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● *Original Contribution*

TOWARD DEEP BRAIN MONITORING WITH SUPERFICIAL EEG SENSORS PLUS NEUROMODULATORY FOCUSED ULTRASOUND

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Abstract—Noninvasive recordings of electrophysiological activity have limited anatomic specificity and depth. We hypothesized that spatially tagging a small volume of brain with a unique electroencephalography (EEG) signal induced by pulsed focused ultrasound could overcome those limitations. As a first step toward testing this hypothesis, we applied transcranial ultrasound (2 MHz, 200-ms pulses applied at 1050 Hz for 1 s at a spatial peak temporal average intensity of 1.4 W/cm^2) to the brains of anesthetized rats while simultaneously recording EEG signals. We observed a significant 1050-Hz electrophysiological signal only when ultrasound was applied to a living brain. Moreover, amplitude demodulation of the EEG signal at 1050 Hz yielded measurement of gamma band (>30 Hz) brain activity consistent with direct measurements of that activity. These results represent preliminary support for use of pulsed focused ultrasound as a spatial tagging mechanism for non-invasive EEG-based mapping of deep brain activity with high spatial resolution. (E-mail: doumitt@uw.edu) **© 2016 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Focused ultrasound, EEG, Brain activity.**

INTRODUCTION

Management of neurologic injuries and disorders, such as traumatic brain injury, epilepsy and depression, as well as control of neuroprosthetic devices, can require monitoring of the brain's electrical activity. Electroencephalography (EEG) exemplifies such monitoring, as it facilitates tracking of different stages of convulsive status epilepticus, important for early treatment (Maganti and Rutecki 2013). Here, we offer first steps toward a means of monitoring focal and deep brain activity through a combination of EEG and transcranially delivered pulsed focused ultrasound (pFU). This combination would permit extracranial monitoring of focal brain function, avoiding invasive procedures such as electrocorticography.

Current EEG techniques based on external electrodes can collect electrophysiological data at frequencies up to 1 kHz (Fedele et al. 2015; Telenczuk et al. 2011; Teleńczuk et al. 2015), with the bulk of measured endogenous activity occurring below 100 Hz (Darvas et al. 2010; Darvas et al. 2013; Smith et al. 2014; Dalal et al. 2008; Cheyne et al. 2008; Ball et al. 2008). However, extra-cranial electrodes can measure focal EEG signals only for superficial brain structures, with variable resolution and localization accuracy (Darvas et al. 2004). This leaves deep brain function inaccessible to external monitoring (Maganti and Rutecki 2013; Wennberg and Cheyne 2014). Barriers include signal non-uniqueness (superficial electrophysiological signals can arrive simultaneously with signals from deep brain), noise induced by motion artifacts and the intrinsically weak nature of deep brain signals. The alternative, electrocorticography, an intra-cranially placed electrophysiological monitoring system, solves this problem but at obvious cost.

Ultrasound has been used to temporarily and non-destructively activate or inhibit central neural circuits. Using a cat model, the Fry brothers (*e.g.,* Fry 1958) showed that unfocused 1-MHz ultrasound reversibly and repeatably suppressed electrophysiologically measured brain activity. This result is consistent with a contemporary and more exhaustive study (Ballantine et al., 1960), as well as the work of Vykhodtseva and Koroleva (1986) who generated cortical spreading

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depression in rat brain using ultrasound. Recently, Tyler et al. (Tyler et al. 2008; Tufail et al. 2010, 2011) showed that pulsed, low-intensity and low-frequency (0.5 MHz) unfocused ultrasound could activate neural circuits in mouse brain, through several means: direct measurement of action potentials within brain slice preparations, in intact brain and direct observation of peripheral motor function. King et al. (2013, 2014) and Younan et al. (2013) produced comparable observations through a greater range of ultrasound frequencies. Yoo et al. (2011) observed that transcranial pFU (at 0.69 MHz) could create functional changes in rabbit brain, including excitatory effects when applied to motor cortex, measurable with functional magnetic resonance imaging.

