Pyramidal Cell Loss in the Cornu Ammonis of 32-day-old Female Rats Following Exposure to a 900 Megahertz Electromagnetic Field During Prenatal Days 13–21

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ABSTRACT

The number of studies reporting that the electromagnetic field (EMF) emitted by mobile phones affects human health is increasing by the day. In previous studies we reported that a 900 megahertz (MHz) EMF applied throughout the prenatal period reduced the number of pyramidal cells in the cornu ammonis of rat pups in the postnatal period. In this study we investigated the effect of a 900 MHz EMF applied on days 13-21 of the prenatal period on the number of pyramidal cells in the cornu ammonis of rat pups in the postnatal period. For that purpose, pregnant rats were divided into experimental and control groups. Experimental group pregnant rats were exposed to the effect of a 900 MHz EMF on days 13-21 of pregnancy. No procedure was applied to the control group. Newborn female rat pups were added to the study, and no procedure was performed on these after birth. Five newborn female rats were obtained from the experimental group and six from the control group. All female rat pups were decapitated on the postnatal 32nd day, and histological procedures were performed on the brain tissues. Sections were stained with Cresyl fast violet. The optical dissector technique was used to estimate the total number of pyramidal cells in the cornu ammonis. Sections of cornu ammonis were subjected to histopathological evaluations. Our results showed that exposure to 900 MHz EMF during prenatal days 13-21 led to a significant decrease in the number of pyramidal cells in the cornu ammonis of the experimental group female rat pups ($P<0.05$). Histopathological examination revealed picnotic cells in the cornu ammonis in experimental female rat pups. The pyramidal cell loss in the cornu ammonis may therefore be attributed to exposure to 900 MHz EMF in days 13-21 of the prenatal period.

Key Words: pyramidal cell, cornu ammonis, newborn female rats, electromagnetic field, stereology

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Introduction

Although the number of studies reporting the adverse effects on living tissue and human health of the electromagnetic field (EMF) emitted by mobile phones is growing by the day (Mausset *et al.*, 2001; 2004; Salford *et al.*, 2003; Hancı *et al.*, 2013), widespread advertizing campaigns are constantly reinforcing the idea that such phones are harmless. However, this does not alter the fact that intracranial structures can be affected by the EMF emitted by mobile phones, the most important communications tool of our day. Mobile phones are used in close proximity to the brain. Studies have shown that EMF can cause cell losses in the brain and brain-associated structures, and that functional losses

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can appear as a result (Bas *et al.*, 2009a; 2009b).

Decreases in neuron numbers and neuronal damage in the cortex, cerebellum, hippocampus and basal ganglia in the brains of animals exposed to 900 megahertz (MHz) EMF have been reported in several studies (Mausset *et al.*, 2001; 2004; Salford *et al.*, 2003). One study on the subject using stereological techniques reported that EMF resulted in a decrease in pyramidal cells in the adult female rat hippocampus (Bas *et al.*, 2009a). Some studies have also reported that EMF applied in the prenatal period causes a decrease in the number of pyramidal cells in the cornu ammonis of newborn rats (Bas *et al.*, 2009b), and affects granular cell development, and that the cellular loss arising due to EMF can inhibit neuron development (Odaci *et al.*, 2008). However, the effect of exposure to EMF on the development of the CNS, and particularly in terms of prenatal development during days 13-21, is still unclear.

The optical dissector technique is a stereological method used in the calculation of numbers of countable structures (cells, glomerules, etc.) at the microscopic level and described as unbiased and effective (Bas *et al.*, 2009a; 2009b; Aktürk *et al.*, 2013; İkinci *et al.*, 2013). The method makes it possible to obtain numerical data for an entire structure on the basis of numerical data from a small part selected using systematic random sampling (Sahin *et al.*, 2002; Odaci *et al.*, 2004; Tunc *et al.*, 2007; Kaplan *et al.*, 2012a; 2012b; İkinci *et al.*, 2013; Aktürk *et al.*, 2013). The optical dissector method makes it possible to obtain an unbiased estimate of any numerical quantity irrespective of factors arising from causes such as particle size and distribution, orientation of particles, section thickness, tissue shrinkage or swelling. These attributes make the optical dissector techniques efficient and bias-free for calculating particle numbers in tissue sections at the microscopic level (Kaplan *et al.* 2012a; 2012b). We therefore used the optic dissector method in this study in the calculation of pyramidal cell numbers in the hippocampus.

