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Impressions of the chronic 900-MHz electromagnetic field in the prenatal period on Purkinje cells in male rat pup cerebella: is it worth mentioning?

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SUMMARY

OBJECTIVE: The aim of this study was to examine the changes on the Purkinje cells in the cerebella of male rat pups born to pregnant dams that were exposed to an electromagnetic field in the prenatal period.

METHODS: The first stage of the study involved 12 Sprague-Dawley rats, 6 male and 6 female, weighing between 180 and 250 g. The female rats in the experimental group were exposed to a 900-MHz electromagnetic field for 1 h at the same time every day, and no procedure was performed on the control group. Following pregnancy, six male pups from each group were divided into experimental and control groups without any procedure on the pups. After 2 months, they were sacrificed and their cerebella were removed. Histopathologically, following routine processing and fixation procedures, the cerebella were embedded in the tissue blocks. The sections taken from these blocks were stained with cresyl violet. The Purkinje cells in the cerebella were then counted on sections using the optical dissector method on an image analysis system.

RESULTS: The estimation of number of the Purkinje cells in the groups revealed more cells in rats in the control group than in the experimental group. Histopathologically, Purkinje cells exhibited a normal morphological structure in the control group, while the cells in the experimental group showed damage.

CONCLUSIONS: It might be asserted that the exposure of mothers to an electromagnetic field in the prenatal period may affect the development of Purkinje cells in the pup cerebella.

KEYWORDS: Electromagnetic fields. Purkinje cells. Cerebellum. Anatomy. Anatomic pathology. Histopathology. Pathology. Surgery.

INTRODUCTION

The usage of wireless communication devices has become a ubiquitous part of daily personal and professional life[1], resulting in an augmented public exposure to radiofrequency electromagnetic radiation (RF-EMF)[2]. Long-term exposure to RF-EMF emitted by various electronic devices has been propounded to result in hazardous biological and health effects[3].

Despite the controversy, deleterious effects such as changes in intracellular calcium homeostasis, neuronal damage, and impairments in neurotransmitter systems have been reported in association with the biological effects of RF-EMF exposure on the central nervous system[4]. Population-based studies have revealed that the neurodevelopment of the offspring exposed to RF-EMF during pregnancy is adversely affected, with a greater risk of emotional and behavioral difficulties[5]. Several epidemiological studies have raised the question of the risk of increasing glioma and acoustic neuroma among these intensive users[6]. The International Commission on Non-Ionizing Radiation Protection recommends animal model evaluation to assess the health risks to humans caused by electromagnetic radiation[7].

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Adolescence in rodents has been classified as early adolescence (preadolescent, postnatal 21–34), mid-adolescence (periadolescent, postnatal 34–46), and late adolescence (young adults, postnatal 46–59)[8]. This study purposed to allow male rat pups to live to adulthood (postnatal 60) in order to investigate whether or not any change occurs in the Purkinje cells of male pups born to dams that were exposed to long-term, chronic EMF in the prenatal period. This time frame may be compared to the preadolescent period in humans. At the end of the study period, the Purkinje cells in the male rat pup cerebella would be subjected to histopathological examination, and the number of the Purkinje cells would be calculated using the stereological method.

METHODS

Ethical aspects

This study was approved by the Research and Ethics Committee of Experimental Animals linked to the Ordu University, Ordu, Turkey, under the approval number 82676388.3.16/2020.

Study design

All procedures in the experiment were performed in conformity with the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health. In all, 12 Sprague-Dawley rats, 6 male and 6 female, weighing 180–250 g, were used. Throughout the study period, the rats were housed in plastic transparent cages, in a 12:12 day:night cycle in a temperature-controlled environment (22±1°C) with 55% humidity. The rats were then allowed to mate for a 24-h period. The following morning, vaginal smears were collected, with the saline solution being injected into the vaginas using a Pasteur pipette. The solution was then collected back and examined under a light microscope.

