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The effects of acute and chronic exposure to 900 MHz radiofrequency radiation on auditory brainstem response in adult rats

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ABSTRACT

The aim of this study was to determine the effects of short and long-term RFR exposure on ABR by evaluating lipid peroxidation and antioxidant status in adult rats. Sixty male albino Wistar rats were randomly divided into four groups. S1:1 week sham, S10:10 weeks sham, E1:1 week RFR, E10:10 weeks RFR. Experimental group rats were exposed to RFR 2 h/day, 5 days/week during the test period. Sham rats were kept in the same conditions without RFR. After the experiment, ABRs were recorded from the mastoids of rats using tone burst acoustic stimuli. Biochemical investigations in rat brain and ultrastructural analysis in temporal cortex were performed. ABR wave I latency prolonged in E1-group and shortened in E10-group compared to their shams. TBARS level increased in E1-group, decreased in E10-group, on the contrary, SOD and CAT activities and GSH level decreased in E1-group, increased in E10-group compared to their sham groups. Edema was present in the neuron and astrocyte cytoplasms and astrocyte end-feet in both E1 and E10 groups. Our results suggest that 900 MHz RFR may have negative effects on the auditory system in acute exposure and no adverse effects in chronic exposure without weekends.

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KEYWORDS
Radiofrequency radiation; auditory brainstem response; lipid peroxidation; antioxidant

Introduction

By the technological development, the use of wireless phones and RFR emitting devices have been raised at a rapid rate and an increasing number of subjects are exposed to RFR. More specifically due to the close proximity of mobile phones to the user's ear and head, the brain is exposed to relatively high specific absorption rates (SAR) compared to the other organs and thus it is more likely to be affected by mobile phone irradiation. Also, several studies suggest that occupational exposure to RFR may be associated with an increased risk of neurodegenerative diseases (Jiang et al. 2016; Obajuluwa et al. 2017).

Although the effects of electromagnetic waves on the nervous system have been examined previously (Jiang et al. 2016; Obajuluwa et al. 2017), there is limited knowledge concerning the adverse effects of RF radiation on brain physiology and function. Besides, there are some publications showing that the RFR emitted from mobile phones can change the electrical activity of the brain. In one of them, 895 MHz RFR caused a decrease in the electroencephalogram (EEG) alpha band power (Croft et al. 2008). In another study, it was reported that third-generation (3 G) mobile phones had no effect on alpha power while the radiation from the second generation (2 G) mobile phones increased it (Croft et al. 2010). Investigations on event-related potentials (ERP) revealed that 2 G mobile phones increased the amplitude of the P50 component (Papageorgiou et al. 2006), decreased the amplitudes of the P300 and N100 components, and increased their latencies (Hamblin et al. 2004; Maby et al. 2004). These varying findings may result from the diversity of experimental design, exposure settings, applied frequency, and exposure duration.

The extensive worldwide use of mobile phones increased the problem of interaction of electromagnetic fields with the auditory system (Çeliker et al. 2017; Kim et al. 2019). However, there have been few studies related to its effect on the auditory system. In most of them, the effect of RFR emitted by mobile phone was investigated by recording possible psycho-acoustical or electrophysiological changes in the auditory system (Çeliker et al. 2017; Kim et al. 2019). Those studies showed little evidence of the RFR effects on the auditory brainstem and middle latency responses (Arai et al. 2003). In a human study, tympanometry, distortion product otoacoustic emission (DPOAE) and ABR have been studied to prove that long-term (using mobile

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phone for more than 1 year) and intensive (more than 60 minutes per day) use of portable telephones may cause damage to the auditory cortex and cochlea (Panda et al. 2011). In another electrophysiological study, people were briefly exposed to 900 MHz global systems for mobile communications (GSM)-like (SAR = 1 W/kg) electromagnetic field and EEG, visual evoked potential (VEP) and ABR records were taken from these people. The findings of this study have shown that short-term exposure to electromagnetic fields does not sharply influence human cognitive function (Kleinlogel et al. 2008).

Exposure to RFR may lead to an increase in free radicals which have been proposed in many pathophysiological processes (Jonwal et al. 2018; Li et al. 2018). Oxidative stress, which is caused by an imbalance between free radical production and antioxidant defenses, has been shown to be associated with the damage of RFR exposure. It is well known that one of the important consequences of oxidative stress is the peroxidation of membrane lipids (Bedir et al. 2018; Comelekoglu et al. 2018). Thus, lipid peroxidation (LPO) was supposed as the major biochemical alteration which produces marked damage to the structure and function of cell membranes in these tissues (Saikhedkar et al. 2014). Many studies have been performed on people and animals exposed to mobile phones at different frequencies and durations (Bedir et al. 2018; Comelekoglu et al. 2018). In a study conducted, rats were exposed to the mobile phone for 40 and 60 days (4 hours per day/whole body mean SAR value 0.043–0.135 W/kg), and the increased levels of xanthine oxidase (XO) activity, protein carbonyl and malondialdehyde (MDA), and decreased catalase (CAT) activity in the brains of rats were indicated (Sokolovic et al. 2008). Thus, it could be expected that RFR induced-LPO may cause alterations in brain functions.

Moreover, there is no comprehensive study to date about the possible effects of short- and long-term exposure of RFR on ABR. Hence, the aim of the current study was to examine the duration effects of 900 MHz RFR on auditory brainstem responses and to assess lipid peroxidation and antioxidant status of RFR exposed rats.

Materials and methods

Animals

All experimental processes performed on rats were conducted in accordance with the standards established by the Institutional Animal Care and Use Committee of Akdeniz University. Three months aged male albino Wistar rats, weighing between 250 and 300 g, were used in the experiment. Rats were kept in plastic cages (four rats per cage) and given food and water *ad libitum*. Animals were maintained at 12 h light-dark cycles and a constant temperature of 23 ± 1°C at all times.

