

Neural Activity Modulation via Ultrasound Stimulation Measured on Multi-Channel Electrodes

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Abstract— Recently ultrasound stimulation of the brain is getting a major attention as an external means of safe and effective brain stimulation to treat neurological diseases. Although it is known that ultrasound affects neural activities in the brain, the fundamental principles of ultrasound stimulation are not clearly elucidated yet. In this study, as an initial attempt to investigate the mechanism of neural activity modulation by ultrasound, we studied the changes in action potentials of cultured hippocampal neurons of rats using multi-electrode arrays. From our results, it was observed that ultrasound stimulation increases the frequencies of action potentials (i.e., the number of spikes), supporting the direct facilitation of neural activities by ultrasound.

Index Terms—Neural Activity Modulation, Ultrasound Brain Stimulation, Hippocampal Neurons, Spike Activities, Multi-electrode Arrays

I. INTRODUCTION

Recently, ultrasound brain stimulation (US) is getting a major attention because of its focusing ability with less side effects. Although brain stimulation techniques such as Transcranial Direct Current Stimulation (tDCS) and Transcranial Magnetic Stimulation (TMS) have been clinically approved and known to be effective on some neurological diseases, their lack of focusing abilities and side

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effects are known due to direct current or magnetic stimulation.

So far, investigations on US on the brain have been carried out on three different levels: namely, small animal brains, human brains, and tissues respectively. Most studies have been done on the brain of small animals. In 1964, Manlapaz et al. observed that US effectively relieved the seizures and abnormal electroencephalographic patterns in the small animal brains through the extermination of the epileptogenic focus by US [1]. In 2010, Tufail et al. showed changes of Local Field Potentials from the primary motor cortex of the rat's brain by low-frequency US [2]. In 2011, Min et al. showed significantly decreased occurrence of epileptic EEG bursts after sonication stimulation on the epilepsy-induced rats [3].

There have been relatively few studies of US on the human brains. In [4], electrophysiological observations show that transcranial focused ultrasound stimulation beams targeted to S1 could focally modulate the sensory evoked brain activity and cortical functions.

However, to understand the fundamental principle of the ultrasound induced neuromodulation, it is essential to investigate its influence on the cell and tissues levels of the brain. At the tissue levels, Khraiche et al. in 2008 reported an increase in the frequency of action potential (i.e., the number of spikes) with US at 7.75MHz using multi-electrode arrays (MEA) [5]. In 2009, Muratore et al. observed the increase activity by stimulating cultured hippocampal slices of rats with ultrasound of 4.04MHz for 1ms [6]. In 2011, Muratore et al. showed similar excitatory response at Cornu Ammonis 1 and Dentate Gyrus regions by stimulating the hippocampal tissue with ultrasound of 4.04MHz for 100ms, [7]. In 2005, Tsui et al. experimented with ultrasound intensities of 1 to 3W on neural tissue and concluded that the compound action potential amplitude was increased by US of 1W [8]. So far there have been no studies at the cell level as far as we know, although it is critical for investigations of the basic mechanisms of US.

In this study, as an initial attempt to investigate the mechanism of ultrasound induced neuromodulation, we studied the changes of neural activities via US on the primary cultured hippocampal neurons of rats using MEAs. From our results, we observed changes of action potentials in the cultured hippocampal cells due to US. In most channels of MEA, neural activities were increased via US and in most channels neural activity decreased after US. Our results support the effect of US on the small animal and human brains. Based on our results, further investigation on the principle mechanisms of US should be possible.

