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Research Article

Effects of Different Duration Time of Exposure to 2100 MHz Electromagnetic Radiation on Behaviour and Hippocampal Levels of Protein Kinases on Rats

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Summary

Objectives: The aim of the present study was to investigate the effects of acute and chronic 2100 MHz radiofrequency-electromagnetic radiation (RF-EMR) exposure on passive avoidance behaviour and hippocampal levels of protein kinases including CaMKIIα, PKA, and p44/42 MAPK from NMDA dependent pathway in Wistar rats.
Materials and Methods: Rats were divided into the following groups: Sham-exposed rats and rats exposed to 2100 MHz RF-EMR for 2 h/day for 1 and 10 weeks, respectively. Passive avoidance task was used as a behavioural method. The hippocampal levels of selected kinases were measured using Western Blotting technique.
Results: Findings demonstrated that both acute and chronic exposure to 2100 MHz RF-EMR can impair the memory retention with less effect on chronic group of rats. In addition, hippocampal levels of selected protein kinases were higher in chronic groups than acute groups.
Conclusion: Different duration times of 2100 MHz RF-EMR exposure has different effects on both passive avoidance behaviour of rats and hippocampal levels of selected protein kinases.

Key words: RF-EMR, hippocampus, behaviour, protein kinases, rat

Sıçanlarda 2100 MHz Elektromanyetik Radyasyona Farklı Maruziyet Sürelerinin Davranış ve Protein Kinazların Hipokampal Seviyeleri Üzerine Etkileri

Özet

Amaç: Sunulan çalışmanın amacı, 2100 MHz radyofrekans-elektromanyetik radyasyona (RF-EMR) akut ve kronik maruziyetin pasif sakınma davranışına ve NMDA bağımlı yolaktaki protein kinazlardan CaMKIIα, PKA, ve p44/42 MAPK'nın hipokampal seviyeleri üzerine etkilerini Wistar sıçanlarında araştırmaktır.
Materyal ve Metod: Sıçanlar sham maruziyet grubu, 2100 MHz RF-EMR'a 1 ve 10 hafta günde 2 saat maruz bırakılan sıçanlar olarak gruplara ayrılmıştır. Pasif sakınma testi davranışsal metod olarak kullanılmıştır. Seçilen kinazların hipokampal seviyeleri Western Blot tekniği ile ölçülmüştür.
Bulgular: Bulgular, 2100 MHz RF-EMR'a hem akut hem de kronik maruziyetin hafızanın sağlamlaştırılmasında bozukluğa neden olabileceğini ve bu etkinin de kronik sıçan grubunda daha az olduğunu göstermiştir. Buna ilaveten, seçilen protein kinazların hipokampal seviyeleri akut gruba kıyasla kronik grupta daha yüksektir.
Sonuç: 2100 MHz RF-EMR'a farklı maruziyet sürelerinin hem sıçanların pasif sakınma davranışına hem de seçilen protein kinazların hipokampal seviyelerine farklı etkileri vardır.

Anahtar Kelimeler: RF-EMR, hipokampus, davranış, protein kinaz, sıçan

INTRODUCTION

The tremendous increase in the use of mobile communications has raised interest about the potential effects of electromagnetic radiation (EMR) exposure on the human organs, especially the brain (1). One of the affected brain regions is the hippocampus, which plays a major role in learning and memory functions. Experimental animal studies have shown that radiofrequency electromagnetic radiation (RF-EMR) exposure can affect cognitive functions and the behaviour of animals (1-3). For example, the potentially devastating effects of 60 min 8 mT, 50 Hz electromagnetic field exposure on learning and information acquisition in passive avoidance learning task in both male and female mice have been demonstrated by Foroozandeh et al. (3). It has been reported in another experimental study that mobile phone (900/1800 MHz) RF-EMR exposure leads to impaired spatial memory performance in the Morris water maze task in Wistar rats (2). In another study of the same research group, they confirmed that mobile phone (0.9 GHz/1.8 GHz) RF-EMR exposure significantly altered the passive avoidance behaviour and hippocampal morphology in rats.

