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Evaluation of cognitive functions and EEG records in rats exposed to 2.45 GHz electromagnetic field

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► ***Original article***

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ABSTRACT

Background*:** Electromagnetic fields may primarily affect cognitive functions. It has not been elucidated how electromagnetic radiation affects the brain, particularly in the young age group. We aimed to examine the cognitive function, expression of N-methyl -D-aspartate receptors (NMDA), and EEG alterations in weaned rats exposed to a 2.45 GHz electromagnetic field. ***Materials and Methods: Twenty-one weaned (21 days old) male Wistar Albino rats were divided into two groups as experimental group (n=12) and control group (n=9). Animals in the experimental group were exposed to a 2.45 GHz electromagnetic field for one hour a day for more than 28 days. At the end of this period, rats were subjected to training and learning test using Morris Water Maze. After obtaining EEG records, hippocampi were removed. 2A and 2B subunits of NMDA receptors were studied in hippocampal homogenates using the Western Blot method. ***Results*:** There were no statistically significant differences between the two groups in measures of latency to target quadrant, time spent in the target quadrant, and average swim speed as compared in Morris water maze. However, the time to arrive at the visible platform was significantly longer in experimental animals. There were no statistically significant differences in expression of 2A and 2B subunits of NMDA receptors between the two groups. Evaluation of EEG records revealed that spike frequency was significantly higher and time to first spike was significantly shorter in the experimental group. ***Conclusion:*** These results indicated that a 2.45 GHz electromagnetic field might negatively affect EEG, motivation, and attention, particularly in the young age group.

INTRODUCTION

Several natural and artificial sources spread electromagnetic energy as electromagnetic waves (EMW). Mainly, 2.45 GHz field spreads throughout a large area [(1)]. The electromagnetic environment is caused by natural radiation, and electromagnetic fields (EMF) produced intentionally or unintentionally by people during the operation of electrical appliances [(2)]. The harm caused by external EMW sources depends on its energy, type of interaction with the tissue, amount of energy absorbed by the tissue, and exposure time [(1)].

The most important factors determining the behavior in mammals include obtaining new information on environmental conditions and keeping this. Learning is the process of obtaining information on the environment while memory encodes, stores, and retrieves the obtained information [(3)]. Spatial memory is the part of memory responsible for recording and processing sensorial data about an organism's environment, mainly using visual and proprioceptive senses. Mammals usually need a hippocampus with a particular function of the CA1 region to create spatial properties and data. Spatial memory needs both N-methyl-D-aspartate (NMDA) and Alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors. NMDA receptors are needed to reinforce information, whereas AMPA receptors are needed to recall information. NMDA receptors play an essential role in synaptic functions in the central nervous system (CNS) [(4,5)]. The electromagnetic field has been shown to cause reduction in norepinephrine and dopamine

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synthesis in the hypothalamus, abnormal neuronal growth, reduction in Purkinje cells when the size of the electromagnetic waves that are primarily emitted by the wireless networks reaching 2.45 GHz, increased temperature of brain cells, changes in the frequency of electroencephalography (EEG), increase in the permeability of blood-brain barrier, myelin degeneration and glial cell proliferation [6]. There are limited data in the literature investigating the effects of EMF on the brain, especially during the neonatal and childhood period. This study aimed to examine the effects of 2.45 GHz EMF on brain development by investigating cognitive function, expression of NMDA receptors, an indicator of cognitive function, and EEG findings in weaned rats.

MATERIALS AND METHODS

The study was conducted at Suleyman Demirel University (SDU), Medical Faculty, Experimental Animal Production and Experimental Research Center, Biochemistry Department, Research Laboratory, and the laboratory of Biophysics Department. The study followed the rules of the ethics committee for using the experimental animals for scientific purposes after obtaining approval from SDU, Medical Faculty, Animal Ethics Committee Experimental Animals (registration number: 12-03 and date of registration: 28.06.2011).

Experimental animals

In this study, twenty-one weaned (21 days of age) male Wistar Albino rats were used. Rats were divided into the experimental group (n=12) and the control group (n=9). A monopole antenna and a plexiglass cage were used to harbor each rat to ensure electromagnetic field exposure.

