Ultrasound in Med. & Biol., Vol. ■, No. ■, pp. 1–19, 2016

0301-5629/$ - see front matter

http://dx.doi.org/10.1016/j.ultrasmedbio.2016.02.012

● *Original Contribution*

FREQUENCY DEPENDENCE OF ULTRASOUND NEUROSTIMULATION IN THE MOUSE BRAIN

PATRICK PEIYONG YE,* JULIAN R. BROWN,† and KIM BUTTS PAULY‡

*Department of Bioengineering, Stanford University, Stanford, CA, USA; †Howard Hughes Medical Institute, Department of Neurobiology, Stanford University, Stanford, CA, USA; and ‡Department of Radiology, Stanford University, Stanford, CA, USA

(*Received* 28 *October* 2015; *revised* 15 *February* 2016; *in final form* 16 *February* 2016)

Abstract—Ultrasound neuromodulation holds promise as a non-invasive technique for neuromodulation of the central nervous system. However, much remains to be determined about how the technique can be transformed into a useful technology, including the effect of ultrasound frequency. Previous studies have demonstrated neuromodulation *in vivo* using frequencies <1 MHz, with a trend toward improved efficacy with lower frequency. However, using higher frequencies could offer improved ultrasound spatial resolution. We investigate the ultrasound neuromodulation effects in mice at various frequencies both below and above 1 MHz. We find that frequencies up to 2.9 MHz can still be effective for generating motor responses, but we also confirm that as frequency increases, sonications require significantly more intensity to achieve equivalent efficacy. We argue that our results provide evidence that favors either a particle displacement or a cavitation-based mechanism for the phenomenon of ultrasound neuromodulation.
(E-mail: ppye@stanford.edu)

Key Words: **Ultrasound neuromodulation, Ultrasound neurostimulation, Brain stimulation, Cavitation, Radiation force, Particle displacement, Electromyography (EMG).**

INTRODUCTION

As a technique for non-invasive stimulation of the brain, ultrasound neuromodulation has received rapidly increasing interest in recent years for two main reasons: first, encouraging developments in the use of ultrasound neuromodulation *in vivo* have raised the possibility of using the technique more extensively in a wide variety of research and perhaps even therapeutic applications; second, alternative neuromodulation technologies face significant technical limitations. Electrical stimulation using implanted electrodes are precise and effective, but require invasive procedures for placement. Transcranial direct current stimulation and transcranial magnetic stimulation are both non-invasive techniques but suffer from low spatial resolution and are unable to reach deep targets (Wagner et al. 2007). Optogenetic techniques have high spatial resolution and precise cell-type specificity, but rely on methods of genetic vector delivery that have yet to be approved for widespread human use (Fenno et al. 2011).

In contrast, ultrasound has been shown to be a non-invasive, safe and spatially specific method for neuromodulation in various animal models. Ultrasound neuromodulation has been successfully performed transcranially in various animal species, including mice (King et al. 2013, 2014; Mehić et al. 2014; Tufail et al. 2010), rats (Kim et al. 2012, 2014, 2015; Min et al. 2011; Younan et al. 2013), sheep (Lee et al. 2016), monkeys (Deffieux et al. 2013) and even humans (Lee et al. 2015; Legon et al. 2014). Histologic techniques have confirmed that it is possible to deliver sonications powerful enough to elicit ultrasound neuromodulation without causing damage to tissues (Tufail et al. 2010; Yoo et al. 2011). At higher intensities, focused ultrasound is already used to reach deep, subcortical targets for ablation applications (Fry et al. 1954; Wang et al. 2015), and several studies have demonstrated non-destructive spatially specific ultrasound neuromodulation (Fry et al. 1958; King et al. 2014; Mehić et al. 2014; Yoo et al. 2011).

The vast majority of published transcranial *in vivo* neuromodulation studies have been performed using relatively low ultrasound frequencies of less than 1 MHz. The use of such frequencies has generally been necessary because attenuation in tissues and the skull increases with frequency. Excessive attenuation is undesirable because it can both lead to potentially harmful heating effects in the attenuating tissues and reduce the ability to

Address correspondence to: Patrick Peiyong Ye, 443 Via Ortega, Room 119, Stanford, CA 94305, USA. E-mail: ppye@stanford.edu

reach deeper structures. In addition, within the window of frequencies below 1 MHz, at least three *in vivo* studies have observed decreasing neuromodulation efficacy at the upper end of the frequency range tested that could not be attributed to heating effects. Tufail et al. (2010) observed decreasing normalized electromyogram (EMG) amplitudes of ultrasound-evoked contractions with increased frequency (250–500 kHz), while King et al. (2013) reported that increased intensities were required at higher frequencies to obtain the same success rate (250–600 kHz). More recently, Kim et al. (2014) observed a 30%–40% lower threshold for inducing motor responses at 350 kHz than at 650 kHz. These trends have also been observed in the peripheral nervous system over a more extensive frequency range that goes beyond 1 MHz; Gavrilov et al. (1976) demonstrated that sonicating at higher frequencies up to 2.67 MHz required higher intensity to produce threshold sensations in human fingers.

