Original Article

Effects of Cellular Phone Electromagnetic Field Exposure on the Hippocampi of Rats in Childhood and Adolescence

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Abstract

Objective: The effects of the electromagnetic fields (EMFs) emitted from cell phones on living organisms and human health have become one of the most important topics for research because cell phones are widely used, even at early ages, all over the world. In this study, it was aimed to reveal the effects of exposure to EMFs emitted from cell phones on the hippocampus region of the brain during childhood and adolescence. **Materials and Methods:** In the study, newborn rats were divided into six groups as control 1–21, EMF 1–21, control 21–60, EMF 21–60, control 1–60, and EMF 1–60. The rats in the EMF groups were exposed to an EMF emitted from cell phones placed in cages every day. No procedure was performed in the control (C) groups. Sections taken from the brain tissues were evaluated using histopathologic, stereologic, and immunohistochemical methods. **Results:** According to the stereologic analysis results we obtained from the study, there was a significant decrease in the number of pyramidal cells and hippocampus volume in the EMF 1–60 group ($P < 0.05$). In the histopathologic examinations of the brain sections, it was observed that there were many damaged neurons with darkly stained cytoplasms among normal pyramidal cells in all age groups exposed to EMF. In addition, caspase 3 immunoreactivity was found to be statistically significantly increased in the EMF 1–60 group compared with all other groups ($P < 0.05$). **Conclusion:** Chronic cell phone exposure from birth to the end of adolescence causes neuronal damage and volume reduction in the developing hippocampus.

Keywords: *Adolescent, cell phone, child, electromagnetic field, hippocampus*

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Introduction

Having entered our lives in parallel with the rapid developments in technology, electronic devices have facilitated our lives and brought some health problems due to the electromagnetic fields (EMFs) they emit. The devices that we are most exposed to EMFs are mobile phones, an indispensable tool in daily life. Due to the widespread use of mobile phones all over the world and the decreasing age of mobile phone usage, the effects of EMFs emitted from mobile phones on living organisms have become one of the most important research topics.[1,2]

Keeping mobile phones close to the ear and head area causes the central and peripheral nervous systems that coordinate vital functions to be affected by EMFs. Numerous experimental and clinical studies have been conducted to investigate the effects of mobile phone EMFs on the nervous system.[1-3] In these studies, it was reported that the effect of EMFs caused many changes that might affect life depending on exposure time, the intensity of the electric field and age, and that these were manifested by morphologic, physiologic, and behavioral changes.[4-6] One of the structures that EMFs target is the brain, one of our most sensitive organs.[7] Studies on the effects of EMFs on the brain have reported that

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these effects were also related to the age of the exposed individual.[1,5,8] One of the reasons for this has been explained as children and adolescents having a much thinner skull thickness as compared with adults and EMF absorption rates thus being higher than in adults.[2]

An important part of the brain, the hippocampus, provides control of various functions such as memory, learning, and affectivity. These important functions of the hippocampus may be affected when it is continuously exposed to drugs, chemicals, or any environmental factors.[9-11] Many studies have shown that one of the most important factors affecting these functions of the hippocampus is EMFs.[4,12,13]

Considering the scientific literature, it has been observed that the effect of EMF exposure on the hippocampus is not yet fully understood and most studies on this subject are on brain development in the prenatal period.[4,6,13,14] There are a limited number of studies on how the hippocampus, which continues to develop in the postnatal period, is affected by EMF exposure in different age groups, especially from birth to adulthood. However, the age of cell phone use in the world is decreasing with each passing year, and experts report that the age of first exposure to EMFs from mobile phones is down to 1 year. Especially during the development period, the biologic and maturation processes of the brain are very sensitive to external factors. Therefore, exposure to EMFs, especially during the development period of the brain, is a concern.[15] It would be correct to suppose that these disadvantages can take many years to be revealed, so possible damage to be observed in future can be prevented by taking precautions today. For these reasons, further studies are needed to fully reveal the biologic effects of EMFs on different stages of brain development in postnatal life and to determine whether it causes permanent damage. In this study, we hypothesized that exposure to cellular phone EMFs during childhood and adolescence would cause morphologic damage and neuron loss in the hippocampus of rats.

