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Suppression of EEG visual-evoked potentials in rats via neuromodulatory focused ultrasound

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Abstract

We investigated the use of pulsed low-intensity focused ultrasound (FUS) to suppress the visual neural response induced by light stimulation in rodents. FUS was administered transcranially to the rat visual cortex using different acoustic intensities and pulsing duty cycles. The visual evoked potentials (VEP) generated by an external strobe light stimulation were measured three times before, once during, and five times after the sonication. The VEP magnitude was suppressed during the sonication using a 5% duty cycle (pulse-repetition frequency of 100 Hz) and spatial-peak pulse-average acoustic intensity of 3 W/cm^2; however, this suppressive effect was not present when a lower acoustic intensity and duty cycle were used. The application of a higher intensity and duty cycle resulted in a slight elevation of VEP magnitude, which suggested excitatory neuromodulation. Our findings demonstrate that the application of pulsed FUS to the region-specific brain area not only suppresses its excitability, but also can enhance the excitability depending on the acoustic intensity and rate of energy deposition. This bimodal feature of FUS-mediated neuromodulation, which has been predicted by numerical models on neural membrane capacitance change by the external acoustic pressure waves, suggests its versatility for neurotherapeutic applications.

Keywords

focused ultrasound; neuromodulation; suppression; EEG; ultrasound; neurotherapeutics

Introduction

Non-pharmacological modulation of the regional brain function has been sought after as a treatment option for various drug-resistant neurological and psychiatric disorders [1]. Invasive electrical brain stimulation modalities, such as electrocorticography (ECoG) [2] or deep brain stimulation (DBS) [3], require the surgical placement of electrodes in the brain. As for non-invasive alternatives, transcranial direct current stimulation (tDCS) and transcranial magnetic stimulation (TMS) have been used [4]. However, these techniques

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lack spatial resolution and depth penetration that limit therapeutic applications involving deep-tissue targeting [4,5].

The need to develop a more refined non-invasive method has led to the introduction of focused ultrasound (FUS) and subsequent studies ensued in hopes of utilizing it as a potential tool for modulating the cortical activity [5]. The administration of a pulsed FUS, compared to the continuous sonication, to the brain not only reduces the acoustic energy deposition but also is believed to create distinct mechano-electrical effects on the neuronal cell membrane by changing its capacitance as well as transmembrane ion currents, which consequently leads to neural stimulation [6,7]. Subsequent animal studies were implemented to characterize the effects of different FUS parameters on inducing functional neuromodulation [8,9]. Specifically, a recent study by Kim *et al.* [10] showed the existence of an optimal pulsing scheme that elicits excitatory neural activity.

Although most of the current studies have focused on examining the excitatory effects of FUS [11], some studies have reported neural suppression. For example, the suppression of the visual cortex activity by FUS was shown *via* the reduction of the signal amplitude of the visual evoked potentials (VEP) and blood-oxygenation-level-dependent (BOLD) functional magnetic resonance imaging (fMRI) signals in rabbits [5]. Epileptographic electroencephalography (EEG) activity from chemically-induced epileptic models in rats was also suppressed by the FUS sonication to the thalamic area [12]. However, the range of sonication pulsing parameters in these studies was rather limited, and further studies were needed to elucidate the pulsing parameters that would induce suppressive neural activity. Here, we examined the varying effects of sonication parameters on regulating the excitability of the visual cortex in rats based on the changes made in the amplitude of the VEP that were elicited by the external light stimulation. Specifically, the involvement of acoustic intensity and duty cycle of the pulsing schemes in achieving functional suppression of regional brain activity was examined.