II. METHODS

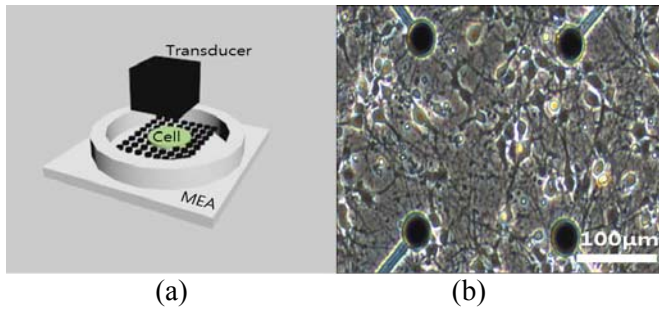


Fig. 1 (a) MEA experimental setup, (b) Hippocampal neurons on MEA

A. Cell Culture

In this study, primary hippocampal neurons were obtained from the brain of embryonic 18-day gestation Sprague Dawley rats. Hippocampi were dissociated and seeded at the density of 600cells/mm². Serum-free neurobasal media (GIBCO®, CA USA) supplemented with 2% B27 (GIBCO®) and 1% glutamax (GIBCO®) was used as a culture media and maintained at 37°C in a 5% CO₂ and 95% air humidified atmosphere [9].

We used microelectrode arrays (Multichannel System GmbH, Reutlingen Germany) to record neural activities. The MEA has 60 electrodes with 200 µm spacing and 30 µm in diameter. The electrode material is titanium nitride on the indium-tin oxide (ITO) conductor lines and the insulation material is silicon nitride. After cleaning with Terg-a-zyme detergent (Sigma-Aldrich, inc., St. Louis USA), coating was done with poly-D-lysine (Sigma-Aldrich, inc.) to promote cell adhesion.

B. Ultrasound Experiments

We used an ultrasound pulser (MKPR-1025, MKC Korea co., Korea) and a transducer (TKS Co., Korea) having a center frequency of 0.5MHz and Crystal element size of 10×10mm². Pulse repetition frequency (PRF) was set at 380~400Hz, Pulse duration (PD) of 2.097µs, spatial-peak pulse-average intensity (I_{SPPA}) of 18.24×10⁻⁵ W/cm², spatial-peak temporal-average intensity (I_{SPTA}) of 98.72×10⁻⁵ mW/cm², and maximum pressure of 11.52KPa. The pulser intensity was measured using the acoustic intensity measurement system (AST01, Onda Corp., Synnyvale, CA, USA) and hydrophone (HGL-0200, Onda Corp., Synnyvale, CA, USA).

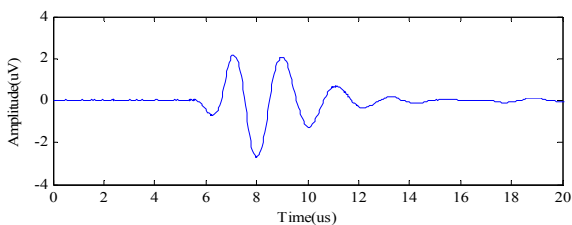


Fig. 2 Ultrasound waveform used in stimulation

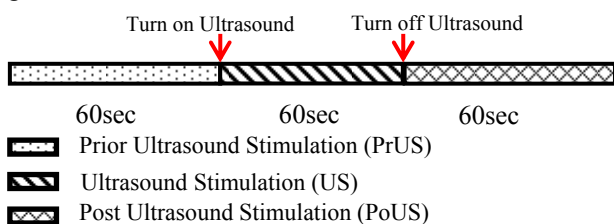


Fig. 3 Experimental protocol

Fig. 2 shows an ultrasound pulse waveform used in stimulation. We divided our experiments into two sessions: one for control and the other stimulation. In the control session, experiments consist of a three-minute recording with no US.

In the stimulation session, an entire three min. session was divided into three one min. sub-sessions. Fig. 3 shows our experiment protocol. During the first min. sub-session, no stimulation was applied (Prior Ultrasound Stimulation, PrUS), in the next one min. sub-session ultrasound stimulation was applied (US), and finally in the last one min. sub-session, there was no US (Post Ultrasound Stimulation, PoUS). In addition, to compare the neural activity under different ultrasound intensities (i.e., to examine the changes of neural activities under different ultrasound doses), the stimulation session experiments were repeated with three different ultrasound intensities with one-hour resting time between each experiment. All MEA data was acquired at a sampling frequency of 25 KHz.

C. Spike detection

To extract neural spikes, the recorded signals were first band-pass filtered between 300~3000Hz using the 4th ordered band pass filter. The threshold for spikes detection was set using the following [10],

$$\text{Threshold} = 6 * \text{median} \left(\frac{\text{abs}(X)}{0.6745} \right) \quad (1)$$

where X is the root mean square (RMS) value of the noise. We detected the spikes and compared the number of spikes in each period (i.e. PrUS, US, and PoUS).

