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● *Original Contribution*

IMAGE-GUIDED FOCUSED ULTRASOUND-MEDIATED REGIONAL BRAIN STIMULATION IN SHEEP

Wonhye Lee,* Stephanie D. Lee,* Michael Y. Park,* Lori Foley,† Erin Purcell-Estabrook,† Hyungmin Kim,*‡ Krisztina Fischer,* Lee-So Maeng,§ and Seung-Schik Yoo*

*Department of Radiology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA; †Invasive Cardiovascular Experimental Laboratory, Brigham and Women's Hospital, Boston, Massachusetts, USA; ‡Center for Bionics, Korea Institute of Science and Technology, Seoul, Korea; and §Incheon St. Mary's Hospital, The Catholic University of Korea, Incheon, Korea

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Abstract—Non-invasive brain stimulation using focused ultrasound has largely been carried out in small animals. In the present study, we applied stimulatory focused ultrasound transcranially to the primary sensorimotor (SM1) and visual (V1) brain areas in sheep (Dorset, all female, n = 8), under the guidance of magnetic resonance imaging, and examined the electrophysiologic responses. By use of a 250-kHz focused ultrasound transducer, the area was sonicated in pulsed mode (tone-burst duration of 1 ms, duty cycle of 50%) for 300 ms. The acoustic intensity at the focal target was varied up to a spatial peak pulse-average intensity (I_{sppa}) of 14.3 W/cm^2. Sonication of SM1 elicited electromyographic responses from the contralateral hind leg, whereas stimulation of V1 generated electroencephalographic potentials. These responses were detected only above a certain acoustic intensity, and the threshold intensity, as well as the degree of responses, varied among sheep. Post-sonication animal behavior was normal, but minor microhemorrhages were observed from the V1 areas exposed to highly repetitive sonication (every second for ≥500 times for electroencephalographic measurements, I_{sppa} = 6.6–10.5 W/cm^2, mechanical index = 0.9–1.2). Our results suggest the potential translational utility of focused ultrasound as a new brain stimulation modality, yet also call for caution in the use of an excessive number of sonications. (E-mail: yoo@bwh.harvard.edu)

Key Words: **Focused ultrasound, Brain stimulation, Large animal model, Sheep, Sensorimotor area, Visual area.**

INTRODUCTION

The development of a method that enables modulation of regional brain activity is sought after as a potential neurotherapeutic modality for neurologic and psychiatric disorders (George and Aston-Jones 2010; Hoy and Fitzgerald 2010), as well as a tool for functional brain mapping (Hallett 2000; Min et al. 2011b). Deep brain stimulation (DBS) and epidural cortical stimulation (EpCS) can modulate the region-specific function of the brain, but the range of utilization is limited because of the invasive surgeries required (Hoy and Fitzgerald 2010). Non-invasive techniques, such as transcranial direct current stimulation (tDCS) and transcranial magnetic stimulation (TMS), lack spatial specificity and penetration depth (Fregni and Pascual-Leone 2007; Loo and Mitchell 2005). Optogenetic techniques are capable of controlling the neural activity in the brain on a cellular level (Deisseroth 2011; Miesenböck 2009), yet the genetic modification of neurons needed to introduce the stimulatory response to an external light stimulus, along with the limited transcranial penetration of the stimulatory light, may impede its prompt utilization in humans.

Focused ultrasound (FUS) techniques deliver acoustic pressure waves to a small, localized area (on the order of a few millimeters in diameter) of biological tissue (Fry et al. 1955; Fry and Fry 1960; Hynynen et al. 1996; Jolesz et al. 2005; Lele 1962; Lynn et al. 1942; Vallancien et al. 1992; Yang et al. 1992). Advancement in FUS technology has enabled the transcranial application of highly focused ultrasound to region-specific brain areas in a non-invasive manner (Elias et al. 2013; Hynynen et al. 2004; Martin et al. 2009). With advantages of spatial specificity

