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A Physiological Marker for Deep Brain Ultrasonic Neuromodulation

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ABSTRACT

Objectives: Transcranial ultrasound neuromodulation (TUSN) is a noninvasive and spatially specific therapy that promises to deliver treatments tailored to the specific needs of individuals. To fulfill this promise, each treatment must be modified to adequately correct for variation across individual skulls and neural anatomy. This study examines the use of ultrasound-induced voltage potentials (measured with electroencephalography [EEG]) to guide TUSN therapies.

Materials and Methods: We measured EEG responses in two awake nonhuman primates during sonication of 12 targets surrounding two deep brain nuclei, the left and right lateral geniculate nucleus.

Results: We report reliable ultrasound evoked potentials measured with EEG after the deep brain ultrasonic modulation in nonhuman primates. Robust responses are observed after just ten repetitions of the ultrasonic stimuli. Moreover, these potentials are only evoked for specific deep brain targets. Furthermore, a behavioral study in one subject shows a direct correspondence between the target with maximal EEG response and ultrasound-based modulation of visual choice behavior. Thus, this study provides evidence for the feasibility of EEG-based guidance for ultrasound neuromodulation therapies.

Keywords: Deep brain, evoked potentials, noninvasive, stimulation, ultrasound

INTRODUCTION

Through its capacity to perturb deep brain regions with millimeter and microsecond precision, transcranial ultrasound promises to deliver precise treatment of neurologic disorders.[1] Recent studies have indicated the capacity of ultrasound to modulate visual circuits, mood networks, motivation, and disease of brain function in both nonhuman primate and human subjects.[2–12] These results have led to an explosion of interest in clinical trials using ultrasound to treat depression,[13,14] epilepsy,[15] chronic pain,[16] and other conditions.[17]

Widespread clinical adoption of this approach would benefit from protocols that deliver repeatable intensity to the target anatomy. Variations in the human skull cause aberration and attenuation of the ultrasound signal in a manner that is unique in each patient.[18–20] These variations in skull properties introduce at least a four-fold variation[19,21] in the intensity delivered to the target across patients.

One solution to this problem is to measure the threshold at which ultrasound evokes changes in neural activity in each patient and then adjust the acoustic intensity relative to this threshold. Transcranial magnetic stimulation (TMS) treatments, for example, use a similar approach, in which treatment intensities are calibrated by measuring the energy needed to elicit a response in the motor cortex.[22] Results in small animal models suggest that such an approach also should be possible for transcranial ultrasound. In rodents, ultrasound can elicit motor evoked potentials and overt muscle contractions.[23] However, these effects have yet to be observed in larger animal studies, which have instead relied on changes in behavior[3,5–7] or changes in the amplitude of electroencephalography (EEG) potentials evoked by other modalities (eg, TMS[9]) to infer the effects of ultrasound on neural tissue.

This study shows the capacity of ultrasound to evoke a transient EEG response in the awake primate brain. The evoked potential is specific to the targeted neural anatomy and independent of the location of the transducer—the placement of which varied across the two subjects. This result lays the groundwork for the development of patient-specific calibration methods to address the variation in patient skulls that has so far precluded the delivery of a controlled and consistent acoustic intensity into the brain.

MATERIALS AND METHODS

Animals

Two male macaque monkeys (*Macaca mulatta*, subjects B and H, ages seven and six years, and weight 15.0 and 10.8 kg, respectively) participated in the study. All procedures were conducted as approved by the Institutional Animal Care and Use Committee of the University of Utah.

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Measurement of Ultrasound Evoked Potentials

The stimulation of the deep brain regions was achieved using Remus (Fig. 1a), a system for remote ultrasound delivery into the brain.[2–4,24] In Remus, a 256-element phased array transducer is affixed to the head of an awake and head-fixed nonhuman primate (NHP) subject. Each NHP subject is implanted with four titanium pins that enable the placement of a custom three-dimensional (3D) frame that simultaneously provides for head fixation and placement of the transducer array. The pins, attached by titanium screws to the skull, also provide a strong intracranial electrical connection. Head fixation is achieved by affixing the frame to a primate chair (Crist Instrument Company, Hagerstons, MD). Ultrasound is delivered through the intact skin, muscle, and skull. The transducer array has a center frequency of 650 kHz but was driven at 480 kHz for this study. There is evidence that lower frequencies are more effective at eliciting neural responses,[23,25–27] and 480 kHz was selected as the lowest frequency at which the array gives sufficient pressure output.

