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Electromagnetic Biology and Medicine

ISSN: 1536-8378 (Print) 1536-8386 (Online) Journal homepage: www.tandfonline.com/journals/iebm20

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To cite this article: Suleyman Dasdag, Mehmet Zulkuf Akdag, Mehmet Bashan, Veysi Kizmaz, Nurten Erdal, Mehmet Emin Erdal, Mehmet Tughan Kiziltug & Korkut Yegin (2022) Role of 2.4 GHz radiofrequency radiation emitted from Wi-Fi on some miRNA and faty acids composition in brain, Electromagnetic Biology and Medicine, 41:3, 281-292, DOI: 10.1080/15368378.2022.2065682

To link to this article: https://doi.org/10.1080/15368378.2022.2065682

Published online: 17 Apr 2022.

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ELECTROMAGNETIC BIOLOGY AND MEDICINE
2022, VOL. 41, NO. 3, 281–292
https://doi.org/10.1080/15368378.2022.2065682

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ORIGINAL ARTICLE

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Role of 2.4 GHz radiofrequency radiation emitted from Wi-Fi on some miRNA and fatty acids composition in brain

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ABSTRACT

The purpose of this study is to investigate the effects of 2.4 GHz Wi-Fi exposure, which is continuously used in the internet connection by mobile phones, computers and other wireless equipment, on microRNA and membrane and depot fatty acid composition of brain cells. Sixteen Wistar Albino rats were divided equally into two groups such as sham and exposure. The rats in the experimental group (n = 8) were exposed to 2.4 GHz RFR emitted from a Wi-Fi generator for 24 h/day for one year. The animals in the control group (n = 8) were kept under the same conditions as the experimental group, but the Wi-Fi generator was turned off. At the end of the study, rats were sacrificed and brains were removed to analyze miRNA expression and membrane and depot fatty acids of brain cells. We analyzed the situation of ten different miRNA expressions and nineteen fatty acid patterns in this study. We observed that long-term and excessive exposure of 2.4 GHz Wi-Fi radiation increased rno-miR-181a-5p, phosphatidylserine (PS) and triacylglycerol (TAG) in the brain. In conclusion, 2.4 GHz Wi-Fi exposure has the potential to alter rno-miR-181a-5p expression and the fatty acid percentage of some membrane lipids such as phospholipid (PL), phosphatidylserine (PS) and triacylglycerol (TAG), which are depot fats in the brain. However, the uncontrolled use of RFRs, whose use and diversity have reached incredible levels with each passing day and which are increasing in the future, may be paving the way for many diseases that we cannot connect with today.

ARTICLE HISTORY
Received 20 January 2022
Accepted 31 March 2022

KEYWORDS
2.4 GHz Wi-Fi exposure; wireless communication; miRNAs; fatty acid composition of membrane and depot fats; brain

Introduction

As it is known, the International Agency for Research on Cancer in 2011 classified radiofrequency radiations (RFRs) as possible carcinogen (2B) to humans, opening this field for further investigation (International Agency for Research on Cancer (IARC) 2011). However, we have very limited information on the relation between microRNAs (miRNAs, miRs), fatty acids in brain and RFRs, which is very important to understand the effects of electromagnetic pollution emitted by all wireless equipment that is uncontrolled and widely used in our daily life. In our previous works we indicated that long term and excessive use of 900 MHz and 2.4 GHz RFR radiofrequency radiation altered microRNA expression in brain (Dasdag et al., 2015a; 2015b). We investigated the effects of 900 MHz and 2.4 GHz RFR on some miRNAs namely, rno-miR-9-5p, rno-miR-29a-3p, rno-miR-106b-5p, rno-miR-107 and rno-miR-125a-3p in brain. In the 900 MHz exposure study, rats were exposed to 900 MHz RF radiation for 3 h per day (7 days a week) for 12 months (one year) in the exposure group and we found that 900 MHz RFR exposure only decreased rno-miR107 expression (Dasdag et al., 2015a). In the 2.4 GHz Wi-Fi exposure study, rats were exposed to 2.4 GHz RFRs emitted from Wi Fi generator for 24 hours a day for 12 months (one year) in the exposure group and we found that one-year exposure of 2.4 GHz Wi-Fi radiation altered expression of some miRNAs such as rno-miR-106b-5p and rno-miR-107. The results of this study indicated that rno-miR-107 expression was 3.3 times and rno-miR-106b-5p expression was 3.65 times lower in the exposure group than the control group (Dasdag et al., 2015b).We also investigated the effects

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This article was originally published with errors, which have now been corrected in the online version. Please see Correction (https://doi.org/10.1080/15368378.2022.2088659)
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of 900 MHz RFR with same exposure setup on ten miRNAs in 2019 and found that chronic exposure of RFR under investigation has potential to increase expression of rno-miR-145-5p (Dasdag et al., 2019).

Kumar R et al. (2021) exposed rats to 900 (5.84×10^{-4} W/kg), 1800 (5.94×10^{-4} W/kg) and 2.4 GHz (6.4×10^{-4} W/kg) RFRs for 2h/d for one, three and six-months and stated that "900–2450 MHz exposure led to hypermethylation of histone (H3K9) protein by upregulating euchromatic histone methyltransfer-ase1 enzyme, whereas hypomethylation of DNA (5-Mc) by downregulating DNA methyltransferase1 enzyme with increasing microwave frequency as well as exposure duration of Wistar rat" (Kumar et al., 2021).

Another important parameter in terms of electromagnetic pollution is the relation between RFR exposure and membrane fatty acids, which we have also very limited knowledge in this field.

It is stated that "pain sensation can be changed upon the effect of extremely weak chemical and physical signals, having intensity even less than thermal threshold and non-linear dose-dependent character. Therefore, such a cellular target must have a quantum-mechanical nature" Ayrapetyan S (2018). However, Dutta et al indicated that "RFR affects both calcium-ion release and acetylcholinesterase activity in nervous system-derived cells in culture in a common dose-dependent manner" Dutta et al (1992). As it is also reported, "primary target for non-thermal biological effects of weak intensity radiation, is skin water contents the dissociation molecules of which have quantum sensitive characters. The product of water molecules dissociation which forming ROS serve one of important messengers trough which circulating blood modulate the metabolism of different tissues. Since by fundamental work by Blackman et al in 1982 is known that in realization non-thermal effect of EHF EMF key role have Ca exchange which is involvement in great number of fundamental cell processes, such as Lipids exchange and nucleic acids metabolisms (Ziskin MC, 2006; Blackman et al (1982); Brini and Carafoli 2009)". Sert et al. (2002) investigated the effect of 3 h/d exposure of the 0.8 mT magnetic field on the membrane fatty acids of testes and found that the fatty acid of the testis cell membrane phospholipids was decreased in the exposure group. It is similarly indicated that twenty minutes of 900 MHz RFR exposure (short term) do not alter the percentage of fatty acids in brain and testes (Dasdag et al. 2004, 2003). Recently, Oyarce et al. (2021) detected the main changes of some fatty acid, ergosterol and antioxidant activity of metabolites in the fungus Serpulahimantioide exposed to 2.5 GHz Wi-Fi exposure. They observed an increment in the content of some fatty acids and ergosterol and finally stated that "Direct exposure to Wi-Fi radiation produces oxidative damage by modifying cellular components in the fubgus S. himantioides" (Oyarce et al. 2021). If the World Health Organization (WHO) do not lead to put realistic educational and protective mechanisms, it is certain that human beings will continue to use wireless communication tools in incredible amounts and therefore will be exposed to more and more electromagnetic pollution with each passing day by ignoring health hazards. To draw attention to the subject, the aim of this study is to reveal the effects of long-term exposure of 2.4 GHz Wi-Fi exposure on some miRNAs such as rno-miR-22-3p, rno-miR-24-1- 3p, rno-miR-132-3p, rno-miR-145-5p, rno-miR-181a-5p, rno-miR-186-5p, rno-miR-195-5p, rno-miR-219a-5p, rno-miR-221-3p, rno-miR-222-3p and fatty acids in brain tissue.

