Fabrication of a Miniature Figure-of-Eight Coil for Micromagnetic Stimulation on Neuronal Tissue

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Abstract – Micromagnetic stimulation (μMS) uses submillimeter coils to exert a magnetic stimulation on microscopic areas of neuronal tissues. Although various techniques related to μMS coils have been developed, few studies have tried the figure-of-eight geometry, commonly used for localized magnetic stimulation in medical fields. In this study, we fabricated a miniature figure-of-eight (mFo8) coil by arranging two microcoils and confirmed that an intermediate frequency (85 kHz) magnetic field (MF) exposure could be generated over 10 mT in a microscopic area. In addition, we evaluated the stimulus effects on a cultured neuronal network and found that MF exposure by using the mFo8 coil can suppress the synchronized bursting activity. On the basis on these results, we highlighted the applicability of the fabricated mFo8 coil for μMS on neuronal networks.

1. Introduction

Micromagnetic stimulation (μMS) is a localized magnetic stimulation technique that uses submillimeter-order coils ("microcoils"). The advantages of this technology are that the magnetic field (MF) distribution and MF-induced stimulus effects can be localized by reducing the size of coils [1], and precise magnetic stimulation can be achieved inside the specimen by enabling penetration of the targeted brain region [2–5]. The μMS can also eliminate thermal effects [6] due to low conductivity and suppress inflammatory reactions in combination with biocompatible materials [7]. However, regarding the design of microcoils, few studies have performed μMS experiments with figure-of-eight coils that are widely used for localized magnetic stimulation [8]. The feature of this magnetic stimulation method with the figure-of-eight coil is that a localized and strong current distribution can be induced at the intersection of coils along a certain direction. By constructing microcoils with the figure-of-eight shape, further localization of the stimulus area and control of the stimulus direction can be achieved. To fabricate a miniature figure-of-eight (mFo8) coil, we contacted two microcoils arranged in a pattern of eight. We also evaluated the distribution of the MF generated from the mFo8 coil and the heat generation characteristics during MF exposure experiments. In addition, cultured neuronal networks were exposed to MF by using the mFo8 coils to verify whether the device can induce responses evoked by magnetic stimulus. These experiments validated the effectiveness of the mFo8 coil for μMS experiments.

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2. Materials and Methods

To fabricate mFo8 coils for μMS, we used a three-axis micrometer stage (RollerBlock 13 M/M, Thorlabs Japan, Tokyo, Japan) to join two chip inductors (LQP18MN33NG02D, Murata, Kyoto, Japan) to produce the figure-of-eight shape with a size of 2 mm × 2 mm, as shown in Figure 1A. Two electroporation electrodes (CUY611P7-2, Nepa Gene, Chiba, Japan) were coupled to both ends of the chip inductor to energize the coils. To bond the chip inductor to the electrodes, we used a UV irradiation system (NS-ULEDN-102CT, NS-Lighting, Osaka, Japan) and UV-curing optical adhesive (NOA 61, Norland Products, Jamesburg, NJ), and the entire mFo8 coil and electrode section were used, as shown in Figure 1B. A coating with UV-cured resin prevented any contact between the metal part of the electrode and the culture medium.

To evaluate the MF exposure performance of the fabricated mFo8 coils, the MF intensity and distribution near the coil were observed. A function generator (WF 1973, NF Corporation, Kanagawa, Japan) and a high-speed bipolar power supply (HAS 4051, NF Corporation) were used to energize the mFo8 coil, and an 85 kHz sinusoidal wave with a 200 to 2000 Vpp(peak to peak voltage) was applied to both ends of the coil. The MF intensity was measured by using a gauss meter (5180, F.W. BELL (OECO), Milwaukie, OR) and an attached ultrafine diameter MF measurement probe (STD18-0404, F.W. BELL (OECO)). The MF distribution near the coil was measured by scanning the probe at various positions near the coil surface. In addition, the temperature elevation of the coil surface was also evaluated because the mFo8 coil must be placed near or in contact with the biological tissue during the exposure experiment. To evaluate the surface temperature of the mFo8 coil during MF exposure experiments, a fiber-optic thermometer (FL-2000, Anritsu, Kanagawa, Japan) was used to confirm the temperature change over time before, during, and after exposure to the MF.

