The effects of 50 Hz magnetic field exposure on DNA damage and cellular functions in various neurogenic cells

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ABSTRACT
Epidemiological studies have indicated a possible association between extremely low-frequency magnetic field (ELF-MF) exposure and the risk of nervous system diseases. However, laboratory studies have not provided consistent results for clarifying this association, despite many years of studies. In this study, we have systematically investigated the effects of 50 Hz MF exposure on DNA damage and cellular functions in both neurogenic tumor cell lines (U251, A172, SH-SY5Y) and primary cultured neurogenic cells from rats (astrocytes, microglia, cortical neurons). The results showed that exposure to a 50 Hz MF at 2.0 mT for up to 24 h did not influence γH2AX foci formation (an early marker of DNA double-strand breaks) in any of six different neurogenic cells. Exposure to a 50 Hz MF did not affect cell cycle progression, cell proliferation or cell viability in neurogenic tumor U251, A172 or SH-SY5Y cells. Furthermore, the MF exposure for 24 h did not significantly affect the secretion of cytokines (TNF-α, IL-6 or IL-1β) in astrocytes or microglia, or the phagocytic activity of microglia. In addition, MF exposure for 1 h per day did not significantly influence expression levels of microtubule-associated protein tau, microtubule-associated protein 2, postsynaptic density 95 or gephyrin in cortical neurons, indicating an absence of effects of MF exposure on the development of cortical neurons. In conclusion, our data suggest that exposure to a 50 Hz MF at 2.0 mT did not elicit DNA damage effects or abnormal cellular functions in the neurogenic cells studied.

KEYWORDS: neurogenic cell, magnetic fields, DNA damage, cellular functions

INTRODUCTION
Increased exposure to the extremely low-frequency magnetic fields (ELF-MFs) generated by power lines and household electrical devices has raised public concerns about possible adverse effects on human health. The International Agency for Research on Cancer (IARC) classified ELF-MFs as a possibly carcinogenic to humans (Group 2B) in 2002 [1]. The carcinogenic potential or genotoxic effects of ELF-MFs have been assessed experimentally in various model systems; however, the results have been controversial [2, 3]. In addition to cancer, some epidemiological studies have suggested that ELF-MF exposure is associated with an increased risk of other diseases, including neurodegenerative diseases, cardiovascular diseases, and adverse pregnancy outcomes [4–7]. However, other epidemiological studies have indicated a lack of any association [8–10], and laboratory studies have not provided consistent evidence supporting the epidemiological observations of associated increased disease risk [11, 12]. Generally, the biological effects induced by low-intensity ELF-MFs have not been determined, and the potential underlying mechanisms remain unclear.

It has been assumed that the nervous system is one of the more sensitive systems in terms of response to ELF-MF exposure [13, 14]. Several epidemiological studies have suggested a possible association between ELF-MF exposure and risk of nervous system diseases, such as brain tumors [15], neurodegenerative diseases [7]...
and other neurological disorders [16, 17], but the findings have been inconclusive. Regarding laboratory investigations, previous in vivo studies have focused on the effects of ELF-MFs on behavior, cognitive functions, and neurotransmitter systems in the brain [18–21]. A number of in vitro studies have been conducted to investigate the biological effects of ELF-MF exposure in neurogenic cells, including cellular functions [22], genotoxicity [23], gene/protein expression [24] and neurogenesis [25]. However, the results from laboratory studies have largely been inconsistent and even controversial [26], and the data have not clarified the associations between ELF-MF exposure and the risk of nervous system diseases. This may be due primarily to the various research models, exposure conditions, and experimental protocols adopted by different groups [26]. Therefore, the biological responses of the nervous system and of neurogenic cells to ELF-MFs require further investigation.

Here, we devised a system for investigating the effects of 50 Hz MF exposure on DNA damage and cellular functions in both neurogenic tumor cell lines (U251, A172, SH-SY5Y) and primary cultured neurogenic cells from rats (astrocytes, microglia, cortical neurons). To make the biological effects induced by ELF-MFs readily comparable, we exposed various neurogenic cells to the same standardized exposure set-up with the same exposure parameters, and evaluated the biological end points using the same methods used by a line of researchers. To evaluate the effects of 50 Hz MF exposure on DNA damage, we first examined γH2AX foci formation, an early marker of DNA double-strand breaks (DSBs) [27], in six different types of neurogenic cells. Because the neurogenic tumor cells are proliferative, we assessed the effects of 50 Hz MF exposure on cell cycle progression, cell proliferation, and cell viability in U251, A172 and SH-SY5Y cells. Considering the diverse functions of the various primary cultured neurogenic cells, we also investigated the immunological roles of astrocytes and microglia, and neuronal development in cortical neurons after 50 Hz MF exposure.