Materials and methods

Subjects and animal care

Sixteen Wistar Albino adult male rats were acquired from the Medical Science Application and Research Center of Dicle University. The rats were fed with standard pelleted food (TAVAS Inc., Adana, Turkey) in a standard Plexiglas cage. The average animal weight, which was determined to be 313 ± 2.5 g for each group at the beginning of the study, was 348 ± 28.8 g at the end of the study. Rats were equally divided into two groups such as sham exposed (n = 8) and exposure (n = 8) and kept on a 14/10 h light/dark schedule. During the study, the ambient temperature (22°C) and the relative humidity (45%) were maintained in the normal range for these animals. All animal procedures were in agreement with the Principles of Laboratory Animal Care and the rules of Scientific and Ethics Committee of Dicle University Health Research Center.

Exposure and field measurements

A pulsed signal generator, which could output Wi-Fi signals at 2.45 GHz, was used in the exposure system. Sham (n= 8) and experimental group animals (n= 8) were maintained free in their respective plexiglass cages to resume their daily activities without any restrictions. The experimental group, however, were exposed to 2.45 GHz RFR, about 50 cm below the transmitting antenna of the signal generator, every day (24 h/d) for one year.

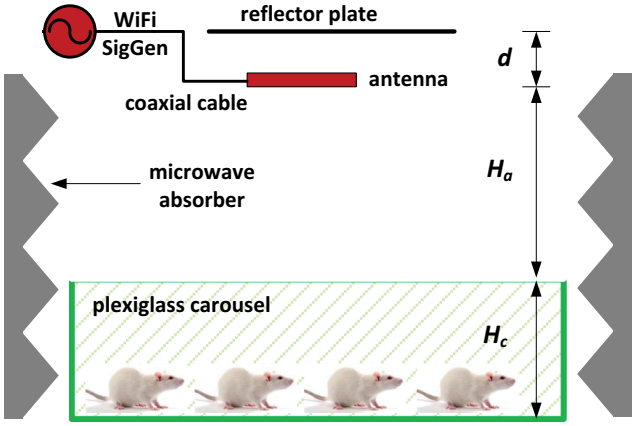


Figure 1. Experimental setup side view. The carousel length and width are 32 cm and 55 cm, respectively. H_c = 20 cm, H_a = 50 cm and d = 2.5 cm. Sixteen rats were free to move inside the carousel.

Similar to experimental group, animals in the control group lived 50 cm below the antenna of the generator (24h/d) for one year, except the generator was turned off. At the end of last exposure, the rats were intraperitoneally administered a combination of 6 mg/kg of 2% xylazine hydrochloride (Rompun) and 75 mg/kg ketamine hydrochloride (Ketalar) for anesthesia. Afterward, the brain of each rat were removed for the analysis.

Experiment setup is displayed in Fig. 1. Electromagnetic power density and the electrical field inside the Plexiglas cage were measured at several points using a field probe EMR 300 (NARDA, Pfullingen, Germany). To minimize coupling from unwanted radiation sources, four sides of the cage were covered with electromagnetic pyramidal absorber backed by metal sheets to isolate the test setup.

Specific absorption rate (SAR)

The Wi-Fi signal generator with a peak power of 100 mW was connected to a stub antenna, and the stub antenna was placed in front of a flat reflector to reduce outside radiation and to convey electromagnetic energy towards the Plexiglas carousel where rats were kept. The antenna was approximately 4 λ away from the rats, where λ is the free space propagation wavelength at 2.45 GHz. With this separation, far-field conditions, which replicated typical Wi-Fi antenna radiation, were formed. At the far field of an antenna, electromagnetic energy can be modelled as a uniform plane wave. To calculate SAR within the rats, first, the electric field generated by the Wi-Fi signal source was measured at several locations of the carousel with a calibrated electric field probe. Then, these measured electric field values were compared with the simulated electric field values where the whole experimental setup was formed in 3D electromagnetic field solver. CST Microwave Studio (CST AG, Germany) was used for electromagnetic field solutions and SAR calculations. Although CST seems similar to well-known finite-difference time domain solvers, it solves Maxwell's equation by the finite-integration technique employed on non-orthogonal grids. The rat voxel model used in CST was based on a computed tomography of a rat, and that model was used in SAR simulations. In SAR averaging calculations, the IEEE/IEC 62704 standard was used.

Lipid extraction and separation of lipid fractions

Total lipids were extracted with chloroform–methanol (2/1 v/v) (Folch et al. 1957). During the extraction process, autoxidation of unsaturated fatty acids was minimized by adding 50 µL of 2% butylated hydroxytoluene (BHT) in chloroform (Stanley-Samuelson and Dadd 1983). Nonlipid contaminants were removed by extraction with 0.88% aqueous KCl (Folch et al. 1957). The extract was evaporated to dryness under a stream of nitrogen at room temperature. Thin layer chromatography (TLC) was used for separation of lipid classes. The petroleum ether/diethyl ether/glacial acetic acid (80/20/1, v/v) mobile phase was used to separate phospholipid and triacylglycerol fractions (Mangold 1969). For separation of phospholipid subclasses such as phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine, TLC plates were completely wetted in 1.8% boric acid prepared in 100% ethanol, dried for 5 min and activated for 15 min at 100°C. The lipid extracts were loaded on the plates and rapidly placed in the chromatography tank containing chloroform/ethanol/water/triethylamine (30/35/7/35, v/v/v/v) (Vaden et al. 2005). The TLC plates were then dried at room temperature. The PL classes were detected under UV light after having sprayed 2′,7′-dichlorofluorescein on the TLC plates. The PL classes were then scrapped into separate test tubes, and their constitutive fatty acids were transmethylated through the addition of acidified (sulfuric acid, 1%) methanol for 2 h at 85°C [11]. The resulting fatty acid methyl esters (FAMEs) were extracted with hexane.

