High-resolution, focused ultrasound-mediated neuromodulation and detailed analysis of electromyography characteristics reveals a high degree of spatial specificity in elicited responses in mice *in vivo*

Christian Aurup[1], Hermes A. S. Kamimura[1], and Elisa E. Konofagou[1, 2]
Department of Biomedical Engineering[1], Radiology[2]
Columbia University
New York, NY, USA
Email: ek2191@columbia.edu

***Abstract*— Focused ultrasound (FUS) has demonstrated the ability to modulate nervous system activity in animals. Recently, higher frequency focused transducers have been used to successfully elicit motor responses in mice in vivo. Despite this success, quantitative analyses of the temporal and magnitude-related characteristics of FUS elicited motor responses measured with electromyography (EMG) and the spatial specificity of those responses have been poorly described. In this study, we use a focused transducer with high spatial resolution (1mm lateral focal diameter) and EMG to evaluate six quantitative EMG response characteristics. Results demonstrate that these responses are highly specific to the brain volume acted upon by the FUS.**

Keywords—focused ultrasound, neuromodulation, mice

I. Introduction

The targeted modulation of brain activity is a powerful tool for the treatment and research of neurological disorders. Ultrasound has been shown to modulate activity in excitable tissue for many decades [1, 2] but resurfaced only recently when ultrasound-induced neuronal activation was shown in ex vivo brain slices using fluorescence imaging [3]. Ultrasound has since been successful in eliciting a variety of responses in many animal models including rabbits [4], rats [5], non-human primates [6], sheep [7], and humans [8, 9]. Ultrasonic neuromodulation has demonstrated extensive success in rodents. However, rodents have innately small brains relative to the size of the acoustic foci used most prior studies [10, 11, 12]. It was therefore a necessary progression to tailor ultrasound properties to improve spatial resolution to millimeter resolution [13, 14, 15]. Most rodent studies utilized electromyography (EMG) to evaluate the efficacy of FUS neuromodulation in generating motor responses but were limited to a subset of EMG characteristics including response latency, duration, or success rate while other studies used less quantitative metrics like video analysis. Importantly, ultrasound can elicit motor responses following sonication of the motor cortex [10, 12, 14] and posterior regions including the somatosensory cortex [13, 14, 15], however the differences between such responses have not yet been adequately quantified.

The limited number of motor response characteristics quantified in literature informed the current investigation aimed at determining whether the spatial-specificity of such responses applies to additional temporal and magnitude-related characteristics. Adding dimensions to the array of EMG quantifications improves the ability to classify differences between motor responses that are not clear when evaluating only the latency and duration of responses. Additionally, identifying how distinct brain regions respond differentially to ultrasound provides more evidence for understanding the underlying mechanisms of [16] and the possible regional sensitivity [17] to ultrasonic neuromodulation. In this study, we use a 2 MHz FUS transducer with high focal resolution (1mm lateral diamter) and four-limb EMG to quantify six different motor response characteristics and evaluate how their expression manifests depending on the brain region and exact neural volume targeted by the focused ultrasound.

II. Experimental setup

All animal procedures in this experiment were reviewed and approved by the Institutional Animal Care and Use Committee of Columbia University. Wild-type mice (C57BL-6) were anesthetized with an intraperitoneal injection of pentobarbital sodium (65 mg/kg). The hair was removed from the scalp and limbs using an electric razor and depilatory cream. The subject was mounted in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) and placed on an electric heating pad to maintain body temperature. An pulseoximeter (MouseOx Plus, Starr Life Sciences Corp., Torrington, CT, USA) was placed on the thigh and used to determine level of anesthesia. Bipolar EMG electrodes were placed in each limb. The EMG and pulseoximetry signals were acquired with an acquisition board (MP150, Biopac Systems, Inc. Santa Barbara, CA, USA). Degassed acoustically transparent gel was placed on the scalp.

A single-element FUS transducer was driven by a function generator with its signal amplified by a linear RF amplifier. The transducer casing had an attached coupling cone filled with degassed water. The transducer was acoustically coupled to the mouse head using a water tank. Three sonication regions (Figure 1), each consisting of eight targets, were chosen from spatially separate areas of the cortex. Each target was sonicated 10 times, each for a duration of 300ms with an interstimulus interval of 5 seconds to mitigate thermal accumulation. The 1.94 MHz focused ultrasound transducer used for neuromodulation was driven at 2 MHz. Previous work

demonstrated that using a 1 kHz pulse repetition frequency, 50% duty cycle, and an acoustic intensity of 97 W/cm2 (1.76 MPa peak negative pressure) could elicit robust motor responses. These acoustic parameters were used throughout the study.