Study design

After the adaptation process (3 days handling), rats were randomly divided into four groups (n = 15 per group): Group 1: Sham-exposed rats for 1 week (S1); Group 2: Sham-exposed rats for 10 weeks (S10); Group 3: Rats exposed to 900 MHz RFR for 1 week (E1); Group 4: Rats exposed to 900 MHz RFR for 10 weeks (E10). During the experiment, each animal was kept in a plexiglass cage with air holes to ease breathing and minimize the increase in body temperature. In the literature, various RFR exposure durations have been applied to experimental subjects (Alkis et al. 2019; Ammari et al. 2010; Croft et al. 2010; Kim et al. 2019). In our study, we formed groups of 1 and 10 weeks to see the short- and long-term effects of RFR. We determined RFR application duration as 5 days/week and 2 hours/day similar to the study published by Ammari M. et al (Ammari et al. 2010). Experimental groups were exposed to 900 MHz RFR emitted from the signal generator for defined durations for 1 (E1) and 10 (E10) weeks. Sham rats were housed in the same room under the same conditions with an equal time period without RFR.

Electromagnetic radiation exposure

The exposure system was shown in Figure 1. In this system, a RF generator (GSM Simulator 900 MHz; Everest Company, Adapazari, Turkey), which produces 900 MHz RFR, was used to present the exposure of GSM. The output power of the generator was adjusted to 5 W during the exposure. Besides the carrier frequency was 900 MHz, the modulation frequency was 217 Hz, the pulse width was 0.577 msec, and the power range of the generator was 0–10 W. The setup consisting of 10 carousels with 10 rats, each restrained in a radially positioned tube, was exposed to the RFR emanating from the antenna at the center. The rats were kept with their snouts at a distance of 3.5 cm from the monopole antenna. The tubes restrained the movement of the rats to such an extent as to get well-defined exposure conditions without immobilizing them. The entire carousels were placed in a shielded room to prevent from other disturbance generating sources. In the experiment the electric field strengths were measured by EMR300 with an appropriate probe (Narda, Germany). In the shielded room, the electric field background level was between

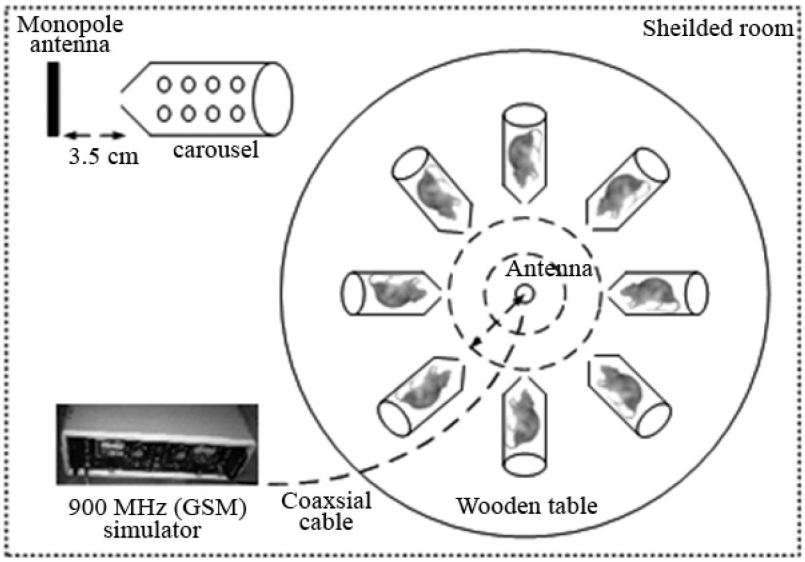


Figure 1. Electromagnetic field exposure system.

0.02 and 0.2 V/m. Also background magnetic field was measured between 0.01 and 0.03 μT by Hioki 3470 Magnetic Field Hitester (Hioki E. E. Corp., Japan) with an appropriate probe. In the "signal-on" position, the measured electric-field strength over the rat's head, positioned 3.5 cm away from the antenna, was 35.5 V/m. The average whole-body SAR value was 5.284 mW/kg. The SAR value for the brain was on the average of 0.66 W/kg. The numerical computation was performed by using the Finite Difference Time Domain (FDTD) Method (Hyun and Oh 2006; LeBlanc et al. 2000). The FDTD code with perfectly matched layer absorbing boundaries was used to measure SAR value on the brain of Wistar rats. Electric field strength, conductivity, and density of the model for each voxel were used for the estimation of the SAR value. Electrical properties, dielectric constant, and conductivity were used from the literature (Gabriel 2005; Peyman et al. 2001). The applied carousel setup procedure of the present study was in accordance with the setup procedure of the other studies in the literature (Burkhardt et al. 1997; Fritze et al. 1997; Schönborn et al. 2004).

The body temperatures of rats were observed by rectal measurements prior to and after all experimental sessions. The RFR exposure did not lead to any rectal temperature increment.

ABR recordings

Hearing functions of all animals were evaluated by using the ABR at the day after the RFR exposure or sham exposure. Rats were anesthetized with a mixture of ketamine (50 mg/kg) and xylazine (10 mg/kg) intraperitoneally and then laid on to a heating pad, to keep up body temperature at 37°C, and placed in a sound-attenuating and electromagnetically shielded cabin. Subdermal stainless steel needle electrodes (Medelec Insert earphone Dual 54455/A) were placed at the ipsilateral mastoid (non-inverting, +) of the test ear, the vertex (inverting, -), and tail (ground) of the rats. A Labat Audiological Platform (Scorze, Italy) auditory data acquisition system, equipped with a computer-based digital signal processing package and software (Labat Audiological Platform, Scorze, Italy) was used for stimulus generation and data acquisition. The acoustic stimuli were supplied via a dynamic transducer connected to a 10 cm plastic tube that was placed into the external auditory canal of the test ear. Tone bursts (2 ms duration, 1 ms rise/fall time, Blackman envelope, alternating starting phase) were presented at 8 kHz and 16 kHz frequencies at varying intensity levels. ABR responses were filtered (100–3.000 Hz bandpass, 50 Hz notch filter), digitized (10 kHz sampling rate), and averaged over 700–800 samples at each frequency. Threshold testing began with the stimulus presented at 70 dB SPL in order to generate a clear ABR waveform; then the intensity was reduced in 10 dB steps until the ABR response disappeared. The ABR threshold was defined as the lowest intensity at which the fifth wave of ABR could be detected. The thresholds were confirmed by two investigators.

