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REPORT

Effects of Single and Repeated Exposure to a 50-Hz 2-mT Electromagnetic Field on Primary Cultured Hippocampal Neurons

Ying Zeng[1] · Yunyun Shen[1,4] · Ling Hong[1] · Yanfeng Chen[1] · Xiaofang Shi[2] · Qunli Zeng[1] · Peilin Yu[3]

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Abstract The prevalence of domestic and industrial electrical appliances has raised concerns about the health risk of extremely low-frequency magnetic fields (ELF-MFs). At present, the effects of ELF-MFs on the central nervous system are still highly controversial, and few studies have investigated its effects on cultured neurons. Here, we evaluated the biological effects of different patterns of ELF-MF exposure on primary cultured hippocampal neurons in terms of viability, apoptosis, genomic instability, and oxidative stress. The results showed that repeated exposure to 50-Hz 2-mT ELF-MF for 8 h per day after different times in culture decreased the viability and increased the production of intracellular reactive oxidative species in hippocampal neurons. The mechanism was potentially related to the up-regulation of Nox2 expression. Moreover, none of the repeated exposure patterns had significant effects on DNA damage, apoptosis, or autophagy, which suggested that ELF-MF exposure has no severe biological consequences in cultured hippocampal neurons.

Keywords ELF-MF · Primary cultured hippocampal neurons · Oxidative stress · Cell viability

Ying Zeng and Yunyun Shen have contributed equally to this work.

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✉ Qunli Zeng
zengqunli@hotmail.com

✉ Peilin Yu
yupeilin@zju.edu.cn

[1] Bioelectromagnetics Laboratory, Department of Occupational and Environmental Health, School of Public Health, Zhejiang University, Hangzhou 310058, China

[2] Department of Neurobiology, Zhejiang University School of Medicine, Hangzhou 310058, China

[3] Department of Toxicology, School of Public Health, Zhejiang University, Hangzhou 310058, China

[4] Institute of Cognitive Neuroscience and Department of Psychology, Zhejiang Sci-Tech University, Hangzhou 310018, China

Introduction

The essential use of domestic electrical appliances and industrial power lines result in residential and occupational exposure to electromagnetic fields (EMFs). The World Health Organization initiated the International EMF Project in 1996 to drive the development of EMF-related research and assess its health effects [1]. Extremely low-frequency magnetic fields (ELF-MFs) from 1 Hz up to 100 kHz, which emanate from power lines, household appliances, and transport systems, have been classified as a possible carcinogen (Group 2B) in humans [2], especially brain-derived tumors [3]. Recent studies have even suggested a contribution of ELF-MFs to the etiology of neurodegenerative disorders [4]. For example, some epidemiological research has reported that ELF-MF exposure could be a risk factor for senile dementia and combined motor neuron diseases, amyotrophic lateral sclerosis, and Alzheimer's disease [5, 6]. It has also been suggested that memory and learning in mice is impaired after exposure to EMFs [7]. However, the etiopathogenesis of these neurodegenerative diseases initiated by ELF-MF exposure is still poorly known. To further evaluate the effects of ELF-MFs and their mechanisms in the central nervous system (CNS), many studies with different cells,

intensities, and patterns of exposure have been performed. The results revealed that ELF-MF exposure can elicit redox and trophic responses in rat cortical neurons [8], induce oxidative stress in mouse cerebellum [9], cause DNA damage in mouse brain [10], and increase neuronal nitric oxide synthase activation in rat brain [11]. Moreover, pre-exposure to ELF-MFs has been reported to have synergistic effects with some chemical reagents, such as altering the cellular responses in retinoic acid-treated human neuroblastoma cells [12], increasing the menadione-induced DNA damage and mitochondrial activity in SH-SY5Y cells [13], and sensitizing SH-SY5Y cells to a pro-Parkinson's disease toxin [14].

However, there have also been negative results among the investigations in the CNS with ELF-MF exposure, for example that exposure to a 50-Hz ELF-MF does not have neurotoxic effects in PC12 cells [15]. Increasing numbers of reports have even pointed out that ELF-MFs can be neuroprotective under different pathological conditions. For example, they save rat cerebellar granule neurons from apoptosis [16] and reduce neuronal death and the activation of astrocytes and microglia subjected to global cerebral ischemia [17]. Based on these findings, the effects of ELF-MFs on the CNS depend on the intensity and pattern of exposure used in different brain regions and cell lines. And few studies to date have investigated the effects of ELF-MFs on primary cultured hippocampal neurons, which are crucial for learning and memory.

In this study, we set out to evaluate the effects of different patterns of ELF-MF exposure (single and repeated) on primary cultured hippocampal neurons at different developmental stages by assessing viability, apoptosis, genomic instability, and oxidative stress, in order to provide better insight into the general effects of ELF-MFs on the CNS.