There are two main types of cell in the hippocampus, pyramidal and granular. Most pyramidal cells are found in the cornu ammonis and develop on the 19th embryonic day (Rodier, 1980; Weinstock, 2001; Sakatani *et al.*, 2002). Various agents that induce cell death in the prenatal period, such as drugs, chemicals and inadequate nutrition, will also prevent the differentiation of neural stem cells into neurons (Tunc *et al.*, 2007). This will lead to compromise of neurogenesis and therefore to impairments in development in the adult brain (Salford *et al.*, 2003). Studies show that EMF may be one such agent. The purpose of this study was therefore to determine whether or not the cerebral development of female rat pups was affected by exposure to the effect of EMF on days 13-21 in the prenatal period. Number of pyramidal cells in the hippocampus of the female rat pups in the study was calculated using the optic dissector method.

Material and methods

Animals, groups and experimental procedures

The first part of the experiment involved 16 male and 16 female Wistar albino rats (the latter exhibiting two regular cycles, aged 6-8 weeks and weighing 180-250 g). These were provided by the Karadeniz Technical University Surgery Research Center (KTUSRC). Rats were allowed to acclimatize for 48 h on arrival at the laboratory. Male and female rats were housed separately in plastic cages during this time. Rats were kept in standard plastic cages on sawdust bedding in a 12:12 h light/dark cycle in a temperature-controlled animal room (22 ± 1 °C) in the laboratory. Ad libitum access to food – a commercially balanced diet – and water was permitted. Rats were rested on the same day and then mated separately overnight. The females were separated from the males the following morning. Vaginal smears were performed in order to identify pregnant animals. Rats with sperm in their smear specimens were regarded as pregnant. That day was designated as gestation day 0. Six animals were classified as pregnant and these were moved into individual cages for the duration of the gestation period. Pregnant rats were divided at random into two groups of three members each, the experimental and control groups. EMF of 900 MHz was applied to the experimental group for 60 min/day, 2:30-3:30 p.m., on days 13 to 21 of the gestation period. With the exception of exposure to EMF, the groups were kept in different cages in the same room throughout the experiment. An EMF exposure room designed for the purpose was used for the application of EMF. Six female pups were selected at random from the control group (2, 1, and 3 pups from control group rats

1, 2, and 3, respectively) and five from the experimental rats (2, 2, and 1 pups from EMF rats 1, 2, and 3, respectively).

Surgical procedures were performed in the KTUSRC, and histological procedures at the Karadeniz Technical University Medical Faculty Histology and Embryology Department Laboratory. All rats were weighed on postnatal day 32 sacrificed on the same day by decapitation under deep anesthesia (Ketalar 50 mg/kg). The brains were removed and weighed on a sensitive scale. These were then processed through graded alcohols and xylene and fixed in paraffin. Paraffin sections from the hippocampus were taken using a fully automatic microtome (Leica RM 2255, Leica Instruments, Nussloch, Germany). Disposable metal microtome blades (Type N35, Feather Company, Osaka, Japan) with a cutting (knife bevel) angle of approximately 5° were used to obtain 30-µm-thick serial sections in a coronal plane from the blocked tissues. Sections were sampled using the optical dissector counting technique representing a combination of systematic random sampling and the optical dissector method (West *et al.*, 1991; Bas *et al.*, 2009a; 2009b; Kaplan *et al.*, 2012a; 2012b). Each sampled section of brain hemisphere including hippocampus was placed on slides coated with a gelatin-formaldehyde mixture and stained with Cresyl violet. A research light microscope (Olympus, BX51, Japan) was used to perform histopathological examination of the stained sections. Photographs were obtained using an Olympus DP 71 (Japan) camera microscope in the Histology and Embryology Department stereology laboratory. All experiments were performed in accordance with institutional guidelines. The Local Animal Ethics Committee of Karadeniz Technical University approved the protocol and pain or discomforts were minimized as much as possible.