Magnetic resonance (MR) thermometry—performed only once—is used to validate targeting. Targeting of the lateral geniculate nucleus (LGN) has been shown previously.[2,3,24] Briefly, the subject was placed in a sphinx position, and a custom 3D printed stand was mounted to a head frame that was affixed to the subject's skull through four titanium pins. MR thermometry was performed simultaneously with high-energy ultrasound sonications (sufficient to increase the temperature at target by approximately 2 °C). The MR imaging was acquired with a 3D-gradient recalled segmented echo planar imaging pulse sequence; repetition time = 24 milliseconds, echo time = 11 milliseconds, bandwidth = 592 Hz/pixel, flip angle 12, echo train length = 5 with monopolar readout, field of view = 144 × 117 × 36 mm, resolution 1.5 × 1.5 × 3.0 mm, acquisition time 4.6 seconds per dynamic image.

The steering settings that caused a temperature increase within each LGN were saved for use in future trials. It is important to note that neuromodulation pulses are much shorter and have much lower duty cycles; thus, heating is not expected in those experiments (Discussion).

Ultrasound evoked potentials were measured across six sessions in subject B and eight sessions in subject H. The total number of trials delivered to each animal was the same (60 sonications per target). Some of the sessions in subject H were shorter because he was less willing to sit for the full session duration. After head fixation, each session proceeded in a dark room to minimize the influence of visual stimuli on the EEG recordings. We measured the EEG response during sonication of 24 targets arranged in two grids with three rows and four columns each. The grids were centered on the left and right LGN (Fig. 1b). During a session, each target was sonicated five to ten times using a randomization without replacement approach. Sonication of the left and right hemisphere was strictly interleaved. All steering was strictly programmatic—the position of the transducer was identical across targets and sessions.

In each session, the time between sonications was random, with a mean of 17 seconds. The maximum delay was 50 seconds, and the minimum delay was 14 seconds. The standard deviation was 4 seconds. The average session length in subject B (six total sessions) was 70 minutes, ranging from a minimum of 66 minutes to a maximum of 73 minutes. Each target was sonicated ten times per session. The average session length in subject H (eight total sessions) was 50 minutes, ranging from a minimum of 33 minutes to a maximum of 68 minutes. In four of the eight sessions, each target was sonicated ten times, and in the other four sessions, each target was sonicated five times. Thus, each target was sonicated a total of 60 times in both subjects. At the conclusion of each session, subjects were returned to their home environment—no other data were acquired during these sessions. A juice reward was delivered 8 seconds after each sonication to keep the subject content throughout the procedure.

Electroencephalography

The EEG response was measured using alligator clips that were electrically connected to the titanium pins that attach the frame to the subject's head (Fig. 1). This method of recording EEG signals has been reported previously.[3] The titanium pins are connected to the skull through titanium screws and thus provide a strong electrical connection to the brain. The placement of the pins is primarily driven by stability requirements for head fixation, but the approximate 10/20 EEG electrode positions are P3 and P4 for the rear pins and FP1 and FP2 for the front pins.

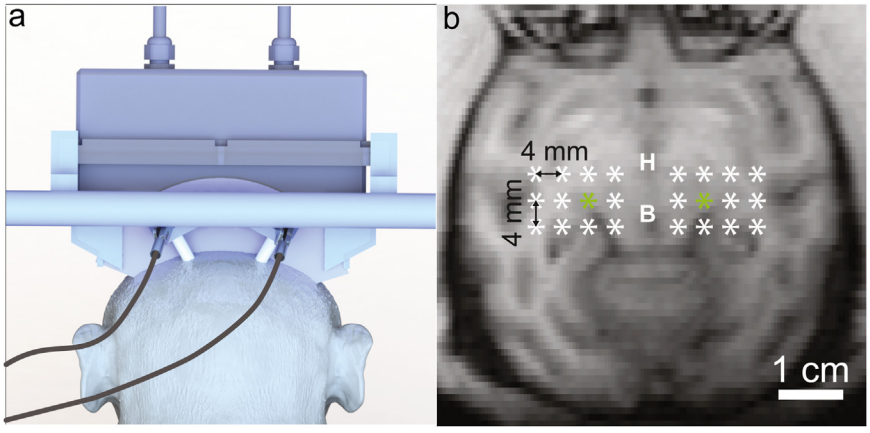

Figure 1. Experimental design. a. Apparatus: Remus, a remote ultrasound system described in,[2,3] delivers ultrasound to deep brain regions in awake, behaving NHPs. Implanted head holder pins serve as electrodes for EEG recordings. b. Deep brain targets: the target locations surrounding the left and right LGN (green). The targets are taken from a single axial plane and varied by 4 mm in the front/back and left/right dimensions. The B and H mark the approximate locations of the array's geometric focus within the sonication plane in subjects B and H, respectively. Relative to the transducer, the estimated depth of the plane containing the LGN is 58 mm in subject B and 51 mm in subject H. [Color figure can be viewed at www.neuromodulationjournal.org]

