Ultrasound in Med. & Biol., Vol. ■■, No. ■■, pp. ■■–■■, 2018

0301-5629/$ - see front matter

ELSEVIER

https://doi.org/10.1016/j.ultrasmedbio.2018.01.010

● *Original Contribution*

FOCUSED ULTRASOUND-INDUCED SUPPRESSION OF AUDITORY EVOKED POTENTIALS *IN VIVO*

Dianne Daniels,* Shirley Sharabi,* David Last,* David Guez,* Sharona Salomon,* Zion Zivli,[†,‡] David Castel,[‡,§] Alex Volovick,[¶] Javier Grinfeld,[¶] Itay Rachmilevich,[¶] Talia Amar,[¶] Sigal Liraz-Zaltsman,**[,††] Narek Sargsyan,[‡‡] Yael Mardor,*[,‡] and Sagi Harnof[†,‡]

* Advanced Technology Center, Sheba Medical Center, Ramat-Gan, Israel; [†] Neurosurgery Department, Sheba Medical Center, Ramat-Gan, Israel; [‡] Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel; [§] Neufeld Cardiac Research Institute, Sheba Medical Center, Ramat-Gan, Israel; [¶] InSightec Ltd., Haifa, Israel; ** The Joseph Sagol Neuroscience Center, Sheba Medical Center, Ramat-Gan, Israel; [††] School of Pharmacy, The Hebrew University of Jerusalem, Jerusalem, Israel; and [‡‡] Faculty of Medicine, St. Georges University, London, United Kingdom

(*Received* 26 *October* 2016; *revised* 29 *September* 2017; *in final form* 12 *January* 2018)

Abstract—The goal of this study was to determine the feasibility of focused ultrasound–based neuromodulation affecting auditory evoked potentials (AEPs) in animals. Focused ultrasound–induced suppression of AEPs was performed in 22 rats and 5 pigs: Repetitive sounds were produced, and the induced AEPs were recorded before and repeatedly after FUS treatment of the auditory pathway. All treated animals exhibited a decrease in AEP amplitude post-treatment in contrast to animals undergoing the sham treatment. Suppression was weaker for rats treated at 2.3 W/cm² (amplitudes decreased to 59.8 ± 3.3% of baseline) than rats treated at 4.6 W/cm² (36.9 ± 7.5%, *p* < 0.001). Amplitudes of the treated pigs decreased to 27.7 ± 5.9% of baseline. This effect lasted between 30 min and 1 mo in most treated animals. No evidence of heating during treatment or later brain damage/edema was observed. These results demonstrate the feasibility of inducing significant neuromodulation with non-thermal, non-invasive, reversible focused ultrasound. The long recovery times may have clinical implications. (E-mail: dianne.daniels@sheba.health.gov.il)

Key Words: **Focused ultrasound, Neuromodulation, Auditory evoked potentials.**

INTRODUCTION

Direct modulation of neural activity has been of considerable relevance in the treatment of neurologic and psychiatric disease (Aubry and Tanter 2016; Tyler 2011) Current non-invasive neuromodulation methods, such as transcranial magnetic stimulation and direct current stimulation, have contributed to the expansion of our knowledge of the brain by probing spatio-temporal characteristics of specific neural substrates (Kim et al. 2014). Because of their limitations of relatively poor spatial specificity and penetration depth, increased attention has been devoted to the application of non-invasive neuromodulation induced by focus ultrasound (FUS), which provides high spatial resolution together with efficient penetration into deep tissues.

The neuromodulatory potential of FUS was suggested by the pioneering work of Fry et al. (1958), which found that FUS administered to the lateral geniculate nuclei of the thalamus reversibly inhibited the visual pathway in cats. Recent studies have reported the excitatory and suppressive neuromodulatory properties of FUS not only in the central nervous system (Bystritsky et al. 2015; Kim et al. 2015), but also in the peripheral nervous system (Juan et al. 2014). To achieve the desired modulation, a wide range of ultrasound pulse schemes and frequencies have been explored (Hainsworth and Stricker 1971; King et al. 2013; Lee et al. 2016). The neuromodulatory effects of FUS have been revealed *via* electrophysiological recordings, such as electroencephalography (EEG) (Kim et al. 2015; Lee et al. 2016; Min et al. 2011) and electromyography (EMG) (King et al. 2013; Ye et al. 2016), and using imaging techniques such as functional magnetic resonance imaging (MRI) (Kim et al. 2014; Yoo et al. 2011) and positron emission tomography–computed tomography

Address correspondence to: Dianne Daniels, The Advanced Technology Center, Sheba Medical Center, Tel-Hashomer, 52621, Israel. E-mail: dianne.daniels@sheba.health.gov.il

(PET-CT) (Kim et al. 2013). FUS-mediated functional modulation was also revealed by direct measurement of extracellular levels of neurotransmitters and metabolic changes (Yang et al. 2012).