The most prominent neurotransmitter associated with learning and memory functions in the hippocampus is glutamate, which exerts its signalling function by binding to hippocampal glutamate receptors, one of which is called as N-methyl-D-aspartate (NMDA) receptor (4). The major intracellular signalling pathway implemented in both short- and long-term memory formations in the hippocampus is the NMDA-dependent pathway, which includes the activation of protein kinases, notably Ca2+/calmodulin-dependent protein kinase II (CaMKII), protein kinase A (PKA), and mitogen-activated protein kinase (MAPK) (5).

There are several behavioural studies on rats mainly focused on differential effects of different waveforms and different duration time of exposure to the cognitive functions, especially learning and memory (6-8). However, little is known about the effects of different duration times of RF-EMR exposure on both passive avoidance behaviour of rats and hippocampal levels of kinases from NMDA receptor related signalling pathways. Therefore, in the present study, we tried to evaluate the effects of both acute (1 week) and chronic (10 weeks) 2100 MHz RF-EMR exposure on passive avoidance behaviour of rats and hippocampal levels of selected kinases including CaMKIIα, PKA, and p44/42 MAPK. To indirectly measure the activity of protein kinases, their phosphorylated forms were also assessed since protein phosphorylation has a key role in triggering the synaptic changes underlying learning and memory (9).

MATERIAL AND METHODS

Animals

Inbred young male albino Wistar rats (n=60) aged 3 months, weighing between 200 to 250 g were kept in home cages in groups of four under a constant temperature (23±1°C) and a 12/12 h light/dark cycle. They were given food and water ad libitum. The animal care procedures and all experimental manipulations were pursued in accordance with the Institutional Animal Care and Use Committee in Akdeniz University, Antalya, Turkey (Number of the Ethical Approval: 2014.02.06).

Study design

After five days of handling process, all 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 2100 MHz RF-EMR for 1 week (2100-1); Group 4: rats exposed to 2100 MHz RF-EMR for 10 weeks (2100-10). Then, all rats in each group including sham-exposed rats were placed in a

plexiglass tube with air holes to facilitate breathing and minimize the rise in body temperature. "2100-1" and "2100-10" group of rats were exposed to 2100 MHz RF-EMR emitted from the signal generator for 2 h per day for 1 and 10 weeks, respectively. Sham-exposed rats were housed in a same room under the same conditions with equal time period, except that the generator was turned off. During data collection and analysis, experimenters were blind to rat experimental group membership.

Electromagnetic field exposure

The exposure system was presented in Figure 1. A RF generator (UMTS Simulator 2100 MHz; Everest Company, Adapazari, Turkey), which produces 2100 MHz RF radiation, was used to represent exposure of Universal Mobile Telecommunications System (UMTS). Peak power of the generator was fixed at 1.5 W during exposure. The carrier frequency was 2100 MHz, the modulation frequency was 217 Hz, pulse width was 577 ms, and the maximal peak power was 0-2W. The system was placed on a wooden table and the monopole antenna of the generator was placed at the center of plexiglass carousels to provide equal exposure to the rats aligned around the antenna. The distance of the rats to the monopole antenna was 10 cm, and the generator terminal output was 1.5 W. The electric field strengths were measured by EMR300 m with appropriate probe (Narda, Germany). The electric field background level in the shielded room was between 0.02-0.2 V/m. The average whole-body SAR was 128 mW/kg. The SAR for the brain was in average of 0.27 W/kg. The numerical computation was performed using Finitte Difference Time Domain (FDTD) Method (10). Electrical properties, conductivity and dielectric constant were taken from the literature (11,12).

