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Stereological examination of the effect on the hippocampal dentate gyrus granule cells number of rats subjected to wireless internet during prenatal period: A preliminary study

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Abstract

As a result of industrialisation and the many recent advances in technology, we are being intensely subjected to non-ionised radiation sources. As such, the effect of non-ionised radiation on human tissue is now being researched. In this study, the effects of Wi-Fi waves found in some third-generation mobile phones to facilitate internet connection on the brain hippocampal dentate gyrus granule cell quantity of rats during the prenatal period was stereologically examined. During the study, mated albino rats were subjected to Wi-Fi throughout pregnancy. A month after giving birth, six rats from each group, for a total of 12 rats, was sacrificed under perfusion and anaesthesia. Their skulls were opened and brains were removed to undergo routine checks in which the granule cells were counted. Strategic sections were defined, and every 45th section couple (with a thickness of 5 µm) was taken and dyed with haematoxylin and eosin (H&E) and cresyl violet stain. The granule cells were counted using a combination of the stereological disector method and Cavalieri's principle. Then, the results were analysed using the Mann–Whitney U test. No statistically significant difference has been observed between the groups ($p > 0.05$). The findings were discussed in light of the relevant literature, and it was determined that throughout pregnancy, Wi-Fi modem device did not result in any changes in the dentate gyrus granule cell count of the hippocampus of postnatal rats' brains.

Keywords: number of granule cells, pregnancy, prenatal rat, stereology, wi-fi

1. Introduction

In recent years, studies on human health in relation to electromagnetic fields (EMF) have investigated the health effects that may arise from low-level, long-term EMF exposure throughout a human's lifetime. Radiofrequency (RF) waves have two types of effect mechanisms: thermal and non-thermal. If there is a level of RF application that can cause a heat increase in biological structures, this first leads to heat increase in the tissue and then to biological changes due to this heat increase. Currently, the number of studies investigating the effects of RF radiation on the nervous system is consistently increasing. In particular, the question of whether the effect of exposure to EMFs on the blood–brain barrier is thermally induced is being discussed by researchers. Exposure to high levels of RF energy causes damage to the nervous system's structure and function. In studies conducted on isolated brain tissue, it has been found that there are different causes, other than thermal mechanisms, influencing neurological electroencephalography (EEG) changes, blood–brain barrier permeability, and calcium flow changes (1). Exposure to RF radiation sources has been reported to cause various pathological conditions such as headaches, insomnia and memory loss (2). Today, with the rapid development of technology, exposure to RF radiation sources has become inevitable. These sources include radars, wireless communication systems, microwave ovens, base stations, mobile phones, televisions and radios (3). There is increasing number of studies stating that radiofrequency waves emitted from EMF sources, which have become an indispensable part of our lives today, have harmful effects on human health (4-7).

Studies analyzing the role of RF in the development of leukemia, lymphoma, other cancers, chromosomal disturbances, behavior disorders, learning difficulties in children, decreased reproduction, blood–brain barrier permeability, cell and DNA synthesis, and increased brain electrical activity (i.e., EEG) have also gained prevalence in recent years. It has also been observed that hormones are affected; carbohydrate, nucleic acid and protein metabolism changes occur; cellular respiration is decreased; structural changes occur; tissue and cell hormonal response is changed; and immune response to different antigens is affected (3).

Increased basal corticosteroid levels, decreased locomotor activity and neuronal damage occurs, with abnormal brain

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functioning (8), as well as increased sympathetic activity (9) have been shown in rat brains exposed to EMFs of different intensities (10). However, other studies contend that EMFs have no negative effect on tissues (11). Alzheimer's and Parkinson's diseases, as well as various neurological disorders, have been found to be more common in radio operators exposed to EMF and others who work with data processing devices, telephone lines, substations and power stations (12). Most studies have focused on the development of brain tumors through the use of mobile telephones. A significant increase in the risk of brain tumors has been observed in mobile and cordless telephone users over the past 10 years, as well as an increase in glioma (especially astrocytoma), acoustic neuroma and meningioma tumors (13-19).

It has been reported that EMF affects fertility in rats, causes genetic disorders, causes cellular and molecular changes in rat brains, affects neuroendocrine system, is associated with cancer formation, affects brain functions and may cause many neurological effects (20). Another study concluded that EMF decreased melatonin hormone production and could lead to depression by decreasing melatonin levels. In addition, the study noted that the EMF emitted by household appliances affects the secretion of neuroendocrine products and causes a deterioration of sleep phases (21). In 1997, Lai et al. reported that exposure to RF waves produces DNA fractures in rat brain cells (22).