Materials and Methods

Animals

This study was conducted with the approval of the Ondokuz Mayıs University Animal Experiments Local Ethics Committee. In the study, a total of 36 male *Wistar albino* newborn rats were obtained from Ondokuz Mayıs University Experimental Animals Application and Research Center. All animals were maintained and fed in a sterile experimental animal unit environment with a 12 h light-dark cycle at 55°C–60°C humidity and 19°C–22°C room temperature. Standard rat chow and tap water were given to all animals.

Study design

The animals were divided into six groups with an equal number of animals in each group ($n = 6$). When forming the groups, the age groups of the rats were considered as childhood between the postnatal 1st and 21st days, and adolescence between the 21st and 60th days, based on previous studies.[16-18]

It was found that five animals were required for each group to obtain 95% power at 5% significance (1−β) using the G*Power 3.1 program. However, considering the death probability of the animals, the number of subjects per group was determined as six.

The groups were evaluated considering the cell phone electromagnetic radiation that babies are directly or indirectly exposed to during childhood and adolescence in the period from the birth until they become adult individuals.

- Control 1–21: Newborn animals were routinely maintained for 21 days after their birth, but no other procedures were performed
- Control 21–60: Animals of 21 days were routinely maintained until the 60th day when they were sacrificed, and no other procedures were performed
- Control 1–60: Newborn animals were routinely maintained for 60 days after their birth, but no other procedures were performed
- EMF 1–21: Newborn animals were exposed to the EMF emitted by a cell phone placed in the cage until 21 days from the day of birth as the phone played YouTube videos for 2 h a day every day (the phones were put on silent mode to prevent the animals from getting stressed due to the sound), it was called every 10 min for 8 h and was set on standby at other times
- EMF 21–60: Newborn animals were exposed to the EMF emitted by a cell phone placed in the cage starting from when they were 21 days old and until 60 days from the day of birth as the phone played YouTube videos for 2 h a day every day (the phones were put on silent mode to prevent the animals from getting stressed due to the sound), it was called every 10 min for 8 h, and was set on stand-by at other times
- EMF 1–60: Newborn animals were exposed to the EMF emitted by a cell phone placed in the cage until 60 days from the day of birth as the phone played YouTube videos for 2 h a day every day (the phones were put on silent mode to prevent the animals from getting stressed due to the sound), it was called every 10 min for 8 h and was set on standby at other times.

In the study, cell phones using the Global System for Mobile Communications (GSM) systems were used to produce EMFs. The cell phones had a head area-specific

absorption rate (SAR) value of 1.18 W/kg and a body SAR value of 1.12, and each had a carrier frequency band of 890–915 MHz; the actual emission during the experiments was not measured. The cell phone in each cage was placed in a special section in the middle and 1 cm below the cage. Only three rats per group were placed in each cage, and the rats were allowed to move freely in the cages during the experiment. Groups exposed and not exposed to cell phones were kept in separate rooms.

Histologic analyses

Perfusion and tissue preparation

One day after the last EMF application, all rats were anesthetized using ketamine hydrochloride (40–50 mg/kg; Alfazyne®, Egevet, Turkey), sacrificed by intracardiac perfusion, and brain tissues were removed. After the removed tissues were fixed with 4% formaldehyde, routine histologic procedures were performed and they were embedded in paraffin blocks. For stereologic and histologic analyses, serial sections of 5 and 20 μm were obtained from paraffin blocks using a rotary microtome (Leica RM 2135; Leica Instruments, Nussloch, Germany). These sections were stained with cresyl violet.

Stereological analyses

Estimation of the total number of pyramidal neurons

The total number of pyramidal cells in the cornu ammonis (CA) region of the hippocampus was estimated with the optical dissector method using a Stereo Investigator analysis system (version 7.0, Microbrightfield, Colchester, VT). This system consists of a digital camera microscope, a motorized system that moves the microscope platform, and an advanced computer that incorporates the software that controls their use. Based on our previous stereologic studies on the hippocampus, serial sections of paraffin were obtained in the sagittal plane with a one in six sample interval using the systematic random sampling method. A 900/40,000 μm^2 area sampling fraction and 20 μm section sampling fraction were used to count the numbers of pyramidal neurons in the hippocampus.[19]