Chromatographic conditions

Fatty acid methyl esters were separated by capillary gas chromatography using a SHIMADZU GC 2010 PLUS equipped with a flame ionization detector (FID) and a fused silica capillary column (BP-21-Polyethylene

Glycol (terephthalic acid treated)) (J & W Scientific, Folsom, CA, USA, 25 m × 0.32 mm internal diameter × 0.25 μm film thickness). Helium was used as the carrier gas. Gas flow rates: helium, 0.5 mL min^{-1}; hydrogen, 30 mL min^{-1}; dry air, 400 mL min^{-1}. The temperature program used was as follows. The initial temperature (170°C) was kept for 8 min, raised to 190°C at a rate of 2°C min^{-1} and kept at 190°C for 17 min and raised to 220°C at a rate of 10°C min^{-1}, and maintained at this temperature for 10 min. The injection and the detector temperatures were 250°C. The split ratio was 20/1. The FAMEs identification and quantification were performed through comparison of retention times (identification) and peak areas (quantification) with a calibration solution composed of a known quantity of each pure FAME (Sigma-Aldrich Chemicals, St., Louis, MO, USA). The amount of fatty acids was given as a percentage.

Fatty acid analysis

Saturated Fatty Acids (SFAs) such as decanoic acid (10:0), lauric acid (12:0), myristic acid (14:0), pentadecanoic acid (15:0), palmitic acid (16:0), heptadecanoic acid (17:0), stearic acid (18:0) and eicosanoic acid (20:0); Monounsaturated Fatty Acid (MUFAs) such as palmitoleic acid (16:1 n-7), oleic acid (18:1 n-9) and eisosenoic acid (20:1 n-9); Polyunsaturated Fatty Acids (PUFAs) such as linoleic acid (18:2 n-6), α- linolenic acid (18:3 n-3), eicosadienoic acid (20:2 n-6), eicosatrienoic acid (20:3 n-6), arachidonic acid (20:4 n-6), eicosapentaenoic acid (20:5 n-3), docosapentaenoic acid (22:5 n-3) and docosahexaenoic acid (22:6 n-3) in total nineteen fatty acids were analysed in this study. In general, fatty acid compositions such as phospholipid (PL), triacylglycerol (TAG) and PL fractions such as phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylserine (PS) and phosphatidylethanolamine (PE) were evaluated in this study.

RNA extraction

Total RNA was extracted from rat brain tissue using Tri-Reagent (Sigma).

Reverse transcriptase PCR reactions (RT-PCR)

Reverse transcriptase reactions contained 5 μl of extracted total RNA, 50 nM stem-loop RT primer, 5X RT buffer, 0.25 mM each of dNTPs, 50 units of modified M-MuLV Reverse Transcriptase (Thermo Scientific, Vilnius, Lithuania), 25 units of RiboLock RNase inhibitor (Thermo Scientific, Vilnius, Lithuania) and nuclease-free water to a total reaction volume of 15 μl. The reaction was performed on an automated Thermal Cycler (Techne Flexigene, Cambridge, UK). RT-PCR conditions for 30 min at 16°C, 30 min at 42°C and 5 min at 85°C were used and then held at 4°C.

Quantitative-Comparative C_T ($\Delta\Delta C_T$) real-time PCR

Quantitative-comparative C_T ($\Delta\Delta C_T$) Real-time PCR was performed in an ABI Prism 7500 Real-Time PCR System (Applied Biosystems) using the SDS 2.0.6 software. The specific primers and fluorogenic ZNA™ probes (Paris et al. 2010) for the microRNAs were designed using Primer Express 3.0 software (Applied Biosystems) and are listed in Table 1. rno-miR-26b-5p was used as an endogenous control microRNA. The mixed RNA created from the sham group was used as a reference RNA sample. Primers and probes were purchased from Metabion International AG, D-82152 Martinsried/Deutschland. The 25 μl PCR included 3 μl of RT-PCR, 12.5 μl of 2× TaqMan Universal PCR Master Mix (Applied Biosystems), 900 nmol of each primer (Primer F and Universal Primer R) and 200 nmol TaqMan probe. The reactions were incubated in a 96-well plate of preincubation at 50°C for 2 min and at 95°C for 10 min, followed by 40 cycles at 95°C for 15 sec and at 60°C for 90 sec. All reactions were run in triplicate (Table 1).

Analyzed microRNA expression

In this study, we investigated effects of 2.4 GHz Wi-Fi exposure on some microRNAs such as rno-miR-22-3p, rno-miR-24-1- 3p, rno-miR-132-3p, rno-miR-145-5p, rno-miR-181a-5p, rno-miR-186-5p, rno-miR-195-5p, rno-miR-219a-5p, rno-miR-221-3p and rno-miR -222-3p.

Statistical analyses

Statistical analyses were carried out using SPSS 18.0 software for Windows. The *t* test was used to determine whether there are any statistically significant differences between the means of control and Wi-Fi-exposed groups. The data in the tables were expressed as mean ± standard error (SE). The miRNA data were processed and analyzed using the statistical package STATISTICA 13 for Windows. Normality assumption of $2^{-\Delta\Delta CT}$ values was checked by the Shapiro–Wilk test. Since the assumption of normality was met, $2^{-\Delta\Delta CT}$ values were expressed as mean and standard deviation, the comparisons between groups were performed using an

Table 1. Primer/probe sequences of the miRNA analyzed by quantitative RT-PCR and Real-Time PCR.

