Multiscale Investigation of Low Intensity Transcranial Focused Ultrasound Neuromodulation in *in-vivo* Rodent Models

A Thesis

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BY

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Abstract

Transcranial focused ultrasound (tFUS) is a noninvasive neuromodulation method that modulates neural activity using mechanical pressure waves. tFUS has emerged as a promising noninvasive neuromodulation method with millimeter scale resolution and propensity to stimulate deep structures. Researchers have reported tFUS induced short term excitation or inhibition at cortical or deep brain. Currently, there are no reports of long term effects elicited by tFUS. The ability to use tFUS to non-invasively induce long term changes in the brain expands the clinical utility of tFUS. In order to explore the long term effects of tFUS on synaptic connectivity, we first evaluated our setup by examining the ability of tFUS to reliably induce short term changes to *in vivo* rats. After establishing our setup, we applied pulsed ultrasound to encode temporal information into the hippocampus to induce long term depression in 5 adult rats. Further investigations are needed to explore the underlying mechanisms.

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INTRODUCTION

Clinically, both invasive and noninvasive neuromodulation methods are applied to the central nervous system (CNS) to treat a wide range of diseases: movement disorders, psychiatric disorders, epilepsy, pain, etc. Regardless of the high effectiveness of treatments, for example in deep brain stimulation (DBS), neuromodulation remains to be only prevalent in patient subpopulations that do not respond to medication or other therapies, as a last resource. The limitation is due to the requisite of highly invasive mediations to achieve required spatial resolutions and depth of stimulation. Currently, the most focal noninvasive neuromodulation technique, transcranial magnetic stimulation, has spatial resolution larger than several centimeters, and stimulation depth around 2 centimeters. tFUS has recently emerged as a promising noninvasive neuromodulation method that rivals the efficacy of DBS with millimeter scale spatial resolution and up to 15 cm stimulation depth without the needs of neural surgery and brain implantation. However, since tFUS utilizes high frequency sound waves rather than electric or magnetic fields, the mechanisms of neural activation are not understood. Therefore, to translate this technology into a clinical therapy, there is a need to characterize the neural responses of an intact central nervous system triggered by tFUS stimulation.

The challenge with tFUS as a neuromodulation technique is the use of appropriate stimulation parameters to overcome the attenuation of ultrasound intensity through the skull without causing heating within the tissue. However, similar to the field of ultrasound imaging, there is an expansive tFUS parameter space to explore and optimize. In order to fully characterize the neural responses to tFUS, we developed a multiscale neural recording method to obtain simultaneous high density scalp EEG (27 channels) and extracellular recordings at the tFUS focal zone as well as a 3-dimensional ex-vivo acoustic

mapping system to characterize the tFUS pressure distributions transmitted through the skull.

In this project, we utilized our unique approach of multiscale neural recording with high spatial-temporal resolutions to first investigate tFUS parameters to achieve reliable short-term brain responses in focal regions, then we applied this setup to explore the induction of long-term synaptic changes in the brain. Results from our pilot study show promising new directions for the use of tFUS in clinics.

BACKGROUND

tFUS Mechanisms

Neuromodulation in the CNS is applied clinically to treat many disease states, from movement disorders to psychological disorders, epilepsy, pain, etc. [1]. The patient population reaches to 7 million in just a few of the representative diseases treated by CNS neuromodulation including: Parkinson's disease, epilepsy, and persistent depression disorder [2]. For many of these diseases, neuromodulation is the only long term and effective treatment. However, neuromodulation remains prevalent in only patient subpopulations that do not respond to medication or other therapies due to the risks for invasive interventions in order to achieve optimal spatial resolution and stimulation depth. **There is a need for a noninvasive neuromodulation method that can achieve equivalent or improved clinical efficacy shown by invasive neuromodulation methods.**

Although, we do not fully understand the mechanisms of many neuromodulation methods, the concept of using pressure waves to elicit electrical activity of neurons is not as intuitive as some other modalities. There are three main working hypotheses on the mechanism of action of ultrasound neural modulation. The first is the passive interaction of mechanical waves with the neuronal membrane, causing transient cavitation, acoustic streaming, and other disruptions to the neuronal membrane. The transient perturbation to the neuronal membrane temporarily changes the membrane charge polarization, capacitance, and resistance, hence changing the excitability of the neural tissue [3, 4].

Another hypothesized mechanism involves the interaction between tFUS and voltage gated or mechanically sensitive ion channels. Many cellular components naturally sense and transduce forces in neurons such as membrane proteins, microtubules, actin filaments, the plasma membrane, and the extracellular matrix. Of note are the voltage

gated ion channels (VGCs) in the neuron membrane. At rest, the subunits in the VGCs are in the closed conformation with low ionic conductance. In the presence of a mechanical stimulant, the VGCs change in conformation due to changes in plasma membrane tension and density, or the forces exerted by the cellular anchors of the VGCs, leading to an increase in channel conductance. The radiation force exerted by tFUS resonates pore-forming ion channels and leads to conformational changes between the protein subunits, hence allowing ions to flow through the ion channels along the concentration gradient across the cell membrane [5]. A subset of ion channels are more responsive to the mechanical stimuli, which triggers the opening of ion channels and leads to changes in intracellular ionic concentrations, therefore leading to neuron suppression or activation based on the type of channel responding. Examples of these ion channels are: transient receptor potential (TRP) channels, two-pore domain potassium channels (TREK), etc. [5-8].

The last hypothesis is the activation of neural tissue due to changes in local tissue temperature, hence changing the thermodynamics of signal propagation. High intensity focused ultrasound is currently used as a clinical therapy to create local brain lesions to ablate neural tissue by causing acute rise in local brain temperature. Thus, heating effects and damage to neural tissue need to be carefully monitored [5]. The FDA guideline of maximum temperature rise in tissue for diagnostic imaging is around 1.5 degrees Celsius.

The feasibility of ultrasound modulated neural activity has been demonstrated by the seminal work by the Fry brothers as early as the 1960s [9]. In the past decade, due to advancements in neuroscience and ultrasound technology, ultrasound neuromodulation has reemerged as a leading field in noninvasive neuromodulation. Recent work has found strong *in vivo* responses to low frequency tFUS at safe intensity levels to directly stimulate

and modulate cortical or subcortical neural activity [10]. tFUS has been shown to have success in suppression of epileptic seizures [11]. Many groups have achieved success using fMRI-guided tFUS in human subjects to improve cognitive function or activations similar with somatosensory evoked potential [12]. In addition, many groups have used tFUS to elicit motor cortex evoked movements in mice, rats, rabbits, and sheep [13-15]. Although tFUS stimulation has been able to observe consistent and location specific activation of all limbs, studies in rats have not yet shown reliable motor evoked responses [16-18]. The inconsistency in results suggest the presence of state dependent evoked responses and the need for parameter space optimization.