Biochemical investigations

Animals were sacrificed after ABR recordings at the same time period (9:00 am and 12:00 am). Brains of

rats were perfused transcardially with heparinized saline and removed immediately in an ice-cold buffer. The brain tissues were homogenized separately in 2–5 ml HEPES buffer for superoxide dismutase (SOD) enzyme activity, 50 mM potassium phosphate, pH 7.0, containing 1 mM EDTA per gram tissue for CAT enzyme activity, glutathione (GSH), and thiobarbituric acid-reactive substances (TBARS). All tissues were rapidly sonicated in a thermally regulated sonicator (Branson Sonifier 250, G. Heinemann Ultraschall- und Labortechnik, Germany) for 1 min. The sonicated samples were divided into aliquots and stored frozen at −80° C until assay determinations.

TBARS assay

A part of sonicated samples was centrifuged at 14,000 g for 10 min at 4°C in an eppendorf microcentrifuge (Biofuge 15 R, Heraeus Sepatech, Osterode, Germany). The supernatants of centrifuged samples were used for the assay of TBARS measurements. Levels of TBARS were measured by a fluorimetric method described by Wasowicz et al. (Wasowicz et al. 1993), using 1,1,3,3-tetraethoxypropane as a standard. The results were given as µmol/g protein.

Determination of SOD activity

Superoxide dismutase activities of brain tissues were measured using SOD assay kit (Cat #706002; Cayman Chemical, AnnArbor, MI, USA) according to manufacturer's instruction. This kit benefits from a tetrazolium salt for the detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD was defined as the amount of enzyme needed to produce 50% dismutation of superoxide radical. The SOD activity was stated in kUnit per g protein.

Determination of CAT activity

Brain catalase activity was measured using CAT assay kit (Cat #707002; Cayman Chemical, AnnArbor, MI, USA) according to manufacturer's instruction. This kit utilizes the peroxidatic function of CAT for determination of enzyme activity. Briefly, the method bases on the reaction of the enzyme with methanol in the presence of an optimal concentration of H_2O_2. The formaldehyde produced is measured colorimetrically with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald) as the chromogen. One unit of CAT was defined as the amount of enzyme that causes the formation of 1.0 nmol of formaldehyde per minute at 25°C. The CAT activity was stated in kUnit per g protein.

Measurement of total GSH

Glutathione levels were measured in brain tissues by a commercially available GSH assay kit (Cayman Chemical, Cat #703002 Ann Arbor, MI, USA). Supernatants were deproteinated in 10% metaphosphoric acid (Sigma Aldrich, Steinheim, Switzerland). The GSSG was reduced to GSH by GSH reductase in the assay cocktail of the kit containing 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), glucose-6-phosphate dehydrogenase, GSH reductase, Nicotinamide adenine dinucleotide phosphate (NADP+) and glucose-6-phosphate. The sulfhydryl group of GSH reacts with DTNB to give a yellow-colored 5-thio-2-nitrobenzoic acid (TNB) which is measured at an absorbance of 405 nm. The values of total GSH for each sample were calculated from their respective slopes using a GSSG or GSH standard curve. Total GSH levels were stated in µmol per g protein.

Determination of protein

Protein concentrations in brain tissues were measured spectrophotometrically (Shimadzu RF-5500, Kyoto, Japan) by a protein assay reagent kit (Pierce, Rockford, IL) via a modified Bradford method (Bradford 1976). Bovine serum albumin was used as a standard.

Transmission electron microscopic (TEM) evaluation

For electron microscopic evaluation, after ABR recordings temporal cortexes were dissected from each group. Samples were fixed with 4% glutaraldehyde in 0.1 M Sorensen's phosphate buffer solution (pH 7.3) and post-fixed with 2% osmium tetraoxide in the same buffered solution. After dehydration through a graded series of ethanol, they were embedded in epoxy resin (Araldite CY212, Agar Scientific Ltd, Stansted, UK). Semi-thin (1 µm) and ultrathin (40–60 nm) sections were cut with an ultramicrotome (Leica ultracut, UCT, Leica MZ6, England). Semi-thin sections were stained with toluidine blue and were examined with a light microscope (Olympus CX41). Afterward, ultrathin sections were contrasted with uranyl acetate and lead citrate and were examined with a transmission electron microscope (Zeiss LEO 906E, Germany). For

ultrastructural analysis of temporal cortex, neurons, astrocytes, and vessels were randomly selected and examined with TEM.

Statistical analysis

The results were expressed as the mean ± standard error of the mean. Statistical significance was determined as $p < .05$. The differences between the groups were analyzed with one-way analysis of variance (ANOVA) and all pairwise multiple comparisons were evaluated by posthoc Tukey test for ABR and biochemical data.

Results

Auditory brainstem response

ABR thresholds

There were no statistically significant differences between the ABR threshold values of all groups at 8 kHz and 16 kHz auditory stimuli.

ABR latencies

ABR latencies for all groups were given in Tables 1 and 2 and representative waveforms of ABRs were presented in Figures 2 and 3.

ABR latencies obtained with 8 kHz and 60 dB SPL tone burst auditory stimuli (Table 1) were as follows: The mean latency of wave I was significantly prolonged in the E1 group compared to the S1 group. In the chronic groups, it was significantly shortened in the E10 group according to the S10 group. Besides there was not a significant difference in wave I latency between E1 and E10 groups. In addition, there were no significant differences between the groups in interpeak latencies of I–III, III–V and I–V waves.

ABR latencies obtained with 16 kHz and 60 dB SPL ton burst auditory stimuli (Table 2) were as follows: Wave I latency statistically significantly prolonged in the E1 group compared to the S1 group. In the chronic groups, it shortened in the E10 group compared to the S10 group. But there was not a significant difference in wave I latency between E1 and E10 groups. In addition, there were no significant differences in interpeak latencies of waves I–III, III–V, and I–V among groups.