**MATERIAL AND METHODS**

**Animal ethics**

All procedures for the isolation of rat primary cultured neurogenic cells, including astrocytes, microglia and cortical neurons, were reviewed and approved by the Animal Ethics Committee at the affiliated institutions of the authors. Considerable effort was made to reduce animal suffering and the number of animals used.

**Exposure system**

The exposure system (sXc-ELF) used in the present study was designed by the Foundation for Information Technologies in Society (ITTS, Zurich, Switzerland) [28]. Briefly, two identical chambers containing a series of Helmholz coils were placed inside a cell culture incubator (Heraeus, Chicago, IL) to ensure stable and consistent environmental conditions (37°C, 5% CO2) (Fig. 1A). One chamber was for the sham control group (without ELF-MF exposure) and the other was for the experimental group (with ELF-MF exposure). The exposure set-up was monitored by a computer to control the exposure parameters, including frequency of ELF (e.g. 50 Hz), exposure intensity and exposure time. The cells were exposed to a 50 Hz sinusoidal MF at 2.0 mT for varying durations (Fig. 1B). The 50 Hz MF exposure intensity of 2.0 mT was selected at twice the reference limit for occupational exposure (1.0 mT) set by the International Commission on Non-Ionizing Radiation Protection (ICNIRP). The temperature variance between the chambers for sham and MF exposure did not exceed 0.1°C.

**Cell culture**

Human glioblastoma cells (U251 and A172) and neuroblastoma cells (SH-SY5Y) were obtained from the cell bank of the Shanghai Institutes for Biological Sciences (Shanghai, China) and were cultured in high-glucose Dulbecco’s modified eagle medium (DMEM, Invitrogen, Carlsbad, CA) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco, Carlsbad, CA).

Primary newborn Sprague-Dawley rat mixed glia cells were isolated from the cerebral cortex of newborn rats (1-day-old) as described by Chen et al. [29], with minor modification. Briefly, the newborn rats were decapitated and immersed in ice-cold Hank’s balanced salt solution (HBSS, Invitrogen). After carefully removing the meninges, tissues were incubated in 0.25% trypsin-EDTA (Invitrogen) for 15 min at 37°C, followed by mechanical trituration in Minima essential medium (MEM, Invitrogen) supplemented with 10% FBS, 100 units/ml penicillin (Invitrogen) and 100 μg/ml streptomycin (Invitrogen). The cells were collected by centrifugation at 1000 rpm for 5 min, and then 10 ml of cell suspension cultures (7 × 10⁵ cells/ml) were plated in T75 flasks coated with poly-L-lysine (PLL, Sigma, St Louis, MO). The medium was replenished on the second day after plating and was changed every 3 days. At Day 7 in vitro (DIV7), microglia were collected by shaking the flasks manually for 10 min, and the remaining mixed glial cells were cultured in fresh complete medium. At DIV14, the culture flasks were shaken for 18 h at 240 rpm to remove microglia and oligodendrocytes, then astrocytes were subcultured in DMEM containing 10% FBS at a density of 1 × 10⁵/dish in 35 mm Petri dishes. Microglia and astrocytes were identified via immunofluorescent staining with ionized calcium-binding adaptor molecule 1 (Iba1) and glial fibrillary acidic protein (GFAP), respectively. The indirect immunofluorescent staining for identification of microglia and astrocytes was carried out with mouse anti-Iba1 (sc-32725; Santa Cruz Biotecnology, Santa Cruz, CA; diluted 1:400) and mouse anti-GFAP antibody (TA336707; Zhongshan Golden Bridge Biotechnology, Beijing, China; diluted 1:800), respectively.

