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● *Original Contribution*

KETAMINE INHIBITS ULTRASOUND STIMULATION-INDUCED NEUROMODULATION BY BLOCKING CORTICAL NEURON ACTIVITY

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Abstract—Ultrasound (US) can be used to noninvasively stimulate brain activity. However, reproducible motor responses evoked by US are only elicited when the animal is in a light state of anesthesia. The present study investigated the effects of ketamine on US-induced motor responses and cortical neuronal activity. US was applied to the motor cortex of mice, and motor responses were evaluated based on robustness scores. Cortical neuronal activity was observed by fluorescence calcium imaging. US-induced motor responses were inhibited more than 20 min after ketamine injection, and US-triggered Ca^{2+} transients in cortical neurons were effectively blocked by ketamine. Our results indicate that ketamine suppresses US-triggered Ca^{2+} transients in cortical neurons and, therefore, inhibits US-induced motor responses in a deep anesthetic state. (E-mail: iyoun@kist.re.kr)

Key Words: **Ultrasound stimulation, Neuromodulation, Ketamine, Cortical neuron.**

INTRODUCTION

Noninvasive brain stimulation (NIBS) has been investigated for the study of brain functions. Brain excitability can be regulated by NIBS techniques, such as transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) (Dayan et al. 2013). TMS uses magnetic coils to induce electric current in the brain *via* electromagnetic induction and can be used to activate or suppress brain function by modulating the stimulation frequency. tDCS induces reversible changes in neural activity *via* direct current stimulation, and anodal stimulation induces excitatory changes in targeted brain regions, whereas cathodal stimulation causes inhibitory changes. Both TMS and tDCS are considered safe for human application and have been widely used to explore and modulate human brain functions. However, these methods have various limitations, such as an insufficient spatial resolution to localize changes in regional brain activity and the inability to penetrate deep brain structures (Bystritsky et al. 2011; Sanguinetti et al. 2014).

Ultrasound (US) stimulation is an alternative NIBS method that provides superior spatial specificity and penetration depth compared with other NIBS methods (Kim et al. 2014). US delivers acoustic energy through the skull to a specific focus area of the brain, and the transmitted acoustic energy can induce biological effects on the targeted tissue. US can excite and suppress neuronal activity without causing heat or mechanical damage, and many studies have investigated modulating the excitability of the brain *via* US. For example, Na^+ and Ca^{2+} transients in hippocampal pyramidal neurons were triggered *via* low-intensity and low-frequency pulsed US, which altered the electrical activity of neurons (Tyler et al. 2008). Transcranial pulsed US is also capable of stimulating intact mouse brains, such as the motor cortex and hippocampus, and such motor responses can be reliably evoked by US with no damage to the brain (Tufail et al. 2010). In addition, a previous study transiently modulated the motor and visual areas in the rabbit brain by focused US (FUS) while brain functions were observed through electrophysiological recordings and functional magnetic resonance imaging (Yoo et al. 2011). These studies demonstrate that US can be safely and reliably used to modulate brain activity.

Various parameters of US, such as duty cycle (DC), tone-burst duration (TBD), pulse-repetition frequency (PRF), sonication duration (SD), acoustic intensity (AI), and fundamental frequency (FF), have been investigated to achieve the desired effects of brain stimulation (Kim et al. 2014; Mehic et al. 2014; Min et al. 2011; Tufail et al.

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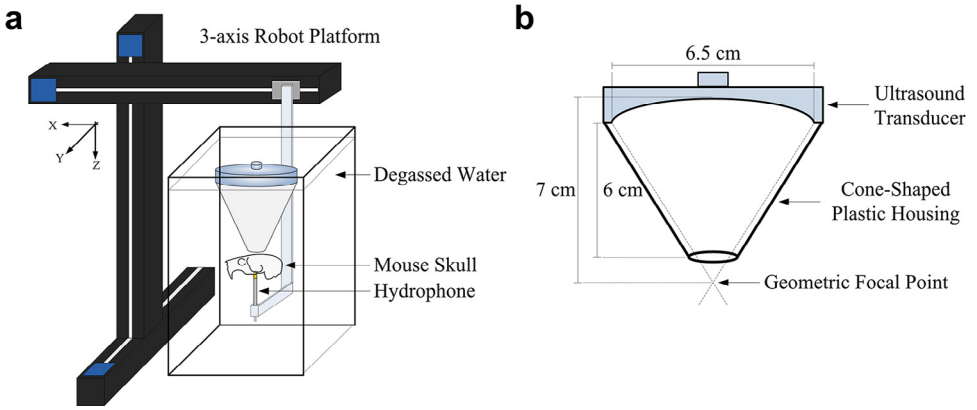