Address correspondence to: Seung-Schik Yoo, Department of Radiology, Brigham and Women's Hospital, Harvard Medical School, 75 Francis Street, Boston, MA 02115, USA. E-mail: yoo@bwh.harvard.edu

and depth penetration over existing methods, FUS has been investigated as a new mode of brain stimulation (Bystritsky et al. 2011; Tufail et al. 2011; Yoo et al. 2011). After early seminal work by Fry et al. (1958), who reported that the sonication of the lateral geniculate nucleus of cats can temporarily modify visual evoked potentials (VEPs), the neuromodulatory effects of ultrasound were illustrated by sonicating excised *ex vivo* rodent brain tissue (Bachtold et al. 1998; Rinaldi et al. 1991; Tyler et al. 2008). Subsequent *in vivo* studies have revealed that FUS applied to region-specific brain areas reversibly modulates the excitability of the motor and visual areas in rabbits (Yoo et al. 2011), stimulates the various motor areas (Mehić et al. 2014), suppresses epileptic electroencephalogram (EEG) activity (Min et al. 2011a) and alters the extracellular levels of neurotransmitters in rats (Min et al. 2011b; Yang et al. 2012). The effects of sonication parameters on the effectiveness of neuromodulation have also been investigated using small animals (Kim et al. 2014, 2015; King et al. 2013). Although the stimulatory effects of FUS have been reported in humans (Lee et al. 2015; Legon et al. 2014) and non-human primates (Deffieux et al. 2013), studies on large animals are warranted to validate the stimulatory findings from small animals, as well as to establish important translational tolerability information for human studies.

As the size of the acoustic focus and concomitant stimulatory area is small (Kim et al. 2013), the use of large animal species (with large brain volumes) is helpful in validating the stimulatory effects of FUS on a discrete region-specific area of the brain. Furthermore, the effect of acoustic reverberations, which may result in less accurate spatial localization of the acoustic energy in a small cranium (Younan et al. 2013), is of less concern in larger cranial structures. In the study described here, we explored the administration of transcranial FUS to region-specific (*i.e.*, primary sensorimotor [SM1] and visual [V1]) cortical areas of sheep. Sheep were chosen as a study model because of their large brain volume with distinct neuroanatomic structures. Unlike pigs (having a flat and thick skull), sheep have a relatively round skull with a thickness (on the order of 4–5 mm) similar to that of humans. Also, its availability in various brain disease/injury models, such as stroke (Boltze et al. 2008), epilepsy (Stypulkowski et al. 2014) and brain injury (Van den Heuvel et al. 1999), makes sheep an attractive species for translational research of FUS.

The hypothesis tested in the present study is that pulsed application of the FUS transcranially delivered to the SM1 and V1 of the sheep brain would stimulate the regional brain tissue. Our aim was to illustrate that the stimulation elicits corresponding electromyogram (EMG)-based motor evoked potentials (MEPs) and EEG-based VEPs. To distinguish the VEPs elicited by the FUS from the traditional nomenclature describing the EEG potentials evoked by external visual stimulation, a term, sonication-triggered VEPs (sVEPs), was employed throughout the text. Placement of the FUS focus at the desired SM1 and V1 areas was achieved using anatomic and functional magnetic resonance imaging (MRI) data obtained from each sheep brain to promote spatial accuracy of the sonication. Different acoustic intensities (AIs) were applied to probe their effect on the magnitude of the evoked potentials. We also assessed the behavior of each animal at different time points for up to 2 mo after sonication and conducted histologic analysis on the sonicated brain tissue.

METHODS

Animal preparation

All animal procedures were performed under the approval of and according to the ethical standards set forth by the Harvard Medical Area Standing Committee. Each sheep (Dorset, all female, weight = 32.6 ± 4.4 kg, mean ± SD, 25–38 kg, n = 8, numbered S1 through S8 herein) underwent two separate procedures: (i) identification of the anatomic and functional locations of the SM1 and V1 areas for sonication using MRI, and (ii) FUS stimulation sessions. For all procedures, the animals were sedated and anesthetized with Telazol (tiletamine [*N*-methyl-D-aspartate, NMDA receptor antagonist] + zolazepam, initial dose 2–4 mg/kg intravenously plus additional doses as needed to maintain anesthesia during each experimental procedure). Inhalant anesthetics (such as isoflurane) were not used because these can alter cerebral blood hemodynamic responses (Matta et al. 1999; Reiz et al. 1983), which would confound the results from functional MRI, possibly resulting in negative BOLD (blood oxygenation level-dependent) signals (Tsurugizawa et al. 2010). Although different anesthetic agents can be used for imaging and sonication procedures, we opted to use the same anesthetic procedures to maintain similar experimental conditions. The relatively shallow anesthetic depth of Telazol does not require intubation/forced ventilation of the animals. Adequate veterinary support, such as monitoring of peripheral capillary oxygen saturation level (S_pO_2) and heart rate (Table 1), was provided by certified veterinary staff during the procedures (each could contain multiple MRI/FUS sessions). We also monitored for the normal range of respiratory rate (16–34 breaths/min).

Functional and anatomic neuroimaging data acquisition and processing for sonication planning

For accurate guidance of the acoustic focal area to the individual functional neuroanatomy of the sheep,