



ORIGINAL ARTICLE

Effects of Electromagnetic Radiation from Smartphones on Learning Ability and Hippocampal Progenitor Cell Proliferation in Mice

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Abstract

Objectives: Nonionizing radiation is emitted from electronic devices, such as smartphones. In this study, we intended to elucidate the effect of electromagnetic radiation from smartphones on spatial working memory and progenitor cell proliferation in the hippocampus.

Methods: Both male and female mice were randomly separated into two groups (radiated and control) and the radiated group was exposed to electromagnetic radiation for 9 weeks and 11 weeks for male and female mice, respectively. Spatial working memory was examined with a Y maze, and proliferation of hippocampal progenitor cells were examined by 5-bromo-2'-deoxyuridine administration and immunohistochemical detection.

Results: When spatial working memory on a Y maze was examined in the 9th week, there was no significant difference in the spontaneous alternation score on the Y maze between the two groups. In addition, there was no significant difference in hippocampal progenitor cell proliferation. However, immunoreactivity to glial fibrillary acidic protein was increased in exposed animals. Next, to test the effect of recovery following chronic radiation exposure, the remaining female mice were further exposed to electromagnetic radiation for 2 more weeks (total 11 weeks), and spontaneous alternation was tested 4 weeks later. In this experiment, although there was no significant difference in the spontaneous alternation scores, the number of arm entry was significantly increased.

Conclusion: These data indicate that although chronic electromagnetic radiation does not affect spatial working memory and hippocampal progenitor cell proliferation it can mediate astrocyte activation in the hippocampus and delayed hyperactivity-like behavior.

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1. Introduction

Nonionizing electromagnetic radiation is energy that is given off from energy sources, including power lines, mobile phones, common electrical devices, and some types of machinery. This type of radiation differs from ionizing radiation, such as gamma rays, X-rays, and ultraviolet light, which exhibit high-frequency waves and have enough energy to liberate an electron from molecules [1]. Although nonionizing radiation has a lower frequency and is generally considered safe, accumulating evidence suggests that some types of nonionizing electromagnetic radiation have enough energy to harm living tissues. Especially, in recent years, the number of smartphone users has tremendously increased; thus, concerns and debates regarding the effects on human health of nonionizing radiation from smartphones have emerged. Importantly, unless some protective measures, such as hands-free or Bluetooth, are employed, the majority of people speak over smartphones with them near the users' heads. Therefore, it is an urgent issue to elucidate the effect of nonionizing radiation from smartphones or mobile phones on development and brain function.

In this study, to elucidate the effect of chronic exposure to electromagnetic radiation from smartphones, we examined adult progenitor cell proliferation in the hippocampus, as well as spatial learning ability using a Y maze in mice.

2. Materials and methods

2.1. Animals

Male and female C57BL/6 mice (8 wk old, Koatec, Kyongki-do, Korea) were housed at a standard temperature ($22 \pm 1^\circ\text{C}$), humidity ($50 \pm 5\%$), and in a light-controlled environment (lights on from 8:00 AM to 8:00 PM) with *ad libitum* access to food and water. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the Catholic University of Daegu (IACUC-2012-34).

2.2. Exposure to electromagnetic radiation

Mice were divided into two groups: animals from the exposed group were housed in a cage that was placed on the smartphone (Galaxy K, Samsung Electronics, South Korea) and it was maintained on operating mode throughout the study. To imitate the condition of daily smartphone use in life, we called and let mice hear the sound from the smartphone for 10 minutes a day. In the control group, animals were housed under normal conditions without electromagnetic radiation exposure. Mice were exposed to electromagnetic radiation for 9 weeks, and spontaneous alternation was tested. To verify the effect of recovery following electromagnetic radiation exposure, female mice were further exposed to

electromagnetic radiation for 2 more weeks. After the smartphone was removed, female mice were housed under normal conditions for 4 weeks to provide a recovery period, and spontaneous alternation was tested (Figure 1).