The EEG signal was recorded on the two rear pins (alligator clips connected to the front pins served as ground). The impedance between the channels and ground was consistently <1 kΩ. We recorded the EEG signals using a 128-channel recording system (RHS2000, Intan Technologies, Los Angeles, CA). EEG processing was performed by custom, in-house MATLAB scripts. Each signal was low-pass filtered at 7.6 kHz and sampled at 20 kHz. The recorded signal showed strong interference at 60 Hz and at the second and third harmonics of 60 Hz; thus, notch filters at 60, 120, and 180 Hz with a 4-Hz bandwidth were applied to the raw data before averaging. Trials in which the filtered EEG signal exceeded 500 μV were excluded given such high signals are not likely to be physiological. The temporal EEG response is then the EEG signal recorded in the 1 second after sonication, averaged across all sonications not excluded by the 500 μV threshold.

Behavior

In a separate experiment, we tested whether the strength of the ultrasound evoked potential detected in EEG could guide ultrasound neuromodulation of awake behavior. In subject B, we selected four targets—the two targets in each hemisphere with a maximal (active sonication) and minimal (control) EEG response. We then measured whether the strength of the ultrasound evoked potential predicted the behavioral effects of ultrasound neuromodulation delivered to each target.

Behavioral modulation was measured with a visual discrimination task that has been described in detail elsewhere.[2,5] Briefly, the subject fixates on a central target. After a random delay, targets appear in the right/left visual hemifield, separated by a random delay between −90 and 90 milliseconds (negative delays mean the right target appeared first whereas positive delays mean the left target appeared first). The subject is rewarded if they look at the target that appeared first within a 1.5 second period.

One sonication is delivered in each behavioral session (behavioral sessions were performed separately from EEG sessions and did not begin until after all EEG sessions were complete). We measured behavioral changes in 16 total sessions, four sessions per anatomical target (targets with maximal and minimal responses in both the left and right hemispheres). The sonication follows a baseline period of 375 trials. The task continues without interruption during and after the sonication, and the animal is then allowed to work until they are satiated (water restriction is used to aid training of the subjects). The subject's behavior is quantified by fitting a sigmoid to their choice behavior and identifying the delay at which the subject has equal preference for the left/right target. Changes in behavior are then quantified by the subject's preference for the left target at the baseline delay. Behavioral trials were performed in a prospective study after EEG measurements. The location of the sonication was guided by the EEG result.

Ultrasound Parameters

The ultrasound parameters for the ultrasound evoked potential experiments and the behavioral experiments are listed in Table 1. The parameters designed to elicit an ultrasound evoked potential (UEP) were selected to match a prior study in which changes in EEG rhythms were observed in subjects who were anesthetized.[4] The UEP parameters have a shorter pulse duration than those in existing studies of ultrasound neuromodulation in humans, but a higher time average, spatial peak intensity (I_{SPTA}) across that 100-millisecond duration (15 W/cm^2).[1] The I_{SPTA} across the entire session (averaged over the full 33–70 minute session time) was quite low (90 mW/cm^2).

In contrast to the parameters selected to elicit UEPs, the parameters used in the behavioral study were designed to cause sustained changes in awake behavior. Thus, we selected sonication parameters for the behavioral experiments from a prior study showing changes in visual choice behavior during sonication of the LGN.[3] It is noteworthy that the total energy delivered by these sonications is two orders of magnitude less than the energy used during thermometry. Thus, no significant heating is expected (Discussion).

In situ pressure was estimated by derating the free field intensity by a factor of 21%. The derivation of this estimate has been described previously.[2,3] Briefly, MR thermometry data in two NHP subjects (subject B and a subject not included in this study) were combined with a simplified version of Pennes' bioheat equation[28] to estimate the pressure at target. The average derating factor across the two subjects was 21%.

The half-power beamwidth (HPBW) of the transducer when steered to the approximate location of the LGN (11, 3, and 15 mm from the natural focus in the left/right, anterior/posterior, and superior/inferior dimensions, respectively) was measured in water using a hydrophone. The resulting HPBW measured in free field was 1.5, 5, and 5 mm in the left/right, anterior/posterior, and superior/inferior dimensions, respectively.

RESULTS

We found that brief pulses of transcranial focused ultrasound applied to deep brain targets of NHPs elicited target-specific evoked EEG potentials. The targeting was achieved electronically; the transducer was fixed to the head always in the same location. Figure 2 presents the UEPs for each of the 24 targets (Fig. 1). Each axis provides the average response to the 60 stimuli after removing trials in which the EEG voltage is >500 μV. The resulting number of averaged trials is shown in the bottom left of each plot. In both subjects, ultrasound elicited the strongest responses in the target located 4 mm medial and 4 mm posterior to the assumed location of the LGN (Fig. 2). Relative to the onset of the ultrasound, the

Table 1. Sonication Parameters for the UEP and Behavioral Experiments.