Passive avoidance task

After the exposure process, all rats were subjected to passive avoidance task by the method of Bures et al. (13). with modifications. The apparatus has two compartments, a rectangular light compartment and a dark compartment with a grid floor. The connection between the two compartments can be closed with a sliding door made of plexiglass. In the acquisition trial, each rat was placed individually in the light compartment and the time taken to enter the dark compartment was measured. As soon as the rat entered the dark compartment, the sliding door was closed and an electrofootshock (1 mA for 5 s) was delivered through the grid floor. The rat was then returned to its own cage waiting for the retention trial. The retention trial was carried out after 24 h. The rat was placed in the light compartment. The sliding door was kept open during this period. The latency time required for rat to enter the dark compartment was recorded. Absence of entry into the dark compartment within 300 s indicated positive memory retention.

Tissue sample preparation

After passive avoidance task, rats were anesthetized under urethane and brains were perfused transcardially with heparinized saline. Then, the perfused brains were rapidly removed, hippocampi was dissected and homogenized by ultrasonication in the presence of protease inhibitors. Protein concentrations were measured at 595 nm by a modified Bradford assay (14) using Coomassie Plus reagent with bovine serum albumin as a standard (Pierce Chemical).

Western blot protein analysis

For determination of CaMKIIα, pCaMKIIα, PKA, pPKA, p44/42 MAPK, phospho p44/42 MAPK expressions, 7.5 µg (for pCAMKIIα, pPKA) and 5 µg (for CAMKIIα, PKA, p44/42 MAPK, phospho p44/42 MAPK) of hippocampal samples were run on 6-7.5% polyacrylamide gels. Primary antibodies were applied at a dilution of 1:1000 (CaMKIIα, SantaCruz; pPKA, BD Bioscience; p44/42 MAPK,

Cell Signalling), 1:2000 (phospho p44/42 MAPK, Cell Signalling), 1:5000 (PKA, BD Bioscience) and 1:250 (pCAMKIIα, SantaCruz) while secondary alkaline phosphatase conjugated antibody (SantaCruz) used at a dilution of 1:1000 (pCaMKIIα), 1:2000 (pPKA, p44/42 MAPK) and 1:5000 (CaMKIIα, PKA, phospho p44/42 MAPK) in 5% dried non-fat milk in TBS-T buffer. A monoclonal antibody directed against B-actin and α-Tubulin was used as a control to normalize protein expression levels. The final images were photographed using a computer-based gel imaging instrument (VilberLourmat) with Infinity-Capt version 12.9 software. Immunoreactive protein bands were then quantified by densitometric scanning method using an Image J software package.

Statistical analysis

To evaluate the data, One-way ANOVA with all pairwise multiple comparison procedure done by Fisher's LSD (least significant difference) post hoc test was applied. A p value less than or equal to 0.05 was considered statistically significant. The statistical package SPSS v.20 was used for statistical analyses.

