HHS Public Access

Author manuscript

Brain Stimul. Author manuscript; available in PMC 2023 July 10.

Published in final edited form as:

Brain Stimul. 2023 ; 16(3): 798–805. doi:10.1016/j.brs.2023.04.012.

Sustained modulation of primate deep brain circuits with focused ultrasonic waves

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Abstract

Background: Transcranial focused ultrasound has the potential to noninvasively modulate deep brain circuits and impart sustained, neuroplastic effects.

Objective: Bring the approach closer to translations by demonstrating sustained modulation of deep brain circuits and choice behavior in task-performing non-human primates.

Methods: Low-intensity transcranial ultrasound of 30 s in duration was delivered in a controlled manner into deep brain targets (left or right lateral geniculate nucleus; LGN) of non-human primates while the subjects decided whether a left or a right visual target appeared first. While the animals performed the task, we recorded intracranial EEG from occipital screws. The ultrasound was delivered into the deep brain targets daily for a period of more than 6 months.

Results: The brief stimulation induced effects on choice behavior that persisted up to 15 minutes and were specific to the sonicated target. Stimulation of the left/right LGN increased the proportion of rightward/leftward choices. These effects were accompanied by an increase in gamma activity over visual cortex. The contralateral effect on choice behavior and the increase in gamma, compared to sham stimulation, suggest that the stimulation excited the target neural circuits. There were no detrimental effects on the animals' discrimination performance over the months-long course of the stimulation.

Conclusion: This study demonstrates that brief, 30-s ultrasonic stimulation induces neuroplastic effects specifically in the target deep brain circuits, and that the stimulation can be applied

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CRediT authorship contribution statement

J.K. and T.W. developed the concept.
T.W., M.W., and H.O. collected the data.
T.W. and M.W. analyzed the data.
T.W. and J.K. wrote the paper, and all authors edited the paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.brs.2023.04.012.

daily without detrimental effects. These findings encourage repeated applications of transcranial ultrasound to malfunctioning deep brain circuits in humans with the goal of providing a durable therapeutic reset.

Keywords

Noninvasive; Transcranial; Ultrasound; Deep brain; Lateral geniculate nucleus; Choice; Sustained neuromodulation; Visual discrimination; Gamma

Introduction

Mental and neurological disorders are frequently resistant to existing treatments [1–8]. Targeted neuromodulation has provided treatment options for some of the patients but existing approaches—such as deep brain stimulation, electroconvulsive therapy, transcranial magnetic stimulation, or transcranial direct/alternating current stimulation—are either invasive or lack the capacity to directly modulate specific deep brain regions [9,10]. These limitations have yielded variable response [11], with only a subset of patients responding to current neurostimulation modalities.

Transcranial low-intensity focused ultrasound can noninvasively modulate deep brain circuits with millimeter-level precision [12] and thus provide a direct and selective access to the malfunctioning circuits.

Therapeutic applications require the capacity to systematically induce durable changes in the neural circuits that at the same time are strong enough to manifest in behavior. Ultrasound is capable of inducing durable changes in target circuits [22–29]. However, thus far, the studies demonstrating such effects have either required anesthesia [22–24], which suppresses neuromodulation effects and has limited practical use, or used single-element transducers, which make it difficult to target deep brain structures precisely and reproducibly.

To address these shortcomings, this study applied ultrasonic phased arrays [20] to achieve precise and controlled modulation of deep brain circuits in awake, task-performing non-human primates. The approach enabled us to reproducibly deliver ultrasound into deep brain targets over the course of several months while evaluating the effectiveness and safety of the stimulation.

Methods

Animals

Four male macaque monkeys (*macaca mulatta*, subjects B, E, C, and H, ages 5, 7, 8, and 8 years and weight 12.0, 9.5, 13.5, and 10.0 kg respectively) participated in the study. Subjects B and E participated in the behavioral and neural studies. Subjects C and H were used to obtain the anatomical safety data. All procedures were conducted as approved by the Institutional Animal Care and Use Committee of the University of Utah.

Modulation of choice behavior

This study leverages Remus [20], a phased array system that programmatically delivers ultrasound into specific deep brain targets. The system has been described in detail previously [20]; the following paragraph provides a summary.

In this approach, a custom head frame is attached to the subject using four titanium pins that are surgically attached to the skull. The attachment is mediated using titanium screws (Gray Matter Research, Bozeman, MT) that traverse the thickness of the skull. The placement of the pins was driven by frame stability and can thus vary from subject to subject. In subject B, the rear pins are located approximately 7 mm anterior to the Lambdoid ridge and 23 mm to the left and 21 mm to the right of the midline. The corresponding locations in subject E were 14, 13, and 15 mm, respectively. To ensure head fixation, the frame is mounted to a primate chair (Crist Instrument Company, Hagerstons, MD) using two steel bars. The frame houses a 256-element ultrasound transducer array (Doppler Electronic Technologies, Guangzhou, China [20]). Acoustic coupling is mediated using a cryogel [30]. This setup makes it possible to reproducibly mount the array into the same location from session to session while collecting behavioral and neural data under head fixation. Using this system, the ultrasound is targeted into specific deep brain regions electronically, using a software command [20]. No movement of the transducer or the subject is necessary.

We targeted the left and right lateral geniculate nucleus (LGN), the entry neural structures that pass visual information from the eyes to the brain. We validated the targeting of the LGN using MR thermometry. Details about the MR acquisition sequence are provided in [20]. Briefly, sonications delivered during MR thermometry intentionally elicit a temperature rise on the order of 2–3 degrees. Because thermometry sonication uses higher intensity than neuromodulation, the temperature rise observed during the thermometry is not indicative of a temperature rise during neuromodulatory sonications. The thermometry sonications were performed at the center frequency of the transducer (650 kHz). There was a minimum period of three months between the initial thermometry and the subsequent collection of the behavioral data. The low temperature rise and the relatively long temporal offset between the thermometry and the data collection make it unlikely that the thermometry could influence the neuromodulation results.