Area measurement of sections and estimation of hippocampal volume

The Cavalieri principle is a special method used to estimate the volume of tissues or organs. In this study, the Cavalieri principle was used to estimate the volumes using the same stereology work station. The same sets of consecutive sections of brain tissues were used for the volume estimation of hippocampal regions. The first preparation of the sections sampled from the hippocampi in all brain tissues was placed on the microscope platform, and then, drawings were made on the section according to the color changes of the outline of the hippocampus to be measured. In the next step, the point grid with systematic point strings separated by equal intervals was placed on the section at a random angle.[20,21]

The frequency of this point grid is determined according to an appropriate error coefficient value. In the later stages, the points on the hippocampus were marked with colored markers, the reference volume of each section on the preparation was automatically measured by the special software, and the values were recorded according to the reference volume formula specified below.[20,21]

$$\hat{V} = t \times \frac{a}{p} \times \sum_{i=1}^{m} P_i$$

The expressions in this formula are as follows: V reference volume, t section thickness or distance between corresponding surfaces, a/p the area represented by a point, ΣP the total number of points for the hippocampus section in a preparation.[20,21]

The same procedures were applied for the other preparation sections in all groups. Finally, the total volume calculation was made automatically by the software in accordance with the Cavalieri principle and according to the total volume formula below:[20,21]

$$\hat{V}_{\leftarrow toplam} = V_1 + V_2 + \ldots V_n$$

The symbols of the formula are as follows:

V_{Total} = total volume

V_1 = volume of the first preparation section

V_2 = volume of the second preparation section

V_n = volume of the last preparation section.

Immunohistochemical analysis

Five-micrometer-thick sections were taken from the brain tissues and transferred on positive-charged slides. The caspase 3 immunohistochemical reactivity for the sections was stained using an automated method on a VENTANA Bench Mark GX System (Ventana Medical Systems, Inc.). Antigenic determinant areas for caspase 3 were unmasked in citrate buffer with steam for 60 min. Caspase-3 antibody (Santacruz; sc-65497) and a mouse monoclonal IgG2a (kappa light chain) were used with a dilution of 1:80. The sections were incubated in the antibody solution at 37°C for 32 min. An UltraView Universal DAB Detection Kit (Roche) was used for detecting primary antibodies. The specificity of the staining was confirmed by the inclusion of negative control sections processed in the

absence of the primary antibody in the same tissues.[22] Immunoreactivity for caspase 3 was evaluated under a light microscope.

Six high-powered (×400) areas were randomly selected in each section. Digitized images were obtained from these selected areas using a digital video analysis system (Leica, DFC 450C and DM 2500, Leica Application Suite Version 4). Caspase 3 (+)-stained cells were counted in each area, and this number was divided by the total number of cells. An average of six areas was estimated for each sample. The proportion of stained cells in each area was evaluated as follows: no stained cells: 0, <25% stained cells: 1, 25%–50% stained cells: 2, and >50% stained cells: 3.

Staining intensity was scored as follows: none: 0, low: 1, moderate: 2, and severe: 3. An immunostaining intensity distribution index (IIDI) for each sample was calculated by the following formula:

IIDI = stained cells X staining intensity.

The mean of six fields was IIDI for the sample.[23]

Statistical analyses

The numerical data of the groups obtained from our study were evaluated statistically using the SPSS software (SPSS version 17.0; SPSS Inc., Chicago, IL, USA). One-way analysis of variance tests were performed for the comparison of each group according to the normality of distribution of variables. When there was a significant difference among groups, *post hoc* comparisons were made using the Tukey test. Data are expressed as mean ± standard deviation. $P < 0.05$ was considered statistically significant.

Results

Stereologic results

Total pyramidal cell number in the cornu ammonis

The number of pyramidal neurons obtained from stereologic estimates in the hippocampus of the rats in all groups is shown in Figure 1. As a result of statistical analysis, there was a decrease in the number of neurons in the hippocampus of rats in the EMF 1–21 groups compared with the C 1–21 group, but this difference was not statistically significant ($P > 0.05$). Similarly, although the number of neurons in the hippocampus of the rats in the EMF 21–60 groups was less than in the C 21–60 group, this decrease was not statistically significant ($P > 0.05$). However, it was determined that the number of neurons in the hippocampus of the rats in the EMF 1–60 group was statistically significantly decreased compared with the C 1–60 group ($P < 0.05$).