miRNA Name	*miRNA Gene No	*miRNA Nucleotide Sequences No	Primer and Probe Sequences
rno-miR-26b-5p	100314229	NR-031831.1	RT 5′-GTCGTATGCAGTGCAGGGTCCGAGGTATTCGCACTGCATACGACACCTAT-3′ F 5′-GCCGCTTCAAGTAATTCAGG-3′ PR 5′-FAM-TG(pdC)ATA(pdC)GA(pdC)A(pdC)CTATCC-ZNA4-BHQ-1-3′
rno-miR-22-3p	100314001	NR-031824.1	RT 5′-GTCGTATGCAGTGCAGGGTCCGAGGTATTCGCACTGCATACGACACAGTT-3′ F 5′-GCCGCAAGCTGCCAGTT-3′ PR 5′-FAM -TG(pdC)ATA(pdC)GA(pdC)A(pdC)AGTT(pdC)T- ZNA4-BHQ-1-3′
rno-miR-24-1-3p	100314003	NR-031827.1	RT 5′-GTCGTATGCAGTGCAGGGTCCGAGGTATTCGCACTGCATACGACCTGTTC-3′ F 5′-GCCGCTGGCTCAGTTCAG-3′ PR 5′- FAM -TG(pdC)ATA(pdC)GA(pdC)CTGTTCCT- ZNA4-BHQ-1-3′
rno-miR-132-3p	100314029	NR-031878.1	RT 5′-GTCGTATGCAGTGCAGGGTCCGAGGTATTCGCACTGCATACGACCGACCA-3′ F 5′-GCCGCTAACAGTCTACAGCCA-3′ PR 5′-FAM-TG(pdC)ATA(pdC)GACCGACCA-ZNA4-BHQ-1-3′
rno-miR-145-5p	100314036	NR-031891.1	RT 5′-GTCGTATGCAGTGCAGGGTCCGAGGTATTCGCACTGCATACGACAGGGAT-3′ F 5′-GCCGCGTCCAGTTTTCC-3′ PR 5′-FAM-TG(pdC)ATA(pdC)GA(pdC) AGGGAT-ZNA4-BHQ-1-3′
rno-miR-181a-5p	100314195	NR-031926.1	RT 5′-GTCGTATGCAGTGCAGGGTCCGAGGTATTCGCACTGCATACGACACTCAC-3′ F 5′-GCCGCAACATTCAACGCTGTCG-3′ PR 5′- FAM-TG(pdC)ATA(pdC)GA(pdC) ACT(pdC)ACCGA-BHQ-1-3′
rno-miR-186-5p	100314043	NR-031904.1	RT 5′-GTCGTATGCAGTGCAGGGTCCGAGGTATTCGCACTGCATACGACAGCCCA-3′ F 5′-GCCGCCAAAGAATTCTCCTT-3′ PR 5′-FAM-TG(pdC)ATA(pdC)GACAGCCCA-ZNA4-BHQ-1-3′
rno-miR-195-5p	100314281	NR-031912.1	RT 5′-GTCGTATGCAGTGCAGGGTCCGAGGTATTCGCACTGCATACGACGCCAAT-3′ F 5′-GCCGCTAGCAGCACAGAAAT-3′ PR 5′-FAM-TG(pdC)ATA(pdC)GACGCCAAT-ZNA4-BHQ-1-3′
rno-miR-219a-5p	100314058	NR-031932.1	RT 5′-GTCGTATGCAGTGCAGGGTCCGAGGTATTCGCACTGCATACGACAGAATT-3′ F 5′-GCCGCTGATTGTCCAAACG-3′ PR 5′-FAM-TG(pdC)ATA(pdC)GA(pdC)AGAATTGC-ZNA4-BHQ-1-3′
rno-miR-221-3p	100314163	NR-031934.1	RT 5′-GTCGTATGCAGTGCAGGGTCCGAGGTATTCGCACTGCATACGACGAAACC-3′ F 5′-GCCGCAGCTACATTGTCTGCTG-3′ PR 5′-FAM-TG(pdC)ATA(pdC)GA(pdC) GAAA(pdC)CCA-BHQ-1-3′
rno-miR-222-3p	100314059	NR-031935.1	RT 5′-GTCGTATGCAGTGCAGGGTCCGAGGTATTCGCACTGCATACGACACCCAG-3′ F 5′-GCCGCAGCTACATCTGGCTA-3′ PR 5′-FAM-TG(pdC)ATA(pdC)GA(pdC) ACC(pdC)AGTAGC-BHQ-1-3′
miR-Universal R			R 5′-GTGCAGGGTCCGAGGTAT-3′

* https://www.ncbi.nlm.nih.gov/gene, Abbreviations: F: Forward, R: Reverse, PR: Probe, RT: Reverse Transcriptase Primer, pdC: Substitution of C-5 propynyl-dC (pdC) for dC is an effective strategy to enhance base pairing. Using these base substitutions, duplex stability and melting temperatures are raised by C-5 propynyl-C 2.8 per substitution, ZNA4: Zip Nucleic Acids: ZNA probes provide broad flexibility in assay design and represent an effective alternative to Minor Groove Binder (MGB)- and Locked Nucleic Acid (LNA)-containing oligonucleotides.

independent t-test and the significance values were adjusted for multiplicity using Benjamini-Hochberg adjustment (Benjamini and Hochberg 1995; Rai et al. 2012). The error-bar graph was used to represent data distribution of rno-miR-22-3p, rno-miR-24-1-3p, rno-miR-132-3p, rno-miR-145-5p, rno-miR-181a-5p, rno-miR-186-5p, rno-miR-195-5p, rno-miR-219a-5p, rno-miR-221-3p and rno-miR-222-3p variables according to the groups. Significant differences (two-tailed *P*) less than 0.05 were regarded as significant.

Results

Phospholipid (PL) fatty acid composition in brain

2.4 GHz radiofrequency radiation increased docosapentaenoic acid (22:5 n-3) and decreased docosahexaenoic acid (22:6 n-3), which are members of n-3 Polyunsaturated Fatty Acids (n-3 PUFAs). Results are given in Figure 2 and Table 2.

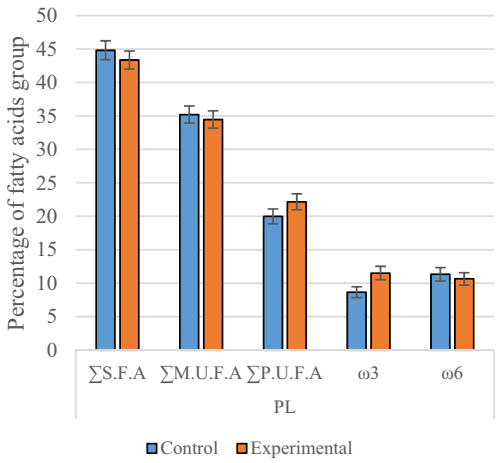


Figure 2. Percentage of fatty acid groups of brain phospholipid in control and experiment animals.

Triacylglycerol (TAG) fatty acid composition in brain

Since 2.4 GHz radiofrequency radiation increased linoleic acid (18:2 n-6), therefore, ∑PUFA and ∑n-6 PUFA also increased. Results are shown in Figure 3 and Table 2.

Table 2. Fatty acid composition of brain PL, TAG and PL subclasses (%).

