

Research Article

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The effects of short-term and long-term 2100 MHz radiofrequency radiation on adult rat auditory brainstem response

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Abstract: Although mobile phones that work with RFR provide very important benefits for our lives, they may have negative effects. Namely, side effects, such as headaches, sleep disorders, dizziness, lower sperm quality, changes in brain potentials, an increase in oxidative stress levels, and a decrease in antioxidant parameters, have been reported due to mobile phone use. Accordingly, the aim of this research is to investigate the effects of acute and chronic 2100 MHz radiofrequency radiation (RFR) exposure on the auditory brainstem response (ABR) in adult rats. Study groups ($n = 10$ rats): Sh-1: sham for 1 week; Sh-10: sham for 10 weeks; 2100-1: 2100 MHz for 1 week; and 2100-10: 2100 MHz for 10 weeks. RFR groups were applied for 2 h/day (5 day/week) 2100 MHz RFR, whereas sham groups were kept under identical circumstances without RFR. ABR were recorded, and biochemical and ultrastructural examinations in the rat brain were carried out. In the acute RFR group, the latencies of all ABR waves were prolonged compared to the sham group. In the acute RFR group, brain 4-hydroxynonenal, thiobarbituric acid reactive substances, and protein carbonyl content levels increased and catalase and superoxide dismutase activities decreased compared to the acute sham group. Edema in acute RFR group neurons, astrocytes, astrocytic end-feet, and mitochondrial damage in astrocytes were observed. Our data imply that acute exposure to 2100 MHz RFR may have adverse impacts on the

auditory system, while chronic exposure with certain rest days has no harmful effects.

Keywords: auditory brainstem response, lipid peroxidation, protein oxidation, radiofrequency radiation, superoxide dismutase

1 Introduction

New devices, such as cell phones, are coming into our lives every day as a result of technological advancements. Radiofrequency radiation (RFR) is used by cell phones. Despite the fact that RFR belongs to the category of non-ionizing radiation, questions about its impacts on health are becoming more and more prevalent as a result of an increase in the number of RFR-emitting devices as well as the usage of mobile phones adjacent to the brain and ear. Accordingly, the number of studies on this topic has increased, and some of these studies have indicated that RFR may have adverse effects on human health [1–3]. In one of these studies, it was reported that oxidative damage may increase and DNA damage may occur in the brain tissue of rats administered RFR for 6 months [2]. In another study, a decrease in EEG alpha band power was shown in people exposed to mobile phone RFR [3].

RFR is known to be a form of electromagnetic radiation. The effects of RFR on the brain have been studied for decades, but there are still many questions that require further investigation. As cellular devices are positioned near the ear, a survey is necessary to examine the interaction between RFR and the auditory system. There are few studies on this topic and numerous issues to investigate. Psycho-acoustic or electrophysiological recordings have been used in the majority of studies to attempt to illustrate the impact of RFR on the auditory system [4–6]. Auditory brainstem response (ABR) tests revealed statistically significant differences in two studies using 900 MHz RFR on rats and rabbits [5]. In contrast, in a study where postnatal mice were exposed to 1850 MHz RFR, no significant difference was observed in ABR tests [6,7]. Besides, N100 and P200

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auditory-evoked potentials and mismatch negativity did not change significantly in a study involving healthy individuals exposed to RFR at a short-term 3G frequency (1947 MHz) [4].

Several studies indicate that exposure to RFR may potentially elevate the levels of free radicals in the body [8,9]. The imbalance between the body's antioxidant molecules and reactive oxygen species (ROS) is known as oxidative stress. This results in the formation of ROS, which is a subset of free radicals that contain oxygen, subsequently causing damage to proteins, cellular membranes, and DNA. Examples of ROS include superoxide anion radical (O_2^-), hydroxyl radical (OH), hydrogen peroxide (H_2O_2), and lipid peroxyl radical (LOO). The existence of one or more unpaired electrons makes these compounds unstable. ROS are generated through various mechanisms, including mitochondrial oxidative phosphorylation, exposure to radiation, and inflammatory processes [10]. When ROS interact with cellular proteins and lipids, they induce protein oxidation and lipid peroxidation, resulting in cellular damage. When ROS accumulate in abundance, the human body employs antioxidant molecules, including catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione (GSH) to defend itself. Numerous studies on humans and experimental animals have examined the effects of RFR on oxidative stress [8,9,11]. In one of these studies, rats were exposed to 1966.1 MHz RFR (2 h/day) for 16 weeks, and it was discovered that ROS, lipid peroxide, and protein carbonyl levels increased, while total antioxidant capacity decreased in the hippocampus [8]. In an independent investigation, rats were subjected to 2600 MHz RFR for a duration of 30 days (5 day/week, 30 min/day), and it was revealed that GSH, GPx, and SOD levels decreased, while malondialdehyde (MDA) and nitrogen oxide ($NO(x)$) levels increased in the rat brain [9].

In addition, there is no published research on the effects of short- and long-term administration of 2100 MHz RFR on ABR in rats. Therefore, the purpose of this research was to clarify the impact of 2100 MHz RFR on ABR with respect to exposure duration, as well as the connection between ABR and oxidative stress markers like protein oxidation, lipid peroxidation, and brain antioxidant status.

2 Materials and methods

2.1 Animals and study design

In the experiment, albino male Wistar rats that were 3 months old and had a weight ranging from 250 to 300 g were utilized. The rats were housed in plastic cages, with a

total of four rats per cage, and were provided with unimpeded access to food and water. The animals were housed in a controlled environment with a temperature of $23 \pm 1^\circ\text{C}$ and subjected to 12-h cycles of light and darkness.