What remains unexplored in the field is the use of tFUS to encode temporal information into the brain in order to change synaptic plasticity in vivo. With the wide parameter space and penetration depth of tFUS, tFUS is an optimal stimulation technique for temporal encoding in the hippocampus.

Ultrasound Interactions with Tissue

In order to study the interactions between ultrasound and the neural tissue, we introduce metrics to quantify the ultrasound energy and transmission. Ultrasound waves are sound/pressure waves at frequencies above the human audible spectrum, greater than 20 kHz. Ultrasound waveforms can be characterized by several parameters: fundamental frequency, intensity, pulse waveforms, and pulse repetition frequency. The frequency and intensity of the ultrasound waves determine how much energy is delivered through the skull and into the brain. According to some computational models, only the pulse repetition frequency determined whether tFUS stimulates or suppresses neural activity; however, there is limited experimental data to validate this model [3].

Many studies have demonstrated ultrasound's ability to reversibly excite or suppress neural tissue [9, 19, 20]. The challenge remains in focusing the ultrasound wave through the skull. Transmission of ultrasound is mainly affected by frequency of the ultrasound wave (f), the ultrasound wavelength (λ), the incidence angle between the waves and the surface (θ_i), the speed of sound of the medium (c), the density of medium (ρ), and the attenuation of the tissue (α). The ratio of ultrasound intensity transmitted to the desired brain region of interest (T) can be modeled by the equations below [21]:

$$T = \left[\frac{t1 \cdot t2 \cdot e^{\frac{i2\pi}{\lambda}}}{\left(1 + r1 \cdot r2 \cdot e^{\frac{i4\pi}{\lambda}}\right)} \cdot e^{-2\alpha l} \right]^2 \quad \text{Eq (1)}$$

Where t1, t2 are the transmission coefficients of the ultrasound transducer and the skull, r1, r2 are the reflection coefficients for these layers, and l is the distance between the transducer and target. The results of the model is shown in Figure 1a. Simply stated, there exists an ultrasound frequency that has optimal transmission through a rat skull at a specific thickness, 1mm. Equation (1) assumes ultrasound waves are harmonic plane waves at normal incidence. The local maxima are located at multiples of the quarter wavelength ($l = n \cdot \frac{\lambda}{4}$) where n = all integers. However, as the ultrasound frequency increase, the attenuation of the ultrasound intensity increases exponentially. The attenuation in skull bone is given by $\alpha = \alpha_0 \cdot f^{2.1}$, where α_0 is an temperature dependent factor [21].

Similarly, the incident angle of the ultrasound with respect to the skull surface determines the characteristic of ultrasound waves that propagate into the brain. Figure 1b. illustrates the transmission coefficient of the ultrasound based on incident angle. The objective is to maximize longitudinal waves and minimize transverse waves since transverse waves will be trapped within the skull layers and dissipated quickly. However,

for this experiment we are employing a single concave focused ultrasound transducer which emits ultrasound waves with a multitude of incident angles, as shown in Figure 1C. Therefore, for precise measurement of transmitted ultrasound intensity, an ex-vivo skull scanning system has been developed for intensity quantifications.

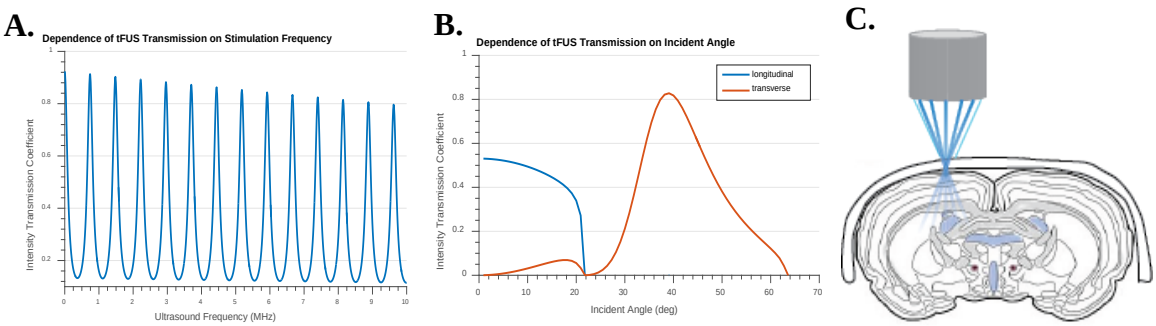

Figure 1. Modeling of ultrasound transmission through the rat skull at different stimulation frequencies and incident angles. **A.** Effect of plane wave ultrasound intrinsic frequency on intensity transmission coefficient. **B.** Effect of plane wave ultrasound incident angle on intensity transmission coefficient. **C.** Illustration on focused ultrasound incident angles.

Currently, the FDA has no specific guidelines of acoustic exposure for tFUS neuromodulation. Thus, the acoustic exposure guidelines for ultrasound imaging is used as a comparable limit. The limits set are 720 mW/cm^2 spatial peak temporal average intensity (I_{SPTA}) and 190 W/cm^2 spatial peak pulse average intensity (I_{SPPA}) [22]. All exposure levels under these guidelines are deemed safe for human use. These intensities can be calculated by the equations (2) and (3) [23].

$$I_{SPTA} = \frac{I_{TA}}{A} \cdot \frac{I_{SP}}{I_{SA}} \qquad \text{Eq (2)}$$

$$I_{SPPA} = \frac{I_{TA}}{A} \cdot \frac{I_{SP}}{I_{SA}} \cdot \frac{1}{duty\ factor} \qquad \text{Eq (3)}$$

The intensity measurements are shown in Figure 2. In addition to intensity levels, the mechanical index (MI) and thermal index (TI) are used to quantify the potential tissue damage due to mechanical or thermal heating effects. Shown in equation (4), the MI limits mechanical damage due to cavitation effects, primarily determined by the spatial-

peak/temporal-peak negative pressure of the attenuated rarefaction pressure (p_r) and frequency of ultrasound (f). The constant C_{MI} is 1 $MPa/MHz^{1/2}$ in order to make MI dimensionless [24].

$$MI = \frac{p_r(MPa)}{C_{MI}\sqrt{f(MHz)}} \quad \text{Eq (4)}$$

$$TI = \frac{W_p}{W_{deg}} \quad \text{Eq (5)}$$

TI is determined based on the ratio between the attenuated output power (W_p), and the ultrasonic power required to raise the target tissue by 1˚C (W_{deg}). The FDA safety limits for MI is 1.9 and, in the presence of bone, the near field TI limit is 3 for transcranial imaging [23]. Throughout this proposal, all intensity values are kept within the FDA limits in order to ensure safety of subjects and smooth transition into clinical approach.