Prenatal irradiation may also increase the risk of cancer in childhood. It is for this reason that pregnant women should avoid having diagnostic X-ray tests in the abdomen until delivery. Should an embryo or fetus be exposed to high doses, serious deformities or death may occur. The threshold for these effects is between 0.1 and 1 Sievert (Sv), or higher, depending on the pregnancy period (23). It has been shown that when female rats are exposed to an EMA field while pregnant, this exposure can affect the development of Purkinje cells in the cerebellum of the fetus; the results of this pathological effect persist after the postnatal period (24).

There was a significant decrease in glutathione peroxidase (GSH-Px), glutathione (GSH) and antioxidant vitamin concentrations in the brain and liver of rat pups exposed to 2.45 GHz of Wi-Fi during pregnancy 1 hour, 5 days a week. It has been reported that oxidative damage due to EMF radiation is more likely to occur than is to be (25). In a study on the determination of oxidative DNA damage and malondialdehyde (MDA) levels in brain tissue in rats exposed to a 2100 Hz EM field at intervals of between 10 and 40 days, an increase in DNA damage and a decrease in MDA levels were observed after 10 days. At the end of 40 days, there was a decrease in DNA damage, and this result was attributed to the adaptation of the repair mechanism (26).

According to our review of the relevant research, no studies have been found to satisfactorily investigate the effect of Wi-Fi on dentate granule cells using a stereological method. Therefore, in this preliminary study, we planned to evaluate the effects of Wi-Fi, which is found in some third-generation mobile phones, on the number of dentate gyrus granule cells in rat brain hippocampus in the prenatal period.

2. Material and Methods

2.1. Specimens

Ethical permission required for the study was obtained by Ethic Committee from Van Yüzüncü Yıl University, with the decision numbers 2016/04 and 05/05/2016. Experimental materials were obtained from the Experimental Animals Unit of Van Yüzüncü Yıl University.

The study was conducted in the stereology laboratory of the Department of Medical Histology and Embryology, Faculty of Medicine, Yüzüncü Yıl University. Nine healthy female and three healthy male Wistar albino rats weighing an average of 220 g were randomly used during the reproductive period. Appropriate room temperature and humidity, normal tap water and standard rat feed were given in a light/dark environment. Three female rats and one male rat were taken into separate plexiglass (polypropylene) cages for mating. Male rats were kept in cages for 48 hours for mating and were then removed from the cages. The female rats were weighed daily for one week. Those with an increase in their weight were accepted as pregnant; only rats that were pregnant were included in the study. In the first and second cages (Group A), three rats were exposed to Wi-Fi for 1 hour/day in a lead-coated private room completely isolated from external EMF radiation; the control (Group B) groups were not exposed to Wi-Fi. After four weeks, six of the new-born pups were selected as the control group and six were used as the experimental group (Table 1).

2.2. Methods

For Wi-Fi exposure during pregnancy, for 1 hour per day, the pregnant rats were placed in the middle of a 33 x 60 x 20 cm3 standard rat cage (Fig. 1). TP-link, TD-W8961N model with a multi-directional fixed antenna system, a 2.45 GHz radio frequency, an operating voltage of 9V and a working current of 0.85A; the exposure to Wi-Fi modem devices occurred at a maximum bandwidth of 300 Mbps (Fig. 2). Throughout the operating time, the frequency verification of the RF waves from the Wi-Fi system was performed using the Good Will GSP-730 150 KHz ~ 3 GHz Spectrum Analyser (Fig. 3). The frequency stabilization of the EMF waves was checked.

After birth, the pups were fed with breast milk until they were approximately one month old. On postpartum day 30, to all rats with 50 mg/kg of Ketamin was administered to six randomly selected male and female rats, which were anesthetised using deep anaesthesia. Transcardiac perfusion was then performed from the left heart via a cannula. For perfusion, general anaesthetics were provided to the rats by intraperitoneal ketamine injection, and the rats were fixed to the operating table in the supine position. The rats were

divided into two by a mid-sagittal incision of the sternum, the diaphragm was released and the rib cages were opened. A three-cannulated syringe needle was inserted 2–3 mm into the left ventricle. Then, 0.5 mg heparin was administered, and a 2–3 mm incision was made in the right atrium to drain the blood. When the blood started to discharge, saline was given; specifically, 100 ml of 0.9% NaCl solution was administered until the fluid coming out of the right atrium became clear (within 5–8 min). After clarification, 10% neutral buffered formaldehyde solution was administered. Muscle twitching was observed with the introduction of fixation fluid so that the fixative reached the tissues and perfusion was maintained by the neutral buffered formaldehyde. After perfusion, the cervical dislocation method was used, and the rats were decapitated. With the help of clamps, the head bones were broken from the superstructure in small dimensions, and their brains were made visible. Brains were excised of the brainstem level (Fig. 4).