Fig. 1. Schematic of the experimental setup for measuring acoustic pressure fields. (a) The acoustic pressure field was scanned with and without the skull, using a three-axis robot platform. (b) Schematic of the cone-shaped plastic housing.

2010; Yoo et al. 2011). Considering the complementary aspects of acoustic frequency (*i.e.,* the use of a lower FF provides superior penetrability while sacrificing spatial specificity), an FF 0.25–0.7 MHz is recommended as an optimal acoustic frequency for intact brain stimulation to modulate brain activity (Tufail et al. 2011). Additionally, stimulation waveforms of US with a TBD 0.16–0.57 ms, a PRF 1.2–3.0 kHz and an SD 21–333 ms can be used to directly stimulate action potentials in intact brain circuits (Tufail et al. 2010). In another study, tail movements were elicited by US at a minimum AI threshold using stimulation waveforms with a TBD between 1 and 5 ms, 50 % DC and 300 ms SD at 0.35 MHz FF (Kim et al. 2014). One of the major contributing factors to modulate brain activity effectively by US is the state of anesthesia. Previous studies have assessed stimulation parameters for modulating brain activity in animal patients under a light anesthetic state, where the anesthetic state was assessed by physiologic cues such as an irregular respiration rate and responsiveness pinch stimulation (King et al. 2013; Mehic et al. 2014; Younan et al. 2013). However, it is difficult to identify the optimal anesthetic state for inducing robust and reliable motor responses by US on the basis of these physiologic cues. Additionally, in a deep anesthetic state, it is difficult to stimulate brain activity and induce motor responses by US. The reason why motor responses cannot be induced by US while in a deep anesthetic state has not been elucidated.

In this study, we sought to investigate the effect of anesthesia on US-evoked motor responses and neuronal activity. US was performed to evoke motor responses in ketamine/xylazine-anesthetized mice, and tail movements were evaluated as motor responses. Ketamine is a dissociative anesthetic agent that is widely used to induce anesthesia in laboratory animals. Ketamine acts as a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist that interacts with voltage-gated ion channels and synaptic transmissions in the peripheral and central nervous systems (Schnoebel et al. 2005). To explore whether ketamine affects the activity of cortical neurons associated with US, fluorescence calcium imaging was used to measure the changes in intracellular Ca^{2+} concentrations in cortical neurons stimulated by US before and after ketamine treatment.

MATERIALS AND METHODS

Ultrasound stimulation setup

US was generated by a single-element FUS transducer (EofE Ultrasonics Co., Ltd, Gyeonggi-do, Korea) with a diameter of 6.5 cm, a focal length of 7 cm and an FF of 350 kHz. Two function generators were serially connected to generate bursts of pulsed waves, where the first function generator (AFG3022 B, Tektronix, Beaverton, OR, USA) regulated the PRF, the SD and the inter-stimulus interval (ISI). The second function generator (33210 A, Agilent, Santa Clara, CA, USA) controlled the FF, the TBD and the AI. The linear power amplifier (AG 1021, T&C Power Conversion, Inc., Rochester, NY, USA) received sine waves from the second function generator and then provided the amplified voltage output to the transducer.

Acoustic-intensity measurement

As the US waves propagate through the tissue, acoustic pressure is attenuated because of scattering and absorption. Bone has a higher attenuation coefficient compared with other soft tissues, and therefore, energy loss typically increases as waves pass through bone (Pinton et al. 2012). For example, the animal's skull is known as a significant barrier to AI transmission. The attenuation of AI because of a rat's skull is approximately 12.1% and 16.8% for 350-kHz FF and 650-kHz FF, respectively (Kim et al. 2014), and approximately 11% for 320-kHz FF (Younan et al. 2013). In contrast, AI attenuation because of prop-