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The effect of caffeine on neuron number of rats exposed to 900-MHz electromagnetic field

Aydın HİM[1,*], Nimet Burcu DENİZ[2], Mehmet Emin ÖNGER[3]

[1]Department of Biophysics, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey
[2]Department of Neuroscience, Health Sciences Institute, Ondokuz Mayıs University, Samsun, Turkey
[3]Department of Histology and Embryology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey

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Abstract: Previous studies have shown that the electromagnetic field (EMF) emitted by cell phones decreased the number of neurons in some parts of the brain. It was also shown that caffeine protects the nerve cells. This study examines if caffeine has any effects on preventing neuron loss in rats exposed to EMF. Rats were exposed to a 900-MHz EMF for 60 min a day for 28 days. The rats that were exposed to EMF were fed 1 mg/L caffeinated water instead of tap water. The rats in the sham group were put inside the exposure chamber but were not exposed to EMF. The changes in the hippocampal and cerebellar neuron numbers were examined with stereological histological methods. EMF application caused a significant decrease in the number of the cerebellar Purkinje neurons and the hippocampal pyramidal neurons. In the group that received caffeine with EMF exposure, the decrease in neuron number was prevented in both the hippocampus and the cerebellum. The results show that caffeine protects the cerebellum and the hippocampus from neural damage induced by EMF exposure.

Key words: Electromagnetic field, rat, Purkinje cell, pyramidal neuron, stereology

1. Introduction

Recently, an increasing number of studies have investigated whether an electromagnetic field (EMF) has harmful effects on human health. The number of masses influenced by the radiofrequency waves radiated by base stations and cell phones increases as time goes by. In particular cell phones, which provide useful services to humans, are thought to have many harmful effects (1). The effects of EMF on the tissues and organs of human beings are frequently investigated (2). The most outstanding of these are studies that present the effects of EMF on central nervous system diseases and brain functions.

Radio waves that occupy the frequency range from 3 kHz to 300 GHz are forms of electromagnetic energy. The devices working in this frequency range form an impact area called radiofrequency EMF. Cell phones operate in the frequency range of 800–2600 MHz. The third- and fourth-generation mobile phones use microwaves of 1800–2100 MHz whereas second-generation phones operate at 900 MHz, which is still the most commonly used mobile communication system (3).

Since developing tissues and organs are very sensitive to harmful agents, the potential harmful effects of microwaves on children have been assessed in the agenda of the World Health Organization since 2006, and continuous studies are being conducted on the subject (4). A wide-scale case-control study conducted by two different research centers has examined the relationship between brain tumors and cell phone use. One of these studies found a significant association between cell phone use and malignant brain tumors (4). On the other hand, another study reported that cell phone use of 10 years did not increase the risk for acoustic neuroma, and it was also mentioned that 10-year cell phone use was not long enough for neuroma development. (5). Exposure to 900-MHz EMF was shown to decrease the number of Purkinje cells in the cerebellum (3).

Caffeine (1,3,7-trimethylxanthine), is a psychostimulant consumed in great amounts in Western countries (6). This purine alkaloid, which has a formula of $C_8H_{10}N_4O_2$, is found in coffee, tea, energy drinks, various soft drinks, and cacao. In addition, it is naturally found in the fruits, seeds, and leaves of many plants (7).

Although it changes from person to person, people generally consume some caffeine every day without even realizing it. A brewed coffee drink of 437 mL contains an average of 188 mg of caffeine. The caffeine concentration in coffee beans can differ between 0.01 mg/g and 19.9 mg/g.

* Correspondence: aydinhim@omu.edu.tr

According to the Centre for Addiction and Mental Health, the average Canadian consumes 210–238 mg/day of caffeine (8).

