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Soft Magnetic Material Based Localized Magnetic Stimulation to Cultured Neuronal Cells and Modulation of Network Activities

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Abstract— **Magnetic stimulation is able to modulate the neuronal network activity using the non-invasive magnetically induced current. However, it is unknown how stimulation modulates the neuronal network activity. Therefore, we considered that precise stimulation and evaluation of the modulation of network activities in the vicinity of stimulated sites is required. Here, to establish precisely magnetic stimulation, we developed a Mu-metal that has high magnetic permeability soft magnetic material based localized magnetic stimulation (LMS) system with micro-fabricated dual cell-culture chambers. And, combining this device with a microelectrode array (MEA) permitted the evaluation of the stimulus effects at the stimulated and non-stimulated sites. Here, the dual cell-culture chambers were arranged in a concentric circle manner. Between the inner and outer chambers, 4, 8 and 12 connecting microfluid channels were fabricated using polydimethylsiloxane (PDMS). Rat cortical neurons were separately cultured in outer and inner chambers. Through the micro-conduits, functional synaptic connections were formed. Mu-metal was aligned along the outer circle, which allowed us of focal magnetic stimulation to the cells in the outer chamber. Applying low frequency magnetic field to the Mu-metal, induced currents were generated and the electrical activity of the cells in the outer chamber was modified depending on the stimulation intensity. Following the modified activity in the outer circles, the cells in the inner chamber also showed slightly depressed activity patterns. These results suggested that our system would be promising for highly regulated neural stimulation.**

I. Introduction

MAGNETIC stimulation has been used for some medical investigation for a long time. Especially, the treatment of the central nervous system (CNS) disorder using a magnetic stimulation is one of the innovative fields in the clinical techniques. In recent years, some disorders such as Parkinson's disease (PD) [1,2], epilepsy [3], and stroke rehabilitation [4] were treated with the magnetic stimulation and some reports expected the therapeutic efficacy of magnetic stimulation about CNS disorders. On the other hand, presently, the mechanisms that modulate the neuronal network activities by magnetic stimulation are unknown, and to reveal the dangerousness of magnetic stimulation the immediate and detailed research is required [5]. Here, we supposed that the difficulty of the evaluation of network activity in the stimulating site made the unclear mechanisms about stimulation effects. In addition, magnetic stimulation is produced by the electrical current which made alternating magnetic field, so localization of electrical stimulation and discrimination of the stimulated area are difficult. Then, although improvement of spatial resolution is tried by using the coil of figure-8 or double cone type, the reduction of a resolution according to the distance from and diameter of coils is not avoided. Moreover, these problems produce the physical constraint that related with the troubles of magnetic stimulation trial which used small samples, such as a cultured cells and test animals. Actually, in the research using laboratory animals or cultured neuronal cells has about 10 to 50 time difference of stimulation threshold [6]. And the sensitivity of stimulation is suggested which related with the difference of the single or multiple neuronal cells [7]. Therefore, the development of magnetic stimulation method for the cultured cells and the determining of exact stimulation threshold are important theme for the elucidation of stimulation mechanisms.

Here, we developed the regional magnetic stimulation method for cultured neuronal cells. For details, we proposed the stimulation method which can focus the external magnetic field to the random region using Mu-metal which is a soft magnetic material, and performed the numerical analysis and experimental trials. Moreover, we evaluated the network activities in the stimulated and non-stimulated sites during LMS by using the dual-cell culture chambers. And we examined the effect of LMS on the synchronized periodic bursting of cortical neuronal network using this stimulating method and the extracellular recording method of MEA.

II. Materials and Methods

A. Localized Magnetic Stimulation (LMS) System

In order to stimulate the local region of a cultured neuronal network, we applied the induced current which is generated in the vicinity of soft magnetic material in the low frequency magnetic fields (Fig.1.A). Here, LMS system is constituted by the alternating current magnetic field generator and soft magnetic material (Fig.1.B). To generate the low frequency magnetic fields, we used the high-speed bipolar power supply (BWS40-7.5, Takasago Co., Ltd.), the function generator (SG-4111, Iwasaki Co., Ltd.), and the air core coil (Jantzen Co., Ltd.). Mu-metal (Nilaco Co., Ltd.) was fabricated to the