We trained two non-human primates (NHPs) to perform an established visual discrimination task that is often used in neurology and neuroscience [17,20,31,32]. In this task, a subject first acquires a central fixation target. After a random delay, one target appears in either the left or right visual hemifield. A second target then appears in the opposite hemifield after a delay that is randomized between 0 and 90 ms. The subject is rewarded for looking at the target that appeared first. Subjects received a liquid reward with a probability $0.5. \leq p \leq 1$ if they looked at the target that appeared first. When both targets appeared at the same time, the subjects were rewarded for either choice. The subjects' eye position was monitored using an eye tracker (Eyelink, SR Research, Ottawa, Canada). This task enables the assessment of both the magnitude (i.e., the proportion of left/right choices) and the polarity (i.e., the dominance of left over the right choices or reversely) of the neuromodulatory effects [17]. For instance, because the LGN encodes visual information of the contralateral

visual hemifield, a contralateral/ipsilateral bias is indicative of an excitatory/inhibitory effect [17,31,32].

Protocol

Sonication of the right and left LGN was interleaved across sessions. The left LGN was sonicated in 55 sessions (35 in subject B and 20 in subject E) and the right LGN in 55 sessions (36 in subject B and 19 in subject E). Changes in behavior were quantified by fitting a sigmoid function to each subject's average choice behavior [17]. In each session, baseline behavior was measured in the five-minute period immediately prior to the stimulation. In this baseline time window, we establish the delay between the onset of the two targets at which the subject showed equal preference for either target. Changes in the subject's behavior in subsequent five-minute time windows were quantified by measuring the subject's preference for the leftward target at the baseline point of equal preference.

The subjects completed a minimum of 600 trials to provide an adequate baseline before the stimulation. The subjects generally worked daily throughout the work week, over a period of eight months. Subject B performed the task for 1–2 hours while Subject E typically worked less than 1 hour. Thus, there are less data available with increasing session time.

We evaluated effects of active sham sonications. The sham sonications used the same parameters as verum but the ultrasound was not focused into the LGN. Instead, the individual delays for each of the 256 elements were set randomly.

Including sham sonications, a total of 79 sessions in subject B and 45 sessions in subject E were collected. Sessions in which the subject worked for less than five minutes were excluded. If a subject performed less than 1 trial per 10 seconds within a five minute window, that window was also excluded.

Subjects gradually adapted to the stimulation (Fig. 5). In month 5, we therefore paused the data collection. Specifically, subject E did not participate in any behavioral sessions at this time and subject B did not receive any sonications with an I_{SPTA} of greater than 0.6 W/cm^2 (data from those sonications are not included in this study). The pause lasted 75 days for subject E and 57 days for subject B. Subject E showed shallower psychometric curve following the pause compared with his performance before the pause. Therefore, in this subject, we increased the time delays between the onsets of the two targets from an initial set of [–50,–25,0,25,50] to [–60,–30,0,30,60].

EEG recordings

The EEG signals were recorded by attaching alligator clips to the surgically implanted pins. Because the securing screws traverse the thickness of the skull, the recorded EEG signals may be classified as intracranial EEG. The left front pin was used as the reference and the right as the ground. The signals from the two rear pins were averaged together. The impedance between the channels was consistently below 1 kΩ.

The signals were recorded using a 128-channel recording system (RHS2000, Intan Technologies, Los Angeles, CA). The signals were low-pass filtered at 7.6 kHz, sampled

at 20 kHz, and notch-filtered at 60 Hz. Power in the alpha (7–12 Hz), beta (12–30 Hz), and gamma (30–70 Hz) bands was established using the Fourier transform performed on 500 ms windows (no overlap). Windows in which the EEG signal exceeded 100 μV were excluded. For each frequency band, a baseline value was determined by averaging the five minute window prior to the sonication. Changes in each band as a percentage of the baseline were computed in 5 minute windows every 2.5 minutes. The effect of sonication on the alpha, beta, and gamma rhythms is measured as the difference between the change in each band measured after verum sonication and the changes measured after the sham sonications. The EEG recordings encompassed a total of 53 of verum sonication (18 and 7 sonications of the left LGN in subject B and E, respectively, and 21 and 7 sonications of the right LGN) and 9 sham sonications (5 in subject B and 4 in subject E). The number of sessions in which the EEG was recorded was lower than the number of behavioral sessions due to availability of the acquisition system.

During sonication, the ultrasound caused an electrical artifact. Thus, Fig. 1D shows only the data acquired during the five minute windows following the ultrasound offset. The statistical analysis likewise includes only data acquired after the sonication. This ensured that this analysis was not affected by an electrical stimulation artifact.

Stimulation parameters

All neuromodulation stimuli had a center frequency of 480 kHz. Each sonication lasted 30 seconds and consisted of a train of 30 ms pulses. The individual parameters of the waveform are provided in Table 1 and Table 2.

Averaged across the two subjects, the estimated pressure at focus was 46% of the free field value (estimated using MR thermometry according to the methods outlined in [20]). Thus, the values given in Table 1 and Table 2 assume that the *in-situ* intensity is de-rated by a factor of 21% relative to the free-field intensity.

Initial parameters were selected by titrating the pressure level until observing robust effects on the choice behavior. We varied additional aspects of the waveform found to be important in previous studies. However, subjects' behavioral adaptation to the stimulation diminished our statistical power. Therefore, this article does not make claims about the relative effectiveness of the individual stimulation waveforms.

Beam scanning

The half power beamwidth of the acoustic focus measured in a water tank is 1, 3.75, and 3.75 mm in the left/right, anterior/posterior, and superior/inferior dimensions, respectively [20]. The approximate dimensions of the LGN are 5, 7, and 6 mm in the same respective dimensions [33]. To ensure adequate coverage of the LGN given the relatively narrow focus of the ultrasound, we electronically focused the beam into five adjacent locations. Specifically, the original central target was complemented by targets ±1.5 mm in the left/right dimension and ±3 mm in the inferior/superior dimension. The timing of the ultrasound in each coordinate was set to maintain a uniform duty cycle throughout the stimulus. This strategy quintupled the effective duty cycle of the transducer array. Table 1 and Table 2 give the parameters for which beam scanning was applied.