Following the process of adaptation, the rats were separated into four distinct groups, wherein each group comprised 10 rats: Sh-1: rats subjected to a sham exposure for 1 week; Sh-10: rats subjected to a sham exposure for 10 weeks; 2100-1: rats subjected to a 1-week exposure to 2100 MHz RFR; and 2100-10: rats subjected to a 10-week exposure to 2100 MHz RFR. Throughout the experiment, every animal was confined in a plexiglass cage equipped with ventilation apertures to enhance respiration and mitigate the elevation of body temperature. Experimental groups, 2100-1 and 2100-10, were exposed (for 5 day/week, 2 h/day) to 2100 MHz RFR radiation from the signal generator for 1 and 10 weeks, respectively. Sham group rats were maintained in an identical environment with an equal measure of duration without RFR.

2.2 RFR exposure

In this study, the experimental apparatus and RFR application were identical to those previously reported [5]. However, the RFR frequency, the distance of the rats from the antenna, and therefore, the specific absorption rate (SAR) values were different. In this system, a 2100 MHz radiofrequency generator (Universal Mobile Telecommunication System [UMTS] Simulator 2100 MHz; Everest Company, Adapazari, Türkiye) was used to simulate exposure to UMTS radiation. The electric-field strength measured above the rat's head in the "signal-on" condition was found to be 35.2 V/m. The average SARs for the whole body and brain were 128 mW/kg and 0.27 W/kg, respectively. The computational analysis was conducted utilizing the finite difference time domain method [12]. Published sources were utilized to obtain data on electrical properties, dielectric constant, and conductivity [13,14].

2.3 ABR recordings

On the day after the experiment, the auditory abilities of all animals were assessed using the ABR test. The rats were administered an intraperitoneal mixture of xylazine (10 mg/kg) and ketamine (50 mg/kg) for anesthesia.

Afterward, the subjects were positioned on a heating pad to maintain a body temperature of 37°C and kept in a

cabin that reduces sound and electromagnetic interference. The rats had a surgical procedure in which stainless steel needle electrodes (54455/A, Insert Earphone Dual, Medelec) were inserted at the vertex (–, inverting), the ipsilateral mastoid (+, non-inverting) of the test ear, and the tail (ground). The stimulus production and data gathering were performed using a Labat Audiological Platform (Italy, Scorze), which included a computer-based digital signal processing software (Labat Audiological Platform, Italy, Scorze). An active transducer, linked to a 10 cm plastic tube, was put into the outer ear canal of the ear being tested to transmit sound waves. Various strengths of tone bursts (Blackman envelope, 1 ms rise/fall time, 2 ms duration, alternating starting phase) were delivered at frequencies of 8 and 16 kHz. The ABR responses were filtered using a bandpass filter with a range of 100–3000 Hz and a notch filter at 50 Hz. They were then digitized at a sampling rate of 10 kHz and averaged using 700–800 samples at each frequency. To produce a distinct ABR waveform, the process of threshold testing commenced by presenting the stimulus at 70 dB SPL. The intensity was systematically lowered in 10 dB intervals until the ABR response was no longer detectable. The ABR threshold was established as the lowest intensity necessary to detect the fifth ABR wave. Two researchers have verified the established limits.

2.4 Biochemical investigations

The animals were euthanized the day after ABR recordings and brain tissues were harvested by surgical procedures without tissue damage. Then brain tissues were placed in appropriate solutions in order to get homogenates, which are needed for SOD, CAT, and GPx activities and 4-hydroxynonenal (4-HNE), thiobarbituric acid reactive substances (TBARS), and protein carbonyl content assays. All tissues were sonicated for one minute in a sonicator (Branson Sonifier 250, Germany) with temperature control. The samples that had been sonicated were then centrifuged in an Eppendorf microcentrifuge (Heraeus Sepatech, Biofuge 15R, Osterode, Germany) at $14000 \times g$ for 10 min at 4°C. The supernatants of centrifuged samples were used for biochemical investigations.

2.4.1 TBARS assay

Wasowicz et al. outlined a fluorimetric technique for quantifying TBARS concentrations by employing 1,1,3,3-tetraethoxypropane as a reference standard [15]. The TBARS concentrations were determined using this method. The findings were presented in units of $\mu\text{mol/gram}$ of protein.

2.4.2 4-Hydroxynonenal assay

The protein samples were analyzed for the presence of an HNE adduct using the 4-HNE Adduct ELISA Kit (Cat # STA-838; Cell Biolabs Inc., OxiSelect, CA, USA), following the instructions provided by the manufacturer. The concentration of HNE adduct was measured in $\mu\text{g/mg}$ of protein.

2.4.3 Quantification of protein carbonyl levels in tissues

Using a protein carbonyl assay kit (Catalog # 1005020; Cayman Chemical, Ann Arbor, MI, USA), protein-bound carbonyls were quantified with respect to the manufacturer's instructions. The results were computed utilizing an extinction coefficient of $22 \text{ mM}^{-1} \text{ cm}^{-1}$ for aliphatic hydrazones and were presented as nmol/mg protein.

2.4.4 Measurement of SOD activity

The brain tissue's SOD activity was quantified using a SOD test kit (Cat # 706002; Cayman Chemical), as per the guidelines provided by the manufacturer. The activity of SOD was quantified as kU/g of protein.

2.4.5 Measurement of CAT activity

The activity of brain CAT was quantified using a CAT test kit (Cat # 707002, Cayman Chemical), according to the manufacturer's instructions. At 25°C, a single unit of CAT was determined to be the quantity of enzyme that generates 1.0 nmol of formaldehyde within a minute. The CAT activity level was quantified in kU/g of protein.

2.4.6 Measurement of GPx activity

The activity of GPx was quantified using an assay kit (Cat # 703102, Cayman Chemical) based on the guidelines provided by the manufacturer. The quantification of GPx activity is determined by a linked reaction that includes the involvement of glutathione reductase. The activity of GPx was quantified in kU/g of protein.