Biochemical findings

TBARS levels

Brain TBARS results of sham and experimental groups were shown in Table 3. It was observed that brain TBARS value significantly increased in E1 group with respect to the S1 group. In chronic groups, it decreased significantly in the E10 group with respect to S10 group. However, there was not a significant difference in between E1 and E10 groups.

SOD activity

The brain SOD activities of all groups were given in Table 3. As a result of the statistical evaluations, brain SOD activity significantly decreased in the E1 group according to the S1 group. The SOD activity of E10 group in brain tissues significantly increased compared to both S10 and E1 groups.

CAT activity

Brain CAT activities of all groups were shown in Table 3. It was observed that brain CAT activity decreased significantly in the E1 group with respect to the S1 group. CAT activity of E10 group compared to both S10 and E1 groups increased significantly.

GSH levels

The brain GSH values of sham and experimental groups were summarized in Table 3. As a result of the evaluation of GSH values between the acute groups, it was found that there was a significant decrease in the E1 group according to the S1 group. Besides mean GSH value of E10 group significantly increased compared to S10 and E1 groups.

TEM findings

Brain TEM images of all the groups were given in Figures 4, 5 and 6.

Table 1. Latencies of ABR components for all groups at 8 kHz and 60 dB SPL auditory stimuli.

Latency(ms)	S1	E1	S10	E10
Wave I	1.93 ± 0.02	2.04 ± 0.03*	2.1 ± 0.02	2 ± 0.03**
Wave II	2.82 ± 0.04	2.88 ± 0.03	2.88 ± 0.02	2.86 ± 0.05
Wave III	3.44 ± 0.05	3.52 ± 0.04	3.52 ± 0.03	3.54 ± 0.07
Wave IV	4.45 ± 0.06	4.58 ± 0.04	4.62 ± 0.04	4.56 ± 0.09
Wave V	5.63 ± 0.09	5.74 ± 0.06	5.62 ± 0.06	5.61 ± 0.15
I–III interpeak latency	1.51 ± 0.03	1.49 ± 0.02	1.44 ± 0.03	1.50 ± 0.06
III–V interpeak latency	2.19 ± 0.08	2.21 ± 0.05	2.10 ± 0.05	2.07 ± 0.12
I–V interpeak latency	3.69 ± 0.09	3.7 ± 0.06	3.54 ± 0.07	3.57 ± 0.13

The results were given as mean ± SEM. * $P < 0.05$ according to S1, ** $p < 0.05$ according to S10.

Table 2. Latencies of ABR components of all experimental groups at 16 kHz and 60 dB SPL auditory stimuli.

Latency(ms)	S1	E1	S10	E10
Wave I	2.01 ± 0.02	2.07 ± 0.01*	2.22 ± 0.04	2.01 ± 0.07**
Wave II	2.92 ± 0.03	2.96 ± 0.04	3.18 ± 0.05	3.04 ± 0.07
Wave III	3.48 ± 0.04	3.48 ± 0.04	3.61 ± 0.03	3.64 ± 0.11
Wave IV	4.40 ± 0.05	4.41 ± 0.08	4.71 ± 0.05	4.56 ± 0.11
Wave V	5.53 ± 0.05	5.54 ± 0.07	5.83 ± 0.06	5.69 ± 0.13
I–III interpeak latency	1.46 ± 0.03	1.41 ± 0.04	1.4 ± 0.04	1.62 ± 0.17
III–V interpeak latency	2.06 ± 0.06	2.06 ± 0.10	2.22 ± 0.07	2.05 ± 0.10
I–V interpeak latency	3.52 ± 0.06	3.47 ± 0.07	3.61 ± 0.04	3.68 ± 0.18

The results were given as mean ± SEM. * $P < 0.05$ according to S1, ** $p < 0.05$ according to S10.

Table 3. Biochemical parameters for all of the groups.

Groups	TBARS μmol/g protein	SOD kU/g protein	CAT kU/g protein	GSH μmol/g protein
S1	1.39 ± 0.25	0.37 ± 0.05	5.67 ± 0.67	1.45 ± 0.27
E1	2.6 ± 0.33*	0.19 ± 0.03*	3.87 ± 0.45*	0.64 ± 0.13*
S10	6.37 ± 0.66	0.21 ± 0.03	2.52 ± 0.21	1.36 ± 0.17
E10	2.86 ± 0.97**	0.35 ± 0.05**,#	5.76 ± 0.64**,#	2.93 ± 0.44**,#

The results were given as mean ±SEM. * $p < 0.05$ according to S1, ** $p < 0.05$ according to S10, # $p < 0.05$ according to E1.

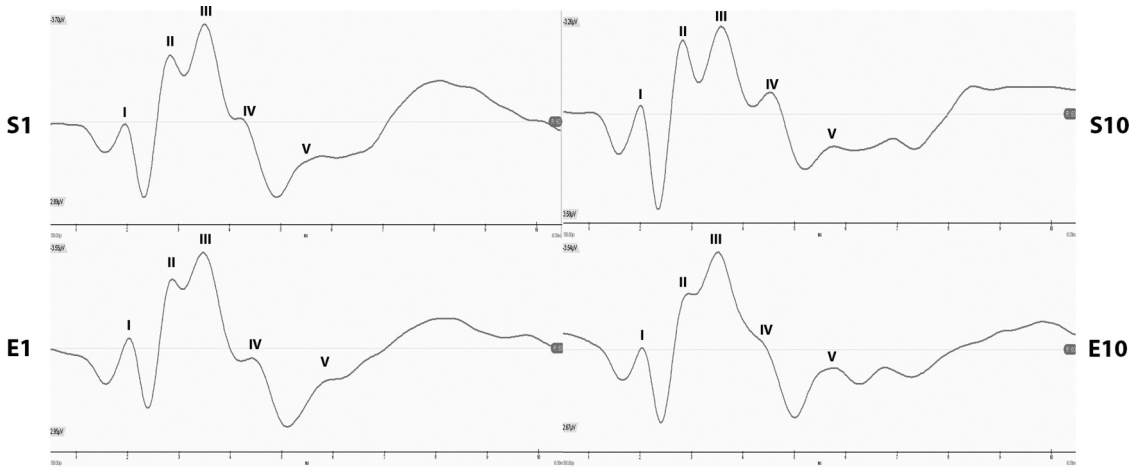


Figure 2. ABR samples of sham and experimental groups recorded at 8 kHz 60 dB SPL auditory stimuli.