Safety

The reproducible daily stimulation of the LGN over the course of eight months provides crucial information on the safety of sustained ultrasonic neuromodulation in the primate brain. The task used in this study has been used in neurology for more than a century as a sensitive readout of the impact of stroke or other perturbations of visual regions [20,31,32,34]. Specifically, damage to the LGN would manifest as a decrease in the subject's visual discrimination accuracy [20,31,32,34]. Thus, the subjects' discrimination accuracy measured during the baseline period across the individual sessions provides a functional assessment of the stimulation safety.

We evaluated safety also at the anatomical level, using gadolinium-enhanced MRI. We took T1- and T2-weighted images. T1-weighted gadolinium-enhanced images are commonly used as evidence of intact or disrupted blood-brain barrier [35–38] as gadolinium does not cross the barrier by default. T2-weighted gadolinium-enhanced images are sensitive to edema formation [37,39]. The MRI data collection followed a previous protocol [39]. T1 (3D volumetric interpolated breath-hold examination (VIBE), TR/TE=4.46/1.42 s, 192×132×80 mm field of view, 1 mm isotropic voxels, readout bandwidth 490 Hz/pixel, 5 averages, 3:55 minutes) and T2 (Sampling Perfection with Application optimized Contrast using different flip angle Evolution, TR/TE=4000/179 s, 192×136×80 mm field of view, 1 mm isotropic voxels, readout bandwidth 789 Hz/pixel, Turbo factor 165, 1 averages, 3:30 minutes) images were acquired before sonication. We then insonated (480 kHz, 1.5 MPa amplitude, 30 ms pulse duration, 10% duty cycle, 1 minute total duration) the left LGN and immediately injected gadolinium contrast agent (gadoteridol, ProHance, Bracco, Milan Italy, concentration of 279.3 mg/ml) at a dose of 0.15 ml/kg. Repeated T1 and T2 scans were taken for 30 minutes following the sonication.

Statistical analyses

A two-way ANOVA was used to establish the difference in behavior between the two stimulation targets. The ANOVA was evaluated in time windows of 0.5–5.5, 5.5–10.5, and 10.5–15.5 minutes following the offset of the stimulation. The windows start at 0.5 in order to exclude trials performed during the stimulation. Similarly, a four-way ANOVA was used to establish changes in EEG rhythms resulting from sonication. The ANOVA analyzed the data in the same time windows (0.5–5.5, 5.5–10.5, and 10.5–15.5 minutes following sonication onset) as the behavioral data. The ANOVA analyzed the difference in changes in alpha, beta, and gamma resulting from verum and sham sonications, using factors of LGN side (left or right LGN), recording side (whether the signal was recorded by the right or left rear pin), sample time, and frequency band.

Results

Noninvasive, sustained, and reversible neuromodulation

We found that a single 30-second stimulation of the LGN induced a sustained but reversible contralateral bias in the subjects' visual choice behavior (Fig. 1). In particular, stimulation of the left/right LGN (Fig. 1A) induced a rightward/leftward bias in the subjects' choices (Fig. 1B). On average (Fig. 1C), the effects outlived the stimulation by up to 15 minutes (final

time point for which the effect in Fig. 1C is significant; two-tailed t-test, $p < 0.05$). The contralateral effect on the animals' choices suggests that ultrasound excited the stimulated circuits [17].

We quantified the effect on choice behavior using a two-way ANOVA, with factors sonicated side and time. We specifically evaluated the effects following the ultrasound delivery to circumvent potential stimulation artifacts. The effect of the sonicated side was highly significant ($F(1, 46) = 13.7$, $p = 0.00058$; $n = 12$ and $n = 9$ for right and left LGN stimulation, respectively). Time or its interaction with side were non-significant. The analysis included 12 sonications of the right LGN (8 in subject B, 4 in subject E) and 9 sonications of the left LGN (3 in subject B, 6 in subject E). These data include early sessions before the subjects adapted to the stimulation. The inclusion of all sessions (35 and 20 sessions for left LGN in subjects B and E respectively and 36 and 19 for right LGN) preserved the effect ($F(1, 297) = 7.57$, $p = 0.0063$) but reduced its magnitude (Fig. S1).

The effect on choice behavior was accompanied by changes of neural rhythms recorded over visual cortex (Fig. 1D). A four-way ANOVA, with factors of signal frequency, sonicated LGN, recording side (whether the signal was recorded over the left or right visual cortex), and time, identified a significant effect of signal frequency ($F(2, 16) = 127.21$, $p < 0.001$), with an increase in gamma and decrease in alpha and beta activity relative to the sham sonication (Fig. 1D). The time at which the signal was acquired was also significant ($F(2, 16) = 34.48$, $p < 0.001$) as was the interaction between time and both recording side and signal frequency (time × recording side $F(2, 16) = 11.3$, $p < 0.001$; time × signal frequency $F(4, 16 = 8.24$. $p < 0.001$). The stimulated LGN side and the recording side were also significant, both individually and in their interaction (sonicated LGN: $F(1, 16) = 4.88$, $p = 0.042$; recording side: $F(1, 16) = 37.81$, $p = < 0.001$; LGN side × recording side $F(2, 16) = 7.77$, $p = 0.013$). The interaction between the sonicated LGN side and the recording side corroborates the behavioral findings that the ultrasound produced side-specific stimulation effects.