Materials and Methods

Primary Hippocampal Neuronal Culture

All animal use procedures were approved by the Committee for the Care and Use of Laboratory Animals at Zhejiang University. Hippocampal neurons were prepared from embryonic E17–E18 Sprague-Dawley rats. After intraperitoneal anesthesia with 10% chloral hydrate (1 mL/250 g body weight), prenatal pups were removed from the uterus, decapitated to expose brain tissue under a dissecting microscope with sterile scissors and forceps. Brain hemispheres were separated, and surrounding tissues around hippocampus were gently pulled away. Then the hippocampal tissue was carefully chopped and digested in 0.25% trypsin (Sigma, St. Louis, MO) for 18 min at 37 °C with gentle shaking. After removing the trypsin solution, hippocampal cells were dissociated and plated at 3×10^6–4×10^6 cells per 35-mm dish coated with poly-L-lysine (Sigma) containing Dulbecco's modified Eagle's medium (Gibco, Carlsbad, CA) supplemented with 10% horse serum (Gibco), 1 mmol/L glutamine (Gibco), and 1% penicillin/streptomycin (Genom, Hangzhou, China) at 37°C under a humidified atmosphere containing 5% CO_2. After culture for 2 h, the medium was replaced with Neurobasal medium supplemented with 2% B27 (Gibco), 1% penicillin/streptomycin, and 0.25% glutamine. Thereafter, half of the culture medium was replaced by fresh medium twice per week.

Exposure System

The exposure system was designed by the Foundation for Research on Information Technologies in Society and has been used routinely in our lab [18, 19]. Briefly, the apparatus consists of two identical coil systems placed in a $25\times25\times25$ cm^3 μ-metal box with a thickness of 1.3 mm and operated at a frequency of 50 Hz. The boxes are laid beside each other in a conventional incubator. Each of the two coil systems is composed of four square subcoils (side length 20 cm) arranged symmetrically around the coil center. Paired wires form the coils, which are connected in parallel for exposure or anti-parallel for sham exposure as the control. Two fans are mounted on each box to guarantee atmospheric exchange. The uncertainty for the applied magnetic field is <4%. Details can be found in the description by Schuderer *et al*. [20].

Exposure

In the single-exposure pattern, exposure groups were each placed in a chamber with a 50-Hz 2-mT ELF-MF once for 30 min, 8 h, or 24 h at DIV 7 or DIV 14 (days *in vitro*). Meanwhile, sham groups were also placed in a sham chamber but without ELF-MF exposure. In the repeated-exposure pattern, cells were exposed to a 50-Hz 2-mT ELF-MF for 30 min or 8 h every day in the morning from DIV 1 to DIV 7 or from DIV 7 to DIV 14. Meanwhile, sham groups were simultaneously placed in the sham chamber without ELF-MF exposure.

Cell Viability

Viability was monitored using a Cell Count Kit (CCK-8; Dojindo, Kumamoto, Japan). Cells were plated at 1×10^4 cells/mL in 96-well plates. After exposure, each well was incubated with 100 μL CCK-8 for 3 h at 37°C and measured as absorbance at 450 nm using a full-wavelength multifunction scanning reader (Varioskan Flash; Thermo

Scientific, Boston, MA). The viability was calculated from the relative absorbance and expressed as a percentage of control.

Western Blotting

After exposure, neurons were washed in cold phosphate-buffered saline (PBS), followed by homogenization in RIPA lysis buffer (Beyotime, Shanghai, China) with a protease inhibitor mixture (Roche Applied Science, Basel, Switzerland). Equal amounts of protein were loaded into the wells for 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. After separation, the proteins were transferred onto PVDF membranes (Whatman, Buckinghamshire, UK) at 300 mA for 90 min. Following blocking with 5% skim milk (Gibco), the membrane was incubated with primary antibodies (Nox2, Abcam, Cambridge, UK, 1:1000 dilution; LC3, Sigma, St. Louis, MO, 1:1000 dilution; or GAPDH, Beyotime, Shanghai, China, 1:2000 dilution) at room temperature (RT) for 2 h at 4°C overnight and then incubated with secondary antibodies at RT for 1 h. Blots were semi-quantified using the Odyssey infrared imaging system (LI-COR, Lincoln, NE).

Measurement of ROS Production

Neurons were washed 3 times with PBS and incubated with 10 μmol/L dichloro-dihydro-fluorescein diacetate (DCFH-DA; Life Technologies, Carlsbad, CA) at 37 °C for 30 min. Then two washes in PBS were followed by fluorescence imaging at 520-nm emission induced by 485-nm excitation, determined with a full-wavelength multifunction scanning reader (Varioskan Flash; Thermo Scientific, Boston, MA). The intensity of the control well was assumed to be 100% and the data are presented as a percentage of control.

Immunofluorescence Staining

Cells were cultured on coverslips coated with poly-L-lysine (Sigma). After exposure, the cells were fixed in 4% paraformaldehyde for 15 min at 4°C, and permeabilized in 0.5% Triton X-100 for 15 min at 4°C. Non-specific binding sites were blocked with goat serum (Zhongshan Golden Bridge Biotechnology, Beijing, China) for 2 h at RT. The cells were then incubated with a mouse monoclonal anti-γH2AX (H2A histone family, member X) antibody (Millipore, Billerica, MA; diluted 1:1000) for 2 h and TRITC-conjugated goat-anti-mouse secondary antibody (Zhongshan Golden Bridge Biotechnology, diluted 1:300) for 1 h at RT. Thereafter, the samples were incubated with 4′,6-diamidino-2-phenylindole (DAPI, Sigma) and stained for 15 min to visualize nuclei. Each step was followed by 3 washes for 5 min in PBS. Then, the coverslip was removed from the dish and mounted on a glass slide. Images were captured on an Olympus AX70 fluorescence microscope (Olympus, Center Valley, PA).