More recently, we (Mehić et al. 2014) used a high-frequency (2-MHz) system capable of focused delivery of very low-frequency ultrasound (modulated focused ultrasound), as well as pFU, to demonstrate spatial variability of transcranial activation of brain circuits in mice on length scales of 1 mm, observed through induction of repeatable peripheral motor function. Deffieux et al. (2013) also found reversible changes in macaque visual function after application of pFU at 0.32 MHz to their prefrontal cortex. Finally, Legon et al. (2014) found modulation of the function of human primary somatosensory cortex with transcranial pFU delivered at 0.5 MHz, with further analysis of the associated EEG signals reported by Mueller et al. (2014) and discussed below.

Of note, none of these studies report adverse events, determined through assay of histologic or electrophysiological changes of brain or changes in grossly observed behavior. This is true despite the fact that several of these studies (*e.g.,* King et al. 2013, 2014; Mehić et al. 2014; Tufail et al. 2011; Yoo et al. 2011) used, among several protocols, ultrasound with a spatial peak and temporal average intensity value greater than that given by FDA guidelines for diagnostic ultrasound and then applied that ultrasound multiple times to the same location in the brain (*e.g.,* Yoo et al. 2011). This is likely because, although some of these protocols employ ultrasound above FDA guidelines for intensity, those protocols fall well below the values used without acoustic contrast agents to create blood–brain barrier disruption (*e.g.,* $\sim$ 80 W/cm^2 spatial and temporal average intensity applied for 0.2 s, as found by Mesiwala et al., 2002).

All of the studies cited above that used EEG did so in order to measure brain activity, generally at less than 50 Hz, induced by application of ultrasound to the brain. In contrast to the present work, no studies to our knowledge have reported measurements of brain activity at the high frequencies associated with ultrasound application, at the 1050-Hz pulsed repetition frequency of our ultrasound, or have sought to extract low-frequency brain activity from those signals. Such analysis is of interest because we seek, long term, to facilitate use of external EEG to monitor deep, focal brain function by "tagging" that activity with a unique, detectable, high-frequency electrical signal generated by application of pFU to the brain volume of interest. Here we describe completion of a first step toward this goal: successful demonstration that transcranially delivered pFU could generate from within rat brain a unique, high-frequency EEG signal measured extra-cranially, whose analysis yields measures of low-frequency brain activity consistent with their direct, extra-cranial measurement.

METHODS

Tissue phantom and EEG implantation

We sought to test the interaction between our ultrasound delivery system and the EEG electrodes *in vitro*, before *in viv*o studies. The medium used consisted of saline-based 4% alginate gel with mechanical properties (density and ultrasound attenuation) similar to rat brain while also being comparably conductive. We inserted eight steel needle tip thin-wire EEG electrodes (Ambu Neuroline Subdermal 27 G, Cadwell, Kennewick, WA) into the gel in the same positioning and pattern by which we would insert them into a rat head (Fig. 1) with approximately 3–4 mm between electrodes in the rostral/caudal direction, each line approximately 4 mm from the midline. We also placed reference and ground electrodes. After electrode insertion, five locations were chosen for application of ultrasound: both outside and within the two rows of electrodes, as well as beside or directly above the electrodes. Using this *in vitro* experimental setup, we mirrored our *in vivo* ultrasound trial protocol with 50 instead of 100 trials, consistent with the low variance of our results. We also performed a test in which we decoupled the transducer from the alginate gel to only allow any electromagnetic fields associated with the transducer to affect the electrode montage.

In vivo *methods*

All animal procedures were approved by the University of Washington Institutional Animal Care and Use Committee (IACUC), IACUC protocol number 4084-06.

Overview. We worked with four Sprague Dawley rats aged 8 wk and weighing approximately 270 g. Isoflurane anesthesia was used at a 5% induction rate and then kept at 2%, with 2 L/min oxygen flow at 100%. Toe and tail pinches were administered to insure adequate depth of anesthesia. After recording baseline brain activity without application of ultrasound, we then applied ultrasound to the brain while recording brain activity. Next, we injected the animals with a lethal overdose of