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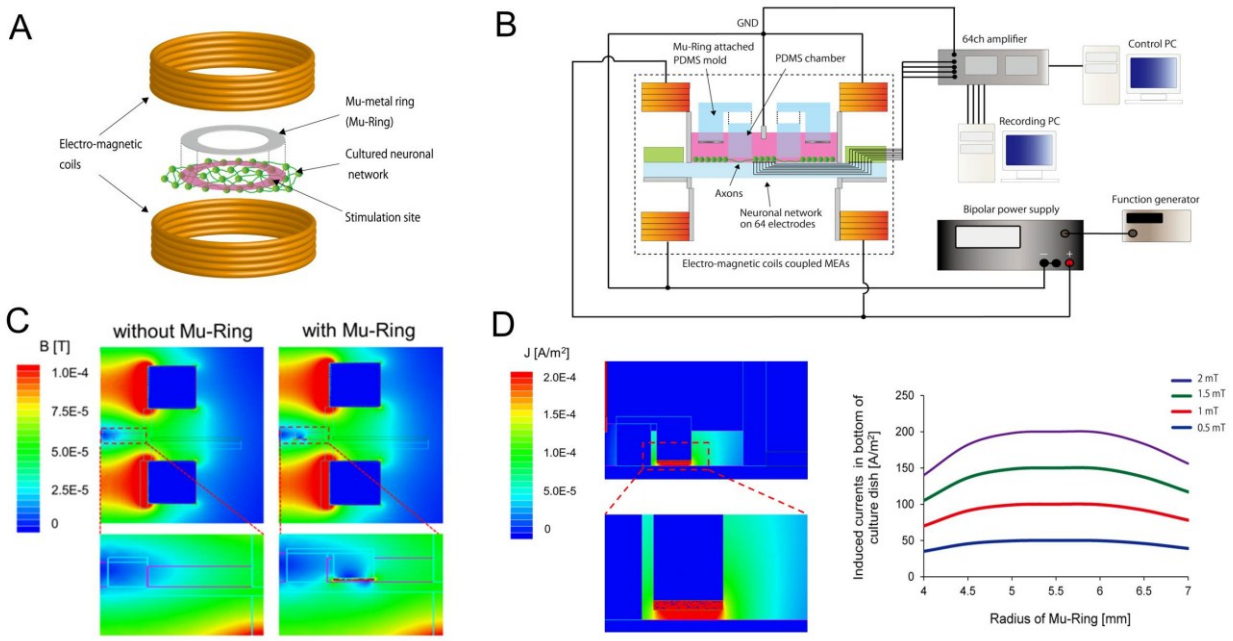

Fig. 1. Method of localized magnetic stimulation to cultured neuronal cells. (A) Schematic of localized magnetic stimulation using Mu-Ring. (B) System for the localized magnetic stimulation and the extracellular voltage recording of non-stimulated neuronal cells. (C) Numerical simulation of magnetic flux density in the cell culture dish with or without Mu-Ring. (D) Numerical simulation of induced current density in cell culture dish and variability of induced current density in bottom of Mu-metal.

concentric circle shape (briefly, Mu-Ring). Mu-Ring has 8 mm internal diameter, 14 mm outside diameter and 125 μm in thickness. In addition, we used the Maxwell 2D (Ansoft) which is electromagnetic field analysis software for the evaluation of the distribution of magnetic flux density and induced current density. At the time of magnetic field exposure to Mu-Ring, we used the two couple of air core coils (internal diameter: 24 mm, outside diameter: 48 mm, about 100 turns) and set up the Mu-Ring on the middle place of two coils. And to prevent the invasion of the induced current in non-stimulation site, the high magnetic density points were focusing in the outside of Mu-Ring for a stimulation period by two coils which had fully-reversed electrical currents (Fig.1.C). By this method, we tried the localized and non-invasive stimulation on the cultured neuronal networks under the Mu-Ring (Fig.1.D). Moreover, we evaluated the temporal response of the spontaneous activities during the LMS by using the optimally patterned MEA in the stimulation or non-stimulated site.