	PL		TAG		PC		PI		PS		PE	
Fatty Acids	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental
10:0[C]	0.14 ± 0.01a	0.11 ± 0.03a	0.79 ± 0.06a	1.07 ± 0.16b	-	-	-	-	-	-	-	-
12:0	0.07 ± 0.02a	0.06 ± 0.02a	0.42 ± 0.03a	0.40 ± 0.02a	0.07 ± 0.02a	0.04 ± 0.02b	0.22 ± 0.02a	0.25 ± 0.02a	0.11 ± 0.03a	0.04 ± 0.02b	0.08 ± 0.02a	0.05 ± 0.03a
14:0	0.24 ± 0.01a	0.55 ± 0.05b	0.78 ± 0.05a	0.50 ± 0.07a	0.20 ± 0.01a	0.21 ± 0.01a	0.44 ± 0.03a	0.41 ± 0.15a	0.13 ± 0.04a	0.13 ± 0.04a	0.10 ± 0.04a	0.12 ± 0.03a
15:0	0.06 ± 0.02a	0.06 ± 0.02a	0.16 ± 0.01a	0.08 ± 0.02b	0.13 ± 0.04a	0.11 ± 0.03a	0.09 ± 0.01a	0.07 ± 0.02a	0.03 ± 0.02a	0.03 ± 0.02a	0.08 ± 0.02a	0.08 ± 0.02a
16:0	23.28 ± 1.22a	21.41 ± 1.12a	17.26 ± 1.17a	18.11 ± 1.18a	45.00 ± 1.45a	46.49 ± 1.47a	20.74 ± 1.13a	20.77 ± 1.13a	6.94 ± 0.69a	4.39 ± 0.41b	8.70 ± 0.85a	8.54 ± 0.81a
17:0	0.21 ± 0.01a	0.21 ± 0.01a	0.23 ± 0.01a	0.11 ± 0.03b	0.26 ± 0.02a	0.19 ± 0.01a	0.15 ± 0.03a	0.24 ± 0.02b	0.09 ± 0.01a	0.20 ± 0.01b	0.29 ± 0.02a	0.18 ± 0.01a
18:0	20.49 ± 1.18a	20.51 ± 1.22a	5.56 ± 0.51a	3.82 ± 0.20b	12.99 ± 1.02a	13.57 ± 1.03a	25.34 ± 1.27a	25.27 ± 1.33a	39.84 ± 1.33a	47.84 ± 1.49b	25.39 ± 1.27a	24.39 ± 1.14a
20:0	0.31 ± 0.02a	0.47 ± 0.04a	0.22 ± 0.01a	0.13 ± 0.04a	0.14 ± 0.01a	0.14 ± 0.01a	0.38 ± 0.02a	0.41 ± 0.15a	0.38 ± 0.02a	0.50 ± 0.07b	0.11 ± 0.03a	0.13 ± 0.04a
∑S.F.A	**44.82** ± 1.42a	**43.37** ± 1.34a	**25.44** ± 1.13a	**24.22** ± 1.24a	**58.78** ± 1.57a	**60.74** ± 1.62a	**47.37** ± 1.48a	**47.42** ± 1.46a	**47.51** ± 1.44a	**53.14** ± 1.52b	**34.73** ± 1.30a	**33.50** ± 1.29a
16:1 n-7	0.44 ± 0.03a	0.44 ± 0.03a	0.90 ± 0.09a	0.67 ± 0.07a	0.33 ± 0.02a	0.33 ± 0.02a	0.96 ± 0.10a	0.98 ± 0.09a	0.11 ± 0.03a	0.04 ± 0.02b	0.15 ± 0.03a	0.24 ± 0.02b
18:1 n-9	33.53 ± 1.32a	32.26 ± 1.30a	58.89 ± 1.55a	57.32 ± 1.63a	30.29 ± 1.44a	30.30 ± 1.19a	18.88 ± 1.16a	18.97 ± 1.08a	31.61 ± 1.19a	27.07 ± 1.40a	26.33 ± 1.13a	27.21 ± 1.27a
20:1 n-9	1.22 ± 0.33a	1.77 ± 0.12a	0.31 ± 0.02a	0.13 ± 0.04b	0.89 ± 0.29a	0.63 ± 0.17b	0.44 ± 0.03a	0.38 ± 0.02a	1.36 ± 0.11a	1.62 ± 0.17a	3.81 ± 0.35a	4.68 ± 0.44a
∑M.U.F.A	**35.20** ± 1.28a	**34.47** ± 1.29a	**60.10** ± 1.63a	**58.12** ± 1.58a	**31.51** ± 1.30a	**31.26** ± 1.35a	**20.29** ± 1.22a	**20.33** ± 1.29a	**33.08** ± 1.37a	**28.73** ± 1.30a	**30.29** ± 1.14a	**32.14** ± 1.35a
18:2 n-6	3.58 ± 0.32a	2.17 ± 0.20a	12.73 ± 1.04a	16.22 ± 1.08a	1.88 ± 0.13a	0.74 ± 0.16b	1.88 ± 0.13a	1.93 ± 0.17a	3.42 ± 0.15a	0.40 ± 0.02b	1.28 ± 0.12a	0.71 ± 0.06b
18:3 n-3	0.11 ± 0.03a	0.03 ± 0.02b	0.56 ± 0.03a	0.78 ± 0.05a	0.02 ± 0.01a	0.01 ± 0.01a	0.13 ± 0.04a	0.14 ± 0.01a	0.10 ± 0.04a	0.01 ± 0.01b	0.06 ± 0.02a	0.09 ± 0.01a
20:2 n-6	0.14 ± 0.01a	0.16 ± 0.01a	0.15 ± 0.03a	0.03 ± 0.02b	0.10 ± 0.04a	0.05 ± 0.03b	0.17 ± 0.06a	0.20 ± 0.01a	0.10 ± 0.04a	0.13 ± 0.04a	0.21 ± 0.01a	0.25 ± 0.02a
20:3 n-6	0.16 ± 0.01a	0.22 ± 0.02a	0.01 ± 0.01a	0.003 ± 0.01b	0.11 ± 0.03a	0.06 ± 0.02b	0.19 ± 0.01a	0.19 ± 0.01a	0.19 ± 0.01a	0.32 ± 0.02b	0.23 ± 0.01a	0.32 ± 0.02a
20:4 n-6	7.45 ± 0.77a	8.10 ± 0.71a	0.66 ± 0.22a	0.39 ± 0.03b	4.90 ± 0.38a	4.68 ± 0.44a	24.32 ± 1.27a	24.05 ± 1.10a	3.13 ± 0.33a	3.62 ± 0.32a	14.96 ± 1.05a	14.81 ± 1.05a
20:5 n-3	0.36 ± 0.03a	0.34 ± 0.03a	0.08 ± 0.22a	0.03 ± 0.02b	0.11 ± 0.03a	0.14 ± 0.01a	0.73 ± 0.06a	1.11 ± 0.19b	0.56 ± 0.04a	1.23 ± 0.30b	0.02 ± 0.01a	0.24 ± 0.02b
22:5 n-3	0.43 ± 0.04a	9.07 ± 0.91b	0.05 ± 0.03a	0.04 ± 0.02a	0.04 ± 0.02a	0.11 ± 0.03b	1.30 ± 0.22a	1.18 ± 0.11a	0.35 ± 0.02a	0.40 ± 0.02a	0.17 ± 0.06a	0.32 ± 0.02b
22:6 n-3	7.76 ± 0.55a	2.08 ± 0.12b	0.22 ± 0.02a	0.19 ± 0.01a	2.55 ± 0.12a	2.21 ± 0.29a	3.62 ± 0.32a	3.47 ± 0.34a	11.57 ± 0.97a	12.02 ± 1.02a	18.04 ± 1.09a	17.64 ± 1.07a
∑P.U.F.A	**19.99** ± 1.10a	**22.16** ± 1.20a	**14.47** ± 1.17a	**17.66** ± 1.09a	**9.71** ± 0.84a	**8.00** ± 0.80a	**32.34** ± 1.33a	**32.26** ± 1.30a	**19.42** ± 1.09a	**18.12** ± 1.09a	**34.98** ± 1.31a	**34.37** ± 1.33a
∑ω3	8.66 ± 0.81a	11.51 ± 1.01a	0.92 ± 0.10a	1.03 ± 0.12a	2.72 ± 0.21a	2.46 ± 0.30a	5.78 ± 0.56a	5.89 ± 0.56a	12.57 ± 1.02a	13.65 ± 1.03a	18.29 ± 1.07a	18.29 ± 1.08a
∑ω6	11.33 ± 1.01a	10.65 ± 0.93a	13.55 ± 1.14a	16.63 ± 1.13a	6.99 ± 0.63a	5.53 ± 0.15a	26.56 ± 1.32a	26.36 ± 1.13a	6.84 ± 0.62a	4.48 ± 0.42a	16.69 ± 1.16a	16.08 ± 1.06a
ω3/ω6	0.76	1.08	0.07	0.06	0.39	0.44	0.22	0.22	1.84	3.05	1.10	1.14