Experimental Group: Animals were exposed to a 2.45 GHz magnetic field for 1 hour a day for more than 28 days.

Control Group: Animals were kept in the cages without any exposure.

Environment and conditions for the project

Rats were kept in a dedicated EMF-proof room at optimum conditions of 20-25°C temperature and 45-60% relative humidity by exposing them to light for 12 to 16 hours a day. Subjects were fed with standard coarse pellet feed (Korkuteli Yem Gıda Sanayi Ticaret AS, *Antalya*), which contains a sufficient amount of animal and vegetable protein, vitamins, and minerals, and enables to measure the amount of consumed feed. Ambient temperature and air-conditioning were adjusted by SDU Research Laboratory for Animal Experiments.

Exposure system and dosimetric design

A 2400 MHz center frequency (2300 MHz-2600 MHz tuned) RF generator (Set Elektronik A. S. Sakarya, Turkey) capable of delivering 1 W RMS power to 50 Ohm output load was used as the EMF source. Restrained albino rats were exposed, as shown in figure 1.

Figure 1. Monopole antenna placement in the carousel exposure setup. All animals were aligned at an equal distance from the central antenna.

The monopole antenna of this device has an impedance of 50 Ohm and is positioned vertically in the middle of the carousel experiment setup. The largest radiation pattern of the antenna is in the direction of the nose of all animals. The test transmitter can also adjust the waveform of the electromagnetic wave given to the antenna as pulsed/continuous and the power as 0.1W-1W. Thus, the desired electromagnetic field strength can be created in the near or far field of the antenna. Electromagnetic Field Meter (Extech Instruments Corporation, USA) was used for electric field value and electromagnetic field intensity measurements during the experiment. The experiments were conducted in the electromagnetically isolated room in Suleyman Demirel University, Experimental Animals Laboratory. The shielding effectiveness at the operating frequency of the room is measured as 80 dB.

For technical measurements such as all electromagnetic field repetitions and disturbances, the infrastructure facilities of Suleyman Demirel University, Electrical and Electronics Engineering department laboratory were used. The adjustable output power of the RF generator was adjusted and fixed to measure 15 (V/m) electric fields at the nasal tips of rats. The distance from the monopole antenna of the device to the nose of all rats was measured as 25 cm. Thus, the largest homogeneous electric field value that can be measured at the point closest to the heads of all rats is 15 (V/m).

In the two-dimensional plane, the RF energy radiating from the monopole antenna travels 25 cm in the air and reaches the target tissue by penetrating 1 mm inside the rat's head. At the operating frequency, the wavelength in the air is about 12 cm. According to the basic principles of electromagnetics,

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in this case, the near field/far field boundary is 1.2 cm. In other words, the characteristic impedance of the environment during exposure can be taken as 377 Ohm.

RF power intensity should be 0.6 (W/m2) or 0.06 (mW/cm2) as the closest and largest measured electric field to the target tissue in the propagation environment is set to 15 (V/m). Indeed, the measured value at this point was also 0.61 (W/m2). To find the local SAR value induced in the tissue at this frequency and power density, the software was developed to calculate the attenuation constant of the RF energy as it travels in the tissue and the electric field that should be local in the tissue. As it is known, RF energy weakens rapidly in good conductive tissues. In the far field region, this attenuation is inversely proportional to the square of the distance. Although the attenuation constant depends on the tissue's dielectric property, the tissue's water content increases the conductivity, which increases the SAR induced in the tissue.

In our example, target tissues are found in the rat brain. Peyman *et al.* [7] give electrical properties of different tissues of different living things at microwave frequencies. Using this reference, we found the relative dielectric constant is 42, conductivity is 1.25 (S/m). The tissue's attenuation constant is calculated as 36 (Neper/m) according to the basic electromagnetic information. The used GUI screenshot is shown in figure 2.

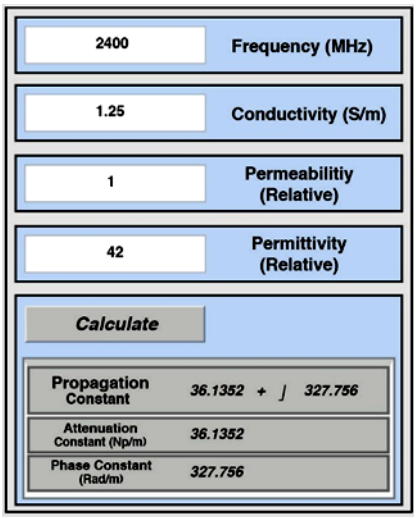

Figure 2. Attenuation constant and internal electric field values were obtained by GUI of software.