B. Cell Patterning using Dual-Cell Culture Chamber

For separation of stimulated and non-stimulated region, the dual cell-culture chamber was fabricated by using the polydimethylsiloxane (PDMS) (Silpot 184, Toray). Here, we prepared the microfluid channel (height: 4-10 μm, width: 100 μm) in the bottom of chambers using the SU-8 (3005, Microchem Co., Ltd.) mold and PDMS. By using this structure, we tried to separate the cell body in the inside and outside of chambers and connect two neuronal networks with only axons. Here, the isolation of the neuronal cells referred to the procedure of the previous work [8]. Briefly, the cerebrocortical tissue isolated from Wistar Rat embryo (18-19 days old) were treated enzymatically by the 0.5 % Trypsin solution (Sigma-Aldrich), and were dissociated physically by using a Dulbecco's Modified Eagle Medium (DMEM) (Gibco) which contain 10 % Fatal Bovine Serum (FBS) (Gibco), 5 % Horse Serum (HS) (Gibco), 1 % Penicillin-Streptomycin (Gibco). The extracted neuronal and glial cells were re-plated into the inner or outer region of dual-cell culture chambers which arranged on the polyethyleneimine (Sigma-Aldrich) coated MEA by density of 5×10^4 cells/mm^2. Moreover, we performed all the culture cells under 37℃, 5 % CO2, and 95% water-vapor conditions.

C. Extracellular Recording using Microelectrode Array

During LMS stimulation, the spontaneous activities of stimulated and non-stimulated site were recorded by MEA. Here, the electrodes patterns were designed with each evaluation method. In particular, the circle type electrodes were set the outer part of chamber and used for evaluation of stimulated site. On the other hand, the square type electrodes were set the inner chamber used for evaluation of non-stimulated site. In addition, all spontaneous activities observed from 64 electrodes were divided to the signals which have a frequency component of 100-2000 Hz by band pass filter (NF). After detection the signals were amplified 100000 times by the amplifier. And the detected signals were recorded on the 25 kHz sampling using the LabVIEW software (National Instruments) and the A/D conversion board (National Instruments). At the end, the recorded data

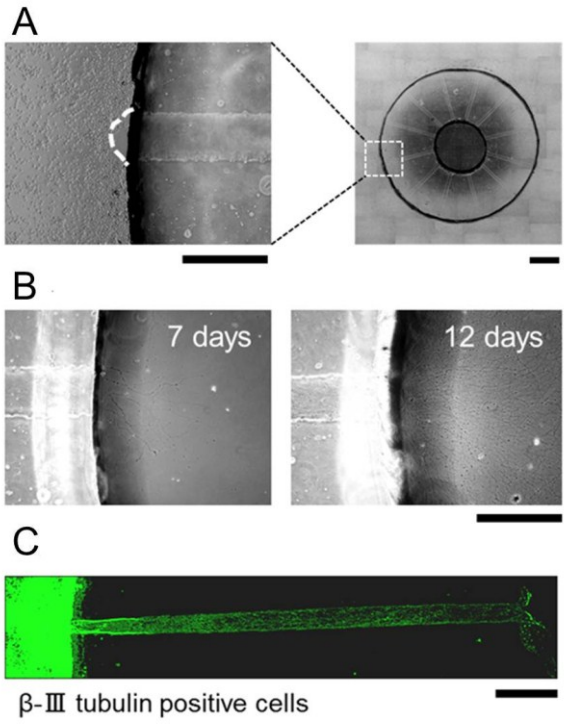

Fig.2. Axonal outgrowth in the microfluid channels. (A) The phase contrast images of the dual cell-culture chamber. (B) Intruded axons in the inner chamber. (C) β-Ⅲ tubulin positive cells in a microfluid channel.

were visualized by spike raster plot analysis using PV-WAVE software (Visual Numerics).

III. Results and Discussion

A. Axonal outgrowth in the microfluidic channels

First, to test the axonal outgrowth of neuronal cells, we plated the neuronal cells to the outer part of chamber and performed immunofluorescence staining. And, neuronal cells which placed in the outer part of chamber were collected near the channel using the surface tension and the hydraulic pressure difference (Fig.2.A). Here, the neurites extended from the outer part of chamber reached to the inner chamber during 7 to 12 days in culture (Fig.2.B). In addition, these neurites has β-Ⅲ tubulin positive parts (Fig.2.C). From these results, the axonal interaction of the separated neuronal networks was suggested.

