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Pernicious effects of long-term, continuous 900-MHz electromagnetic field throughout adolescence on hippocampus morphology, biochemistry and pyramidal neuron numbers in 60-day-old Sprague Dawley male rats☆

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ABSTRACT

The central nervous system (CNS) begins developing in the intrauterine period, a process that continues until adulthood. Contact with chemical substances, drugs or environmental agents such as electromagnetic field (EMF) during adolescence therefore has the potential to disturb the development of the morphological architecture of components of the CNS (such as the hippocampus). The hippocampus is essential to such diverse functions as memory acquisition and integration and spatial maneuvering. EMF can result in severe damage to both the morphology of the hippocampus and its principal functions during adolescence. Although children and adolescents undergo greater exposure to EMF than adults, the information currently available regarding the effects of exposure to EMF during this period is as yet insufficient. This study investigated the 60-day-old male rat hippocampus following exposure to 900 megahertz (MHz) EMF throughout the adolescent period using stereological, histopathological and biochemical analysis techniques. Eighteen male Sprague Dawley rats aged 21 days were assigned into control, sham and EMF groups on a random basis. No procedure was performed on the control group rats. The EMF group (EMFGr) was exposed to a 900-MHz EMF for 1 h daily from beginning to end of adolescence. The sham group rats were held in the EMF cage but were not exposed to EMF. All rats were sacrificed at 60 days of age. Their brains were extracted and halved. The left hemispheres were set aside for biochemical analyses and the right hemispheres were subjected to stereological and histopathological evaluation. Histopathological examination revealed increased numbers of pyknotic neurons with black or dark blue cytoplasm on EMFGr slides stained with cresyl violet. Stereological analyses revealed fewer pyramidal neurons in EMFGr than in the other two groups. Biochemical analyses showed an increase in malondialdehyde and glutathione levels, but a decrease in catalase levels in EMFGr. Our results indicate that oxidative stress-related morphological damage and pyramidal neuron loss may be observed in the rat hippocampus following exposure to 900-MHz EMF throughout the adolescent period.

1. Introduction

The hippocampus is an essential component of the limbic system and of such diverse functions as memory acquisition and integration and spatial maneuvering (Squire, 2004; Moser et al., 2008; Yau et al., 2015). Long-term memory is created by processing

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new, short-term memories and storing these on a temporary basis before permanent deposition in the cortex (Yau et al., 2015). Studies show that the hippocampus plays a crucial role in this temporary deposition and in the recovery of contextual fear memory for up to 2–3 weeks following the original learning process (Kim and Fanselow, 1992; Yau et al., 2015). Therefore, in addition to compromising the normal morphological architecture of the hippocampus, there is also a strong probability that exposure to long-term drugs, chemical agents or any environmental factor will also impair important functions such as these. Studies have shown that electromagnetic field (EMF) may be one such agent (Bas et al., 2009a,b; Baş et al., 2013).

Previous studies have reported that exposure to EMF may cause numerous changes capable of affecting the normal life cycle, depending on the length of exposure and the intensity of the electrical field, and that these may be seen in the form of morphological (Bas et al., 2009a,b; Baş et al., 2013), physiological or behavioral changes (Salford et al., 2003; Baş et al., 2013; İkinci et al., 2013; Odacı et al., 2013). One of the main sources of EMF capable of causing these changes in humans is the cell phone. Due to their being held close to the head in particular, it has been suggested that EMF originating from cell phones can have an adverse impact on the brain and associated structures (Davis et al., 2013).

Many studies have suggested that the results of animal studies can be directly equated with the equivalent developmental stage in humans, irrespective of whether analysis is performed in the fetal, prenatal, or postnatal periods (Rodier, 1980; Jacobson, 1991; Odaci et al., 2004). For example, the neonatal stage of the development of the cornu ammonis (CA) in rats is equivalent to the third trimester of CA development in humans (Dobbing, 1970; Dobbing and Sands, 1973; Rodier, 1980; Jacobson, 1991). Therefore, although there is disagreement concerning whether rat and human studies can be compared directly with one another, the findings of studies involving rats exposed to the effect of a 900-megahertz (MHz) EMF throughout pregnancy will certainly make a significant contribution to the literature concerning the same stage of development in humans.