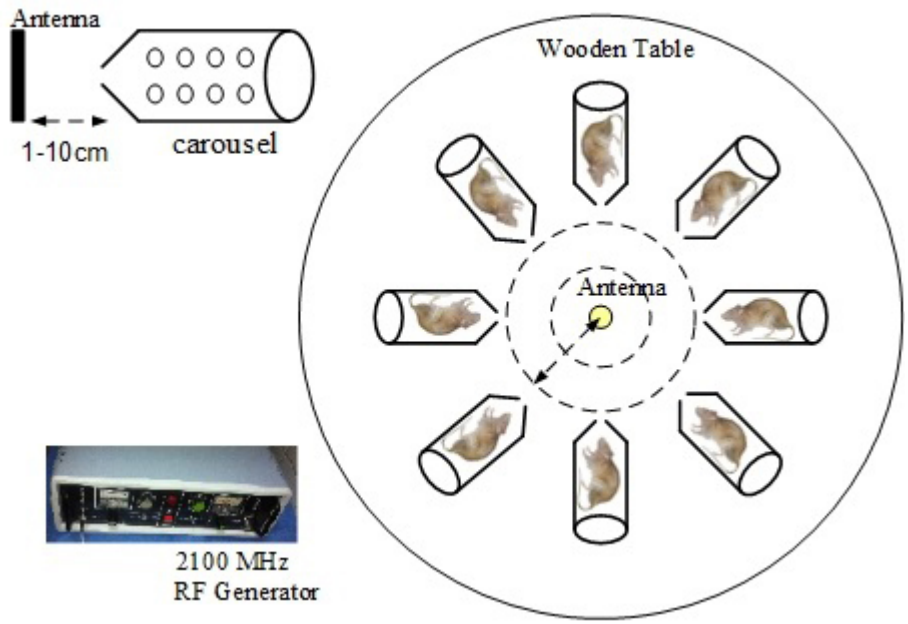

Figure 1: *Electromagnetic field exposure system (217 Hz pulse modulated-2100 MHz).*

RESULTS

Passive avoidance task

The latency time required for rat to enter the dark compartment during the retention trial was demonstrated by Figure 2. During the retention trial, the entrance latency to the dark compartment was significantly less for RF-EMR exposed rats as compared to sham-exposed rats (p=0.001). The mean value of entrance latency of chronic group of rats is higher than the mean value of entrance latency of acute group of rats (105.73 and 59.93, respectively).

Western blot protein expression assays

In the Western blot analysis of CaMKIIα and pCaMKIIα, proteins were detected as bands of 50 kDa, PKA and pPKA were detected as bands of 53 kDa, and p44/42 MAPK and phopsho p44/42 MAPK were detected as bands of 42,44 kDa (Figure 3A). Relative protein expressions were determined by comparing band intensities with that of B-Actin and α-Tubulin which were detected at the position corresponding to a molecular weight of 42 kDa and 55 kDa, and were used as the internal control (Figure 3A).

The differences in the levels of both CaMKIIα and pCaMKIIα among rat groups were illustrated by Figure 3B. There was a significantly higher expression of both hippocampal CaMKIIα and pCaMKIIα in "2100-1" and "2100-10" groups as compared to "S-1" and "S-10" groups (p=0.001). Hippocampal levels of both CaMKIIα and pCaMKIIα were approximately one and a half time high in "2100-10" groups as compared to "2100-1" groups. In addition, mean numbers of pCaMKIIα level (2.34±0.04 and 3.28±0.01, respectively) were significantly ($p \leq 0.001$) higher than mean numbers of CaMKIIα level (2.21±0.02 and 2.86±0.03, respectively) in "2100-1" and "2100-10" rat groups (Figure 3B).

Western blot data of PKA and pPKA was revealed that hippocampal levels of both kinases were significantly higher in "2100-1" and "2100-10" groups as compared to "S-1" and "S-10" groups ($p \leq 0.001$ respectively) (Figure 3C). Hippocampal levels of both PKA and pPKA were approximately one and a half time high in "2100-10" groups as compared to "2100-1" groups. In "2100-1" and "2100-10" rat groups, mean numbers of pPKA level (2.65±0.06 and 3.83±0.06, respectively) were significantly ($p \leq 0.001$) higher than mean numbers of PKA level (2.18±0.09 and 3.34 ± 0.04, respectively) (Figure 3C).

As shown in Figure 3D, quantitative immunoblot analysis of p44/42 MAPK and phospho p44/42 MAPK levels in the hippocampus of rats revealed significantly higher expression of both p44/42 MAPK and phospho p44/42 MAPK in "2100-1" and "2100-10" groups as compared to "S-1" and "S-10" groups ($p \leq 0.001$, respectively). Hippocampal levels of both p44/42 MAPK and phospho p44/42 MAPK were approximately one and a half time high in "2100-10" groups as compared to "2100-1" groups. In "2100-1" and "2100-10" rat groups, mean numbers of phospho p44/42 MAPK level (2.36±0.05 and 3.35±0.04, respectively) were significantly ($p \leq 0.001$) higher than mean numbers of p44/42 MAPK level (2.19±0.1 and 3.11±0.05, respectively) (Figure 3D).

