ORIGINAL ARTICLE

The Effect of Non-Ionizing Electromagnetic Fields in The Range of 2.4 GHz on Memory, Thermal Sensitivity and Serum Protein in Male Rats

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Abstract OBJECTIVES: Electromagnetic fields (EMFs) have been demonstrated to influence animal and human behavior in various ways, including learning, pain perception (nociception or analgesia), and behaviors. The study aimed to investigate the effects of electromagnetic fields (EMFs) on learning, memory, and serum protein, and their analgesic activity in male rats.

METHODS: Adult rats were randomly divided into four groups (exposed and control). The exposed groups of adult rats were exposed to EMF (4 mW/cm², 2.45 GHz) for 45 minutes daily for four (the Exp I group) and eight (the Exp II group) consecutive days. The behavioral tests were conducted 24 hours following the EMF application, including collecting their blood plasma.

RESULTS: There was no significant difference in body weight between the control and exposed groups. Both exposed groups spend more time and distance than the control and sham groups in terms of spatial memory. The number of entries and percentage of time spent increased dramatically in the open-field test (OFT) compared to the control and sham groups. Our data indicate that non-ionizing EMF radiation affected serum protein considerably in the Exp II group after eight days. This group had strong analgesic efficacy in the hot-plate test (p < 0.0001).

CONCLUSION: The current variations in the A/G ratio and impairment in spatial memory in the Exp II may contribute to the documented harmful consequences of 2.45 GHz radiation devices.

Abbreviations:

Electromagnetic field (EMF), Morris water maze (MWM), open-field test (OFT), Radio frequency electromagnetic field (RF-EMF).

INTRODUCTION

Modern mobile phones (with frequencies ranging from 1800 MHz to 2200 MHz), laptops (with frequency ranges ranging from 1000 MHz to 3600 MHz), and wireless networks employ microwave radiation operating at high frequencies (2.45 GHz) (Kivrak, Yurt, Kaplan, Alkan, & Altun, 2017). This century has seen a dramatic rise in the importance of electronic gadgets in everyday life. Aside from being time-saving, they can also lead to various health issues (Kivrak et al. 2017). Studies in progress have shown that the resonant frequency of electromagnetic fields (EMFs) exposure can modify physiological processes, such as protein levels, proliferation, changing dipole distribution, the permeability of cellular membranes, and the transfer of calcium, sodium, and potassium ions (Kula, Sobczak, Grabowska-Bochenek, & Piskorska, 1999; Verginadis et al. 2012). Moreover, over the last several decades, numerous epidemiological and experimental research have produced contradicting findings on the possible effects of EMFs on the neurological system (Yang et al. 2012). Hence, the potential neurobiological impacts of EMF exposure are widely contested (Yang et al. 2012). EMFs with varied strengths are commonly employed in homes, businesses, and public locations (Mahmoudi et al. 2018). As evidenced in regional variations in cerebral blood flow, exposure to pulse-modulated radio frequency (RF) EMF equivalent to those generated by mobile phones affects brain physiology (Huber et al. 2005). As demonstrated lately, short-term exposure to mobile phone jammers reduces blood sugar levels in adult male rats (Shekoohi Shooli et al. 2016). These modifications may affect biochemical processes within the cell, hence altering the serum's biochemical characteristics and enzyme activity. The impact of these devices can also vary depending on the device's proximity and the surrounding environment (Yazdanpanahi, Namazi, Shojaeifard, Nematollahii, & Pourahmad, 2020). Nonionizing radiation's effects on the brain can shift over time (Othman et al. 2021). For this study, we were concerned about shorttime exposure at different times. On the other hand, EMF demonstrated positive influences when utilized to treat cognitive problems (Khajei et al. 2021). Therefore, research on blood serum and related parameters in the electromagnetic field seems necessary. Plasma contains a variety of proteins, two of the most significant of which are albumin and globulin. Plasma albumin is a component of colloid osmotic pressure and one of the plasma's antioxidants. Globulins are found in inflammation and immunology parameters in plasma. Albumin is the primary target of plasma protein oxidation (Cenesiz, Atakişi, Akar, Önbilgin, & Ormancı, 2011). During inflammation, both albumin and globulin play crucial roles (Liang, Li, Tang, & Liu, 2019). For example, in extremely low frequency EMF radiation, Eraslan et al. (2004) found no significant difference (p > 0.05) between levels of albumin, globulin, and total protein when male rats were subjected to EMF with a frequency of 60 Hz (5 mT) for eight hours (Eraslan *et al.* 2004). Hence, more research needs to be focused on high EMF frequency.

