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Exposure to extremely low frequency electromagnetic fields alters the calcium dynamics of cultured entorhinal cortex neurons

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ABSTRACT

Previous studies have revealed that extremely low frequency electromagnetic field (ELF-EMF) exposure affects neuronal dendritic spine density and NMDAR and AMPAR subunit expressions in the entorhinal cortex (EC). Although calcium signaling has a critical role in control of EC neuronal functions, however, it is still unclear whether the ELF-EMF exposure affects the EC neuronal calcium homeostasis. In the present study, using whole-cell recording and calcium imaging, we record the whole-cell inward currents that contain the voltage-gated calcium currents and show that ELF-EMF (50 Hz, 1 mT or 3 mT, lasting 24 h) exposure does not influence these currents. Next, we specifically isolate the high-voltage activated (HVA) and low-voltage activated (LVA) calcium channels-induced currents. Similarly, the activation and inactivation characteristics of these membrane calcium channels are also not influenced by ELF-EMF. Importantly, ELF-EMF exposure reduces the maximum amplitude of the high-K^+-evoked calcium elevation in EC neurons, which is abolished by thapsigargin, a Ca^{2+} ATPase inhibitor, to empty the intracellular calcium stores of EC neurons. Together, these findings indicate that ELF-EMF exposure specifically influences the intracellular calcium dynamics of cultural EC neurons via a calcium channel-independent mechanism.

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1. Introduction

The extremely low-frequency electromagnetic field (ELF-EMF), primarily produced by the electrical wires and equipments, is one of the prominent types of pollutions in modern industrial society (Lacy-Hulbert et al., 1998). Given that exposure to ELF-EMF occurs throughout a person's entire life, concerns about the health risks associated with exposure are accumulating (Hardell and Sage, 2008; Kheifets et al., 2006). Indeed, it has been reported that the ELF-EMF exposure affects the neuronal functions in the hippocampus and prefrontal cortex, both of which are closely related to the learning and memory. In the hippocampus, ELF-EMF (1–60 Hz, 0.05–0.56 mT) disrupted neuronal rhythmic slow activity (Bawin et al., 1996) and altered the neuronal Ca^{2+} signaling events, leading to the aberrant NMDA receptor activities (Manikonda et al., 2007). In the prefrontal cortex, ELF-EMF (50 Hz, 0.1–1 mT) exposure induced the increase of 5-HT2A receptor density (Janac et al., 2009) and affected neurotrophic signaling (Di Loreto et al., 2009) and anti-oxidative enzymatic activity (Falone et al., 2008). The entorhinal cortex (EC) has been recognized as the major relay station between the neocortex and hippocampus (Van Cauter et al., 2013; van Strien et al., 2009). It contains spatial information-related functional cells and plays an essential role in the spatial learning and memory (Burak, 2014; Si and Treves, 2013; Yartsev et al., 2011). Previous studies in our laboratory have found that EC neurons are also vulnerable to the ELF-EMF. ELF-EMF (50 Hz, 0.5 mT) exposure reduced the dendritic spine density of the EC neurons and affected their NMDAR and AMPAR subunit expressions, although it did not affect the rat spatial learning ability (Li et al., 2014; Xiong et al., 2013).

Calcium signaling plays an important role in controlling a variety of EC neuronal functions, including neurotransmitter release, membrane excitability and gene expression (Berridge, 1998). Because of the key role of Ca^{2+} signaling in cellular functions, the cytosolic Ca^{2+} is rigorously and largely controlled by the membrane calcium channels and intracellular calcium dynamics in normal condition (Mills, 1991; Ross, 1989). Dysfunctions of calcium channels or intracellular calcium dynamics can disrupt the calcium homeostasis, which is involved in the pathogenesis of cognitive-related diseases (Small, 2009; Supnet and Bezprozvanny, 2010). Interestingly, the regulatory machineries

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that control the calcium homeostasis are affected by a number of environmental factors. Among these factors, ionizing and microwave radiation have attracted great scientific interest. In rat whole-brain synaptosomes, gamma irradiation reduced KCl-stimulated voltage-dependent uptake of Ca^{2+} via the inhibition of protein kinase C activity (Kandasamy and Harris, 1992). In stem cell-derived neuronal cells and dorsal root ganglion neurons, microwave radiation significantly increased the cytosolic Ca^{2+} concentration through its influence on N-type calcium channels and TRPV1 channels, respectively (Ghazizadeh and Naziroglu, 2014; Titushkin et al., 2009). Moreover, it has been shown that radiofrequency radiation and microwave affected the Ca^{2+} efflux from human neuroblastoma cells (Dutta et al., 1989, 1984).

Although the calcium dynamics is a principal mediator of many vital neuronal activities and sensitive to environmental factors (Berridge, 1998), it is still unclear whether the ELF-EMF exposure affects the EC neuronal calcium homeostasis. In the present study, using whole-cell patch clamp recordings and calcium imaging, we investigate the effects of ELF-EMF (50 Hz, 1 mT or 3 mT) with exposure duration of 24 h on the EC neuronal membrane ion channels, especially calcium channels, and the intracellular calcium dynamics. We found ELF-EMF exposure specifically influenced the intracellular calcium dynamics, but not the membrane calcium channel activities. These results reveal a novel interaction mechanism between ELF-EMF and the EC, which might partially interpret the ELF-EMF-induced changes in cellular functions.