2.3. Spontaneous alternation in a Y maze

A Y maze was made from black Plexiglas and consisted of three arms with an angle of 120° between each arm. Spontaneous alternation consists of sequential entry into all three arms. Percent alternation was calculated by dividing the number of alternations by the number of possible alternations [number of alternation / (number of total arm entries - 2)].

2.4. Injection of 5-bromo-2'-deoxyuridine and immunohistochemistry

To label proliferating cells, mice received an intraperitoneal (i.p.) injection of 5-bromo-2'-deoxyuridine (BrdU; 100 mg/kg, dissolved in saline; Sigma Aldrich, St. Louis, MO, USA) and were sacrificed 1 day later. A BrdU immunohistochemistry method was described by Choi et al [2]. To label glial fibrillary acidic protein (GFAP) and CD68, sections were blocked with 10% normal goat serum, followed by overnight incubation with a mouse monoclonal anti-GFAP antibody (1:1,000; Millipore, Temecula, CA, USA) or a monoclonal anti-CD68 antibody (1:500; Biolegend, San Diego, CA, USA). After several washes with phosphate buffered saline, sections were incubated (2 h at room temperature) with secondary antibodies conjugated with horseradish peroxidase (1:1,000; Jackson ImmunoResearch, West Grove, PA, USA) and developed

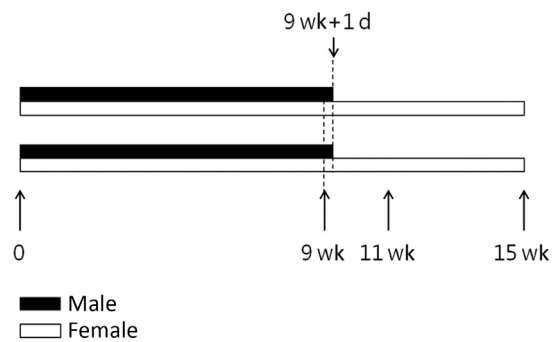


Figure 1. Summary of experimental schedule. Male and female mice were divided into control and exposed groups. Mice from the exposed group were exposed to electromagnetic radiation for 9 weeks and spatial working memory was measured. One hour after measurement 5-bromo-2'-deoxyuridine (BrdU) was injected into male mice from both groups and BrdU-injected animals were sacrificed 1 day later. Female mice were housed 2 more weeks under electromagnetic radiation or control conditions and housed for 4 more weeks without electromagnetic radiation to give the exposed group recovery. At the 15th week spatial working memory was measured again with a Y maze.

using diaminobenzidine (0.1%, Sigma Aldrich) and hydrogen peroxidase (0.005%, Junsei Chemical, Tokyo, Japan).

2.5. Cell quantitation

The density of BrdU-positive cells was quantitated as described by Choi et al [2]. Briefly, cells were counted (bilaterally) in three dorsal hippocampal sections (AP coordinate of the first dorsal-most section: $-1.40 \mu\text{m}$) separated by $160\text{-}\mu\text{m}$ intervals and averaged for each animal. To measure the granule cell layer/subgranular zone area, photomicrographs of sections were captured ($10\times$) and quantitation was performed using Photoshop (Adobe Systems Incorporated, San Jose, CA, USA). The granule cell layers/subgranular zones of both the upper and lower blades were outlined and the area was measured. Volume was counted in three sections using the coordinates described above. The number of cells was expressed as the mean \pm standard error of the mean from five mice for each group. Cell counts were analyzed statistically using the Student *t* test, and significance was accepted at $p < 0.05$.