B. Effects of LMS on the Neuronal Network Activities in the Stimulation Site

In this study, we focused the temporal and spatial dependence of network activities which were stimulated by LMS. Here, we arranged the position and the shape of MEA to set near the Mu-Ring, and evaluated the modulation of network activities in stimulated site (Fig.3.A). After one minute exposure of 0.5 Hz sinusoidal stimulation, the spontaneous activities were significantly increased compared with non-stimulated activities in near the stimulation site by student's t-test in statistical analysis (Fig.3.B). Moreover, for observation of the similar stimulation response, we repeated the stimulation to the neuronal networks in the same condition (Fig.3.C). Here, the response activities of a certain electrode during repeating stimulation are shown in Fig.3.C. The spontaneous activities which applied continuous LMS were disappeared about 3-5 seconds immediately after the stimulation. In addition, from the results of spike raster plot analysis, this stimulus-related effect was observed from almost all electrodes (Fig.3.D). On the other hand, the effects of stimulation did not continually in the entire period, and the persistence times were different in each electrode. Therefore, in near the stimulation site, the spatial response was similar, but temporal response was different in each stimulus.

C. Effects of LMS on the Neuronal Network Activities in the Non-Stimulation Site

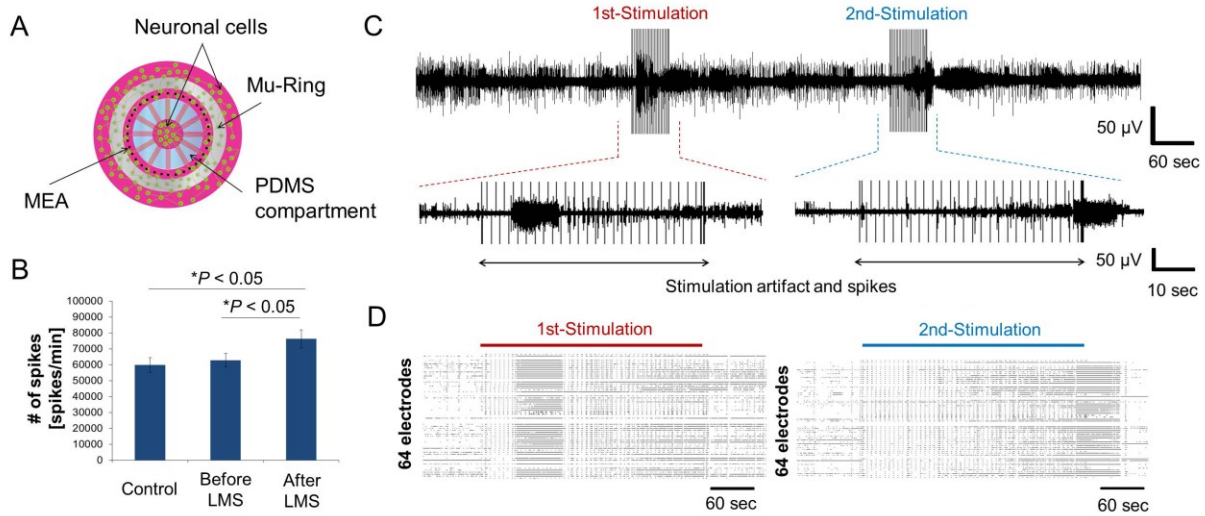

Fig.3. Effects of LMS on the neuronal network activities in the stimulated site. (A) Method of the stimulation and the extracellular recording in the vicinity of stimulation site. (B) Total number of spikes detected all microelectrodes per minute at the before and after of LMS. (n=4, ±SD, *$P < 0.05$) (C) Stimulation responses of spontaneous activities by repetitive LMS. (D) Raster plot of spontaneous activities which is detected from 64 electrodes during

