

Hippocampal Culture Stimulus with 4-Megahertz Ultrasound

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Abstract. Among current modalities, ultrasound uniquely offers both millisecond and millimeter accuracy in noninvasively stimulating brain tissue. In addition, by sweeping the ultrasound beam within the refractory period of the neuronal tissue, ultrasonic neuromodulation can be adapted to target extended or multiply connected regions with quasi-simultaneity. Towards the development of this safe brain stimulus technique, the response of rat hippocampal cultures to ultrasound was investigated. Hippocampal slices, 0.4-mm thick, were obtained from 8-day old Sprague Dawley rats and cultured for 6 days. The in vitro cultures were exposed to multiple 100-ms 4.04-MHz ultrasound pulses from a 42-mm diameter, 90-mm spherical cap transducer. Peak pressure ranged from 0 through about 77 kPa. Responses in the form of electrical potentials from a sixty channel electrode array were digitized and recorded. The DG and CA1 regions of the hippocampus exhibited similar ultrasonically-evoked field potentials.

Keywords: neuromodulation, brain, stimulus, response, evoked potential, refractory period

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INTRODUCTION

Ultrasonic backscatter reveals that acoustic radiation force elastography is capable of moving tissue reliably and repeatedly over a displacement less than the diameter of a single cell [1]. Optical microscopy of insonified cultured cells (PC12 cells from the rat adrenal gland) confirms this and demonstrates reversible distortion [2]. These observations are consistent with the early work of Wood [3] and more recent studies by Haake [4] and Lee [5] and their coworkers. Structural changes suggest accompanying functional changes. Neuronal tissue responses to insonification have been reported peripherally [6,7] and centrally [8,9,10], although the underlying mechanism (whether radiation force directly, radiation force via extracellular and intracellular streaming, cavitation, or heating) has not yet been determined.

An understanding of the spatial and temporal characteristics of the neuronal response to ultrasonic stimulus is critical for the development of future clinical applications of ultrasonic neuromodulation. The rat hippocampal culture in combination with a multi-channel electrode array offers a precise means of spatially and temporally tracking responses to ultrasonic stimuli. To investigate how different hippocampal regions responded to the same stimulus, additional data from a previously reported experiment [2,10] were analyzed.

METHODS

A systems diagram of the apparatus is shown in Fig. 1. Full experimental details were presented previously [2,10] and are summarized here. Hippocampal slices were obtained from 8-day old Sprague Dawley rats and cultured for 6 days [2]. The in vitro cultures were exposed to multiple 100-ms 4.04-MHz ultrasound pulses from a 42-mm diameter, 90-mm spherical cap transducer. The free field peak pressure in water was estimated to be about 77 kPa with a ruggedized hydrophone (Onda Corp., Sunnyvale CA USA, model HNA-0400 [11]). The full width at half power of the focal region was calculated to be about 1 mm transversally. The angle of incidence of the beam was about 45°. Stimuli proceeded from low (sham) to high (77 kPa). Three sweeps were made at each dose.

Responses in the form of electrical potentials from a sixty channel electrode array (Multi Channel Systems GmbH, Reutlingen Germany) were digitized and recorded [2]. The ultrasonically-evoked field potentials were averaged over three sweeps and two hippocampal regions determined with optical microscopy: CA₁ (Cornu Ammonis, mostly pyramidal cells) and DG (Dentate Gyrus, mostly granular cells). The transducer was fixed in one position and not moved among regions or between trials.