Fig. 1. Wi-Fi application setup

Fig. 2. 300Mbps Wireless N ADSL2 + Modem Router

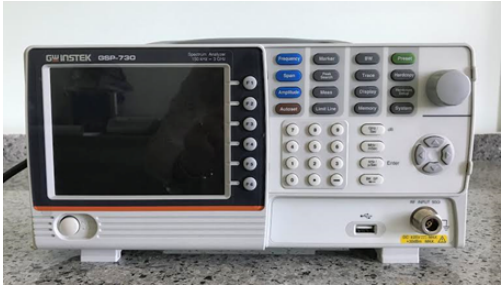

Fig. 3. Spectrum Analyzer

The brains obtained from the experimental and control groups were kept in 10% buffered formaldehyde for at least 72 hours, and the left hemispheres were separated for routine fixation, follow-up and embedding using histological methods. The frontal sections were taken using a rotary microtome (Leica RM 2125, Leica instrument, Nussloch, Germany), and each 45th section of the paraffin blocks (5 µm thick) was placed on a slide and then in an oven. Sections stained with haematoxylin and eosin (H&E) and cresyl violet were dropped onto the coverslip with entellan and allowed to dry. Stereological, the dentate gyrus granule cell count used a combination of the disector method and Cavalieri's principle. The Shtereom 1.5 software as used for volume measurement (Fig. 5). The results were compared statistically.

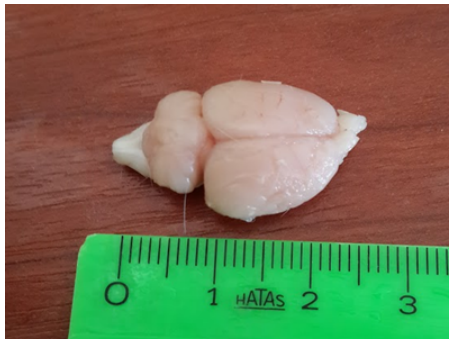

Fig. 4. Whole rat brain for one month

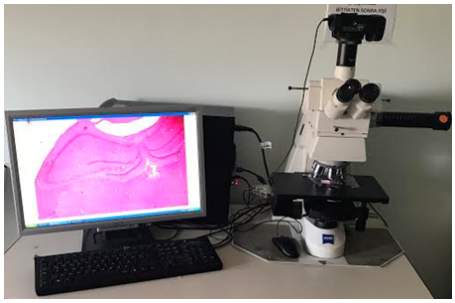

Fig. 5. Counting light microscope with camera and computer

2.3. Stereology for light microscopy

Brain tissue samples were first detected in 10% formaldehyde solution using light microscopy. After fixation, the tissue samples were placed in cassettes and washed under under a stream of water. In the follow-up process, tissues were passed through an increasing series of alcohols to remove water, passed through a xylene series for clarification and embedded in molten paraffin. Sections of 5 µm thickness obtained from prepared paraffin blocks were stained with H&E and cresyl violet. The sections were evaluated and photographed using the ZEISS (Göttingen, GERMANY) computer-assisted imaging system AxioVision 4.8 (Figs. 5–7). The approximate brain weights (g) of the control and experimental group animals were measured. Sections taken from the all experimental groups were kept in an oven at 80°C for about 30 minutes; then, xylene was taken for 5 minutes three times and paraffin-free. The tissues were then passed through a decreasing series of alcohols for 5 minutes three times. After washing in the stream of water, and stained in Harris haematoxylin for 1–3 minutes and the slides were washed in the stream of water. The Slides were then immersed in

ammonia solution three times and washed until turbidity disappeared. The slides were kept in eosin for 2–5 minutes, washed again in the stream of water and then passed through the alcohol and xylene for 5 minutes; they were sealed by dropping them into Entellan. The combination of the disector method and Cavalieri's principle was used for the calculation of the number of granule cells (27, 28). In particular, the physical dissector counting method was used in our study (29).