Values are provided as mean ± SE; SFA saturated fatty acid, MUFA monounsaturated fatty acid, PUFA polyunsaturated fatty acid. Significant differences at a level of $p < 0.05$ are designated by 'a' and 'b' for PC, PE, PI and PS; a and b for PC, PE, PI and PS in control and experiment animals

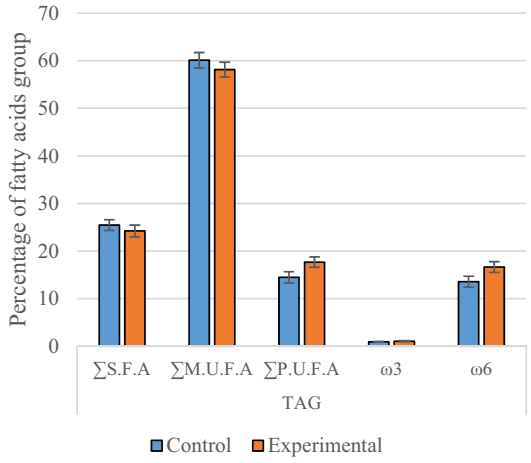


Figure 3. Percentage of fatty acid groups of brain triacylglycerol in control and experiment animals.

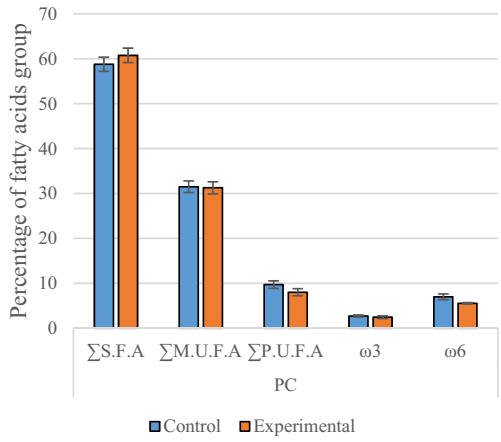


Figure 4. Percentage of fatty acid groups of brain phosphatidylcholine in control and experiment animals.

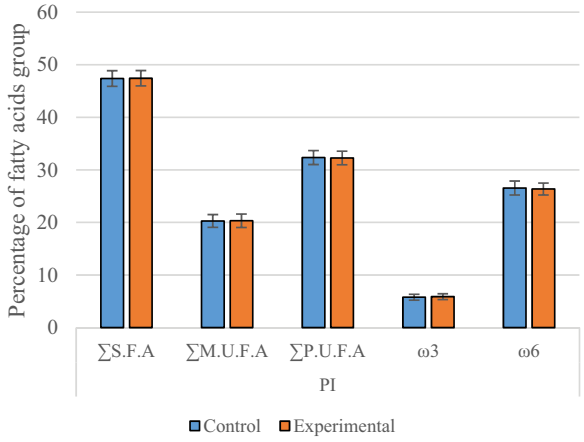


Figure 5. Percentage of fatty acid groups of brain phosphatidylinositol in control and experiment animals.

PL subclasses of fatty acid composition in brain

Phosphatidylcholine (PC) fatty acid composition

Any differences were not observed between the 2.4 GHz Wi-Fi exposure group and sham animals in terms of PC in brain. Results are given in Figure 4 and Table 2

Phosphatidylinositol (PI) fatty acid composition

Any differences were not observed between the 2.4 GHz Wi-Fi exposure group and sham animals in terms of PI fatty acid composition in brain. Results are given in Figure 5 and Table 2.

Phosphatidylserine (PS) fatty acid composition

2.4 GHz Wi-fi increased the percentage of 18:0, which is the member of Saturated Fatty Acids (SFAs). However, 2.4 GHz Wi-fi decreased the percentage of 18:1 n-9, which is a member of Monounsaturated Fatty Acids (MUFAs) and 18:2 n-6 which is the member of n-6 Polyunsaturated Fatty Acid (n-6 PUFAs). Results are given in Figure 6 and Table 2.

Phosphatidylethanolamine (PE) fatty acid composition

Any differences were not observed between the 2.4 GHz Wi-Fi exposure group and sham animals in terms of PE fatty acid composition in brain. Results are given in Figure 7 and Table 2.

In summary, 2.4 GHz Wi-Fi exposure has potential to change the fatty acid composition of the membrane lipids phospholipid and phosphatidylserine with triacylglycerol, the storage lipid in brain.

The percentage of fatty acids in sham and exposed groups is given in Table 2.

microRNA expression results

As it is stated above, we evaluated the effect of 2.4 GHz RFR exposure on ten miRNAs. At the end of the study, we observed that only miR_181a_5p increased by 2.4 GHz Wi-Fi exposure (Table 3).

The results of this study showed that the long-term 2.4 GHz Wi-Fi exposure increased expression of rno-miR-181a-5p ($p = 0, 007743$), while other microRNAs investigated in this study were not altered ($p > .05$).

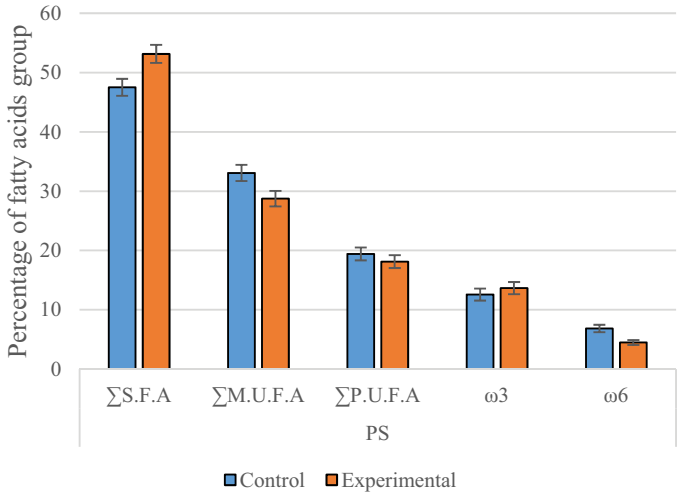


Figure 6. Percentage of fatty acid groups of brain phosphatidylserine in control and experiment animals.

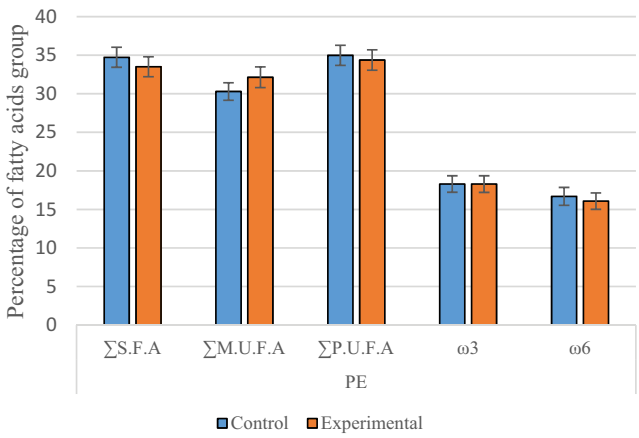


Figure 7. Percentage of fatty acid groups of brain phosphatidylethanolamine in control and experiment animals.