3. Results

3.1. The effect of electromagnetic radiation on the Y maze task

First, to elucidate the effect of nonionizing radiation from smartphones on spatial working memory, we measured spontaneous alternations using a Y maze. For this purpose, 4-week-old mice were randomly separated into two groups, and animals from one group were exposed to electromagnetic radiation emitted by a smartphone for 9 weeks (exposed group: 5 males and 8 females). The control group mice (5 males and 10 females) were housed under standard conditions without any electromagnetic radiation from the smartphone. In this experiment, the percent of alternation was not significantly different between the two groups (Figure 2A). In addition, there was no significant

difference in arm entry (Figure 2B). These data indicate that chronic electromagnetic radiation exposure from smartphones for 9 weeks does not affect spatial working memory in mice.

3.2. The effect of electromagnetic radiation on progenitor cell proliferation in the dentate gyrus

Next, to elucidate whether electromagnetic radiation emitted by a smartphone affects progenitor cell proliferation in the dentate gyrus, male mice from both groups were injected with BrdU (100 mg/kg) 1 day after the Y maze test and transcardially perfused 24 hours later. As shown in Figure 3, although the density of BrdU in the subgranular zone of the exposed group was slightly higher than that of the control animals, there was no significant difference between the two groups. These data also indicate that exposure to electromagnetic radiation for 9 weeks does not have any significant influence on progenitor cell proliferation in the subgranular zone.

3.3. The effect of electromagnetic radiation on glial reaction in the hippocampus

Next, to identify whether chronic exposure to electromagnetic radiation has an effect on glial reaction immunoreactivity to GFAP, a marker of astrocytes, and CD68, a marker of microglia/macrophages, was examined. For this study, sections from the controls or from the mice exposed to electromagnetic radiation for 9 weeks were stained, and, as shown in Figure 4, increased immunoreactivity to GFAP was observed in the hippocampus, especially in the CA1 subfield. However, there was no difference in immunoreactivity to CD68 (data not shown).

3.4. The effect of recovery after electromagnetic radiation on the Y maze task

Finally, we tested whether recovery following chronic exposure to electromagnetic radiation affects

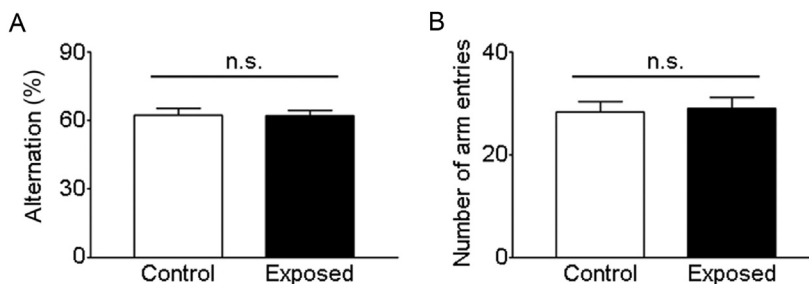


Figure 2. Spatial working memory examined after electromagnetic exposure for 9 weeks. (A) Spontaneous alternation score was not significantly different between control and exposed groups. Data were collected from 5 males and 10 females from the control group, and 5 males and 8 females from the exposed group. (B) The number of arm entry was not significantly different between control and exposed groups. n.s. = not significant.

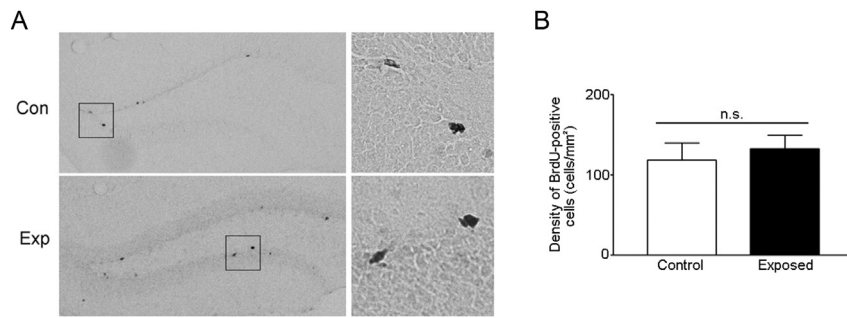


Figure 3. Hippocampal progenitor cell proliferation. Hippocampal progenitor cell proliferation was monitored following intraperitoneal injection of 5-bromo-2'-deoxyuridine (BrdU). (A) Representative images show the expression pattern of BrdU in the subgranular zone of the hippocampus from control and exposed groups. (B) Mean density of BrdU-labeled cells were not significantly different between two groups suggesting that exposure to the electromagnetic radiation does not affect progenitor cell proliferation in the hippocampus. BrdU = 5-bromo-2'-deoxyuridine; con = control; exp = exposed; n.s. = not significant.

