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Ipsi- and contralateral motor response using ultrasound-induced neurostimulation in deeply anesthetized mice

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Abstract

Ultrasound neurostimulation has been proven capable of eliciting motor responses. However, the studies in sedated rodents presented problems with target specificity due to the use of low ultrasound frequencies (<700 kHz). Here, we show that focused ultrasound (FUS) in mega-Hz range was able to evoke motor responses in mice under deep anesthesia. Contralateral movements of the hind limbs were observed when sonications were carried out at +2 mm of Lambda and ±2 mm lateral of midline in three mice. Moreover, stimulating other regions of the somatosensory and cerebellum induced trunk and ipsilateral limb movements in all six mice.

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Keywords: Evoked motor response; neurostimulation; ultrasound

1. Introduction

Neuronal activity can be induced by modulating currents in the brain using electrodes, creating induced currents using magnetic fields or activating genetically modified neurons with light. Recently, the capability of ultrasound of driving neuronal activity has been reported where mice hippocampal slices in culture were stimulated with pulsed mechanical waves in the kHz-range (Tyler *et al.*, 2008).

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In vivo studies with rats, mice, and rabbits have shown the capability of ultrasound of evoking motor responses. The main pointed out advantages of this technique are the target specificity of the stimulated region, the noninvasive procedure, and the capability of stimulating deeper regions. However, the use of kHz-range has failed in showing contralateral and ipsilateral differences during experiments with mice and rats. In addition, the use of anesthesia in motor response studies such as isoflurane (King *et al.*, 2013), known to suppress the motor cortex activity (Kawaguchi *et al.*, 1996), or the mixture of ketamine and xylazine (Younan *et al.*, 2013) have shown drawbacks with the spontaneous movements or working time.

In studies with monkeys, ultrasound neurostimulation was capable of modulating cognitive response in antisaccade tasks (Deffieux *et al.*, 2013). In humans, ultrasound neurostimulation reduced pain and it was associated with changes in mood (Hameroff *et al.*, 2013). The development of this technique intend to offer an alternative to the current techniques such as Deep Brain Stimulation (DBS), Transcranial Magnetic Stimulation (TMS) and optogenetics. Thus, the ultrasound neurostimulation may be used for brain mapping and in the treatment of various psychiatric disorders and neurological diseases.

In this study, ultrasound neurostimulation was performed in mice using the mega-Hertz range. The use of higher frequencies intended to provide a more confined focus region, thus allowing the targeted specific stimulation of the brain. The experiments were performed using deeper levels of anesthesia to avoid spontaneous movements.

2. *In vivo* ultrasound neurostimulation

The mice (mass: 24 g, sex: male, C57BL/6, Harlan, Indianapolis, IN, USA) were anesthetized with intraperitoneal injection of sodium pentobarbital (65 mg/kg). After 30 minutes, when the animals were under the anesthesia effect, they were positioned in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) with a mask delivering oxygen constantly at 0.8 L/min (SurgiVet, Smiths Medical PM Inc., Waukesha, WI, USA). The scalp and neck of the animals were shaved and a water bath was used to acoustically couple the transducer to the animal's head (Fig. 1). The anesthesia effect was monitored by paw pinches and heart rate (HR) and respiratory rates (RR) (MouseOx Plus, Starr Life Sciences Corp., Torrington, Connecticut, USA). A 10 MHz pulse-echo transducer (focal depth: 60 mm, radius 11.2 mm; Olympus NDT, Waltham, MA, USA) was used to position the focus in the brain using Lambda as the reference. A focused 1.9 MHz transducer (radius: 30 mm; Imasonic SAS, Voray-sur-lOgnon, France) was used for the sonication. The transducer was driven by a function generator (33220A, Agilent Technologies, Palo Alto, CA, USA) through a 50-dB power amplifier (ENI Inc., Rochester, NY, USA) using the acoustic parameters shown in the Table 1. The transducer attached to a 3D positioning system (VXM, Velmex Inc, New York, USA) was centered at -2 mm to the Lambda suture on the midline and moved in a randomized pattern within a 6x6 mm grid (1 mm spacing). Once responsive regions were found, a threshold study using an electromyography (EMG) system (BN-EMG2, Biopac Systems Inc., Santa Barbara, CA) was performed to find the minimum acoustic pressure needed to evoke motor response. The muscles activity of the biceps femoris were monitored using 26-gauge needles implanted 5-mm apart in both hind limbs. Videos recorded the evoked motor response (EOS Rebel T3i, Canon, Melville, NY, USA).

Table 1. Acoustic parameters for FUS neurostimulation.

Parameters	Value
Frequency	1.9 MHz
Pressure range	1.74 – 4.7 MPa
Pulse repetition frequency	1 kHz
Sonication duration	1 s
Interval interstimulus	1 s
Duty cycle	50%
Number of shots	10

The sonication started approximately 30 minutes after the anesthesia injection when the HR and RR were less than 250 bpm (beats per minute) and 60 brpm (breaths per minute), respectively. The evoked limb movements were recorded with video cameras. Each animal remained unresponsive to pedal pinches throughout both the sonication and the sham studies. No spontaneous movements were observed when HR and RR were less than 350 bpm and 90 brpm, respectively. The sodium pentobarbital allowed us to perform the ultrasound neurostimulation for a period of 45 to 80 minutes, longer than ketamine/xylazine anesthesia (reportedly ~30 min).