Cerebral cortex neurons in primary culture were prepared from E18-day-old Sprague-Dawley rat embryos. Meninges were removed and cortices were cut into pieces and dissociated by 0.05% trypsin-EDTA for 15 min, then plated in 35 mm Petri dishes coated with PLL in DMEM supplemented with 10% horse serum (HS, Gibco). After 4 h incubation, unattached cells and debris were removed by replacing the medium with Neurobasal medium (Gibco) supplemented with 2% B27 supplement (Invitrogen), 1% L-glutamine (Invitrogen), 100 units/ml penicillin and 100 μg/ml streptomycin. Three days after plating, 2.5 μM of cytosine arabinoside (Sigma) was added to the culture to inhibit the growth of non-neuronal cells. The medium was half renewed every 3 days. Cortical neurons were identified via immunofluorescent staining with microtubule-associated protein 2 (MAP2). The immunofluorescent staining was performed...
using mouse anti-MAP2 (ab11268; Abcam, Cambridge, UK; diluted 1:500).

**Immunofluorescent detection of γH2AX**

The neurogenic tumor cell lines (U251, A172 and SH-SY5Y), astrocytes and microglia were exposed or sham-exposed to 50 Hz MFs for 1, 6 or 24 h after pre-culture for 24 h. The cortical neurons were exposed or sham-exposed to 50 Hz MFs for 1, 6 or 24 h at DIV7. As positive controls, the neurogenic tumor cells (U251, A172 and SH-SY5Y) and the primary cultured neurogenic cells (astrocytes, microglia and cortical neurons) were treated with 0.1 µM of 4-Nitro-Quinoline-1-Oxide (4NQO, Sigma), a carcinogenic chemical...
Neurogenic cell responses to 50 Hz MF exposure

that induces DNA damage. Immunofluorescence staining of γH2AX was carried out as previously described [30]. Primary mouse anti-γH2AX antibodies (Millipore, Temecula, CA; diluted 1:1000) were used. Alexa Fluor® 546-conjugated goat anti-mouse IgG (Zhongshan Goldenbridge Biotechnology, Beijing, China; diluted 1:300) was used as the secondary antibody. The dye 4', 6-diamidino-2-phenylindole (DAPI, Sigma, St Louis, MO) was used to stain the cell nuclei. Over 200 cells were scored from 5 to 10 randomly selected observation fields. The number of γH2AX foci per cell and the percentage of γH2AX foci-positive cells were used as the indicators of DNA DSBs.

Cell cycle analysis
After 50 Hz MF exposure, the neurogenic tumor cells were cultured for an additional 0, 6 or 24 h, detached with 0.25% trypsin-EDTA, subsequently resuspended in 70% ethanol and stored at −20°C until measurement. The cells were stained with 500 μl of phosphate-buffered saline (PBS) containing 50 mM of propidium iodide (Sigma) and 10 mg/ml of RNase A (Thermo Scientific, Waltham, MA) for 30 min at room temperature in the dark. The cell cycle progression was assessed by flow cytometry, and the percentage of cells at the G0/G1, S and G2/M phases were determined using Wincycle32 software (Beckman Coulter, Brea, CA). As a positive control, cells were treated with 2.0 μM of 4NQO for 1 h to inhibit cell cycle progression.

Cell proliferation analysis
After 50 Hz MF exposure, the neurogenic tumor cells were cultured for an additional 0, 24 or 48 h, and the number of cells detached by 0.25% trypsin-EDTA was manually counted using a hemocytometer. As a positive control, cells were treated with 2.0 μM of 4NQO for 1 h to inhibit cell proliferation.

Cell viability analysis
After 50 Hz MF exposure, the neurogenic tumor cells were continuously cultured for 0, 24 or 48 h. The cell viability was determined using Cell Counting Kit-8 (CCK-8, Dojindo Molecular Technologies, Kumamoto, Japan). The CCK-8 reagent was added (10 μl per well) and the cells were then incubated for 2.5 h. The optical density (OD) value at a wavelength of 450 nm was determined using a Varioskan Flash Multimode Reader (Thermo Scientific). As a positive control, the cells were treated with 2.0 μM of 4NQO for 1 h to inhibit cell viability.