2.4.7 Protein analysis

Brain tissue protein concentrations were quantified using spectrophotometry (RF-5500, Shimadzu, Kyoto, Japan),

employing a modified Bradford method [16] and a Pierce protein assay reagent kit (Rockford, IL). The standard utilized was bovine serum albumin.

2.5 Transmission electron microscopy (TEM) analysis

Subsequent to ABR recordings, the temporal cortices were excised from the rat brains of each group and analyzed using an electron microscope. Electron microscopic investigations were conducted using the methodology outlined in our prior study [5]. Neurons, astrocytes, and blood vessels from the temporal cortex of each group were randomly selected for ultrastructural evaluation via TEM.

2.6 Statistical analysis

The findings were presented as the mean $\pm$ standard error of the mean (SEM). The significance level was established as $p < 0.05$. One-way analysis of variance was utilized to analyze the group differences, and the post hoc Tukey test was employed to assess all pairwise multiple comparisons for ABR and biochemical data.

3 Results

3.1 ABR findings

3.1.1 2100 MHz RFR did not alter ABR thresholds

There were no significant variations in the ABR threshold values for auditory stimuli at 8 and 16 kHz between the acute and chronic groups.

3.1.2 2100 MHz RFR prolonged ABR latencies in the acute group

The ABR latencies obtained from tone bursts at 8 kHz and 60 dB SPL across all groups are presented in Table 1. In comparison to the Sh-1 group, the mean latencies of all ABR waves (waves I, II, III, IV, and V) were significantly longer in the 2100-1 group. In the chronic groups, there were no significant variations in the average latencies of these ABR waves. Furthermore, there were no notable variations in the interpeak I–III, III–V, and I–V latencies between the acute and chronic groups.

The ABR latencies obtained from tone bursts at 16 kHz and 60 dB SPL across all groups are presented in Table 2 and were comparable to our 8 kHz and 60 dB SPL results.

3.2 Biochemical findings

3.2.1 2100 MHz RFR increased lipid peroxidation and protein oxidation in the acute group

In our study, we discovered that the levels of brain TBARS, 4-HNE, and protein carbonyl content were noticeably increased in the 2100-1 group compared to the Sh-1 group. However, there was no meaningful difference between the Sh-10 and 2100-10 groups (Figure 1).

3.2.2 2100 MHz RFR reduced antioxidant levels (SOD and CAT) in the acute group

Compared to the Sh-1 group, brain SOD and CAT activities were discovered to be substantially lower in the 2100-1 group. Nevertheless, there was no discernible difference between the Sh-10 and 2100-10 groups (Figure 2).

Table 1: ABR component latencies of the sham and RFR groups in response to 8 kHz and 60 dB SPL auditory stimuli

Latency(ms)	Sh-1	2100-1	Sh-10	2100-10
Wave I	1.94 $\pm$ 0.01	2.14 $\pm$ 0.03*	2.07 $\pm$ 0.02	2.01 $\pm$ 0.03
Wave II	2.82 $\pm$ 0.03	2.94 $\pm$ 0.03*	2.87 $\pm$ 0.02	2.86 $\pm$ 0.03
Wave III	3.43 $\pm$ 0.03	3.60 $\pm$ 0.04*	3.54 $\pm$ 0.03	3.50 $\pm$ 0.03
Wave IV	4.37 $\pm$ 0.05	4.73 $\pm$ 0.06*	4.62 $\pm$ 0.04	4.56 $\pm$ 0.05
Wave V	5.50 $\pm$ 0.09	5.75 $\pm$ 0.08*	5.60 $\pm$ 0.06	5.59 $\pm$ 0.06
I–III interpeak latency	1.49 $\pm$ 0.02	1.47 $\pm$ 0.03	1.47 $\pm$ 0.03	1.49 $\pm$ 0.01
III–V interpeak latency	2.07 $\pm$ 0.06	2.15 $\pm$ 0.04	2.06 $\pm$ 0.05	2.09 $\pm$ 0.04
I–V interpeak latency	3.56 $\pm$ 0.08	3.61 $\pm$ 0.07	3.53 $\pm$ 0.06	3.58 $\pm$ 0.05

Data were presented as mean $\pm$ SEM. * $p < 0.05$ compared to Sh-1.

Table 2: ABR component latencies of the sham and RFR groups in response to 16 kHz and 60 dB SPL auditory stimuli

Latency(ms)	Sh-1	2100-1	Sh-10	2100-10
Wave I	2.02 ± 0.03	2.45 ± 0.06*	2.21 ± 0.06	2.06 ± 0.04
Wave II	2.95 ± 0.03	3.35 ± 0.05*	3.08 ± 0.08	2.98 ± 0.06
Wave III	3.50 ± 0.03	3.83 ± 0.06*	3.61 ± 0.04	3.51 ± 0.07
Wave IV	4.44 ± 0.06	4.84 ± 0.07*	4.66 ± 0.10	4.59 ± 0.05
Wave V	5.53 ± 0.05	5.87 ± 0.08*	5.77 ± 0.08	5.67 ± 0.07
I–III interpeak latency	1.48 ± 0.03	1.38 ± 0.04	1.40 ± 0.03	1.45 ± 0.09
III–V interpeak latency	2.04 ± 0.05	2.04 ± 0.09	2.16 ± 0.06	2.15 ± 0.11
I–V interpeak latency	3.52 ± 0.04	3.42 ± 0.09	3.56 ± 0.04	3.61 ± 0.07

Data were presented as mean ± SEM. * $p < 0.05$ compared to Sh-1.

3.2.3 2100 MHz RFR did not alter GPx activity

Our statistical analysis showed that the brain GPx activity of the Sh-1 and 2100-1 groups did not differ significantly. Similarly, there was no discernible difference in brain GPx activity between the Sh-10 and 2100-10 groups (Figure 2).