The rats identified as pregnant on the first day were placed into the EMF group. The three rats in that group were then exposed to 900-MHz EMF for 1 h between 08:00 and 09:00 a.m. every day. The EMF applied to the rats in the EMF group was maintained throughout pregnancy. No sham group was established since the rats in both groups were housed under identical conditions. The G*Power 3.1.9.2 statistical software, the F-tests ANOVA, and the fixed-effects, omnibus, and one-way module were used to calculate the sample size. This analysis determined a sample size of six rats in each group in this study.

At the end of pregnancy, the rats in the EMF gave birth to 5, 7, and 4 pups, respectively (a total of 16), 7 of which were male. The rats in the control group gave birth to 5 and 8 pups (a total of 13), 6 of which were male. After 2 months, the rats included in the study were anesthetized through ketamine (60 mg/kg body weight, Sigma Chemical Comp, St. Louis, MO, USA)/xylazine (5 mg/kg body weight, Sigma Chemical Comp, St. Louis, MO, USA) injection at a 1/5 ratio. Then they were sacrificed and their cerebella were extracted and placed into 10% formaldehyde.

Electromagnetic field application

EMF application to the pregnant rats in the experimental group was performed according to previous studies. Briefly, the EMF application system consisted of an oscillator with an ultrahigh-frequency uninterrupted power source (1218-BV Lockable Oscillator, 900–2000 MHz, General Radio Company, Concord, MA, USA, Serial No. 1483). The study was performed with an uninterrupted power source (1267-B Regulated Power Supply, General Radio Company, Concord, MA, USA, Serial No. 903) (set to an approximate output of 300 mW and 900 MHz frequency) and a Plexiglas cage specially designed for the study (30×42×52 cm). The oscillator was attached to a half-wave antenna made from a copper rod of 1×150 mm in size through a coaxial cable. The antenna was placed in the center of the cage at a depth of 110 mm from the open top. The mean location intensity was calculated using a measurement device with a range of 100 kHz to 2.5 GHz (Chauvin Arnoux CA43 Isotropic Electric Field Intensity Measurement Device). This EMF represents the threshold values for a single source determined in Global System for Mobile Communications (GSM)-900 base stations.

Histopathological and stereological procedures

After the cerebella had been stored in 10% buffered formaldehyde for 1 week, they were removed. The surrounding tissues were dissected, and fixation procedures were performed. The cerebella were processed through a varying series of alcohol and xylene and embedded in paraffin blocks. Sections were next taken from these blocks using a rotary microtome (Leica RM 2135, Leica Instruments, Nussloch, Germany). Samples cut to 30 µm thickness in the sagittal plane with 1/7 sampling were taken using single-use metal microtome blades (Type N35, Feather Company, Osaka, Japan) with a 5° blade angle. The sections were then placed onto gelatin-formaldehyde-covered slides and stained with cresyl violet. The stained slides were then covered with Entellan medium (Merck 107961 Entellan new for microscopy) and subjected to histopathological examination (Olympus, BX51, Japan).

Consistent with previous studies, the number of Purkinje cells in the cerebellum sections placed onto the slides was

estimated in an unbiased manner using the optical dissector method. A pilot study was conducted to determine the research strategy. Sampling was continued by taking every seventh section until the end of the procedure. The equipment used for cell counting with the optical dissector technique included a light microscope (Leica DM4000 B, Germany), a computer, a computer-controlled motorized joystick (Prior ProScan, USA), a digital camera (JVC, Japan), and an electronic microactuator (Heidenhain, Germany). The system was entirely controlled by the Stereo investigator software (version 9, MicroBrightField Inc., USA). The cell counting was conducted on a computer screen using a 100× Leica HCX Plan Apo lens (NA=1.135). Total number of neurons in the cerebellum was calculated using the formula $N=\sum Q.\frac{1}{ssf}.\frac{1}{asf}.\frac{1}{tsf}$ (N: total number of neurons; ΣQ: total number of dissector neurons; ssf; section sampling fraction; asf: area sampling fraction; and tsf: thickness sampling fraction). The number of cells sampled was validated by calculating the coefficient of error and coefficient of variation values which were obtained from previous reports in order to estimate the efficiency of the sampling strategy[9].