Since the external electric field at the closest point to the tissue was 15 (V/m), the average electric field inside the tissue would have been measured as 14.46 (V/m). We use this calculated field value and electrical properties at 2400 MHz. The average SAR value is found to be 0.26 (W/kg) or 260 (mW/kg) as the tissue conductivity is given as 1.25 (S/m). Thus, all animals were exposed to a local average SAR of 260 (mW/kg) in target tissues at 0.06 (mW/cm^2) microwave power density.

Morris water maze and learning tests

A water maze is a circular pool of 150 cm diameter made of galvanic metal with white-colored internal surfaces. The laboratory containing the water maze was illuminated with halogen lamps placed in four corners of the maze with their lights reflected on the ceiling. Fixed visual cues (table, chair, desk) were placed around the water maze. Before the experiment, the water maze was filled with water, heated to 23 °C, and painted with the non-toxic yellow dye. The maze was divided into four quadrants as "quadrant 1", "quadrant 2", "quadrant 3", and "quadrant 4" using the SMART version 2.5 computer program. Quadrant 4 was selected as the target quadrant, and a hidden platform was placed in this quadrant submerged 2 cm below the water surface. Each rat was released into the water facing the maze's wall from a randomly selected different quadrant each time. Each rat was then given one minute to find the platform, and if the rat failed, it was guided to the platform and allowed to remain on it for 30 seconds on the first day and 15 seconds thereafter to provide rats to familiarize the place and learn their target. Once the rats located the platform, they were permitted to remain on it for 30 seconds on the first day. All the exercises were recorded using an overhead video camera (Sony SSC-DC398P, Japan). Each rat was subjected to 20 exercises composed of 5 training sessions per day over 4 days and learned to find the platform. Intervals of at least 20 minutes were left between the exercises within the day for each rat. On Day 5, the platform was removed from the target quadrant where it was placed during the training, and the time spent in the target quadrant was recorded for each rat (Probe Trial). The “Visible Platform Test” was performed following this test, which measures the memory or learning level, the "Visible Platform Test" was performed. The water level was lowered to make the platform visible in this test. Then, the rats were released into the water from quadrant 4, while the target quadrant and platform were moved to the other quadrants each time. We applied this procedure to eliminate the direct or indirect effects of 28-day EMF application, including a decrease in vision and/or motivational factors and an increase in animals' anxiety in the water, thus decreasing the desire to escape [8].

EEG

EEG records were taken in all animals using a polygraph (ADI Instruments, Australia) before sacrifice. Animals were immobilized with stereotaxic methods to prevent noise during recording. All records were taken in a container called a Faraday cage which is covered with an electrically conductive metal and protects the internal volume against external electric fields. Stainless steel electrodes were placed on animals before the recording. The negative electrode was attached to the frontal cortex, the

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positive electrode to the parietal cortex, and the grounding electrode to the root of the tail. Animals were connected to the amplifier of the recorder with a polygraph system and XA-400 quad-channel differential amplifier (ML 870, ADI Instruments) via a micro connector. EEG biopotential was recorded using ADI Instruments X-chart 5 computer program (time constant 1 s, Low-pass filter 50 Hz, High-pass filter 1 Hz, Range 200 μV). Basal EEG records were taken for 5 minutes after the placement of electrodes. EEG records were evaluated based on the records obtained after this period expired.

Obtaining the brain tissue

After EEG recording, rats were sacrificed under anesthesia with 10% ketamine (90 mg/kg) (Alfamin, Alfasan IBV) - 2% xylazine (10 mg/kg) (Alfazin, Alfasan IBV). Brain tissue and the hippocampus were removed from the skull and put on an ice battery wetted with cold phosphate buffer. They were then immediately transferred into Eppendorf tubes filled with phosphate buffer and sent to the Biochemistry Laboratory.