Our formula as: $N = \Sigma p \times \bar{Q}^{-} \times k \times \frac{(a/p)}{a(frame)}$

N = Total number of particles; ∑ p = Total number of points; $\bar{Q}^{-}$ = Average numbers of disector granule neuron counts; k = Section thickness: 5µm; a/p = Area represented by a point: 5625 µm²; a frame = Unbiased counting frame area: 1.525,68 µm²

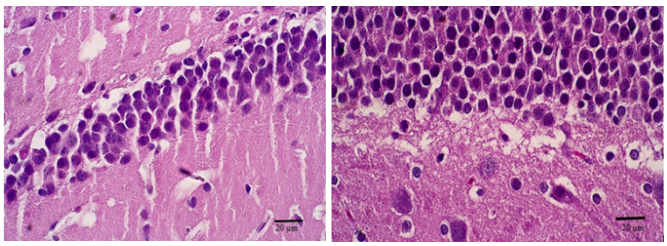

Fig. 6. Dentate gyrus granule cells (Wi-Fi) (H&E) (x63)

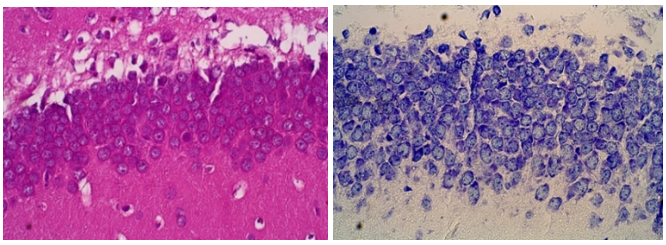

Fig. 7. Dentate gyrus granule cells (Control) (H&E) (x63) and (Cresyl Viole) (x63)

2.4. Calculation of error and coefficient variance

According to the standard stereological approach, the coefficient of variation (CV) and coefficient of error (CE) are taken into consideration to determine the optimal sample size in each group. The following formula was used to calculate the CE (29, 30). The stereological estimation values based on our results are given in Table 1.

Table 1. Calculation of error coefficient (CE) in a sample

Section	Q	Qi.Qi	Qi.Qi+1	Qi.Qi+2
1	25	625	950	250
2	38	1444	380	532
3	10	100	140	180
4	14	196	252	98
5	18	324	126	270
6	7	49	105	0
7	15	225	0	0

NOISE:127 VAR (srs):8.441666667 Total Var:135.4416667
CE:0.091637329 % NOISE: 93.7673045

2.5. Statistical analysis

Data were compared non-parametrically using the Mann–Whitney U test (Table 2) ($p > 0.05$). The statistical significance level was set at 5%, and the SPSS statistical package program was used for the calculations.

3. Results

In this study, the dentate gyrus granule cells of one-month-old offspring of both control rats and subject rats exposed to Wi-Fi during pregnancy were counted (Fig. 1–2). The mean brain weights of the control group were 1.08 ± 0.12 g and of the experimental group rats were 1.15 ± 0.18 g. The difference in the total number of granule cells between the subjects and controls was not statistically significant. Therefore, the one-sample Kolmogorov–Smirnov test was not required for the distribution of the data. Data were compared non-parametrically using the Mann–Whitney U test. There was no statistically significant difference between the two groups in terms of N values ($p > 0.05$) (Table 2) or granule cell numbers (CE and CV values) (Table 3).

Table 2. Descriptive statistics about the total number of granule neurons

	N	Medyan	Mean	SEM	Min.	Max.	P
Control	6	1246173	1541285,17	835819,357	654868	2890018	
Wi-Fi	6	1941799	1861036,67	1019873,304	727315	3435868	0.566
Total	12		1701160,92	904554,176	654868	3435868	

4. Discussion

Wireless Internet is widely used in schools, hospitals, libraries, cafeterias and homes. There are a limited number of studies reporting the effects of this radiation interval on the human system (31). However, there is no detailed study investigating the effects of wireless Internet-induced EMFs on foetal development. This study was conducted to investigate the prenatal effects of EMF exposure associated with wireless Internet use in pregnant rats using a stereological method. In our study, the number of dentate gyrus granule cells in the brain hippocampus of postnatal rat pups exposed to Wi-Fi as foetuses was calculated using the stereological method.

The limbic system is the action system of the brain, which consists of a group of nuclei connected to each other for the regulation of memory and mood. This neuron system evaluates rewards and has an important place in causing motivation. The limbic system plays a role in the autonomic functions of the brainstem, the combination of conscious and unconscious behaviours, the facilitation of the retrieval of information and the biological rhythm. The hippocampus, which is one of the most important parts of the limbic system,

takes part in organizing and recalling memories (32, 33). The hippocampus is seen to be more related to long-term memory and is responsible for the transformation of short-term memory into a long-term state (34). Many stimuli from the cortical center are introduced through the entorhinal cortex; the stimuli are transmitted to the dentate gyrus—which is a thin, scalloped cortical strip—and from there to the hippocampus. Thus, the dentate gyrus serves as a station for the transfer of information to the hippocampus (35).