Enzyme-linked immunosorbent assay of TNF-α, IL-6 and IL-1β
Levels of TNF-α, IL-6 and IL-1β in the cell culture medium for astrocytes and microglia were measured using rat enzyme-linked immunosorbent assay (ELISA) kits (Raybiotech, Atlanta, GA) according to the manufacturers’ protocols. Briefly, 100 μl of standards or culture medium was added to each well of the plate and incubated at room temperature for 2.5 h. The medium was removed and the wells were washed three times with washing buffer, followed by incubating with 100 μl of prepared biotin-conjugated primary antibody for 1 h. Following three washes, the wells were incubated with 100 μl of prepared streptavidin-conjugated secondary antibody solution for 45 min. After three washes, each well was subsequently incubated with 100 μl of 3,3′,5,5′-tetramethylbenzidine (TMB) one-step substrate reagent for 30 min. This reaction was stopped with 50 μl of stop solution. Absorbance values were immediately measured at 450 nm using a Varioskan Flash Multimode Reader. The cells were treated with 1.0 μg/ml of lipopolysaccharide (LPS, Sigma) for 1 h as a positive control.

Phagocytosis assay
Microglia were plated on glass coverslips in 35 mm Petri dishes at a density of 2 × 10⁵ cells/dish. Cells were left to adhere overnight and exposed or sham-exposed to 50 Hz MFs for 24 h. Then, 1.0 μl of carboxylate-modified fluorescent microspheres (1.0 μm diameter) [Nile Red fluorescent (535/575), Invitrogen] was added to the culture for 1 h at 37°C. The medium was removed, and the cold PBS was added to arrest microspheres uptake. Then, the cells were fixed in 4% formaldehyde for 15 min. The number of microspheres per cell was counted for >60 cells per sample on a Nikon microscope (Nikon, Tokyo, Japan). The phagocytic activity was calculated using the following indicators: average number of microspheres/cell, the percentage of positive cells with microspheres, and the percentage of cells with various number of microspheres. Microglia were treated with 1.0 μg/ml of LPS for 1 h as a positive control.

Neuron staining for morphological analysis
For morphological analysis, the cortical neurons were stained with microtubule-associated protein tau (Tau) and MAP2 as markers of axon and dendrites, respectively, to evaluate the neurite outgrowth, and were stained with postsynaptic density 95 (PSD95) and gephyrin as markers of excitatory and inhibitory synapses respectively, to assess synaptogenesis. The cortical neurons were plated on a coverslip at a density of 4 × 10⁶ cells per 35 mm dish, and exposed or sham-exposed to 50 Hz MFs for 1 h per day from DIV1 to DIV14. As positive controls, neurons were treated with 1.0 μg/ml of brefeldin A (an inhibitor of axonal growth, MedChem Express, Monmouth Junction, NJ) at DIV2, 10 μM of LY294002 (an inhibitor of dendrite development, Cell Signaling Technology, Danvers, MA) at DIV4 and 10 μM of U0126 (an inhibitor of synaptogenesis, Cell Signaling Technology) at DIV10, respectively. Immunofluorescence staining of axon, dendrites and synapses were carried out at DIV3, DIV7 and DIV14, respectively. Immediately after exposure, the cells were fixed in 4% paraformaldehyde for 15 min at 4°C, and permeabilized using 0.25% Triton X-100 for 5 min at 4°C. The cells were pre-incubated in the blocking reagent for 2 h and then incubated in primary antibodies overnight at 4°C, including mouse anti-Tau (4019; Cell Signaling Technology; diluted 1:800), mouse anti-MAP2 (ab11268; Abcam; diluted 1:500), mouse anti-PSD95 (ab2723; Abcam; diluted 1:200) and mouse anti-gephyrin (147011; Synaptic Systems, Gottingen, Germany; diluted 1:400). Thereafter, cells were incubated with goat-anti-mouse secondary antibody (Zhongshan Golden Bridge Biotechnology; diluted 1:300) for 2 h at room temperature.
Image analysis of neuron morphology
For quantification of axon length, axon branch length and branch number, the neurons were fixed at DIV3, and images of neurons were acquired with a ×20 objective under a Nikon confocal microscope. Axon lengths were measured from the cell body to the distal extent of the central region of the growth cone. An axon was defined as the process that was at least double the length of the next longest minor process. For determination of axon versus branch, the axon was defined as the process that remained parallel to the initial axon segment extending from the cell body. Branches were defined as processes extending at orthogonal angles to the axon. These counts were performed blindly using MetaMorph software (Molecular Devices, Sunnyvale, CA).

For analysis of dendritic morphology, the neurons were fixed at DIV7, and images were taken with a ×20 objective under a Nikon confocal microscope. The length of dendrites and the branch, branch number, and Sholl analysis for each neuron were measured using Imaris software (Bitplane, South Windsor, CT).

