

Real-Time Detection of Neuronal Network Activity Under 85 kHz Band High-Intensity Intermediate Frequency-Magnetic Field Exposure

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Abstract – Evaluation of the biological effects of intermediate frequency–magnetic field (IF-MF) exposure on the human body is important for developing high-power wireless power transfer technologies. However, the neuronal responses to high-intensity IF-MF exposure, which exceeds the biological bases of safety limits at the 85 kHz band, are currently inadequate. In this study, a nonconductive fiber-optic recording system is developed for real-time detection of neuronal network activities under 80 mT (RMS) in 82 kHz high-intensity IF-MF exposure environments. The experimental results revealed that the stimulus-evoked responses to IF-MF exposure were not observed. Conversely, the potentiation of neuronal activity was observed after IF-MF exposure. These results suggest that the proposed experimental system can evaluate the threshold of synaptic modulation using IF-MF exposure at a band of 85 kHz.

1. Introduction

Wireless power transfer (WPT) is a technology that transmits electrical energy through space. Recently, WPT with high energy, exceeding 100 kW in the 85 kHz band (79 kHz to 90 kHz), is being considered for

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application in electric buses and trucks [1]. In such applications, when an individual unexpectedly approaches the vicinity of such devices, they could potentially be exposed to an intermediate frequency–magnetic field (IF-MF), which exceeds the international guideline limitations. These guidelines are developed by the International Commission on Non-Ionizing Radiation Protection (ICNIRP) to protect humans against electromagnetic fields, and the limits are based on a threshold of stimulus or thermal effects [2, 3]. These limits protect humans from the adverse effects of electromagnetic field exposure. However, experimental data for human safety against short-term exposure, exceeding the ICNIRP guidelines, are inadequate. Therefore, a high-intensity IF-MF exposure system that can generate an 82 mT (RMS) IF-MF at 85 kHz bandwidth [4, 5] is developed. The system is expected to be used in the WPT charging of electric vehicles. The proposed system generates 80 mT (RMS) IF-MF at 82 kHz on 400 A current. This value of current is approximately 3000 times higher than the current reference levels (27 μ T at 82 kHz) set by the ICNIRP low-frequency guidelines [2] for the general public. In addition, the effects of the WPT system–related IF-MF exposure on rodents were evaluated, and the gene expression remained unaffected [6, 7]. Conversely, limited studies have explored the biological bases of the adverse effects defined by international guidelines, such as the thresholds of nerve stimulation or brain function. Therefore, knowledge of the adverse effects of electromagnetic fields on the nervous system, which reinforces the biological bases for the limitations of the international guidelines used in high-power WPT systems, must be acquired.

In this study, an optical recording system that detects the effects of IF-MF exposure on a neuronal network function in the central nervous system is constructed. In addition, the modulation of neuronal network activity, immediately after IF-MF exposure on neuronal networks by using a developed recording system, is evaluated. Based on these experimental results, the effectiveness of the proposed system for evaluating the effects of high-intensity IF-MF exposure in the 85 kHz band on the nervous system is discussed.

2. Materials and Methods

For the IF-MF exposure experiments, neuronal networks generated by human-induced pluripotent stem (hiPS) cell–derived neuronal progenitor cells (Repro-

Neuro, RCDN001N, REPROCELL) were used. The hiPS cell-derived neuronal progenitor cells were stored in a liquid nitrogen container and thawed on the day of differentiation induction. Before seeding the hiPS cell-derived neuronal progenitor cells, the surface of the culture dish was coated with two cell adhesion substrate solutions formulated for cell culture, that is, poly-L-lysine (P8920, Sigma-Aldrich) and a coating solution (ReproNeuro Coat, RCDN201, REPROCELL), on a 10 mm × 10 mm × 1 mm quartz glass plate. The cell culture was performed in a CO₂ incubator capable of maintaining 37°C and a 5% CO₂ concentrated environment. The cell adhesion substrate solution was removed on the day of seeding, and a specialized culture medium (ReproNeuro MQ, RCDN102, REPROCELL), which is a solution for differentiation into human neurons, was added to the culture dishes. Thereafter, the hiPS cell-derived neuronal progenitor cells were removed from the liquid nitrogen container and rapidly thawed in a water bath. Cell suspension with a seeding density of 3×10^5 cells per 60 mm of the culture dish was prepared using a basal medium (DMEM/F12, Thermo Fisher Scientific). The hiPS cell-derived neuronal progenitor cells were induced to differentiate into human neurons by replacing half the ReproNeuro MQ medium on the third, seventh, and 14th days.