Table 3. Control and Wi-Fi granule cells number, CE and CV values

Control	N	N-CE	Wi-Fi	N	N-CE
1- Control	1.012.140	0.08	1- Wi-Fi	3.435.868	0.05
2- Control	1.279.756	0.07	2- Wi-Fi	1.748.736	0.06
3- Control	1.212.589	0.07	3- Wi-Fi	727.315	0.07
4- Control	2.198.340	0.07	4- Wi-Fi	2.323.301	0.06
5- Control	2.890.018	0.07	5- Wi-Fi	796.138	0.07
6- Control	654.868	0.09	6- Wi-Fi	2.134.862	0.05
Mean	1.541.285	0.07	Mean	1.861.036	0.06

CV Control groups: 0.49 CV Wi-Fi groups: 0.5

It has been reported that prolonged exposure to a low-intensity EM field has effects on cancer formation, biomolecule synthesis and cell division in both physiological and biochemical cells and tissues. It has been observed that as a result of such exposure, hormones are affected; carbohydrate, nucleic acid and protein metabolism are different; cellular respiration is decreased; structural changes occur; tissues' and cells' hormonal responses are changed; and immune responses to different antigens are affected (3).

Radioactive waves can adversely affect brain structure and physiology by damaging cell membranes and organelles at both the cellular and molecular levels. Daniels et al. (2009) exposed rat brains to EMF resonance waves, observing that locomotor activity decreased, basal corticosterone levels increased and neuronal damage and abnormal brain functioning occurred (8). Wilen et al. (2006) showed that increased sympathetic activity occurred with exposure to EM waves (9). In a study of rat astrocytes and capillary endothelial cells in porcine brains conducted by Schirmacher et al. (2000), cells were exposed to a 1.86 GHz EMF (4). Another study found that the increase in blood–brain barrier permeability was significantly higher in cells exposed to EMF compared to the control group (10). As our study was not conducted at the molecular level, the findings cannot be compared. Odacı et al. (2008) showed that EMFs inhibit the formation and differentiation of neural stem cells during embryonic development and showed a statistically significant decrease in the number of dentate gyrus granule cells of prenatal rats exposed to an EMF (900 MHz) ($p < 0.01$) (36). Rağbetli et al. (2010) and Sonmez et al. (2010) exposed female rats to a 900 MHz wave frequency EMF and evaluated the number of Purkinje cells in the cerebellum that was it found that the number of Purkinje cells was decreased in rats exposed to an EMF (37-40). This may be due to the fact that the distance between the antenna and the subjects was as small as 1 cm and due to the difficult of keeping the subject's distance from the antenna constant in a narrow area.

Schmitz et al. (2002) observed a decrease in the number of hippocampal granule cells due to prenatal stress in female rat hippocampus (41). Alchalabi et al. (2016) reported that prolonged exposure to EMFs during pregnancy leads to chronic stress and has adverse effects on prenatal and postnatal development (42). These probable effects were not observed in our study because there was no long-term exposure. In addition, the stress factor was not relevant because the animals were free to roam about in our experimental setup.

Sangun et al. (2015) observed postnatal growth restriction and puberty delay in rats—especially female rats—exposed to an EM field of 2450 MHz in the prenatal period (5). They also found that total oxidant state and oxidative stress index values increased in brain and ovarian tissues. Tök et al. (2014) reported that melatonin has beneficial effects on lipid peroxidation and the regulation of TSH values in rats exposed to Wi-Fi (2.45 GHz; 60 min/day) (41). In our study, we could not compare with biochemical results because only the number of dentate gyrus granule cells of the hippocampus was calculated.

In contrast to the mentioned effects of EMFs, some studies have reported that EMFs have no harmful effects on biological systems and tissues (11). Takahashi et al. (2010), for example, reported that whole-body exposure to a 2.14 GHz EMF during pregnancy and lactation did not have any negative effects on the development of the pregnancy or of the rat pups themselves (42). Ohtani et al. (2015) observed that long-term exposure to RF EMFs in developing rats had no adverse effects on the number and activation of T cells (43). Rağbetli et al., (2010) observed no change in cerebellum granule cell count in postnatal offspring of mice exposed to cell phones (890–915 MHz) during pregnancy (37). Sambucci et al. (2010) did not observe any effect on prenatal effects in mice exposed to Wi-Fi signals during pregnancy (44). Rağbetli et al. (2009) observed no change in the number of hippocampal pyramidal cells in prenatal mice exposed to cell phones (890–915 MHz) (45). Our study results are similar to these results. However, in our study, the prenatal effect was examined differently.