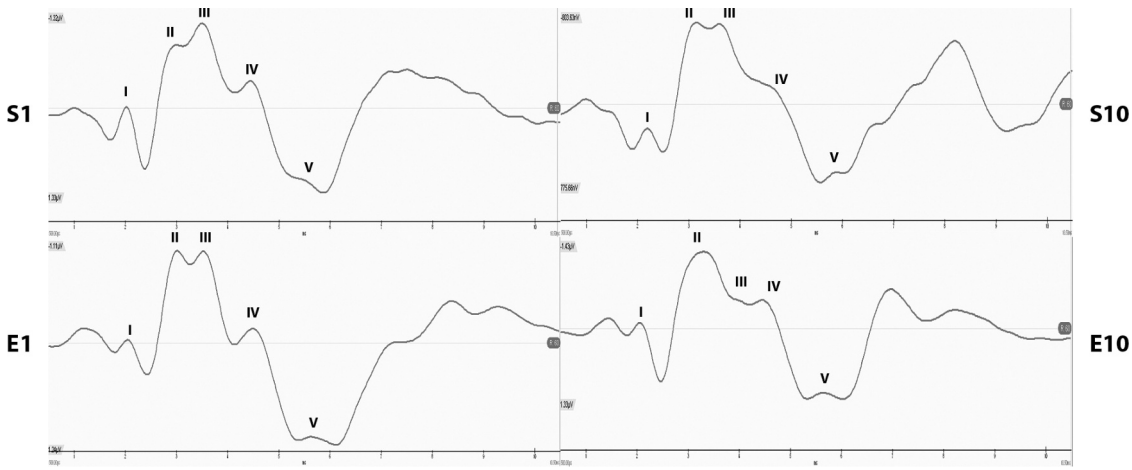


Figure 3. ABR samples of sham and experimental groups recorded at 16 kHz 60 Db SPL auditory stimuli.

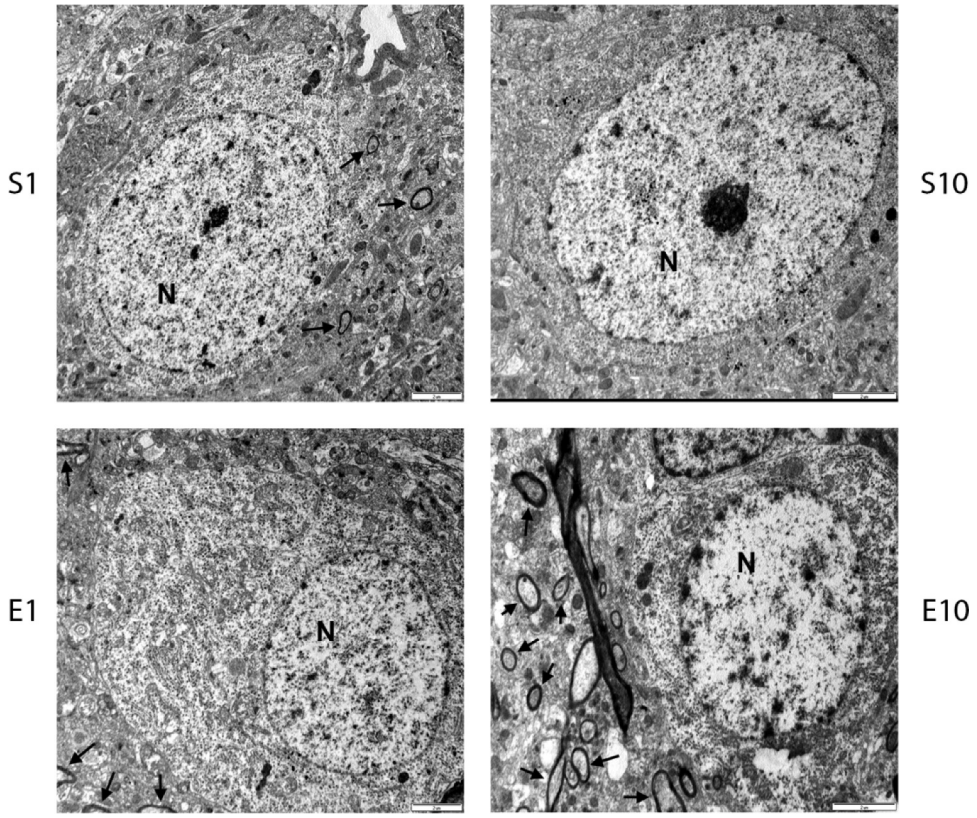


Figure 4. TEM images of sham and experimental groups' temporal cortex neurons. N: Nucleus, Arrow: Myelin, Scale bars: 2 µm.

The neuron ultrastructure in E1 group (eukaryotic nucleus, continuity of nuclear membrane integrity, the normal structure of polyribosomes and granular endoplasmic reticulum) was similar to those of the S1 group. Besides the loss of electro-density due to edema was seen in neuroplasma. Edema of the neuropil, especially of the astrocyte end-feet, formed by the glial and neuronal extensions around the neuron has been observed. Similar structural deterioration in astrocytes has also been observed in the glial membrane which is formed by perivascular astrocytic end-feet. Endothelial cell membrane and myelin structures were in a normal structure. Similar to the above findings, vacuolization due to edema and loss of normal structure in the astrocyte cytoplasm were determined. The astrocytic cytoplasm lost its electron density due to edema, whereas the nucleus was in a normal heterochromatin structure.