2. Materials and methods

2.1. Primary EC neuron culture

All experimental procedures involving animals were in accordance with the guidelines for the care and use of laboratory animals in the Third Military Medical University. Postnatal 0-day-old Sprague–Dawley rats obtained from the Center of Animal Laboratory of the Third Military Medical University were soaked in alcohol for acute disinfection and then decapitated to remove out the brain. After isolating the cerebellum and removing the meninges, the rostral parts of the EC were cut away. Each pair of EC (left and right) from a rat was used for a single culture. The dissected tissue pieces were then collected in 1 ml ice-cold Hank's balanced salt solution (composition in g/L: 8 NaCl, 0.4 KCl, 0.06 NaH_2PO_4, 0.0475 NaH_2PO_4, 1 D-glucose, pH 7.2, Hyclone). Next, trypsin (750 μl, 0.25%, Sigma) was added to the solution. After incubation at 37 °C for 20 min, the trypsin was inactivated with 750 μl cold fetal calf serum. Then, the mixed solution was centrifuged at 1800 rpm for 5 min. After removing the supernatant, fresh neurobasal medium (1 ml, Gibco), which can help to suppress glial cells, was added to resuspend the cells. Dissociation of tissue fragments was achieved by gentle trituration using a fire-polished Pasteur pipette. Next, cells from the each pair of EC were seeded on a single culture dish, respectively, and all cultures were maintained for 6 hours at 37 °C in 5% CO_2 in neurobasal medium (1 ml, Gibco) supplemented with 2.5 mg/ml B27 (2%), 2 mM L-glutamine, 160 U/ml penicillin, and 200 U/ml streptomycin. The medium was changed 6 h after cell dissection and subsequently every third day for 12 days.

2.2. The exposure procedure to ELF-EMF

After the 12 day culture period, cultured EC neurons were divided into sham exposure or ELF-EMF exposure (sinusoidal waveform, 50 Hz, 1 or 3 mT) groups and subjected to a 24 h exposure procedure, in which sham or ELF-EMF exposure was applied alternately, 5 min on and 10 min off. After the completion of the exposure at the thirteenth day (DIV13), the cultural neurons were immediately used for the whole-cell patch clamp recording or the calcium imaging experiments. As described previously, this kind of intermittent exposure pattern but not continuous may not only mimic real life exposure conditions to the time-varying magnetic fields that are the primary sources of ELF-EMF, but also achieve the possibly greatest effects of ELF-EMF (Focke et al., 2010). During the exposure procedure, each one or two cultures exposed to ELF-EMF was always accompanied by a sham-exposed culture through an sXcELF system (IT'IS Foundation, Switzerland) at the same time. In brief, the exposure system consisted of two four-coil setups (2 coils with 56 windings, 2 coils with 50 windings), each of which was placed inside a Mu-metal box. The currents in the bifilar coils could be switched between parallel for field exposure and non-parallel for sham control. Thus the field exposure and sham exposure can be achieved at the same time. Two fans were mounted per box to guarantee enough atmospheric exchange within the exposure chambers. Both setups were placed inside a commercial incubator (Heracell 240i, Thermo scientific) to ensure constant environmental conditions (37 °C, 5% CO_2, 95% humidity). In addition, the temperature was monitored at the location of the culture dishes with Pt100 probes and maintained at 37.0–37.5 °C during exposure. The temperature difference between the chambers did not exceed 0.1 °C. Thus the possible thermal effects could be ruled out. A current source was developed based on four audio amplifiers (Agilent Technologies, Zurich, Switzerland) and applied to the bifilar coils to allow magnetic fields up to 3.5 mT. The field could be arbitrarily varied in the frequency range from 3 to 1250 Hz by a computer controlled arbitrary function generator. The entire system has been optimized for homogeneous field distribution, maximum field strength, minimum temperature increase and minimum vibrations. Non-uniformity of the magnetic field is $<1\%$ for all possible petri dish locations. And the fields and all sensors were continuously monitored.

2.3. Whole-cell patch clamp recording

With the aid of an upright microscope (BX51WI, Olympus) equipped with a 40× water immersion objective and a video imaging camera, visualized whole-cell patch clamp recordings in voltage clamp mode were made on cultured EC neurons immediately and up to 4 h after finishing the 24 h sham or ELF-EMF exposure procedure at room temperature (22–25 °C). After giga-ohm seal formation and patch rupture, the membrane potential of neurons before and after the voltage pulse stimulus were held at −70 or −80 mV for whole-cell current or calcium channel activity recordings, respectively. The current signal was amplified using an Axopatch 200B amplifier (Molecular Device) with a low cutoff frequency of 1 or 2 kHz and stored in a computer with a sampling frequency of 10 or 20 kHz for off-line analysis. Throughout the entire experiment, membrane capacitance and series resistance were continually monitored. Although the series resistance was not compensated, the recorded neuron was included in the final analysis only if the series resistance did not exceed 20 MΩ and did not change over 15%. In the off-line analysis session, the scaled P/N leak subtraction was applied routinely to correct for passive membrane current ($N=4$ or 5).

For whole-cell current recordings, the culture medium was replaced by artificial cerebrospinal fluid (ACSF, composition in mM: 126 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 1.3 $MgSO_4$, 26 $NaHCO_3$, 2 $CaCl_2$, 10 D-glucose) before the recording sessions. The patch pipette (2.5–5 MΩ) was filled with normal K^+-based internal solution (composition in mM: 130 K-gluconate, 5 KCl, 2 $MgCl_2$, 10 HEPES, 0.1 EGTA, 2 Na-ATP, 0.3 Na_2-GTP, 4 Na_2-phosphocreatine, adjusted to pH 7.25 with 1 mM KOH). After finishing the recordings, tetrodotoxin (TTX, 1 μM)+tetraethylammonium (TEA, 10 mM)+4-aminopyridine (4-AP, 5 mM) were

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