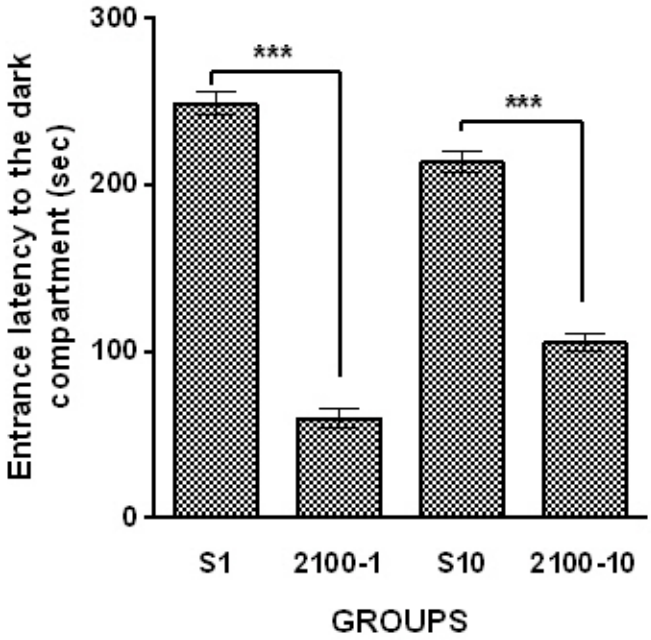

Figure 2 *Passive avoidance responses in retention trial as an entrance latency time (s) in all groups.*