Active Sham control

Thus far, there are two controls for potential generic artifacts associated with ultrasound [40,41]. First, the effects outlive the stimulation by up to 15 minutes (Fig. 1C; Fig. S1). Second, the delivery of focused ultrasound into the left and the right LGN elicited effects of opposite polarities (Fig. 1C; Fig. S1). We included a third control in the form of active sham sonication. The sham sonication delivered into the brain the same amount of acoustic energy but the phases applied to each element of the transducer were set at random, thus creating an unfocused beam. There was no significant effect following the sham stimulation (Fig. 2).

Safety

The reproducible targeting of the LGN from session to session enabled us to evaluate the long-term safety of the stimulation. We assessed the safety at the functional and anatomical levels.

At the functional level, we evaluated the accuracy of the visual discrimination behavior over the individual stimulation sessions. Damage to the LGN would manifest as a decrease in the

subject's visual discrimination accuracy [31,32,34]. Yet, the subjects showed either stable or increased accuracy over time (Fig. 3). There was a significant increase in the discrimination performance in subject B (Pearson's correlation between accuracy and time, $r = 0.50$, $p < 0.001$).

At the anatomical level, we evaluated the safety of the stimulation using contrast-enhanced MRI imaging. Contrast-enhanced T1 images acquired before and after sonication showed no evidence of perturbation of the blood-brain barrier (Fig. 4). Specifically, yellow overlays, which indicated signal changes greater than 10% [35–38], were only observed in regions with low signal (skull and ventricles). No significant increase was observed in the stimulated LGN. These data suggest that the blood-brain barrier remained intact [35–38]. In addition, T2-weighted contrast-enhanced images, which have been used to evaluate formation of edema [37,39] also did not show a signal increase within the LGN or elsewhere in the brain. Together, these behavioral and anatomical data suggest that the sustained ultrasonic stimulation of the deep brain targets was safe.

Adaptation

The stimulation was applied to the subjects over the course of eight months. As a consequence, the monkeys learned to compensate for the stimulatory effects. A compensation was expected because an ultrasound-induced bias decreases the discrimination accuracy and therefore the reward income for the animals. Fig. 5 shows the average choice preference, pooled over left and right LGN stimulation session, as a function of the stimulation month. The effects were strong early during the stimulation and gradually declined with each stimulation month. Given this finding, at month 5, we paused the data collection (see Methods for details). Resuming the stimulation in month 6, the effect and its adaptation were replicated (Fig. 5).

Stimulation parameters

As a part of this chronic stimulation study, we aimed to investigate the effects of individual stimulation parameters (Table 1 and 2). Unfortunately, the observed adaptation to the stimulation (Fig. 5) reduced the power of this analysis, and so any claims on relative advantages of some parameters over others would be inconclusive.

Discussion

This study demonstrates that transcranial focused ultrasound can safely induce sustained stimulation of deep brain circuits in primates. Just thirty seconds of low-intensity ultrasound produced 15-minute long effects on the subjects' choice behavior.

Unique aspects

Effective and safe treatments of brain disorders require an approach that modulates deep brain targets, does so precisely, in a sustained manner, and in subjects who are not anesthetized. This study is unique in that it fulfills all four requirements. We have modulated two deep brain targets, the left and the right LGN. These targets were modulated at high spatial precision using an ultrasonic phased array. We have demonstrated that the effects of

brief stimulation trains outlast the stimulation—a key premise for inducing a durable reset of malfunctioning circuits. And finally, the stimulation was performed in subjects who were not anesthetized. Anesthesia should be avoided as it impairs cortical excitability—including excitability by ultrasound [42]—and limits widespread applications in humans. One prior study demonstrated durable effects in the deep brain of awake primates [28], but the study used a single-element transducer, which has limited spatial precision and must be physically moved to target distinct brain regions. Precise and systematic applications will require phased arrays, such as the one developed for these purposes here.

Durable effects

The finding that relatively short, 30 s trains of stimulation induce durable effects within the target circuits align with previous findings, which report effects on NHP behavior [28], resting-state fMRI [22–24], and motor evoked potentials in humans [29]. The present study applied the stimulation systematically to two distinct targets with predicable changes in choice behavior. This allowed us to assess the polarity of the sustained effects. Specifically, the contralateral bias in choice behavior suggested that the ultrasound excited the stimulated circuits.

The duration of the effects reported in this study is on par with the effects elicited by trains of transranial magnetic stimulation (TMS) pulses of comparable duration [43]. This is a notable finding given that TMS applied to the brain for about 40 minutes per day for several weeks can induce persistent improvements in depressive symptoms [44]. Ultrasound could be applied in a similar, repeated mode, with the key advantage that it can directly modulate the diseased deep brain targets. TMS is thought to rely on an indirect modulation of deep brain circuits. Direct modulation of specific deep brain regions with ultrasound is expected to increase the efficacy of the treatments while limiting off-target side-effects.

Involved mechanisms

The result reported here is in line with a recent mechanistic study that recorded discharge activity of primary rat cortical neurons in response to low-intensity ultrasound [45]. A 40-s stimulation increased the neuronal excitability for up to 6 hours. The protocol used a neuronal culture and different ultrasound parameters (center frequency of 200 kHz compared to 480 kHz; duty cycle of 50% compared to duty cycles of less than 15%; and a pulse duration of 100 ms instead of 30 ms), but both studies demonstrate that ultrasound can excite neural tissues in a sustained manner.

Existing studies have demonstrated that ultrasound mechanically activates ion channels in neurons and glial cells [46]. The activation is in part due to the acoustic radiation force [47–50] and could also be due to the displacement caused by the cycle-by-cycle variability in the acoustic pressure waveforms [50,51]. The cycle-by-cycle displacements could further lead to cavitation-related phenomena, such as intramembrane cavitation [52]. The effects of ultrasound on nervous tissue can be transient or durable, depending on stimulus duration. Stimulus durations of at least several seconds, such as 30 s used here, tend to produce durable changes in the stimulated circuits [22–25]. These neuroplastic effects are, at least in part, due to stimulation of astrocytes and subsequent modulation of NMDA receptor

activity [53]. Future studies that vary the ultrasound parameters systematically will provide the means to distinguish between the physical forces and cell-type-specific [54] bioeffects.