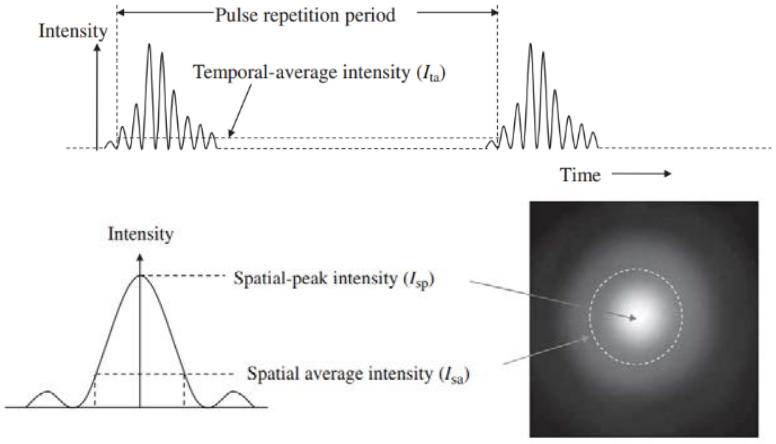

Figure 2. Graphical representation of time averaged intensity (I_{TA}), spatial peak intensity (I_{SP}) and spatial average intensity (I_{SA}) [1].

Long-Term Synaptic Changes

Long-term potentiation (LTP) and depression (LTD) has been widely studied in neuroscience as a mechanism of memory storage and emotion processing in the hippocampus [25]. LTP Literature in LTP has been shown that when a brief tetanic stimulation is applied at the Schaffer collaterals an increase in the EPSP amplitudes is

seen in the CA1 pyramidal cells, as well as through the medial performant path to the dentate gyrus [26-29]. This illustrates the immense ability for the brain to use frequency encoding in order to set up long term information storage.

The mechanism of action is mainly due to activation of *N*-methyl-d-aspartate (NMDA)-type glutamate receptors. Early phase of LTP is mediated by AMPA-type glutamate receptors. The addition of AMPA receptors increase the strength of synaptic connection. Overtime more as the depolarization in the postsynaptic terminal become for negative, NMDA receptors become activated as a coincidence detector for the simultaneous activation of pre and postsynaptic neurons. NMDA receptors in turn activates a signaling cascade of transcriptions in the postsynaptic neuron [30].

Tetanus electrical stimulations are typically used to induce long-term synaptic changes in the hippocampus. Several trains of high frequency tetanus electrical stimulations applied at 50 to 100 Hz for 1 second can induce LTP by causing a strong increase in presynaptic EPSP enough to activate the NMDA receptor. Similarly, low frequency tetanus electrical stimulation is applied at 1-3Hz for several minutes in order to induce LTP. Here, the low frequency tetanus only leads to a modest increase in presynaptic EPSP, which is not enough to activate NMDA receptors but leads to prolonged increase in postsynaptic calcium concentration due to repetitive stimulation. The small increase in calcium concentration triggers LTD [31-33].

METHODS

Ultrasound Stimulation Setup

In this project, ultrasound is delivered with a single element ultrasound transducer (V389, Olympus, USA) at 500 kHz. A custom 3D printed collimator filled with ultrasound coupling gel is used to deliver tFUS to the rat skull, see Figure 3. The length of the collimator is designed to match the 55mm focal length of the transducer. The incident angle and collimator output diameter are both modular. The transducer is controlled by function generators to create tFUS waveforms (33220 A, Keysight Technologies, USA). The signals are then amplified with a high power amplifier (2100L, E&I RF Amplifier, USA) and delivered to the ultrasound transducer.

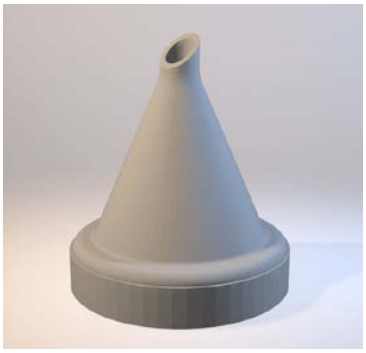

Figure 3. Design of tFUS collimator with 45 degrees incident angle.

Multiscale Recording System

In order to characterize neural responses to ultrasound neuromodulation, we need a holistic and wide scale method to examine the neural responses to tFUS. This investigation is made feasible by the multimodal neural recording system that our group has pioneered. We customized a high density 26-channel rodent scalp EEG cap which contains a window designed for the insertion of intracranial electrodes and tFUS stimulation. This setup allows us to introduce intracranial electrode arrays and tFUS focused at the target region while simultaneously recording scalp EEG recordings (Figure 4). This set up allows high resolution monitoring of the local and global brain state, especially in the cortical regions of the rodent brain. This multiscale system allows us to leverage on our lab's expertise on the analysis of EEG source imaging (ESI) [34-37]. The unique custom designed EEG cap allows for simultaneous recording of scalp EEG,

intracranial recordings, and tFUS stimulation in *in vivo* anesthetized rats, shown in Figure 4.

To gather recordings, rats are anesthetized with ketamine (75 mg/kg) and xylazine (10 mg/kg) cocktails in accordance to the University of Minnessota IACUC regulations. Rat scalps are prepared by shaving and applying hair removal gels over the entire scalp. Rats are place on stereotaxic frames with heart rate, temperature and toe pinch responses monitored at every 15-30 minutes. EEG caps electrodes are Ag/AgCl electrodes shaped to approximately 4 mm^2 in area, distributed evenly across the rat scalp. Electrode gel is applied under each electrode to improve electrical conductance with the shaved scalp. To insert intracranial electrodes, local craniotomies are conducted over calculated locations based on rat brain atlas and subject specific MRI's. Intracranial electrodes are inserted at 30-50 degrees angles in order to record from tFUS stimulation targets. NeuroNexus electrode arrays are used for intracranial recordings.