TUNEL Assay

TUNEL assays were performed with an *in situ* cell death detection kit (TMR red, Roche Diagnostics, Basel, Switzerland). After exposure, cells were rinsed three times with cold PBS, then fixed in 4% paraformaldehyde at RT for 1 h. Fixed cells were then rinsed in PBS, followed by 0.1% Triton X-100 at 4 °C for 2 min. The TUNEL reaction mixture was added and incubated in a humidified chamber at 37°C for 1 h in the dark. Then the cells were incubated with DAPI for 20 min in the dark at RT followed by three washes with PBS. The images were captured on an Olympus AX70 fluorescence microscope (Olympus, Center Valley, PA). Data are presented as the ratio of apoptotic neurons (red) to total neurons (blue).

Data Analysis

SPSS 17.0 was used for statistical analysis. All data are expressed as mean ± SEM, and are representative of at least three independent experiments. A two-tailed Student's *t*-test was applied to determine the significance of differences. $P < 0.05$ was considered to be statistically significant.

Results

Repeated Exposure to an ELF-MF Decreases Viability of Hippocampal Neurons

To assess the purity of primary cultured hippocampal neurons, cells were fixed at DIV7 and a protein specific for neuron dendrites, microtubule-associated protein 2, was used as a marker of neurons. The purity of the neurons was >95% (Fig. S1). The cultured neurons at different stages were then exposed to different patterns of 50 Hz ELF-MF at 2 mT, and CCK-8 assays were used to evaluate their viability. A single exposure on DIV7 (Fig. 1A) and DIV14 (Fig. 1B) for 30 min, 8 h, or 24 h did not affect viability (Fig. 1), while repeated exposure from DIV1 to DIV7 (Fig. 1C) and DIV7 to DIV14 (Fig. 1D) (8 h/day) significantly decreased the viability. Interestingly, unlike the 30 min/day exposure during DIV1-7, which had no effect on viability, it was increased in the DIV7-14 group. These findings indicate that changes in exposure time and stage influence the effect of ELF-MFs on cell viability.

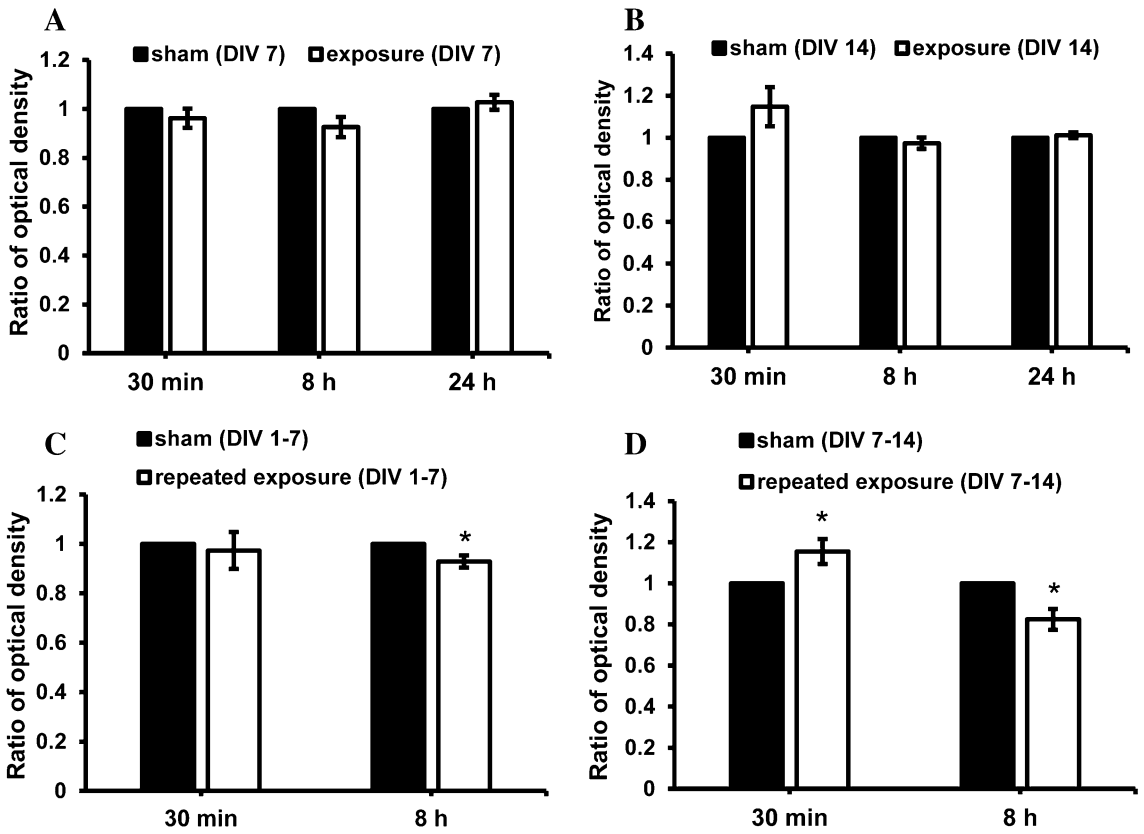

Fig. 1 Repeated exposure to an ELF-MF induces cytotoxicity in hippocampal neurons. Cell viability as measured with CCK-8 assays in groups with a single exposure on DIV7 (**A**) and DIV14 (**B**), and repeated exposure from DIV1 to DIV7 (**C**) and DIV7 to DIV14 (**D**) (mean ± SEM, $n = 3$; $^*P < 0.05$ *vs* sham exposure, *t*-test).