Coffee is rapidly absorbed through the gastrointestinal tract right after it is consumed and it reaches the maximum level in blood 30–60 min after it is consumed (9). Caffeine has the effects of inhibiting lipid peroxidation and decreasing the production of reactive oxygen species (10). In addition, chronic caffeine intake can decrease oxidative stress and improve mitochondrial function in a few neurotoxic situations. In a study conducted with rats, it was shown that caffeine reversed the oxidative stress and weakened inflammation caused by d-galactose, which is a compound that may cause aging in rat brains. In addition, caffeine was shown to increase glutathione S-transferase activity and prevent the deterioration of red blood cells and apoptosis (11). In addition, due to being a strong assembler of hydroxyl radicals, the effects of caffeine on neurodegenerative disorders have been examined on a large scale in the last decade (12).

The objective of this study was to assess the neuroprotective effects of caffeine in rats exposed to 900-MHz EMF through histological and stereological methods.

2. Materials and methods

Following approval by the local ethics committee for animal research (HAYDEK/35, 2015), the study was carried out according to the principles of animal research regulations. Thirty Wistar albino adult male rats weighing 250–300 g were included in the study. During the study, the rats were kept in the experimental animal unit with a 12-h/12-h light/dark cycle at a room temperature of 22 ± 3 °C and humidity of 55%–60%. The animals were allowed to consume food and tap water ad libitum. The rats were randomly divided into five groups (each with 6 rats) as Control, Sham, Caffeine, EMF, and EMF + Caffeine. The EMF group was subjected to EMF for 60 min per day for 28 days whereas the Control, Sham, and Caffeine groups were not. The EMF exposure was performed according to Onger et al. (13). Rats in a Plexiglas cage were exposed to 900-MHz EMF produced by a monopole antenna placed in the middle of the cage and powered with an EMF generator (SET ELEC. CO. 900/1800 Lab Test Transmitter, İstanbul, Turkey). The EMF strength near the heads of the rats was measured with an EMF strength meter (Extech, Nashua, NH, USA). The rats of the Caffeine group were fed with water containing 1 mg/L caffeine (Sigma-Aldrich, Gillingham, UK), instead of tap water. The rats were euthanized under anesthesia with ketamine HCl at the end of the 28th day and histological tissue preparation steps were performed.

After 28 days, all groups were anesthetized with ketamine (80 mg/kg) (Ketalar, Pfizer, İstanbul, Turkey) and xylazine (10 mg/kg) injection (i.p.) (Rompun, Bayer, İstanbul, Turkey). Intracardiac perfusion was performed with physiological saline solution and neutral formalin, respectively. Brain and cerebellum samples were embedded in paraffin blocks after tissue processing steps. A pilot study was conducted to establish a strategic plan before the sectioning. As a result of the pilot study, it was considered appropriate to obtain sections of the right hemisphere in the transverse plane with 1/6 sampling and in thickness of 25 μm.

Cell counts were performed on sections of the brain and cerebellum tissues using an unbiased counting frame measuring 60 mm × 40 mm for the number of pyramidal neurons in the hippocampal region and 70 mm × 70 mm for the Purkinje cell count. Total Purkinje cell number in the cerebellum and total hippocampal pyramidal neuron number in (CA)1, CA2, and CA3 were estimated using the optical fractionator method in the cerebellar area using a Stereoinvestigator work station (Stereoinvestigator 9.0. MicroBrieldField, Colchester, VT, USA).

Quantitative results of samples were compared using the Mann–Whitney U test. Mean values were considered to be different when $P < 0.05$. All statistical analyses were performed using SPSS 20.0 for Mac (IBM Corp., Armonk, NY, USA).