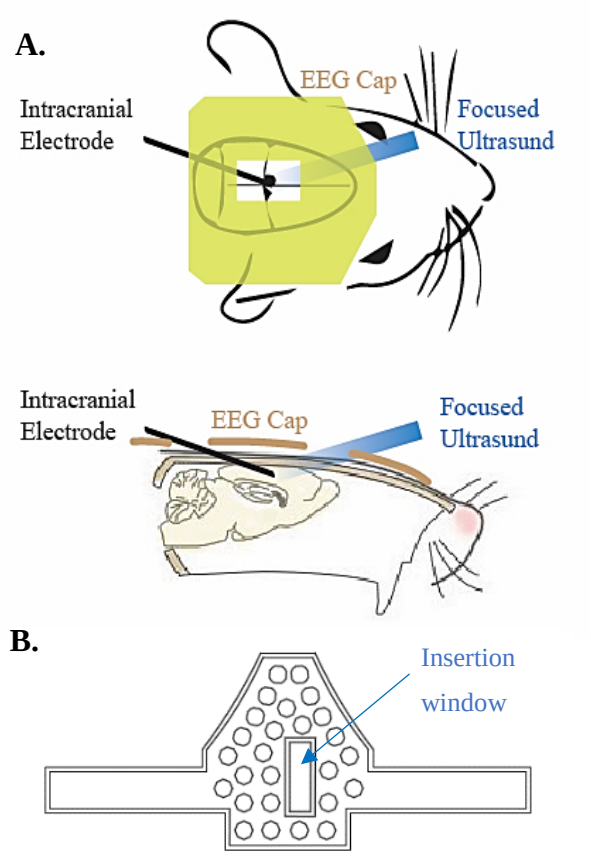

Figure 4. A. multimodal neural image system set up on rats. **B.** Electrode cap electrode configuration. An open window is designed for electrode and tFUS collimator insertion.

The goal of the multimodal recording system is to cross-validate the neural activations evoked by tFUS stimulation. Our modality allows us to gather simultaneous 26

channel global EEG, local field potential (LFP), and neural spikes in anesthetized rodents. We have been able to consistently detect acute neural activations due to tFUS stimulation in both the motor and somatosensory cortices of rat brains. Subject specific CT and MRI images are used to co-register skull thicknesses and brain shapes, Figure 5. The EEG information presents high temporal resolution information on the global neural network, identification of the topography of areas that have elevated activation compared to baseline levels, and global field powers quantifies the amount of total activity in the field. However, EEG alone is difficult to quantify the neural activation patterns of tFUS, mainly due to the low spatial specificity nature of EEG.

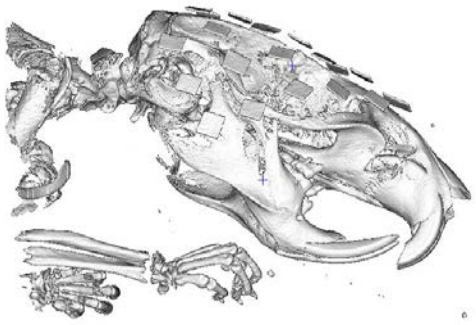

Figure 5. CT image of rat skull with distributed EEG electrodes.

Sample Data and Data Processing

Figures 6C and D are example recordings from tFUS stimulation target site in the local neural environment 1 mm below cortex. LFP are extracted from the 1-300 Hz frequency band, and spikes are recorded from the 300-6000 Hz frequency range. Electrical noise is notch filtered at 60 Hz. Since our recording arrays are capable of multi-unit recordings, offline spike sorting is important to separate single unit spikes in each electrode channel. Features such as spike amplitude, shape and inter-spike intervals are used in the cluster identification and separated using principle component analysis. Spike sorting is performed offline using the standard deviation of noise as detection thresholds and clustered using a k-means cluster analysis and principle component analysis. We observed significant rises in LFP amplitude and spiking frequency when compared to sham conditions. The post stimulus time histograms (PSTH) is constructed by the

alignment of multiple trials of tFUS stimulation to the onset of tFUS at time of 0 seconds. Figure 6C illustrate increases in neuron spiking time locked to the tFUS stimulation duration, while sham conditions only observe tonic, spontaneous firing. Interestingly, raster plot of spikes during tFUS stimulation shows initial trials firing at tonic spiking patterns, which reorganizes and aligns to the tFUS stimulation duration overtime.

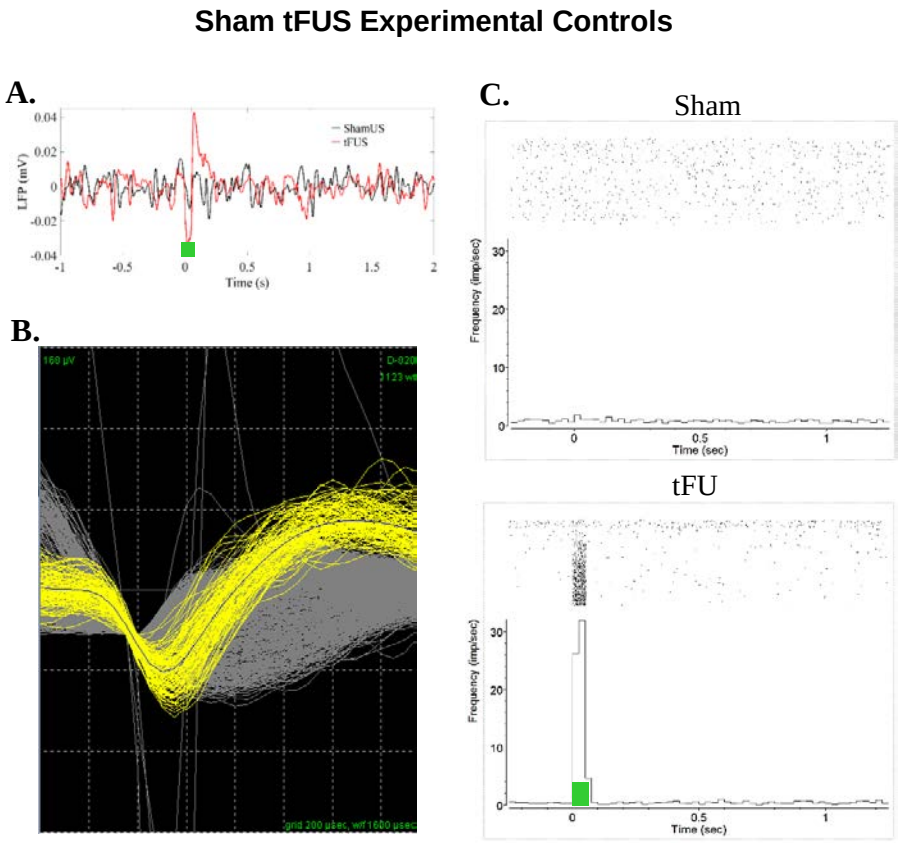

Figure 6. Example intracranial recordings from rat somatosensory cortex. **A.** LFP recording with tFUS and under sham conditions. **B.** An example of sorted spike recorded in one channel of electrode array. **C.** Neural spike raster plot and post stimulus time histograms. Raster plots are arranged in order of time progression of each trial, first to last trial ordered from top to bottom. Green bars represent tFUS stimulation, n = 300 trials.