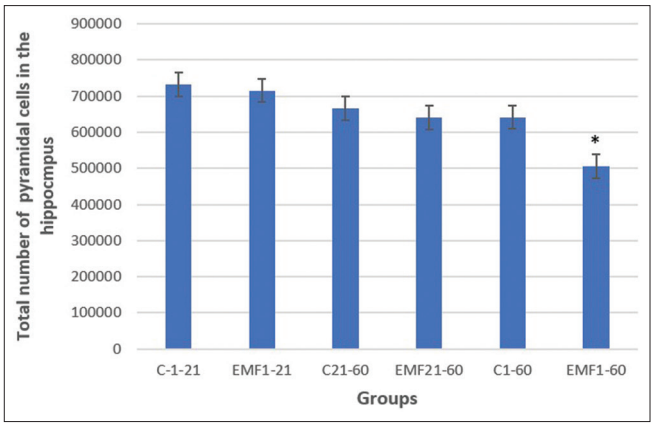

Figure 1: The total number of pyramidal neurons in the hippocampus CA region of rats in all groups. *A significant difference compared with all other groups ($P < 0.05$)

Hippocampus total volumes

The hippocampus volumes of the rats in all groups were calculated using stereologic methods [Figure 2]. After statistically evaluating the data obtained, no significant difference was found between the C 1–21 and EMF 1–21 groups ($P > 0.05$). In addition, no significant difference was found between the C 21–60 and EMF 21–60 groups ($P > 0.05$). The hippocampus volumes of the EMF 1–60 group were significantly decreased compared with the EMF 21–60, C 21–60, and the C 1–60 groups ($P < 0.05$).

Histopathologic results

Control groups

Hippocampus samples obtained from C 1-21 [Figure 3a and b], C 21–60 [Figure 3e and f], and C 1–60 [Figure 3i and j] rats had a histologic view that reflected the known mature hippocampus structure. Both the hippocampus regions divided into different areas as CA1, CA2, and CA3, and the crescent-shaped gyrus dentatus region adjacent to these regions could easily be observed. The hippocampus neurons were pyramid shaped with achromatic nuclei and basophilic cytoplasms. Each neuron was observed to have one or two nuclei. There was no difference in histologic appearance in the hippocampus sections taken from rats in all control groups (C 1–21, C 21–60, C 1–60).

Electromagnetic field groups

Examining the general structure of the hippocampus of the rats in all EMF groups, they had a histologic appearance that reflected the known mature hippocampus structure. The overall appearance of the EMF 1–21 [Figure 3c and d] and EMF 21–60 [Figure 3g and h] groups was similar. In both groups, nuclei that had lost their euchromatic properties and neurons with narrow and dark stained cytoplasm

were found in the hippocampi. In particular, the boundaries of the neurons that were almost degenerated could not be observed. The number of damaged neurons with dark-stained nuclei and cytoplasm in the hippocampus sections of the rats in the EMF 1–60 group [Figure 3k and l] was greater than the rats in all other groups. The cell boundaries were not apparent in most neurons in this group, and most had shrunk. In addition, the sections had a significant decrease in cell counts compared with the other groups, along with a decrease in cell density.

Immunohistochemical results

An IIDI was calculated for the animals in each group and the data obtained were compared statistically [Figures 4 and 5]. As a result of statistical analysis, the IIDI value of EMF 1–21 group was higher than in the C 1–21 group, but this difference was not statistically significant ($P > 0.05$). Similarly, the IIDI value of the EMF 21–60 group was higher than the in the C 21–60 group, but this difference was not statistically significant ($P > 0.05$). The IIDI value of the EMF 1–60 group was statistically significantly higher than in all other groups ($P < 0.05$).

