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Response Analysis of Brain Nerves System Cultivation Cell by Ultrasound

超音波照射刺激による脳神経系培養細胞の応答解析

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1. Introduction

Ultrasound neuromodulation is currently expected as a method of rehabilitating the body paralyzed by cerebral infarction or stroke [1]. As a prior study several research groups have made the rats sleep on anesthesia, ultrasound stimulation is given, and the motion of the tail and lower limbs is successfully induced [2]. On the other hand, it was reported that the induction of motion of rats by ultrasonic stimulation is not triggered by irradiation of ultrasound to the brain, but the effect that rat's auditory cortex senses sound [3].

In this research, we analyze the response of cells by ultrasound stimulation using cultured cells, and ultimately aim to clarify the mechanism of ultrasonic neuromodulation.

2. Materials and Methods

Cells were taken out from cerebral cortex of 19-day-old rat fetus and cells cultured for about 27-35 days were irradiated with ultrasonic waves and the response was analyzed. The following experimental system was constructed with the purpose of analyzing Ca^{2+} response to ultrasound stimulation of nerve cells and glial cells (Fig.1).

Neurons and glial cells were excised from rat fetal cerebral cortex and cultured on dishes. Changes in intracellular Ca^{2+} concentration were detected using the fluorescent probe Fluo-8 AM. Fluorescent images of cell groups were acquired using a microscope, a confocal scanner unit, a laser oscillator, and a camera.

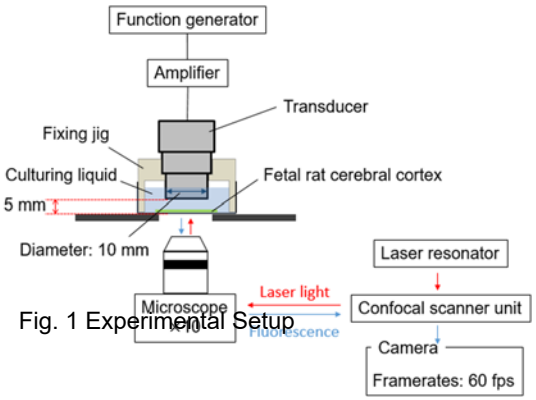

Fig. 1 Experimental Setup

3. Results and Discussion

3.1 Difference in ultrasound stimulation response between nerve cells and glia cells

In order to confirm the difference in responses of neurons and glial cells to ultrasound irradiation, ultrasound irradiation experiments were performed after selectively staining only neurons. Cultured cells were irradiated with ultrasound having a frequency: 1.5 MHz, a sound pressure: 0.97 MPa, and an irradiation time: 41.7 ms, and image capture was performed at a frame rate of 1 fps. It shows how it responds at that time (Fig. 1, 2). The numbers shown in the upper left of the images in Fig. 1, 2 are the numbers of consecutive images, and a total of 60 images were taken. It was irradiated between 29 th and 30 th images. Also, it

shows the state of luminance change of the continuous image (Fig. 3).

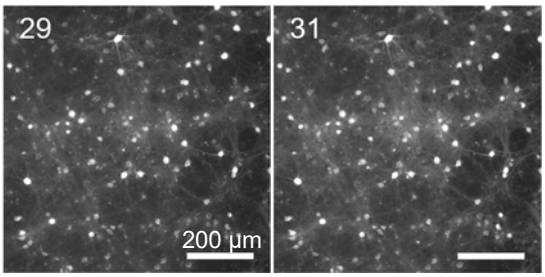

Fig. 1 Consecutive images of cells stained with Fluo-8.

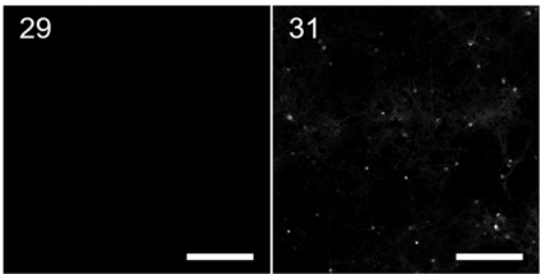

Fig. 2 Continuous image obtained by difference processing with the first image as background.

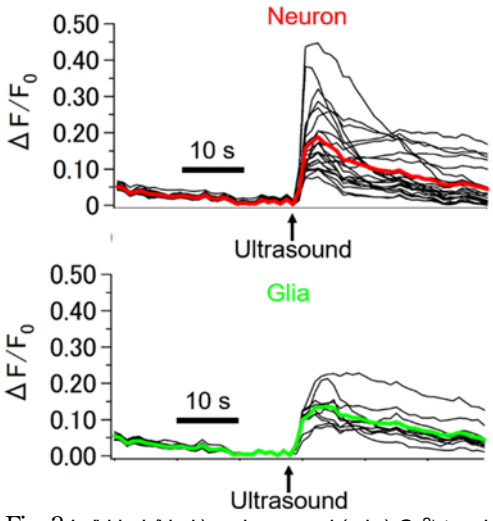

Fig. 3 Individual (black) and averaged (color) Ca^{2+} transients of neurons (red) and glial cells (green)

3.2 Frequency dependence of ultrasound stimulation response

Ca^{2+} responses of cells to different frequencies of ultrasound were observed and evaluated for frequency dependence. Three kinds of frequencies of 0.5 (N = 11), 1.5 (N = 6) and 8 (N = 5) MHz were used. Ultrasound waves with an irradiation time of 41.7 ms were irradiated while varying the sound pressure in the range of 0.067-0.29, 0.19-1.43, 0.13-1.4 MPa, respectively. The response probability in each sound pressure zone is compiled for each frequency by partitioning the sound pressure range. It was suggested that 0.5 MHz compared to 1.5 and 8 MHz can cause a response with higher probability at lower sound pressure.

4. Conclusion

In this study, to clarify the mechanism of ultrasound neuromodulation, Ca^{2+} response to ultrasound stimulation of cultured neurons and glial cells was observed and the following findings were obtained. In the range of the time resolution observed, it was suggested that both neurons and glial cells have an acceptance mechanism for ultrasound stimulation. It was suggested that the higher the sound pressure of the irradiated ultrasound and the lower the frequency, the more likely it is that the Ca^{2+} response of the cell is caused.

References

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