In humans, granule cells begin to develop in the sixth week of the embryonic period (46). In rats, the primary critical period occurs from the last week of pregnancy to the

tenth day after birth (47). In this study, the application of the EMF was based on this period. Prenatal administration was performed, and the rats were expected to be one month old at the time of their evaluation. Therefore, there was no loss or error in terms of application time. According to our results, no statistically significant difference was observed in the number of hippocampus dentate gyrus granule cells.

In the prenatal period, it was observed that Wi-Fi increased the number of granule cells, but this was not statistically significant. Therefore, the wireless modem had no anyone affect. The distance of the Wi-Fi modem device to the subjects was 33 cm in diameter, and no significant effect was observed due to the presence of an EMF at this distance. No histopathological effect of Wi-Fi on the hippocampus was observed. It can be said that the Wi-Fi effect decreases as you move away from the modem device. This is especially true for distances greater than 10 cm.

A Wi-Fi device with multi-directional antenna system was used (TP-link, TD-W8961N model, 2.45 GHz radio frequency, 9V operating voltage, 0.85A operating current, 300 Mbps maximum bandwidth and values conforming to international standards). It is possible that no negative effects were observed because the Wi-Fi system used complies with certain requirements and standards. As such, the results of the current study, and those of other genetic, biochemical and physiological studies, should be supported with further research. Another reason why Wi-Fi has no numerical effect on the hippocampus dentate gyrus granule cells in stereological and statistical terms may be due to the fact that the RF radiation from Wi-Fi cannot directly lead to ionization. The RF waves used in this study are non-ionizing frequencies.

Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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Ethical Approval

Ethical permission required for the study was obtained by Ethic Committee from Van Yüzüncü Yıl University, with the decision numbers 2016/04 and 05/05/2016. Experimental materials were obtained from the Experimental Animals Unit of Van Yüzüncü Yıl University.

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Authors' contributions

Concept: M.Ç.R., T.Ç., Design: M.Ç.R., T.Ç., Data Collection or Processing: B.Ö., Analysis or Interpretation: B.Ö., Literature Search:B.Ö., Writing: M.Ç.R., B.Ö., T.Ç.

References

1. Sırav B, Seyhan N. Effects of GSM modulation radio-frequency radiation on permeability of blood-brain barrier in male and female rats. Journal of Chemical Neuroanatomy. 2016; 75(123-127.
2. Ahlbom IC, Cardis E, Green A, Linet M, Savitz D and Swerdlow. AReview of the epidemiological literature on EMF and health. Environ Health Perspect. 2001; 109, 6, 911-933.
3. Seyhan N. Elektromanyetik Kirlilik Ve Sağlığımız. Nöropsikiyatri Arşivi. 2010; 47: 158-61.
4. Schirmacher A, Winters S, Fischer S, Goeke J, Galla HJ, Kullnick U. et al. Electromagnetic fields (1.8 GHz) increase the permeability to sucrose of the blood-brain barrier in vitro. Bioelectromagnetics. 2000; 21, 5: 338-345.
5. Sangun O, Dundar B, Darici H, Comlekci S, Kumbul Doguc D, Celik S. The effects of long-term exposure to a 2450 Mhz electromagnetic field on growth and pubertal development in female wistar rats. Electromagn Biol Med. 2015; 34, 1: 63–71.
6. Foster, K.R., Moulder, J.E. Wi-Fi and health: review of current status of research.Health Phys. 2013; 105: 561–565.
7. Pall ML. Wi-Fi is an important threat to human health. Environmental Research. 2018; 164 405–416.
8. Daniels WM, Pitout IL, Afullo TJ and Mabandla MV. The effect of electromagnetic radiation in the mobile phone range on the behaviour of the rat. Metab Brain Dis. 2009; 24, 4: 629-641.
9. Wilen J, Johansson A, Kalezic N, Lyskov E and Sandstrom M Psychophysiological tests and provocation of subjects with mobile phone related symptoms. Bioelectromagnetics. 2006; 27, 3: 204-214.
10. Kuloğlu M ve Korkmaz S. Cep telefonu ve baz istasyonlarının nöropsikolojik etkileri. New Symposium Journal. 2011; 49, 2: 99-105.
11. Chung MK, Lee SJ, Kim YB, Park SC, Shin DH, Kim SH et al. Evaluation of spermatogenesis and fertility in F1 male rats after in utero and neonatal exposure to extremely low frequency electromagnetic fields. Asian J Androl. 2005; 7, 2: 189-94.
12. Sunay Ç. Teknolojiyle birlikte gelen sorun elektromanyetik kirlilik. Bilim ve Teknik Dergisi.2000; Ocak, 386.
13. Hardell L, Mild Kh, Carlberg M and Söderqvist F. Tumour risk associated with use of cellular telephones or cordless desktop telephones. World Journal of Surgical Oncology. 2006; 4: 74.
14. Khurana V, Moulder J and Orton C. There is currently enough evidence and technology available to warrant taking immediate steps to reduce exposure of consumers to cell-phone-related electromagnetic radiation. Med. Phys. 2008; 35, 12: 5203-5206.
15. Röösli M, Lörtscher M, Egger M, Pfluger D, Schreier N, Lörtscher E et al. Mortality from neurodegenerative disease and exposure to extremely low-frequency magnetic fields: 31 years of observations on Swiss railway employees. Neuroepidemiology.2007; 28 (4) pp. 197-206.
16. He WB, Zhang JL, Hu JF, Zhang Y, Machida T, Chen NH Effects of glucocorticoids on age-related impairments of hippocampal structure and function in mice. Cell Mol Neurobiol.2008; 28, 2, 277–291.
17. Kundi M. The controversy about a possible relationship between mobile phone use and cancer. Environ Health Perspect.2009; 117, 3, 316–24.

