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916 MHz electromagnetic field exposure affects rat behavior and hippocampal neuronal discharge☆

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Abstract

Wistar rats were exposed to a 916 MHz, 10 W/m^2 mobile phone electromagnetic field for 6 hours a day, 5 days a week. Average completion times in an eight-arm radial maze were longer in the exposed rats than control rats after 4–5 weeks of exposure. Error rates in the exposed rats were greater than the control rats at 6 weeks. Hippocampal neurons from the exposed rats showed irregular firing patterns during the experiment, and they exhibited decreased spiking activity 6–9 weeks compared with that after 2–5 weeks of exposure. These results indicate that 916 MHz electromagnetic fields influence learning and memory in rats during exposure, but long-term effects are not obvious.

Key Words

electromagnetic field; Wistar rats; maze learning; feeding behavior; neurons; neural regeneration

Research Highlights

Rats exposed to a 916 MHz 10 W/m^2 electromagnetic field exhibited higher completion times and error rates in an eight-arm radial maze. The hippocampal neurons of exposed rats consistently presented an irregular firing pattern and significantly decreased discharge frequency 6–9 weeks after exposure.

Abbreviations

GSM, Global System for Mobile

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INTRODUCTION

The expansion of mobile communications has generated concerns about the possible health risks of electromagnetic fields, including potential adverse effects on the human brain. Mobile phones are often close to the head during a call, and the brain may thus be exposed to microwaves. Although there have been a large number of studies[1-3], it is not easy to determine the long-term effects of exposure to electromagnetic fields on human brain function because of methodological limitations. Rat models are usually adopted to investigate the long-term effects of 900 MHz electromagnetic fields, the typical mobile phone frequency.

Some studies indicated that electromagnetic fields may alter histopathological parameters[4], morphological maturation of neural cells[5], and cause some behavioral disorders in rats[6]. Eberhardt *et al* [7] observed that albumin extravasation and its uptake into neurons and dark neurons in the rat brain were enhanced after exposure to microwaves from Global System for Mobile Communications (GSM) mobile phones. Previous studies have verified that prenatal and postnatal exposure to 900 MHz electromagnetic fields induced hippocampal pyramidal cell loss in both the newborn rats and the adult female rats[5, 8]. Extended exposure to 900 MHz electromagnetic fields led to fewer Purkinje cells in the female rat cerebellum[9] and adversely affected the rat brain[10]. Zhu *et al* [11] showed that microwaves emitted from mobile phones led to significant cell death in cultures and more brain neuronal cells that stained positive *in vivo*. Memory functions in rats were significantly impaired by GSM 900 MHz microwave exposure[12]. These rats exhibited decreased locomotor activity, increased grooming, and a tendency towards increased basal corticosterone levels. Some studies provided no evidence that electromagnetic fields affect spatial working memory[13], hearing[14], blood-brain barrier permeability, or neuronal degeneration[15]. GSM 900 MHz did not affect either basal Ca^{2+} homeostasis or evoked Ca^{2+} signals[16] and no albumin immunoreactivity was observed in the brains of the exposed rats[17]. Other studies found no significant alterations of histopathological parameters[18], morphological features or the physiology of cortical synaptosomes[19] in exposed rats. Additional examinations in rats of the effects of whole-body exposure to GSM-950 MHz electromagnetic fields on the acquisition and consolidation of spatial memory and long-term potentiation in the dentate gyrus did not detect significant effects of the exposure[20]. One study even found significantly improved learning and memory in rats after 5 weeks of exposure to radiofrequency electromagnetic fields[21]. The studies conducted so far have yielded an equivocal relationship between exposure to electromagnetic field radiation and brain activity.

In this study, we attempted to determine whether rat behavior is impaired by exposure to electromagnetic fields, and the mechanisms underlying the persistence of these impairments.

RESULTS

Quantitative analysis of experimental animals

Twenty Wister rats were used at the beginning of this experiment. After eight-arm radial maze training, the 10 rats that met our criteria were equally and randomly divided into the control and exposure groups. One rat from each group was implanted with electrode arrays in the hippocampal CA1 region. However, the electrode array fell off of the control rat during the experiments. Therefore, only neuronal signals from the exposed rat were recorded. The first 9 weeks of data were analyzed, even though the experiments lasted 10 weeks, because two exposed rats unexpectedly did not finish the maze experiment within the 10th week.