Loss of electro-density due to edema was seen in neuroplasma in E10 group similar to E1 group. The neuron nucleus (eukaryotic nucleus) was similar to those of the S10 group. The cytoplasmic impairment of the astrocytic end-feet due to severe edema and edema and cytoplasmic degradation have been detected in neuron-astrocyte vicinity. Although endothelium and myelin structures were normal, dilatations due to edema and cytoplasmic disruptions were seen in the astrocyte end-feet around the vein. The astrocytic findings were similar to those of group E1. The cytoplasm with vacuolization was electrolucent in appearance and the nuclei were in normal heterochromatin structure.

Discussion

Communication devices such as mobile phones have become indispensable elements of our life because of the importance of keeping an important place in all aspects of human life and technological progress. Although mobile phones and base stations used for communication make life easier, it is thought that RFR may have negative effects on human health (Dasdag et al. 2012; Kesari et al. 2011; Sokolovic et al. 2008).

The biochemical parameters have been evaluated in most of the studies on the effect of RFR on the brain (Alkis et al. 2019; Karimi et al. 2019). There are a few publications in the literature that show these changes by electrophysiological studies (Gok et al. 2014; Hidisoglu et al. 2016). ABR is a sensitive and reliable method used in the functional investigation of the auditory system and in the evaluation of auditory pathologies (Gilles et al. 2016; Maslin et al. 2015; Monsell 2004). For this

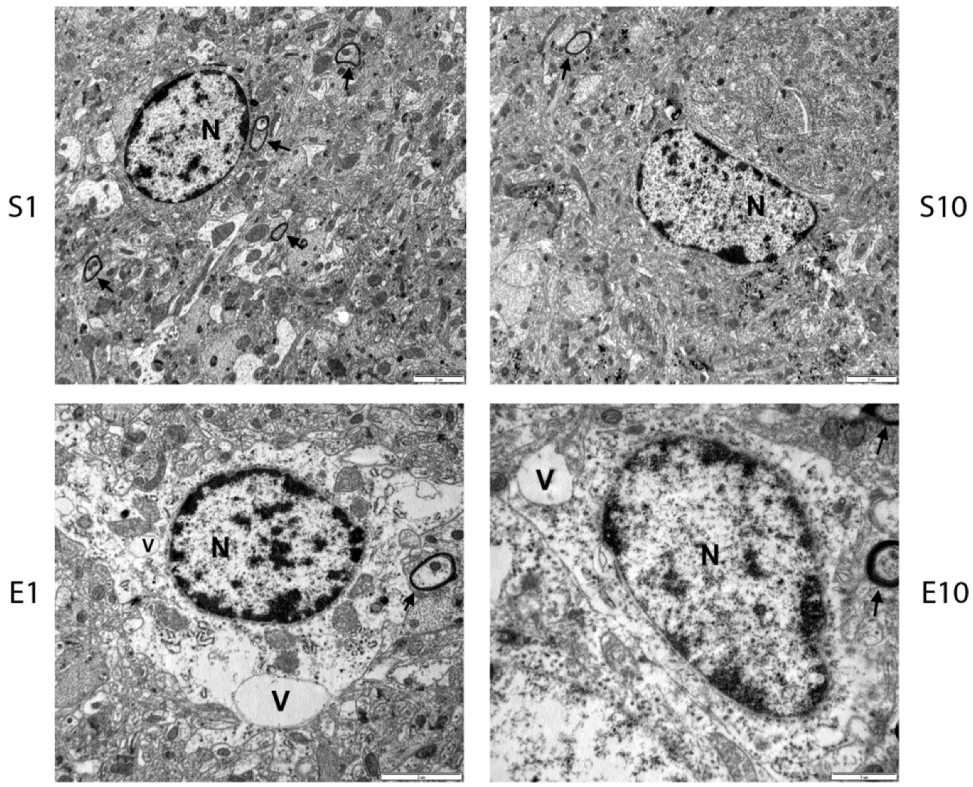


Figure 5. TEM images of sham and experimental groups' temporal cortex astrocytes. N: Nucleus, V: Vacuole, Arrow: Myelin. Scale bars of S1, S10, E1 images: 2 µm, Scale bar of E10 image 1 µm.

reason, this study was planned to clarify the role and mechanism of lipid peroxidation in the changes of electromagnetic radiation in ABR.

In our study when hearing thresholds were evaluated, no significant differences were observed at both 8 kHz and 16 kHz frequencies. In a study, 900 MHz RFR with SAR 4 W/kg was administered to the guinea pigs for 2 months (1 h/day and 5 days/week), and no significant difference between the ABR thresholds of the experimental and control groups was reported (Aran et al. 2004). The ABR threshold results of E10 and E1 groups of our study were compatible with the work by Aran JM et al (Aran et al. 2004). When ABR latencies recorded at 8 kHz and 16 kHz 60 dB SPL were evaluated, it was seen that the latencies of all ABR waves of E1 group were longer than those of S1 group and this prolongation was significant at wave I. In chronic groups, it has been found that the latencies of the ABR waves of E10 group tend to be shorter than those of the S10 group and this shortening was significant at wave I. Besides there were no significant differences in between E1 and E10 groups. On the other hand, when I–III, III–V, and I–V interpeak latencies were evaluated, no significant differences were observed at both 8 kHz and 16 kHz. When we survey the literature, there are a few studies which are related to RFR and ABR (Özgür et al. 2015; Stefanics et al. 2007). In one of those studies rats were administered RFR at 1800 MHz frequency, 3.47 mW power for 24 hours a day for 30 days, and ABRs reported that there were no significant differences between the experimental group and the control group latencies and interpeak latencies (Özgür et al. 2015). In our study, the chronic group results of the wave latencies except for wave I and interpeak latencies are similar to those obtained by Ozgur et al (Özgür et al. 2015). In another study by Stefanics G. et al. (Stefanics et al. 2007), it was reported that there was no difference in latency of ABR waves when young and healthy people were exposed to 900 MHz mobile phones for 10 minutes. In our study, the results obtained in other waves except wave I in the acute RFR group coincide with this study. The contradictions regarding the wave I latency may be due to our exposure duration, SAR value or RF frequency is different with those studies (Özgür et al. 2015; Stefanics et al. 2007). ABR waves of rats are generally related to the activation of the auditory nerve, cochlear nuclei, superior olivary complex and lateral lemniscus (waves I, II, III and IV, respectively) (Chen and Chen 1991). It has been previously shown that wave I of ABR could be attenuated or eliminated by lesions in the auditory nerve (Chen and Chen 1991; Rosahl et al. 2000). Besides, in their study, Lee J.E. et al. found 4-HNE-mediated

