

# Pupil dilation and motor response elicitation by ultrasound neuromodulation

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**Abstract**— Focused ultrasound (FUS) neuromodulation has been previously proposed as a promising technique to drive neuronal activity. Here, we explored motor- and cognitive-related brain regions of mice by targeting specific brain structures using FUS neuromodulation in the mega-Hz range under a specific type of anesthesia. Contralateral motor responses were observed showing successful target specificity of the FUS neuromodulation achieved with 1.9 MHz. Higher acoustic pressures increased the success rate from 20% (at the threshold, 1.45 MPa) to 70% (1.79 MPa). The estimated latency measured by electromyography was  $266 \pm 37$  ms. Pupil dilation was observed when neuromodulating regions in the brain covering the superior colliculus and other anxiety-related structures such as hippocampus and locus coeruleus. This study demonstrated the capability of FUS to modulate target specific regions in the brain including pupil dilation induced by FUS for the first time. Furthermore, evoked responses by cognitive regions demonstrated the capability of FUS to modulate deeper structures in the brain.

**Keywords**— cognitive response, motor response, transcranial therapeutic ultrasound, ultrasound neuromodulation

## I. INTRODUCTION

Focused ultrasound (FUS) neuromodulation is a promising technique to drive neuronal activity. It has been demonstrated the activation of ion channels by pulsing ultrasound on a mice hippocampal slice in culture [1]. FUS neuromodulation has been presented as an alternative to overcome limitations of other techniques such as implantation of electrodes when using deep brain stimulation, the poor spatial resolution (~1cm), inadequate depth penetration and short lasting effects (milliseconds) of transcranial magnetic stimulation, and the gene modification required by optogenetics [2,3].

Bilateral muscular activation has been demonstrated when sonicating motor and somatosensory cortices with frequencies less than 690 kHz [4-8]. High-level cognitive behavior has been modulated in monkeys using 320 kHz [9]. The use of higher frequency produces higher focal spatial resolution, which has been proven capable of improving the target specificity [10]. However, consistent contralateral and ipsilateral muscular activation was not observed.

In addition to observing motor responses by activating the cortex, pupil dilation can be a parameter to track neuronal activation of structures localized in deeper regions in the brain. The iris muscles are directly innervated by brain structures associated with cognitive and emotional processing [11]. Thus, changes in pupil size are associated e.g. with mental activity [12,13] and anxiety [14].

Here, we investigated the activation of motor- and cognitive-related brain regions of mice using FUS neuromodulation. The objectives were to demonstrate target specificity of 1.9 MHz on the motor activation and the capability of FUS to activate deeper structures in the brain associated with cognitive responses.

## II. MATERIAL AND METHODS

### A. Animal preparation

All procedures with mice were approved and conducted in accordance with the Columbia University Institutional Animal Care and Use Committee. The experiments were performed in wild-type adult male mice (strain: C57BL/6, masses: 20-28 g; Harlan, Indianapolis, IN, USA) anesthetized with intraperitoneal (IP) injection of sodium pentobarbital (65 mg/kg). After IP injection, the animals were remained in the cage for 20 to 30 minutes before immobilization within a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). The fur on head and throat was removed. The heart and breathing rates were recorded during the sonication using a sensor positioned on the throat (MouseOx Plus, Starr Life Sciences Corp., Torrington, Connecticut, USA). The animal's body temperature was maintained at approximately 40 °C using a heating pad and oxygen at 0.5 L/min was continuously delivery during the whole procedure (SurgiVet, Smiths Medical PM, Inc., Waukesha, Wisconsin, USA).

### B. Ultrasound neuromodulation

The sonications were performed using a single-element FUS transducer driven by a function generator (33220A, Agilent Technologies, Palo Alto, CA, USA) through a 50 dB power amplifier (ENI Inc., Rochester, NY, USA). The transducer was fixed in a 3D positioning system (VXM,

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This study was supported in part by NIH (R01EB009041 and R01AG038961) and FAPESP (2011/10809-6 and 2013/08116-8). The opinions, assumptions, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of FAPESP.