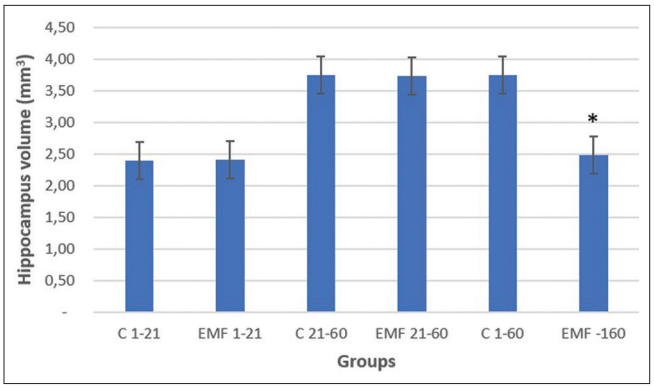

Figure 2: The graph shows the hippocampus volumes in all groups. *Significant difference compared with the C 21–60, C 1–60, and EMF 21–60 groups ($P < 0.05$)

Discussion

This study investigated the effect of EMF exposure during childhood and adolescence on the developing hippocampus in rats using stereologic, immunohistochemical, and histopathologic methods. There are many studies in the literature investigating the effects of EMF exposure on the central nervous system.[1,6,24] Unlike other studies, the cell phone radiation exposure model applied to rats in this study was designed by taking the daily cell phone radiation exposure time of children and adolescents during everyday life. Therefore, this is original research.

The stereologic analysis results of this study demonstrated that the number of pyramidal cells and hippocampus volume decreased significantly in the EMF 1–60 group. In histopathologic examinations, numerous damaged neurons with dark-stained cytoplasms and reduced morphology were observed among normal pyramidal cells in all age groups exposed to EMF.

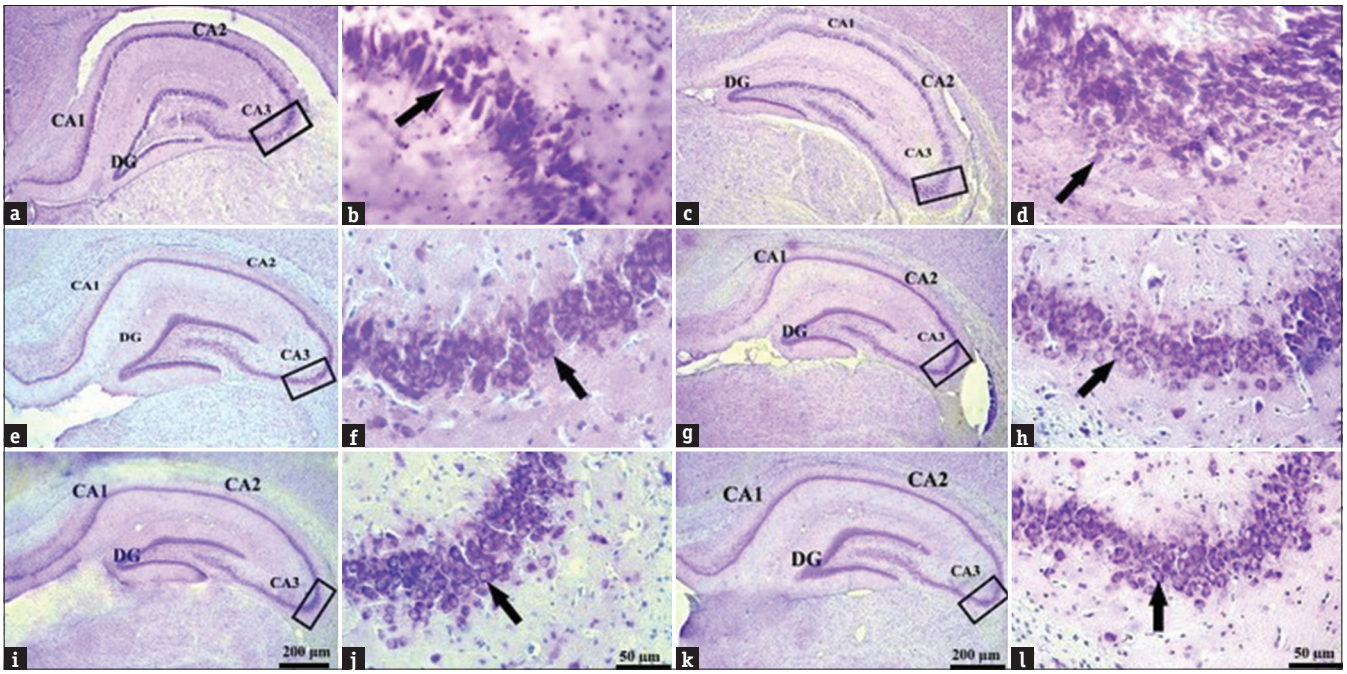

Figure 3: Light microscopic sections obtained from the hippocampus of rats in all groups. (a and b) C 1–21 group (c and d) EMF 1–21 group (e and f) C 21–60 group (g and h) EMF 21–60 group (i and j) C 1–60 group (k and l) EMF 1–60. CA1, CA2 and CA3: Hippocampus regions; DG: Dentate gyrus; black arrow: Neuron; staining: Cresyl violet, A, C, E, G, I, ×40; B, D, F, H, J, ×400