In this study, we used cultured neuronal networks created from neurons derived from human-induced pluripotent stem cells (ReproNeuro, ReproCell, Kanagawa, Japan) as target samples to evaluate the stimulus response to MF exposure. The cultured neuronal networks were developed by using the dedicated medium (ReproNeuro MQ Medium, ReproCell) and B-27 Plus Neuronal Culture System (A3653401,

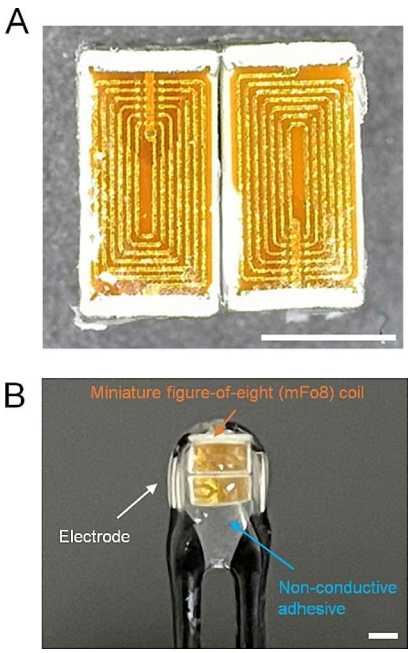

Figure 1. Appearance of the minimFo8 coil. Shape of (A) the mFo8 coil fabricated by coupling two chip inductor coils and (B) the mFo8 coil fabricated by a coupling of two electrodes. Scale bar = 1 mm.

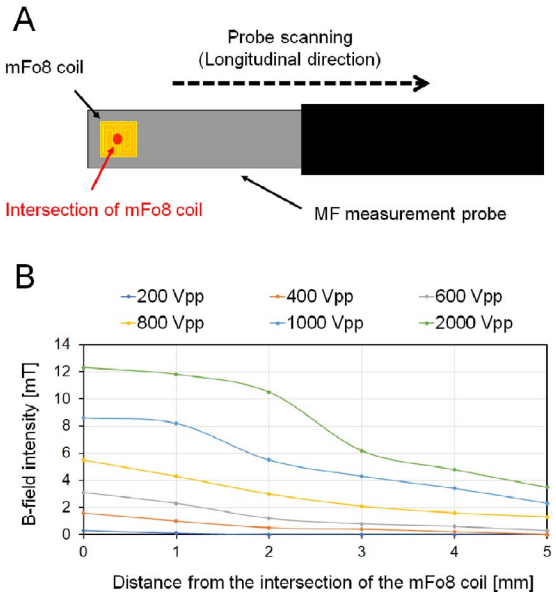

Figure 2. Evaluation of the MF distribution generated by the mFo8 coil. (A) A schematic of the MF measurement method. (B) Relationship between the input voltage value and the distance from the intersection of the mFo8 coil.

Thermo Fisher Scientific, Waltham, MA) that contained the 2% GlutaMAX supplement (35050061, Thermo Fisher Scientific) and 1% penicillin–streptomycin (15140122, Thermo Fisher Scientific). Using 96-well plates (MS-9096 U, Sumitomo Bakelite, Tokyo, Japan) with low-adhesion substrates during the differentiation induction process, cell clumps of human-cultured neuronal networks with three-dimensional structures were formed. The calcium fluorescent indicator (Fluo-8 AM, AAT Bioquest, Sunnyvale, CA) was used to evaluate the stimulus response induced by MF exposure. A fluorescence imaging system combined with a fluorescence microscope (Eclipse Ti, Nikon, Tokyo, Japan), cooled charge-coupled device camera (ImagEM, Hamamatsu, Shizuoka, Japan), and the associated software (Aquacosmos 2.6, Hamamatsu) were used to detect the intracellular calcium dynamics. The response stimulated by MF exposure was evaluated by using previously published methods [9]. For MF exposure of cultured human neuronal networks, the mFo8 coils were positioned in the vicinity of the cells by using a micromanipulator (MC-35 A, Narishige, Tokyo, Japan), and micropositioning was performed under fluorescence microscopy. In our experiments, the cultured cells were placed at the coil intersection, the magnetic stimulation spot in the figure-of-eight coil.