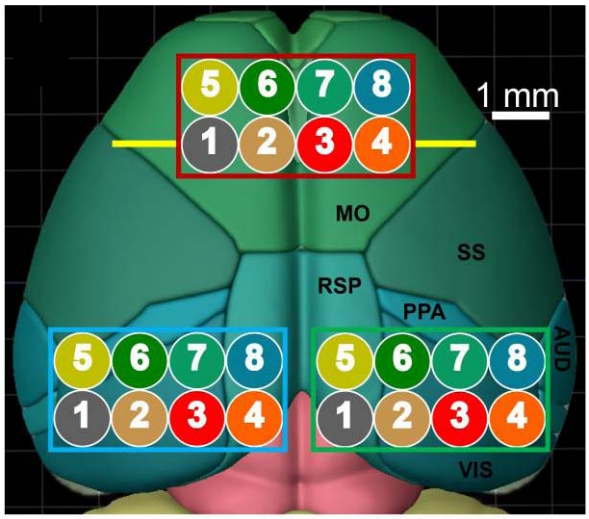

Figure 1: The three sonicated regions are shown (motor region in red, left posterior region in blue, and right posterior in green), each with 8 non-overlapping brain targets. MO: motor cortex, RSP: retrosplenial cortex, SS: somatosensory cortex, PPA: posterior parietal association area, VIS: visual cortex, AUD: auditory cortex.

Bipolar EMG electrodes were made in-house using the procedure outlined by Tufail et al. 2011 using 0.0018" PTFE insulated stainless steel wire (California Fine Wire, Grover Beach, CA). The electrodes were implanted into the tripceps brachii and biceps femoris in the forelimbs and hind limbs, respectively. Ground reference electrodes were implanted underneath the skin of the back. Signals were sent through EMG specific amplifier modules (EMG100C in hind limbs and EMG2-R in forelimbs, Biopac Systems, Inc., Goleta, CA) with bandpass filtering between 500 Hz and 5 kHz with 2000x gain.

The temporal EMG characteristics evaluated are the response latency, response duration, the time until the signal reached 50% of its total energy, and the time until the signal energy reaches its maximum. Response latency is defined as the time between ultrasound stimulus onset and the time at which the EMG signal envelope crosses the set threshold. The response duration is the total time the signal is above the threshold during the 3 second epoch following FUS application. The 50% energy time ($T_{50\%}$) is the time difference between the calculated latency and the time at which the signal reaches 50% of its total energy. The peak energy time is the elapsed time between the calculated latency and the signal envelope reaches its peak value.

Two magnitude-related characteristics are also determined. The total signal energy is calculated by taking the integral of the EMG signal envelope over the 3 second epoch following FUS application. The second measure of robustness is the peak magnitude of the EMG envelope following FUS application. Each magnitude characteristic value is normalized by the median value for a given region.

III. Results

The response latencies, durations, peak energy time, and $T_{50\%}$ were found to differ significantly among a set of eight regional targets in 76%, 80%, 48%, and 68% of the sonicated regions, respectively. Figure 2 provides a representative example demonstrating how the temporal components can vary within a region. Additionally, of the regions where ANOVA revealed differences among the targets, pairwise comparisons of response characteristics identified significant differences in at least 75% of those regions for all characteristics. The mean and median temporal characteristics are summarized for the hind and forelimbs in Table 1.