Table 3. Results of microRNAs expression investigated in this study.

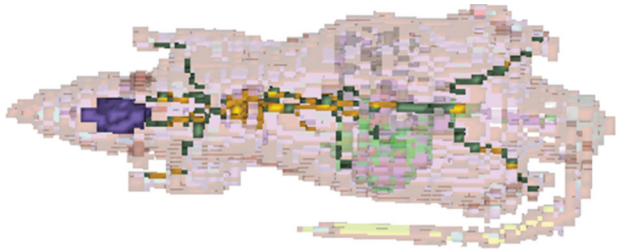
microRNAs	Sham Mean	Std. Dev. ±	2.4 GHz Mean	Std. Dev. ±	p
rno-miR-22-3p	0.678560	0.296382	0.756667	0.126383	0.529866
rno-miR-24-1-3p	0.649322	0.230953	0.633689	0.134501	0.877750
rno-miR-132-3p	1.073392	0.534161	1.361540	0.216013	0.206306
rno-miR-145-5p	1.051213	0.632334	1.623565	0.599016	0.096452
rno-miR-181a-5p	0.483891	0.140160	0.666881	0.066802	0.007743
rno-miR-186-5p	0.499652	0,.159833	0.522221	0.108539	0.757931
rno-miR-195-5p	0.593268	0.171782	0.521081	0.150385	0.405576
rno-miR-219a-5p	0.271842	0.099724	0.253847	0.360437	0.908345
rno-miR-221-3p	0.281981	0.142452	0.315691	0.085829	0.595539
rno-miR-222-3p	0,427805	0,263579	0.547197	0.88440	0.338093

SAR (Specific Absorption Rate) result

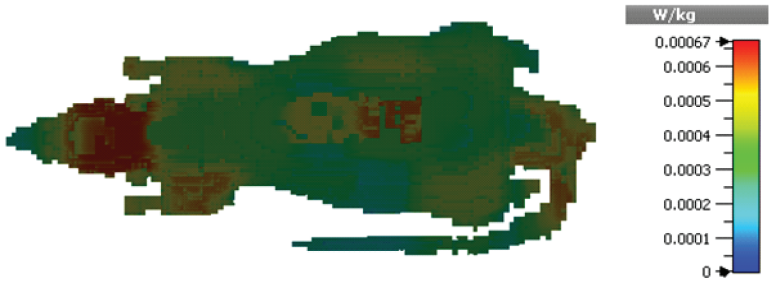
Brain, cerebral fluid, blood arteries and blood of the simulated rat are shown in Figure 8 a. Coronal SAR distribution for 10 g averaging and saggital SAR distribution for 1 g averaging are shown in Figure 7 b and c. Maximum SAR levels in those regions were 1543 μW/kg and 655 μW/kg, for 1 g and 10 g averaging, respectively. Whole body total SAR and maximum point SAR were calculated to be 328 μW/kg and 7197 μW/kg, respectively.

Discussion

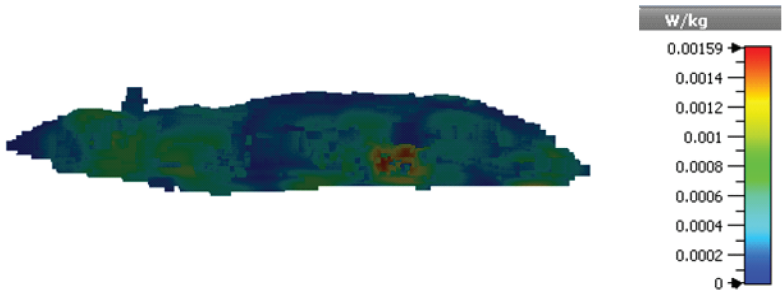
It is reported that "The discovery of microRNAs has revealed an unexpected and spectacular additional level of fine tuning of the genome, and how genes are used again and again in different combinations to generate the complexity that forms, for instance, the brain". Although, microRNAs are abundantly expressed in the brain, relatively little is known about the multiple functions of these RNA molecules in the nervous system. MicroRNA pathways play major roles in the proliferation, differentiation, function and maintenance of neuronal cells. Several intriguing studies have linked microRNAs as major regulators of the neuronal phenotype and have implicated specific microRNAs in the regulation of synapse formation and plasticity. Dysfunction of microRNA pathways is also slowly emerging as a potential important contributor to the pathogenesis of major neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease" (De and Christen 2010; He´bert et al. 2010). It is also stated that miRNA research has mainly focused on cancer (He´bert et al. 2010). As it is known, miRNAs were recently very popular; however, the study performed to reveal effects of RFR exposure on miRNAs is very limited as stated above. Under radiofrequency radiation, the relationship between RFR and various diseases, especially cancer, is underestimated. For instance, Dasdag et al. (2020) summarized that "The



(a)



(b)



(c)

Figure 8. Rat SAR simulations: a) SAR model, b) coronal cut for 10 g averaging and c) saggital cut for 1 g averaging.

Table 4. Changes in fatty acid composition triggered by Wi-Fi exposure.

Fatty Acid	PL	TAG	PC	PI	PS	PE
10:0		Increased				
12:0			Decreased		Decreased	
14:0	Decreased					
15:0		Decreased				
16:0					Decreased	
17:0		Decreased			Increased	
18:0		Decreased			Increased	
20:0					Increased	
∑S.F.A					Increased	
20:1 n-9		Decreased	Decreased			
18:2 n-6			Decreased		Decreased	Decreased
18:3 n-3	Decreased				Decreased	
20:2 n-6		Decreased				
20:3 n-6		Decreased			Increased	
20:4 n-6		Decreased				
20:5 n-3		Decreased		Increased	Increased	Increased
22:5 n-3		Increased	Increased			Increased
22:6 n-3	Decreased					

results of the study investigating the relationship between Alzheimer's disease (AD) and electromagnetic fields were still contradictory". Whereas many researchers suggest that some electromagnetic radiation has prepared the ground for AD, others claim that RF radiation has a positive effect on the disease in question" (Lin et al. 2021). Therefore, it may be too late when the evidence between certain diseases and electromagnetic pollution is established in "the future.

The results of our previous studies, which are in a limited number of studies examining the relationship between RFRs and microRNAs, revealed that this radiation has potential to predispose to different diseases. Although the results obtained from animal studies must be taken into consideration by the relevant persons or organizations, there is a need for comprehensive studies. However, the results of our previous studies on microRNAs indicated that 900 MHz RFR emitted from mobile phones has potential to decrease rno-miR107 expression and increase rno-miR-145-5p in brain. However, we also observed that miR-107 expression was 3.3 times and miR-106b-5p expression was 3.65 times lower in the 2.4 GHz Wi-Fi exposure group. The relation between the alteration in rno-miR107, rno-miR -145-5p and miR-106b-5p and some diseases has been discussed in detail in our previous studies. In these studies, we observed that rno-miR107 is more sensitive to both RFR radiation more than two different miRNAs (Dasdag et al. 2015a, 2015b; Dasdag et al. 2019).