For the measurement of synapse density, the neurons were fixed at DIV14, and images were acquired with a ×60 objective under a Nikon confocal microscope. PSD95 and gephyrin cluster density in neurons were quantified using MetaMorph software. The background fluorescence of each channel seen in the dendrites was subtracted, and the maximum intensities of the fluorophores channels were normalized [32]. A total of 60–70 randomly selected neurons were analyzed from three independent experiments for each ELF-MF exposure condition (e.g. different exposure duration). Density values were calculated as number of clusters per micrometer of dendrite length.

Statistical analysis
All computations were performed with Statistical Package for the Social Sciences (SPSS) 16.0 for Windows. Data are presented as the means ± standard errors of the mean (SEMs). After the normality test, statistical analysis was performed with Student’s t-test or the rank sum test between 50 Hz MF exposure and sham exposure groups, or between positive control and control groups. A P < 0.05 was considered statistically significant.

RESULTS
Effects of 50 Hz MF exposure on γH2AX foci formation in neurogenic cells
To determine the effects of 50 Hz MF exposure on γH2AX foci formation, the cells were subjected to immunofluorescence staining after 50 Hz MF exposure at 2.0 mT for 1, 6 or 24 h. The results showed that 50 Hz MF exposure did not affect the average number of γH2AX foci per cell or the percentage of γH2AX foci-positive cells in U251, A172 or SH-SY5Y cells (Fig. 2A–C, Supplementary Fig. S1). However, treatment with 0.1 μM of 4NQO significantly induced γH2AX foci formation in each neurogenic tumor cell line (Supplementary Fig. S2).

For primary cultured neurogenic cells, exposure for 1, 6 or 24 h did not significantly increase γH2AX foci formation in astrocytes, microglia, or cortical neurons, while treatment with 0.1 μM of 4NQO significantly increased γH2AX foci formation in each type of cells (Fig. 3A–C, Supplementary Fig. S3). Taken together, our results demonstrated that 50 Hz MF exposure did not significantly affect γH2AX foci formation in these neurogenic cells (Supplementary Fig. S4).

Effects of 50 Hz MF exposure on cellular behaviors in neurogenic tumor cells
To evaluate whether 50 Hz MF exposure could affect cellular behaviors, we examined cell cycle progression, cell proliferation, and cell viability, all of which are involved in cancer progression, in these neurogenic tumor cells. First, the cell cycle distribution at 0, 6 and 24 h after MF exposure was analyzed by flow cytometry. As shown in Fig. 4 and Supplementary Fig. S5, no significant difference in the percentage of cells at G0/G1, S or G2/M phase was observed between 50 Hz MF- and sham-exposed groups in U251, A172 or SH-SY5Y cells, suggesting that 50 Hz MFs did not affect cell cycle progression.

We next examined the effects of 50 Hz MF exposure on cell proliferation, using a hemocytometer, and found that exposure for 24 h did not change cell proliferation in U251, A172 or SH-SY5Y cells (Fig. 5A). Moreover, no significant difference in cell proliferation was observed between 50 Hz MF- and sham-exposed groups when the cells were subjected to prolonged incubation for 24 or 48 h after exposure (Fig. 5A). Meanwhile, we tested the effects of MF exposure on cell viability using the CCK-8 assay. No significant change was found in cell viability between 50 Hz MF- and sham-exposed groups after 24 h of MF exposure or incubation for an additional 24 or 48 h after the exposure in U251, A172 or SH-SY5Y cells (Fig. 5B).

As positive controls, treatment with 2.0 μM of 4NQO significantly influenced cell cycle progression, inhibited cell proliferation and reduced cell viability in U251, A172 and SH-SY5Y cells. These data demonstrated that a 50 Hz MF under current exposure conditions did not significantly influence cellular behaviors in U251, A172 or SH-SY5Y cells.