Experiment	I_{SPPA}	P	DC	PRF	PRI	PD	F	MI	SD
UEP	31	1.0	50	200	5	2.5	0.48	1.4	100
Behavior	7.7	0.5	14.4	4.8	208	30	0.48	0.72	30,000

Left to right columns: I_{SPPA} (W/cm^2), DC (%), P (MPa), PRF (Hz), (PRI; milliseconds), PD (milliseconds), F (MHz), MI, SD (milliseconds).
DC, duty cycle; F, center frequency; I_{SPPA}, spatial peak pulse average intensity; MI, mechanical index; P, pressure; PD, pulse duration; PRF, pulse repetition frequency; PRI, pulse repetition interval; SD, sonication duration.

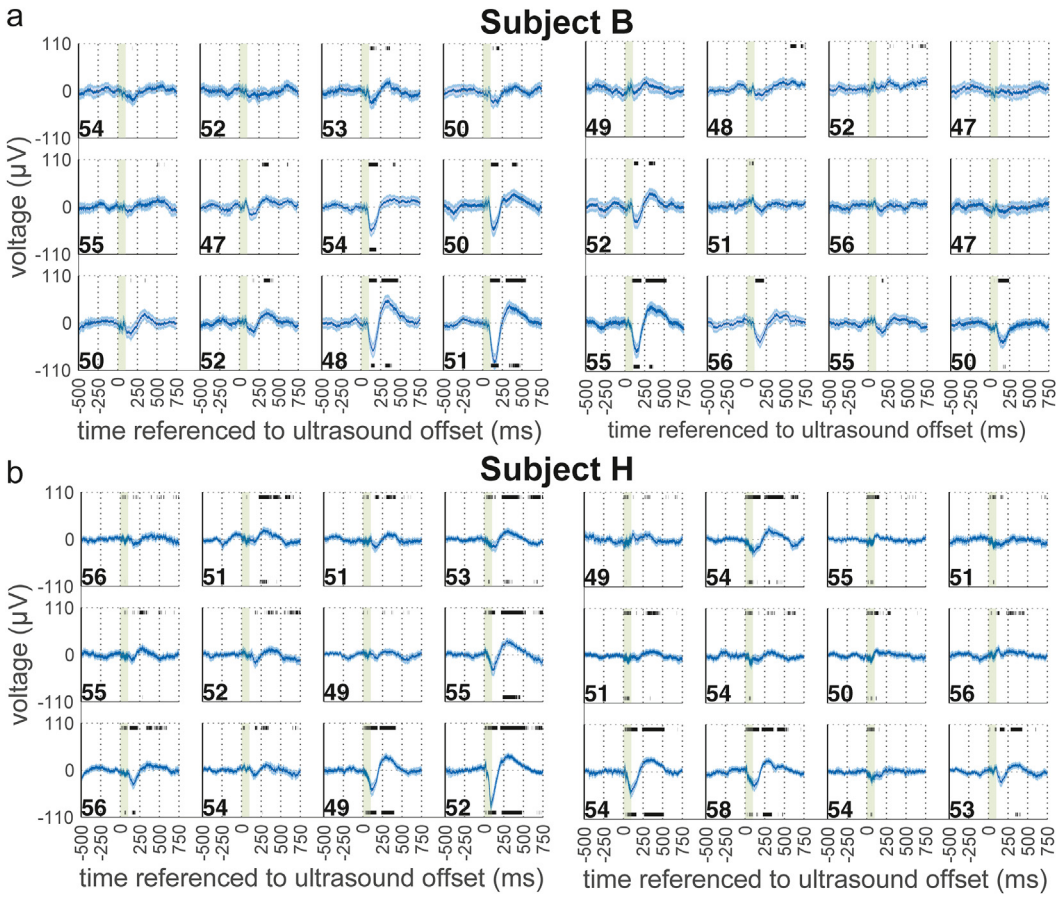

Figure 2. Deep brain region-specific UEPs. The EEG responses (mean ± SEM) as a function of time at the 24 target locations shown in Figure 1. The green rectangle shows the 100-millisecond sonication. Each plot is referenced to the stimulus onset. Results for subject B are shown in panel a, and results for subject H are shown in panel b. The numbers in the bottom left of each axis give the number of averages used to create each plot (after removing traces in which the EEG >500 μV). Time points at which a two-tailed *t*-test reveals a significant deviation from the baseline measurement (average value between −500 and 0 milliseconds) are marked with a black line at the top ($p < 0.05$) and bottom ($p < 0.001$) of each plot. Notably, the strongest UEPs are observed at the same anatomical locations in both subjects. [Color figure can be viewed at www.neuromodulationjournal.org]

latencies of the negative peak ranged from 139 to 180 milliseconds in subject B and 86 to 115 milliseconds in subject H. This spatially confined response was consistent across subjects, despite differences in the placement of the ultrasonic transducer (Fig. 1b). This result indicates the capacity of ultrasound to directly perturb neural activity in deep brain targets.