3. Results

Total pyramidal neuron numbers in the hippocampus and total number of Purkinje cells in the cerebellum in experimental groups are shown in Figure 1 and Figure 2, respectively. There was no significant difference between the Control and Sham groups ($P > 0.05$). Exposing the rats to 900-MHz EMF caused a significant decrease in the number of both the pyramidal neurons in the hippocampus and Purkinje neurons in the cerebellum ($P < 0.001$, Control vs. EMF groups). The addition of caffeine to the drinking water of the rats exposed to EMF prevented

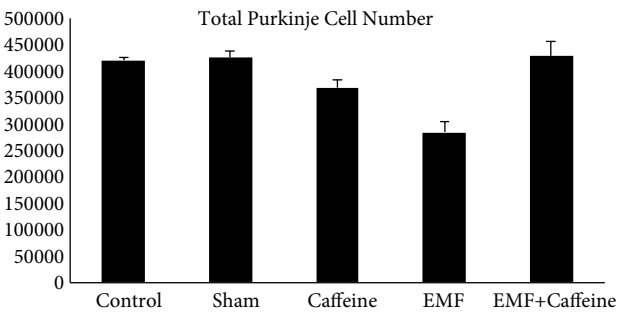

Figure 1. Total number of hippocampal pyramidal neurons in experimental groups. EMF, Electromagnetic field; a, Control vs. Sham, $P > 0.05$; b, Control vs. Caffeine, $P > 0.05$; c, Control vs. EMF, $P < 0.001$; d, Control vs. EMF + Caffeine, $P > 0.05$; e, EMF vs. EMF + Caffeine, $P < 0.01$. Error bars indicate standard error of the mean.

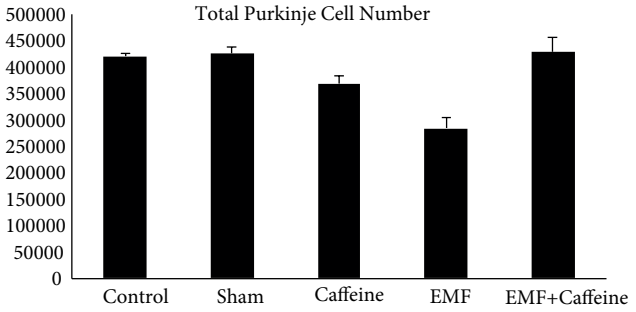

Figure 2. Total number of Purkinje cells in the cerebellum is shown. EMF, Electromagnetic field; a, Control vs. Sham, $P > 0.05$; b, Control vs. Caffeine, $P > 0.05$; c, Control vs. EMF, $P < 0.001$; d, Control vs. EMF + Caffeine, $P > 0.05$; e, EMF vs. EMF + Caffeine, $P < 0.01$. Error bars indicate standard error of the mean.

the decrease in the numbers of both hippocampal pyramidal and cerebellar Purkinje neurons, which were significantly higher than those of the EMF group ($P < 0.01$, EMF vs. EMF + Caffeine groups). There was no significant difference between the Control and EMF + Caffeine groups in terms of the numbers of hippocampal pyramidal and cerebellar Purkinje neurons ($P > 0.05$).

Representative histological images of the hippocampal region and the cerebellum of the experimental groups are presented in Figures 3 and 4, respectively, where pyramidal neuron and Purkinje neuron degenerations are notable in the EMF group, whereas less degeneration was evident in the EMF + Caffeine group.

4. Discussion

Based on the results of studies conducted with humans and experimental animals so far, the International Agency for Research on Cancer has classified radiofrequency EMF as "possibly carcinogenic" (http://www.iarc.fr/en/media-centre/pr/2011/pdfs/pr208_E.pdf). In addition to a great number of studies that have shown the carcinogenic effects of electromagnetic waves on experimental animals, the effects of using cell phones, especially very close to the brain, on the central nervous system have been the subject of many scientific studies. However, despite molecular studies of experimental animals and epidemiologic studies of humans, the effects of radiofrequency EMF on the central nervous system are still a topic for discussion (14).

Even studies conducted with humans or animals have shown that low-frequency cell phones change the activity and morphology of both the peripheral and central nervous system significantly. Among these, the effects on synaptic plasticity, neurotransmitter oscillation, life of neuronal cells, learning, and memory are especially remarkable. In view of the current studies, it can be said that overuse of cell phones may particularly cause long-term significant changes in the structure and function of brain nerve cells (15).