Repeated Exposure to an ELF-MF does not Induce DNA Damage, Apoptosis, or Autophagy in Hippocampal Neurons

DNA damage has been reported in all cerebral areas of the mouse brain exposed to a 50-Hz ELF-MF at 1 mT for 1 or 7 days (15 h/day) [10]. Apoptosis in zebrafish embryos has also been observed after exposure to a sinusoidal ELF-MF at various intensities [21]. In order to determine whether this occurs in our system, we examined the DNA damage and apoptosis levels after repeated exposure. First, immunofluorescent detection of γH2AX was used to assess DNA damage. There were no significant changes in the average numbers of foci per cell between sham and repeated ELF-MF exposure in both the DIV1-7 and DIV7-14 groups (Fig. 2A, B). Then, TUNEL assays to evaluate apoptosis showed no significant increase in the number of TUNEL-positive cells in the DIV1-7 and DIV7-14 groups after repeated exposure (Fig. 2C, D).

Besides, as a double-edged sword having a close relationship with both apoptosis and cell survival, we previously showed that autophagy in fibroblasts is induced by an ELF-MF [18, 19]. Yet few investigations of the autophagic response to ELF-MFs in the CNS have been reported. To determine whether autophagy is involved in the determination of cell fate in our system, autophagy was measured as the protein level of microtubule-associated protein 1 light chain 3-II (LC3-II). Compared to the sham group, the LC3-II protein level showed no significant changes in the DIV1-7 and DIV7-14 groups after repeated ELF-MF exposure (Fig. 2E, F).

In summary, neither 30 min nor 8 h per day repeated ELF-MF exposure induces DNA damage, apoptosis, or autophagy in hippocampal neurons.

Repeated Exposure to an ELF-MF Increases ROS Generation in Hippocampal Neurons

Previous studies have reported that ELF-MFs induce ROS generation in several cell types, such as human SH-SY5Y neuroblastoma cells and Jurkat T cells, so this has been assumed to play a critical role in ELF-MF-induced cytotoxicity and changes in cellular processes [22, 23]. To clarify whether this happens after repeated ELF-MF exposure, the fluorescent dye DCFH-DA was used to assess