spatial working memory. For this purpose, female mice tested using the Y maze above were further exposed to electromagnetic radiation emitted from a smartphone for 2 more weeks (total of 11 weeks); then, smartphones were removed and mice were housed under normal conditions for 4 weeks to provide a recovery period. As shown in Figure 5, the percentage of spontaneous alternation was not significantly different compared with that measured after exposure to electromagnetic radiation for 9 weeks. However, the number of arm entries was significantly increased. These data indicate that chronic exposure to electromagnetic radiation may induce hyperactivity-like behavior later on.

4. Discussion

At present, people are constrainedly exposed to the electromagnetic radiation given off from the electronic devices, including mobile phones and many types of machinery. As electromagnetic waves have been known

to be capable of breaking chemical bonds and harming living tissues, extensive research has been focused on elucidating the impact of electromagnetic radiation on human health. However, in view of the results thus far achieved, it is quite controversial. For example, radiation from mobile phones or electromagnetic fields of generators increased the intracellular concentration of reactive oxygen species, activated apoptotic signaling, and induced marked cell death [3–7]. On the other hand, exposure to electromagnetic fields does not alter apoptosis and even enhances progenitor cell proliferation and its survival in the hippocampus [8–10]. In human studies, as the majority so far has been focused on human health following acute to subchronic exposure to electromagnetic radiation, it is not easy to translate results to the circumstance of smartphone use in real life. Of note is that many people carry smartphones in their pockets all day long and sleep with them next to them or even under the pillow. That being said, it is urgent to evaluate carefully the physiological effect of long-term exposure to the electromagnetic radiation

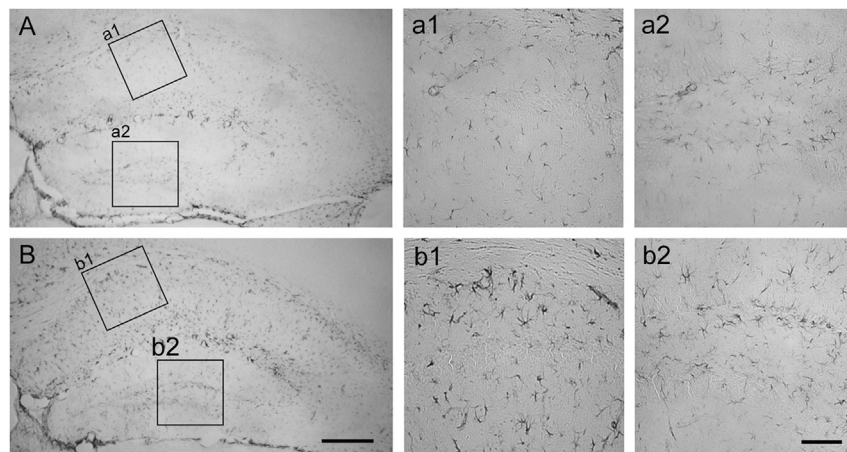


Figure 4. Astrocyte activation by chronic electromagnetic radiation exposure. Compared to control (A), immunoreactivity to glial fibrillary acidic protein (GFAP), a marker of reactive astrocyte, increased in radiated animals (B). Among the hippocampal subregions GFAP immunoreactivity distinctively increased in CA1 area rather than dentate gyrus.

