JOURNAL OF ELECTROMAGNETIC ENGINEERING AND SCIENCE, VOL. 15, NO. 3, 151~157, JUL. 2015

http://dx.doi.org/10.5515/JKIEES.2015.15.3.151
ISSN 2234-8395 (Online) · ISSN 2234-8409 (Print)

The Effect of Sub-chronic Whole-Body Exposure to a 1,950 MHz Electromagnetic Field on the Hippocampus in the Mouse Brain

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Abstract

The increasing use of mobile phones has raised public concern about the possible biological effects of radiofrequency electromagnetic field (RF-EMF) exposure on the human brain. To investigate the potential effect of RF-EMF exposure on the brain, we examined the behaviors and hippocampal morphology of C57BL/6 mice after sub-chronic exposure to RF-EMFs with a relatively high SAR level (5.0 W/kg). We applied a 2-hour daily exposure of WCDMA 1,950 MHz using a reverberation chamber that was designed for whole-body exposure for 60 days. In the behavioral tests, RF-EMF did not alter the physical activity or long-term memory of mice. Moreover, no alteration was found in the neuronal and glial cells in the hippocampus by RF-EMFs. In this study, we showed that sub-chronic whole body RF exposure did not produce memory impairment and hippocampal morphological alteration in C57BL/6 mice.

Key Words: Hippocampus, Memory, Mobile Phone, Radiofrequency Electromagnetic Field, Sub-chronic Exposure.

I. INTRODUCTION

With the exponential increase in the use of mobile phones, public concern about the effect of radiofrequency electromagnetic fields (RF-EMF) on health, particularly on the brain because of its close proximity to mobile phones, has been raised. Because neuronal damage to the cortex, hippocampus, cerebellum, and basal ganglia due to RF exposure has been reported earlier [1], many research groups have investigated whether the RF-EMF from mobile phones can affect the brain in various ways by focusing on biochemical and morphological alterations: cerebral blood flow [2−5], blood–brain barrier permeability [6, 7], neurotransmitter balance [8, 9], and nerve cell damage [1, 10]. In these studies, the possible effects of RF exposure on the nervous system remain controversial. Thus far, researchers have focused on the possibility that using mobile phones may affect cognitive function. Some experimental data have been reported suggesting that RF-EMF may affect me-

Manuscript received April 29, 2015 ; Revised July 6, 2015 ; Accepted July 6, 2015. (ID No. 20150429-018J)
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mory and learning impairment [11−13]. However, other experimental data have shown that RF-EMF does not affect memory and learning function [14, 15] or even has beneficial effects [16, 17].

To clarify the possible effect of RF-EMF on the brain, we applied a 1,950 MHz field with a very high level of SAR (5.0 W/kg) and a relatively long daily exposure (2 hours, continuous) for 60 days in this study. We investigated the effect of RF-EMF on memory function and hippocampal morphology by behavioral tests and histological analyses.

II. Materials and Methods

1. Whole-Body RF-EMF Exposure System

Exposure to RF-EMF of 1,950 MHz was conducted in a reverberation chamber designed to allow whole-body exposure for rodents. The details of the exposure system, including the signal generation, the uniformity of the field dose, and the SAR, were described previously [18]. Fig. 1 shows the simple scheme and a photograph of the exposure system.

2. Animals

All mouse procedures in this study were approved by the Institutional Animal Care and Use Committee of the Korea Institute of Radiological and Medical Sciences (IACUC permit number: KIRAMS2013-67). We used 14 female C57-BL/6 mice at 12 weeks old (Doo-Yeol Biotech Ltd., Seoul, Korea). The animals were randomly assigned to the RF (−), sham control, or RF (+) group, which is the RF-exposed group (n=7 per group). The animals were exposed to 1,950 MHz RF-EMF according to the following schedule: SAR 5.0 W/kg, 2 hr/day, and 5 days/week for 60 days. All animals were placed inside chambers with or without RF-EMF signals.