Radiofrequency EMF of 900 MHz was used in this study, which is the commonly used level for cell phone radio wave frequency in the world. Being exposed to 900-MHz EMF 1 h a day for 28 days caused a significant decrease in the hippocampus pyramidal neuron and cerebellar Purkinje neuron numbers of adult rats. There are a great number of studies reporting that EMF causes neuron damage in different parts of the brain. Bas et al. observed a significant decrease in total pyramidal cell numbers of the brain cortex and hippocampus of rats exposed to 900-MHz EMF when compared with control and sham groups (16). In a study by Odaci et al., it was shown by stereological methods that there were numerical decreases in the granule cells of rats in the dentate gyrus in the prenatal period due to exposure to EMF (17). In a study conducted on in vitro hippocampus sections, Xu et al. showed that exposure to 15 min of 1800-MHz radio waves every day for 8 days caused a decrease in AMPA miniature postsynaptic current and a decrease in PSD95 synapse protein expression, and they asserted that cell phone radio waves can affect synaptic activity level and number of stimulated synapses (18). Exposure of female rats to 900-MHz radio waves for 1 h every day for 28 days was reported to cause a decrease in cerebellum Purkinje neuron number (3). One reason why the cerebellum and especially the hippocampus are affected by exposure to EMF may be the excessive plasticity in these brain areas (19). Despite the negative effects of exposure to EMF on the number of neurons in different areas of the brain, there are also findings that exposure to EMF does not cause a behavioral disorder in experimental animals. Dubreuil et al. reported that being exposed to 900-MHz radio waves for 45 min did not affect the spatial or nonspatial memory performance of rats (20).

Although there are some studies reporting that caffeine may have some negative effects such as causing hypertension, arteriosclerosis, and cancer, there are also studies that reported neuroprotective effects (21). Caffeine was found to decrease dopaminergic neuron loss in the substantia nigra that occurred as a result of MPP+ injected in cerebral ventricles in a rat model of progressive Parkinson disease (12). In another study, giving caffeine and ethanol to rats orally decreased the infarct area in the brain and had a neuroprotective effect in an ischemia model made with middle cerebral artery occlusion in rats (23). In a study by Gohary et al., it was reported that with its suppressive effects on the power amplitude of especially alpha EEG waves in the brain motor cortex, caffeine intake probably decreased the harmful effects of EMF (6). Intraperitoneal caffeine injection has been reported to increase the main antioxidant glutathione level in the