Materials and Methods

Animal Preparation

The animal experiments conducted in this study were in compliance with the standards set forth by the institutional review and was under the approval of the Harvard Medical Area Standing Committee on Animals. Sprague-Dawley rats (all male, $n = 24$) were anesthetized by an intraperitoneal injection of a ketamine/xylazine mixture at 80:10 mg/kg prior to sonication. The fur over the skull region was shaved and two subdermal EEG electrodes (SWE-L-25, Ives EEG Solution, Canada) were then placed under the midline of the frontal and occipital scalp surface, with a distance of approximately 15 mm between the electrodes, to measure the VEP. The ground electrode was placed on the ventral side of the left ear. The rat was positioned on a stereotactic stage (SRP-AR, Narishige, Japan), whereby the head was demobilized using ear bars and a teeth holder. The FUS transducer was then positioned over the rat's head while the acoustic path was coupled to the skull *via* a cone-shaped plastic bag containing degassed water as shown in Fig. 1A. Hydrogel (Aquasonic, Parker Laboratories, Fairfield, NJ) was applied to the interface between the rat's scalp and the water bag. The position of the rat was adjusted relative to the FUS transducer by manipulating the

three-axes platform (MF70, Proxxon, Hickory, NC, and Lab Jack, Edmund Scientifics, Tonawanda, NY) attached to the stage, and the acoustic focus was aimed at the visual cortex under the stereotactic guidance [11].

Sonication Setup

An air-backed, segmented spherical FUS transducer (Channel Industries, Santa Barbara, CA), which has an operating frequency of 350 kHz, was mounted to an articulated arm. A transducer with an outer diameter of 6 cm and a radius-of-curvature of 7 cm was used in the present study. The input signal to drive the transducer was generated from two serially connected function generators (33220A Agilent technologies, Inc., Santa Clara, CA), which was amplified by a linear power amplifier (240L, Electronics and Innovations, LTD, Rochester, NY). Prior to the experiment, the acoustic intensity profile of the transducer around the focus was characterized in *ex vivo* setup using a calibrated needle-type hydrophone (HNR500, Onda, Sunnyvale, CA). The geometry of the neuromodulatory area at the acoustic focus, estimated at the full-width at 90%-maximum, was roughly an elongated ellipsoidal shape (3.7 mm in diameter, 5.6 mm in length). The detailed procedure to map the acoustic pressure field can be found elsewhere [11].

Experimental Design

VEP were measured to investigate the suppressive effects of FUS on the function of the visual cortex. After allowing the rats to adapt to a dark room environment for more than 5 min, 20 ms-long white stroboscopic light stimulation was given 100 times to both eyes in one-second intervals. The detected EEG signals were amplified and averaged by the acquisition system (Scope and PowerLab 8/30, AD Instrument, CO), after applying a 60 Hz notch filter to reduce the effect of ambient electrical signals. We used the magnitude between the first positive and negative peaks of the VEP (*i.e.* P1 - N1 in Fig. 1B), which is the most prominent and shows the least amount of normal variability in latencies and amplitude [13], as the primary measure for the degree of modulation resulting from the sonication. The EEG measurements were taken at approximately 36 min (36.5 ± 8 min; n = 24) after the injection of anesthetics. The VEP data was obtained nine times, every 150 s, representing the pre-sonication baseline periods (noted as B1 through B3, Fig. 1C), the sonication period (noted as S), and the monitoring periods after the sonication (noted as R1 through R5). We conducted repeated sonication sessions on the same animal, but each session was performed on different days with different sonication parameter sets.

The range of sonication parameters included the pulsing parameter that previously showed suppressive effects on rabbits [5] and rats [12], *i.e.* tone-burst duration of 0.5 ms and pulse-repetition frequency of 100 Hz (thus operating at 5% duty cycle). To examine the effects of various acoustic intensities, three different spatial-peak pulse-average intensities (I_{sppa}), *i.e.* 1, 3, and 5 W/cm^2 (n = 10, 9, and 9, respectively), were used at a fixed tone-burst duration of 0.5 ms at 100 Hz pulse-repetition frequency (*i.e.* 5% duty cycle). Next, the effects of different duty cycles (1 and 8.3%; n = 10 and 9, respectively) on modulating the magnitude of the VEP were examined by adopting two different pulse-repetition frequencies (20 and 166 Hz) while maintaining the same acoustic intensity of 3 W/cm^2 I_{sppa} with a tone-burst duration of 0.5 ms. The use of 8.3% duty cycle at 3 W/cm^2 I_{sppa} was adopted to evaluate the