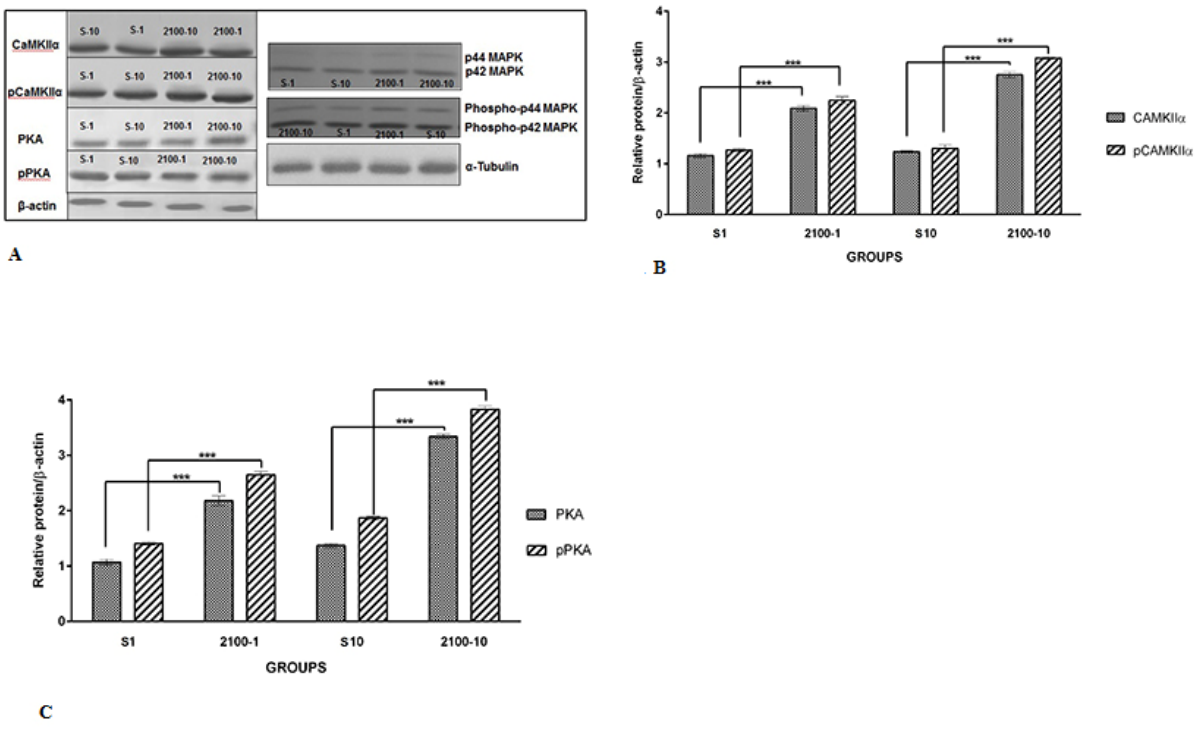

Figure 3 *A: Representative immunoreactive protein bands for CaMKIIα, pCaMKIIα, PKA, pPKA, p44/42 MAPK and Phospho p44/42 MAPK from hippocampus of "S-1", "S-10", "2100-1" and "2100-10" rat groups. The B-actin and α-Tubulin bands were used as an internal control for each gel. Comparison of the levels of B: CaMKIIα and pCaMKIIα; C: PKA and pPKA; and D: p44/42 MAPK and Phospho p44/42 MAPK among rat groups. Band quantifications are expressed as the mean ± SEM of the relative intensity with respect to that of B-actin and α-Tubulin. Error bars denote ± SEM. Asterisks denote the level of significance: ***p<0.001.*

DISCUSSION

The passive avoidance task is a fear-aggravated test used to evaluate learning and memory in rat models. In the present study, RF-EMR exposure significantly affected the memory retention of rats in the passive avoidance test. In comparison to sham-exposed rats, RF-EMR exposed rats showed shorter latency to enter into the dark compartment in the retention trial (24 h after the aversive stimulus). In this trial, acute groups showed shorter entrance latency than chronic groups. This demonstrated that they did not remember the passive avoidance task to some extent on the following day which is an indicator of the impairment of the memory. These findings are in agreement with the results showing the impairing effects of short term RF-EMR exposure on cognitive functions by using different behavioural tasks (3,15-17). For example, Lai et al. showed that 60 min exposure to a 1 mT, 60 Hz extremely low-frequency (ELF) magnetic fields before training, impaired spatial memory in a water maze (16). It was demonstrated that 45 min exposure to a 0.45 mT ELF fields shortly before daily testing sessions in radial maze reduced the acquisition rate of spatial learning (17). Impairment on the consolidation of spatial memory in a water maze after 20 min exposure to an 8 mT, 50 Hz magnetic fields has been provided by Jadidi et al. (15). In addition, the devastating effects of 60 min 8 mT electromagnetic fields exposure on learning and information acquisition in passive avoidance learning task in both male and female mice have been demonstrated by Foroozandeh et al. (3).

In the present study, in comparison to the acute groups, it can be stated that the memory retention was significantly less affected in chronic group of rats. This is consistent with the study suggesting that chronic exposure to magnetic fields possesses positive effects on cognitive function (18). In Liu et al. study, rats were exposed daily for either 1 or 4h to a 50 Hz magnetic field of 2 mT for a period of 4 weeks and findings indicated that chronic exposure to ELF magnetic field exerts a positive effect on spatial learning and memory in the Morris water maze (18). It has been suggested that electromagnetic radiation exposure can alter the Ca2+ homeostasis and related signalling processes (19,20). It is possible that chronic exposure induced the elevation in postsynaptic Ca2+ and thus triggers the induction as well as long-term maintenance of long-term potentiation (LTP), which is one of the cellular foundations of learning and memory (9). The changes in the Ca2+ concentration after being exposed to RF-EMR in the rat hippocampus lead to the increase in the cyclic adenosine monophosphate (cAMP) concentration (21), in addition to the activation of cAMP responsive element binding protein (CREB) which is activated by phosphorylation from various kinases including PKA, CaMKIIα, and p44/42 MAPK (22,23). It has been also indicated that long-term, but not short-term spatial memory is facilitated by the increased level of CREB in the hippocampus (24). Therefore, the improvement in the passive avoidance behaviour of the chronic group of rats in the present study might be partially explained by the result of the modulatory effect of the magnetic field on Ca2+ signalling pathway and components of LTP in the hippocampal neurons.