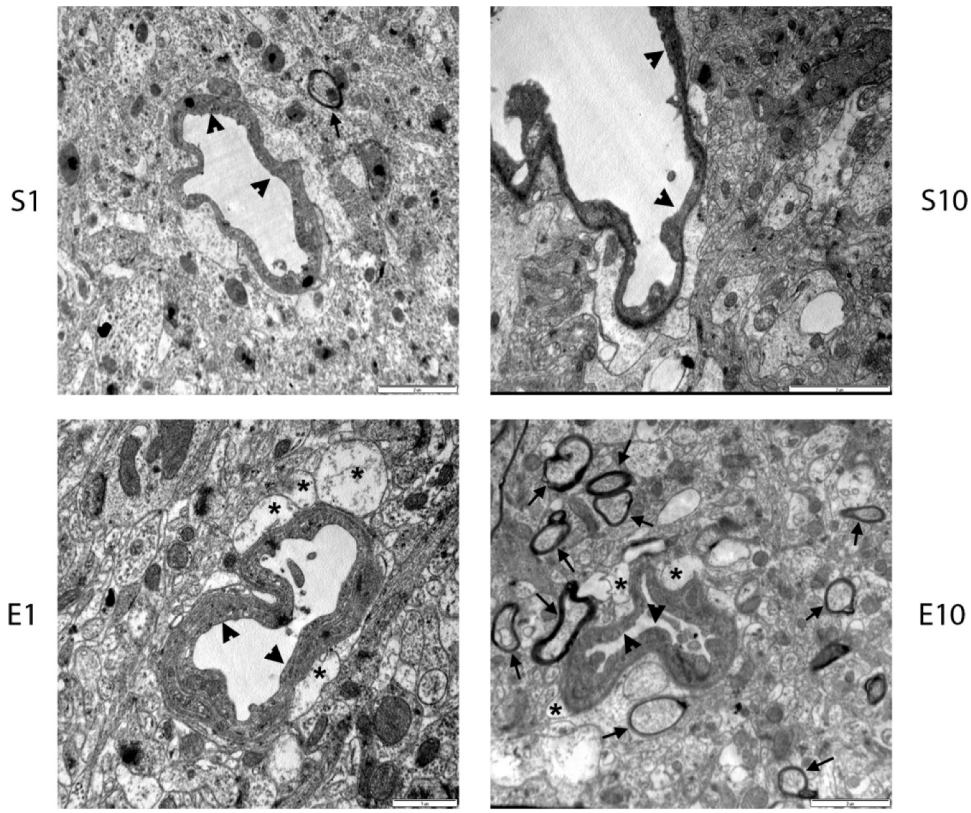


Figure 6. TEM images of sham and experimental groups' temporal cortex vessel circumference. *: Edema, Arrow: Myelin, Arrow head: Endothelial cell membrane. Scale bars of S1, S10, E10 images: 2 µm, Scale bar of E1 image 1 µm.

damage in auditory nerves (Lee et al. 2004). On the other hand in a study which was conducted on rats, Chen B. et al. (Chen et al. 2010) showed that in a group ABR wave I latency prolonged and the level of MDA also increased. Similarly, in our study, prolongation of the wave I latency in the acute RFR group with respect to its sham group may be due to a lesion on the auditory nerve due to an increase in lipid peroxidation. Shortening of the wave I latency in the chronic RFR group according to its sham group may also be due to the improvement in the auditory nerve due to decreased lipid peroxidation in the chronic group.

It is known that free radicals destroy cellular functions by damaging lipids, proteins, and nucleic acids which are building blocks of the cell (Kıvrak et al. 2017; Marjanovic Cermak et al. 2018). The brain, which is rich in unsaturated fatty acids, is more susceptible to free radicals. It is known that lipid peroxidation products resulting from the oxidation of membrane lipids lead to significant changes in membrane functions (Ghanbari et al. 2016; Marjanovic Cermak et al. 2018). For this reason, the TBARS level, which is an indicator of lipid peroxidation, was measured in our study. In our results, the TBARS level increased significantly in E1 group with respect to the S1 group. In chronic groups, TBARS levels decreased significantly in the E10 group with respect to S10 group. However, there was not a significant difference in between E1 and E10 groups. To demonstrate the lipid peroxidative effects of RFR on brain and other tissues, a number of studies have been carried out in humans and experimental animals exposed to RFR at different intensities, frequencies, modulations, and durations (Esmekaya et al. 2016; Ezz et al. 2013; Ilhan et al. 2004; Köylü et al. 2006; Oktem et al. 2005; Sahin et al. 2016; Sokolovic et al. 2008). In one of these studies, 900 MHz RFR (SAR: 0.301 W/kg) was applied to adult female mice for 45 and 60 minutes and they have demonstrated significant MDA elevation in RFR group with respect to the sham group (Esmekaya et al. 2016). Our acute group TBARS result is consistent with this study. In another study 900 MHz RFR (SAR: 0.95 W/kg) was applied to female rats for 4 weeks (40 min/day), and there was no significant change in MDA level between control and experimental group (İmge et al. 2010). Besides in their study, Sahin D. et al. (Sahin et al. 2016) 2100 MHz (whole-body SAR: 0.4 W/kg) RFR applied to female rats for 2 and 8 weeks and they have demonstrated that MDA level did not change significantly in acute groups but in chronic groups, it was significantly lower in the RFR group with

respect to control group. Our chronic group TBARS result is consistent with this study. On the other hand, MDA levels were found to be increased in the brains of the rats exposed to the mobile phone (total body SAR value 0.043–0.135 W/kg) for 4 hours a day for 40 and 60 days (Sokolovic et al. 2008). In our study, the increase in TBARS levels in the acute group with respect to its sham group may be due to decreased antioxidants. On the contrary, the decrease in TBARS level in the chronic group according to its sham group may be due to the increase in antioxidants. Consequently in the literature, there are conflicting results about RFR and lipid peroxidation. This may be due to different RF properties and experimental designs.