Thermal sensitivity has previously been studied using subjective scales or reflex reactions in the hot plate model, which showed conflicting RF-EMF effects on thermal sensitivity (Bodera et al. 2019; Vecsei, Thuroczy, & Hernadi, 2018). In the case of pain, it has been suggested that MF/EMF may be able to influence the effects of opioid drugs by altering the threedimensional structure of water dipoles surrounding receptors and their physical-chemical properties, such as hydration and salvation ability, surface tension, pH, and electro-conductivity (Bodera et al. 2012). Acute exposures to EMFs have consistently reduced analgesia in most investigations (Del Seppia et al. 2007; Verginadis et al. 2012). Exposure to a variety of EMFs has an effect on the specificity of pain (nociception) and the suppression of pain (analgesia) (Verginadis et al. 2012). According to Mathur et al. intermittent RF-EMF exposures (0.4 W/kg, 73.5 MHz, whole-body) increased the emotional component of phasic pain in rats (Mathur, 2008). Hence, the hot plate test is one of the most well-known and commonly used procedures for assessing nociception in rats and mice (Del Seppia et al. 2007; Gunn, Bobeck, Weber, & Morgan, 2011). In the current study, we utilized a hot plate test to determine the reaction time of our rats in various exposed groups and analyzed their blood plasma proteins along with the analgesic effects of EMF. Supraspinally-organized reactions might be assessed quantitatively using this method (Ouadah, Blazy, & Villegier, 2020). Therefore, the reduction in thresholds caused by EMF is most likely due to supraspinal analgesia (Del Seppia et al. 2007). Regarding memory, Greenebaum and Barnes (2018) found that the pulse field of 2.45 Hz microwave radiation leads to cognitive impairment and memory loss (Greenebaum & Barnes, 2018). However, other research indicates that pulsed electromagnetic fields promote axonal renewal and neurite regeneration (Gholami, Riazi, Fathi, Sharafi, & Shahverdi, 2019). As a result, it is critical for the general public, particularly young children who use the wireless internet frequently, to understand the link between EMF and medical diseases such as neurological illnesses and behavioral disorders (Obajuluwa et al. 2017).

The purpose of the present study was to determine whether locating animals immediately after exposure (from day 0 to 4) to EMF or after four days (from day 4 to 8) can affect the cognitive and analgesic activity of exposure to EMF at 2.45 GHz and to evaluate some structural alterations in the serum protein. In this study, we assessed the effects of EMFs on weight gain, memory, and pain. The effects of EMF radiation on animal behavior and memory remain unclear.





MATERIALS AND METHODS

<u>Animals</u>

In the present research, 6-7-week-old Sprague Dawley Wistar male rats weighing 180-250 g were bought from the Razi Vaccine and Serum Research Institute (RVSRI). They were kept in a 12-hour cycle of light and darkness and fed commercial rat chow. Rats were allowed at least one week to become used to the animal groups. All tests were performed following guidelines established by the Malek-Ashtar University Medical Ethics Committee, the Animals (Scientific Procedures) Act of 1986, and related procedures, as well as a recommendation from the National Institutes of Health (NIH) for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). A daily body weight measurement was taken before each treatment. We have made every attempt to alleviate the pain.

EMF application and exposure system

The 2.45 GHz electromagnetic radiation device for live animals incorporated a computer, microwave signal sources, and a two-dimensional moving loading platform. The microwave output of a horn antenna has a power range of 1–200 W. The electromagnetic radiation device includes a power amplifier (ZHL-16W-43+, USA) to amplify the signal generated and a signal generator (the Agilent HP 83732B, USA), which can generate signals in the frequency bandwidth range from 10 MHz to 20 GHz. A horn antenna S-band (model LB-OH-320-10-C-NF) with dimensions of 257×124×164 mm, which can operate in the frequency range of 2 to 4 GHz, a power meter, a radiation box $(20\times30 \text{ cm})$, and a power density measuring device were used. Electromagnetic waves (pulsed modulation) with a frequency of 2.45 GHz and a density of 4 mW/cm3, pulse width (2 ms), and pulse repetition frequency of 500 Hz were irradiated from above on the surface of the radiation cage. The spacing between the horn antenna and the tested animals was 25 cm.