18. Khurana V, Teo C, Kundi M, Hardell L, Carlberg M. Cell phones and brain tumors: a review including the long-term epidemiologic data. Surg. Neurol. 2009; 72, 205–214.

19. Baan R, Grosse Y, Lauby-Secretan B, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L. et al. Carcinogenicity of radiofrequency electromagnetic fields. Lancet Oncol. 2011; 12, 7: 624–6.

20. Balıkçı K, Serhatlioğlu I, Kutlu S, Poyraz M. Effect of electromagnetıc field exposure in vivo on catecholaminergic neurotransmitter concentrations in brain tissues in rats. Medical Sciences.2016; 11(3): 1-8.

21. Kiray M. Elektromanyetik alana maruz kalan sıçan beyin dokusunda oksidatif stres üzerine karnitinin immunohistokimyasal ve yapısal etkileri. Dokuz Eylül Üniversitesi Sağlık Bilimleri Enstitüsü, Histoloji ve Embriyoloji Anabilim Dalı, Doktora Tezi, İzmir.2010.

22. Lai H, Singh NP. Acute exposure to a 60 Hz magnetic field increases DNA strand breaks in rat brain cells. Bioelectromagnetics. 1997; 18:156–165.

23. Türkiye Atom Enerjisi Kurumu. Radyasyon, İnsan ve Çevre. Ankara.2009.

24. Odacı E, Hancı H, İkinci A, Sönmez O.F, Aslan A, Şahin A, Kaya H, Çolakoğlu S, Baş O. Maternal exposure to a continuous 900-MHz electromagnetic field provokes neuronal loss and pathological changes in cerebellum of 32-day-old female rat offspring. Journal of Chemical Neuroanatomy. 2016; Volume 75, Part B, Pages 105-110.

25. Çelik Ö, Kahya MC, Nazıroğlu M. Oxidative stress of brain and liver is increased by Wi-Fi (2.45GHz) exposure of rats during pregnancy and the development of newborns. J. Chemical. Neuroanatomy.2016; 75(Pt B):134-9. doi: 10.1016/j.jchemneu.2015.10.005. Epub 2015 Oct 28.

26. Sahin D, Ozgur E, Guler G, Tomruk A, Unlu I , Sepici-Dinçel A, Seyhan N. The 2100 MHz radiofrequency radiation of a 3G-mobile phone and the DNA oxidative damage in brain Journal of Chemical Neuroanatomy.2016;75: 94–98.

27. Gundersen HJ and Jensen EB. The efficiency of systematic sampling in stereology and its prediction. J Microsc.1987; 147,

28. West MJ, Gundersen HJG. Unbiased stereological estimation of the number of neurons in the human hippocampus. J Comp Neurol. 1990; 296: 1–22.229-63.