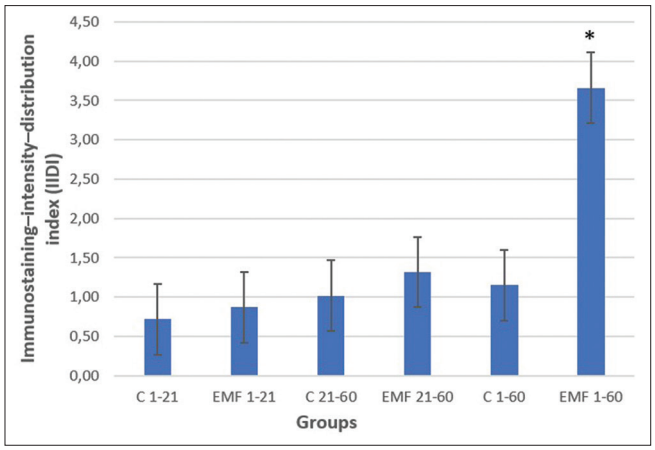

Figure 4: Immunostaining-intensity-distribution index scores for caspase 3 immunoreactivity in the hippocampus of rats in all groups (×1000). *Significant difference compared to all other groups ($P < 0.05$)

Furthermore, the caspase-3 antibody was used to observe the presence of apoptosis in neurons in thin sections taken from the brain tissues of the rats. According to the calculated IIDI results, IIDI values were observed to increase in all EMF groups compared with the control group, but this increase was statistically significant only in the EMF 1–60 group. Histopathologic and immunohistochemical results obtained from the study groups support the results of the stereologic analysis.

Many studies have shown that EMF exposure causes different results depending on age.[1,3,4,6,25] Fetuses and infants are known to have large numbers of stem cells. Embryonic Neural Progenitor Cells (NPCs) play an important role in the development of the fetal brain because they are responsible for the formation of the fetal nervous system.[26] In the reproduction of these stem cells, neuronal and glial cell differentiation and cell death are some of the most important steps. Differentiation to various cells, such as astrocytes and oligodendrocyte cells, is most susceptible to EMF exposure.[27,28] There are studies suggesting a change in the transcript levels of genes associated with apoptosis in embryonic stem cell-derived neurons.[29]

It is known that increased myeloperoxidase and lipid hydroperoxide activity in immature rats are oxidative stress parameters. This indicates that immature rats are more susceptible to EMF radiation exposure than mature rats.[30] Moreover, it was observed that electromagnetic radiation penetrates through the skull by 25% in adults, 50% in adolescents, and 75% in children (the skull thickness of a 5 years old is 1–2 mm, and the absorption rate is 4.49 W/kg; the skull thickness of a 10 years old is 1 mm, and the absorption rate is 3.21 W/kg; the skull thickness of adult is 2 mm, and the absorption rate is 2.93 W/kg). Brain development is much more affected