Experimental design

Four groups of animals (in each group: n = 8) were randomly employed in the current investigation as follows:

- 1. The control group underwent all behavioral tests to check normal memory and learning without exposure to EMF for four days.
- 2. Sham was exposed to switch off of EMF device but at the same container for 45 min per day for eight days. The learning phase for the MWM test started on the fourth day.
- 3. The Exp I group was exposed to 2.45 GHz EMF for four days, starting from day zero.
- 4. The Exp II group was exposed to 2.45 GHz EMF for eight days, but the learning phase started on the fourth day.

<u>Behavioral analysis</u>

Morris water maze (MWM)

According to the selected procedure (four days of training started on day 3 in the water tank, followed

by the seventh day of the experiment as the last day), the MWM began on the third day for the short-term sham group. The maze comprises a circular pool (width of 140 cm, height of 50 cm) filled to a depth of 30 cm with warm water (22–25 °C) and set in a gloomy room with minimal lighting. The pool was filled with water, and the wall of the pool was covered with dark glass. For the long-term group, the tests started on the 26th day and day 30, and a probe trial was conducted. The tank was separated into four quadrants, and an invisible platform (diameter of 10 cm) was placed in the center of one of the quadrants of the tank that extended 1 cm below the water's surface. Rats learned to identify the fixed platform using spatial indicators. Each rat was free to swim for 90 seconds during training days and 60 seconds during the probe trial until it came across and climbed onto the platform, where it remained for fifteen seconds to recall the cue signs on the walls. The video was then processed with the behavioral analysis system (Panlab, Barcelona, Spain) to analyze data on latency (the time the rat was placed on the platform when the platform was identified) and swimming distance. A recall or probe trial was conducted after the last training session on days 7 and 30 to measure the time spent in the target quadrant where the platform had been located during training. The number of times the animal crossed the platform area and the amount of time spent in the target quadrant were recorded and evaluated to determine the animal's spatial memory. After being removed from the pool, the rats were hand dried with a terrycloth towel and put in a warming cage (containing a heating pad set to a low setting beneath a standard shoebox cage) for at least 5 minutes before returning to their home cage. Twenty-four hours following the previous training session, a probe trial was conducted to assess spatial reference memory by removing the platform. Then rats were permitted to swim without restriction in the pool for 60 seconds.

During the retention test (60 s), the platform was removed, and the animal was permitted to look for it. In case rats might not discover the platform inside the 60s (for training days only 90s), they were guided to the platform by hand and permitted to stay there for 15s to learn cue signs on the walls to remember the platform's situation and then their escape latency was acknowledged as 60s. In the retention tests, we assessed four different measures of platform memory; time spent (%) in the platform quadrant, mean distance (cm) to the previous position of the platform, latency (s) to the primary crossing over the prior platform situation, and the number of crossings over the former platform position. As a result, escape latency was utilized to examine rats' learning and memory abilities.

Open-field test (OFT)

Quantifying motor activity was measured using Plexiglas open field boxes (90×90 cm² with a 42 cm height). Black lines were painted on the cardboard on the box's floor, splitting the floor into 18 cm \times 18 cm squares. Gridlines, consisting of four 11 cm distances from each wall, divide open fields into centers and surroundings. Based on dependent measures, time spent in the center, distance traveled in the center, and distance traveled in the center divided by total distance traveled were measured. After an observer blind to the treatment regimen rated each video footage, the number of squares crossed and those reared were recorded. Following the test, each rat was returned to its cage. Each rat was returned to its cage at the end of the test.