effect of sonication parameters that have the same rate of temporal acoustic energy deposition as the sonication given at 5% duty cycle and 5 W/cm^2 I_{sppa} (*i.e.* 250 mW/cm^2 spatial-peak time-average intensity, *i.e.*, I_{spta}). The sonication duration was set to the same duration as the VEP measurement segment (indicated as 'S' in Fig. 1C, *i.e.* 150 s). A control experiment was performed under identical experimental conditions as the FUS experiment, but without any sonication (n = 8). The percentage difference of the VEP magnitudes from each segment, with respect to the ones from the baseline conditions (averaged among the B1–B3 segments), was calculated by removing the signal drift via linear detrending. To statistically evaluate the effects of different sonication parameters, one-way ANOVA followed by *post-hoc* Tukey's test (thus, allowing for multiple comparisons) was performed on the percentage differences of the VEP magnitude across the time segments with respect to the control condition.

The maximum acoustic intensity deposition was 250 mW/cm^2 I_{spta}, having a mechanical index (MI) of 0.75. The mechanical index is generally used to estimate the degree of mechanical bio-effects and is used as a reference to stipulate medical safety regulations for clinical use of ultrasound. The maximum MI used in the present study was much lower than the limit set forth (MI of 1.9 for diagnostic ultrasound scanner) by the Food and Drug Administration (FDA) [14,15].

Results

There were no significant differences in the weights of animals between the control group and each FUS sonication group ($F(5,54)=0.84$, $P > 0.5$). The level of normal variations in the VEP signals from the control group ranged from -2.6 to 2.7% compared to that of the baseline condition. The overall time latency of N1 from the onset of the visual stimulation was measured at 59 ± 7 ms (mean ± s.d.), and there was no difference in time latency between the control group and each FUS sonication group for all time periods ($F(5,54)=1.43$, $P > 0.2$). During the baseline segments (B1 through B3), the level of VEP magnitude was not significantly different from that of the control condition ($P > 0.05$; repeated measures ANOVA using the *post hoc* Dunnett's test, one-sided; $F = 0.49$, the adjusted *d.f.* = 9.19 according to the Greenhouse-Geisser correction; in Fig. 2A).

Varying acoustic intensity (I_{sppa}) at 5% duty cycle

The FUS given at 1 W/cm^2 I_{sppa} did not affect the VEP compared to those of the control group; however, when an acoustic intensity of 3 W/cm^2 I_{sppa} was used, a significant reduction in the VEP peak (*i.e.* -13.2 ± 9.6%, mean ± s.d.) was observed during the sonication period ($P = 0.02$; Fig. 2A). The reduction in VEP restored to the baseline level immediately after the completion of the sonication period (throughout the R1–R5). When a higher acoustic intensity (5 W/cm^2 I_{sppa}) was used, changes in VEP were not detected during the sonication segment, but the level was moderately increased ($P = 0.11$; Fig. 2A, represented by daggers) especially during early post-sonication (R1 and R2). A simple *t*-test (one-tail) between the control and 5 W/cm^2 I_{sppa} conditions supports an increasing trend of VEP in these time segments ($P = 0.06$ for R1 and $P = 0.01$ for R2).

Varying duty cycle at 3 W/cm^2 I_{sppa}

Fig. 2B shows the effects of changing the duty cycle when the acoustic intensity was kept constant at 3W/cm^2 I_{sppa}. The data pertaining to the control condition and 5% duty cycle were also plotted against the 1 and 8.3% duty cycles (Fig. 2B). The use of 1% duty cycle did not have any effects on the VEP signal compared to the control condition. When a higher duty cycle (*i.e.* 8.3%) was used, the suppressive effects on the VEP were not observed. Instead positive changes in VEP magnitude were observed compared to those from the use of 1 or 5% duty cycles ($P = 0.02$). In a subsequent post sonication segment (R1), although statistically marginal ($P = 0.11$), the level of VEP was slightly elevated compared to that of the control condition, which was also supported by the results from the student *t*-test ($P = 0.04$, one-tail).