We hypothesized that the target that evoked the strongest response also would lead to strongest effects on visual choice behavior.[3] In other words, we tested whether this ultrasound evoked response could be used to guide targeting to achieve behavioral effects. Indeed, Figure 3 shows that the evoked-potential–optimized targets lead to notable and significant (time window 17–22 minutes after sonication, $n = 4$ left and $n = 4$ right, two-tailed *t*-test, $p < 0.05$) effects on visual choice behavior (Fig. 3a). No significant effect was observed for control targets ($n = 4$ left, $n = 4$ right; Fig. 3b).

DISCUSSION

We showed a measurable and transient physiological response to ultrasound stimulus of deep brain anatomy. The response is specific to the targeted anatomy and is engaged independently of the location of the transducer array. The latency of the evoked potential suggests that it takes some time for the ultrasound stimulation to elicit a response. This agrees with a prior study in mice that found latencies up to 200 milliseconds when measuring evoked potentials using electromyography.[26] Behavioral data in one subject validate that the strength of the physiological response correlates with modulation of awake behavior. Indeed, we have observed that stimulation of those locations with maximal response led to substantial effects on visual choice behavior. Stimulation of locations without a physiological response did not affect choice behavior. These results provide evidence of the feasibility of developing treatment guidance algorithms that rely on physiological feedback to provide robust, repeatable ultrasound therapies. Such novel approaches could provide insight into both spatial targeting and intensity calibration for transcranial ultrasound neuromodulation (TUSN) therapies.

Control for Artifacts

Studies in rodents, which have a small cranium, have shown that ultrasound can engage auditory or vestibular pathways.[29,30] We controlled for such confounds by working with NHPs, in which the ultrasound focus is well confined regarding the brain dimensions,

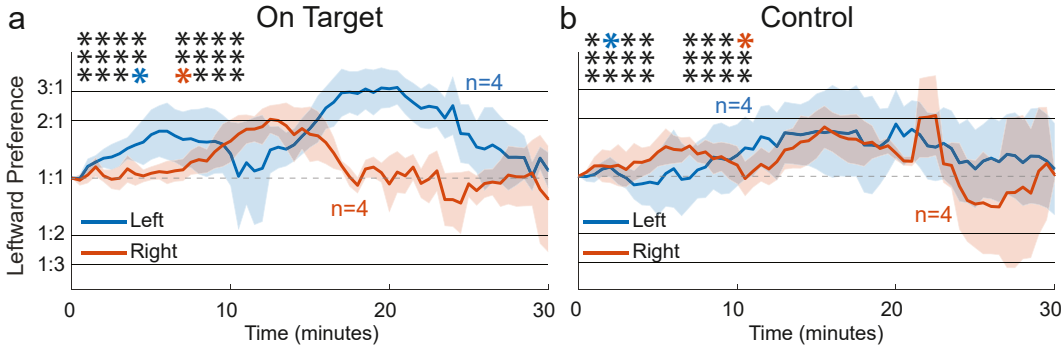

Figure 3. Modulation of visual choice behavior. Choice preference (mean ± SEM) in subject B as a function of time, aligned to stimulus onset, in response to stimulation of the targets that caused maximal EEG response (panel a; blue and red star) and minimal response (panel b; blue and red star). Each curve comprises n = 4 sessions. [Color figure can be viewed at www.neuromodulationjournal.org]

and by evaluating responses to multiple deep brain targets in each session. The energy delivered to both the optimal and the control targets was the same, yet there was a clear target dependence for both the neural and behavioral effects. Thus, the effects cannot be explained by generic potential artifacts.

Nonsignificant Thermal Effects

Previously published thermometry data[2] also allow us to estimate the temperature increase resulting from each 100-millisecond sonication. In that experiment, 5 seconds of continuous ultrasound were delivered at an input voltage of 20 Vp. In the first 2.3 seconds (the time it took to acquire the center of k-space in one thermometry image), the measured temperature increase was approximately 1.8 °C. The sonication in this study delivered 100 milliseconds of 50% duty cycle ultrasound at a voltage of 18.1 Vp. Assuming a linear relationship between input power and temperature increase, we thus expect the sonications in this study to cause a temperature increase of approximately 0.03 °C. Thus, it is unlikely that temperature played a significant role in evoking the EEG response.