29. Howard CV and Reed MG. Unbiased Stereology Three-Dimensional Measurement in Microscopy. BIOS Scientific Publishers, Oxford, UK.1998.

30. Gundersen HJG, Bendtsen TF, Korbo L, Marcussen N, Moller A, Nielsen K, Nyengaard JR, Pakkenberg B, Sorensen FB, Vesterby A and West MJ. Some new simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS. 1988; 96, 5, 379-394.

31. Tok OE ve Ercan F. Cep Telefonlarının Yaydığı Elektromanyetik Dalgaların Sıçan Testis Morfolojisi Üzerine Etkileri. Marmara Üniversitesi Sağlık Bilimleri Enstitüsü Dergisi.2013; 3, 3:138-144.

32. Aktümsek, A. Anatomi ve Fizyoloji (İnsan biyolojisi). Nobel yayın dağı-tım. Ankara.2001.

33. Solomon EP. İnsan Anatomisi ve Fizyolojisine Giriş. Ertuğrul L (Editör). Akademi Basın ve Yayıncılık, 2. Baskı, İstanbul.2008.

34. Albayrak A, Uçak Erden H, Erkal S, Köşgeroğlu N ve Ançel G. Nörolojik Bilimler Hemşireliği. Alter Yayıncılık, Ankara.2006.

35. Waxman SG. Korrelatif Nöroanatomi. Yıldırım M (Editör). Nobel Tıp Kitabevleri, İstanbul.2002.

36. Odacı E, Baş O and Kaplan S. Effects of prenatal exposure to a 900 Mhz electromagnetic field on the dentate gyrus of rats: a stereological and histopathological study. Brain Research. 2008; 1238, 224-229.

37. Rağbetli M.Ç., Aydinlioglu A., Koyun N., Rağbetli C., Bektas Ş., Ozdemir S. The effect of mobile phone on the numberof Purkinje cells: A stereological study, Internatıonal Journal of Radiation Biology. 2010; vol.86, pp.548-554.

38. Sönmez OF, Odacı E, Baş O and Kaplan S. Purkinje cell number decreases in the adult female rat cerebellum following exposure to 900 Mhz electromagnetic field. Brain Research.2010; 1356, 95-101.

39. Schmitz C, Rhodes ME, Bludau M, Kaplan S, Ong P, Ueffing I, Vehoff J, Korr H and Frye CA. Depression: Reduced number of granule cells in the hippocampus of female, but not male, Rats due to prenatal restraint stres. Molecular Psychiatry. 2002; 7, 7: 810-3.

40. Alchalabi ASH, Aklilu E, Aziz AR, Malek F, Ronald SH and Khan MA. Different periods of intrauterine exposure to electromagnetic field: Influence on female rats' fertility, prenatal and postnatal development. Asian Pacific Journal of Reproduction. 2016; 5, 1: 14–23.

41. Tök L, Nazıroğlu M, Doğan S, Kahya MC, Tök Ö. Effects of melatonin on Wi-Fi-induced oxidative stress in lens of rats. Indian Journal of Ophthalmology. 2014; 62, 12-15.

42. Takahashi S, Imai N, Nabae K, Kanako K, Kawai H, Wang J et al. Lack of Adverse Effects of Whole-Body Exposure to a Mobile Telecommunication Electromagnetic Field on the Rat Fetus. Radiation Research. 2010; 173, 3: 362-372.

43. Ohtani S, Ushiyama A, Maeda M, Ogasawara Y, Wang J, Kunugita N et al. The effects of radio-frequency electromagnetic fields on T cell function during development. J Radiat Res. 2015; 56, 3: 467-474.

44. Sambucci M, Laudisi F, Nasta F, Pinto R, Lodato R, Altavista P. et al. Prenatal exposure to non-ionizing radiation: effects of WiFi signals on pregnancy outcome, peripheral B-Cell compartment and antibody production. Radiation Research. 2010 732-740.

45. Rağbetli MÇ, Aydinlioğlu A, Koyun N, Rağbetli C and Karayel M. Effect of prenatal exposure to mobile phone on pyramidal cell numbers in the mouse hippocampus: A stereological study. International Journal of Neuroscience. 2009; 119, 7, 1031-1041.

46. Moore KL, Persaud TVN and Torchia MG. The Developing Human Clinically Oriented Embryology. Elsevier Saunders, 9th Edition, Canada. 2013.

47. Hines M. Prenatal testosterone and gender-related behaviour. European journal of endocrinology. 2006; 155, 1: 115-121.