3. Behavioral Tests

To evaluate the general physical activity, an open-field test was performed in an acrylic chamber (60 cm × 60 cm × 50 cm) for 30 minutes with a video tracking system (Viewer3, BIOSERVE GmbH, Bonn, Germany). Each mouse was allowed to move without restriction in the open field apparatus during the test. The tracking system was recorded, and the

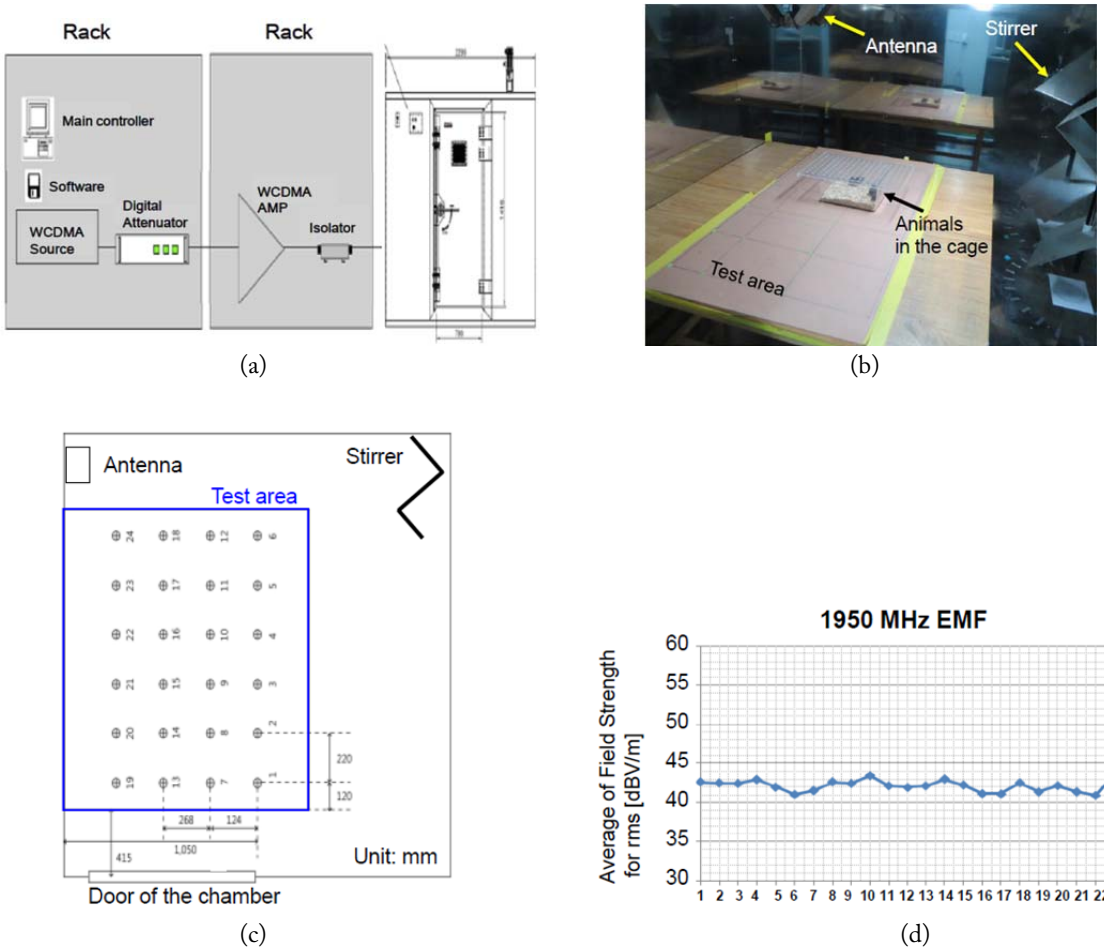

Fig. 1. Radiofrequency electromagnetic field (RF-EMF) exposure system for *in vivo* study. (a) Diagram of the RF exposure system. (b) Photograph of the test area in the reverberation chamber. (c) Measurement points in the test area. (d) Field uniformity measured at 24 points in the test area.

total number of track lengths traveled was quantified.