3.3 Transmission electron microscopic findings

TEM findings for group 2100-1: the temporal cortex neurons of group 2100-1 have similar eukaryotic nuclei and organelles as those of group Sh-1. In contrast, the

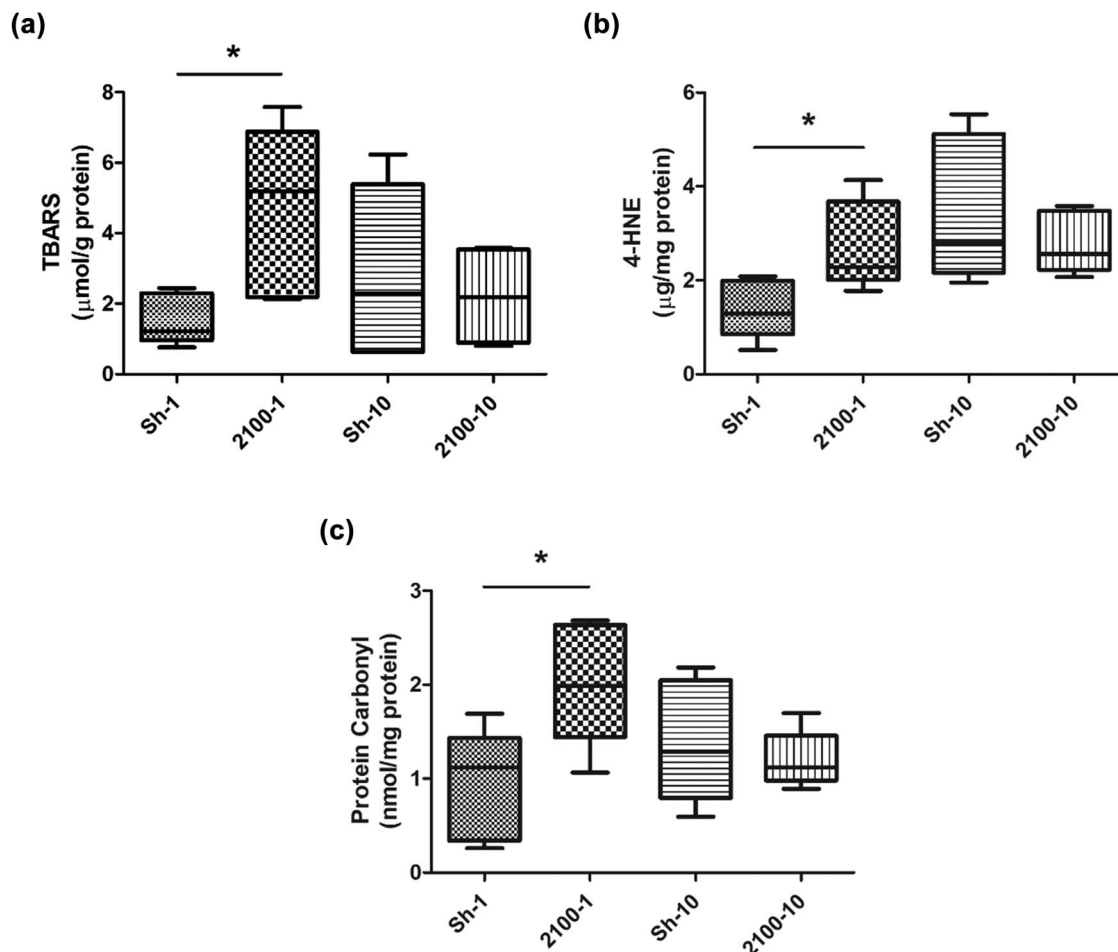


Figure 1: Lipid peroxidation and protein oxidation parameters of the sham and RFR groups. TBARS (a), 4-HNE (b), and protein carbonyl (c) content levels of experimental groups. Data were presented as mean ± SEM. * $p < 0.05$ according to the Sh-1 group.

neuropil exhibited an enlargement of the rough endoplasmic reticulum cisterns and a loss of electron density due to edema (Figure 3b). Besides, edematous loss of electron density and mitochondrial damage were identified in the cytoplasm of astrocytes (Figure 4b). The perivascular astrocytic end feet have also exhibited edematous characteristics (Figure 5b). On the contrary, intact myelin sheaths (Figure 3b) and functional endothelial cell plasma membrane structures (Figure 5b) were identified.

TEM findings for group 2100-10: in temporal cortex neurons, eukaryotic nucleus and cytoplasmic components were similar to those in group Sh-10 (Figure 3d). Besides, normal heterochromatin structure was observed in astrocytic nuclei (Figure 4d). Myelin sheaths (Figure 3d) and endothelial cell plasma membrane structures were normal, but perivascular astrocytic end-feet edema (Figure 5d) was observed.

4 Discussion

The enhanced functionalities of mobile phones have made people more reliant on them in their daily lives. As a result, debate over the possible negative consequences of RFR emitted by mobile phones on human health has increased [9,17,18]. Namely, an increase in oxidant parameters, a decrease in antioxidant parameters, and an increase in apoptosis in brain tissue were reported in a study in which 2600 MHz RFR was applied (for 30 days) to rats [9]. In another study, 1800 MHz RFR was applied to rabbits (for 30, 45, and 60 min), and prolongation of certain ABR components was detected [18]. Several studies have examined biochemical parameters [2,8,9] or electrophysiological parameters [18–20] to find out the effects of RFR on the brain. ABR, one of these electrophysiological parameters, is a reliable and delicate technique for evaluating the function and