To evaluate the functional maturation of the cultured neuronal networks, Neurobasal Plus (NBP) medium (A3582901, Thermo Fisher Scientific) was used as the optimal medium for long-term culture (more than three months). Additionally, 2% (v/v) B-27 Plus Supplement (A3582801, Thermo Fisher Scientific), 1% (v/v) Gibco GlutaMAX Supplement (35050061, Thermo Fisher Scientific), and 1% (v/v) penicillin-streptomycin (P4333, Sigma-Aldrich) were added to the NBP medium. Three weeks after the differentiation induction, the cultured neuronal networks were maintained using the NBP medium, and subsequently half the medium was replaced with the NBP culture solution every two to three days. For evaluation of the neuronal network activity, changes in the intracellular calcium oscillation were captured through a fluorescence imaging system combined with a fluorescence microscope (Eclipse Ti, Nikon) and a cooled CCD camera (ImagEM, Hamamatsu) with its associated software (AQUACOSMOS 2.6, Hamamatsu). These were used to confirm the generation of spontaneous activities or stimulus-evoked responses through electrical stimulation given from outside the cells. Immediately after a 82 kHz sinusoidal electrical stimulation (applied voltage: 1 Vpp), the electrical stimulation-evoked intracellular calcium oscillation was evaluated using a function generator (WF1973, NF Corporation) and a bipolar power supply (HSA4051, NF Corporation).

For the IF-MF exposure experiments, a previously developed high-intensity IF-MF exposure system was employed [4, 5]. A culture dish of 60 mm diameter was placed in the IF-MF exposure coil, and the cultured neuronal networks were placed within a 15 mm radius on the culture dish. Thereafter, the culture dishes were

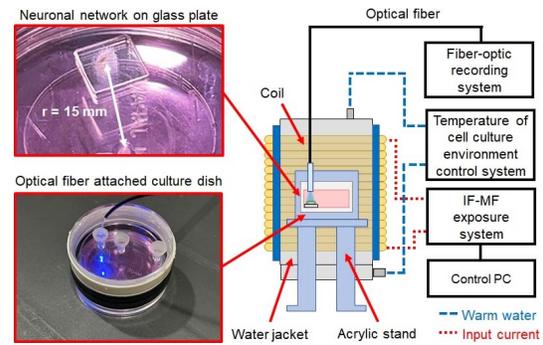


Figure 1. IF-MF exposure and nonconductive fiber-optic recording system with a hiPS cell-derived neuronal network cultured on a quartz glass plate. The culture dish and fiber optic are set in the IF-MF exposure coil for real-time detection of neuronal activity.

placed in a water jacket installed inside the IF-MF exposure coil. The IF-MF exposure experiments were then conducted at a temperature of $37 \pm 0.2^\circ\text{C}$. The IF-MF exposure duration was set to 1 s, for which the repetition time interval was set to 17 min, and fewer than 10 times IF-MF exposures were performed during each experiment. In addition, it was confirmed that the MF distribution in the coil was uniform at the position of the culture dish by numerical estimation during the development of the IF-MF exposure system [4].

To evaluate the effects of IF-MF exposure on neuronal activity, the calcium oscillation and subsequent electrical excitation of neurons were assessed. Quest Fluo-8 AM (AAT Bioquest) of 10 μM , which is used as a calcium fluorescent indicator, was added to the culture medium before the IF-MF exposure experiment. Thereafter, the medium was allowed to activate in the incubator at $37 \pm 0.2^\circ\text{C}$ for at least 30 min. Since the effects of IF-MF exposure on neuronal activity were transient and rapid, a real-time optical recording of the coils exposed to the high-intensity magnetic fields was taken. Hence, a newly developed nonconductive fiber-optic recording system was constructed based on previous studies [8, 9]. It could detect real-time changes in neuronal activity during IF-MF exposure by placing a cell culture dish with a fiber optic connected inside a coil, as shown in Figure 1. Optical data were acquired using a custom-made fiber photometry system (Dric Lenses) at a frame rate of 500 fps. Data required for calculating the rate of change in neuronal activity, before and after IF-MF exposure, were obtained using the LabChart Pro software (V8, ADInstruments). The changes in fluorescence intensity (ΔF) related to the calcium oscillation were represented by a fluorescent intensity ratio ($\Delta F/F$), which was normalized by background fluorescence intensity (F).