As it is discussed above, we investigate the effect of 2.4 GHz Wi-Fi exposure on ten different microRNAs such as rno-miR-22-3p, rno-miR-24-1-3p, rno-miR-132-3p, rno-miR-145-5p, rno-miR -181a-5p, rno-miR-186-5p, rno-miR-195-5p, rno-miR-219a-5p, rno-miR-221-3p and, rno-miR-222-3p in this study. However, we observed that 2.4 GHz RFR only increased rno-miR-181a-5p expression, which is stated that "miR-181a-5p increased the chemical sensitivity of HS578T cells to cisplatin by inhibiting VDR to promote autophagy" (Lin et al. 2021). Therefore, we can state that our previous studies supported the results of this study in terms of indicating the effects of RFRs on miRNA. miR-181a-5p was highly expressed in serum of acute ischemic injury patients" (Song et al., 2021). On the other hand, Liu et al. stated that "miR-181a-5p is a miRNA shown to play an essential role in multiple cancers, such as hepatic cancer, non-small cell lung cancer and human diseases, such as osteoarthritis. The interaction between lncRNA CCAT1 and miR-181a-5p has been demonstrated in endometrial carcinoma" (Liu et all. 2020).

As a result, we can say that it should be focused on the diseases associated with rno-miR107, rno-miR-145-5p, miR-106b-5p and rno-miR-181a-5p expressions in humans exposed to long-term RFR exposure. Illumination of these relationships may also create opportunities to develop new approach various diseases.

As it is known, we also evaluated interaction between 2.4 GHz Wi-Fi exposure and membrane fatty acid of brain tissue in this study. Our previous studies indicated that short-term exposure of RFR does not have potential to alter membrane fatty acids in different tissues. Since our previous studies represent short-term exposure, we have no chance to compare the results of them with the data obtained in this study (Dasdag et al. 2004, 2003; Sert et al. 2002). However, the data of Oyarce et al. (2021) indicated that Wi-Fi exposure has potential to cause an increment in the content of some fatty acids. This means that there is an agreement between the results of this study and Oyarce's data (Oyarce et al. 2021). On the other hand, Fragopoulou et al. (2018) observed some alteration in membrane fatty acids in brain and stated that "The observed changes indicate a membrane remodeling response of the tissue phospholipids after nonionizing radiation exposure, reducing SFA and EPA, while increasing MUFA residues". They also claimed that RFR radiation induces hippocampal lipidome and transcriptome changes that may explain the brain proteome changes and memory deficits". In Fragopoulou's study, some fatty acids such as 16:0, 20.5 ω-3 and SFA decreased, while 16:1 and 18:1–9c increased by RFR exposure (Fragopoulou et al. 2018).

In our study, we also observed that 2.4 GHz Wi-Fi exposure altered some fatty acids. 2.4 GHz exposure caused changes in PL and TAG percentages, but changes in TAG were found to be more pronounced. However, PS, which is subclasses of PL, is affected by RFR more than other subgroups. It is reported that "Phosphatidylserine is subclasses called a phospholipid. It covers and protects the cells in your brain and carries messages between them. Phosphatidylserine plays an important role in keeping the mind and memory sharp. Animal studies suggest that the level of this substance in the brain decreases with age" (Burr and Burr 1930). Therefore, our results on the changes in the fatty acid composition of phosphatidylserine gain an importance because 2.4 GHz WI-Fi exposure decreased percentage of linoleic acid (18:2 n-6). Linoleic acid (LA, 18:2 n-6) is an essential n-6 polyunsaturated fatty acid (PUFA) required for normal growth and development. However, little is known about how this increase in dietary LA affects the brain. It is mainly viewed as an essential precursor to arachidonic acid (20:4 n-6, AA), which is

important for neurodevelopment and other physiological processes (Brenna 2016). A summary of fatty acid alteration triggered by Wi-Fi exposure is given in Table 4. However, although the results we obtained in this study are consistent with the results of Fragopoulou et al. (2018), ours are more pronounced. The reason of this situation sourced from the exposure setup and duration differentiations. For instance, we used a generator to standardize the exposure and investigated long-term/chronic effects of RFRs by exposing animals during day /24 hours) for one year. Whereas they exposed animals to 1800 MHz RFR exposure for 2 hours emitted from a commercial mobile phone is investigated. Since RFR exposure cannot be standardized because of some physical characteristics, using a mobile phone in the study like this is not acceptable. Although this exposure setup was acceptable when studies in this field were started first, they are not realistic today.

On the other hand, It is interestingly reported that "electronically generated electromagnetic fields are coherent, producing very high electric and magnetic forces, which act on the voltage sensor of voltage-gated calcium channels to produce increases in intracellular calcium [Ca^{2+}]i" (Pall. 2022). Blaustein and Lederer reported that "In controlling of intracellular Ca homoesis have key role cGMP-dependent Ca efflux from the cell, which realized by Na/Ca exchange in forward(F) mode and Ca-pump in cell membrane and cAMP-Ca exchange in/Ca exchange in reverse (R) mode". They also stated that "the discovery of multiple Na^+/Ca^{2+} exchanger genes and gene products, and multiple splice variants, as well as exchangers with different coupling ratios [i.e., the 3 Na^+:1Ca^{2+} exchanger a Na^{2+}/Ca^{2+} exchanger and the 4 Na^+:(1Ca^{2+} +1K^+) exchanger] has added to the complexity. We must even consider the possibility that some cells may express more than one Na^+/Ca^{2+} exchanger [or $Na^+/(Ca^{2+} + K^+)$ gene product or alternatively spliced isoform] and that these exchangers may perform different functions" (Blaustein and Lederer, 1999). By Ayrapetyan work have shown that in cell membrane have two family of quantum-sensitive receptors through which the non-thermal effect of non-ionizing radiation on cells are realized they are soluble guanylate cyclase (sGC) and G proteins family of receptors the activation of which leads to stimulate the cAMP –dependent RNa/Ca exchange (Ayrapetyan, 2018). Therefore we hypothesis that the non -thermal radioation-induced changes of physicochemical properties of circulating blood by stimulation of quantum-sensitive membrane receptors induced [Ca]i changes could be primary mechanism for modulation oflipids metabolisms and RNA metabolism. To check this hypothesis will be the aim of future works.

Conclusion

The results of this study revealed that long-term and chronic exposure of 2.4 GHz Wi-Fi radiation increased rno-miR-181a-5p expression and changed the percentage of some fatty acids and its subclasses. Therefore, it should be kept in mind that the radiofrequency radiations emitted by various internet providers, mobile phones and other wireless communication devices that we are exposed to throughout the day have the potential risk. Being cautious is the most reasonable approach. The uncontrolled exposure of RFRs, whose use and diversity have reached incredible levels with each passing day and which are increasing in the future, may be paving the way for many diseases that we cannot connect with today. Therefore, parents have to pay more attention to this risk to protect themselves and especially children and adolescent.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The author(s) reported that there is no funding associated with the work featured in this article.

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