EMF exposure room and application of the electromagnetic field

In order to expose the experimental group to EMF, a special chamber known as the EMF exposure room, used solely for the application of EMF, was prepared in the KTUSRC. Throughout the experiment, no rat was placed inside this room apart from members of the experimental group. Control group and experimental group rats were kept in the same environment apart from during exposure to EMF. The apparatus referred to as the EMF exposure system was prepared in the EMF exposure room. The EMF exposure room and the EMF exposure system it contained were also used in other studies of ours. In brief, the EMF exposure system consisted of an ultra-high-frequency oscillator (1218-BV, Lockable Oscillator, 900–2000 MHz, General Radio Company, Concord, Massachusetts, USA, Serial No. 1483), an uninterrupted power source (1267-B Regulated Power Supply, General Radio Company, Concord Massachusetts, USA, Serial No. 903) (with output power of approximately 300 mW and a frequency adjusted to 900-MHz) and a glass jar produced specially for the study made of Plexiglas (30 cm X 42 cm X 52 cm). The oscillator was attached to a half-wave dipole antenna made from a 1 mm x 15 cm copper rod using a coaxial cable. The antenna was placed inside the central area, approximately 11 cm inside the open surface of the jar. Experimental group rats were placed inside the jar, after which a 900-MHz EMF was applied for 1 h (from 2:30 p.m. to 3:30 p.m. each day). Positional averaging of electrical field intensity was calculated by one of the co-authors (Haydar Kaya) with the help of a wide-range measuring device with a measurement range of 100 kHz-2.5 GHz (Chauvin Arnoux CA43 Isotropic Electrical Field Intensity Meter). The experimental group was exposed to a mean electrical field intensity of 10 V/m inside the jar (0.265 W/m^2). This intensity is similar to the electrical field created in their immediate vicinity by mobile phones in speak mode (mean 1–10 V/m for variables such as model and location of mobile phone, distance from the base station, etc.). These represent the limit values for a single source set out in the Global System for Mobile Communications (GSM)-900 base station systems (Bas *et al.*, 2009a; 2009b; Odaci *et al.*, 2008; Sonmez *et al.*, 2010; Hancı *et al.*, 2013).

Design-based stereological analyses

Stereological analyses were performed blind by one of the co-authors (Orhan Baş). The optical dissector technique (Hausdorf *et al.*, 2008; Kaplan 2012a; 2012b) was employed to estimate the total number of pyramidal cells in the cornu ammonis using a stereological workstation. Stereology workstation system involved Leica stereology software and equipment. The system consists of a computer (Pentium PC, DELL OptiPlex, USA) containing

stereology analysis software Stereo Investigator 9, Computer Assisted Stereological Toolbox-Leica) designed for stereological research by MicroBrightField (USA), a research microscope (Leica, DM4000B-M, Germany), a CCD camera (JVC, Japan), a measuring rod measuring on the Z axis (Heidenhain LIP401 R, Germany), a microcator indicator (Heidenhain ND 221 B, Germany), a computer-controlled step motor whose movements along the microscope try X and Y axes are controlled by PC and a tray joystick (Prior, USA). The sampling and counting schedule of the study sections was determined on the basis of a pilot study. Briefly, in the series to be analyzed was randomly selected. Thereafter, every successive seventh section was taken from the series, giving a 1/7 section-sampling fraction (ssf). Approximately 12-18 sections from each brain are known to be sufficient for estimating total neuron numbers in the optical dissector method for neuron counting (Gundersen and Jensen, 1987; West *et al.*, 1991; Tunc *et al.*, 2007). Once the first section in the series had been selected, section sampling commenced from a random point (between 1 and 7) with alternate sections being collected as reserves. The efficiency of sampling and the appropriate convenient number of sampled cells for total neuron number estimation were corroborated using coefficient of error (CE) and coefficient of variation (CV) (Gundersen and Jensen, 1987; West *et al.*, 1991; Bas 2009b; Odaci *et al.*, 2010). All details of the counting procedure are given in Table 1.

Table 1. Mean values of rats' total pyramidal cell numbers, body and brain weights, CV and CE of stereological analysis and details of the counting procedure for estimation of total pyramidal cell number in the cornu ammonis for control and experimental group rats at 32 days of age.

	Control group pups (n=6)	Experimental group pups (n=5)
Total pyramidal cell number (Mean ± SEM)[a]	513.890 ± 9.960	451.100 ± 17.700*
Body weight (Mean ± SEM)	60.308 ± 0.481	59.900±0.510**
Brain weight (Mean ± SEM)	1.090 ± 0.0151	1.124 ± 0.0133**
CE (Mean)	0.06	0.08
CV	0.04	0.07
Dissector particle number (Mean)	234.83	217.45
Average section thickness (Mean) (µm)	21.90	21.72
Number of steps for counting (Mean)	203.16	197.83
Number of sampled sections (Mean)	14.51	13.83
Counting frame size (μm^2)	900	9000
Area sampling fraction	900 /40000	900 / 40000
Thickness sampling	10 /21.90	10 /21.72

[a] Total pyramidal cell numbers in the CA of the hippocampus in the experimental group pups showed significant differences compared with the control pups. SEM, standard error mean; CE, coefficient of error; CV, coefficient of variation.
*P < 0.05 and ** P > 0.05

Statistical analysis

Descriptive statistics were expressed as mean ± standard error mean (SEM) for the variables. The Mann-Whitney U test was used to compare these variables between the control and experimental groups. Statistical significance was set at 5% for all computations. All statistical analysis was performed using SPSS v.20 (IBM Corp., Armonk, NY, USA) (Sokal and Rohlf, 1995).

Results and discussion

Physical examination and body and brain weights

Physical examination revealed no skeletal anomaly or unexpected findings in either rat group. No significant difference was determined between the groups in terms of pups' body or brain weights on postnatal day 32 (P > 0.05) (Table 1).