Statistical analysis

The normality assumption for all the research data (rat weights, rat cerebellum weight, and the number of rat cerebellar neurons) was tested using the Shapiro-Wilk test ($p>0.05$). Differences were therefore determined using the Student's t-test. The research findings were expressed as mean, standard deviation, minimum & maximum values and p-values <0.05 were regarded as statistically significant. All the statistical calculations were performed using the SPSS software version 22.0 V, demo version.

RESULTS

Histopathological evaluations estimated the Purkinje cell counts, and data concerning the rats' physical examinations and weights are given below.

Histopathological observations

The sections from the cerebella of the 2-month-old male rats from the control and EMF groups were subjected to histopathological examination. The Purkinje cells in the cerebella from the control group of rats exhibited a normal morphological appearance (Figure 1). However, the damage was histopathologically observed in the Purkinje cells from the EMF group of male rats (Figure 2) in the form of dark, pyknotic cytoplasm by cresyl violet staining.

Number of the Purkinje cells

The number of the Purkinje cells was significantly higher in rats in the control group compared to those in the EMF group ($p=0.001$). The relevant number of cells in the control and EMF groups is exhibited in Table 1.

Physical examination and the cerebellum weights

No pathological finding or abnormal external appearance was observed in either the EMF or control groups during the physical examination before sacrifice at the end of the 2 months.

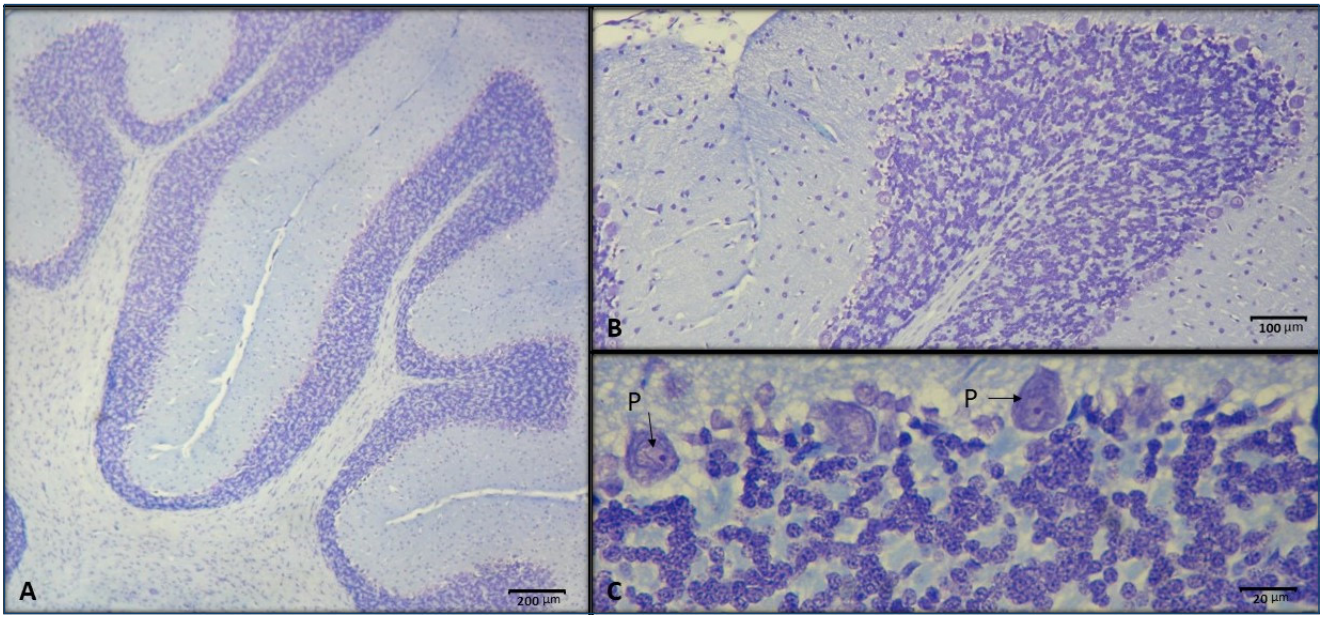

Figure 1. A normal morphological appearance, microphotograph of the cerebellum (cresyl violet staining; original magnifications, A: Bar=200 µm, B: Bar=100 µm, C: Bar=20 µm) P: Purkinje cell.