Control for confounds

This study controls for potential auditory or vestibular artifacts that can be associated with transcranial ultrasound [40,41]. We devised three measures to control for such confounds. First, the behavior and neural activity was assessed only after the ultrasound had been turned off. During that time, there was no stimulation and so there could be no auditory or vestibular confound. Second, the behavioral and electrophysiological effects were stimulation-hemisphere-specific. Since the targeting is achieved entirely electronically, the mechanical setup for the sonication of the left and right LGN is identical, thus keeping any potential generic artifact fixed. Yet, we find effects that are specific to the stimulated target side. Finally, active sham stimulations, which delivered the same energy and waveform as the verum stimulation, did not result in significant effects.

Safety

This study informs on the safety of long-term ultrasound stimulation. The ultrasound was applied to the deep brain targets almost every working day over several months. Moreover, the delivered in situ I_{SPTA} intensity of up to 2 W/cm^2 was higher than the 0.72 W/cm^2 guideline level of the current FDA 510(k) Track 3 recommendations for ultrasound imaging systems [55]. Using a behavioral task that is often used in neurology to detect harm to nervous tissue [31,32,34], we found stable or increasing accuracy in the subjects' discrimination performance over the time of the stimulation regimen. No safety concerns were detected during the chronic stimulation.

Limitations and future work

The study has four limitations. First, in this initial evaluation of effectiveness and safety of chronic deep brain stimulation with ultrasound, we have applied to the deep brain targets stimuli of limited duration (30 seconds). Future studies should test effects of stimuli of much longer duration, e.g., up to session lengths used during TMS stimulation (about 40 minutes). Second, our subjects adapted to the stimulation within about 1 month of stimulation. This is expected given that the adaptation leads to an increased reward income. As a consequence, we were unable to systematically test the effects of the individual stimulation parameters. Future studies should apply the stimulation less frequently, randomize the order in which each US parameter is applied, alternate distinct tasks, or use invasive recording techniques to directly measure neuronal responses. Third, the study does not provide direct insights into the responses of individual cells. It has been demonstrated that ultrasound mechanically activates ion channels in neurons and glial cells [9]. Distinguishing which stimuli modulate which cell types will direct applications of this emerging neuromodulation tool to specific disorders and conditions. Finally, because macaque skulls are thinner than those of humans, this model does not fully represent the difficulties of delivering ultrasound to the human brain. Ultrasound neuromodulation in humans has thus far produced variable response [10], an outcome likely due to the uncertainty in the acoustic dose delivered through the skull of each individual [56]. Thus, robust applications of neuromodulatory ultrasound in clinics will

require an effective approach to accurately correct for the ultrasound attenuation caused by the human skull.

Conclusion

In summary, this study demonstrates effective and safe modulation of circumscribed deep brain circuits with transcranial ultrasound applied to task-performing primates. The study highlights the full potential of the approach, modulating behavior in a sustained manner following stimulation of deep brain circuits in non-anesthetized subjects. These results encourage applications of this precise and noninvasive tool to modulate malfunctioning deep brain circuits in humans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was funded by grants from the NIH (R00NS100986, RF1NS128569, and F32MH123019), the Focused Ultrasound Foundation, and by the Margolis Foundation. We thank Caroline Garrett and Tyler Davis for making this work possible.

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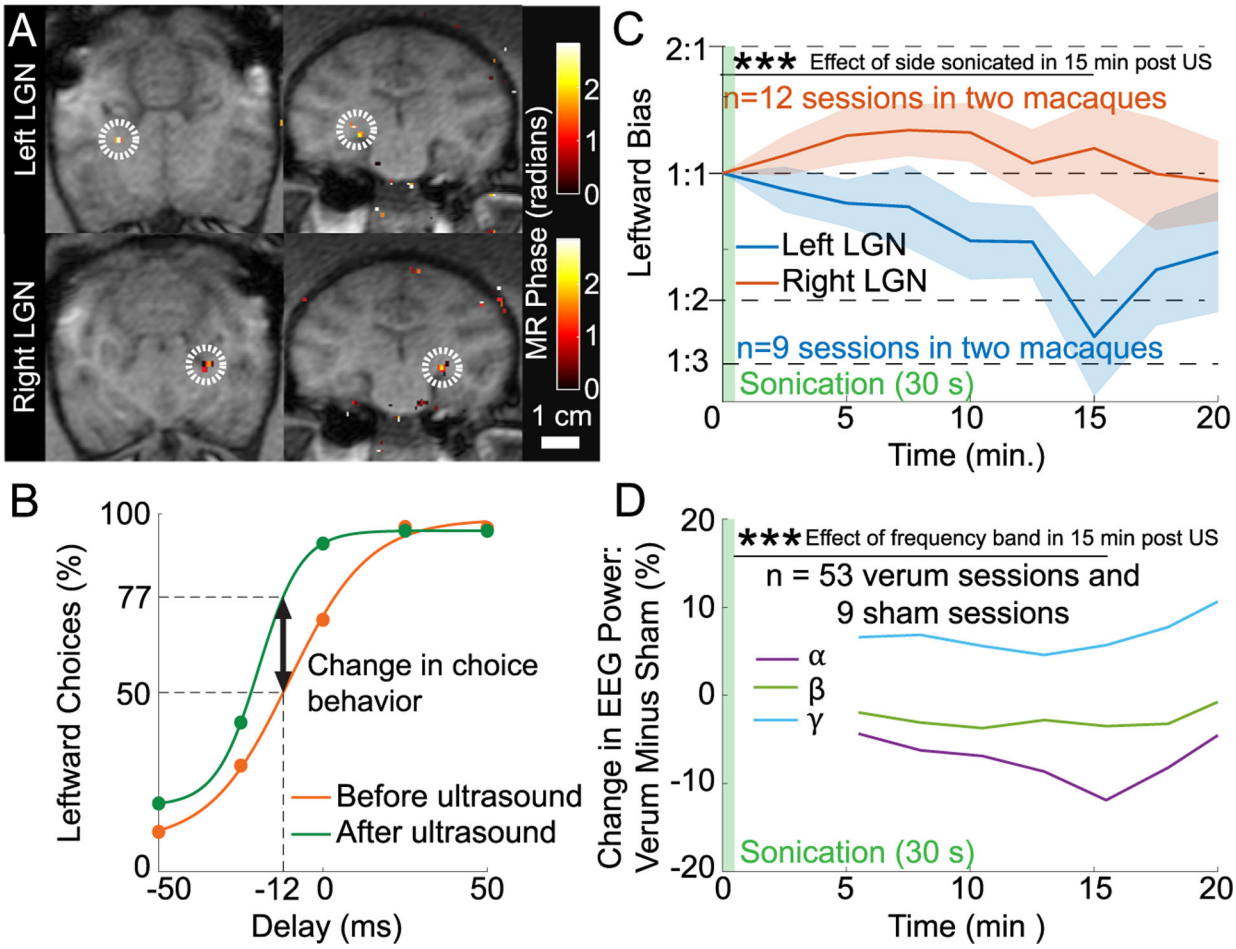