Discussion

In the present study, we used an external photic stimulation to activate the visual cortex of the rodent brain, and administered pulsed low-intensity FUS with varying acoustic intensities and duty cycles to suppress the light-induced neural activity. The pulsing parameters that successfully suppressed the VEP in the current study were 3 W/cm^2 I_{sppa} and 5% duty cycle (tone-burst duration of 0.5 ms and pulse-repetition frequency of 100 Hz), which is comparable to the suppressive FUS parameters used for suppressing visual cortex activity in rabbits (3.3 W/cm^2 I_{sppa}) [5] and chemically-induced epileptographic EEG signals in rodents (2.6 W/cm^2 I_{sppa}) [12]. The observed suppressive neural activity was shown only during the sonication period (Fig. 2), whereas a lasting post-FUS-mediated suppression (approximately 10 min) was reported with the sonication of the rabbit visual cortex [5]. Although the definite cause for this discrepancy is unclear, we conjecture that the use of different animal models, brain regions, and fundamental frequencies (as well as the difference in acoustic focal size), might have been contributing factors, and calls for additional investigations.

When the FUS was given below a certain degree of temporally-averaged acoustic intensity (*i.e.* 30 and 50 mW/cm^2 I_{spta} resulting from the use of 3 W/cm^2 $I_{sppa,}$ at 1% duty cycles and 1 W/cm^2 I_{sppa} at 5% duty cycle respectively), the VEP was not affected by the sonication. These results suggest a presence of a threshold for inducing modulatory effects, which is in accordance with previous experimental data [9]. When FUS was administered with a higher duty cycle (*i.e.*, 8.3%) at 3 W/cm^2 I_{sppa}, the magnitude of the VEP increased and remained elevated beyond the sonication duration. This excitatory behavior was also observed when a higher level of acoustic energy (*i.e.*, 5 W/cm^2 I_{sppa}) was used with 5% duty cycle. This differential outcome on neural behaviors implies a possible dependency of acoustic intensity and duty cycle, in addition to the overall energy deposition to the brain, on determining the neuromodulatory effects. These findings are in a good agreement with the recent theoretical model that predicted the differential modulatory outcome of the brain tissue under exposure to acoustic pulsation [16]. According to the model, which probed the brain tissue excitability via neural membrane capacitance changes due to exposure to acoustic pressure waves [7], 'slow' inhibitory interneurons are more susceptible to the exposure to temporally-sparse acoustic stimuli (*i.e.* equivalent to the use of low duty cycle on the order of 5%) *via* T-type

voltage-gated Calcium channels, and consequently suppress the overall excitability of the brain tissue. On the other hand, the use of a higher duty cycle favors the recruitment of excitatory neurons (e.g., pyramidal cells), which make the neural tissue readily excited. Further study is warranted to assess more detailed effects of pulsing parameters, including the effects of acoustic intensity and duty cycle (especially in the range < 10%), on inducing the bi-modal (*i.e.* suppressive or excitatory) neuromodulatory features of the FUS.

Although not shown with statistically robust significance, it is also notable that the level of VEP, once increased, remained elevated beyond the sonication period itself (R1 and R2 in the case of 5 W/cm^2 I_{sppa} at 5% duty cycle and R1 in the case of 8.3 W/cm^2 I_{sppa} at 3% duty cycle; Fig. 2). From a clinical aspect, a prolonged neuromodulatory effect, regardless of the suppressive or excitatory nature, may be conducive to inducing lasting changes to neurons. For example, this capability can be utilized in the context of suppressing epileptic or epileptogenic activities [12]. However, the phenomena should be met with caution due to the possibility that FUS may produce an unintended and uncontrolled hyperexcitatory state, possibly inciting seizures, when administered with excessive intensity, duration, or duty cycle.

The critical element in the practical use of neuromodulatory FUS in neurotherapeutics is identifying specific pulsing parameters that are most effective in eliciting a desired neural activity without causing neural damage. Possibility of tissue damage, cavitation or disruption of blood-brain-barrier from the pulsing parameters used in the present study would be extremely slim considering the fact that the acoustic intensities used and the corresponding MIs were much lower than the FDA limit on clinical ultrasound imagers [14,15].