Effects of 50 Hz MF exposure on cytokine release in astrocytes and microglia
Astrocytes and microglia play major immunological/inflammatory roles in the central nervous system (CNS). Therefore, we investigate whether a 50 Hz MF exposure could influence the secretion of pro-inflammatory cytokines in astrocytes or microglia. The results showed that a 50 Hz MF exposure for 24 h did not alter the release of cytokines TNF-α, IL-6 or IL-1β in astrocytes. Nevertheless, LPS treatment for 1 h increased TNF-α, IL-6 and IL-1β levels in astrocytes (Fig. 6A). In microglia, TNF-α, IL-6 and IL-1β levels were not significantly changed by a 50 Hz MF exposure for 24 h. LPS treatment for 1 h led to an increase in TNF-α and IL-6 in microglia, but no significant change in IL-1β level was found between LPS treatment and control groups, suggesting differing effects of LPS on the various cytokines in microglia (Fig. 6B).

Effects of a 50 Hz MF exposure on microglial phagocytic activity
To examine the effects of a 50 Hz MF exposure on microglial phagocytic activity, microglia were exposed to a 50 Hz MF for 24 h, and the
phagocytosis of fluorescence-labeled microspheres inside cells was calculated under fluorescent microscope. No significant difference was found in the average number of microspheres per cell between the sham and exposed groups (Fig. 7A and B). Furthermore, the percentage of microsphere-positive cells and the percentage of cells with various numbers of microspheres per cell were analyzed; no significant change was found in either (Fig. 7C and D).

Effects of a 50 Hz MF exposure on morphological development in neurons

To investigate the effect of a 50 Hz MF exposure on neuronal morphological maturation, primary cortical neurons were exposed to a 50 Hz MF from DIV1 to DIV14 (1 h per day) and subjected to morphological analysis at DIV3, DIV7 and DIV14. We first examined axon morphology at DIV3 by staining for Tau and found no difference in axon length, axon branch length, or branch number between 50 Hz MF- and sham-exposed groups, while treatment with 1.0 µg/ml of brefeldin A at DIV2 significantly affected the axonal growth (Fig. 8A). Furthermore, we analyzed dendritic morphological development at DIV7 by staining for MAP2. The results showed that a 50 Hz MF exposure did not significantly change the total dendritic length or primary dendritic length, but slightly decreased the secondary and tertiary dendritic length, though the difference did not reach significance. Comparisons of the average number of branches showed a slight, but not statistically significant, increase in the number of primary dendrites in MF-exposed neurons, while no significant difference was observed in the number of secondary or tertiary dendrites between 50 Hz MF- and sham-exposed groups. As a positive control, treatment with 10 µM of LY294002 at DIV4 significantly inhibited dendrite growth.

To explore the prolonged effect of a 50 Hz MF exposure, we further determined the density of PSD95 and gephyrin clusters, as indicators of synaptogenesis. No difference in the density of PSD95-stained puncta on the dendrites was observed between the 50 Hz MF- and sham-exposed groups (Fig. 8C). The density of gephyrin-stained puncta on the dendrites was slightly but not statistically significantly decreased in the MF-exposed neurons. As a
positive control, treatment with 10 µM of U0126 at DIV10 significantly inhibited synaptogenesis.

In brief, a 50 Hz MF exposure under the current conditions did not significantly influence morphological development in cortical neurons.

**DISCUSSION**

In this study, we systematically evaluated the DNA damage and cellular function responses to a 50 Hz MF exposure in neurogenic cells under well-controlled experimental conditions. Our data showed that exposure to a 50 Hz MF at 2.0 mT did not induce significant changes in γH2AX foci formation in six neural cell types. The exposure did not affect cell cycle progression, cell proliferation, or cell viability in U251, A172 or SH-SY5Y cells. Furthermore, exposure to 2.0 mT of a 50 Hz MF did not significantly affect the secretion of cytokines TNF-α, IL-6 or IL-1β in astrocytes or microglia, or phagocytic activity of microglia. For primary cortical neurons, the exposure did not change the morphological indicators of axon, dendrites or synapses.