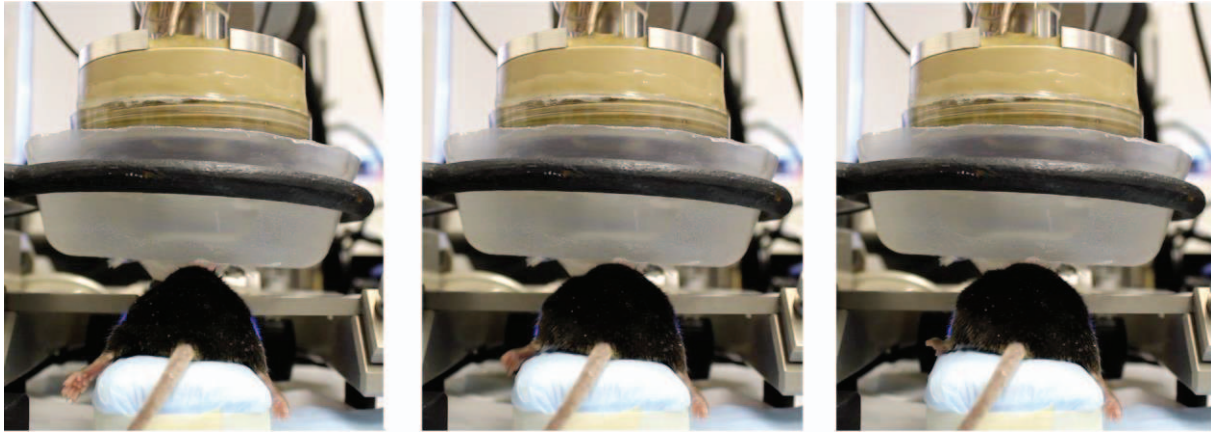


Fig. 1 – Video frames displaying contralateral movement of left hindlimb evoked by FUS neuromodulation at AP = +2 mm and ML = +2 mm.

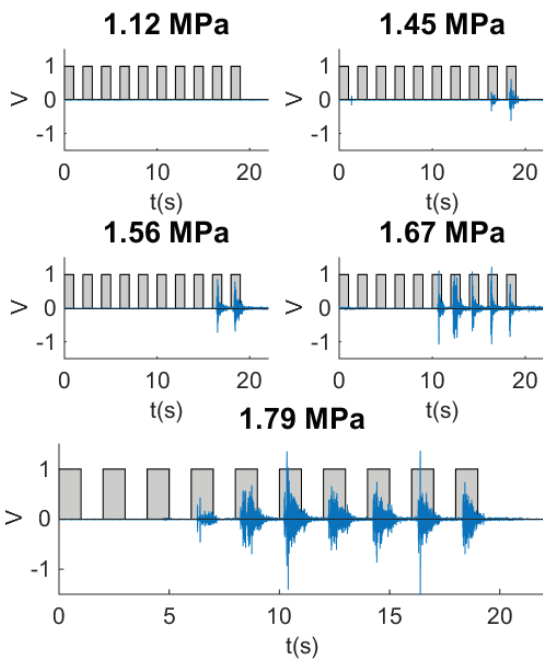


Fig. 2 – EMG of contralateral movement of left hindlimb evoked by FUS neuromodulation at AP = +2 mm and ML = +2 mm.

Velmex Inc, New York, USA) and acoustically coupled to the animal's head using a water container. The transducer was moved randomly within a scanning grid of 8 by 8 mm with a resolution of 1 mm, centered at anterior/posterior (AP) = - 2 mm and medial/lateral (ML) = 0 mm from lambda mice skull landmark. The sonications of motor- and cognitive-related brain regions were carried out at 1.9 MHz with 50% duty cycle, pulse repetition frequency of 1 kHz, pulse duration of 1s, interval interstimulus of 1s for 10 repetitions.

### C. Evoked responses monitoring

Muscle activity was recorded using electromyography (EMG) (BN-EMG2, Biopac Systems Inc., Santa Barbara, CA, USA) with 26-gauge electrodes placed 5-mm apart in the biceps femoris in both hindlimbs and the ground electrode on the tail. Hindlimbs and tail evoked motor responses were recorded on videos (EOS Rebel T3i, Canon, Melville, NY, USA). Eye movements and pupil dilation were recorded by a high-resolution camera focused at the right eye of the mice (DMK 23U618, The Imaging Source, Bremen, Germany).

### D. Safety evaluation

Five mice were sonicated with 1.93 MPa at AP = +2 mm and ML = +2 mm and with 3.0 MPa at AP = +2 mm and ML = -2 mm. After sonication, the mice were transcardially perfused and fixed in 4% paraformaldehyde. The brains were harvested, paraffin embedded, and sectioned at 6- $\mu$ m thickness with 180- $\mu$ m distance between sections. The sections were stained with Hematoxylin and Eosin (H&E) and evaluated in a light microscope searching for red blood cell extravasation and cell or tissue loss.

## III. RESULTS

Motor responses including paws and tail movements were recorded on videos synchronized with the FUS. Fig. 1 shows contralateral movement of left hindlimb evoked by FUS neurostimulation at AP = +2 mm and ML = +2 mm. Contralateral muscle activities were recorded on EMG for sonications carried out at both sides at AP= +2 mm and ML=  $\pm$ 2 mm. Fig. 2 shows EMG signals for contralateral evoked response of the left hindlimb obtained for sonications at different pressure levels performed on the right hemisphere. The minimum pressure to elicit movements was 1.45 MPa (calibrated using an excised skull). Higher pressures increased the success rate from 20% (at the threshold, 1.45 MPa) to 70% (1.79 MPa). The estimated latency was  $266 \pm 37$  ms. Ipsilateral movements were observed in other regions of the sensory cortex that varied among animals.