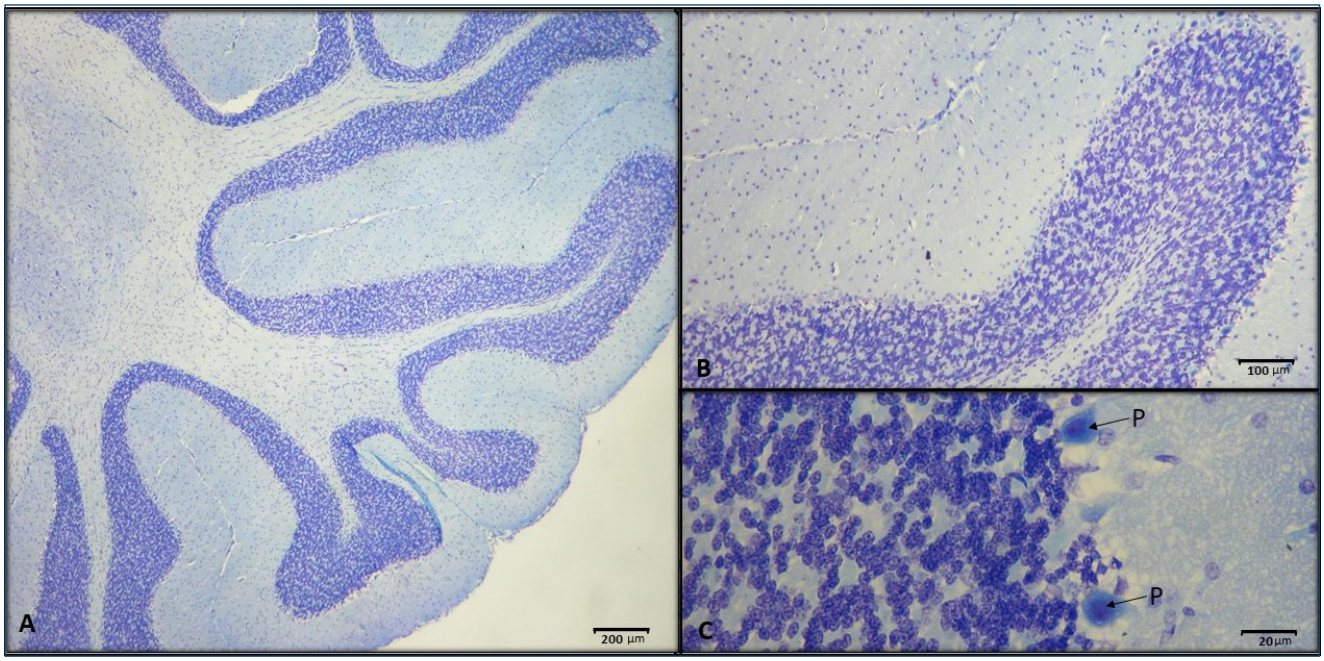

Figure 2. The damage, which was revealed in the Purkinje cells in the form of dark, pyknotic cytoplasm, microphotograph of the cerebellum (cresyl violet staining; original magnifications, A: Bar=200 µm, B: Bar=100 µm, C: Bar=20 µm) P: Purkinje cell.

At the end of the experiment, no statistically significant difference was observed in terms of the body weight or cerebellum weight between the two groups (p>0.05) (Table 1).

Table 1. Optical dissector analysis data for the estimation of total number of the Purkinje cells in the cerebella.