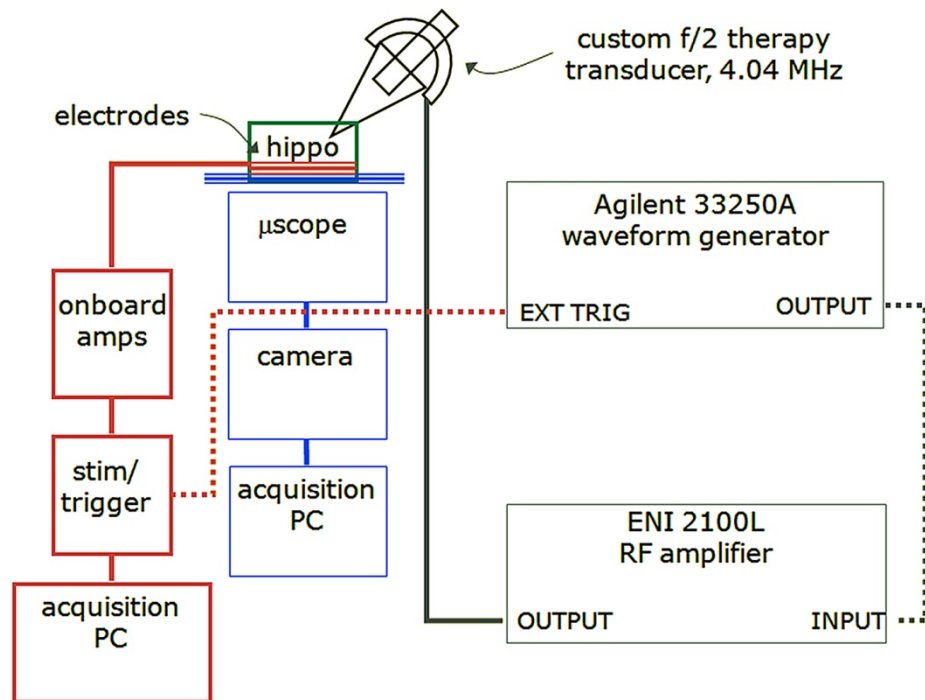


FIGURE 1. Neuromodulation apparatus used to record electrical activity from a rat hippocampal culture in response to ultrasonic stimuli.

RESULTS

The Dentate Gyrus and Cornu Ammonis regions of the hippocampus exhibit similar dose-response profiles (Fig. 2): a quieting of the system at very low dose (5.5 kPa), an apparent threshold (between 20 and 48 kPa), and possible fatigue at the highest dose (77 kPa), which was the last stimulus.