Histopathological observations

The histological section appearances of the control and experimental group pups are shown in Figure 1. The number of pyramidal cells in the experimental group pups was significantly lower compared to the control group pups. Cell loss with picnotic cells in the experimental group pups (Figure 1 D, E, F) is clearly visible, while pyramidal cell morphology in the control group pups was normal (Figure 1 A, B, C).

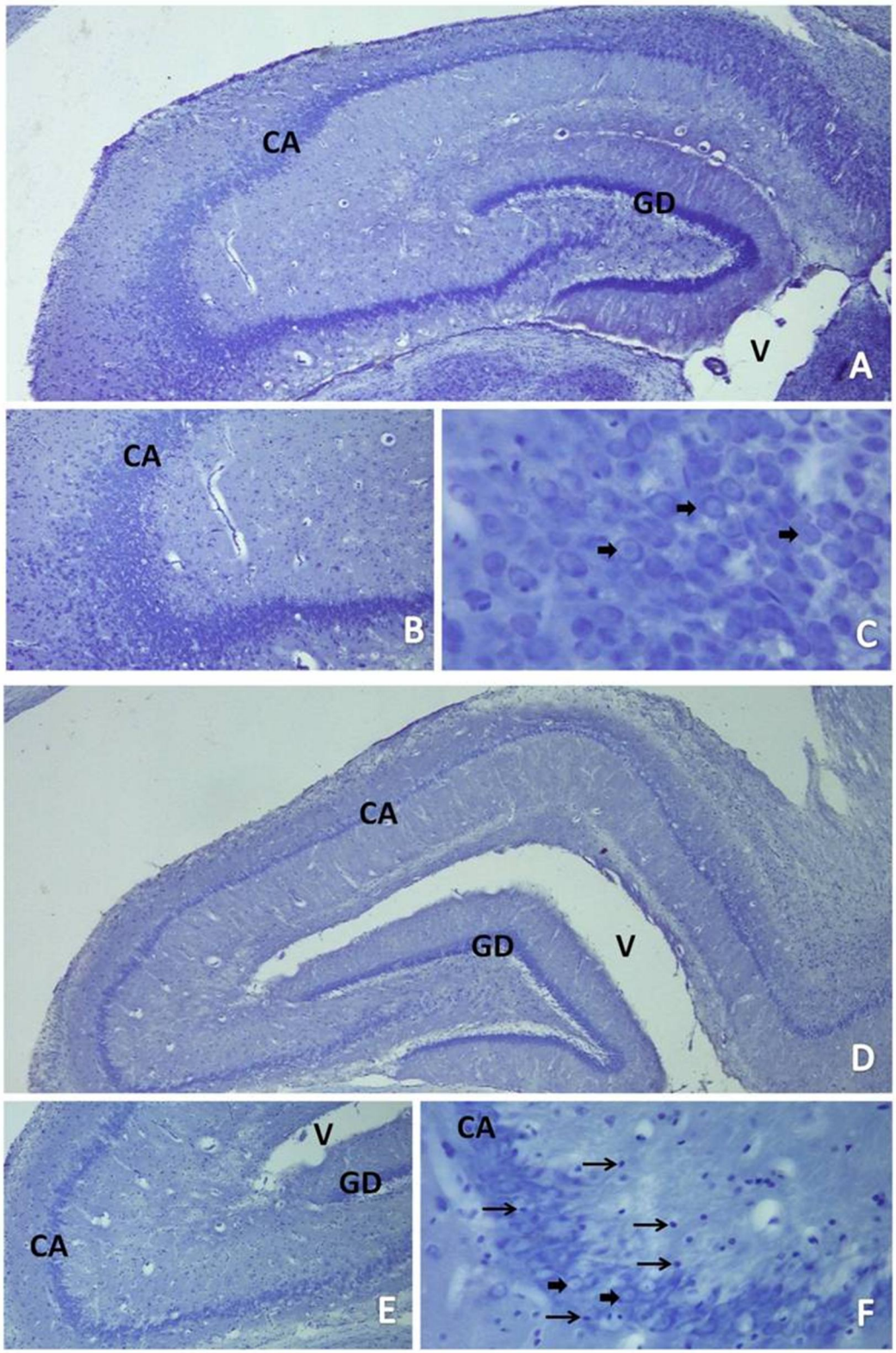

Figure 1. Representative photomicrographs of the hippocampus sections from the control group pups (A, B, C) and experimental group pups (D, E, F). While the morphology of pyramidal cells (thick arrow) in the cornu ammonis in the control group pups was normal (C), picnotic cells (arrows) among the pyramidal cells (thick arrow) of the cornu ammonis are easily visible in the experimental group pups (F). It should be noted that such a result may not be directly evident from simple qualitative observation of the tissue sections, since the orientation of the cutting may result in a very different sectioned surface area for each sampled area. Substantial neuron loss may therefore not be seen in such images. Additionally, such images may not be informative since the picture may vary depending on the section plane, tissue shrinkage or swelling, or the area of the images taken. CA, cornu ammonis; DG, dentate gyrus; V, ventricle, (Cresyl violet staining) (A and D, X 40; B and E, X 100; C and F, X 400).