3. Results and Discussion

A functional evaluation of the neuronal networks prior to the IF-MF exposure experiments was performed. As per Figure 2, 1) the cultured neuronal network contained beta-III tubulin, which is a neuron-

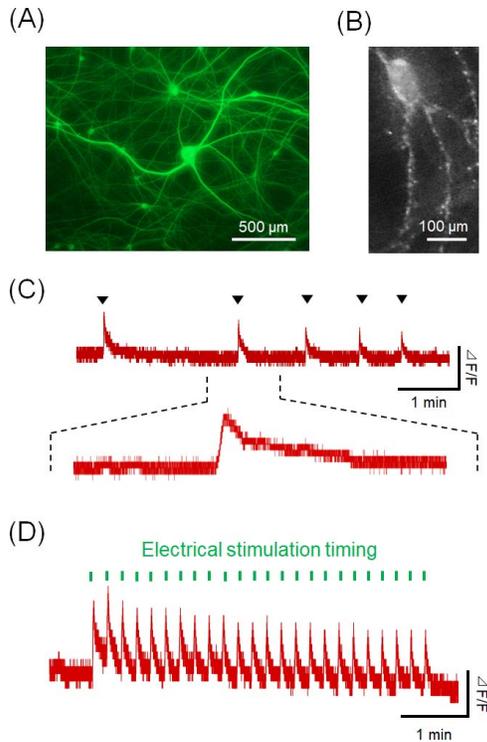


Figure 2. Functional evaluation of a hiPS cell-derived neuronal network with (A) beta-III tubulin and (B) synapsin-I-positive neurons, (C) spontaneous calcium oscillation, and (D) electrical stimulation-evoked calcium oscillation (green dots indicated the stimulation timing).

specific membrane protein; 2) the nerve fibers contained synapsin I, which is a synapse-specific membrane protein; 3) the neuronal network generated spontaneous calcium oscillations; and 4) the electrical stimulation induced membrane excitation-related calcium influx in the neuronal network. Thus, the differentiated human neuronal networks construct functional networks and are responsive to electrical stimulation.

The results of evaluating the stimulus-evoked responses in different cultured neuronal networks after the high-intensity IF-MF exposure are shown in Figure 3. The cultured neuronal networks, exhibiting spontaneous electrical activities, were exposed to 80 mT (RMS) IF-MF at 82 kHz. The stimulus-evoked responses in any sample could not be confirmed immediately after (<5 s) IF-MF exposure. Neuronal activity was continuously observed after IF-MF exposure, and no clear excitation or inhibition of neuronal activity was observed. Typically, stimulus-evoked responses are caused by the excitation of the neuron cell membrane, which occurs within 1 s after electrical stimulation. Therefore, no direct stimulus-evoked responses due to membrane excitation under the experimental conditions were observed.

Conversely, repeated IF-MF exposure to one group induced an increase in spontaneous neuronal activity compared to the nonexposed groups, as shown in Figure 4. Therefore, the frequency of neuronal

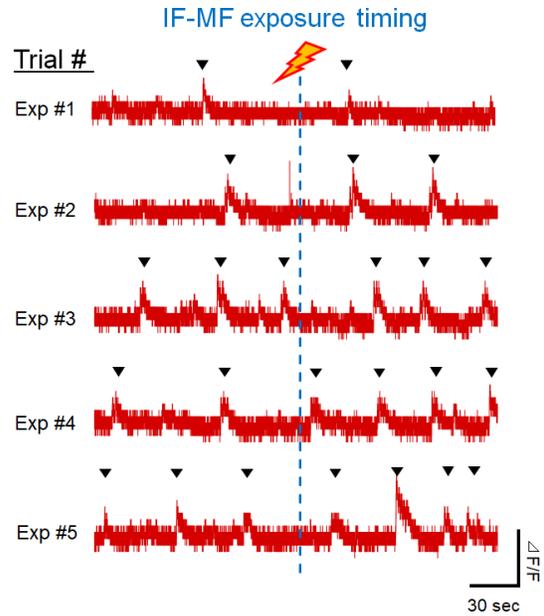


Figure 3. Evaluation of 80 mT (RMS) at 82 kHz IF-MF exposure stimulus-evoked response using the proposed nonconductive fiber-optic recording system. The inverse triangles indicate the spontaneous neuronal activities.