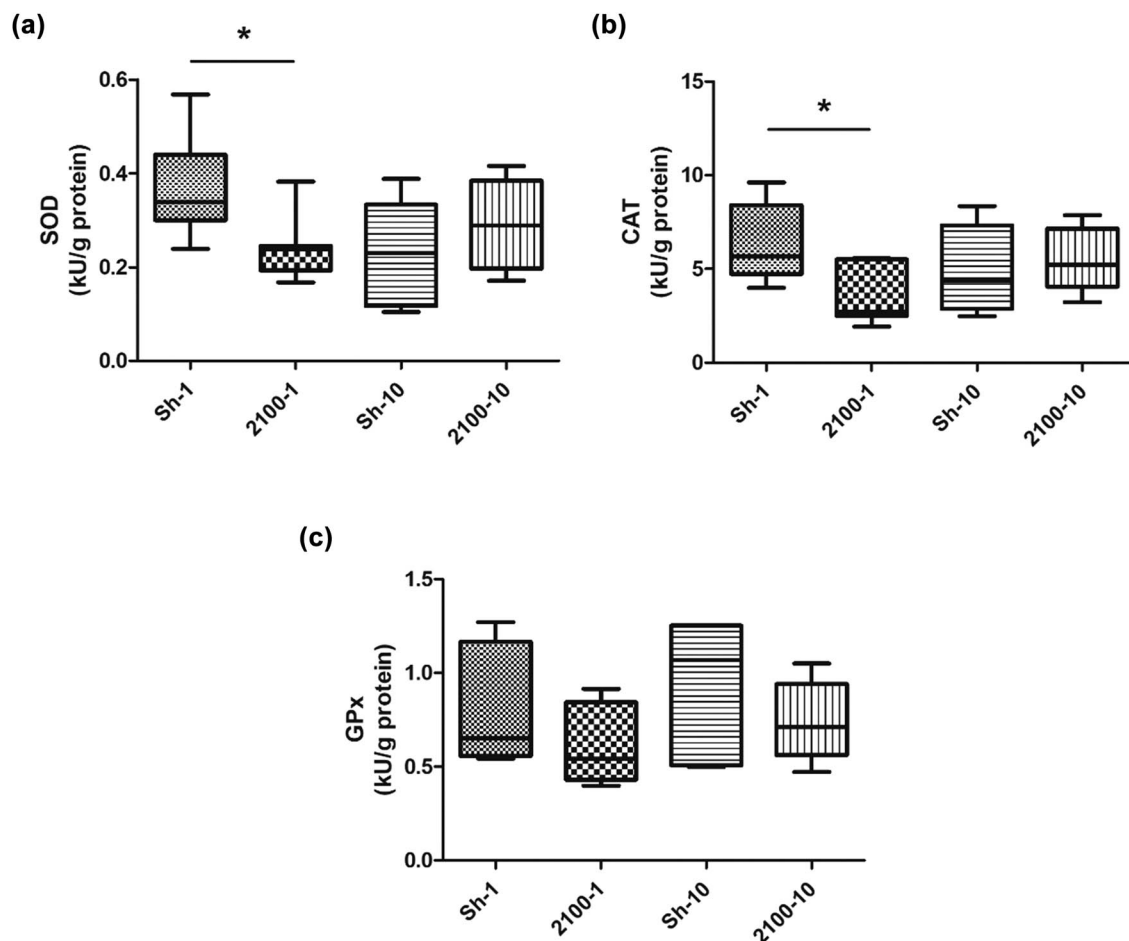


Figure 2: Antioxidant system parameters of the sham and RFR groups. SOD (a), CAT (b), and GPx (c) activity levels of the experimental groups. Data were presented as mean $\pm$ SEM. * $p < 0.05$ according to the Sh-1 group.