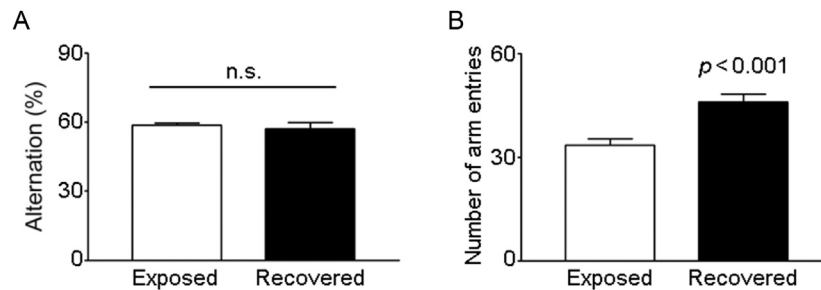


Figure 5. Spatial working memory following 4 week-recovery following electromagnetic radiation. (A) The spontaneous alternation score was not significantly difference between two groups suggesting that 4 week long recovery following electromagnetic radiation does not affect spatial working memory. (B) The number of arm entry of exposed-recovered group was significantly higher compared with that of the exposed group. These data suggest that electromagnetic radiation induces hyperactivity-like behavior in mice. Data were collected from 8~10 mice in each group. n.s. = not significant.

from smartphones. To this end, male and female mice were exposed to the electromagnetic field for 9 weeks and 11 weeks, respectively, and spatial working memory and progenitor cell proliferation in the dentate gyrus were examined.

To assess spatial working memory, we employed a spontaneous alternation test with a Y maze apparatus. In this experiment, exposure to the radiation field for 9 weeks did not significantly change the percentage of spontaneous alternation or the number of arm entries in both male and female animals. In addition, there was no significant difference in progenitor cell proliferation in the dentate gyrus. As adult neurogenesis in the dentate gyrus has been well known to play an important role in spatial working memory, these behavioral and physiological data indicate that chronic exposure to electromagnetic radiation does not affect spatial working memory in mice.

Recently, it was reported that prenatal exposure to electromagnetic radiation resulted in hyperactivity in the open field test or the light-dark box test in rodents [11,12]. In addition, prenatal and, to a lesser degree, postnatal exposure to cell phones is associated with hyperactivity problems around the age of school entry [13]. These reports led us to test whether recovery after chronic electromagnetic radiation exposure affects spatial working memory and/or animal behavior. To answer these questions, we exposed electromagnetic radiation to female mice for 11 weeks and provided a recovery period under normal housing condition for 4 weeks. To our surprise, although the percentage of spontaneous alternation was not affected, the number of arm entries was significantly increased by providing a recovery for 4 weeks following an 11-week-long period of exposure to a radiation field. These data further support the previous evidence showing that exposure to electromagnetic radiation may be able to induce delayed hyperactivity-like behavior.

In our study, there was no significant difference in progenitor cell proliferation in the hippocampus and in spatial working memory. However, some reports

indicate that electromagnetic radiation influences progenitor cell proliferation and/or spatial working memory [14,15]. The reason for the discord is unknown, although it is worth noting that we provided exposure to electromagnetic radiation for a relatively long term. Interestingly, recent data indicate that although electromagnetic fields influence learning and memory in rodents, the animals can adapt to long-term exposure [16]. In addition, long-term whole-body exposure to an electromagnetic field from a mobile phone does not cause any adverse effects on memory function and development [17]. Therefore, it is tempting to hypothesize that our nervous system can adapt to long-term exposure to electromagnetic radiation from smartphones. However, we cannot exclude the possibility that acute whole-body exposure to electromagnetic radiation changes working memory and/or hippocampal progenitor cell proliferation. Further study will be needed to elucidate this hypothesis.

In summary, we provided herein further solid evidence supporting the hypothesis that chronic exposure to electromagnetic radiation may induce delayed hyperactivity-like behavior without affecting spatial working memory and hippocampal progenitor cell proliferation.

Conflicts of interest

The authors have no conflicts of interest to declare.

Acknowledgments

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