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Non-conductive and miniature fiber-optic imaging system for real-time detection of neuronal activity in time-varying electromagnetic fields

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ABSTRACT

Establishing an appropriate threshold value for neuronal modulation by time-varying electromagnetic field (EMF) exposure is important for developing international guidelines to protect against the potential health effects, and to design a variety of medical devices. However, it is technically difficult to achieve real-time detection of neuronal activity under repetitive and long-term exposure to EMF. For this purpose, we developed a non-conductive, miniature, and flexible fiber-optic imaging system that does not affect the electromagnetic noise, induction heating, or vibration in a high-intensity and repetitive time-varying EMF exposure. Using the proposed system, we succeeded at real-time detection of spontaneous Ca^{2+} oscillations in single neuronal and glial cells, as well as synchronized bursting activities of multiple neuronal networks at a micrometer-scale and millisecond-order spatiotemporal resolution during long-term EMF exposure (sinusoidal wave, 20 kHz, 8.6 mT, > 30 min). The results indicated that short-term (< 5 min) exposure-related neuronal modulation was not detectable; however, long-term (15–30 min) exposure was observed to depress neuronal activities. In addition, the simultaneous and real-time recording of neuronal activity and the environmental temperature revealed that the neuronal modulation was accompanied by a 0.5–1 °C rise in the temperature of the culture medium induced by the heat generation of exposure coils. These findings suggest that our real-time imaging system can be used for precise evaluation of the threshold values and clarification of the mechanisms of neuronal modulation induced by time-varying EMF exposure.

1. Introduction

Time-varying and sufficiently strong electromagnetic field (EMF) can provide a stimulus to or exert a thermal effect on living tissues. In some human subjects, it has been confirmed that the low-frequency (1 Hz–100 kHz) EMF exposure stimulates the neuronal cells that constitute the central or peripheral nervous system (D'Arsonval, 1896; Thompson, 1910; Lövsund et al., 1979, 1980; Barker et al., 1985; Day et al., 1987; Cohen et al., 1990; Budinger et al., 1991; Bourland et al., 1999; Nyenhuis et al., 2001; Stefan et al., 2002, 2006). The threshold value of EMF exposure-related nerve stimulation serves as an important biological indicator to develop an international guideline for prevention of the adverse health effects (ICNIRP 1998, 2010; IEEE, 2002, 2005), or design of a variety of medical devices (Nyenhuis et al., 2001); however, the detailed mechanisms of nerve stimulation by EMF exposure are not clear. In fact, based on the lack of scientific understanding of a neuronal modulation by EMF exposure, the World Health Organization (WHO) Environmental Health Criteria 238 (EHC 238) recommended the elucidation of a threshold value of multiple networks within the nervous system (WHO, 2007). Furthermore, the threshold values detected in several experiments have been considerably variable compared with the estimation results (Bawin et al., 1984, 1986; Jefferys, 1995; Saunders 2003; Francis et al., 2003; Weinberg et al., 2012). Therefore, it is necessary to establish a precise analytical method that can be applied to a variety of EMF exposure-inducing devices to investigate the threshold values and mechanisms of neuronal modulation by EMF exposure.

For precise evaluations of biological threshold values of nerve stimulation, responses to stimuli must be detected on a millisecond-order time scale and a cellular-level resolution. In addition, to evaluate the neuronal modulation, we must monitor the neuronal activity over a long time within the EMF exposure environment. Previous studies have evaluated the short-term magnetic nerve stimulation response with a single cell resolution by electrical recording using microelectrodes (Meyer et al., 2009; Pashut et al., 2014; Saito et al., 2014) and optical recording using a fluorescence microscope (Rotem and Moses, 2008;

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Murphy et al., 2016); these methods enable the detection of neuronal activity with a high spatiotemporal resolution. However, these experimental methods could not be applied to repetitive and long-term time-varying EMF exposure environments, which cause electromagnetic noise, induction heating, and vibration of the recording systems. Electromagnetic noise captured through the electrodes affects the signal-to-noise (S/N) ratio in the recording data. Induction heating of the microscope body which includes metal components leads to an increase in the temperature of the EMF-exposed area. Moreover, these recording systems cause magnetostriction in an EMF exposure environment.

To overcome these experimental problems, we developed a novel real-time and cellular-level fluorescence detection method that can be applied to investigate the mechanisms of neuronal modulation under high-intensity and long-term time-varying EMF exposure. Here, we built a system that can detect the fluorescence dynamics of cultured neuronal networks in the narrow space within a time-varying EMF exposure coil, using non-conductive and miniature fiber-optic probes. Using the system, we examined the real-time detection of intracellular Ca^{2+} dynamics accompanying the spontaneous and synchronized excitation of neuronal cells and networks in an area that was sufficiently distant from the EMF exposure. In addition, we performed the real-time detection of neuronal activity during high-intensity and long-term exposure to an induction heating-related intermediate frequency EMF by using our recording system. Based on these results, we describe the advantages of our novel platform for investigating the mechanisms of neuronal modulation in response to the time-varying EMF exposure of living tissues.