To assess the response to aversive stimulus and long-term memory, a passive avoidance test was conducted. An apparatus with two divided rooms and a grilled floor to conduct an electric shock was used. A gate that could be moved up and down automatically was placed between the two rooms. For adaptation to the apparatus, the mice traveled freely between the rooms through the opened gate for 5 minutes. The next day, each mouse was placed in one room and kept in the dark for 30 seconds. Then, the light was turned on, and the gate was moved simultaneously. As the mouse traveled to the other room to avoid the bright light, the gate was shut down, and an electrical shock impulse (1 mA, 5 seconds) was transmitted through the grill. After 24 hours, the same trial was conducted without the electrical shock, and the latency time to cross over to the other room was recorded.

4. Immunohistochemistry

After the termination of the behavioral test, the animals were euthanized, and the brains were fixed in 4% paraformaldehyde for 48 hours, paraffin-embedded, and sectioned at 5 μm intervals. Immunohistochemistry was conducted using the Vectastain Elite ABC kit (Vector Laboratories Inc., Burlingame, CA, USA) following the manufacturer's protocol. For antigen retrieval, the sections were placed in a citrate buffer (pH 6.0) and heated in boiling water for 30 minutes. The sections were then placed in 0.3% H_2O_2 in absolute methanol for 15 minutes at room temperature to block the endogenous peroxidase. The sections were blocked with normal horse serum (Vector Laboratories Inc.), incubated for 1 hour at room temperature with anti-NeuN (1:100, Novus), anti-PCNA (1:100, Santa Cruz), anti-GFAP (1:100, Abcam) or anti-Iba1 (1:100, Wako), and incubated with biotinylated goat anti-rabbit IgG (Vector Laboratories Inc.) for 30 minutes at room temperature. Immunoreaction with an avidin–biotin peroxidase complex was then performed for 30 minutes at room temperature. The peroxidase reaction was developed using the DAB kit (Vector Laboratories Inc.). As a control, the primary antibody was omitted for several test sections in each experiment. The sections were counterstained with Harris' hematoxylin prior to mounting.

5. Statistical Analysis

Data were analyzed using the two-tailed *t*-test for comparison in GraphPad (version 6.0). All data are presented as the mean ± standard error of the mean (SEM). Significance was taken when the *p*-value was less than 0.05.

III. Results and Discussion

In this study, we investigated the effect of RF-EMFs at 1,950 MHz on the hippocampus of C57BL/6 mice using a whole-body exposure system. As RF-EMF could influence neuronal activity by heat depending on the RF exposure method [19, 20], we applied a whole-body exposure system that did not induce an increase in body temperature through the free movement of animals and a ventilation system. We confirmed that the air temperature in the reverberation chamber was maintained at 20℃±3℃ and that the body temperature did not increase by over 0.5℃ within the normal range during the 2 hours daily RF exposure.

After the termination of RF-EMF exposure, the mice were subjected to behavioral testing. First, the physical activity of the experimental animals was measured by an open-field test. The open-field test is a common method of evaluating general activity [21], which is represented by the total traveled distance of each animal. No difference was found between the sham and RF-exposed groups (Fig. 2(a)). Then, a passive avoidance test was performed to assess long-term memory. Passive avoidance tests have been used in several studies to assess memory or retention, as well as retrieval, during or after other treatments [22, 23]. Although mice flee naturally into dark chambers because of their inborn light-phobic habit, mice with memory of an electric shock upon entering a dark room 24 hours previously have been found to stay longer under the light than mice with impaired memory. In the passive avoidance test, RF-EMF did not alter the latency time in the dark room (Fig. 2(b)). These data indicated that RF-EMF did not affect the memory function of mice.

Next, we performed a morphological analysis of the hippocampus following sham or RF exposure. The hippocampus is known to be involved in the cognitive function and that neuronal and glial alteration can cause cognitive impairment [24,