Cellular DNA damage repair plays a crucial role in cell fate determination that is related to human health. DNA damage in neurogenic cells could lead to some nervous system diseases, including the development of neurodegenerative diseases and neurogenic tumors [33, 34]. Although some epidemiological studies have suggested a possible association between ELF-MF exposure and the risk of brain tumor [15, 35–38], the genotoxic effects of ELF-MF have rarely been investigated in neurogenic cells. Previous studies have examined the effects of ELF-MF exposure on genotoxicity in neurogenic cell lines by a variety of methods. An early study showed that exposure to 60 Hz MF at 2.0 mT did not produce any increased γH2AX expression, DNA fragmentation, or aneuploidy formation in mouse hippocampal HT22 cells [23]. Exposure to 50 Hz MF at 100 µT did not induce DNA damage or micronucleus formation in SH-SY5Y cells, but pre-exposure to a MF altered cellular responses to menadione [39]. Koyama et al. reported that exposure to a 60 Hz MF at 5.0 mT alone did not significantly increase apurinic/apyrimidinic (AP) site formation in genomic DNA in A172 cells, whereas the exposure could enhance chemically induced formation.
of AP sites in A172 cells [40]. Consistent with the results of previous studies, our data demonstrated that exposure to a 50 Hz MF at 2.0 mT alone did not significantly affect γH2AX foci formation in neurogenic cells. We did not evaluate the induction of γH2AX foci formation by a 50 Hz MF exposure combined with chemical agents; thus, whether 50 Hz MF exposure affects other environmental stimuli–influenced neurogenic cellular DNA damage requires further investigation.

Cellular behaviors, including cell cycle progression, cell proliferation and cellular activity, play critical roles in cancer promotion and progression. Thus, in vitro assessments of cell behavior provide a way of evaluating the effects of ELF-MF exposure on cancer development. Marcantonio et al. reported that a 50 Hz MF at 1.0 mT did not elicit alterations in cell cycle progression or cellular viability in neuroblastoma BE(2)C cells [41]. Previous studies have investigated the effects of ELF-MF exposure on cellular proliferation in various neurogenic cell types, such as SH-SY5Y cells, but have shown inconsistent results with respect to cellular behaviors [42, 43]. Sulpizio et al. reported that 1.0 mT of 50 Hz MF altered the proliferative status, cell growth pattern, and cytoskeletal organization of proliferating SH-SY5Y cells, due to significant changes in the expression levels of common proteins involved in cellular defense mechanisms, organization and biogenesis [42]. A study by Benassi et al. demonstrated that 1.0 mT of a 50 Hz MF did not affect cell survival, shape or morphology of SH-SY5Y cells, but that it significantly impairs redox homeostasis and thiol content, triggering an increase in protein carbonylation [43]. ELF-MF exposure appeared to enhance proliferative ability in neuroblastoma NB69 cells [44] and astrocytoma 132–1N1 cells [45]. However, the present study showed that 50 Hz MF at 2.0 mT did not significantly affect cell cycle progression, cell proliferation or cellular viability in neurogenic cell lines, including U251, A172 and SH-SY5Y cells. These inconsistencies may be due to the various cell lines used in the various studies.

Astrocytes are responsible for the production of cytokines and chemokines under diverse pathological CNS insults, including Alzheimer’s disease, multiple sclerosis, Parkinson’s disease, and brain injury/trauma [46]. However, studies on the cellular responses of primary cultured astrocytes to a 50 Hz MF are very limited. Bodega et al. demonstrated that exposure to 1.0 mT of a 50 Hz sinusoidal MF had no significant effect on the stress, cytoskeletal protein levels, or proliferation in primary cultured astrocytes [47]. In this

Fig. 4. Effects of exposure to 50 Hz MF on cell cycle progression in neurogenic tumor cells. The proportions of cells at each phase of the cell cycle were measured by flow cytometry at 0, 6 and 24 h after 50 Hz MF exposure for 24 h, or after 2.0 μM 4NQO for 1 h as a positive control. (A–C) Histograms show percentages of cells at different phases of the cell cycle in U251 (A), A172 (B) and SH-SY5Y (C) cells at 0 h (upper), 6 h (middle) and 24 h (lower) after 24 h exposure. Error bars indicate SEMs for three independent experiments. *P < 0.05 and **P < 0.01 compared with sham group (Rank sum test).
study, our data showed that a 50 Hz MF exposure for 24 h did not affect the release of TNF-α, IL-6 or IL-1β in primary cultured astrocytes. Thus, the current available studies do not suggest that a 50 Hz MF exposure could alter the functions in primary cultured astrocytes.