One of the most commonly used frequencies in cell phone communications is 900 MHz (Dubreuil et al., 2002; Bas et al., 2009a,b). This study therefore investigated the effect of 900-MHz EMF. The findings from previous studies of ours suggest that 900-MHz EMF compromises the development and morphological structures of CNS tissues (Odaci et al., 2008; Odacı et al., 2013; İkinci et al., 2013; Sonmez et al., 2010). Examination of previous research on the subject shows that there have been insufficient studies using histopathological, biochemical and stereological techniques to investigate changes occurring in the hippocampus of male rats exposed to the effect of continuous 900-MHz EMF throughout all of adolescence. This study was therefore planned accordingly.

2. Materials and methods

2.1. Animals and study protocols

All procedures were compatible with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health. Written permission is a legal requirement for any animal experiment in Turkey. The Karadeniz Technical University (KTU) Animal Care and Ethics Committee provided formal, written confirmation that all surgical and other experimental procedures had been revised and approved by members of the KTU Ethical Committee.

Male rats were obtained from the KTU Experimental Animals Surgical Research and Application Center. On the first day of the study these were 21 days old and weighed between 28.3 and 43.9 g. Rats were kept in standard plastic laboratory cages under standard laboratory conditions at room temperature ($22 \pm 2^{\circ}C$) and humidity ($50\% \pm 10$) and in a 12 h light/12 h dark cycle. Standard rat chow (Bayramoğlu Feed and Flour Industry Trading Corporation, Erzurum, Turkey) and tap water were provided ad libitum for all animals during the experiments.

Eighteen Sprague Dawley male rats were used. These were randomly assigned into one of three equal groups, CNGr, SHMGr and EMFGr. All groups were kept in the same laboratory room, defined as the resting room, but in different cages, under the same environmental conditions, apart from during sham and EMF exposure. During sham and EMF exposure, the SHMGr and EMFGr rats were moved to another room specifically designed for EMF application, defined as the EMF exposure room. Following EMF or sham exposure, the rats were returned to the resting room. The CNGr rats received no sham or EMF exposure during the study and remained in the resting room in their cage throughout the study period. The SHMGr rats were taken to the EMF exposure room for 1 h at the same time (10:00–11:00 a.m.) every day between postnatal days 21 and 59, inclusive, but were not exposed to EMF in the EMF cage. Following sham exposure, the SHMGr rats were returned to the resting room. The EMFGr rats were then moved into the EMF exposure room, where they were exposed to 900-MHz EMF in the EMF cage for 1 h at the same time (11:00–12:00 a.m.) every day between postnatal days 21 and 59, inclusive.

2.2. Sham and EMF applications

SHMGr rats were placed inside the EMF cage every day for sham administration before the EMF group rats were placed in the cage. All SHMGr rats were placed inside the EMF cage together during sham application. Once the sham administration was complete the SHMGr rats were removed from the EMF cage and returned to their cages inside the resting room. Rat feces and other residual substances were then removed from the EMF cage using tap water and an unscented cleaning product. EMFGr rats were subsequently placed inside the EMF cage and exposed to 900–MHz EMF. All EMFGr rats were placed inside the EMF cage together during EMF application. After the EMF procedure the EMFGr rats were removed from the EMF cage and returned to their cage inside the resting room. SHMGr and EMFGr rats were permitted to move around freely inside the EMF cage during applications. The internal temperature and humidity levels in the EMF cage during EMF and sham applications were measured using a digital thermometer (YCOM KMN-303, Yu Yau Shuanghe Electron Instrument Co., Ltd. Zhejing-China). Temperature and humidity levels were similar inside the EMF cage during sham and EMF applications. Temperature inside the EMF cage was $21.9 \pm 1.09\,^{\circ}C$ during sham application compared to $21.82 \pm 1.01\,^{\circ}C$ during EMF applications. Humidity levels inside the EMF cage were $58.6\% \pm 5.72$ during sham application and $58.4\% \pm 5.41$ during EMF application.

2.3. EMF administration system

An EMF administration system was used to expose the EMFGr rats to 900-MHz EMF. The system was designed by the authors and was identical to those used in previous studies and described in detail elsewhere (Baş et al., 2013; Hancı et al., 2013, 2015; Türedi et al., 2015; Topal et al., 2015; İkinci et al., 2013; Odacı et al., 2013, 2015; Odacı and Özyılmaz, 2015). Briefly, it involved an oscillator (1218-BV, ultra-high frequency lockable oscillator, 900–2000 MHz, General Radio Company, Concord, Massachusetts, USA, Serial No. 1483), a constant electric power source (1267–B Regulated Power Supply, General Radio Company, Concord, Massachusetts, USA,