Bioeffects of Low Dose Ultrasound on Neuronal Cell Function

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***Abstract*—A growing body of work demonstrates the therapeutic value of sub-ablation ultrasound on various tissues. If ultrasound can safely manipulate neuronal tissue, then it might be possible to use it to treat neurodegenerative diseases. Anticipating such future applications, we investigated reversible bioeffects of very low dose focused ultrasound on neuronal cell function in vitro. A rat hippocampal slice was cultured for 6 days and transferred to the well of a 60-channel multi-electrode array. An f/2.1 ultrasound transducer with a water-filled coupling cone was focused on a culture and excited with 100-μs, 4.04-MHz pulses ranging from 5.5 kPa through 77 kPa. Ultrasonically-evoked field potentials with biphasic amplitudes as high as 329.1 μV_{pp} were observed in the dentate gyrus region. There appears to be a stimulus threshold that lies between an incident ultrasonic pressure of 20 and 48 kPa.**

Keywords-ultrasonics; bioeffects; neuroscience; hippocampus; dentate gyrus, electrophysiology; evoked potential

I. INTRODUCTION

Therapy is an induced tissue state change that benefits the organism. Ultrasonically-induced state changes range from quasi-reversible (e.g., enhanced blood flow and enhanced membrane permeability) through irreversible (e.g., ablation for cardiac conduction blocks or tumor debulking). Quasi-reversible, sub-ablation therapy is of particular interest in treating diseases that are not immediately life-threatening; the reduced invasiveness favorably balances the risk-benefit calculus. This is especially true for early stage neurodegenerative diseases and chronic peripheral pain with a neural locus, where avoidance of permanent damage is foremost.

Peripheral nerve conduction blockage by insonation was reported by Gavrilov and co-workers [1-3]. Vaitakunas [4] reported a spectrum of peripheral nerve bioeffects, in which low levels of ultrasound enhance conduction, intermediate levels block conduction temporarily, and high levels ablate the nerve and block conduction permanently.

In the central nervous system, stimulus-response studies typically report field potentials, viz., voltage measurements of the electric field arising from an aggregate of cellular action potentials. Central nervous system bioeffects have been reported by Bachtold et al. [5] who demonstrated depression and enhancement of electrically evoked field potentials following insonation. Vykhodtseva and Koroleva [6] demonstrated reduced rat brain firing rates in vivo following insonation. Previously, we reported preliminary results of structural and functional effects of low-intensity ultrasound on neurological tissues in vitro [7], demonstrating biphasic ultrasonically-evoked field potentials in the rat hippocampus. Here we present the dose dependence of this response in the dentate gyrus region of the hippocampus.

II. METHODS

Culture preparation methods and transducer parameters, briefly summarized here, were described in detail elsewhere [6]. Under IACUC guidelines [8], a 400-μm thick hippocampal slice was obtained from an 8-day Sprague Dawley rat and cultured for 6 days. The culture was placed in a 60-channel electrode array well controlled by MC_Rack software (version 3.5.1.0, Multi-Channel System GmbH, Reutlingen Germany). The culture was irrigated with artificial cerebrospinal fluid (ACSF) saturated with a 95:5 O:CO_2 mixture [9]. An inverted optical microscope (Olympus America Inc., Center Valley PA USA) was used to determine the position of the hippocampal structures relative to channel electrodes.

A 42-mm diameter, 90-mm focal length ultrasound transducer with a 90-mm long degassed-water-filled resin cone, capped at the distal end with a latex membrane, was focused on the culture at about a 45° angle of incidence. The transducer was driven with 100-μs 4.04-MHz pulses of various amplitudes from a waveform generator, a radiofrequency amplifier (model 2100L, ENI, Rochester NY USA), and a custom impedance-matching network. The maximum incident ultrasonic radiation pressure was estimated with a needle hydrophone (model HNA-0400, Onda Corp., Sunnyvale CA USA) to be 77 kPa.

Radiation force balance readings [10] were used to scale the pressure estimates at lower intensities.

Three stimulus-response sweeps were recorded at each pressure. The radiation pressure was increased between each set of sweeps. Irrigation was turned off during the recordings in order to avoid streaming from the irrigation pumps. The irrigation fluid was changed between the stimulus-off configuration (0 kPa) and the lowest insonation level (5.5 kPa). The elapsed time between the onset of the 0 kPa and 5.5 kPa stimuli was approximately 5 minutes, and between the onset of the stimuli at other levels, approximately 90 s.