In order to study the short term effects of tFUS, stimulation procedures were randomly shuffled to prevent the cumulative effects of long-term exposure to tFUS. The baseline neural activity will be recorded before any tFUS stimulation and used as a

standard to compare with sham conditions. Many different sham conditions were applied to eliminate possible confounding factors that can lead to neural activation. Sham tFUS conditions was applied with the ultrasound transducer turned 180 degrees away from the stimulation site. With no ultrasound waves directed into the skull, the neural activities recorded is anticipated to be only due to spontaneous activities and the animal's auditory percept of the ultrasound. Note, although the fundamental frequencies of the ultrasound is on the orders of MHz, the lower frequency PRF results in audible artifacts during the active segment of the ultrasound pulse. The sham condition was also compared to the baseline recording to determine significant difference and the degree of deviation between sham and a naive brain.

Another type of control procedure is done by applying tFUS on the scalp far away from the region of interest with the intracranial electrode. During this control, we anticipate on observing activation in the EEG, but no significant activation in the intracranial electrode. This control will help eliminate the possibility of neural activation by vibrations that propagate in the skull due to the transverse ultrasound waves that are transmitted and trapped in the skull layer.

Ex vivo Skull Mapping

The spatial distribution of ultrasound waveforms after transmission through the skull is difficult to model due to the inhomogeneity of the rat skull density and speed of sound as well as the aperture of the skull. There has been evidence of tFUS penetration into deep brain regions in rodents [13, 38]. In order to examine whether our tFUS stimulation is able to reach our targets, we have developed a three-dimensional ex-vivo pressure mapping system that uses a water submerged needle hydrophone (HNR500, Onda, USA) to map out the spatial-temporal pressure profiles of ultrasound transmitted

through an excised skull. The results of the ex-vivo intensity mapping can be matched with the corresponding electrophysiology data to attain a better understanding of the brain responses to tFUS.

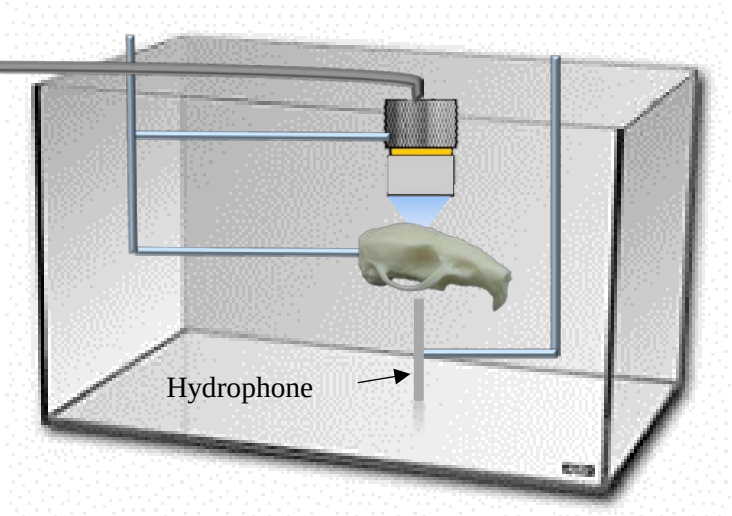

Figure 7. *Ex vivo* skull scanning system. tFUS is applied to the *ex vivo* skull, submerged in a water tank. A needle hydrophone on a 3D motor is used to scan the ultrasound intensities distributed through the skull.

To characterize the ultrasound intensity values and focal region, a needle hydrophone was placed beneath an ex-vivo skull and recorded ultrasound pressure values at discrete locations along the skull. Skulls were dissected from euthanized animals, fixed in formalin and preserved in ethanol before used in ex vivo scans. Scans are reformed within 2 weeks of skull extraction in order to prevent prolonged degradation overtime that can change the acoustic properties of the skull. This set up allows us to quantify the amount of energy delivered to the brain, which varies vastly due to the inhomogeneity and aperture of the skull. As seen in Figure 7, the system can be set up to mimic the exact conditions of the ultrasound set up. tFUS is delivered through a collimator to the target skull location. The values received from the hydrophone is used to create a pressure map of the ultrasound pressures that are transmitted across the skull.

Long-Term Depression Setup

In addition to our multiscale neural recordings, we have also applied intracranial electrical stimulation to induce excitatory post synaptic potentials (EPSP). Rats are anesthetized with ketamine (75 mg/kg) and xylazine (10 mg/kg) cocktails through intraperitoneal injections. Rats are skin prepped and placed on stereotaxic frames with heart rate, temperature and toe pinch responses monitored at every 15-30 minutes. Two burr holes were drilled at the surface of the skull according to atlas locations. As seen in Figure 8, animals were implanted with acute stimulating electrodes in the medial perforant path (mPP), containing the presynaptic input from the entorino cortex to the dentate gyrus (DG) [26, 39].

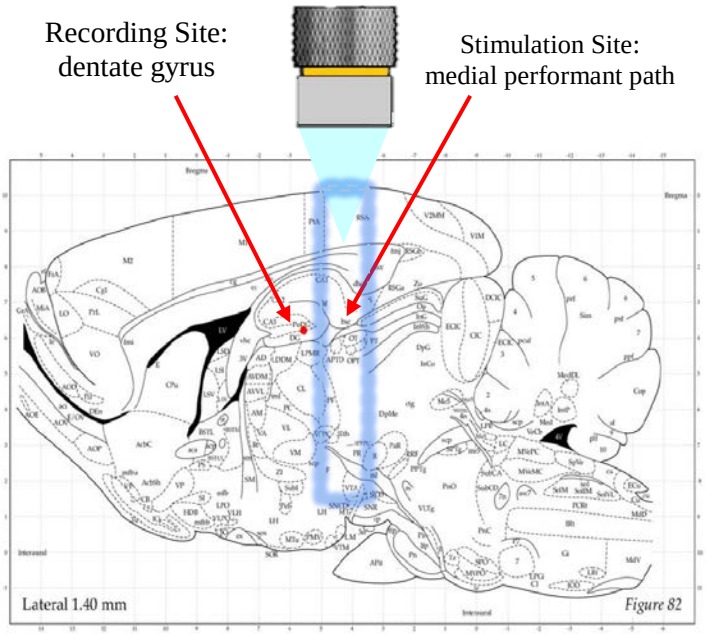

Figure 8. Long-term synaptic connectivity stimulation setup. Stimulation and recording electrode positions overlaid on rat brain atlas. Blue shaded regions denote approximate ultrasound beam location.