Cell numbers in the cornu ammonis of the hippocampus

Pyramidal cell numbers in the cornu ammonis of the hippocampus were estimated in both groups. If the largest nuclear profile came into focus within the unbiased virtual counting frames spaced randomly and systematically throughout the delineated regions, the pyramidal cells were counted as dissector particles (Bas *et al.*, 2009b). Estimated total pyramidal cell numbers were calculated on the basis of the number of pyramidal cells counted and the sampling probability (Gundersen, 1986; West *et al.*, 1991; Bas *et al.*, 2009b). The total number of pyramidal cells in the cornu ammonis was significantly lower in the experimental group pups compared to the controls ($P < 0.05$) (Table 1).

The fact that in addition to making verbal communication possible, mobile phones are also important tools for accessing the virtual world in the form of social media raises a number of problems. The most important of these is the effect on human health of the EMF they emit. It is not easy to answer this question, which has been posed ever since mobile phones became part of daily life. While some scientists maintain that the EMF emitted by mobile phones can have serious effects on human health, others maintain the exact opposite (Bas *et al.*, 2009a; 2009b; Sudan *et al.*, 2012; Hao *et al.*, 2013).

One of the questions to which an answer is sought is which in period, childhood; youth or old age, the EMF emitted by mobile phones has the greatest effect on human health. Studies have suggested that the brain and structures related to the brain are more affected by the EMF emitted by mobile phones in childhood and youth, when development of the nervous system is not yet complete (Bas *et al.*, 2009b). In addition to this effect being associated with stage of development, it is also associated with children and young people exhibiting greater interest in mobile phone use. This is because mobile phones used when the need arises in adulthood are widely used to pass the time, access social media and for other purposes, such as entertainment, in childhood and youth (Söderqvist *et al.*, 2007; 2008). Studies emphasize that because children start using mobile phones at earlier ages they are exposed for longer periods to the EMF these emit, and may therefore suffer greater harm (Schuz, 2005).

Another subject of research is whether or not the embryo and fetus are affected by the EMF resulting from mobile phone use in pregnancy (Odaci *et al.*, 2008; Bas *et al.*, 2009b). Based on the fact that cells, tissue and systems in the developmental stage are more sensitive to external agents, some researchers (Rodier, 1980) have investigated the effect of EMF emitted by mobile phones on the developing embryo and fetus in the prenatal period. Studies on the subject have reported important findings. One study reported that 900 MHz EMF can induce cell death, and that this can inhibit neural stem cell differentiation into neurons in the embryonic period (Salford *et al.*, 2003). That study also reported neuron loss in the cerebral cortex, hippocampus and basal ganglia in rats exposed to EMF (Salford *et al.*, 2003).

Although several studies have investigated the effects of mobile phones on human health, no serious efforts or warnings are made by those responsible for countries' health policies on at least restricting use of mobile phones by children. This may be due to the different results from scientific studies. While some studies report long-term use of mobile phones can lead to severe health problems, and even brain tumors, others have not corroborated this (Hardell *et al.*, 1999; 2006, 2007; Inskip *et al.*, 2001; Lahkola *et al.*, 2008). A specific experimental study of this kind, that examines the effect of prenatal exposure (13-21 of the prenatal term) to EMF on pyramidal cell numbers in the cornu ammonis region of the hippocampus of rats, may provide valuable and useful data regarding the potential prenatal adverse effects on the central nervous system of mobile phone use. We exposed pregnant female rats to 900 MHz EMF, since most mobile phones in Europe operate at a frequency of 900 MHz (Dubreuil *et al.*, 2002, 2003; Koyu *et al.*, 2005; Panagopoulos *et al.*, 2007). Our results revealed cell losses in the cornu ammonis resulting prenatal exposure to 900 MHz EMF. We used the optical dissector counting technique, a combination of systematic random sampling and the optical dissector method, in order to quantify changes taking place in the cornu ammonis following exposure to EMF (West *et al.*, 1991; Bas *et al.*, 2009a; 2009b). To the best of our knowledge, this is the first quantitative report in the literature concerning prenatal (days 13–21) exposure to 900 MHz EMF on the 32-day-old female rat brain.