<u>Analgesic assay</u>

The hot plate test examined nociception (Columbus Instruments, Columbus, OH). When the rat was positioned on a $55 \pm 1^{\circ}$ C plate, the latency to lick its hind paw was assessed (Gunn *et al.* 2011). The plate was surrounded by four Plexiglas barriers, preventing the rat from escaping. When the rat licked its hind paw or did not react within 50 seconds, it was immediately removed from its dish.

Biochemical analysis

Total protein, albumin, and globulin were measured with commercial kits (BioMerieux, France).

Statistical analysis

All results are presented as the mean \pm standard deviation (SD). All statistical analysis was analyzed using GraphPad Prism® 8.1 software for Windows (GraphPad Software, San Diego, CA, USA). The data were analyzed using a one-way one-repeated analysis of variance (ANOVA) with sham groups and exposed groups as two between-subject factors, followed by Sidak's test to compare the groups and time exposure (short-term and long-term). One-way ANOVA was performed using Dunnett's test to compare groups in the learning phase comparison with the first day of the MWM test. The swim paths were assessed utilizing a computerized video-tracking system (Ethovision 3.1; Noldus Information Technology, Wageningen, the Netherlands). It was considered statistically significant only when the *p*-value was less than 0.05, 0.01, 0.001, and 0.0001.

RESULTS

Based on Fig. 1, there was no significant difference in body weight between the control and exposure groups due to the short time of the experiments (Fig. 1B). Also, we did not find a significant difference in weight gain compared to the control (p > 0.05, Fig. 1C).

However, we found a significant difference in the Morris Water Maze test. Following training, the amount of time that passes until the animal steps onto the platform to leave the water (escape latency) and the proportion of time or path length spent in the quadrant, including the platform, is used to determine whether to learn (target quadrant). The daily data graphs showed



Fig. 2. Effect of the electromagnetic field on spatial learning and memory during the training days to find hidden stage (**A**, **B** and **C**); On the fifth (for Exp I) and ninth day (for Exp II), a probe trial was performed to test spatial memory in relation to the following based on stage and non-stage in the MWM test: **D**) escape latency in probe trial; **E**) accumulative distance travelled in the probe trial; **F**) mean velocity for non-stage as compared with stage in MWM in the probe trial day, and **G**) representative swimming traces of the four groups of rats were observed on the probe trial day. One-way ANOVA was used to statistically difference compared to the control group using Dunnett's test, and * p < 0.05, ** p < 0.01, **** p < 0.001, **** p < 0.0001, while ## p < 0.01, ### p < 0.001, and #### p < 0.0001 compared to the Sham group.

that each group had successfully finished the four-day training. Fig. 2A shows that Exp I and Exp II have significant differences (p < 0.0001) compared to the control group in the escape latency. However, the Exp II group increased the escape latency. Exp II spent more time finding the platform for four consecutive days than the other groups, while Exp I declined in the escape latency. The Exp II showed a decline in the mean velocity compared to the control group (p < 0.0001) in its search for the platform. However, no significant difference was found between all groups in the swimming distance (p > 0.05) (Fig. 2C). In addition, experiments were performed in two modes: with a platform, which is an indicator of visual memory performance, and without a platform, which is an indicator of rat spatial memory performance. In the off-platform mode (Fig. 2D-E), time was given for 60 seconds to test spatial memory and whether the rats remembered the platform position. As shown in Fig. 2D, Exp I spent more time finding the platform than other groups (p < 0.01 as compared to the control and sham groups, Fig. 2D), but Exp II spent less time and distance finding the platform the same as the sham and control groups (black columns indicate stage mode) with slightly different (p > 0.05, Fig. 2D and E), whereas, as shown in Fig. 2D-E, the red columns represent the non-stage (without platform) mode compared to the stage mode. In non-stage (platform) mode, the Exp II spends less time and distance than the control and Sham groups. However, the mean velocity in the Exp II group was significantly less than the control (p < 0.0001) and sham (p < 0.0001) groups in non-stage mode (Fig. 2F), where this p-value was not shown in Fig. 2. Rats from the four groups showed representative swimming traces (Fig. 2G).