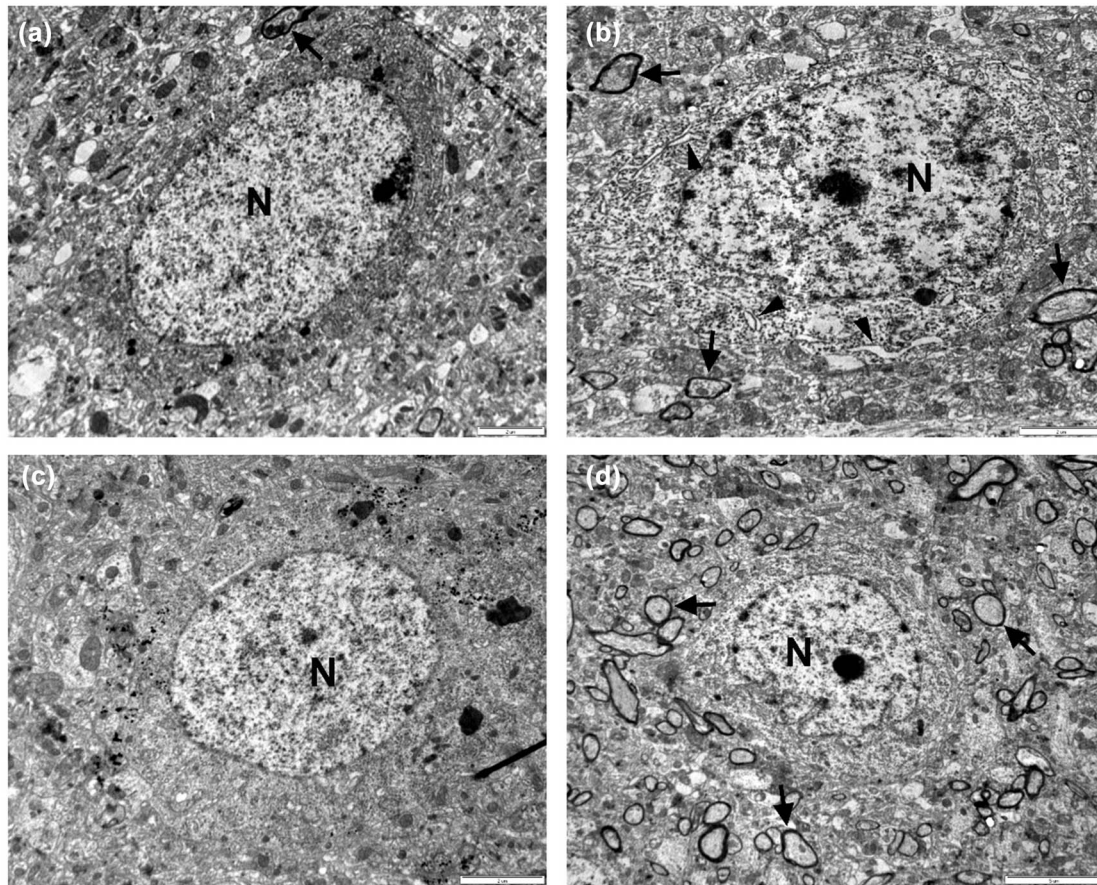


Figure 3: Electron microscopic images of temporal cortex neurons of the sham and RFR groups. (a) Sh-1 group; (b) 2100-1 group; (c) Sh-10 group; and (d) 2100-10 group. N: Neuron nucleus; arrow: Myelin sheath; scale bars: A–C: 2 μ m and D: 5 μ m.

pathology of the auditory system [21]. In this study, it was aimed to examine the effects of 2100 MHz RFR on ABR and the relationship between this effect and lipid peroxidation and protein oxidation.

When the ABR latencies were evaluated in our study, the latencies of all ABR waves (I–V) at 8 and 16 kHz at 60 dB SPL were notably prolonged in the 2100-1 group compared to the Sh-1 group. Furthermore, no significant variations were found in ABR latencies in our chronic groups. A study in which 1800 MHz RFR (SAR = 0.11 mW/kg) was applied to the ears of rabbits for durations of 1, 15, 30, 45, and 60 min revealed that waves I, II, III, IV, and V and interpeak I–III and I–V mean latencies were significantly prolonged in the 60-min group [18]. Additionally, our previous study demonstrated a notable increase in wave I latency to occur in the acute group of rats that were exposed to 900 MHz RFR for 5 days (2 h/day) [5]. In contrast to our previous study, in this study, we applied 2100 MHz RFR, and the prolongation of the latencies of all ABR waves suggests that 2100 MHz RFR may have affected the entire auditory pathway. In a long-term RFR study, rats were continuously exposed to 2100 MHz RFR for 30

days, and wave V and interpeak I–V latencies were measured; however, no significant diversities were determined [20].

We have measured lipid peroxidation (TBARS, 4-HNE) and protein oxidation (protein carbonyl content) to figure out the oxidative effects of 2100 MHz RFR in the rat brain. In this study, the levels of TBARS, 4-HNE, and protein carbonyl increased in the acute group; however, there was no significant distinction observed between the chronic groups. In a study, rats were exposed to 900 MHz RFR for 45 min in one group and 60 min in another, and brain TBARS levels increased in both RFR groups relative to their control groups [22]. In long-term RFR studies, there are contradictory results [2,23]. A study was conducted where rats were subjected to 2100 MHz RFR for a duration of 8 weeks (5 day/week, 6 h/day), resulting in a decrease in the level of MDA (a lipid peroxidation marker) in their brains [23]. In contrast, rats exposed to 1915 MHz RFR for 16 weeks (2 h/day) exhibited an increase in TBARS levels in rat hippocampus [8]. On the other hand, in the literature, there are limited studies related to RFR and protein carbonyl [1,24]. A study applying acute 900 MHz RFR demonstrated no significant difference in protein carbonyl levels in guinea pig liver [24].