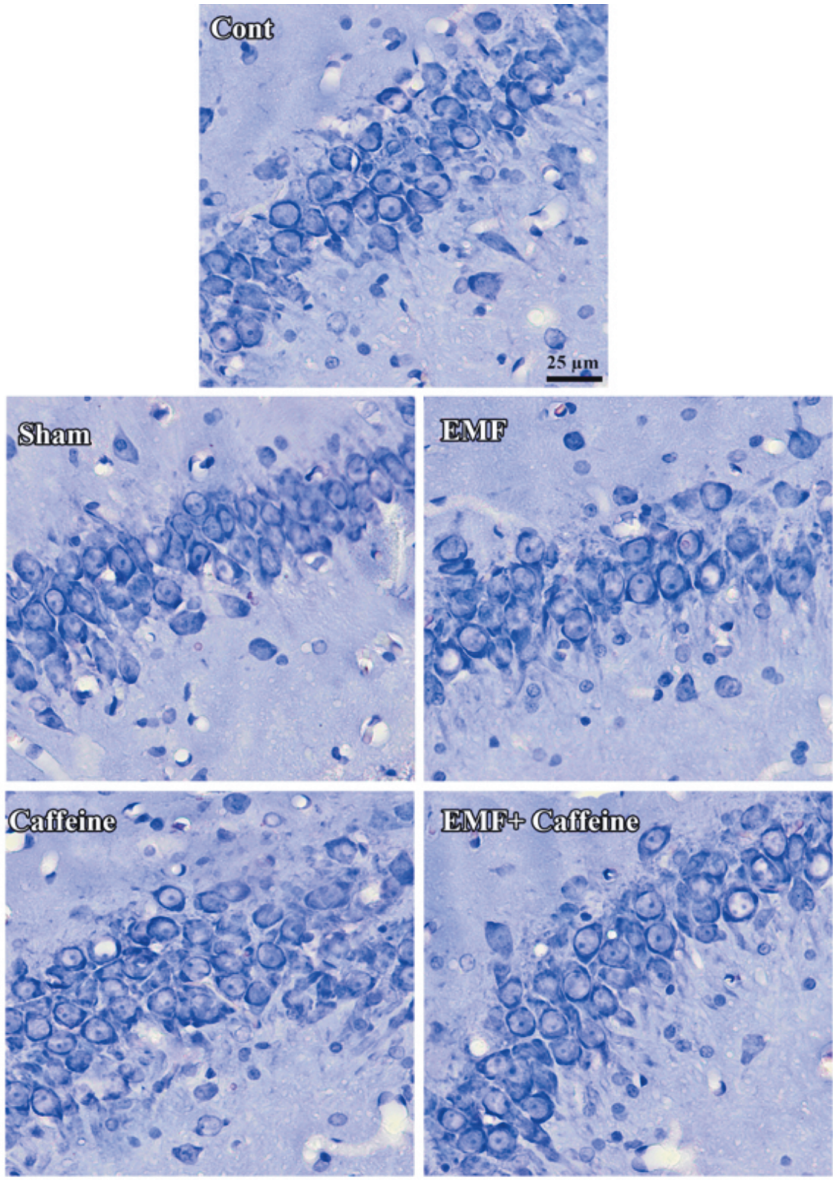

Figure 3. Histologic images of pyramidal neurons in the hippocampal region stained with cresyl violet. Pyramidal neuron degeneration and decrease in number are remarkable in the histological image of the EMF group. Cont, Control; EMF, Electromagnetic field.

hippocampus of rats and thus it was suggested that it could reduce neurodegeneration (11).

Caffeine and its metabolites were shown to have different effects based on the dose and way of application in the treatment of ischemia and based on the model of ischemia. When alcohol and caffeine were administered together orally or intraperitoneally they reduced ischemic brain damage in rats, but this effect was not observed with chronic low-dose alcohol and caffeine therapy. This neuroprotective effect of caffeine is thought to occur due to its inhibitory effect on the receptor pathways and its ability for adaptation (23).

The molecular mechanisms of the neuroprotective effect of caffeine on neurons exposed to EMF are not exactly known. Caffeine essentially shows its effect in the brain as the competitive antagonist of adenosine receptors. The fact that adenosine receptors are expressed at a high rate especially in the hippocampus and cerebellar cortex indicates that the neuroprotective effect of caffeine in these areas may be through adenosine receptors (24). Adenosine receptors modulate the neuronal activity level of the central nervous system by regulating stimulatory and inhibitory neurotransmitter release and the blockage of their receptors with caffeine can change the excitability