Fig. 3A shows the number of entries in the OFT. Using one-way ANOVA followed by Dunnett's test exhibited a significant increase in Exp II (p < 0.001); however, Exp I showed a significant reduction in the number of entries to the center of the arena (p < 0.01) compared to the control group. While Exp I and Exp II showed a significant difference compared to the sham group, p < 0.001 and p < 0.05, respectively. The percentage of time spent follows the same trend as in Fig. 3A. The Exp II group showed a significant difference, along with the Exp I, at p < 0.0001 and p < 0.01, compared to the control group (Fig. 3B). However, no





significant difference was found between all treated groups when counting their steps in the open-field test (p > 0.05, Fig. 3C). Also, Exp II showed important analgesic activity in the hot-plate test (p < 0.0001) by raising the reaction time of rats by 11.5 sec on the ninth day in comparison to the control group (8.8 sec).

The globulin levels in the Exp II group showed a significant decrease (p < 0.01) using one-way ANOVA followed by Dunnett's multiple test. However, the A/G ratio was observed to be considerably increased in the Exp II group (p < 0.01) compared to the control group (Table 1). The EMF radiation did not alter all groups' total protein and albumin levels (p > 0.05).

Discussion

Different electromagnetic fields are thought to have both beneficial and detrimental biological effects (Saliev, Begimbetova, Masoud, & Matkarimov, 2019). In particular, non-thermal EMF radiation exposure at 900 MHz or 2.45 GHz has been shown to cause neuronal malfunction and apoptosis in hippocampus pyramidal cells in rats, both in the short- and longterm (Hu, Zuo, & Li, 2021). However, chronic exposure to 2.45 GHz EMF radiation increases neuronal plasticity and cognitive performance in the vascular This study aimed to determine the effect of EMFs at 2.45 GHz radiation on rats to improve cognitive function, analgesic activity, and health issues on some serum proteins. According to the Morris water maze results, the radiation groups (Exp I and Exp II) spent more time than the control and sham groups trying to find the platform, which indicates the negative effect of the electromagnetic field on the memory of rats. Karimi et al. discovered that when rats were exposed to 2.45 GHz EMF radiation (with a power density of 0.016 mW/cm² and a specific absorption rate of 0.017 w/kg) for 2 hours per day for 40 days, spatial learning and memory were impaired using passive avoidance tests and radial maze. However, they did not find any impact on short-term neuroplasticity (Karimi, Bayat, Haghani, Saadi, & Ghazipour, 2018), as we found similar data in our study. On the other hand, Gokcek-Sarac found that short-term (2 hours per day for seven days) exposure to 2.1 GHz radiation had no significant influence on locomotor activity in the openfield test (Gökçek-Saraç, 2020). Our results confirmed that counting steps in locomotor activity. However, based on the swimming trace shown in Fig. 2F, rats in the Exp II demonstrated remarkable spatial memory performance, indicating that an eight-day exposure

dementia paradigm in rats (Bayat et al. 2021).

| | | 5 1 | | |
|---------|----------------------|-----------------|----------------------|----------------------|
| Groups | Total protein (g/dl) | Albumin (g/dl) | Globulin (g/dl) | A/G ratio |
| Control | 5.67 ± 0.12 | 2.23 ± 0.24 | 1.36 ± 0.50 | 1.65 ± 0.48 |
| Sham | 5.97 ± 0.44 | 2.03 ± 0.13 | 1.04 ± 0.26 | 1.95 ± 0.50 |
| Exp I | 5.72 ± 0.32 | 2.14 ± 0.17 | 1.27 ± 0.32 | 1.69 ± 0.53 |
| Exp II | 5.63 ± 0.28 | 2.15 ± 0.20 | $0.71 \pm 0.21^{**}$ | $3.03 \pm 0.92^{**}$ |
| | | | | |

| | Tab. ' | 1. | Total protein, | albumin, | globulin l | evel and | A:G ra | atio of various | groups | induced with | ı 2.45 | GHz frequ | ency |
|--|--------|----|----------------|----------|------------|----------|--------|-----------------|--------|--------------|--------|-----------|------|
|--|--------|----|----------------|----------|------------|----------|--------|-----------------|--------|--------------|--------|-----------|------|

Data expressed as mean \pm SD (n = 8).

could improve spatial memory latency, one of the primary dependent performance measures on learning trials. On the other hand, cumulative distance has been considered a valuable measurement of spatial learning (Zhang *et al.* 2015).