The comparison of hippocampal levels of PKA, CaMKIIα, and p44/42 MAPK between acute and chronic groups of 2100 MHz exposed rats revealed that hippocampal levels of these kinases were higher in chronic groups than acute groups. Similarly, phosphorylated forms of these kinases were significantly higher in chronic groups. These results are consistent with the passive avoidance behaviour of the chronic group of rats. These findings are in agreement with the results showing increased levels of neuronal enzymes after long term exposure to RF-EMR. For example, in Manikonda et

al. study, long term exposure (90 days) to 50Hz ELF fields caused increased intracellular Ca2+ levels together with increased activities of PKA and calcineurin in hippocampal regions as compared to control group of rats (20). Jadidi et al. confirmed that ELF electromagnetic fields can alter calcium ion homeostasis in neuronal tissue(15). Another research data showed that expression of CaMKII gamma was significantly up-regulated both in hippocampal tissues and nerve growth factor (NGF)-differentiated PC12 cells, which can be activated by the increased Ca2+ influx signal through postsynaptic NMDARs that arise from following 30 mW/cm² microwave exposure (25). In that study, activation of transcription factors including phospho CREB (p-CREB) by activation of CaMKII triggered by glutamate-mediated Ca2+ entry was also observed. Therefore, the increased level of the PKA, and CaMKIIα, and their phosphorylated forms observed in the present study might be related with the increased intracellular Ca2+ and calcineurin levels (20,25).

It has been known that electromagnetic field exposure may have adverse effects on brain tissues by increasing free radicals, causing oxidative stress which may affect cognitive processes and result in behavioural deficits (26). For example, in Cui et al. study, it was found that ELF magnetic fields exposure (1 mT, 50 Hz) induced serious oxidative stress in the hippocampus and impaired hippocampal dependent spatial learning (27). In another study, the levels of brain thiobarbituric acid reactive substances (TBARS) and 4-hydroxy-2-nonenal (4-HNE) which are known as oxidative stress biomarkers were found to be significantly increased in 10-week group that was exposed to 2100 MHz electromagnetic fields (28). Kesari et al. reported that 3G mobile phone exposure (2 h a day for 60 days) on rats induced oxidative stress, causing a transient increase in the level of p38MAPK (29). It has been known that major signal transduction pathway activated by oxidative stress is mitogen-activated protein kinases (MAPK) (30). In light of these findings, the increased level of p44/42 MAPK and its phosphorylated form observed in the present study might be explained by oxidative stress induction via mobile phone exposure.

CONCLUSION

To summarize, findings indicate that both acute and chronic exposure to a 2100 MHz RF-EMR can impair the memory retention with less effect on chronic group of rats. In addition, findings demonstrate that both acute and chronic exposure to a 2100 MHz RF-EMR have different effects on hippocampal levels of selected protein kinases. To our knowledge, this is the first study investigating effects of different duration times of 2100 MHz RF-EMR exposure to both on passive avoidance behaviour and on hippocampal levels of selected protein kinases from NMDA dependent pathway.

Conflict of Interests: The authors declare that they do not have any conflict of interests.

Author Contribution: ÇGS designed the study, HE performed RF-EMR exposure, ÇGS performed behavioural test, tissue sample preparation, protein measurements, Western Blotting, ÇGS collected and analysed all the data, ÇGS wrote the manuscript.

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