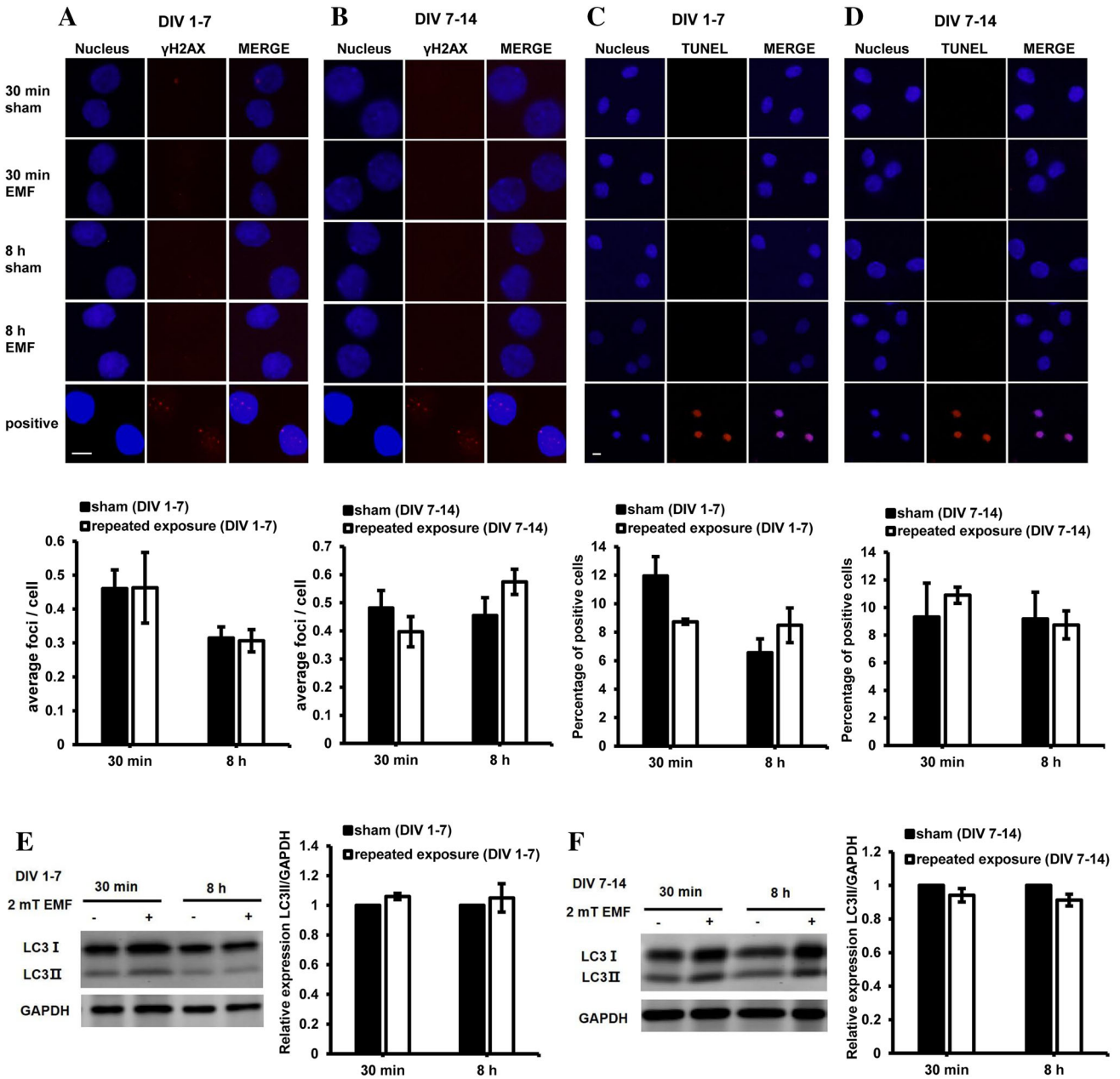

Fig. 2 Repeated exposure to an ELF-MF does not induce DNA damage, apoptosis, or autophagy in hippocampal neurons. **A**, **B** Representative images of γ-H2AX immunofluorescent staining (*upper panel*) and its quantitative analysis (*lower panel*) for hippocampal neurons repeatedly exposed to a 50 Hz 2 mT ELF-MF for 30 min and 8 h per day at DIV1-7 (**A**) and DIV7-14 (**B**). *Scale bar* 10 μm. *Red dots* indicate γ-H2AX foci; nuclei are stained *blue* with DAPI. The positive group was treated with 1 μmol/L 4-nitroquinoline 1-oxide. **C**, **D** Representative images of TUNEL staining (*upper panel*) and its quantitative analysis (*lower panel*) for hippocampal neurons repeatedly exposed to a 50 Hz 2 mT ELF-MF for 30 min and 8 h per day at DIV1-7 (**C**) and DIV7-14 (**D**). Red fluorescence identified TUNEL-positive apoptotic cells. *Scale bar* 10 μm. The positive group was treated with 4 μL 30% H_2O_2. **E, F** Representative western blots (*left panels*) and summary of protein expression of LC3-II (*right panels*) in hippocampal neurons repeatedly exposed to a 50-Hz 2-mT ELF-MF for 30 min and 8 h per day at DIV1-7 (**E**) and DIV7-14 (**F**). Values in **A**–**D** are mean ± SEM of ten samples from five independent experiments. The data in **E** and **F** were from four independent experiments.

ROS production. Under our experimental conditions, repeated ELF-MF exposure at DIV1-7 for 30 min/day induced no changes in ROS generation compared with sham-exposure. In contrast, exposure for 8 h/day at DIV1-7 slightly increased the ROS production (Fig. 3A), and the increase was significant in the DIV7-14 group with 30 min/day and 8 h/day (Fig. 3B). The increase in ROS corresponded with the decreased cell viability, suggesting that ROS generation might be closely associated with ELF-MF-induced cell death.