We recently reported that 900 MHz EMF significantly reduced total pyramidal cell numbers in the cornu ammonis in newborn rats exposed during the prenatal period (Bas *et al.*, 2009b). We also reported the effect of prenatal exposure to 900 MHz EMF on the number of granule cells in the dentate gyrus of 4-week-old rats. The results of that study showed, for the first time in the literature, a cell loss in the dentate gyrus resulting from prenatal EMF, and cell loss can be attributed to chronic prenatal exposure to EMFs (Odaci *et al.*, 2008). However, there are certain differences in terms of experimental method between these two earlier studies of ours (Odaci *et al.*, 2008; Bas *et al.*, 2009b) and the present study. In those two studies we exposed pregnant rats to the effect of 900 MHz EMF on days 1–19 of pregnancy and investigated the number of cells in the newborn rat hippocampus on the 28th day. Additionally, we made no gender distinctions among rat pups in those studies. In the present study, however, female rat pups were exposed to the effect of EMF on days 13–21 and cell number in the cornu ammonis region of the hippocampus were investigated on the postnatal 32nd day.

Another study by our research group investigated the 16-week-old female rat hippocampus following postnatal exposure to 900 MHz EMF investigating the number of pyramidal cells in the cornu ammonis using the optical fractionator technique. In this study, EMF group rats were exposed to 900 MHz EMF (1 h/day for 28 days) in an exposure tube. Results showed that postnatal exposure to EMF resulted in a significant decrease in pyramidal cell numbers in the cornu ammonis in the EMF group. Moreover, cell loss was observed in the cornu ammonis in the EMF group even at qualitative evaluation (Bas *et al.*, 2009a). That study, in which we used the same research method and protocol and rat groups of the same age (Bas *et al.*, 2009a), and another study of ours, revealed a significantly lower total number of Purkinje cells in the cerebellum in the EMF group compared to the control group (Sonmez *et al.*, 2010).

It needs to be stated here that the EMF exposure system in our previous studies (Odaci *et al.*, 2008; Bas *et al.*, 2009a; 2009b; Sonmez *et al.*, 2010) was designed differently to that in the present study. The system consisted of a dipole exposure antenna and a round plastic tube cage. An electromagnetic energy generator was manufactured at the Electromagnetic Compatibility Laboratory at Süleyman Demirel University (Koyu *et al.*, 2005; Ozguner *et al.*, 2005; Köylü *et al.*, 2006; Yildiz *et al.*, 2006). This emitted a 900 MHz continuously-modulated EMF (2W peak output power and 1±04mW/cm2 power density). The rats in the EMF group were placed in close contact above the dipole antenna and then exposed to EMF. They were positioned perpendicular to the dipole antenna, with a distance of 1 cm between them. The rats' heads were aligned along the direction of the antenna, making the rats' long axis perpendicular to the long axis of the antenna (Koyu *et al.*, 2005; Ozguner *et al.*, 2005; Köylü *et al.*, 2006; Yildiz *et al.*, 2006; Odaci *et al.*, 2008; Bas *et al.*, 2009a; 2009b; Sonmez *et al.*, 2010). The EMF exposure system used in the present study, however, was redesigned. As described in the materials and methods section, the redesigned EMF exposure system permitted the rats to move around freely inside the cage. We thus attempted to reduce any stress they might experience due to constriction to a minimum. However, whichever system is used, the common finding from our studies is that there is a decrease in neuron numbers in rats exposed to the effect of 900 MHZ EMF (1 h/day for 28 days). Moreover, this decrease does not vary depending on whether EMF is applied pre- or postnatally.

Conclusion and outlook

The study results show that exposure to 900 MHZ EMF on prenatal days 13-21 caused a decrease in the number of pyramidal cells in the cornu ammonis region of the hippocampus on postnatal day 32. In addition, the presence of picnotic cells in the cornu ammonis region in the experimental group pups suggests that EMF applied on days 13-21 of the prenatal period leads to cell deaths, and that it reduces the number of pyramidal cells with these deaths. Additionally, these findings suggest that long-term exposure to 900 MHz EMF in the prenatal period affects neuron development in the hippocampus, and that this may result in a decrease in cell numbers in the postnatal period. To the best of our knowledge this is the first time our study results have been reported in the literature. It is therefore important, in terms of supporting our findings and contributing to the literature, for further studies on the subject to be performed using different methods.

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References

Aktürk Z, Odacı E, İkinci A, Baş O, Canpolat S, Çolakoğlu S, Sönmez OF. Effect of Ginkgo biloba on brain volume after carotid artery occlusion in rats: a stereological and histopathological study. Turk J Med Sci DOI: 10.3906/sag-1305-40.