Since the introduction of FUS in recent years, we have gained a better understanding of its interactions with biological structures and the mechanism of action of neuromodulation (Plaksin et al. 2016). Major advances in the design of ultrasound applicators (Werner and Martin 2015) and development of imaging modalities have opened a broad spectrum of potential clinical applications of FUS.

We chose to study the neuromodulation effects of non-invasive FUS using auditory evoked potentials (AEPs), as these can be recorded under full anesthesia in small and large animals.

Evoked potentials are discrete and minute electrical potentials that appear in EEG recordings, thus providing a sensitive method for studying the effect of drugs and treatments on neuronal activity (Ehlers et al. 1991; Meador 1995; Noldy et al. 1990). AEPs reflect neuronal activity along the auditory pathway, including in the auditory nerve, cochlear nucleus, superior olive and inferior colliculus of the brainstem. Because AEPs can easily be recorded in deeply anesthetized animals, we used this neurophysiological method to study the feasibility of non-invasive, non-thermal, FUS-induced reversible neuromodulation.

The goal of this study was to determine the feasibility of non-invasive, non-thermal, FUS-based reversible neuromodulation affecting AEPs in small and large animals.

METHODS

The study was approved by Sheba Medical Center's institutional ethics committee for animal experiments.

Animal preparation

FUS-induced suppression of AEPs was studied using 22 rats (Sprague-Dawley, males; weight: 400–500 g, age: 6–8 wk) and 5 pigs (*Sus scrofa domesticus*, females; weight 20–24 kg, age: 3 mo).

All anesthetics were provided by Eliezer Linevitz LTD (Even Yehuda, Israel), unless otherwise stated. Rats were deeply anesthetized with an intramuscular (IM) injection of 600 μL of 0.75 mL/kg ketamine and 1.3 mL/kg xylazine, and their heads were shaved to prevent air bubbles during FUS treatment. The rats were kept under anesthesia during the entire experiment.

Pigs were pre-anesthetized with an IM injection of 10 mg/kg ketamine and 2 mg/kg xylazine and then deeply anesthetized by inhalation of 1%–2.5% isoflurane (Sheba Medical Center, Tel-Hashomer, Israel) in 100% oxygen, followed by intravenous (IV) injection of 4 mg/kg propofol (Sheba Medical Center). Pigs were intubated and ventilated during the entire experiment. For analgesia, animals received 0.1 mg/kg buprenorthine (Vetmarket, Shoham, Israel) before craniectomy. The FUS brain system was designed for penetration of a human skull, which has a larger area and different curvature and is thinner than the pig skull. Therefore, pigs underwent bilateral frontal craniectomy before EEG recordings and treatment, creating an average bone opening of 3×4 cm. The craniectomy site was flushed with copious amounts of saline (0.9% w/v sodium chloride) to ensure that no residual air bubbles or bone fragments were left. Throughout the procedure, oxygenation, respiratory rate and temperature were monitored.

Auditory stimulus

Repetitive sound for inducing AEPs was produced using a waveform generator (Model 395, Wavetek, San Diego, CA, USA). Each pulse consisted of two 5-kHz square wave periods. In rat experiments this pulse was repeated every 151 ms (~7 pulses/s); in pig experiments, repetition was every 251 ms (~4 pulses/s).

Electroencephalography recording

AEPs were recorded using an electroencephalograph (MP150 system with EMG100 cMRI, BIOPAC Systems, Goleta, CA, USA). The readings were triggered by the pulse generator and averaged over 500 repetitions (accumulated within ~1–2 min) of the auditory stimulus to minimize the random background electrical activity.

The recording electrodes (EL254 RT surface electrodes with 4 mm diameter contact area, BIOPAC Systems) were filled with conductive electrode gel (ABRALYT HiCl, EASYCAP, Etterschlag, Germany) and placed as follows: In rats, the positive electrode was placed on the scalp above the frontal cortex (midline) and the negative/ground electrodes behind the left/right earlobes; and in pigs, the positive electrode was placed on the dura (within the craniectomy region) above the parietal cortex (lateral), the negative electrode behind the left earlobe and the ground electrode on the scalp in a midline frontal location.

Magnetic resonance–guided FUS system

Animals were treated using a clinical 230-kHz FUS brain system with 1000 elements, a diameter of 30 cm and a spot diameter of 3 mm (ExAblate4000, InSightec, Tirat Carmel, Israel) embedded within a clinical 1.5-T MRI scanner (Signa HDxt, GE Healthcare, Chicago, IL, USA) and an integrated two-channel head coil (InSightec). To verify that the physiologic effects were non- thermal, temperature maps were generated by the MRI scanner during the treatment. Temperature was calculated using a fast spoiled gradient echo sequence (TE/TR = 13/25 ms, BW = 5.6 kHz, single acquisition time: 3.6 s, spatial resolution: $1.1 \times 1.1 \times 3$ mm) based on the shift in proton resonance frequency, as previously described (Rieke and Pauly 2008).