with more subjects and better controls.

Table 3
Neuropsychological Differences From Pre-to Post-tFUS for Group B using the Wilcoxon Signed Rank Test
Table 3. Wilcoxon Signed Rank Test Outcomes.

Group	Test	*W*	*z*	*p*	*r*
B (n4)	RAVLT	15	2.023	.043	.640
B (n4)	BVMT	26.5	−0.209	0.835	−0.066

Limitations

It is worth noting however, that Lee et al. did see damage on histology with a sonication every second for 500 s, with short interstimulus intervals (ISI) [18]. This makes it possible that one reason we did not see damage is because subjects received fewer sonications. It could also be a combination of different peak pressures and duty cycles, in addition to different ISIs. As such, more work is needed with greater number of sonications to elucidate the effect of number of sonications on histology and to better establish safety.

The size of this study limits its generalizability to a large population, but the results do not indicate substantial risk of tFUS producing damage to brain tissue. The other results have greater limitations. Whether tFUS produces minor changes to neuropsychological function cannot be determined from this study. There is a possibility of histological changes at the molecular level, but this was not studied. There was also possibility for histological effects outside the resected tissue, but due to the nature of the study, these effects could not be assessed. Control of seizures was not evaluated – because temporal lobe epilepsy is usually infrequent and therapeutic effect could not be assessed within this experimental design.

Small methodological and technical differences existed in the first two participants: the maximum intensity used for the first two patients was much lower than the maximum intensity used for the other 6 participants. Further, starting with the third participant, the design of the transducer holder was changed (from a water-filled holder to one using a gel pad) to allow for more positional control and flexibility). This is yet another confound, though all transducers were calibrated to ensure consistent output, and gel pads were designed to minimize absorption. However, in the group analysis of the neuropsychological testing, these subjects were already excluded on the basis of lower maximum intensity.

The actual pressures and intensities in the brain are a function of the thickness and non-uniformity of the patient's skull and may be estimated based on any of several methods [33–37]. Transmission through the temporal bones typically reduces the pressure/intensity at the focus by 10–20 dB, based on our own experiments, and other reports in the literature [33,37,38]. Our study of the

distortion of the beam conducted on cadaver skulls in a water tank indicated that the focal region shifted laterally by 1 mm or less, broadened the beam width by about 1.5 mm, and lengthened the focal region by about 1.5 mm as well [39]. Because computerized tomography was not utilized to obtain information about the patients' skull thickness and shape, the pressures and intensities would have varied slightly from subject to subject.

We do not provide MRI measurements of skull thickness, as these are not ideal, especially since there are other characteristics of bone not visible on MRI can affect ultrasound transmission. Furthermore, the sequence used for targeting is a brief T1-weighted clinical sequence which is not optimal for imaging of skull bone. Since these measurements of skull thickness would not be particularly helpful, and possibly even misleading, we consider this to be a limitation of this study and recommend either CT scans or MR sequences optimized for bone imaging (e.g. ultrashort echo time (UTE) sequences) for future studies.

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CRediT authorship contribution statement

John M. Stern: Conceptualization, Supervision, Funding acquisition, Writing – original draft. **Norman M. Spivak:** Investigation, Writing – original draft, Writing – review & editing, Formal analysis, Project administration. **Sergio A. Becerra:** Software, Formal analysis, Visualization, Resources. **Taylor P. Kuhn:** Software, Formal analysis, Supervision, Writing – review & editing. **Alexander S. Korb:** Methodology, Investigation, Conceptualization, Writing – review & editing, Project administration. **David Kronemyer:** Resources, Investigation, Formal analysis. **Négar Khanlou:** Resources, Formal analysis. **Samuel D. Reyes:** Resources, Formal analysis. **Martin M. Monti:** Software, Supervision. **Caroline Schnakers:** Resources, Investigation. **Patricia Walshaw:** Resources, Investigation. **Inna Keselman:** Formal analysis. **Mark S. Cohen:** Data curation, Resources. **William Yong:** Resources, Formal analysis. **Itzhak Fried:** Investigation, Resources. **Sheldon E. Jordan:** Resources, Software. **Mark E. Schafer:** Conceptualization, Resources, Software, Methodology. **Jerome Engel:** Conceptualization, Supervision. **Alexander Bystritsky:** Conceptualization, Supervision, Funding acquisition.

Declaration of competing interest

Dr. Korb is a Vice-President of BrainSonix Inc. Dr. Schafer is the Chief Technology Officer of BrainSonix Inc. Dr. Bystritsky is the Founder and Chief Executive Officer of BrainSonix Inc. Drs. Korb, Schafer, Bystritsky also own shares in the company. Other authors report no conflicts of interest.

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