A 32-channel recording electrode microarray was inserted at the post synaptic dentate gyrus (DG). Single subthreshold monopolar pulses were delivered at the DG, resulting field excitatory post-synaptic potentials (fEPSP) were recorded at the DG. fEPSP's are filtered from 1-300 Hz. After baseline fEPSP was recorded, tFUS stimulation targeting at mPP was administered through the intact scalp and skull. Total of 5 to 10 minutes of tFUS at fundamental frequency of 0.5 MHz was delivered through the intact rat

skull, stimulation waveforms shown in Figure 9. tFUS is pulsed at two frequencies. tFUS intrinsic frequency is delivered at 0.5MHz to ensure penetration of rat skull and limit stimulation area. The intrinc frequency is pulsed by a 3-10kHz pulse repetition frequency (PRF). This range is selected based on literature values [3,4,11-17]. Since many studies how activation or inhibition at these values, each burst of tFUS applied at 3-10 kHz PRFs is hypothesized to be equivalent of a single pulse in a train of tetanic electrical stimulation. Then, these bursts of tFUS delivered at 3-10 kHz is delivered at 50-100Hz, frequency encoding used for high frequency tetanic stimulation. For this work, PRF will only refer to the 3-10kHz range pulse frequency. The applied ultrasound intensity is under FDA standards (700 mW/cm^2). fEPSP was recorded immediately after cessation of tFUS stimulation to compare to baseline.

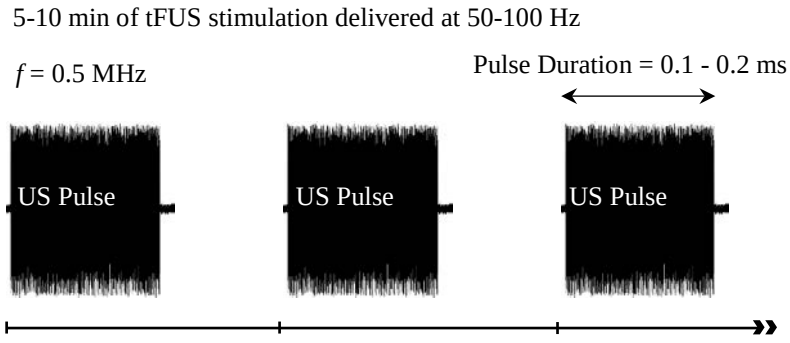

Figure 9. Stimulation parameters for eliciting LTP.

RESULTS

Effect of tFUS on Acute Brain Responses

Ultrasound spatial profile can be tuned to achieve its axial specificity. Single element focused ultrasound transducers are widely used by the ultrasound neuromodulation community. We have also been using such transducers featured with a variety of fundamental frequencies. However, this type of transducers failed to provide a high axial resolution. Pioneers in the community take advantage of this feature to pursue a deep-brain stimulation. In our first study, we are researching to control the sonication depth by steering the incidence angle of the ultrasound beam transmitting through ultrasound collimators. For this reason, we employ 3D printing technique to design and fabricate the customized collimators with different tip angles. The 3D ex vivo skull scanning system is used to evaluate their performance and test whether this collimator technique can be used to practically control the sonication depth. We found that tFUS delivered at a 40 degrees incident angle is able to restrict itself within a shallower area behind the skull than that produced by normal incident ultrasound (see Fig. 10), which would be helpful if only cortical regions are to be targeted in small animals. However, the transcranial ultrasound starts to become disperse, and its pressure/ intensity decreases

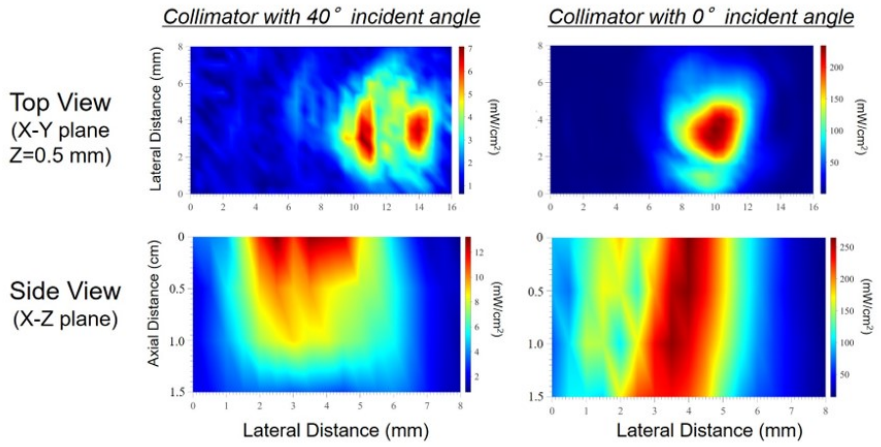

Figure 10. A comparison of ultrasound spatial profiles at different incident angles.

significantly due to the change of wave mode through the skull while maintaining the transmitted ultrasound parameters constant.

In fact, angled incident tFUS is a popular setup among several research groups in the community. The major reason is a compromise with the neural signal detection regime, like *in vivo* optical imaging method. In our multi-scale electrophysiological framework, we are able to avoid such compromise and directly compare the results due to the change of ultrasound incident angles. From our recorded local field potentials, we can evaluate this comparison (presented in Fig. 11). From this initial result, both incident tFUS present a significant acoustically evoked potential comparing to a sham ultrasound condition by flipping ultrasound transducer away from the scalp and a baseline one with ultrasound being shut off. Moreover, the normal incident ultrasound leads to a doubled magnitude comparing to the 40-deg incident case, which indicates a high efficiency of normal incidence in preserving the longitudinal wave mode for relatively higher ultrasound pressure and intensity. Deep brain structures may be also modulated by the normal incident ultrasound. These LFPs are all recorded at the primary somatosensory cortical region.

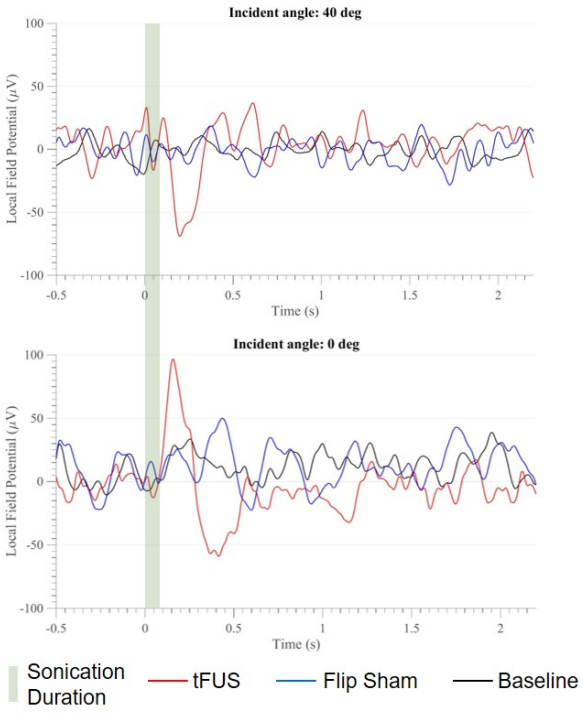

Figure 11. A comparison of local field potentials of different ultrasound incident angles.