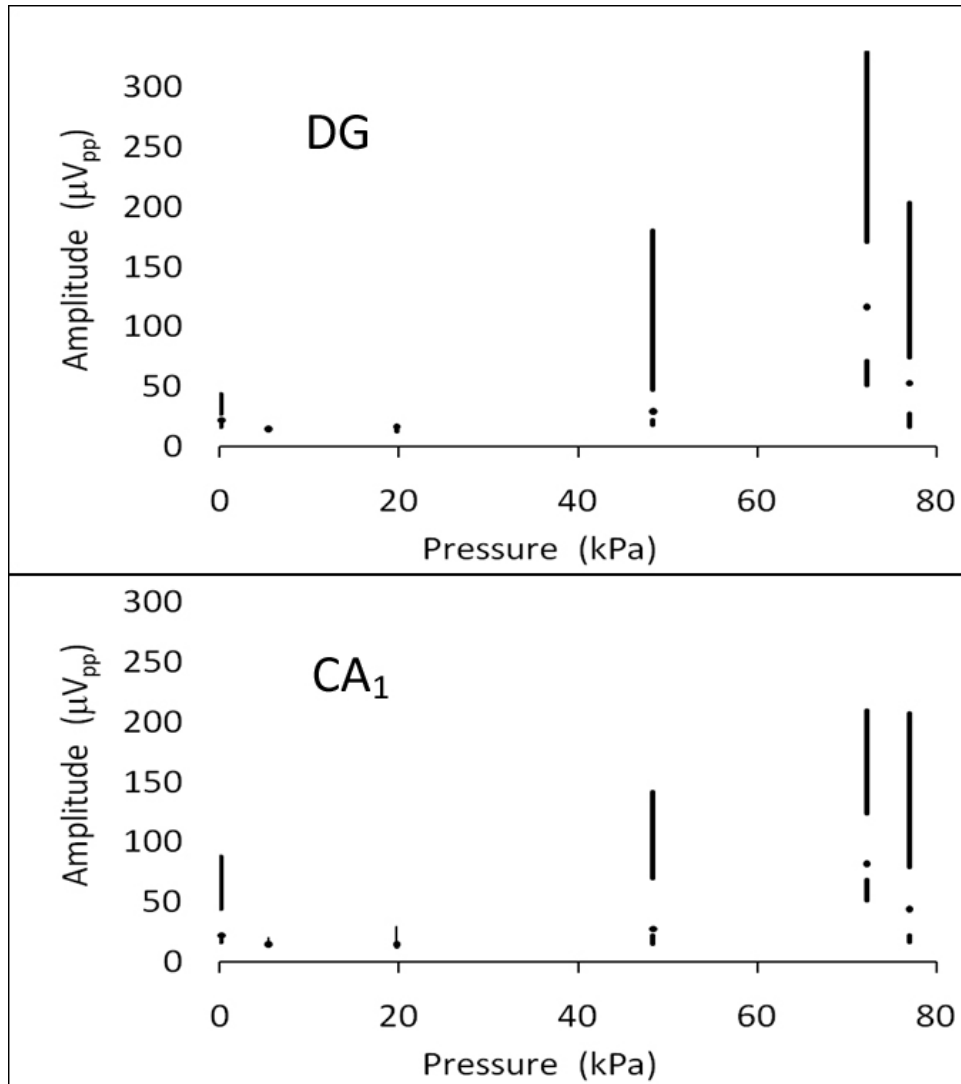


FIGURE 2. Dose-response quartiles of two regions, Dentate Gyrus (adapted from [10]) and Cornu Ammonis, of a rat hippocampal culture to ultrasonic stimuli. Stimulus (shown on horizontal axes) proceeded from low intensity to high intensity. Response (shown on vertical axes), measured peak-to-peak, was the average of three sweeps and the electrodes associated with the hippocampal region. The graphs display minimum, first quartile, median (central dot), third quartile, and maximum values of the response at each dose.

DISCUSSION

Refractory periods in brain tissue range from the Hodgkin-Huxley period of several milliseconds [12], through the fatigue suggested in Fig. 2, through spreading depression of several minutes [8]. Thus, when a small region of the brain tissue is stimulated, neurons in that region will be unresponsive for a time. During this refractory period, the effective area (e.g., the focus) of the incident ultrasound beam can be moved to a new location, stimulating tissue there. By rapidly moving the beam throughout an extended region of interest, it is possible in principle to bring all the tissue within that extended region into refraction simultaneously. Therefore, rather than creating a complicated beam shape, it is instead necessary only to move a simple beam shape rapidly to sweep out the desired complicated shape. Furthermore, by modulating the beam intensity as it sweeps, it is possible to produce discontinuous (or in mathematical terms, “multiply connected”) regions of simultaneous refraction (Fig. 3). Whether these extended and discontinuous regions of refracted neuronal tissue act as stimulated or as inhibited tissue remains to be determined experimentally.

If the discontinuous regions can be stimulated simultaneously (either by rapidly sweeping a beam within the refractory period as described above or by using multiple beams or multiple transducers), it might be possible to achieve Hebb’s criteria [13] and encourage association among the regions. Thus ultrasonic neuromodulation has the potential to become a powerful tool for treating a range of developmental and neurodegenerative disorders.

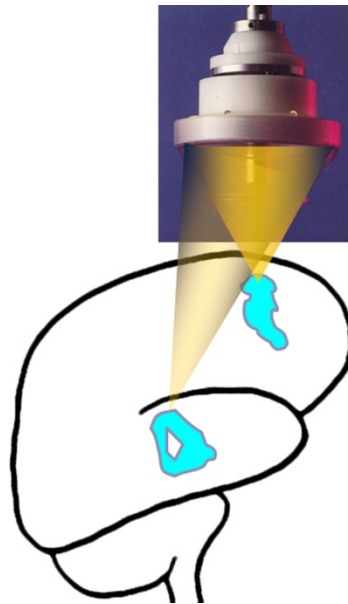


FIGURE 3. Sweeping a simple beam shape throughout an extended region of interest or among discontinuous regions within the refractory period has the potential to bring all of the targeted tissue into refraction simultaneously. This sketch omits the skull, through which the neuromodulation beam presumably can be aimed.

CONCLUSIONS

Ultrasonic stimulus of a hippocampal culture produces a similar response in the Dentate Gyrus and Cornu Ammonis regions. Refractory periods can be exploited to tailor the shape of the stimulated or inhibited regions to the neurobiology rather than to the conveniences of beam formation.

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