From a practical point of view, these trends appear to be unfortunate because sonicating at higher frequency has the potential for achieving more spatially specific effects, given the shorter wavelengths involved and the ability to resolve smaller focal spot sizes. The eventual hope is to demonstrate targeted neuromodulation-based therapies and techniques that focus tightly on specific regions of the brain. One striking example is offered by Menz et al. (2013b), who used a 43 MHz ultrasound transducer to achieve a focal spot size of 87 microns in salamander retina *in vitro*, a scale perhaps necessary for achieving the high spatial resolution required for a retinal prosthesis.

However, the use of pure-tone, high frequencies far above 1 MHz has yet to be found effective *in vivo* for transcranial applications, and even at intermediate frequencies, both the scale and the cause of reduced efficacy associated with increasing frequency remains undetermined. One possibility is that as frequency rises, the focal spot size typically tightens, inevitably changing the amount of stimulated brain tissue. If it were possible to factor out the effect of focal spot size, any frequency dependence could cast light on what remains the most vexing problem in understanding ultrasound neuromodulation: There is still no accepted theory of how it works. Nevertheless, several of the proposed candidate mechanisms for ultrasound neuromodulation, including cavitation and radiation force, are inherently frequency-dependent, but in distinguishably different ways, so at least one of these could play a role in explaining the observed frequency effects. In addition, the notion of particle displacement, in which neurocellular components are buffeted by ultrasound waves, has been proposed as another potential mechanism that could underlie ultrasound neuromodulation (Gavrilov et al. 1976). It is thus of potentially great importance to explore the effects of higher ultrasound frequencies *in vivo*, as these could not only be relevant for optimizing clinical and research applications but could also help to clarify how ultrasound elicits its neuromodulatory effects.

Our goal in this study was to achieve a better understanding of the frequency response of *in vivo* ultrasound neuromodulation. We first sought to quantify the intensities required to achieve effective transcranial neuromodulation for several ultrasound frequencies both above and below 1 MHz. We then aimed to explain differences observed across ultrasound frequencies by examining the contributions of focal spot size, as well as different hypothesized frequency-dependent mechanisms underlying ultrasound neuromodulation.

MATERIALS AND METHODS

Experimental design

Five sets of experiments were performed to examine the effects of ultrasound frequencies ranging from 0.3 to 2.9 MHz (Table 1). Experiment A was designed to determine how much of the previously reported frequency dependence at low frequencies (*i.e.*, below 1 MHz) was due to changing sonication duration. King et al. (2013) varied the ultrasound frequency while simultaneously varying the ultrasound pulse duration; thus, whether the effects observed were due to ultrasound frequency, sonication duration or some combination of the two was left ambiguous. To overcome this ambiguity, we tested the response of mice to continuous wave sonications at four low frequencies (0.3, 0.4, 0.5 and 0.6 MHz) using a constant number of ultrasound cycles (40,000 cycles, as in King et al. 2013) and, in addition, using pulses of constant duration (80 ms). Employing a 0.5 MHz planar transducer with a waveguide similar to King et al. (2013), we sonicated five mice according to a semi-randomized schedule consisting of blocks of sonications (Fig. 1). Within each block, while frequency and duration of each sonication were held constant, four sonications of different intensities and one sham sonication (where the output of the function generator responsible for generating the ultrasound was off) were performed in random order. The blocks themselves were randomly ordered within sets, each chosen to include all combinations of frequency and duration. For each mouse, at least 10 sets were performed resulting in at least 10 trials for each sonication type (*i.e.*, unique combination of intensity, frequency and duration).

We designed experiments B and C to extend our knowledge of the neuromodulatory efficacy of ultrasound *in vivo* to pure-tone frequencies beyond 1 MHz, a regime that as far as we know has not been previously explored *in vivo*. For experiment B, we sonicated five mice at three frequencies (0.6, 1.0 and 1.4 MHz) and at five intensities for each frequency. For experiment C, a different set of