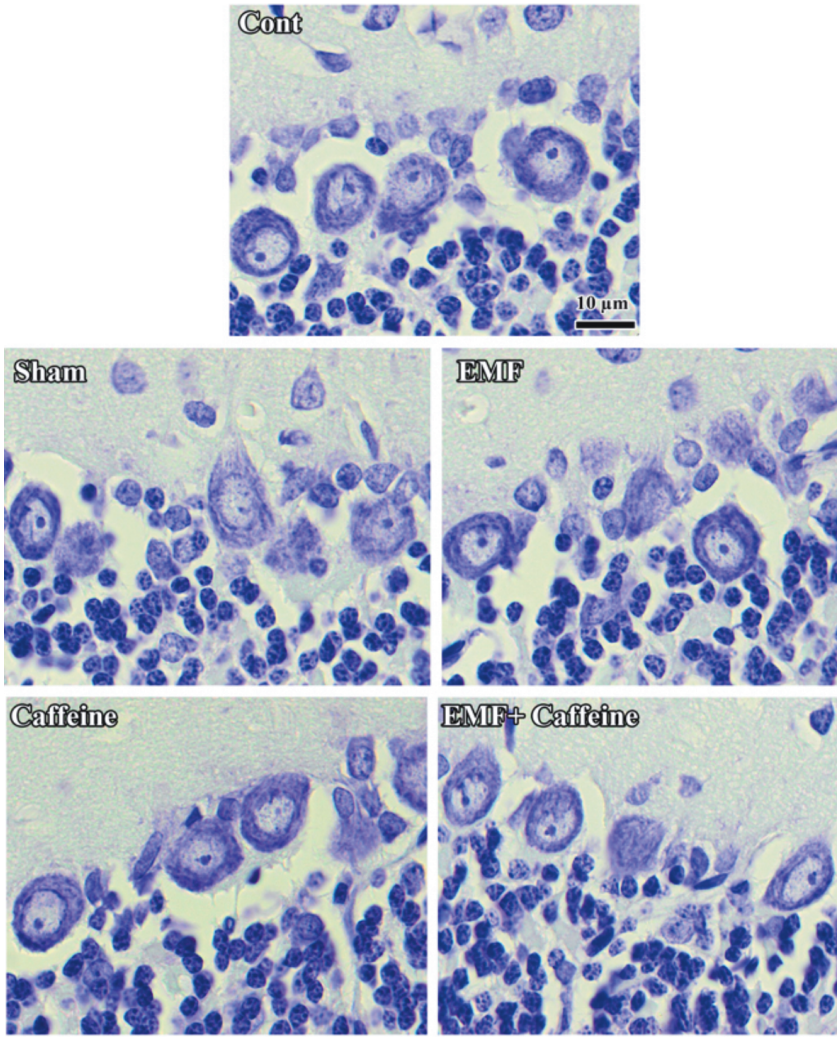

Figure 4. Histological images of the Purkinje cells in the cerebellum stained with cresyl violet. The decrease in Purkinje neuron number and degeneration of the cells are remarkable in the histological image of the EMF group. Cont, Control; EMF, Electromagnetic field.

of neurons that can be involved in neuroprotective mechanisms (21). Chronic caffeine administration causes an increase in the adenosine receptor number in the brain and thus inhibitory adenosine can show a neuroprotective effect by modulating signal transduction pathways (25).

In this study, caffeine was added to the drinking water of rats at a concentration of 1 g/L. This dose of caffeine corresponds to approximately 60–80 mg/kg daily for each rat. This dose is also equivalent to 10–20 mg/kg daily that corresponds to 3–4 cups of coffee consumption in humans (26). Although the amount of caffeine used in rats in this study seems to be high when compared with the dose consumed by people, given that the half-life of caffeine in rats is 1 h, when compared with 5 h in humans, a higher dose is needed in rats to get the same plasma and brain concentration (27). While the daily intake of caffeine differs from society to society and person to person, it is 70–400 mg/day on average (21).

No significant difference was found between the hippocampus and cerebellum neuron numbers and weights of rats given caffeine and those in the control group. Although the effects of long-term caffeine application on animal behavior were not tested in the current study, in light of the literature, its beneficial effects were more often reported rather than negative effects. Long-term caffeine application in experimental animals has been reported to increase spatial learning capacity (28) and to decrease sensitivity to epileptic seizures (29). With advancing age, a decrease occurs in neuron numbers in the different parts of the brain, including the cerebellum (30). There are no studies about the effects of caffeine on the aging-related decrease in the number of neurons; thus, the current

results suggest that caffeine should also be studied for its neuroprotective effects on nonpathological processes such as aging.

The results show that caffeine can reduce neuronal loss in the cerebellum and hippocampus in rats exposed to EMF. Coffee is one of the most consumed beverages worldwide and its consumption has been demonstrated to impact human health. Considering the beneficial properties of caffeine in neurodegenerative conditions and the molecular targets of caffeine in the central nervous system, it is very important to elucidate the effects of caffeine and its metabolites on neuronal cell loss with further studies.

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