3. Results and Discussion

To evaluate the relationship between distance from the intersection of the mFo8 coil and the MF intensity, the MF distribution was evaluated at 1 mm intervals from the intersection of the mFo8 coil to 5 mm in the longitudinal direction by using the probe placement method with the highest average value, as shown in Figure 2A. Here, the MF measurement probe was attached with an mFo8 coil; therefore, the perpendicular direction was not evaluated. The input voltage values were set to be in the range of 200 to 2000 Vpp. The results shown in Figure 2B indicate that the MF intensity increased with the value of the input voltage and the MF distribution decreased, depending on the distance between the probe and the coil. When the distance from the intersection of the coil was more than 1 mm, the MF distribution significantly decreased. The trend of the MF distribution was relatively uniform within a distance of 1 mm or less from the coil center for the high-voltage inputs (1000 and 2000 Vpp). These results indicate that the fabricated mFo8 coil could be used to conduct high-intensity MF exposure experiments in a localized area.

In the MF exposure experiment using the mFo8 coil, a large voltage of up to 2000 Vpp was applied to both ends of the coil. Therefore, the effect of heat generation in the electrode and coil sections must be investigated. In this experiment, a fiber-optic thermometer was placed in contact with the surface of the mFo8 coil to measure the temperature change during and after exposure to the MF in real time. The temperature elevation was evaluated in air when a voltage of 1000 Vpp was applied continuously for 5 min, assuming the maximum exposure conditions in the experiment with cultured cells. Figure 3 shows that the temperature of the mFo8 coil before, during, and after MF exposure remained within the range of ± 0.5 °C. The results indicate that the surface temperature of the mFo8 coil due to energization did not increase the temperature to a

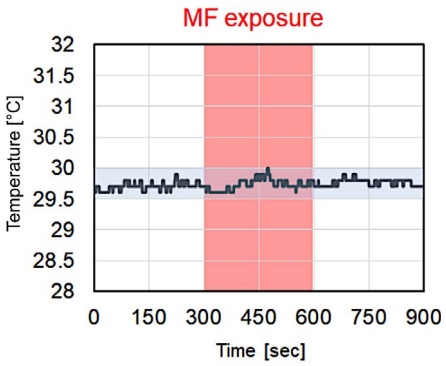

Figure 3. Surface temperature of the mFo8 coil before, during, and after MF exposure.

degree that could affect the cultured cells. Therefore, the heat generation of the coil and electrode sections could be suppressed by the UV-curable adhesive.

Because the surface temperature of the coil did not increase due to the energization, the mFo8 coil was placed in the culture medium, as shown in Figure 4A. To expose the high-intensity MF to the cultured neuronal networks, the positions of the mFo8 coil and the cultured neuronal networks were precisely aligned with the micrometer by using the micromanipulator and a movable stage. As shown in Figure 4B, the shape of the mFo8 coil was confirmed through fluorescence imaging in real time; therefore, we precisely placed the cultured neuronal networks at the intersection of the mFo8 coils where the induced currents are concentrated by simultaneously performing the fluorescence imaging and manipulating the cultured neuronal networks.

Next, Figure 5 shows the effect of MF exposure on the activity of human-cultured neuronal networks. Figure 5A shows an example of MF exposure suppressed by an intracellular calcium oscillation related to the synchronized bursting activity in the cultured neuronal network. The red area in Figure 5A shows the time duration of the 85 kHz sinusoidal wave MF exposure, which was continuously applied for 3 min at an input voltage of 1000 Vpp. Similarly, for the other samples, the number of synchronized bursting activity per minute was analyzed, and the frequencies of bursting activities observed during and after exposure were significantly reduced compared with that before exposure, as shown in Figure 5B ($n = 12$, $\pm$standard deviation, $^{*}P > 0.05$, $^{**}P > 0.001$). These results confirmed the reduction in the frequency of bursting activity during MF exposure, and these suppressive effects were sustained, although a slight recovery occurred after exposure. The trend of the suppression of synchronized bursting activities after MF exposure observed in our experiments was similar to that of a previous study, in which a hippocampal slice from a rat with epileptic seizures was stimulated with a 70 kHz μMS [10]. These results showed that our experimental system by using the fabricated mFo8 coils can be applied for μMS experiments to reveal the stimulus