According to several studies, EMFs may suppress the analgesic activity of drugs in animals. Additionally, there is mounting evidence that some types of magnetotherapy may effectively treat pain (Weintraub, 2003) and thermal sensitivity (Ouadah et al. 2020). For example, Bodera et al. (2012) found that both frequencies (1500 and 1800 MHz) of EMF exposure transiently inhibited tramadol's analgesic efficacy, dramatically shortening paw withdrawal latency in mice administered with this medication 30 minutes after injection (Bodera et al. 2012). In another study, Maillefer and Quock found that continuous-wave (2.45 GHz, 20 mW/cm², and 46 W/kg) exposure to acetic acid abdominal constriction caused analgesia in mice (Maillefer & Quock, 1992). Therefore, our findings suggest that rats exposed to 2.45 GHz for eight days (as we experienced in our study in the Exp II group) may experience analgesic effects comparable to those in the control and sham groups. Although for more details, we need to use metabolomics to detect molecular pathways (Modarresi Chahardehi & Lim, 2022).

Albumin and globulin, two critical components of total blood protein, have gained greater interest as non-invasive predictive indicators for biological impacts (Liang et al. 2019). Hence, animal clinical problems can be diagnosed and prognosticated with early and valuable diagnostic and prognostic data based on changes in albumin, globulin, and the albumin/ globulin ratio (Zaias, Mineau, Cray, Yoon, & Altman, 2009). Albumin acts as an antioxidant, removes pollutants, and prevents the production of amyloid betapeptide fibrils. Several studies have linked low albumin levels to an increased risk of cognitive decline and dementia (Koyama et al. 2016). Also, albumin serves as a significant binding and transport protein in the body, a critical metabolic function (Zaias et al. 2009). However, albumin levels did not change significantly in all groups in this study. Additionally, low albumin levels have been linked to systemic infection, nephrotic syndrome, and chronic liver disease (Suh et al. 2014), and this might lead to tissue damage and cardiovascular disorders (Hassan & Abdelkawi, 2014). In our

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study, albumin levels in various groups did not change compared to the control group, where the globulin level increased significantly only in the Exp II group. There were no statistically significant increases in total protein since albumin and globulin ratios were equal. However, the A/G ratio increased significantly (p < 0.01) in the Exp II group compared to the control group. Koyama et al. (2016) found that the A/G ratio may accurately indicate cognitive deterioration induced by homeostasis disturbance (Koyama et al. 2016). On the other hand, neurotransmitters in many brain areas may become imbalanced due to RF-EMR exposure (Ferreri et al. 2006; Noor, Mohammed, Ahmed, & Radwan, 2011). Cenesiz et al. (2011) observed that when rats were exposed to 900 and 1800 MHz EMF, plasma albumin levels decreased considerably, whereas the globulin levels rose statistically (Cenesiz et al. 2011). However, Taheri et al. found that it has been hypothesized that 4.5 hours of exposure to 2.45 GHz Wi-Fi radiation boosts bacteria's antibiotic susceptibility (Taheri et al. 2015). On the other hand, Wi-Fi at 2.45 GHz was determined to have no adverse effect on DNA (Akdag et al. 2016). Obajuluwa et al. indicated that 2.5 GHz radiation increased anxiety significantly and affected locomotor performance (Obajuluwa et al. 2017). However, we did not find a significant difference in our groups in terms of locomotor performance in the short-term (four and eight days). When combined with the findings of this study, growing evidence shows that starting immediately with electromagnetic fields at 2.45 GHz does not change serum protein levels during the initial days, nor not improve cognitive function. However, after eight days, the exposed group showed better results in exploratory behavior.

CONCLUSION

The present study's findings suggest that while the Exp II group (exposed for eight-day) performed better spatial memory than the Exp I group (four-day), Exp II might change body homeostasis in terms of biochemical analysis. Therefore, it can be noted that EMF radiation emitted from mobile devices, Wi-Fi modems, and other devices in a short time has a negative effect on spatial memory and some blood plasma. Further studies are needed to provide more data based on the different days of exposure.

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