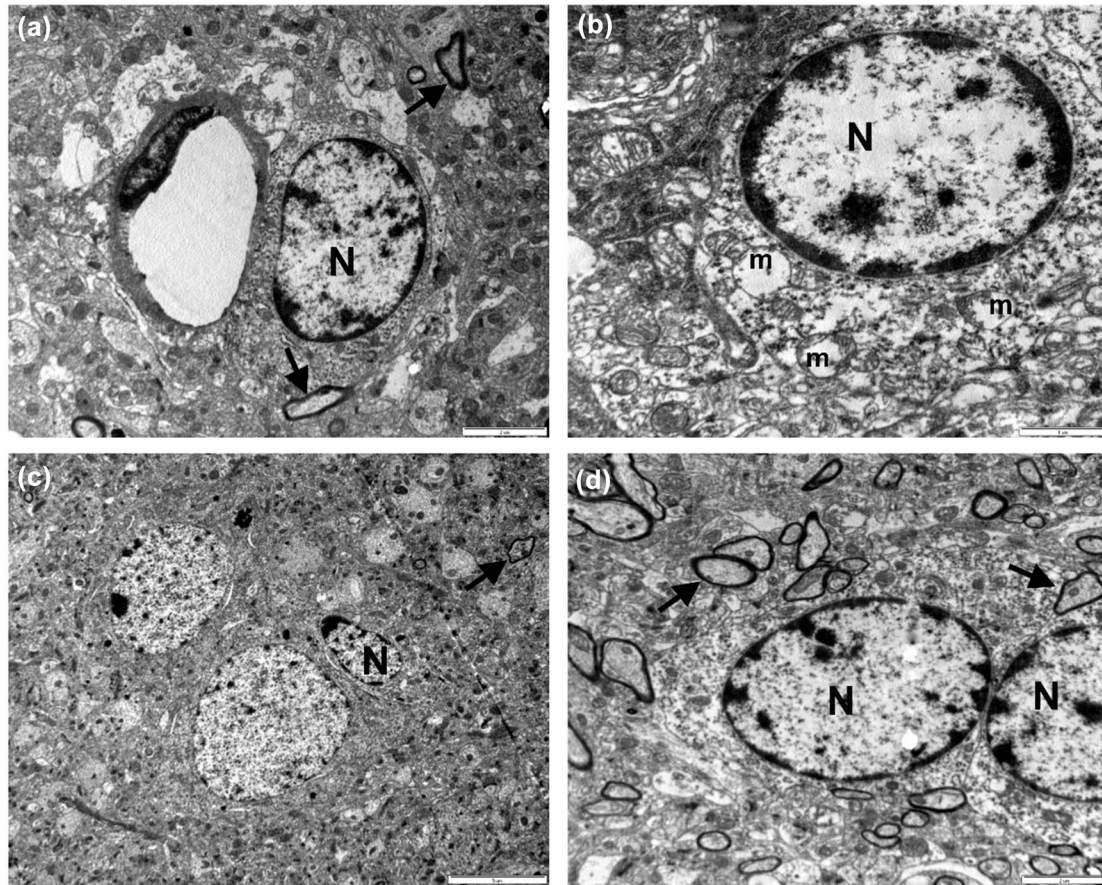


Figure 4: Electron microscopic images of temporal cortex astrocytes of the sham and RFR groups. (a) Sh-1 group; (b) 2100-1 group; (c) Sh-10 group; and (d) 2100-10 group. N: Astrocyte nucleus; arrow: Myelin sheath; m: Mitochondria; scale bars: A and D: 2 μm , B: 1 μm , and C: 5 μm .

Another study conducted on rat brains, utilizing RFR (915 and 2450 MHz) for 30 days, revealed an increase in protein carbonyl levels [1]. The current corpus of literature on this issue is restricted. Furthermore, the findings of some studies exhibit contradictions. Upon careful examination of the studies, it becomes apparent that the observed variations can potentially be attributed to factors such as the frequency of RFR employed, duration of exposure, or the specific tissues under investigation.

The antioxidant defense system, comprising SOD, CAT, and GPx, safeguards cells against the detrimental effects of oxidative stress [25]. In this study, SOD, GPx, and CAT enzymes were analyzed to determine the impacts of 2100 MHz RFR on the antioxidant system. In our research, SOD and CAT enzyme activities were significantly reduced in the acute RFR group; nevertheless, there were no notable differences observed among the chronic groups. Nevertheless, no significant distinction in GPx activity was observed between the acute and chronic groups. It has been reported in the literature that SOD and/or CAT activities decreased in some studies [5,24], increased in one [26], and remained unaffected in another

study [27] that employed 900 MHz RFR. A study revealed no substantial alteration in GPx activity after acute exposure to 835 and 847 MHz RFR [28], whereas another acute study reported an increase with 900 MHz RFR exposure [29]. Our findings regarding acute group SOD, CAT, and GPx activities appear to be consistent with the literature. Long-term studies exploring RFR and SOD, CAT, and GPx activities also produce contradictory findings [1,30,31]. In the literature, it was reported that SOD and/or GPx and/or CAT activities decreased in some studies using RFR [1,17,32] and increased in others [5,30], and no significant difference was observed in another [31]. Our findings regarding SOD and GPx activities in our chronic groups appear consistent with the literature. Besides the findings of our CAT enzyme activity in the chronic groups are inconsistent with the current literature. Upon examination of the study by Imge *et al.* [31], it is evident that their RFR frequency, SAR value, and exposure duration differ from those utilized in our research. In the antioxidant defense system, SOD initially neutralizes 2O_2^- and transforms it into two molecules of H_2O_2 . Subsequently, CAT degrades them, transforming them into H_2O and O_2 .