Digitized (20-kHz, 12-bit) waveforms were recorded from each channel relative to a standard reference channel. The recordings were 300 ms in duration: 100 ms pre-stimulus and 200 ms post-stimulus (excluding an approximately 1-ms blanking period beginning with stimulus onset to avoid amplifier saturation). The peak-to-peak amplitudes of the waveforms on the channels recording from the dentate gyrus (66, 76, 86, 67, 77, 68, and 78) were exported as text files from MC_Rack and imported into an Excel spreadsheet (Microsoft Corp., Redmond WA USA) for analysis.

As a control for system noise, similar recordings were made without the hippocampal slice culture, in two configurations: with only ACSF filling the electrode array well, and with a slice of agarose gel (similar in size to the hippocampal slice) plus ACSF. In addition, a digital multimeter (Fluke Corp., Everett WA USA) was used to estimate the electrical impedance of the latex membrane material that sealed the transducer cone. The multimeter leads were coupled to the latex with normal phosphate buffered saline.

III. Results

Fig. 1 shows aspects of the response of the dentate gyrus region of the rat hippocampal culture to varying ultrasonic

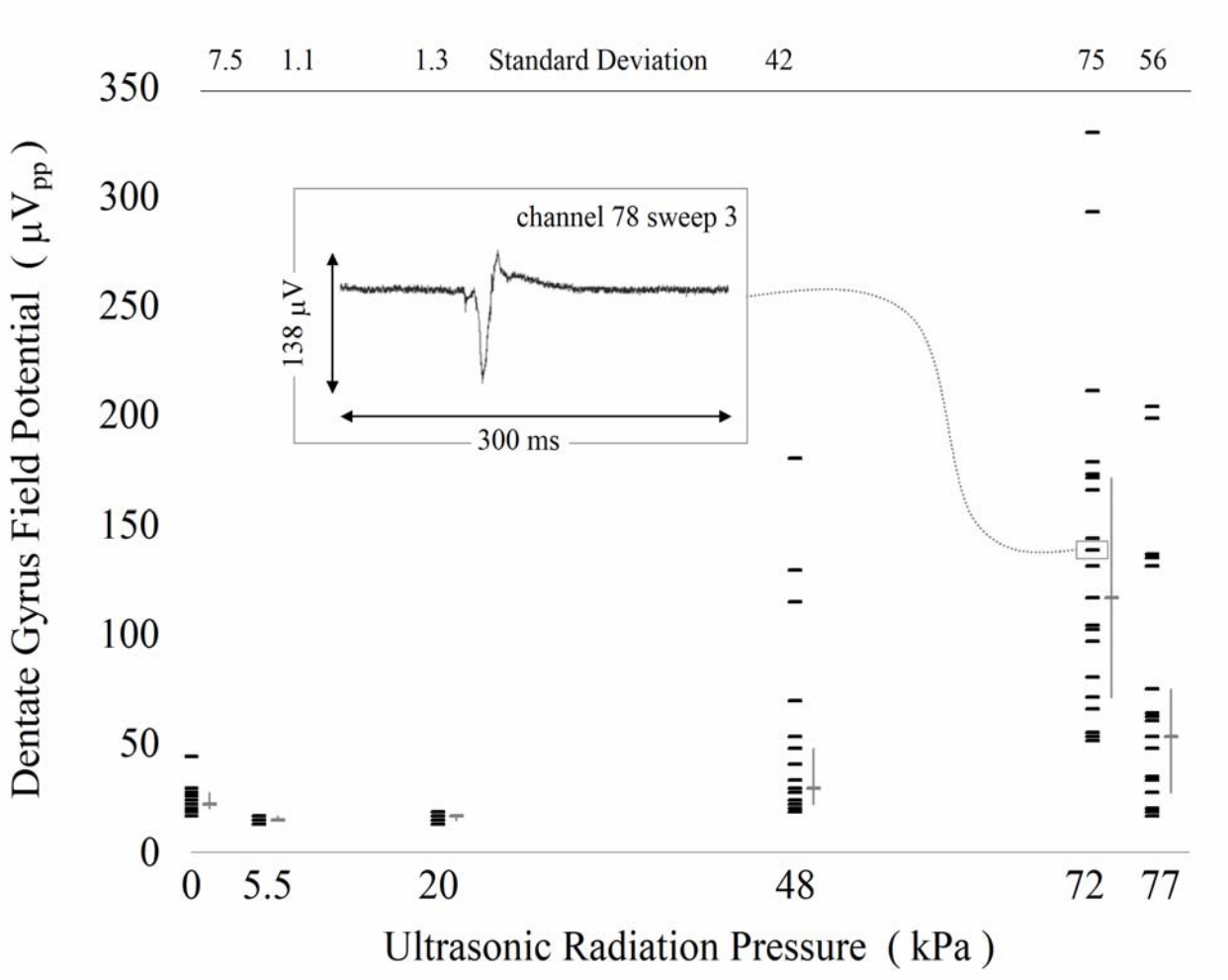

Figure 1. Response of a rat hippocampal slice culture to varying ultrasonic dose. The lower horizontal axis linearly displays the incident ultrasonic radiation pressure, measured with a hydrophone at 77 kPa, and scaled via radiation force balance readings at lower settings. The ultrasonic stimuli were 4.04-MHz, 100-μs pulses from a 42-mm diameter f/2.1 transducer. The focal region was aligned with the hippocampal surface, incident at about 45°. The vertical axis displays the peak-to-peak electrical potentials of the dentate gyrus (DG) channels relative to a standard reference channel. Three sweeps were recorded at each dose, digitized at 20 kHz and 12 bits. The standard deviations of the 21 samples per dose are displayed across the top of the figure. The gray bars to the right of the potentials represent the 1st, 2nd (median), and 3rd quartiles. The inset is a sample waveform from the third recording (or sweep) of channel 78. The recordings were 300 ms in duration: 100 ms pre-stimulus and 200 ms post-stimulus, with an approximately 1-ms blanking period at stimulus onset.