Homogenization of hippocampus samples

Hippocampus samples collected from different rats in the same group were weighed, three hippocampi were combined to represent one sample based on their weights to ensure sufficient protein concentration and then homogenized with cold homogenization buffer in a ratio of 1:5 (50 mm Tris-HCl (pH 7.5), 0.15 M NaCl, 1% Triton X-100, 1 mM EDTA, 1 mM EGTA, 25 μg/ml leupeptin, 25 μg/ml aprotinin, 1 mM sodium orthovanadate, 10 μM benzamidine and 4 mM p-nitrophenyl phosphate). In the first step, homogenization was performed on ice with 18-20 pulses using a Teflon-glass homogenizer, and then the process was completed with sonication (UW-2070 Bandeun Electronic, Germany) for 1 minute. Homogenized samples were centrifuged at 10000 g for 10 minutes at +4 °C. Protein assay was performed from supernatants using the Lowry method [9]. Finally, supernatants were portioned and stored at -80 °C until they were studied.

SDS-PAGE method

The study was based on Laemmli's method [10]. 7.5% lower gel and 4% upper gel were prepared, and the study was run by diluting the tissue homogenate with sample buffer in a ratio of 1:1 to a final protein concentration of 50 μg per well.

Western blot method

First, Biorad Wet/Tank Blotting System (California, USA) and Bio-Rad Power Pac 1000 (California, USA) were used for electrophoresis and immunoblotting procedure. The protein content of samples was obtained 50 μg protein per lane, separated on the gel by SDS-PAGE protocol, and then taken to the transfer tank to transfer into polyvinylidene difluoride membrane (immobilon-P). Following the transfer procedure, membranes were incubated in solutions containing anti NR2A (1/500), anti NR2B (1/500), which were obtained from Millipore (Billerica, MA, USA); and beta-actin (1/5000) overnight, which were obtained from Abcam (Cambridge, USA). Additionally, a prestained molecular weight marker (26,600–180,000 kDa) was provided by Sigma-Aldrich Co. (Steinheim, Germany). After that, membranes were incubated with secondary antibody for 1 hour and held in freshly prepared BCIP/NBT solution until sufficient staining was achieved [11]. The reagents used in western blot analyses were an analytical grade or the highest grade available. Formed bands were scanned using Kodak Image Station 2000 MM (USA) instrument. SDS -PAGE and Western blot analyses were done on 4 independent hippocampus preparations (2-3 animals/group) to obtain at least 50 μg protein per lane [11]. Immunoblotting for β-actin was used as an internal standard to confirm equal protein loading and sample transfer. So the band intensities obtained were normalized by comparing them with β-actin bands of each receptor subunit. The mean of the control group values for each antibody was set to 100, and each value of the experimental groups was proportioned against that of the control group to simplify the values; thus, data were expressed as a percentage of the control group [12] (figure 3).

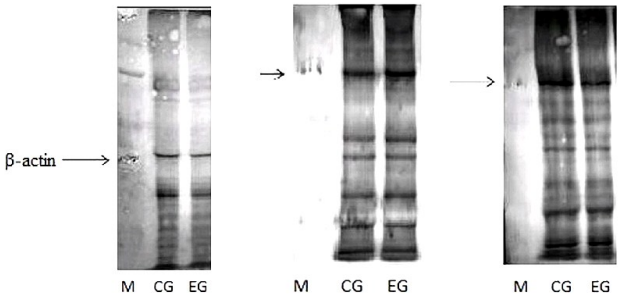

Figure 3. Representative western blotting bands for each receptor from hippocampi homogenates of the experimental and control groups. Notes: Prestained marker and the blotted bands of each receptor on each membrane are denoted with an arrow. M: marker; CG: control group; EG: experiment group.

Statistical analysis

Statistical analyses were performed using SPSS 15 program. Data of groups are expressed as mean and standard deviation. Repeated Measures ANOVA (RMANOVA) was used to evaluate the intragroup day-to-day change. The effect of day/time on the learning ability was evaluated. The Greenhouse-Geisser correction was applied ($p<0.05$). Paired comparisons were made with a T-test to determine the day that caused the significance of the difference. Statistical significance of the difference between the groups was calculated using Bonferroni corrected Mann-Whitney U test, and the Mann-Whitney U test was used to compare the groups' NR2A and NR2B receptor levels. Western Blot results are expressed in % mean ± SEM. Statistical significance value was

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accepted as p<0.05 in 95% confidence interval.