To examine the global effect of tFUS on the rat brain, we also examined source imaging patterns elicited by tFUS. Figure 12A and B are butterfly plots and EEG source images illustrating rises in mean global field potentials 15 ms after tFUS onset and continues until tFUS offset. Source images show initial local activation at the target site, which propagates over time to residual areas such as the auditory cortex.

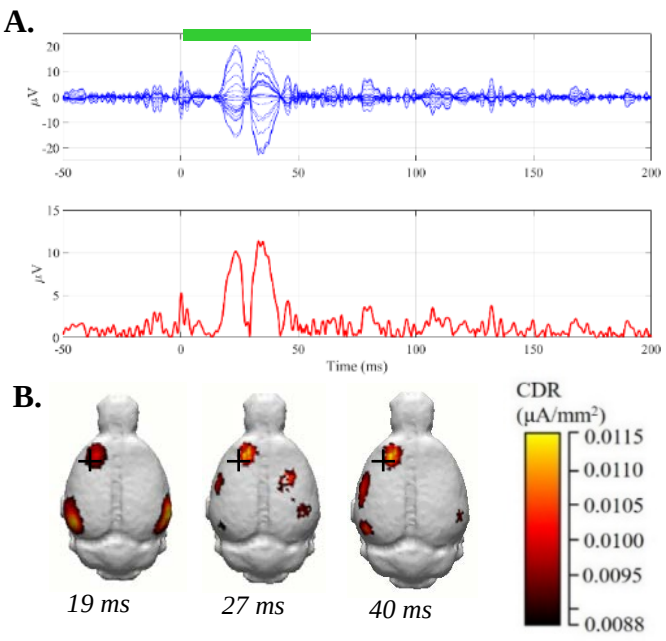

Figure 12. 26-channel rat scalp EEG recording results. **A.** EEG butterfly plot and mean global field potential. **B.** ESI of elicited by tFUS. Green bar represents tFUS stimulation, n = 300 trials.

To better understand the spiking patterns seen in intracranial electrode, histology is performed to characterize the location of the intracranial electrode, shown in Figure 13B. Based on the hematoxylin and eosin stain, the electrode appears to be recording from the stellate neurons in the somatosensory cortex, layer IV.

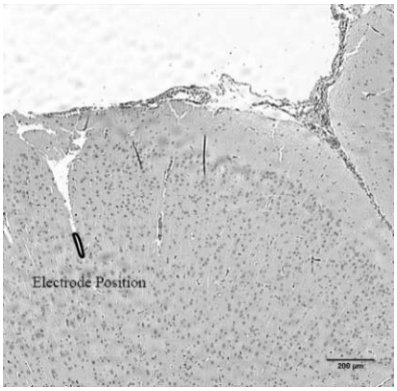

Figure 13. Fixed tissue staining showing intracranial electrode positions.

Effect of tFUS on Long Term Synaptic Plasticity

In this pilot study, we have observed the induction of LTD using pulsed tFUS. The main goal of this set of experiments is to investigate the ability to elicit long-term changes in synaptic strengths. We applied pulsed tFUS stimulation at 50-100 Hz stimulation in order to mimic the effects of long-term potentiation (LTP) in the dentate gyrus. In five out of rats, long term depression (LTD) was observed when tFUS stimulation is applied with PRF of 3-10KHz. LTD is observed to persist 30 min after stimulation. As seen in the Figure 15, fEPSP slope significantly decreases after tFUS stimulation and returns toward baseline overtime. After all tFUS experiments, LTD is elicited using low frequency (1 Hz) electrical tetanus stimulation to validate the correct localization of neural pathway. Sham experiments have been performed to verify whether LTD still occurs without the presence of tFUS. Figure 14 shows no LTD induction without direct tFUS stimulation.

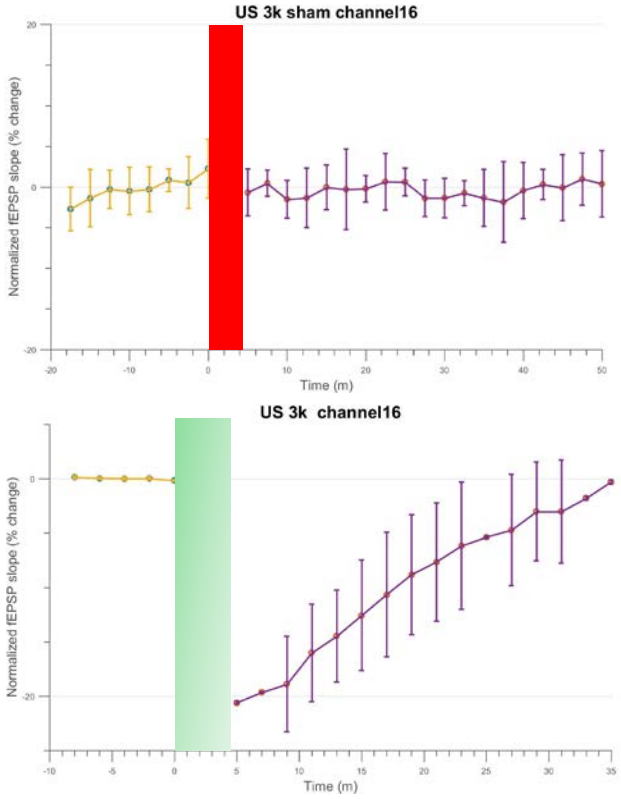

Figure 14. Comparison between sham and tFUS stimulation. Sham tFUS delivered by flipping ultrasound transducer 180 degrees away from skull. All other procedures held constant. Red bar denotes application of sham ultrasound, green bar denotes application of tFUS, both for a duration of 5 minutes.