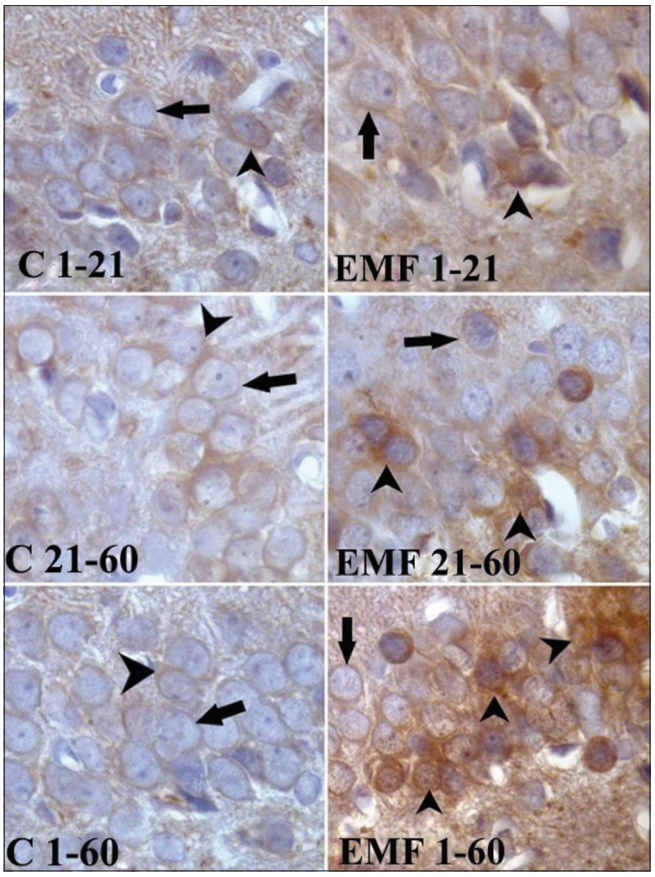

Figure 5: Light micrographs showing caspase 3 immunohistochemical reactivity for hippocampus sections in all groups. Cells in the cornu ammonis region of the hippocampus were marked with the caspase 3 antibody, and those who showed a positive reaction were observed in brown. Black arrowheads point to cells stained with caspase 3 (+), while black arrows indicate cells that are not stained with caspase 3

by EMF exposure in children and adolescents compared with adults.[2]

In the majority of the studies on cell phones, the 900 MHz frequency is used, which is used by most cell phones in GSM, widely used in Europe, as in the present study. In these studies, 900-MHz EMF exposure was reported to alter calcium levels in neurons in the hippocampus of rats, particularly, during childhood and adolescence, to cause oxidative stress and morphologic damage due to oxidative stress, to disrupt the structure of pyramidal and granular cells, and reduce their numbers, and increase glial fibrillary acidic protein expression.[1-3,24,31] GSM-900 MHz cell phones have been shown to alter the permeability of the blood–brain barrier, affect gene expression levels in embryonic stem cells (p53 deficiency), and significantly increase heat shock protein 70 production, and have an age-related effect on neuroimmunity, stress, and behavioral parameters in rats.[32-34] The studies conducted show that oxidative stress due to EMF exposure can induce apoptosis by causing the

production of apoptotic signals in cell membranes of the developing and adult brain, and that it may prevent neural stem cells from differentiating into neurons during embryonic development.[8,35] The results obtained from the EMF 1–60 group in this study are consistent with the literature. The development of morphologic damage in the hippocampus in the EMF 1–60 group may be attributed to the fact that EMF creates oxidative stress in the hippocampus and as a result leads to apoptosis of neuronal stem cells and neurons. Another possible reason may be that EMF prevents neural stem cells from differentiating into neurons.

Although morphologic damage developed in pyramidal cells in the EMF 1–21 and EMF 21–60 groups in this study, there was no statistically significant change in cell number and hippocampus volume. In rats, hippocampal development continues until the fourth postnatal week.[36] The reason for no change in cell number and hippocampus volume may be because neurons undergo apoptosis due to EMF, and that new neurons are generated as neurogenesis continues during this process. Moreover, these differences in results for EMF groups support the hypothesis that the effect of EMF on tissues and organs is related to exposure time and age, as stated in previous studies.[37,38]

However, in several studies conducted on rats in early adolescence and late adolescence periods, EMF was reported to cause oxidative stress in the hippocampus, causing a decrease in the number of pyramidal cells, unlike the results of this study.[1,6,24,39] The inconsistency between the results of this study and other studies may be due to the fact that cell phones were used in this study, whereas other studies used an EMF-emitting system.

Conclusion

Considering the data obtained from this study, it can be stated that the EMF of the cell phones affects the hippocampus differently depending on exposure time, EMF intensity, and age. Cell phones are used more widely by children and adolescents throughout the world, and this indicates that they are more exposed to the EMFs emitted by cell phones than adults. EMF exposure during these periods is a great matter of concern because the biologic and maturation processes of the brain are particularly vulnerable during growth. Therefore, more interdisciplinary studies are required to identify the effects of exposure to cell phone EMFs on brain development more clearly, particularly in childhood and adolescence, and necessary measures should be taken to avoid the harmful effects.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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