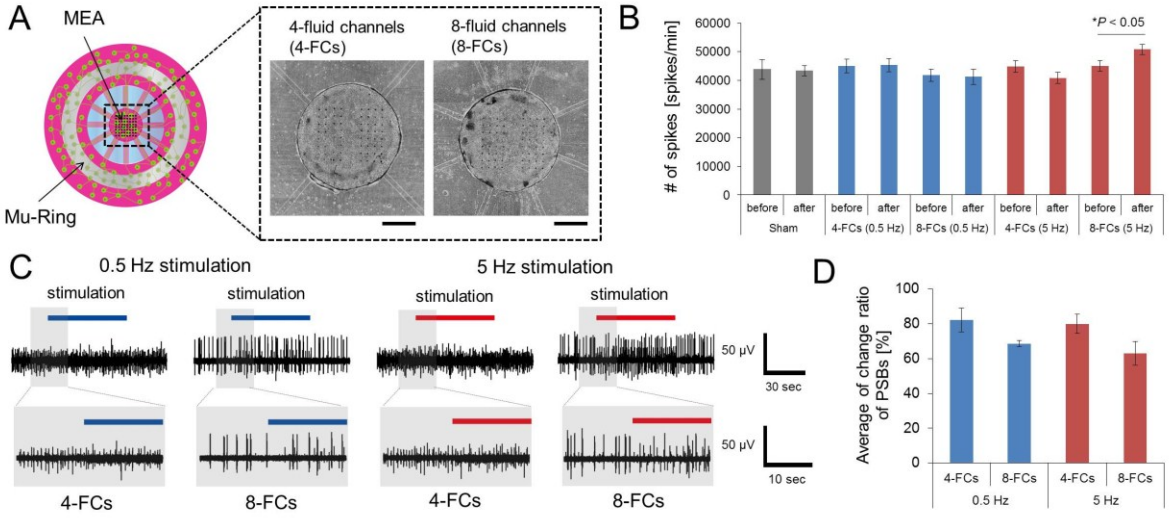

Fig.4. Effects of LMS on the neuronal network activities in the non-stimulated site. (A) Method of the stimulation and the extracellular recording in the non-stimulation site and two types of cell-culture chambers. (B) Effects of two types of stimulations on the different types of neuronal networks and the comparison of the total number of spikes per minute. (n=4, ±SD, $*P < 0.05$) (C) Effects of two type of stimulations and network interactions on the stimulation responses of spontaneous activities (without stimulation artifact). (D) Comparison of change ratio of the periodic synchronized bursting (PSB) activities on the two types of stimulation frequency and neuronal networks during LMS. (N=4, ±SD, $*P < 0.05$).

Next, to examine the propagation of the stimulation effects to the non-stimulated site, so we stimulated the neuronal network of outer part of chamber and recorded the network activity in the inner part which inter-connected with the outer part of chamber. Furthermore, we examined the effects of functional connection on the stimulation effect by using different type of chambers that has different number of microfluidic channels (Here, we used the 4 or 8 microfluidic channels) (Fig.4.A). Here, Fig.4.B is showing the comparative results of the number of spontaneous activities which were stimulated the 0.5 Hz or 5 Hz with 4 or 8 microfluidic channels. When 4 channels, the significant effects were not seen but in 8 channels, the number of spikes was increased after 5 Hz stimulation. Therefore, it was suggested the neuronal network activity of non-stimulated site is affected in the innervation from the stimulated neuronal network. Moreover, this phenomenon was related to the rate of innervation and stimulation frequencies.

Here, to reveal the stimulus dependent activity modulation, the periodic synchronized bursting (PSB) activities during stimulation were compared with non-stimulation periods. In all samples, the PSB activities were reduced immediately after stimulation. However, these durations were short as compared with that of the stimulation sites. In addition, the "high-density axonal innervation" provoked highly depression of PSB activities during stimulation period, but this phenomenon was not related with the stimulation frequency. On the other hand, the number of spikes after stimulation was clearly increased at 5 Hz stimulation, and significant changes were not seen in 0.5 Hz stimulation. Therefore, it was suggested that the high-density axonal innervation is necessary for effective propagation of the activity modulation, and the high-frequency stimulation was performed a crucial function that maintain the stimulus effect.

IV. Conclusion

In this study, we developed the localized magnetic stimulation method for cultured neuronal cells, and evaluated the field dependent effect using MEA. In the stimulation site, the synchronized periodic bursting was inhibited by localized stimulation. Moreover, the responsiveness of the stimulation modulated the non-stimulated neuronal network, and this modulation was changed with network formation and connection. In particular, stimulation responses of non-stimulated site were depending on the rate of axonal intrusion from the stimulated area. And the prolonged stimulation effects were observed from high frequency (5 Hz) stimulation. From these results, it was suggested that both axonal connection rate and stimulation frequency have affected the stimulus dependent network activity modulation.

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