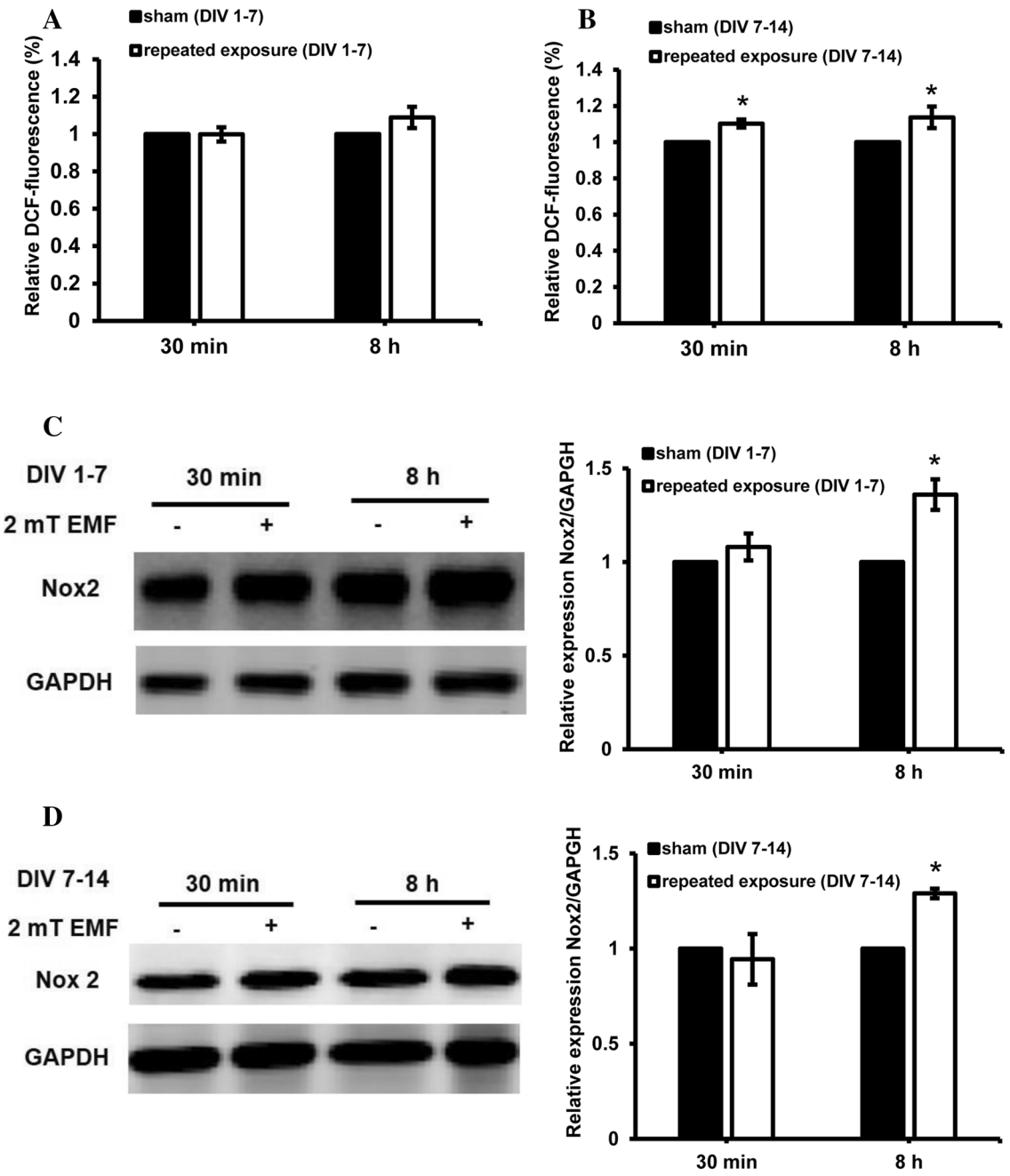

Fig. 3 Repeated exposure to an ELF-MF increases ROS generation in hippocampal neurons. **A, B** Summary of intracellular ROS production in hippocampal neurons repeatedly exposed to a 50-Hz 2-mT ELF-MF for 30 min and 8 h per day at DIV1-7 (**A**) and DIV7-14 (**B**). **C, D** Representative western blots (*left panels*) and summary of Nox2 protein expression (*right panels*) in hippocampal neurons repeatedly exposed to a 50-Hz 2-mT ELF-MF for 30 min and 8 h per day at DIV1-7 (**C**) and DIV7-14 (**D**). Mean ± SEM, $n \geq 3$; $^*P < 0.05$ *vs* sham exposure, *t*-test.

Furthermore, since nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is mainly responsible for the ROS generation associated with neuronal death [24, 25], we tested whether NADPH oxidase is involved in ELF-MF-induced ROS generation. The protein level of the NADPH oxidase subunit Nox2 was measured, as it has been reported to be the most abundantly-expressed subunit in CNS neurons and appears to be a major source of pathological oxidative stress in the CNS [26]. After repeated exposure to the ELF-MF for 8 h/day, the Nox2 protein level was significantly higher in the DIV1-7 and DIV7-14 groups than in the sham-exposure group (Fig. 3C, D), indicating that Nox2 expression is an important factor in producing ROS during repeated ELF-MF exposure.

Discussion

The exposure to ELF-MFs caused by occupational as well as general sources in our environment has dramatically increased, raising concerns about its effects on health. Animal studies have shown that ELF-MF exposure causes behavioral and cognitive disturbances, as well as inducing significant impairment of learning [27, 28], but the mechanism is still poorly defined. Although the study by Cui *et al*. [7] provided evidence for an association between the learning impairment induced by ELF-MF exposure and oxidative stress in the hippocampus, there was no direct evidence of increased oxidative stress in hippocampal neurons. In this study, using two ELF-MF exposure patterns (single and repeated) in primary cultured hippocampal neurons on different days, we found that only repeated exposure to a 50-Hz 2-mT ELF-MF for 8 h per day on DIV1-7 and DIV7-14 decreased the viability and increased intracellular ROS production. Further, we found for the first time up-regulation of Nox2 expression in both of these groups, which probably caused the increased ROS. These results provide conclusive evidence for an association between oxidative stress and ELF-MF-induced functional damage in hippocampal neurons. Meanwhile, our results indicate that the reduction in cell viability induced by an ELF-MF depends on the exposure pattern and also highlights the cumulative effects of repeated exposure.