Limitations and Future Work

This study has several important limitations. First, the study shows a robust, measurable EEG response in only one deep brain region. It is not clear whether other deep brain regions will similarly respond to ultrasound stimulus. Indeed, the study shows that—at least for the parameters used in these experiments—some deep brain regions do not produce a response measurable by EEG electrodes placed over the occipital lobes. Thus, clinical translation of physiological feedback to guide TUSN interventions will depend on whether targets relevant to neurologic disease also produce a reliable response. Therefore, future studies should seek to replicate these findings in both NHP and human subjects and to determine other neural targets that produce an EEG response. A second, related limitation is uncertainty in the specific neural anatomy underlying the responses presented in Figure 2. The maximum response did not occur at the assumed location of the LGN, and the cause of this is unclear. One possibility is that ultrasound interacts more strongly with the white matter tracts posterior to the LGN than with the LGN itself.[31] Another possibility is systematic error in the targeting protocol used to identify the LGN—this could be the result of inaccuracies in the overall protocol or of targeting the wrong portion of the LGN that is larger than the HPBW of the transducer.[3] The EEG readout similarly lacks the spatial specificity required to definitively state that the measured evoked potential results from activity in the visual cortex. Thus, although changes in visual choice behavior show that the target has some effect on visual circuits, this study cannot conclusively state whether the measured evoked potential is a result of direct modulation of the visual system. Future studies could use denser electrode arrays or invasive methods to better identify the specific neuroanatomy involved in the measured response.

The estimated pressure delivered to the target region constitutes another limitation because it is not subject or target specific, it relies on assumptions about the thermal properties of the brain, and it does not account for the temporal and spatial averaging inherent in MR thermometry.[3] Thus, there remains considerable uncertainty in the estimated pressure at target.

Owing to constraints on the NHP subject's time, data showing a correlation between UEPs and ultrasound-induced modulation of choice behavior were performed in only one subject. As a result, the behavioral data can only offer a proof of concept and do not offer definitive evidence of a link between the magnitude of the UEP and the efficacy of altering behavior by sonication of the targeted anatomy. Future work is required to confirm whether the magnitude of the UEP is directly correlated to behavioral changes resulting from TUSN.

CONCLUSIONS

This study shows that ultrasound modulation of deep brain targets can elicit robust evoked potentials. Ultrasound-based modulation of behavior correlates with the magnitude of the evoked potential. A robust EEG response to ultrasound may provide a physiological marker to validate target engagement during neuromodulation procedures. Such validation would greatly enhance the efficacy of neuromodulation protocols that are plagued by uncertainty in the acoustic intensity delivered to the targeted tissue.[19,32]

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Authorship Statements

Taylor D. Webb, Jan Kubanek, and Carter Lybbert developed the concept. Taylor D. Webb designed and implemented the hardware and software setup. Taylor D. Webb, Carter Lybbert, Matthew G. Wilson, and Henrik Odéen acquired the data. Taylor D. Webb analyzed the data and wrote the manuscript, and all authors edited and approved the manuscript.

Conflict of Interest

Jan Kubanek reports that he is the cofounder with equity stake in SPIRE, an ultrasound neuromodulation company. The remaining authors reported no conflict of interest.