activity occurrence, measured 15 min before and after exposure to 80 mT (RMS) IF-MF at 82 kHz, was compared for different samples. The trend of increasing neuronal activity with stimulation frequency was also

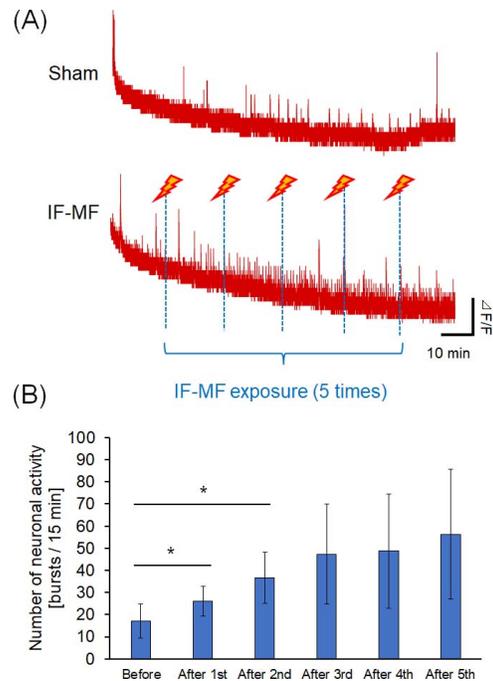


Figure 4. Effects of 80 mT at 82 kHz IF-MF exposure on neuronal activity, where (A) changes in firing patterns during the IF-MF exposure experiment and (B) changes in the number of synchronized bursting activity during repetitive IF-MF exposure are shown. Mean \pm standard deviation, $*P < 0.05$, $N = 3$.

examined. A significant increase in the frequency of neuronal activity after the first and second IF-MF exposure compared to the nonexposed group (mean \pm standard deviation, $*P < 0.05$, $N = 3$) is observed in Figure 4b. This suggests that the defined IF-MF exposure condition may alter the neuronal network activity that does not involve stimulus-evoked responses. After the third IF-MF exposure, the trend of increasing neuronal activity settled to steady-state values, and no significant changes were observed.

To estimate the factors that cause neuronal activity changes observed in the above experimental results, a comparison was made with a stimulus threshold derived from theoretical models described by the IEEE standard C95.-2019 [9]. For estimating the stimulus threshold of the central nervous system, theoretical models were used to calculate the “excitation threshold of central nerve fibers at 10 μm ” and the “threshold of change in brain synaptic activity” in the established model based on the action thresholds [9]. In this study, the hiPS cell-derived neuronal networks had a diameter of 1 μm . Therefore, differences in the axon diameter had to be considered while estimating the excitation threshold of the central nerve fibers. Theoretical models use axons with a diameter of 10 μm . Since the stimulus excitation threshold of the central nerve fibers was inversely proportional to the axon thickness, a correction coefficient of 10 was applied to the thickness of the hiPS cell-derived nerve fibers. The correction coefficient related to the axon thickness was not applied to the threshold for synaptic modulation [9].

Thus, the estimated theoretical stimulus excitation threshold for a nerve fiber of 1 μm diameter and the synaptic modulation threshold for the central nervous system at 82 kHz were 3010 V/m (peak) and 308 V/m (peak), respectively. The electric field intensity induced in the vicinity of the cultured neuronal networks in the magnetic field exposure experiment was approximately 439 V/m (peak) at a frequency (f) of 82 kHz. Furthermore, the magnetic flux density (B) was 113 mT (peak) or 80 mT (RMS), and the radius (r) was 15 mm. When the cultured neuronal network was positioned at 15 mm, the induced electric field strength ($E = \pi f B r$) was approximately 439 V/m (peak). These numerical estimates suggest that the changes observed in neuronal activity during the IF-MF exposure experiments may be due to the effects mediated by the synaptic modulation. Therefore, the proposed high-intensity IF-MF exposure and the nonconductive fiber-optic recording system could detect the IF-MF exposure-related synaptic modulation.

4. Conclusion

In this study, a recording system was constructed to evaluate the presence or absence of stimulus-evoked responses and detect changes in neuronal activity under a high-intensity IF-MF exposure environment. The

experimental results revealed that none of the stimulus-evoked responses were attributable to IF-MF exposure. However, an increase in neuronal activity was confirmed when the threshold for synaptic modulation was surpassed. These results indicate that the proposed system can be used to assess the threshold for synaptic modulation by IF-MF exposure in the 85 kHz band.

5. Acknowledgment

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6. References

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