dose. The lower horizontal axis linearly displays the incident ultrasonic radiation pressure. The vertical axis displays the peak-to-peak electrical potentials of the dentate gyrus. The standard deviations of the peak-to-peak amplitudes of the 21 samples per dose are displayed across the top of the figure. The gray bars to the right of the potentials represent the 1st, 2nd (median), and 3rd quartiles. The inset is a sample waveform from the third recording of channel 78.

Waveforms for 0, 5.5, and 20 kPa stimuli exhibit no evident biphasic shape; the waveforms appear to be noise. The peak noise levels at 5.5 (median amplitude = 14.5 μV_{pp}) and 20 kPa (16.4 μV_{pp}) appear somewhat lower than the noise level at 0 kPa (21.8 μV_{pp}).

Many of the waveforms at 48, 72, and 77 kPa stimuli exhibit biphasic shapes (as seen in the sample waveform shown in the inset of Fig. 1) that are very similar to the electrically-evoked waveforms [7].

At the highest three stimulus levels, the waveform peaks exhibit high median amplitudes (29.1, 116.4, and 52.7 μV_{pp} respectively), large standard deviations (41.8, 74.7, and 56.0 μV_{pp}), and high maximum amplitudes (180, 329.1, and 203.6 μV_{pp}).

No signals, and only very low levels of noise, were detected on any channels recorded in the no-culture configurations. In addition, the DC impedance of the latex membrane exceeded the 50 MΩ limit of the multimeter:

IV. Discussion

The slight trend towards quieter cells at 5.5 and 20 kPa relative to 0 kPa might be statistically insignificant. Should it prove to be a real effect, the cause might be the refreshing of the culture by an exchange of ACSF.

Overall, the graph exhibits a threshold-like aspect; stimuli at and above 48 kPa exhibit robust biphasic responses. This is seen in the median, the range, and the standard deviation of the peaks.

It is possible that the decrease in responsiveness at the highest intensities is due to fatigue. Similar reductions in response amplitude were noted anecdotally but were not addressed systematically in this study. Rinaldi et al. [11] reported a similar ultrasonically-induced fatigue effect on electrically-evoked field potentials.

One possible mechanism for the observed responses is electrical leakage from the apparatus. However, the front surface of the transducer was insulated by over 50 MΩ from the ACSF. Also, recordings without cell cultures exhibited no evident signal. Therefore, electrical leakage seems an unlikely mechanism.

V. Conclusions

Ultrasonically-evoked field potentials have been observed; low-dose ultrasound can stimulate neurons. There appears to be a threshold for the stimulus that lies between an incident ultrasonic pressure of 20 and 48 kPa.

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