Stereological analysis parameters	Control (n=6)	Experimental (n=6)
Dissector particle number (mean)	184.83	165.47
Number of sampled section (mean)	17.81	18.11
Section thickness (mean) (µm)	19.87	20.01
Number of steps for counting (mean)	147.34	128.72
Section sampling fraction (ssf) (coronal)	1/7	1/7
Counting frame size (μm^2)	400	400
Area sampling fraction (asf) ($\mu m^2/\mu m^2$)	400/40,000	400/40,000
Thickness sampling fraction (tsf) (µm/µm)	10/19.87	10/20.01
Coefficient of error	0.7	0.7
Coefficient of variation	0.4	0.4

DISCUSSION

The biological effects of exposure to EMFs have been investigated in several studies, particularly in experimental mouse studies[10]. However, the biological effects of such exposure on the development of the brain and the underlying mechanisms have still not been completely elucidated[2].

Exposure to EMF both before and after birth has been reported to cause various potential impairments in the physiological and morphological structures and behavior of several animal species[11]. Haghani et al.[12] reported the adverse effect of EMF exposure on the central nervous system and investigated the effect of prenatal exposure on the Wistar rat pup cerebellum.

A study investigating the effect of exposure to an ultra-high frequency on occupational burnout syndrome enrolled 115 hospital central workers and 124 administrative personnel in the study group. Levels of oxidative stress biomarkers including malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and total antioxidant capacity were measured. MDA, SOD, and CAT levels in the group exposed to low-frequency EMF were significantly lower than those in the nonexposed group. The prevalence of burnout syndrome and severity of depression were also higher in the former group[13]. These results suggest that oxidative stress induced by RF-EMF can lead to DNA damage in neurons during prolonged exposure to animals. Almost the same results have been found in several other studies[14].

Apoptosis was evaluated with caspase-3 activity in the ventral cochlear nucleus, which is the first hearing place in the brain stem, and neurons and oligodendrocytes at different stages of the postnatal period after 900-MHz EMF application in the prenatal period, and it was stated that shrunken apoptotic neurons and oligodendrocytes with fragmented nuclei were detected in the EMF group. In addition, it has

been reported that varying intensities of caspase-3 expression were observed in the neurons, oligodendrocytes in particular, in EMF study groups[15]. Furthermore, the effects of 900-MHz EMF on the cerebellum were investigated using histopathological and immunohistochemical methods and the results indicated, as a remarkable finding, that the Purkinje cells and granular layer cells in the cerebellum of the EMF study group had pycnotic nuclei, and a significant decrease in their cytoplasmic content was recognized. Caspase-3 induces apoptosis through chromatin condensation and degradation of DNA into nucleosomal subunits[16].

EMF affects not only the nervous system but also other systems. Borzoueisileh et al.[17] exposed rats to 900/1,800-MHz and 2,400-MHz EMF. Their histopathological examination revealed tubular cyst formation, tubular vacuolization, tubular dilatation, tubular atrophy, interstitial inflammation, interstitial bleeding, interstitial edema, lymph vessel dilatation, vascular wall thickening, and blood vessel inflammation in the kidneys.

CONCLUSIONS

Everyone is well aware that mobile devices are an inseparable part of daily life and is also concerned about the deleterious effects. This study investigated the potential deleterious effects of prenatal EMF exposure on individuals to be born subsequently. The effect of the EMF received by the dam in the prenatal period was found to affect the development of the Purkinje cells, a component of the central nervous system, in the pups, and was observed to persist in the postnatal period. Thus, this issue merits further investigation.

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AUTHORS' CONTRIBUTIONS

OB: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft. **IS:** Data curation, Formal analysis, Investigation, Methodology, Software, Supervision, Visualization, Writing – review & editing. **OFMB:** Investigation, Project administration, Resources, Validation, Visualization. **HH:** Investigation, Project administration, Resources, Validation, Visualization. **MD:** Investigation, Project administration, Resources, Validation, Visualization. **DS:** Data curation, Formal analysis, Investigation, Methodology, Software, Supervision, Visualization, Writing – review & editing. **EA:** Investigation, Project administration, Resources, Validation, Visualization. **USS:** Project administration, Resources, Validation, Visualization. **OFS:** Investigation, Resources, Validation, Visualization. **JMSJ:** Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – review & editing.

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ERRATUM

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