2. Materials and methods

2.1. Cell culture

All animal experiments were approved by the Animal Experiments Ethics Committee at the Central Research Institute of Electric Power Industry (CRIEPI) and were performed according to the guidelines for the care and use of laboratory animals. We harvested cells from cortical and hippocampal tissues. Isolation and culture of the neuronal and glial cells were performed using the following methods. Cortical and hippocampal tissues harvested from 18 to 19-day-old Wistar rat embryos (Charles River Laboratories, Japan) were enzymatically dissociated into single cells by a 15–20 min treatment with a 0.5% Trypsin solution (Sigma-Aldrich, USA). A Neurobasal medium (Thermo Fisher Scientific, USA) containing 2% B-27 supplement, 1% GlutaMAX supplement, and 1% penicillin-streptomycin (Pe-St) was used for the cell culture medium.

During the cell seeding process, the neuronal and glial cells were locally patterned along the distribution of the induced currents in the culture medium using a contact printing method for precise assessment of the effects of EMF exposure, as detailed in the Supplementary information.

2.2. Fluorescent imaging and data analysis of neuronal activity

The fluorescent imaging and data analysis methods applied to the neuronal and glial cells and their network activities are described in the Supplementary information.

2.3. Non-conductive fiber-optic imaging (NCFI) system

To attain real-time recording of the neuronal activity in time-varying EMFs, we proposed a fluorescence imaging method that uses a fiber-optic probe consisting of non-conductive materials. A schematic view of the fluorescence method of recording neuronal activity in a magnetic field by using a non-conductive fiber-optic imaging (NCFI) system is shown in Fig. 1A. The NCFI system was built by combining a fluorescence microscope with a NCFI probe consisting of a graded index (GRIN) lens (NA=0.5, diameter=500 μm, length=2.19 mm, NEM-050-06-00–520-S, GrinTech, Germany) attached to a bundle-type optical fiber (diameter=450 ± 30 μm, length=3 m, FIGH-10-350S, Fujikura, Japan). Each side of the GRIN lens and the fiber bundle was bonded using UV-curing adhesive (NOA 63, Norland Products, USA). To reinforce the bond between the GRIN lens and the fiber bundle, the surface was protected by a custom-made alumina sleeve (Phonon Meiwa, Japan) and a cover formed from a polyolefin flexible heat-shrinkable tube (H-X4-080-CLR, Cobalt Polymers, USA) with an inner diameter of 600 μm and an outer diameter of 1.5 mm.

The fluorescence microscope system consisted of a phase microscope (Eclipse Ti-U, Nikon, Japan), a cooled charge-coupled device (CCD) camera (ImagEM, Hamamatsu Photonics, Japan), and a mercury lamp (C-HGFI, Nikon). Using a micromanipulator (MM-3, Narishige, Japan) fixed to the microscope stage, the end of the fiber bundle to which the GRIN lens is not attached was optically coupled to the focal length of the object lens (NA =0.45, WD =4 mm, CFI Plan Apo λ, Nikon) of the phase microscope. A custom-made acrylic holder was used to retain the side attached to the GRIN lens in a Helmholtz coil, and the triaxial point was controlled using a three-axis motorized micromanipulator (EMM-3NV, Narishige) to maintain a sufficient distance from the exposure system. In addition, the tip of the GRIN lens was fixed at a focal distance 60 μm above the specimen. The fluorescent data was captured by using AQUACOSMOS software (Hamamatsu Photonics) with the image acquired from the cooled CCD camera. In addition, to investigate the influence of the magnetic field on the fluorescence detection, we used 20-μm diameter fluorescent microbeads (Fluoresbrite YG Microspheres 20.0 μm, Polysciences, USA) of a size similar to that of a neuronal cell body.

2.4. Time-varying electromagnetic field (EMF) exposure system

A time-varying EMF exposure system was built by combining a Helmholtz coil (TECNO Electric Industry, Japan), a custom-made coil controller (NF, Japan), a high-speed bipolar power supply (Precision Power Amplifier 4520, NF), a function generator (WF1946A, NF), a custom-made acrylic box (Fuji Business, Japan), and a temperature controller (TIZHB, Tokai Hit, Japan). A Helmholtz coil (inner diameter of 30 mm, outer diameter of 120 mm, height of 50 mm, coil separation of 50 mm) achieved vertical and uniaxial exposure to a sinusoidal magnetic field. The resonance frequency of the coil was 20 kHz, and the magnetic flux density that could be generated with the maximum current input was 10 mT.

At the time of EMF exposure, to place a culture dish in the uniform magnetic-field space of the Helmholtz coil, we inserted the specimen into the space using an acrylic vibration isolator. Here, the alignment of the central point of the coil and culture dish was performed with a sub-millimeter tolerance. In addition, to establish an incubation space in the exposure environment, the temperature in the coil (when not energized) was maintained at 37 ± 0.5 °C using a temperature controller mounted inside the acrylic box.

3. Results and discussion

3.1. Fluorescent imaging of stimulus-related activity using NCFI system

To evaluate the performance of the NCFI system, we performed fluorescent imaging of the cell morphology and intracellular Ca^{2+} transients in cultured neuronal networks. The diameter of one of the optical fibers in the fiber bundle of the NCFI probe was 3–4 μm; it had sufficient resolution to visualize the morphology of neuronal and glial cells. The fluorescent images obtained using a phase-contrast microscope with (Fig. 2A) and without (Fig. 2B) the NCFI probe showed that the neuronal and glial cell bodies, which have diameters of 20–30 μm,

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