Fig. 1. Noninvasive, Sustained, and Reversible Modulation of Deep Brain Circuits and Choice Behavior.

(A) Target Validation: Ultrasound targeting of the left and right LGN. Color shows the phase signal associated with MRI thermometry. The left and right columns represent axial and coronal planes, respectively. The corresponding data for the second subject are shown in [20]. **(B) Quantification of the Effects on Choice Behavior:** Example session for right LGN stimulation. The orange and green plots show five minutes of choice behavior before and after the delivery of ultrasound, respectively. In all subsequent behavioral plots, we quantify the proportion of leftward choices at the baseline point of equal preference (dashed line). **(C) Sustained Modulation of Behavior:** Mean±s.e.m. choice preference as a function of time. Sonication of the right (red) or left (blue) LGN induces a persistent contralateral bias in the animals' choices. These data include early sessions before animals adapted to the stimulation (see text for details). The acoustic parameters are described in Table 1 and Table 2 (8 sessions at B1, 3 sessions at B2, 4 sessions at E3, and 6 sessions at E1.) ***: $p < 0.001$: A two-way ANOVA with factors of side sonicated and time detected a significant effect of the stimulated LGN on the choice behavior (see text for details). **(D) Sustained Changes in Neural Rhythms:** Change in alpha, beta, and gamma activity resulting from verum stimulus minus the change resulting from sham stimulus as a function of time. The modulation by

the frequency band was assessed using a three-way ANOVA with factors of frequency band, sonicated side, and recording pin (see text for details); ***: $p < 0.001$.

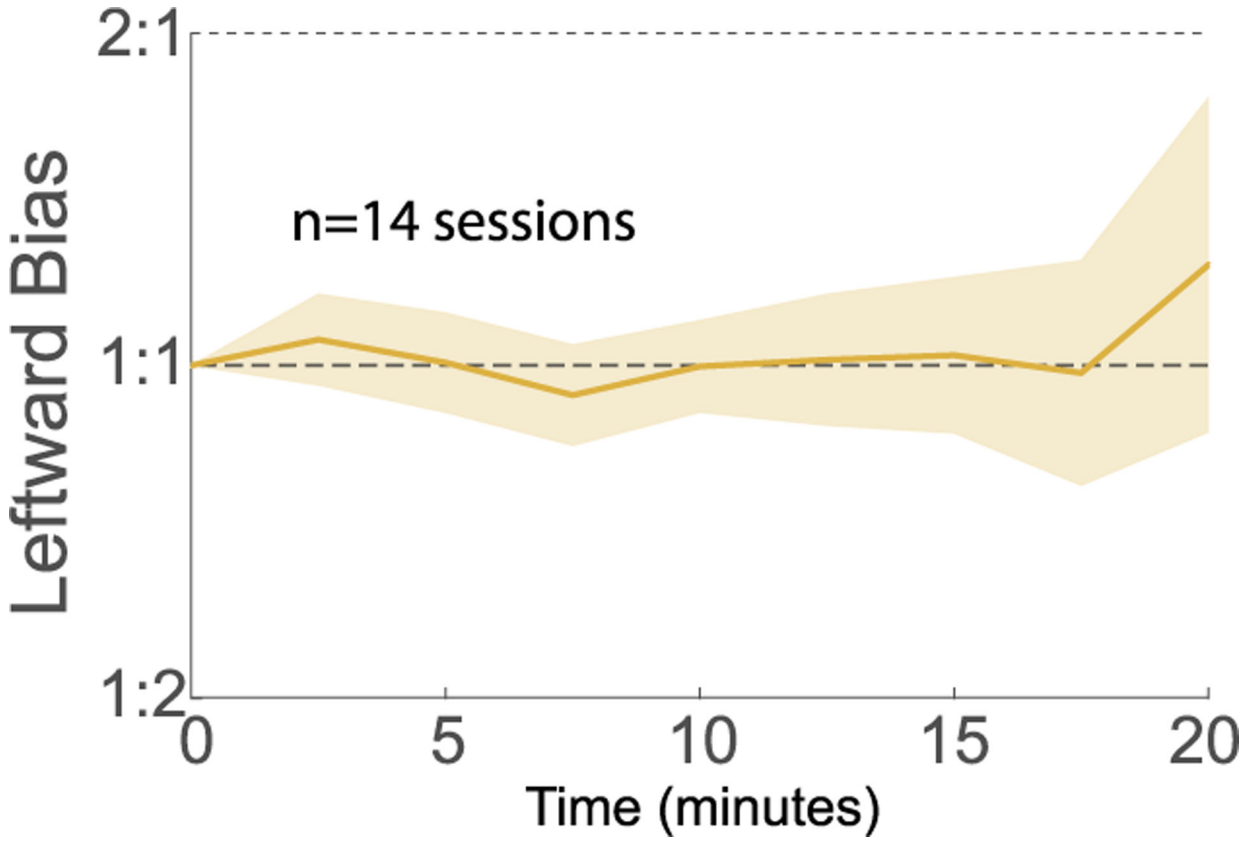

Fig. 2. Active Sham.