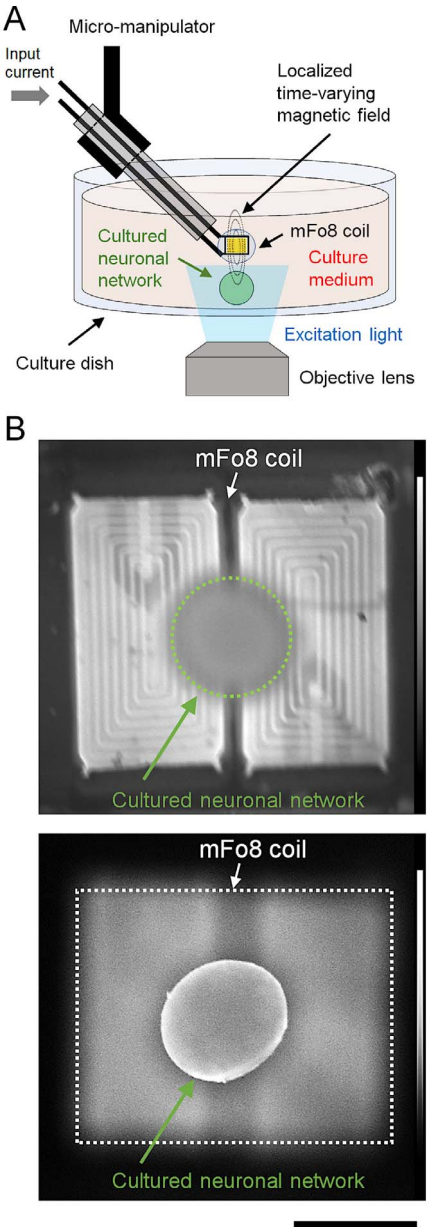

Figure 4. Experimental setup for MF exposure and fluorescent imaging by using mFo8 coils. (A) A schematic diagram of MF exposure and fluorescent imaging. (B) Real-time image of the position of cultured neuronal networks and mFo8 coils during fluorescent imaging.

effects of intermediate-frequency (85 kHz) MF on neuronal networks.

4. Conclusion

In this study, we developed the mFo8coil that generates an intermediate-frequency, high-intensity MF exposure in a microscopic area. The biological experiments using the mFo8 coil showed that the microscopic MF exposure can suppress the synchronized bursting activity patterns in cultured neuronal

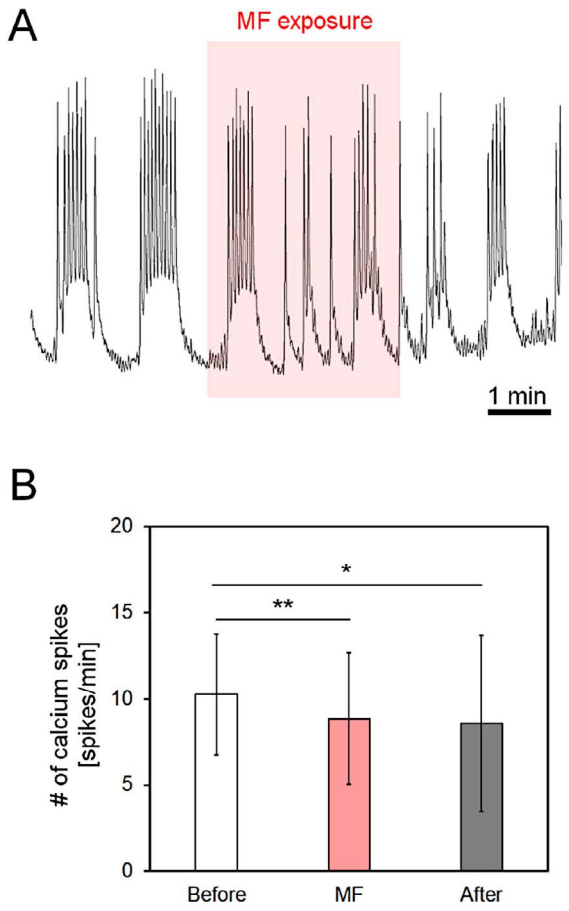

Figure 5. Suppression of the calcium oscillations related to the synchronized bursting activity by MF exposure. (A) Inhibition of synchronized bursting activities during MF exposure. (B) Significant reduction in the number of calcium spikes during and after exposure to MF. $n = 12$, ±standard deviation, $*P > 0.05$, $**P > 0.001$.

tissue. In future research, the local magnetic stimulation will be improved by measuring the MF distribution in more detail and further reducing the coil size. Furthermore, the effect of μMS in deep regions of the body must be verified through in vivo experiments by using individual animals.

5. Acknowledgments

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6. References

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