The neural effects of sonication with different pulsing parameters presented in this study demonstrate the feasibility of transcranially applying FUS to induce bimodal neural modulation. Furthermore, the study suggests the added potential for having the sustained modulatory effects even after the end of FUS sonication. Similar bimodal neural modulatory effects (*i.e.*, either excitation or suppression) have been observed in different brains stimulation modalities such as repetitive TMS (rTMS) and tDCS [4,17]. As it is often difficult to predict the modulatory effects from these techniques (*i.e.*, whether enhancing or suppressing the activity)[18,19], it is important to note that the fundamental neural mechanisms underpinning the efficacy of these techniques are still unknown, and a significant amount of work remains in determining their efficacy and method of deployment. With further studies on pulsing parameters to manipulate regional brain activity, the potential use of low-intensity FUS may be broadened to clinical application and brain mapping research.

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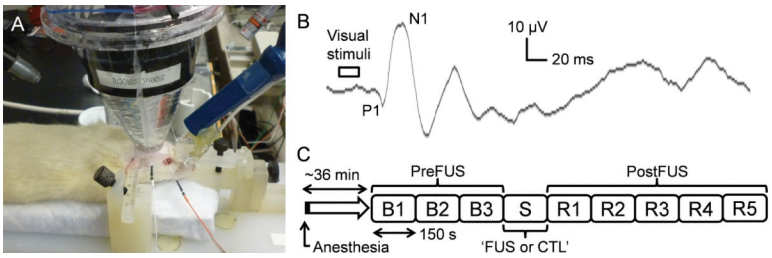

Figure 1.

Illustration of the experimental procedures. (A) An overview of the experimental setup (B) An example of the averaged visual evoked potentials (VEP) (n = 100) measured from an individual rat. Square box shows the timing of the visual stimulation. The first positive and negative peaks of the VEP are indicated as 'P1' and 'N1', respectively. (C) Timeline of the experiment. Boxes indicate VEP sessions with or without FUS sonication: B = Baseline before sonication ('PreFUS'), S = FUS sonication or Control (CTL) without FUS, R = Resting after stimulation ('PostFUS').

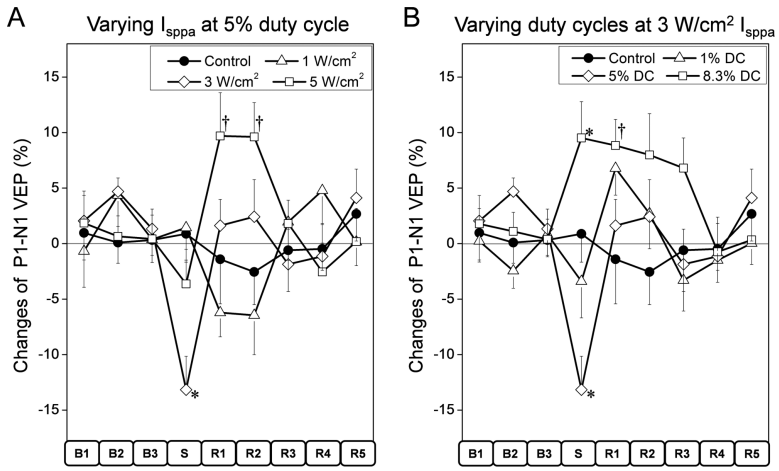

Figure 2.

The percentage changes in the VEP magnitude (P1 - N1, shown with standard error) in different data acquisition blocks (shown in horizontal axis) for (A) acoustic intensities of 1, 3, and 5 W/cm^2 at a fixed duty cycle of 5%, and (B) duty cycles of 1, 5, and 8.3% at a fixed acoustic intensity of 3 W/cm^2. For both (A) and (B), the unsonicated condition was included as a control group. Asterisk (*) indicates the significant group-level difference ($P < 0.05$), while dagger (†) indicates the marginal significance ($P = 0.11$), according to one-way ANOVA followed by *post-hoc* Tukey's test.