Microglia play major immunological/inflammatory roles as macrophages in the CNS, and contribute to the pathophysiology of various neurological disorders, such as neurodegenerative diseases [48]. When activated by various neuropathological stimuli, microglia shift to an ameboid type, phagocytose, and release various...
mediators, such as pro-inflammatory cytokines. Using immortalized human microglial HMO6 cells, Duong et al. found that a 50 Hz MF at 1.0 mT enhanced the survival rate of the microglial cells and protected the cells from oxygen deprivation– and glucose deprivation– induced cell death through regulating calcium ions (Ca\(^{2+}\)) and reactive oxygen species (ROS) levels \[49\]. However, our data indicated that a 50 Hz MF did not affect immune abilities (e.g. release of cytokines and phagocytic activity). Whether the primary cultured and immortalized microglial cells respond differently to a 50 Hz MF awaits further studies.

Accumulating evidence suggests that an ELF-MF can influence the process of learning and memory in both animals \[50–52\] and humans \[53\], but the cellular and molecular mechanisms are still unknown. To evaluate whether an ELF-MF could influence the axonal and dendritic extension and synaptic density, considered the basis of learning and memory, we investigated the effects of chronic exposure to a 50 Hz MF during neuronal development on morphological parameters of primary rat cortical neurons, which are widely used in the field of developmental neurotoxicology \[54\]. Our data demonstrated that a 50 Hz MF did not significantly affect neuronal morphology. Recently, several studies investigated the in vitro neurotoxicity of an ELF-MF in various nerve cells. Sul et al. reported that a 60 Hz MF exposure enhanced the proliferation of cortical neuronal HCN-2 cells, but did not affect cell cycle progression, morphological differentiation, or actin distribution \[55\]. Ma et al. demonstrated that exposure to a 50 Hz MF promoted neuronal differentiation and neurite outgrowth of embryonic neural stem cells (eNSCs) via upregulation of the expression of transient receptor potential canonical 1 (TRPC1) and proneural genes (NeuroD and Ngn1) \[56\]. Luo et al. reported that a 50 Hz MF exposure influenced the intracellular calcium dynamics of cultural entorhinal cortex neurons via a calcium channel–independent mechanism \[57\]. A study by de Groot et al. found that exposure to 1.0 mT of a 50 Hz MF affected stimulation-evoked increases in \([\text{Ca}^{2+}]_i\) and neurite outgrowth in vitro, but these changes were insufficient to affect the development of electrical activity \[58\]. However, it remains unclear whether different neuronal cell types respond differently to ELF-MF exposure, and what the biological consequences of ELF-MF–affected neurite outgrowth are.

**CONCLUSION**

In conclusion, the present study systematically investigated the effects of 50 Hz MF exposure on DNA damage and cellular functions in both neurogenic tumor cell lines and primary cultured neurogenic cells. Our data suggested that exposure to a 50 Hz MF at 2.0 mT does not elicit genotoxic effects or abnormal cellular functions in neurogenic cells.
Fig. 8. Effects of exposure to 50 Hz MF on cortical neuronal morphology development. (A) Images showing the morphology of axons after 2.0 mT of 50 Hz MF exposure for 3 days, 1 h per day. Histograms showing the average axon length per neuron, the average axon branch length per neuron and the average number of branches per axon in the sham and exposure groups. Neurons were treated with 1.0 µg/ml brefeldin A at DIV2 as a positive control. Bar = 20 µm. (B) Images showing the morphology of dendrites after 2.0 mT of 50 Hz MF exposure for 7 days, 1 h per day. Histograms showing the total dendrite length per neuron, the average dendrite branch length per neuron, the average number of branches per neuron and Sholl analysis of the dendrites in the sham and exposure groups. Concentric circles in Sholl analysis are spaced 10 µm apart, ranging from 10 µm to 150 µm from the center of the neuronal soma. Neurons were treated with 10 µM of LY294002 at DIV4 as a positive control. Bar = 20 µm. (C) The images showing the PSD95 (upper) and gephyrin (lower) clusters after 2.0 mT of 50 Hz MF exposure for 14 days, 1 h per day. Histograms showing the density of the PSD95 (upper) and gephyrin (lower) cluster in the sham and exposure groups. Neurons were treated with 10 µM of U0126 at DIV10 as a positive control. More than 20 neurons were analyzed from each experiment. Values shown are means ± SEMs from three independent experiments. Bar = 20 µm (full size) and 4 µm (insert). *P < 0.05 and **P < 0.01 compared with sham group (Student's t-test).
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SUPPLEMENTARY DATA
Supplementary data are available at the Journal of Radiation Research online.

CONFLICT OF INTEREST
The authors declare that there are no conflicts of interest.

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