Interestingly, we found that exposure for 30 min/day at DIV7-14 markedly increased the cell viability. Consistent with our results, Di Loreto *et al*. [8] showed that the viability of cortical neurons significantly increased after ELF-MF exposure, and this was accompanied by a reduction of the glutathione (GSH) level. This was assumed to reflect the overall antioxidant protection in which GSH plays an essential role to avoid oxidative stress-induced cytotoxicity. The GSH content in the brain is believed to change with maturity and aging; its content in the hippocampus first increases to a peak value during the process of maturation before it decreases during aging [29, 30]. In our system, by DIV7 the cells have initiated the process of synaptogenesis and tend to be mature [31]. The increased viability after ELF-MF exposure in the DIV7-14 group precisely reflected the antioxidant-protective effect of higher GSH content in the mature stage, which resists the decreased viability caused by oxidative stress. Besides, the unconventional dose-response phenomenon in our viability experiments resembled a hormesis-like phenomenon in the DNA damage caused by radiofrequency EMFs at a low dose [32]. Hormesis is recognized as an unconventional dose-response relationship characterized by a biphasic response at low and high doses of a chemical, biological molecule, physical stressor, or other initiator of a response. Szili *et al*. [33] also reported that a low ROS level increases cell viability, while a higher ROS level decreases it, which is consistent with our results. Although the molecular mechanism has not been resolved, it is important to explore the role of hormesis in the EMF effects in future studies.

The 50-Hz ELF-MF is non-ionizing radiation produced by the generation, transmission, and application of electricity. The limiting values of 50 Hz ELF-MF provided by the International Commission on Non-Ionizing Radiation Protection are 0.2 mT for public exposure and 1 mT for occupational exposure [34]. Here, using 2 mT (double the occupational exposure limit) in two patterns, we found that neither single nor repeated exposure had significant effects on DNA damage, apoptosis, and autophagy in cultured hippocampal neurons. These results are consistent with previous studies in different tissues and cell lines [13, 18], and suggest the absence of severe biological consequences of ELF-MF (50 Hz, 2 mT) exposure. However, it is worth noting that increasing numbers of studies have shown that ELF-MFs might interfere with DNA-damaging agents [13]. Since human beings are likely to be exposed to environments containing a variety of genotoxic agents, the synergistic effects of ELF-MFs with these substances cannot be ignored.

In conclusion, although ELF-MF exposure causes an oxidative stress-induced decrease of viability in hippocampal neurons, it does not have severe adverse biological consequences, and there might be compensatory mechanisms at the translational or posttranslational level. Therefore, the mechanism underlying the stress response after repeated exposure needs to be further defined.

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References

1. WHO. Extremely low frequency fields environmental health criteria monograph No. 238. World Health Organization 2007, Monograph No. 238.
2. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Non-ionizing radiation, part 1: static and extremely low-frequency (ELF) electric and magnetic fields. IARC Monogr Eval Carcinog Risks Hum 2002, 80: 1–395.
3. Turner MC, Benke G, Bowman JD, Figuerola J, Fleming S, Hours M, *et al*. Occupational exposure to extremely low-frequency magnetic fields and brain tumor risks in the INTEROCC study. Cancer Epidemiol Biomark Prev 2014, 23: 1863–1872.
4. Consales C, Merla C, Marino C, Benassi B. Electromagnetic fields, oxidative stress, and neurodegeneration. Int J Cell Biol 2012, 2012: 683897.