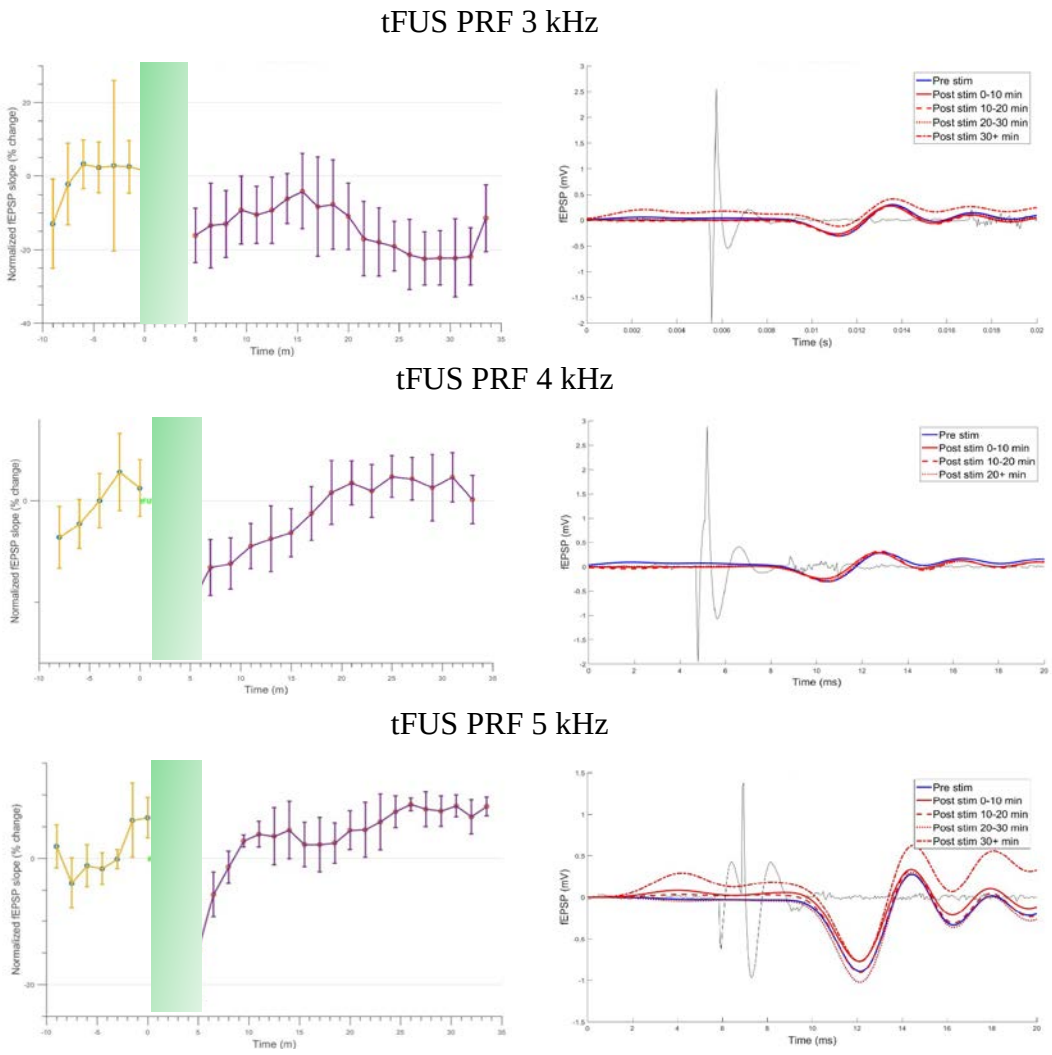

Figure 15. Preliminary results of LTD induced by pulsed tFUS at different PRF. Stimulation at mPP, recording from DG. Left plot, yellow data points indicate baseline fEPSP slope, purple data points indicate fEPSP after tFUS stimulation. Right plot shows the averaged field potentials before (blue) and after (red) tFUS stimulation, black lines show the fEPSP stimulation artifact. Error bars indicate standard deviation of the data across 240 trials. All data shown are from the same recording channel.

Based on our hypothesis, we expected to observe LTP after tFUS stimulation since tFUS was applied at the same frequency as high frequency tetanic stimulation used throughout LTP literature [26, 39]. The observed results did not show LTP, suggesting that the temporal encoding using tFUS does not share the same efficiency and/or mechanism as electrical tetanus stimulation. However, the demonstrated long-term effect is a

promising new feature of tFUS stimulation to be employed as a potential non-invasive therapeutic neuromodulation technique. Our results suggest tFUS can be used to encode time dependent stimulation paradigms into neural networks and non-invasively elicit long-term changes in the strength of synaptic connections.

In order to investigate whether tFUS PRF has an effect on strength of LTD induction, we surveyed a range of PRFs from 3 to 10 kHz. Shown in Figure 16, change in PRF is significant between 3 kHz and 4 kHz and between 3kHz and 5 kHz based on one way ANOVA test with post hoc Tukey procedure to adjust for difference in sample size. Although there are statistical significance between the sample groups, overall we observed LTD across all sample groups. This phenomena suggests tFUS PRF is not correlated with LTP. We hypothesize the reason no LTP was observed could be due to several factors. First, our animals are anesthetized with ketamine and xylazine cocktails. Ketamine is a known NMDA receptor antagonist, therefore it could interfere with NMDA receptor dependent LTP, whereas LTD is not NMDA receptor independent. Furthermore, the location of stimulation could be another variable. If stimulation of tFUS is applied at the postsynaptic terminal, then we could be inducing spike timing dependent plasticity by increasing postsynaptic firing rate, which can result in depression of synaptic connectivity.

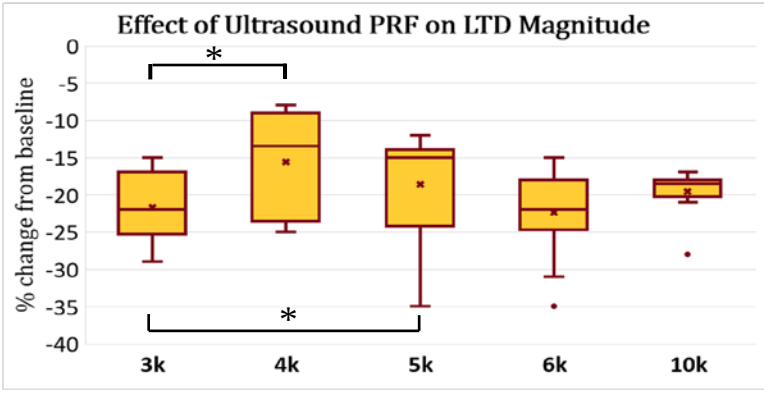

Figure 16. Comparison between sham and tFUS stimulation. Sham tFUS delivered by flipping ultrasound transducer 180 degrees away from skull. All other procedures held constant.

CONCLUSIONS AND FUTURE DIRECTIONS

In this study we first examined the ability of tFUS to elicit reliable acute brain responses. We find that our tFUS stimulation elicits distinct brain responses from sham conditions. Future work will verify that the signals recorded in our system are due to neural activities rather than mechanical movements by applying chemical ion channel blockers to block action potentials. Tetrodotoxin (TTX), is a selective sodium channel blocker that prevents the inflow of sodium into the neuron, hence blocking the initiation of action potentials [40]. Under TTX, we expect to observe no significant neural activation in the local region of injection, signals observed in this immediate area will be attributed to artifacts from mechanical vibrations of tFUS.

After establishing our setup, we applied tFUS to encode temporal information into the rat hippocampus in vivo. Further investigations are needed to explore the mechanisms of action in this phenomenon. To our knowledge, this is the first *in vivo* neuronal evidence that demonstrates the long-term neuromodulatory effects using tFUS. Future work will be conducted on adolescent rat subjects under a different type of anesthetic agent (isoflurane) in order to verify results; further histological evidences such as image comparisons of dendritic spines will also be pursued.

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