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REFERENCES

1. Sarica C, Nankoo JF, Fomenko A, et al. Human studies of transcranial ultrasound neuromodulation: a systematic review of effectiveness and safety. *Brain Stimul*. 2022;15:737–746. https://doi.org/10.1016/j.brs.2022.05.002.
2. Webb TD, Wilson MG, Odéen H, Kubanek J. Remus: system for remote deep brain interventions. *iScience*. 2022;25:105251. https://doi.org/10.1016/j.isci.2022.105251.
3. Webb TD, Wilson MG, Odéen H, Kubanek J. Sustained modulation of primate deep brain circuits with focused ultrasonic waves. *Brain Stimul*. 2023;16:798–805. https://doi.org/10.1016/j.brs.2023.04.012.
4. Webb T, Cheeniyil R, Wilson M, Kubanek J. Remote targeted electrical stimulation. *J Neural Eng*. 2023;20:036030. https://doi.org/10.1088/1741-2552/acd95c.
5. Kubanek J, Brown J, Ye P, Pauly KB, Moore T, Newsome W. Remote, brain region-specific control of choice behavior with ultrasonic waves. *Sci Adv*. 2020;6:eaaz4193. https://doi.org/10.1126/sciadv.aaz4193.
6. Deffieux T, Younan Y, Wattiez N, Tanter M, Pouget P, Aubry JF. Low-intensity focused ultrasound modulates monkey visuomotor behavior. *Curr Biol*. 2013;23:2430–2433. https://doi.org/10.1016/j.cub.2013.10.029.
7. Wattiez N, Constans C, Deffieux T, et al. Transcranial ultrasonic stimulation modulates single-neuron discharge in macaques performing an antisaccade task. *Brain Stimul*. 2017;10:1024–1031. https://doi.org/10.1016/j.brs.2017.07.007.
8. Hameroff S, Trakas M, Duffield C, et al. Transcranial ultrasound (TUS) effects on mental states: a pilot study. *Brain Stimul*. 2013;6:409–415. https://doi.org/10.1016/j.brs.2012.05.002.
9. Gibson BC, Sanguinetti JL, Badran BW, et al. Increased excitability induced in the primary motor cortex by transcranial ultrasound stimulation. *Front Neurol*. 2018;9:1007. https://doi.org/10.3389/fneur.2018.01007.
10. Legon W, Sato TF, Opitz A, et al. Transcranial focused ultrasound modulates the activity of primary somatosensory cortex in humans. *Nat Neurosci*. 2014;17:322–329. https://doi.org/10.1038/nn.3620.
11. Legon W, Ai L, Bansal P, Mueller JK. Neuromodulation with single-element transcranial focused ultrasound in human thalamus. *Hum Brain Mapp*. 2018;39:1995–2006. https://doi.org/10.1002/hbm.23981.
12. Legon W, Bansal P, Tyshynsky R, Ai L, Mueller JK. Transcranial focused ultrasound neuromodulation of the human primary motor cortex. *Sci Rep*. 2018;8:10007. https://doi.org/10.1038/s41598-018-28320-1.
13. Reznik SJ, Sanguinetti JL, Tyler WJ, Daft C, Allen JJ. A double-blind pilot study of transcranial ultrasound (tus) as a five-day intervention: tus mitigates worry among depressed participants. *Neurol Psychiatry Brain Res*. 2020;37:60–66. https://doi.org/10.1016/j.npbr.2020.06.004.
14. Riis TS, Feldman DA, Vonesh LC, et al. Durable effects of deep brain ultrasonic neuromodulation on major depression: a case report. *J Med Case Rep*. 2023;17:449.
15. Lee CC, Chou CC, Hsiao FJ, et al. Pilot study of focused ultrasound for drug-resistant epilepsy. *Epilepsia*. 2022;63:162–175. https://doi.org/10.1111/epi.17105.
16. Pérez-Neri I, González-Aguilar A, Sandoval H, Pineda C, Ríos C. Therapeutic potential of ultrasound neuromodulation in decreasing neuropathic pain: clinical and experimental evidence. *Curr Neuropharmacol*. 2021;19:334–348. https://doi.org/10.2174/1570159X18666200720175253.
17. Nicodemus NE, Becerra S, Kuhn TP, et al. Focused transcranial ultrasound for treatment of neurodegenerative demen- tia. *Alzheimer's and Dementia: translational research and clinical interventions*. 2019;5:374–381. https://doi.org/10.1016/j.trci.2019.06.007.
18. Webb TD, Leung SA, Rosenberg J, et al. Measurements of the relationship between CT hounsfield units and acoustic velocity and how it changes with photon energy and reconstruction method. *IEEE Trans Ultrason Ferroelectr Freq Control*. 2018;65:1111–1124. https://doi.org/10.1109/TUFFC.2018.2827899.
19. Riis TS, Webb TD, Kubanek J. Acoustic properties across the human skull. *Ultrasonics*. 2022;119:106591. https://doi.org/10.1016/j.ultras.2021.106591.
20. Leung SA, Webb TD, Bitton RR, Ghanouni P, Pauly KB. A rapid beam simulation framework for transcranial focused ultrasound. *Sci Rep*. 2019;9:7965. https://doi.org/10.1038/s41598-019-43775-6.
21. Vyas U, Ghanouni P, Halpern CH, Elias J, Pauly KB. Predicting variation in subject thermal response during transcranial magnetic resonance guided focused ultrasound surgery: comparison in seventeen subject datasets. *Med Phys*. 2016;43:5170. https://doi.org/10.1118/1.4955436.
22. Rossini PM, Barker AT, Berardelli A, et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. *Electroencephalogr Clin Neurophysiol*. 1994;91:79–92. https://doi.org/10.1016/0013-4694(94)90029-9.
23. King RL, Brown JR, Newsome WT, Pauly KB. Effective parameters for ultrasound-induced in vivo neurostimulation. *Ultrasound Med Biol*. 2013;39:312–331. https://doi.org/10.1016/j.ultrasmedbio.2012.09.009.
24. Wilson MG, Webb TD, Odéen H, Kubanek J. Remotely controlled drug release in deep brain regions of non-human primates. *J Control Release*. 2024;369:775–785. https://doi.org/10.1016/j.jconrel.
25. Riis T, Kubanek J. Effective ultrasonic stimulation in human peripheral nervous system. *IEEE Trans Biomed Eng*. 2022;69:15–22. https://doi.org/10.1109/TBME.2021.3085170.
26. Ye PP, Brown JR, Pauly KB. Frequency dependence of ultrasound neurostimulation in the mouse brain. *Ultrasound Med Biol*. 2016;42:1512–1530. https://doi.org/10.1016/j.ultrasmedbio.2016.02.012.
27. Kim H, Chiu A, Lee SD, Fischer K, Yoo SS. Focused ultrasound-mediated non-invasive brain stimulation: examination of sonication parameters. *Brain Stimul*. 2014;7:748–756. https://doi.org/10.1016/j.brs.2014.06.011.
28. Pennes HH. Analysis of tissue and arterial blood temperatures in the resting human forearm. *J Appl Physiol*. 1948;1:93–122. https://doi.org/10.1152/jappl.1948.1.2.93.
29. Guo H, Hamilton M, Offutt SJ, et al. Ultrasound produces extensive brain activation via a cochlear pathway. *Neuron*. 2018;98:1020–1030.e4. https://doi.org/10.1016/j.neuron.2018.04.036.
30. Sato T, Shapiro MG, Tsao DY. Ultrasonic neuromodulation causes widespread cortical activation via an indirect auditory mechanism. *Neuron*. 2018;98:1031–1041.e5. https://doi.org/10.1016/j.neuron.2018.05.009.
31. Bancel T, Béranger B, Daniel M, et al. Sustained reduction of essential tremor with low-power non-thermal transcranial focused ultrasound stimulations in humans. *Brain Stimul*. 2024;17:636–647. https://doi.org/10.1016/j.brs.2024.05.003.
32. Webb TD, Fu F, Leung SA, et al. Improving transcranial acoustic targeting: the limits of CT-based velocity estimates and the role of MR. *IEEE Trans Ultrason Ferroelectr Freq Control*. 2022;69:2630–2637. https://doi.org/10.1109/TUFFC.2022.3192224.