Bas O, Odaci E, Kaplan S, Acer N, Ucok K, Colakoglu S. 900 MHz electromagnetic field exposure affects qualitative and quantitative features of hippocampal pyramidal cells in the adult female rat. Brain Res 2009a; 1265: 178-85.

Bas O, Odaci E, Mollaoglu H, Ucok K, Kaplan S. Chronic prenatal exposure to the 900 megahertz electromagnetic field induces pyramidal cell loss in the hippocampus of newborn rats. Toxicol Ind Health 2009b; 25(6): 377-84.

Dubreuil D, Jay T, Edeline JM. Does head-only exposure to GSM 900-MHz electromagnetic fields affect the performance of rats in spatial learning tasks? Behav Brain Res 2002; 129(1-2): 203-10.

Dubreuil D, Jay T, Edeline JM. Head-only exposure to GSM 900-MHz electromagnetic fields does not alter rat's memory in spatial and non-spatial tasks. Behav Brain Res 2003; 145(1-2): 51-61.

Gundersen HJG. Stereology of arbitrary particles. A review of unbiased number and size estimators and the presentation of some new ones in memory of William R Thomson. J Microsc 1986; 143: 3-45.

Gundersen HJG, Jensen EB. The efficacy of systematic sampling in stereology and its prediction. J Microsc 1987; 147: 229-263.

Hancı H, Odacı E, Kaya H, Aliyazıcıoğlu Y, Turan I, Demir S, Colakoğlu S. The effect of prenatal exposure to 900-MHz electromagnetic field on the 21-old-day rat testicle. Reprod Toxicol 2013;42C:203-209. doi: 10.1016/j.reprotox.2013.09.006.

Hao D, Yang L, Chen S, Tong J, Tian Y, Su B, Wu S, Zeng Y. Effects of long-term electromagnetic field exposure on spatial learning and memory in rats. Neurol Sci 2013; 34(2): 157-64.

Hardell L, Näsman A, Påhlson A, Hallquist A, Hansson Mild K. Use of cellular telephones and the risk for brain tumours: a case-control study. Int J Oncol 1999; 15(1); 113-116.

Hardell L, Carlberg M, Hansson Mild K. Pooled analysis of two case-control studies on use of cellular and cordless telephones and the risk for malignant brain tumours diagnosed in 1997–2003. Int Arch Occup Environ Health 2006; 79(8): 630-9.

Hardell L, Carlberg M, Söderqvist F, Mild KH, Morgan LL. Long-term use of cellular phones and brain tumours: increased risk associated with use for Nor =10 years. Occup Environ Med 2007; 64(9): 626-32.

Hausdorf, J, Lemmens, MA, Kaplan, S, Marangoz, C, Milz, S, Odaci, E, Korr H, Schmitz C, Maier M. Extracorporeal shockwave application to the distal femur of rabbits diminishes the number of neurons immunoreactive for substance P in dorsal root ganglia L5. Brain Res 2008; 1207: 96-101.

Inskip PD, Tarone RE, Hatch EE, Wilcosky TC, Shapiro WR, Selker RG, Fine HA, Black PM, Loeffler JS, Linet MS. Cellular-telephone use and brain tumors. N Engl J Med 2001; 344(2): 79-86.

İkinci A, Odacı E, Baş O. Effects of ethyl pyruvate administration on female rats' pyramidal cells of cornu ammonis after brain ischemia: a stereological and histopathological study. Turk J Med Sci 2013; 43(3): 354-361.

Kaplan S, Odacı E, Canan S, Onger ME, Aslan H, Ünal B. The Disector counting technique. NeuroQuantology 2012a; 10: 44-53.

Kaplan S, Canan S, Altunkaynak ME, Odacı E, Aslan H, Unal B. An unbiased way to estimate total quantities: the fractionator technique. NeuroQuantology 2012b; 10: 54-65.

Koyu A, Cesur G, Ozguner F, Akdogan M, Mollaoglu H, Ozen S. Effects of 900 MHz electromagnetic field on TSH and thyroid hormones in rats. Toxicol Lett 2005; 157(3): 257-62.

Köylü H, Mollaoglu H, Ozguner F, Naziroglu M, Delibas N. Melatonin modulates 900 MHz microwave-induced lipid peroxidation changes in rat brain. Toxicol Ind Health 2006; 22(5): 211-6.

Lahkola A, Salminen T, Raitanen J, Heinävaara S, Schoemaker M, Christensen HC, Feychting M, Johansen C, Klæboe L, Lönn S, Swerdlow A, Tynes T, Auvinen A. Meningioma and mobile phone use-a collaborative case-control study in five North European countries. Int J Epidemiol 2008; 37(6): 1304-13.