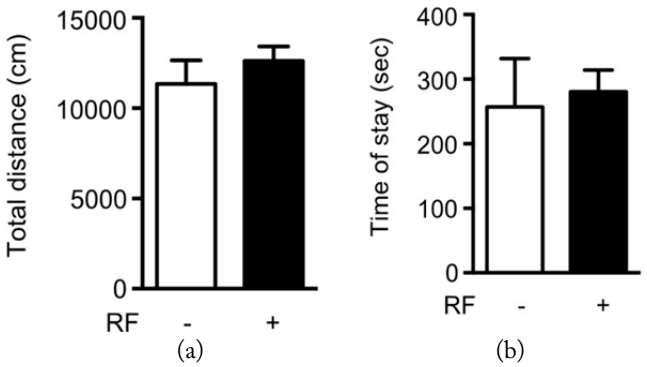

Fig. 2. Effect of radiofrequency electromagnetic field (RF-EMF) on mouse behaviors. (a) Open-field test conducted for general activity, which is represented by total distance traveled in 30 minutes. (b) Passive avoidance test performed to evaluate memory impairment using electric shock. The values are presented as mean±standard error of the mean (SEM). RF(−)=sham control, RF(+)=RF-exposed group.

25]. Therefore, to evaluate the effects of RF-EMF on neurogenesis in the hippocampus, we performed an immunohistochemical assessment of anti-NeuN, a neuronal cell marker, and anti-PCNA, a cell proliferation marker, in a paraffin-embedded hippocampus with or without RF-EMF exposure. The total number of NeuN-positive and PNCA-positive cells was counted in the same-sized area of the dentate gyrus (DG) and region 1 of the hippocampus (CA1) from both groups. No significant difference was found in the NeuN- and PCNA-positive cells between the RF-exposed group and the sham group (Fig. 3).

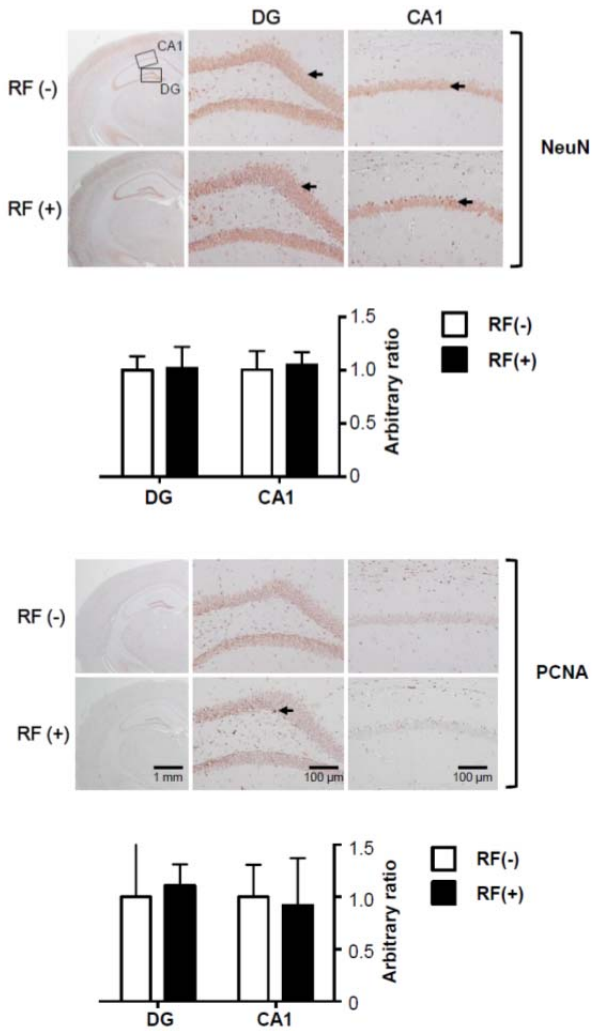

Fig. 3. Effect of radiofrequency electromagnetic field (RF-EMF) on neural cell proliferation. Detection of the NeuN- and PCNA-positive cells in mouse hippocampus by immunohistochemistry. Brain sections were obtained from mice with and without RF-EMF exposure at SAR 5.0 W/kg, 2 hours per day, and 5 days per week for 60 days. Dentate gyrus (DG) and CA1 regions are indicated as block boxes in the hippocampus. Arrows indicate positive-stained cells (colored brown by DAB). The data are presented as mean±standard error of the mean (SEM). RF(−) = sham control, RF(+)=RF-exposed group.