D. Statistical analysis

The statistical significance in the number of action potentials during PrUS and US was evaluated using SPSS (SPSS Statics 21, SPSS, Chicago, IL, USA), the p-value of Paired t-test was 0.05 indicating the significant difference between the number of detected spikes during the PrUS and US conditions.

III. RESULT

For the control session experiments, the average threshold was $-37.59 \pm 7.57 \mu\text{V}$ for 57 channels. Fig. 4 (a) shows the raster plot of the detected action potentials when US was not present during the entire 3 min. period.

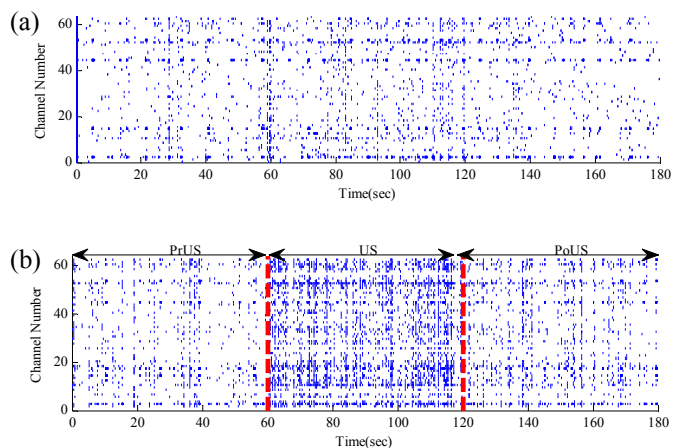


Fig. 4 Raster plots of neural spikes recorded on MEA (a) The control session with no US and (b) The stimulation session of PrUS, US, and PoUS.

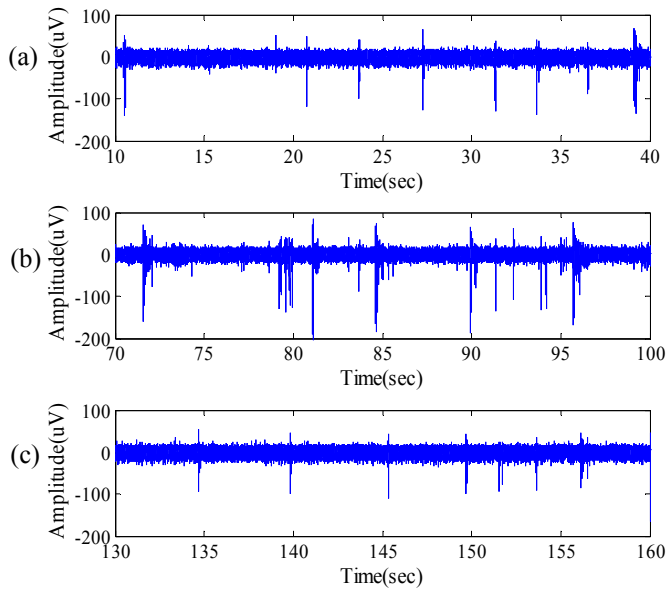


Fig. 5 Recorded neural activity signals on MEA during (a) PrUS, (b) US, and (c) PoUS

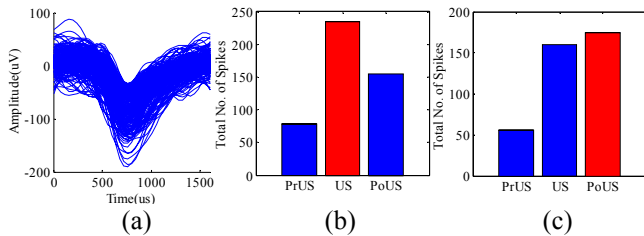


Fig. 6 (a) Overlapped waveforms of detected spikes (b), (c) the numbers of spikes during PrUS, US, and PoUS on two different channels of MEA

Fig. 4 (b) shows the raster plot of the detected spikes during the three sub-sessions of the stimulation session. An increase in the number of spikes was clearly observable during the second sub-session in the period of 60~120 sec. (i.e., US). During PoUS, the number of spikes decreased gradually as shown in Fig. 4 (b).