Same format as in Fig. 1C. Sham sonications delivered into the brain the same amount of energy but in an unfocused manner.

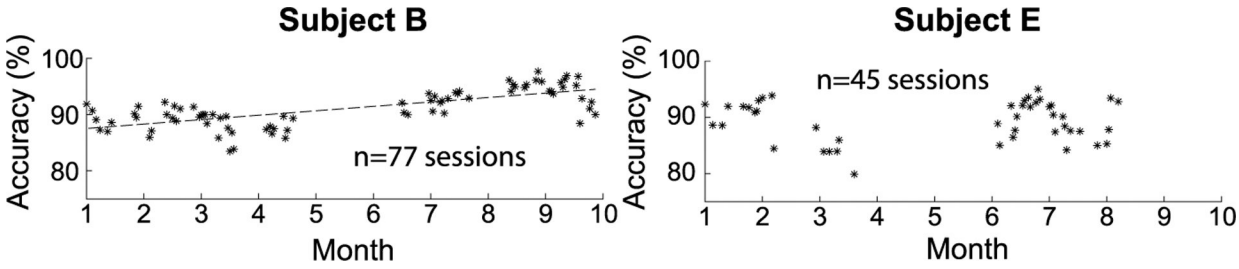

Fig. 3. Chronic Modulation of Primate Deep Brain Circuits with Ultrasound Is Safe.

Repeated application of neuromodulatory ultrasound to deep brain circuits of non-human primates is safe. Significant damage to the LGN in this sensitive visual discrimination task would cause a notable decline in subjects' visual discrimination performance. There was no decline, with both subjects showing steady or increasing discrimination accuracy across successive sessions.

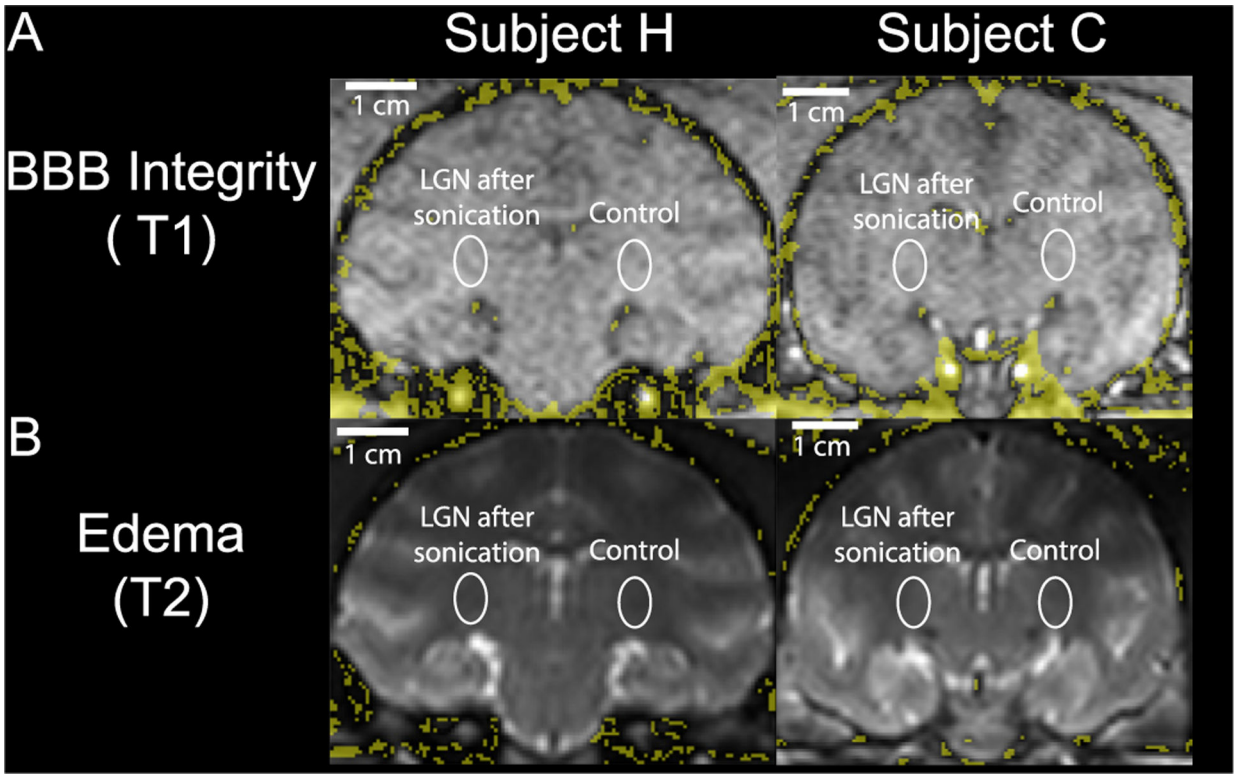

Fig. 4. The stimulation does not disrupt the blood-brain barrier or cause edema.

a) Ultrasound (1.5 MPa, I_{SPTA} of 7 W/cm^2, 1 minute sonication, 30 ms pulse duration, 10% duty cycle) was delivered into the left LGN. Immediately following the sonication, we injected the contrast agent gadoteridol (ProHance, Bracco, 279.3 mg/ml). The top and bottom images show T1-weighted and T2-weighted MRI images, which quantify the difference before and after the administration of ultrasound and gadoteridol. The yellow regions show contrast greater than 10% on the T1 images and regions where the post image was greater than the pre image on the T2 images. The contrast was below this threshold within both LGNs (white circles), which is commonly taken as evidence of intact blood-brain barrier [35–38]. The lack of increased T2 signal in the brain (bottom) provides further evidence of safety as edema due to the ultrasound would result in an increased intensity on a T2 image [37].