COMMENTS

The study by Webb et al, "A Physiological Marker for Deep Brain Ultrasonic Neuromodulation," is interesting and innovative, with potential broader implications to human focused ultrasound (FUS) treatment. In this work, the authors show elicitation of EEG evoked potentials (EP) recorded at the skull after transcranial ultrasound stimuli targeting deep brain regions (lateral geniculate nucleus [LNG]) in two adult macaque monkeys. The EPs termed "ultrasound EPs" have a long latency (85–150 milliseconds) because some time is taken for the ultrasound pulse to elicit an electrical response. The authors could show spatial specificity for the LNG target and maximum ultrasound EP amplitude. In a second experiment in one monkey, the authors could modify a visual choice behavior consistently using LNG FUS that targeted LNG by maximizing the ultrasound EP. In effect, the authors were able to show, in preliminary form, that an ultrasound EP-guided, deep brain FUS was able to modify physiologically relevant behaviors. This topic is of interest to the scientific and clinical FUS community because additional neurophysiological markers for targeting and physiological circuit modification are needed. Currently, human FUS

treatment for tremor and other conditions is magnetic resonance imaging (MRI) guided by anatomical target identification, with MRI thermometry to define temperature, size, and shape of heated tissue. During clinical treatment, intermediate temperature sonications in the range of 49 to 52 °C are used to assess clinical improvement and side-effect profile before definitive lesions; however, reliable responses are not always achieved. It therefore remains valuable to develop physiological tools to complement anatomical targeting because they provide different information about the same circuits. This point is certainly true by analogy in deep brain stimulation when intraoperative neurophysiology for target identification complements anatomical targeting. Although this work requires replication in larger studies, it provides proof of concept that ultrasound evoked EPs may have future application to human FUS treatment, assisting in target identification. Technical barriers to overcome with this approach would include the scalp recording electrodes interfering with FUS delivery and EP recording in an MRI environment due to interference artefacts and MRI incompatibility of equipment, but even taking these challenges into account, this work sets an exciting precedent.

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This piece by Webb et al describes as series of important studies on deep brain ultrasonic neuromodulation in nonhuman primates. It describes EEG responses of the lateral geniculate nucleli and found they could elicit dependable output. While the precise source of the observed signal remains uncertain—whether it truly is generated from the LGNthis study is one of the first to report reliable and reproducible effect. One can hope this helps pave the way for similar work in humans.

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