Fig. 5 shows the MEA signal measured from one channel during the stimulation session. Fig. 5 (a) shows the signal from PrUS. Fig. 5 (b) shows the signal from US, and Fig. 5 (c) from PoUS. The changes in action potentials are clearly visible in the three different sub-sessions.

Fig. 6 (a) shows the overlapped waveform of the detected spikes. Figs. 6 (b) and (c) show the comparison of the number of spikes during PrUS, US, and PoUS. In Fig. 6 (b), the detected numbers of spikes are 78, 235, and 155 from PrUS, US, and PoUS respectively. Again, in Fig. 6 (c), there are 56, 160, and 174 spikes during PrUS, US, and PoUS respectively. It was observed that the occurrence of spikes increased during US than PrUS at most channels (57 out of 60). One channel showed the decrease in frequency of spikes during PoUS as shown in Fig. 6 (b) and in two other channels, the frequency of spikes increased more than that of the US sub-session as shown in Fig. 6 (c).

Table 1. Results of Paired t-test on the number of spikes between PrUS and US ($P < 0.05$)

	N	Mean	SEM	T-value	p-value
PrUS	57	25.54	5.59	-11.21	3.07E-16
US		67.35	8.18		

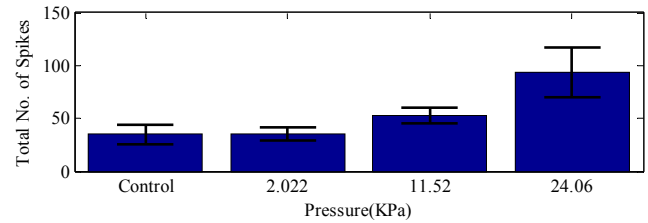


Fig. 7 Number of spikes in response to three different intensities of ultrasound stimulation

The results of the significance test for the occurrence of spikes for all channel ($N=57$) during PrUS and US are given in Table. 1 showing the average number of spikes of 25.54 ± 5.59 during PrUS and 42.17 ± 8.18 during US. The statistical difference in the detected number of spikes between PrUS and US ($T=-11.21$, $P \approx 3.07E-16$), indicating a significant difference.

Fig. 7 shows the frequency of action potentials in the neural cells ($N=4$) with three different stimulation intensities of ultrasound. The number of detected spikes (mean \pm SEM) was 35.22 ± 9.38 during the control session, but 34.91 ± 6.25 during the US sub-sessions with the ultrasound intensity of 2.02Kpa, 52.58 ± 7.39 of 11.52Kpa, and 93.90 ± 23.78 of 24.06Kpa, showing the proportional increase in the number of spikes upon the increase of stimulation ultrasound intensity.

IV. DISCUSSION

Our results show that neural activities can be modulated via low-intensity and low-frequency ultrasound stimulation. The hypothesized mechanism of ultrasound stimulation relies on thermal and/or mechanical effects [11]. However, in our thermal measurements given in the 3 min. duration of ultrasound stimulation, the MEA well temperature rises less than 0.001°C , indicating low-intensity and low-frequency ultrasound induced almost no change in temperature. Therefore assuming the minimal effect of temperature in our experimental settings, it seems that the mechanical influence of ultrasound is greater on the increased neural activities of the cell. The precise mechanisms of US are not clearly elucidated yet and are still under investigation [12-16] and further investigations are needed. We plan to examine the mechanism with neural blockers and calcium imaging techniques.

Although we had observed excitatory responses from the cell during US, we noticed different responses after US (i.e., PoUS). In most channels, the activity decreased with the reduced number of spikes but on few channels the activity increased with more spikes. Our observation matches the observations in [5]. Again further investigation is necessary on the after effect of US.

Our results could serve as the basis for low-intensity and low-frequency ultrasound stimulation of the brain, which offers advantages of non-invasive and high concentration brain stimulation using ultrasound.

V. CONCLUSION

In this study, we observed the modulation in neural activity of the hippocampal neural cells of the rat brain during ultrasound stimulation. Our results could serve as the basis for low-intensity and low-frequency transcranial ultrasound stimulation at the brain level of small animals and humans.

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