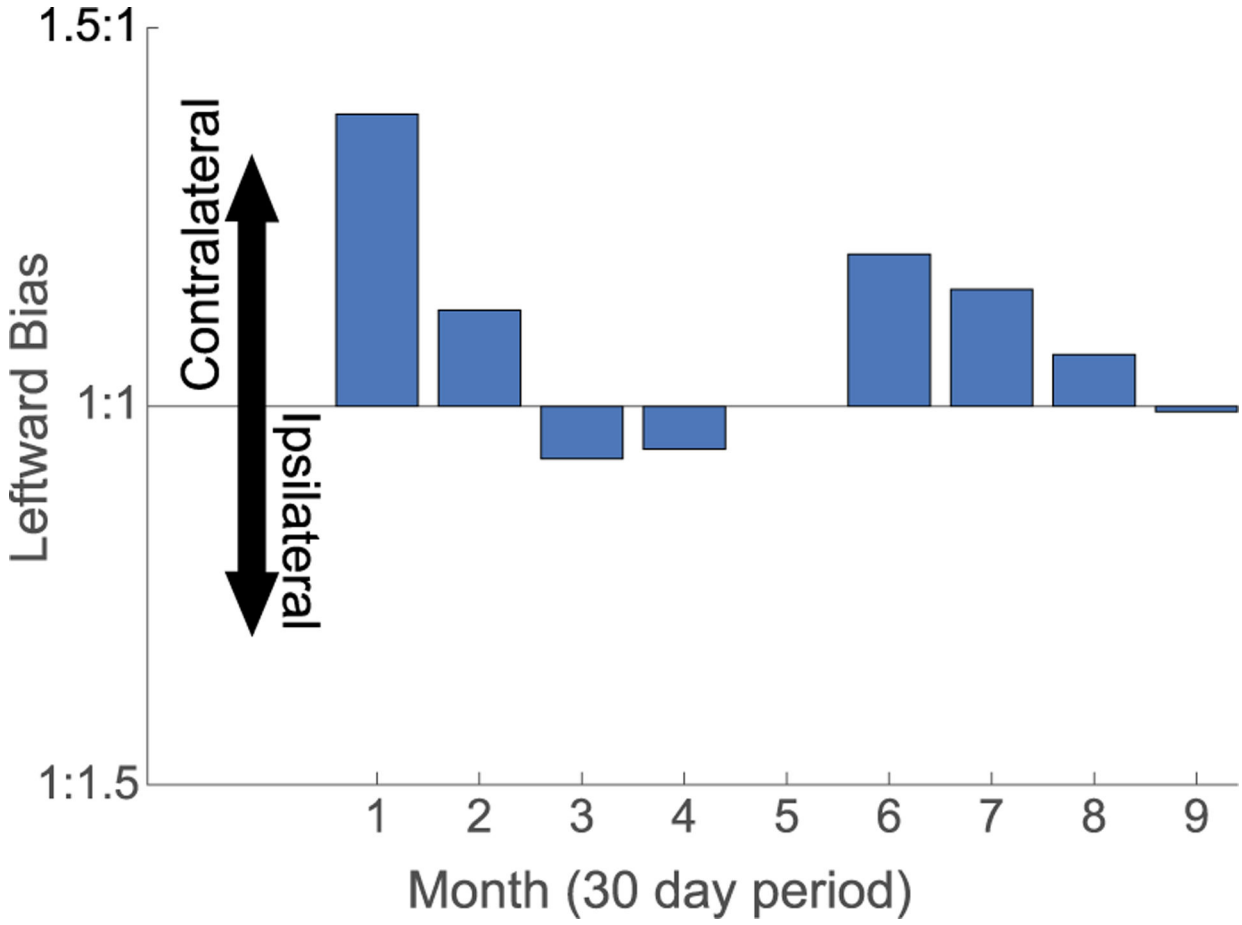

Fig. 5. Behavioral Adaptation.

The effect was prominent in the initial month of stimulation and gradually declined thereafter. Given the adaptation, the stimulation was paused in month 5 (see Methods for details). Details regarding the number of sessions and stimulation parameters for each datapoint are provided in Table S1.

Table 1

Stimulation parameters and the number of sessions delivered in Subject B. Left to right columns: Spatial peak temporal average intensity (I_{SPTA}; W/cm^2), spatial peak pulse average intensity (I_{SPPA}; W/cm^2), duty cycle (DC; %), pressure (P; MPa), pulse repitition frequency (PRF; Hz), pulse duration (PD; ms), mechanical index (MI), the number of sessions recordedduring sonication of the left/right LGN, the number of sham sonications, and whether or not the acoustic beam was scanned throughout the LGN (see Methods details).

Name	I_{SPTA}	I_{SPPA}	P	DC	PRF	PD	MI	Left	Right	Sham	Scan
B1	1	31	1.0	3.6	1.2	30	1.4	8	8	0	No
B2	1	31	1.0	3.6	1.2	30	1.4	8	7	0	Yes
B3	1	8	0.5	14.4	4.8	30	0.7	8	9	8	Yes
B4	2	59	1.4	3.6	1.2	30	2	8	8	0	Yes
B5	2	14	0.7	14.4	4.8	30	1	3	4	0	Yes

Table 2

Stimulation parameters and the number of sessions delivered in Subject E. Same format as in Table 1.

Name	I_{SPTA}	I_{SPPA}	P	DC	PRF	PD	MI	Left	Right	Sham	Scan
E1	1	59	1.4	1.8	0.6	30	2	9	8	0	Yes
E2	2	14	0.7	14.4	4.8	30	1	4	6	0	Yes
E3	2	64	1.4	3.6	1.2	30	2.1	7	5	6	No