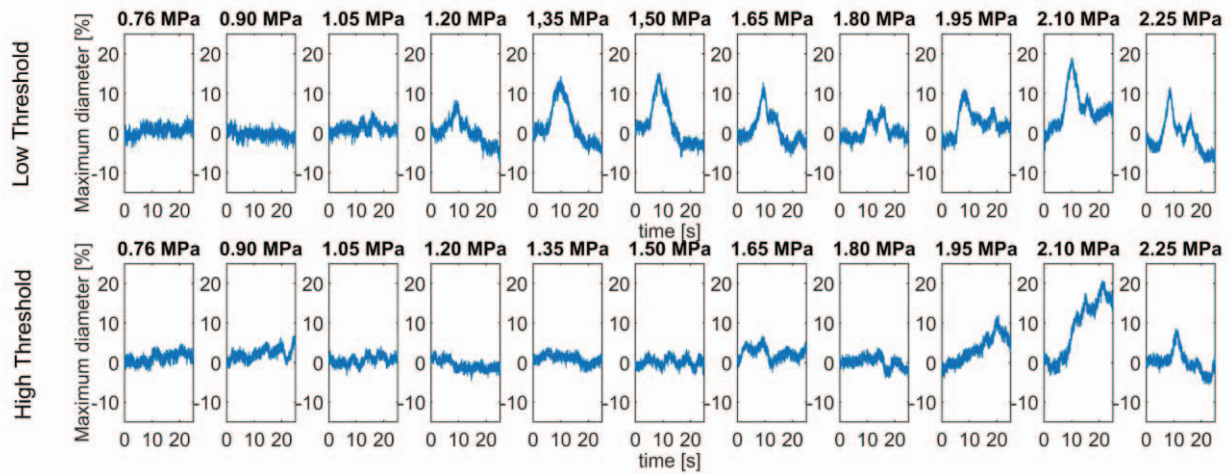


Fig. 3 – Pupil dilation evoked by FUS neurostimulation at different acoustic pressure levels. (Top) Pupil dilation required lower pressure threshold (1.20 MPa) when sonications were carried out at AP = 0 mm and ML =  $\pm 2$  mm covering the superior colliculus region. (Bottom) Pupil dilation required higher pressure threshold (>1.80 MPa) when sonications were carried out at AP = -1 mm and ML =  $\pm 0.8$  mm covering the locus coeruleus.

The sonication of eye-motor related and anxiety related regions of the brain elicited eyeball movements and pupil dilations of up to 20% (Fig. 3). The superior colliculus region (AP = 0 mm and ML =  $\pm 2$  mm) required a lower pressure threshold (1.20 MPa) to elicit pupil dilation. Other regions that are anxiety-related such as the hippocampus (AP = +2 mm and ML = 1.5 to 2.0 at both sides) and locus coeruleus (AP = -1 mm and ML =  $\pm 0.8$  mm) required higher pressure thresholds (>1.8 MPa) to elicit pupil dilations.

#### IV. DISCUSSION

FUS was randomly swept over the mice motor and somatosensory cortices. Once responsive regions were detected, the opposite hemisphere was evaluated seeking for contralateral or ipsilateral responses. The contralateral response observed in this study demonstrates that successful target specificity of the FUS neuromodulation was achieved using frequencies in the MHz-range. However, ipsilateral responses varied among animals. The variation of motor responses may be related to inhibitory effects on the cortex. In addition, as demonstrated by other studies [15], anesthesia affects the synaptic inhibition–excitation balance of cortex, and may be the cause of the variations on the responses observed in this study. Other cause of variation could be the size of the ultrasound focus (8.73 mm long and 1.02 mm diameter) that could simultaneously modulate additional regions that could have a counter-effect.

The pupil dilation response elicited by the FUS neuromodulation has important implications for cognition and demonstrated the capability of FUS to activate deeper regions in the brain. Therefore, this technique may become an important tool for functional brain mapping to understand the brain complex network activity and connectivity.

#### V. CONCLUSION

In this study, we demonstrated the target specificity capability of FUS in the mega-Hz range to control motor responses. Moreover, cognitive regions were explored with FUS neuromodulation to demonstrate the capability of FUS to target deeper regions in the brain. The evoked response variation among animals observed in this study indicates the need of further studies to explore acoustic parameters for the control of the inhibition–excitation balance of neuronal activity as well as further explore the type of anesthesia suitable for the targeted region to be modulated with FUS.

#### ACKNOWLEDGMENTS

We thank Hohetebanhan Getachew, B.S., Yang Liu, Ph.D., and Edward Li for their important input and technical support.

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