5. Hakansson N, Gustavsson P, Johansen C, Floderus B. Neurodegenerative diseases in welders and other workers exposed to high levels of magnetic fields. Epidemiology 2003, 14: 420–426; discussion 427–428.
6. Johansen C. Exposure to electromagnetic fields and risk of central nervous system disease in utility workers. Epidemiology 2000, 11: 539–543.
7. Cui Y, Ge Z, Rizak JD, Zhai C, Zhou Z, Gong S, *et al.* Deficits in water maze performance and oxidative stress in the hippocampus and striatum induced by extremely low frequency magnetic field exposure. PLoS One 2012, 7: e32196.
8. Di Loreto S, Falone S, Caracciolo V, Sebastiani P, D'Alessandro A, Mirabilio A, *et al.* Fifty hertz extremely low-frequency magnetic field exposure elicits redox and trophic response in rat-cortical neurons. J Cell Physiol 2009, 219: 334–343.
9. Chu LY, Lee JH, Nam YS, Lee YJ, Park WH, Lee BC, *et al.* Extremely low frequency magnetic field induces oxidative stress in mouse cerebellum. Gen Physiol Biophys 2011, 30: 415–421.
10. Mariucci G, Villarini M, Moretti M, Taha E, Conte C, Minelli A, *et al.* Brain DNA damage and 70-kDa heat shock protein expression in CD1 mice exposed to extremely low frequency magnetic fields. Int J Radiat Biol 2010, 86: 701–710.
11. Cho SI, Nam YS, Chu LY, Lee JH, Bang JS, Kim HR, *et al.* Extremely low-frequency magnetic fields modulate nitric oxide signaling in rat brain. Bioelectromagnetics 2012, 33: 568–574.
12. Marcantonio P, Del Re B, Franceschini A, Capri M, Lukas S, Bersani F, *et al.* Synergic effect of retinoic acid and extremely low frequency magnetic field exposure on human neuroblastoma cell line BE(2)C. Bioelectromagnetics 2010, 31: 425–433.
13. Luukkonen J, Liimatainen A, Hoyto A, Juutilainen J, Naarala J. Pre-exposure to 50 Hz magnetic fields modifies menadione-induced genotoxic effects in human SH-SY5Y neuroblastoma cells. PLoS One 2011, 6: e18021.
14. Benassi B, Filomeni G, Montagna C, Merla C, Lopresto V, Pinto R, *et al.* Extremely low frequency magnetic field (ELF-MF) exposure sensitizes SH-SY5Y cells to the pro-Parkinson's disease toxin MPP(+). Mol Neurobiol 2016, 53: 4247–4260.
15. de Groot MW, Kock MD, Westerink RH. Assessment of the neurotoxic potential of exposure to 50Hz extremely low frequency electromagnetic fields (ELF-EMF) in naive and chemically stressed PC12 cells. Neurotoxicology 2014, 44: 358–364.
16. Oda T, Koike T. Magnetic field exposure saves rat cerebellar granule neurons from apoptosis in vitro. Neurosci Lett 2004, 365: 83–86.
17. Raus S, Selakovic V, Manojlovic-Stojanoski M, Radenovic L, Prolic Z, Janac B. Response of hippocampal neurons and glial cells to alternating magnetic field in gerbils submitted to global cerebral ischemia. Neurotox Res 2013, 23: 79–91.
18. Chen Y, Hong L, Zeng Y, Shen Y, Zeng Q. Power frequency magnetic fields induced reactive oxygen species-related autophagy in mouse embryonic fibroblasts. Int J Biochem Cell Biol 2014, 57: 108–114.
19. Shen Y, Xia R, Jiang H, Chen Y, Hong L, Yu Y, *et al.* Exposure to 50Hz-sinusoidal electromagnetic field induces DNA damage-independent autophagy. Int J Biochem Cell Biol 2016, 77: 72–79.
20. Schuderer J, Oesch W, Felber N, Spat D, Kuster N. In vitro exposure apparatus for ELF magnetic fields. Bioelectromagnetics 2004, 25: 582–591.
21. Li Y, Liu X, Liu K, Miao W, Zhou C, Li Y, *et al.* Extremely low-frequency magnetic fields induce developmental toxicity and apoptosis in zebrafish (Danio rerio) embryos. Biol Trace Elem Res 2014, 162: 324–332.
22. Luukkonen J, Liimatainen A, Juutilainen J, Naarala J. Induction of genomic instability, oxidative processes, and mitochondrial activity by 50Hz magnetic fields in human SH-SY5Y neuroblastoma cells. Mutat Res 2014, 760: 33–41.
23. Tang R, Xu Y, Ma F, Ren J, Shen S, Du Y, *et al.* Extremely low frequency magnetic fields regulate differentiation of regulatory T cells: Potential role for ROS-mediated inhibition on AKT. Bioelectromagnetics 2016, 37: 89–98.
24. Cheret C, Gervais A, Lelli A, Colin C, Amar L, Ravassard P, *et al.* Neurotoxic activation of microglia is promoted by a nox1-dependent NADPH oxidase. J Neurosci 2008, 28: 12039–12051.
25. Cao L, Chen J, Li M, Qin YY, Sun M, Sheng R, *et al*. Endogenous level of TIGAR in brain is associated with vulnerability of neurons to ischemic injury. Neuroscience bulletin 2015, 31: 527–540.
26. Lam TI, Brennan-Minnella AM, Won SJ, Shen Y, Hefner C, Shi Y, *et al*. Intracellular pH reduction prevents excitotoxic and ischemic neuronal death by inhibiting NADPH oxidase. Proc Natl Acad Sci U S A 2013, 110: E4362–E4368.
27. Che Y, Sun H, Cui Y, Zhou D, Ma Y. Effects of exposure to 50 Hz magnetic field of 1 mT on the performance of detour learning task by chicks. Brain Res Bull 2007, 74: 178–182.
28. Sun H, Che Y, Liu X, Zhou D, Miao Y, Ma Y. Effects of prenatal exposure to a 50-Hz magnetic field on one-trial passive avoidance learning in 1-day-old chicks. Bioelectromagnetics 2010, 31: 150–155.
29. Sasaki T, Senda M, Kim S, Kojima S, Kubodera A. Age-related changes of glutathione content, glucose transport and metabolism, and mitochondrial electron transfer function in mouse brain. Nucl Med Biol 2001, 28: 25–31.
30. Zhang DL, Chen YQ, Jiang X, Ji TT, Mei B. Oxidative damage increased in presenilin1/presenilin2 conditional double knockout mice. Neurosci Bull 2009, 25: 131–137.
31. Bassani S, Cingolani LA, Valnegri P, Folci A, Zapata J, Gianfelice A, *et al*. The X-linked intellectual disability protein TSPAN7 regulates excitatory synapse development and AMPAR trafficking. Neuron 2012, 73: 1143–1158.
32. Sun C, Wei X, Fei Y, Su L, Zhao X, Chen G, *et al*. Mobile phone signal exposure triggers a hormesis-like effect in $Atm^{+/+}$ and $Atm^{-/-}$ mouse embryonic fibroblasts. Scientific Reports 2016, 6.
33. Szili EJ, Harding FJ, Hong S-H, Herrmann F, Voelcker NH, Short RD. The hormesis effect of plasma-elevated intracellular ROS on HaCaT cells. J Phys D Appl Phys 2015, 48: 495401.
34. International Commission on Non-Ionizing Radiation P. Guidelines for limiting exposure to time-varying electric and magnetic fields (1 Hz to 100 kHz). Health Phys 2010, 99: 818–836.