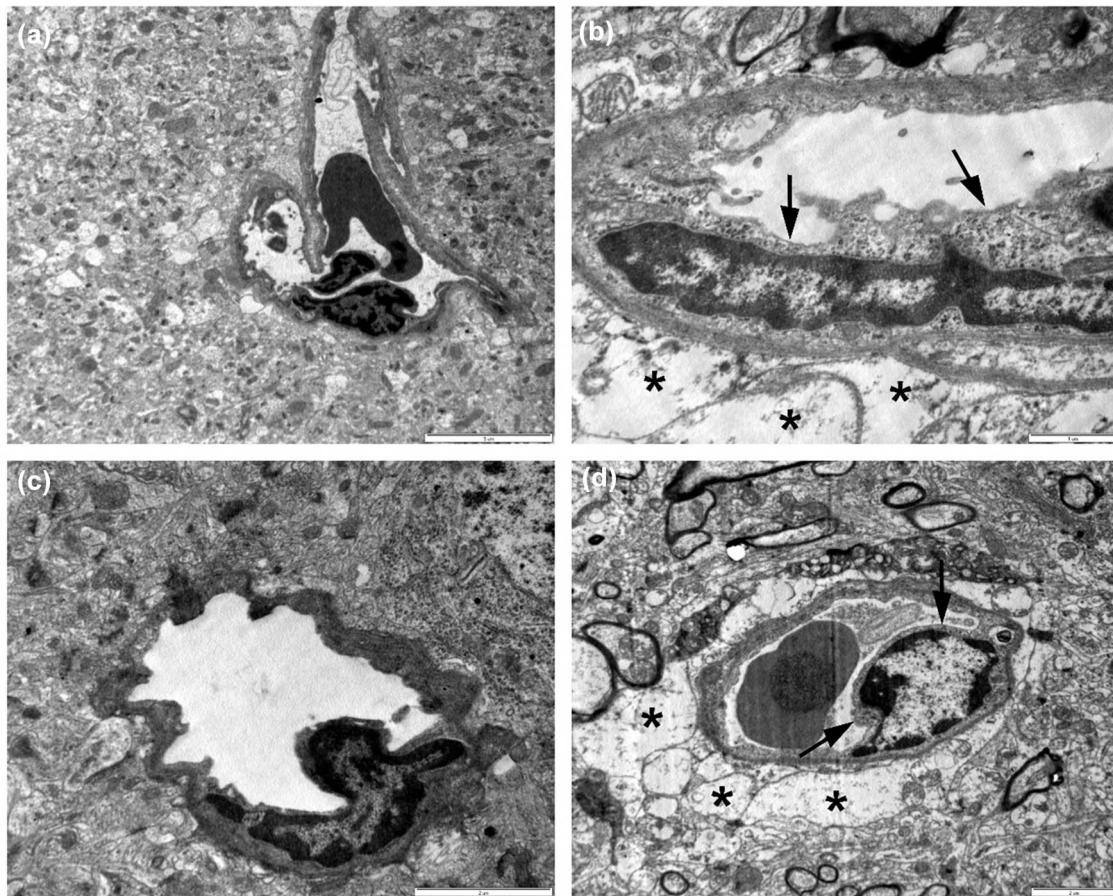


Figure 5: Electron microscopic images of the temporal cortex vessel circumferences of the sham and RFR groups. (a) Sh-1 group; (b) 2100-1 group; (c) Sh-10 group; and (d) 2100-10 group. Arrow: Endothelial cell plasma membrane; *: Edema; scale bars: (a) 5 μm , (b) 1 μm , and (c and d) 2 μm .

Additionally, GPx facilitates the removal of H_2O_2 by modulating the redox state of glutathione [33]. In our study, the lack of substantial variation in GPx activity levels between acute and chronic groups may be attributed to the neutralization of O_2 generated by the RFR predominantly by SOD and CAT. In addition, the lack of significant change in oxidant and antioxidant parameters in our chronic groups may be attributed to the antioxidant defense system effectively balancing the oxidant system, given that RFR was not administered over the weekend, thus eliminating any adverse effects.

In our study, the electron microscopic analysis revealed no significant differences in the acute and chronic RFR groups in terms of neuron nuclei, neuron cytoplasmic components, and myelin sheaths. In acute and chronic RFR groups, edema was observed in astrocytic end feet. Edema was observed also in acute RFR group neurons and astrocytes. Besides, in contrast to chronic groups, mitochondrial damage was observed in acute RFR group astrocytes. A study involving mice exposed to 835 MHz (SAR = 4 W/kg) RFR for 12 weeks (5 h/day) revealed an increase in autophagic structures within hippocampal

neurons, while no significant changes were observed in brain stem neurons [34]. Myelin degeneration was also observed in rats exposed to 1800 MHz (2 h/day, SAR = 1.79 W/kg) RFR during prenatal and postnatal periods in a separate study [35]. In addition, according to a study where rats were exposed to 900 and 1800 MHz RFR for 20 min, blood–brain barrier permeability increased in the RFR groups [36]. Our findings related to neuron nuclei and cytoplasmic components appear to be consistent with the findings of Kim et al. regarding brainstem neurons [34]. Our result regarding the myelin sheath contradicts that of Akakin et al. [35]. This difference may be due to the age of the experimental animals, the applied frequency, or the SAR value. The increased permeability of the blood–brain barrier identified by Sirav and Seyhan [36] may align with our findings on edema. Regarding our finding of mitochondrial damage in the acute RFR group, it is known that mitochondria in eukaryotic cells facilitate aerobic respiration, utilizing oxygen to generate ATP. This process continuously generates ROS along the electron transport chain. Different stress conditions, such as RFR in this

study, can significantly increase mitochondrial ROS production. Excessive production of ROS can lead to oxidative stress and impair mitochondrial function [37,38]. In our study, the correlation between mitochondrial damage in acute RFR group astrocytes and the increase in oxidant parameters, decrease in antioxidant parameters, and elongation in latencies in acute groups is plausible.

In this study, both acute and chronic groups were examined, with RFR exposure administered continuously for 2 h daily. In reality, individuals exhibit varying patterns of mobile phone usage, with some engaging in continuous use for extended periods and others opting for intermittent usage, such as 10 min of use followed by 30 min of inactivity. Appropriate experimental models must be designed and investigated; however, we can speculate as follows: The antioxidant defense system may reduce oxidant damage that remains relatively stable during periods of mobile phone inactivity, suggesting that the negative effects identified in our study could be reduced.

As a result, in our study, although the acute group of rats treated with 2100 MHz RFR exhibited prolonged ABR latencies, no substantial difference was detected in the chronic group. It is possible that the increase in 4-HNE, TBARS, and protein carbonyl levels and the decrease in SOD and CAT activities and mitochondrial damage in the acute group are related to the prolongation of latencies in the acute RFR group. In our study, we found that 2100 MHz RFR had a negative effect on ABR latencies in the acute group but had no effect on the chronic group. Our study's RFR exposure schedule may account for the lack of difference in ABR latency in the chronic group. The chronic group received RFR for 2 h/day, 5 day/week, for 10 weeks, excluding weekends. Therefore, the antioxidant defense system may have mitigated the adverse effects of RFR, which was administered over the week, during the weekends. In conclusion, our study revealed that acute exposure to 2100 MHz RFR could potentially have adverse effects on the auditory system; however, intermittent long-term use may not cause any issues.

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Author contributions: H.E.: conceptualization, methodology, data curation, validation, formal analysis, visualization,

writing – original draft, and reviewing. G.B.: methodology, data curation, and formal analysis. N.D.: conceptualization, methodology, and supervision. D.K.: methodology and data curation. S.O.: conceptualization and methodology.

Conflict of interest: The authors state no conflict of interest.

Ethical approval: This study obtained ethical approval for the experimental animal protocols from the Akdeniz University Local Ethics Committee for Animal Experiments, with the assigned protocol number 1574/2023.04.001.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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