RESULTS

Results of Morris water maze test

Time to find the target platform during the learning period in Morris water maze (days 1 - 4) was evaluated separately for each group; thus, the intragroup day-to-day change and the effect of the day (time) on learning ability were assessed, and statistical significance was found in both groups. Significant differences were observed between the day 1 and 2 (p= 0.0001); day 1 and 3 (p= 0.0001) and, day 1 and 4 (p= 0.0001) for both of the groups. These data demonstrate that both groups learned the system from day 2 (figure 4A, 4B). There was no statistically significant difference in time to find the target quadrant between the groups (figure 5A, 5B, table 1). Intergroup comparison was revealed no significant difference between the groups for all days during the training. Data were evaluated by the Mann -Whitney U test. The results are given in ± SEM.

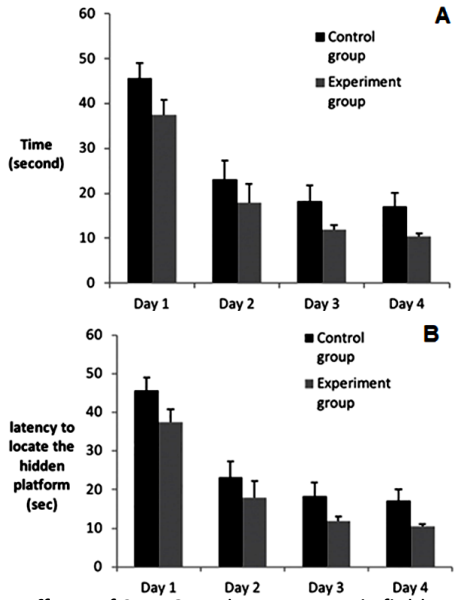

Figure 5. Effects of 2.45 GHz electromagnetic field on spatial memory performance **(A)**; Effects of 2.45 GHz electromagnetic field on latency to locate the hidden platform **(B)** (Intergroup comparison).

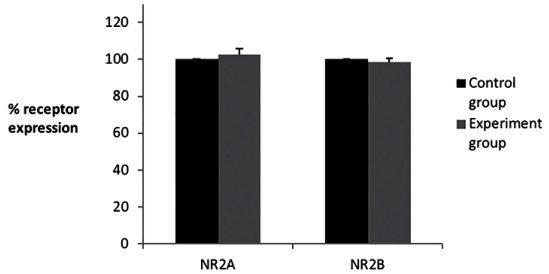

Figure 6. Optic densities of NMDAR subunits: NR2A & NR2B protein expression Protein levels from hippocampus homogenates were assayed by western blotting. The mean of the control group data was assumed as 100, and % concentration values of experiment group was expressed in percent change from control. There were no statistically significant differences between groups (p > 0.05).

Table 1. Learning time and time spent in the target quadrant in Morris water maze.

	Experimental (n=12) time (s) (mean±SEM)	Control (n=6) time (s) (mean±SEM)	p
First Day	37.4±11	45.6±10	.08
Second Day	17.9±14	23.2±12	.22
Third Day	11.9±3	18.2±0	.11
Fourth Day	10.4±2	17.1±8	.25
Target quadrant speed total	14.4±2	15±2	.43

s: second, n: number of rats in the group, SEM: Standard Error of Mean.

Probe test

The platform in the target quadrant was removed after the training period of four days, and time spent in this quadrant was evaluated. As shown in table 2, no statistically significant differences between the groups regarding time spent in the target quadrant and average swim speed were detected.

Table 2. Test data evaluating the memory functions in Morris water maze.

	Experimental (n=12)	Control (n=9)	p
Time spent in the target quadrant (s) (mean±SEM)	30.8 ± 1.59	26.4 ± 1.79	.081
Time to find the visible platform (s) (mean±SEM)	24.7 ± 4.3	13.5 ± 4.3	.039*
Mean speed (cm/s) (mean±SEM)	14.4 ± 0.6	15 ± 0.8	.43

"*" symbol indicates that there is a significant difference between the groups in terms of time to find the visible platform, data were evaluated by Mann-Whitney U test, s: second, cm: centimeter, n: number of rats in the group, SEM: Standard Error of Mean.