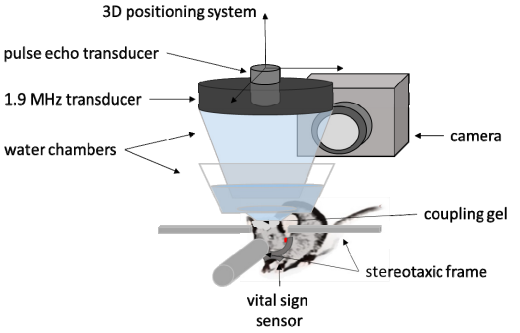

Fig. 1. Experimental setup for focused ultrasound neurostimulation. The mouse was positioned in a stereotaxic frame. 1.9 MHz FUS transducer for the neurostimulation. A pulse-echo transducer and a positioning system for the FUS alignment. Water chambers for acoustic coupling.

The evoked response varied with the animals, but contralateral movements of the hind limbs were consistently observed when sonications were carried out at +2 mm of Lambda and ±2 mm lateral of midline in three mice (Fig. 2). In the representative case shown in Fig. 2 the stimulation of other regions (in green) evoked contralateral movements. Moreover, stimulating other regions of the somatosensory and cerebellum induced trunk and ipsilateral limb movements (in red) in all six mice. No brain damages were found in the H&E analysis performed in 3 mice sonicated with the maximum pressure assessed in this study, 4.7 MPa.

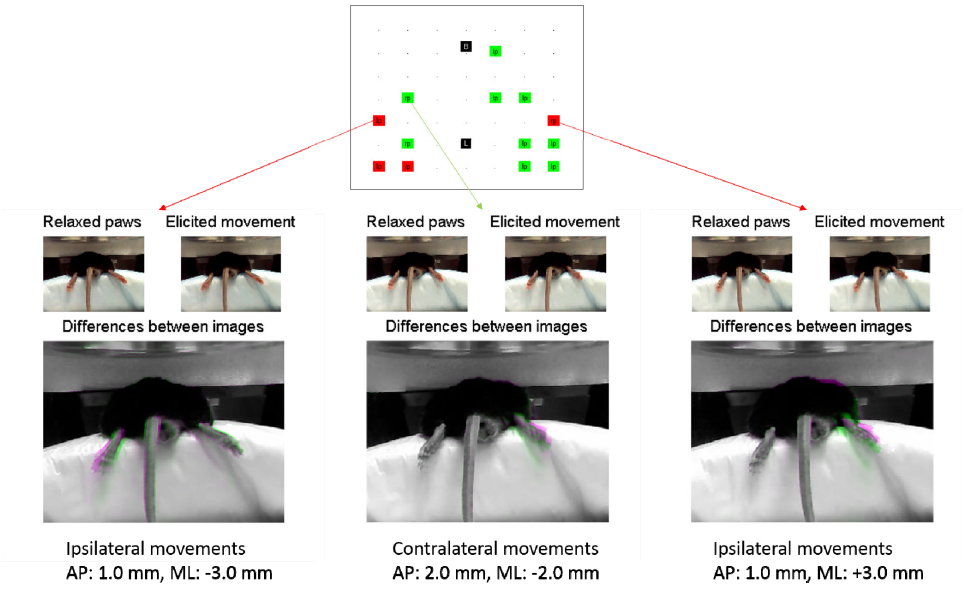

Fig. 2. Examples of ipsilateral and contralateral hind limb movements evoked by focused ultrasound neurostimulation. Green regions in the map represents regions where contralateral movements were observed and red regions represents where ipsilateral movements were observed.

Once responsive regions were found a minimum acoustic pressure to evoke motor response was evaluated with EMG monitoring (Fig. 3). The minimum acoustic pressure to evoke contralateral movements of the right paw was 2.26 MPa. However, the success rate was 20%, which was increased using higher pressures. With 2.78 MPa the success rate was 70% and the latency between the sonication and the evoked motor response was 266 ± 37 ms.

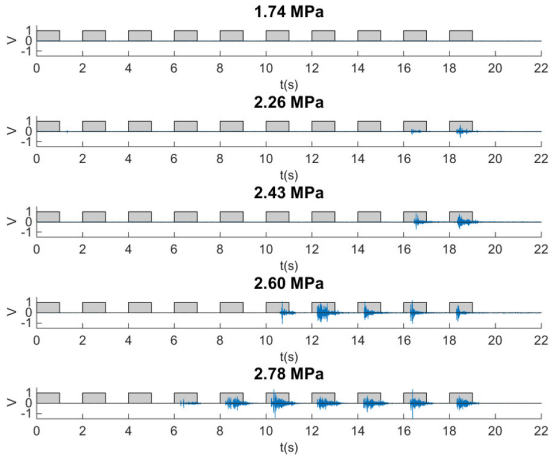

Fig.3. Electromyography signals of the right hind limb during contralateral movements elicited by FUS neurostimulation.

3. Conclusion

Although the use of higher frequencies for neurostimulation requires higher pressures because of the skull attenuation effects, the use of 1.9 MHz focused ultrasound transducer allowed a more confined focus region for neurostimulation. Contralateral and ipsilateral hind limb movements were evoked using a safe range of acoustic pressure, where no brain damages were observed. More studies are necessary to evaluate the capability of the FUS neurostimulation in modulating the activity of deeper structures of the brain. Ongoing studies are being performed to evaluate regions of the brain associated to cognition and behavior.

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