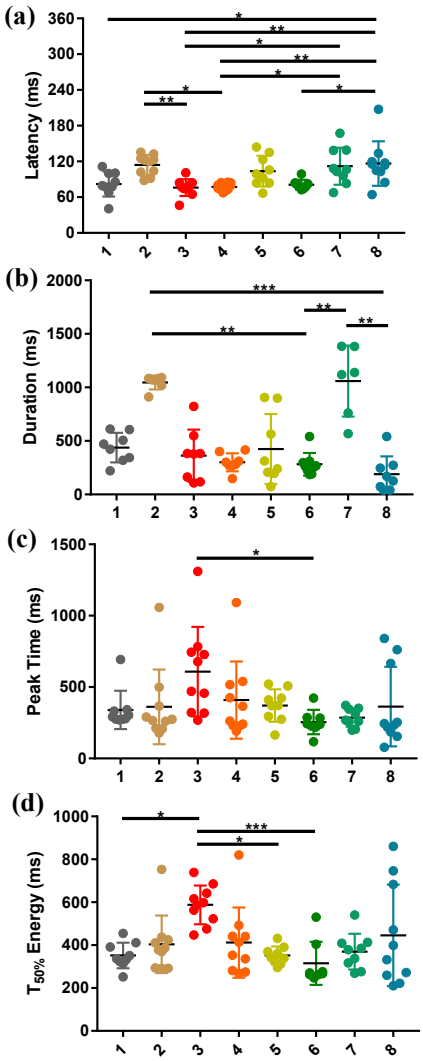

Figure 2: The temporal EMG characteristics recorded in mouse limbs are depicted. (a) The response latencies recorded in the left hindlimb following sonication of the the right posterior region are shown. (b) Evaluation of the response duration measured in the left forelimb following sonication of the motor region. (c) The $T_{50\%}$ times recorded in the right forelimb following sonication of the left posterior are depicted. (d) The peak energy times are shown for recordings from the right hind limb following sonicaiton of the right posterior region. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$)

The total signal energy metric revealed significant differences between regional targets in 84% of regions. The second magnitude-related characteristic evaluated was the peak energy, which yielded significant differences in 64% of regions.

Figure 3 shows representative results for the magnitude characteristics.

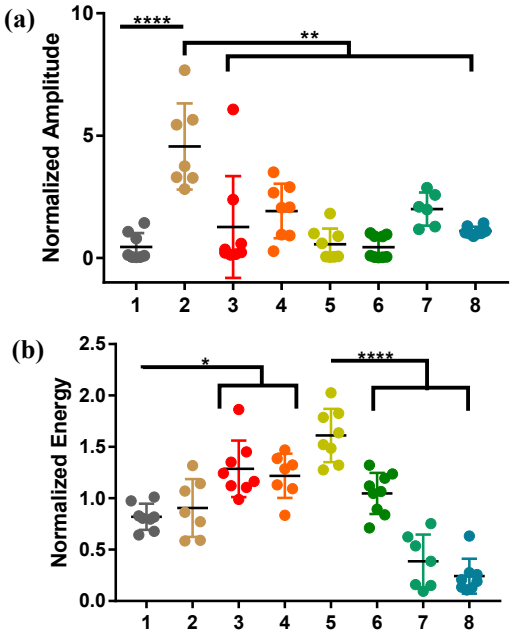

Figure 3: The results for the magnitude characteristics are depicted. Both the (a) peak energy (i.e. amplitude) and (b) signal energy were found to vary remarkably by sonicated brain targets. Both of these example results are from sonicating the right posterior region.

IV. Discussion

Focused ultrasound application to the mouse brain in vivo elicits motor responses detectable with intramuscular EMG. Motor responses can be generated by sonicating neural volumes at multiple brain targets. In this study, six characteristics of EMG responses were evaluated to determine the target specificity of FUS-elicited motor responses. Differences in both temporal and magnitude-related EMG characteristics could be identified between targets separated by as little as 1mm laterally. This result demonstrates the high degree of spatial-specificity of such motor responses. Each of these characteristics are useful in understanding the functioning of the underlying neural volumes.

The temporal characteristics from this study that have been previously evaluated are latency and duration (King 2013, King 2014, Li 2016). The results in this study are consistent with the latency and duration evaluations from these previous works although each study utilizes a different set of acoustic parameters, transducer geometries, and anesthesia types that may inform the subtle differences. The remaining characteristics have yet to be evaluated by other groups.

Most ultrasound-mediated neuromodulation studies in small animals treat sonicated targets as though they are well constrained to the cortex when this is certainly not the case due to the length of the acoustic foci in the axial dimension. The large neural volumes, often extending into the subcortex, exposed to ultrasound contain many neural circuits all with complex excitation-inhibition feedback mechanisms and unknown sensitivities to ultrasound. Additionally, standing waves from skull reverberations may result in localized pressure peaks outside of the predicted focus [18].

V. Conclusion

Focused ultrasound neuromodulation generates spatially specific motor responses. This was demonstrated via the evaluation of six temporal and magnitude-related EMG characteristics, all of which were able to identify response differences between neural volumes. The differences between these characteristics can provide important information on the functioning of the underlying neural circuitry and may be used for functional brain mapping with ultrasound. The observation of no cumulative effects from successive stimuli indicates the lack of a thermal mechanism driving activity. Future work needs to be performed in larger animals.

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