Visible platform test

The time to arrive at the visible platform was significantly longer in experimental animals than in control animals. However, there was no significant difference in swim speed between the groups (Table 2), and this may be explained by experimental animals having negative effects on motivation and attention behavior rather than locomotor activity.

NR2A and NR2B receptor levels by Western blot analysis

In the intergroup evaluation of receptor expressions, the mean optical density of the control group was accepted as 100 separately for both the receptors and optical densities of the experimental group were measured for both receptors, compared to 100 and expressed as a percentage change. No statistically significant difference was found between the two groups in terms of the expression of NR2A and NR2B receptors (p>0.05) (figure 6).

EEG results

EEG records, spike waves, and filtered records obtained from the experimental group (demonstration of Alpha Waves: High cutoff; 12 Hz and Low cutoff; 8 Hz) were recorded. Records obtained from total brain activity revealed that alpha waves accounted for 70% of the record. The amplitude of brain waves ranged between -50 (±5)

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and +50 (±5) millivolt as shown in the experiment protocol (AD Instruments Lab Tutor-EEG Records). EEG records of the control group were consistent with the experiment protocol, while the records of the experimental group revealed waves called 'Spike' which resulted from neuronal discharges and had amplitudes of 2-3 times greater than normal records. The results obtained were evaluated for frequency and latency of spikes. The number of spikes was significantly higher in the experimental group compared to the control group ($p<0.005$). Likewise, when means of spike latencies were compared, spike latency was statistically significantly shorter in the experimental group than the control group ($p<0.005$).

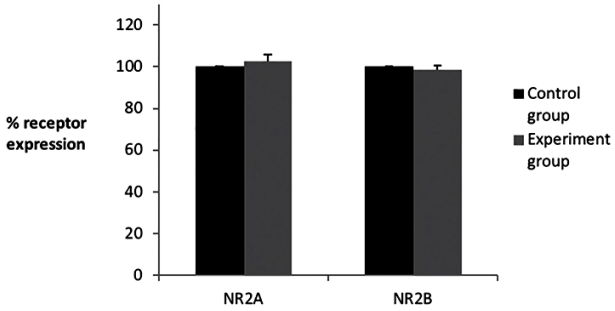

Figure 6. Optic densities of NMDAR subunits: NR2A & NR2B protein expression Protein levels from hippocampus homogenates were assayed by western blotting. The mean of the control group data was assumed as 100, and % concentration values of experiment group was expressed in percent change from control. There were no statistically significant differences between groups ($p > 0.05$).

DISCUSSION

With the technological advances, EMW is increasingly used, particularly 2.45 GHz field (microwave oven, wireless computer, etc.) spreads over a large area. The disrupted blood-brain barrier was detected in the cerebral cortex, thalamus, basal ganglia, hippocampus, cerebellum, midbrain, and medulla of fetal mice exposed to 900 MHz EMF [13]. There are some hypotheses about microwave radiation action on biological systems. The ions, electrons of molecules, ion channels, DNA, RNA, and protein expression can be affected significantly due to reactive oxygen species than it can cause energy metabolism, cell morphology, cell division, and membrane permeability changes. These cytotoxic mechanisms can negatively affect the nervous, cardiovascular, hematopoietic, and reproductive systems [14].

In this study, we found that the number of spikes was statistically significantly increased in EEGs of rats exposed to EMF and the mean spike latency was significantly shorter in these experimental animals. This is the first study conducted in weaned rats in the literature reporting that the use of 2.45 GHz EMF caused changes, demonstrating statistically significant stimulation in EEG records. In the literature, most of the studies examining the effects of EMF on EEG were conducted in humans, while the number of rat studies is limited. Vorobyov *et al.* [15] and Petrova *et al.* [16] reported that EEG changes were observed in rat studies using low-frequency EMF. Thuroczy *et al.* [17] found no EEG changes in their study conducted in rats exposed to 2.45 GHz EMF, whereas Naziroglu and Gumral [18] reported an increase was observed in the number of spikes, although it did not reach statistical significance. An EEG study with ten adults and ten children subjects has shown that 900 MHz affects delta waves [19]. In another study, a mobile phone was placed on the back of subjects' heads, and changes were detected in alpha waves (8–13 Hz) and beta waves (13–32 Hz) during talk mode and active-standby mode [20]. A recent study has shown that third-generation (3G) mobile phone-related EMF does not affect human EEG records [21].