The brain is the most oxygen-consuming organ in the body. Most of the oxygen consumed by the brain is converted to CO_2 and water (Harvey and Ferrier 2011). Under normal conditions, however, some of the oxygen is converted into free radicals such as superoxide ($\cdot O_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl ($\cdot OH$). For this reason, there are various antioxidant mechanisms that will remove the harmful effects of free radicals (Pandya et al. 2013). In our study, SOD and CAT antioxidant enzyme activities and GSH levels were evaluated to demonstrate the effect of RFR on antioxidant enzyme activities. Findings of our study showed that in acute groups SOD and CAT activities and GSH levels decreased significantly in the E1 group compared to the S1 group. In chronic groups, SOD and CAT activities and GSH levels increased significantly in E10 group compared to the S10 group. Also in these three parameters, there were significant increases in E10 group with respect to E1 group. There are incompatible results in the literature regarding the RFR studies associated with antioxidant enzyme activities (Dasdag et al. 2009; Irmak et al. 2002; Kesari et al. 2011; Meral et al. 2007). When antioxidants are evaluated, there are publications showing that CAT enzyme activity is both decreased (Meral et al. 2007) and increased (Dasdag et al. 2009; Kesari et al. 2011) in the result of long-term RFR exposure. Our CAT activity results of chronic groups are consistent with the studies which support the increment. However, no study has been found in the literature to examine the effect of RFR on brain CAT activities for 1 week. When the SOD enzyme was evaluated, the decrease in E1 activity of this enzyme was found in agreement with the study performed by İlhan et al. (Ilhan et al. 2004). On the other hand in a study of 900 MHz RFR applied to rats for 45 days (4 h/day), it was reported that SOD activity in rat testis decreased in RFR group compared to the control group (Jelodar et al. 2013). These data contradicts with SOD activity level of our chronic RFR group. When the GSH level was evaluated, although we could not find the same experimental design for our acute group (such as RFR exposure duration, SAR value, tissue), Esmekaya et al. (Esmekaya et al. 2016) and Elhag et al. (Elhag et al. 2007) reported that GSH level decreased compared to the control group as a result of acute RFR application in mice brain and in rat plasma, respectively. Our GSH level result of acute RFR group is compatible with their results. For long-term RFR application, there are studies saying that there is no difference (Demirel et al. 2012) or there is a decrease (Asl et al. 2020) in GSH level. Our chronic RFR group GSH level finding contradicts with these papers. The reason for both SOD and GSH conflict may be our RFR exposure duration is longer (also without weekends), our SAR value is different or tissue difference. An increase in SOD and CAT activities and GSH levels with a TBARS decrease in E10 group may be an important adaptive response to increased lipid peroxidation. However, these findings we have obtained regarding antioxidant and oxidant status in the E10 group are also compatible with the biochemical findings of Khan R.A.'s study (Khan 2012) in rat brain.

In our ultra-structural analysis, no difference was found between neuron and astrocyte nuclei, mitochondria, granulated endoplasmic reticulum and polyribosomes in acute and chronic groups. In the literature review we conducted, RFR studies with ultra-structural analysis in the rat brain that undergoing RFR were found to be less than satisfactory. In a magnetic field study, 2mT, 60 Hz ELF-MF was applied to rats for 5 days and no significant difference was observed in nuclei and mitochondria in hippocampal neuronal cells (Cho et al. 2012). Our results are consistent with this study. In our study, myelin sheaths were found to be normal in the experimental groups. In the study of Ju Hwa Kim et al. (Kim et al. 2017), 835 MHz RFR (SAR = 4 W/kg) was administered to mice for 12 weeks (5 h/day) and defects were observed in the axon myelin sheaths. As is known, the International Commission on Non-Ionizing Radiation Protection has set the maximum SAR value for human health from 10 MHz-10 GHz to 2 W/kg (ICNIRP 1998). This difference between the study conducted by Kim J.H. et al. (Kim et al. 2017) and our study may be due to their high SAR value and different frequency. In our study, edema was observed in astrocyte end-feet. In a study, Sirav and Seyhan (SİRav and Seyhan 2009) found an increase in blood-brain barrier (BBB) permeability in male rats that were acutely administered 900 and 1800 MHz RFR. As is known, the lanthanum nitrate cannot cross the brain capillaries and is therefore often used to test BBB integrity (Bradbury and Deane 1993; Le Sueur et al. 2004). In another study, 400 pulses

of 1 Hz EMP at 200 kV/m was applied to the rats and it was revealed that the lanthanum dye passed the cerebral capillaries (Gui-Rong et al. 2009). The findings of edema obtained in our study may be related to the increase in permeability of the blood-brain barrier in accordance with these studies. In our study, it was found that edema was less in E10 group than E1 group. This may indicate that the permeability of the blood-brain barrier is reduced and the cells begin to recover.

As a result, ABR latencies were prolonged in the acute administration of 900 MHz RFR and shortened in chronic administration of it with respect to their sham groups. The cause of latency prolongation in acute treatment may be due to the reduced level of GSH and SOD and CAT activity together with increased lipid peroxidation in the E1 group. The latency shortening in chronic administration may be due to an increase in SOD and CAT activities and GSH levels which may well overcome lipid peroxidation. The adverse effects seen in the acute experimental group and not in the chronic experimental group may be related to our RFR application period. Namely in our study, RFR was administered to the chronic experimental group 5 days/week for 10 weeks. RFR was not applied at the weekends. The antioxidant defense system may have ameliorated the adverse effects that occur on weekdays at the weekends. Therefore, it may be beneficial for people to suspend using mobile phones for some days. In conclusion, short-term exposure to RFR caused negative effects on the auditory system, but no side effects due to chronic exposure except weekends were determined.

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Disclosure of interest

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