We also examined the glial cell expression in the hippocampus. We used the glial fibrillary acidic protein (GFAP) as a marker for reactive astrocytes and the ionized calcium-binding adaptor molecule (Iba1) as a marker for activated microglia to detect glial cell damage due to RF-EMF. Astrocytes regulate brain homeostasis and limit brain injury, and increased GFAP expression is a feature of reactive astrocytes [26]. Microglia form a vast network in the brain, and it has a constitutive role in homeostatic surveillance. Resting microglia constitutively express Iba1, which is a specific immunehistochemical marker for these glial cells and is a protein that is up-

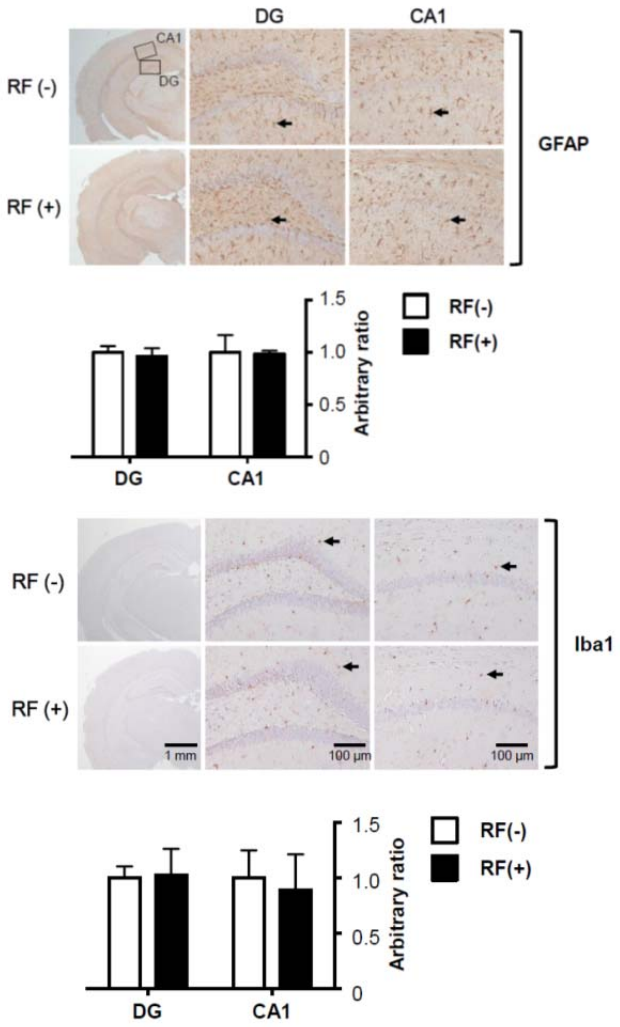

Fig. 4. Effect of radiofrequency electromagnetic field (RF-EMF) on neuroinflammation. Representative photos of astrocytes expressing glial fibrillary acidic protein (GFAP) and microglia-expressing Iba1 in the mouse hippocampus. Hippocampus sections were prepared after sham or RF-EMF exposure for 60 days and stained with GFAP and Iba1 antibodies. Dentate gyrus (DG) and CA1 regions are indicated as block boxes in the hippocampus. Arrows indicate positive-stained cells (colored brown by DAB). The data are presented as mean± standard error of the mean (SEM). RF(−)=sham control, RF(+)=RF-exposed group.

regulated during microglial activation [27]. We observed no difference between the sham and RF-exposed groups (Fig. 4). We found that sub-chronic RF-EMF did not affect the neuronal and glial cells of the mouse hippocampus.

As the cognitive function of the brain is important in human life, public concern about the potential RF-EMF effects on the brain should be clarified. Although many researchers have focused on behavioral models to investigate the effect of RF-EMF on brain function, no such effect has yet been established. In this study, our results show that sub-chronic whole-body exposure to 1,950 MHz at SAR 5.0 W/kg did not induce impairment of memorial behavior and morphological alteration in the hippocampus of C57BL/6 mice. Our study presents basic data to clarify the possible effect of mobile phone use on public health.

This work was supported by the IT R&D program of MSIP/IITP (No. B0138-15-1002; Study on the EMF exposure control in smart society).

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