Mausset AL, de Seze R, Montpeyroux F, Privat A. Effects of radiofrequency exposure on the GABAergic system in the rat cerebellum: clues from semiquantitative immunohistochemistry. Brain Res 2001; 912: 33-46.

Mausset AL, Hirbec H, Bonnefont X, Privat A, Vignon J, de Seze R. Acute exposure to GSM 900-MHz electromagnetic fields induces glial reactivity and biochemical modifications in the rat brain. Neurobiol Dis 2004; 17: 445-454.

Odaci E, Kaplan S, Sahin B, Bas O, Gevrek F, Aygun D, Unal B, Sonmez OF, Colakoglu S, Bilgic S. Effects of low-dose oxcarbazepine administration on developing cerebellum in newborn rat: A stereological study. Neuroscience Research Communications 2004; 34(1): 28-36.

Odaci E, Bas O, Kaplan S. Effects of prenatal exposure to a 900 MHz electromagnetic field on the dentate gyrus of rats: a stereological and histopathological study. Brain Res 2008; 1238: 224-9.

Odaci E, Cihan OF, Aslan H, Ragbetli MC, Kaplan S. Prenatal diclofenac sodium administration increases the number of

Purkinje cells in female rats: a stereological study. Int J Dev Neurosci 2010; 28(2): 145-51.

Ozguner M, Koyu A, Cesur G, Ural M, Ozguner F, Gokcimen A, Delibas N. Biological and morphological effects on the reproductive organ of rats after exposure to electromagnetic field. Saudi Med J 2005; 26(3): 405-10.

Panagopoulos DJ, Chavdoula ED, Nezis IP, Margaritis LH. Cell death induced by GSM 900-MHz and DCS 1800-MHz mobile telephony radiation. Mutat Res 2007; 626(1-2): 69-78.

Rodier PM. Chronology of neuron development: animal studies and their clinical implications. Dev Med Child Neurol 1980; 22(4): 525-45.

Sahin B, Aslan H, Canan S, Kaplan S, Unal B, Odaci E. A stereological estimation of total neuron number and volume of the hippocampus at one and seven day-old chicks. Neuroscience Research Communications 2002; 32(1): 29-38.

Sakatani S, Okada YC, Hirose A. A quantitative evaluation of dominant membrane potential in generation of magnetic field using a pyramidal cell model at hippocampus CA3. Neurocomputing 2002; 44: 153-160.

Salford LG, Brun AE, Eberhardt JL, Malmgren L, Persson BR. Nerve cell damage in mammalian brain after exposure to microwaves from GSM mobile phones. Environ Health Perspect 2003; 111: 881-883.

Schüz J, Böhler E, Berg G, Schlehofer B, Hettinger I, Schlaefer K, Wahrendorf J, Kunna-Grass K, Blettner M. Cellular phones, cordless phones, and the risks of glioma and meningioma (Interphone Study Group, Germany). Am J Epidemiol 2006b; 163(6): 512-20.

Sokal RR, Rohlf FJ. Biometry: the principles and practice of statistics in biological research, Third ed. New York: W.H. Freeman and Co 1995.

Sonmez OF, Odaci E, Bas O, Kaplan S. Purkinje cell number decreases in the adult female rat cerebellum following exposure to 900 MHz electromagnetic field. Brain Res 2010; 1356: 95-101.

Söderqvist F, Hardell L, Carlberg M, Hansson Mild K. Ownership and use of wireless telephones: a population-based study of Swedish children aged 7–14 years. BMC Public Health 2007; 7:105.

Söderqvist F, Carlberg M, Hardell L. Use of wireless telephones and self-reported health symptoms: a population-based study among Swedish adolescents aged 15–19 years. Environ Health 2008; 7:18.

Sudan M, Kheifets L, Arah O, Olsen J, Zeltzer L. Prenatal and Postnatal Cell Phone Exposures and Headaches in Children. Open Pediatr Med Journal 2012; 6(2012): 46-52.

Tunc AT, Aslan H, Turgut M, Ekici F, Odaci E, Kaplan S. Inhibitory effect of pinealectomy on the development of cerebellar granule cells in the chick: a stereological study. Brain Res 2007; 1138: 214-220.

Weinstock M. Alterations induced by gestational stres in brain morphology and behaviour of the offspring. Prog Neurobiol 2001; 65: 427-451.

West MJ, Slomianka L, Gundersen HJG. Unbiased stereological estimation of the total number of neurons in the subdivisions of the rat hippocampus using the optical fractionator. Anat Rec 1991; 231: 482-497.

Yildiz M, Cicek E, Cerci SS, Cerci C, Oral B, Koyu A. Influence of electromagnetic fields and protective effect of CAPE on bone mineral density in rats. Arch Med Res 2006; 37(7): 818-21.