In this study, learning ability was evaluated through time to find the target platform. The time to find the target platform was shorter in the experimental group, but the difference was not statistically significant. This result of the study shows that EMF does not affect learning ability. On the contrary, Wang and Lai [22] found that EMF affects the learning ability by demonstrating a statistically longer learning time in rats exposed to 2450 MHz EMF. Shai *et al.* [23] showed that radiation emitted from mobile jammers affects spatial memory and radiofrequency waves cause behavioral changes in adult rats. Our different results from other studies in the literature evaluating the effect of EMF on learning ability might be caused by rats' different age groups (weaned) in our study.

In this study, memory was evaluated with a probe test, and no statistically significant differences were detected between the groups in terms of time spent in the target quadrant and average swim speed. These findings show that EMF has no negative effect on memory. Although most of the studies in the literature reported that EMF has negative effects on memory, some studies reported results similar to ours [24, 25].

While some clinical studies have reported that mobile phones (900 or 1800 MHz) associated with EMF caused no changes in cognitive functions [26], Barth *et al.* [24] published a meta-analysis containing the results of 19 experimental studies. They reported that EMF slightly affects attention and working memory in humans. In the last meta-analysis published by the same working group, it was reported that mobile phone-associated EMF does not affect human cognitive functions [25]. A published experimental study has shown that 900 MHz EMF affects the behavioral parameters in middle-aged rats [27]. A study examining the effect of EMF exposure on learning ability and memory using a water maze test has reported that learning ability and memory were

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impaired in correlation with the reduction of NR2A expression in the hippocampus [28]. Another study has evaluated the behavioral changes in rats chronically exposed to 2450 MHz EMF and detected hyperactivity and aggression in rats [29].

In the visible platform test, rats exposed to 2.45 GHz EMF reached the platform in a longer time, but no significant difference was found in their swim speed. This finding demonstrates that the negative effects were observed in motivation and attention rather than locomotor activity. When 2450 MHz continuous wave, 2.7 W/kg fields were applied for 420 minutes in a day, locomotor activity and response to acoustic stimuli were decreased in rats [30].

The most studied and known receptor complexes are NMDA receptors. They are involved in sensory messages, the integration of messages, and the coordination and programming of motor functions and activities [31]. Several studies are examining the effects of EMF on NMDA receptors. An experimental study has reported that expressions of NR2A and NR2B receptors in several hippocampal areas were significantly reduced in rats exposed to 1800 MHz EMF, while another study has reported that expressions of NR1, NR2A, and NR2C in the hippocampus were significantly reduced, expression of NR2D was significantly increased. No changes occurred in the expression of NR2B [32, 33]. In this study, no significant difference was observed between expressions of NR2A and NR2B receptors in the two groups.

In conclusion, considering the age of rats, these results show that 2.45 GHz EMF may negatively affect EEG, motivation, and attention, particularly in the young age group. Recommendations may include revising safe exposure limits accepted by international authorities, especially for the young age group. Our findings may be compared with more extensive studies, and necessary actions may be taken about this issue.

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***Conflict of interest*:** None of the authors have potential conflicts of interest to be disclosed.

***Ethical considerations*:** The study followed the rules of the ethics committee for using the experimental animals for scientific purposes after obtaining approval from SDU, Medical Faculty, Animal Ethics Committee Experimental Animals (registration number: 12-03 and date of registration: 28.06.2011).

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Author contributions: Halime Yucel collected the data and wrote the paper, Nihal Olgac Dundar designed the study and analysis, wrote and overview the paper, Duygu Kumbul Doguc contributed the data analysis and together with Fatma Tutku Aksoy performed Morris Water Maze and learning test, retrieval of the brain tissue, and homogenization of hippocampus samples, Cihangir Uguz, Omer Celik, and Mustafa Nazıroglu performed EEG and contributed the data analysis, Selcuk Comlekci and Bumin Dundar performed the exposure system and dosimetric design and overviewed the paper.

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