Electromagnetic field exposure impairs learning and memory in rats

As shown in Figure 1A, the average completion time of the exposed rats was longer than that of the control rats at 4–5 weeks after electromagnetic field exposure ($P < 0.05$). The groups did not show significant differences during the other weeks examined ($P > 0.05$). However, the difference at 6 weeks ($P = 0.054$) approached significance. As displayed in Figure 1B, the average error rate of the exposed rats at 6 weeks was higher than that of control rats ($P < 0.05$), and there were no significant differences during the other weeks ($P > 0.05$). The results indicated that the electromagnetic field did not impair the behavior of the rats in the first 3 weeks, and then the electromagnetic field significantly impaired the rats during weeks 4–6. The behavior of the rats then normalized during weeks 7–9.

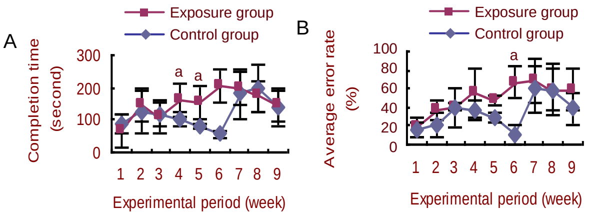

Figure 1 Completion times (A) and error rates (B) of control rats and exposed rats in an eight-arm radial maze experiment.

The data are expressed as mean ± SEM of five rats in each group, and are analyzed using an analysis of independent samples *t*-test. [a]$P < 0.05$, *vs*. control rats.

Hippocampal neurons discharge irregularly in rats undergoing exposure to electromagnetic radiation

The number of neurons, rather than the number of rats, was the unit of analysis in the neuronal studies. Twelve neuronal signals were obtained over the 9 weeks. The hippocampal neurons maintained irregular discharges during the experimental period, which suggested that electromagnetic field exposure did not change their firing pattern.

As exhibited in Figure 2, the average number of hippocampal spikes per second declined during the first 5 weeks, and then plateaued from weeks 6–9. The discharge frequency of hippocampal neurons at 6–9 weeks was significantly lower than at 2–5 weeks ($P < 0.05$).

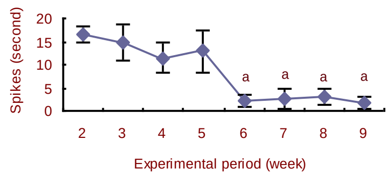

Figure 2 Average discharge number per second in hippocampal neurons across the 9 weeks of electromagnetic field exposure.

The data are expressed as mean ± SEM of 12 neurons at each time point, and are analyzed using a repeated-measures analysis of variance. [a]$P < 0.05$, *vs*. that at 2–5 weeks.

DISCUSSION

Our experiments continued until there were no obvious changes over 3 consecutive weeks. At that point, the Wistar rats had been exposed to the electromagnetic fields for more than 300 hours, which is much longer than in most other studies[5, 22]. Therefore, our measurements may include both cumulative and delayed effects of the electromagnetic field. The eight-arm radial maze is recognized as an excellent model of spatial memory[23], and spatial memory formation and consolidation depend on hippocampus[24]. Accordingly, the maze and neuronal signal data were combined to characterize the effects of electromagnetic field exposure.

Although there were no significant behavioral differences between the control and exposed rats over the first 3 weeks of exposure, clear differences emerged in weeks 4–6. We propose that the decreased spiking activity in exposed rats was probably a result of a partial hippocampal lesion caused by the electromagnetic fields, and it engendered impaired learning at 4–6 weeks. Xu *et al* [25] also revealed microwaves reduced excitatory synaptic activity and the number of excitatory synapses in rat hippocampal neurons, which are findings that are consistent with our results. Martin and Clark[26] pointed out that spatial memories can eventually be acquired by extrahippocampal brain structures, Neural reorganization may allow for compensatory strategies that overcome deficits induced by electromagnetic fields and provide nearly normal task acquisition and subsequent memory retrieval. Perhaps as a result of such a process, the two groups' behavior was similar in both weeks 7–9 and 1–3. The behavioral and neuronal discharge tests suggested that electromagnetic fields affected rat brain function during a specific phase of the exposure period. Both their learning abilities and neuronal discharges were clearly reduced at 6 weeks, which indicates that these processes are related and that rats can adapt to electromagnetic fields over time.

MATERIALS AND METHODS

Design

A randomized controlled animal study.

Time and setting

This experiment was performed at the Experimental Center of College of Biomedical Engineering, South-Central University for Nationalities, China. Data were analyzed at the office of College of Life Science and Bioengineering, Beijing University of Technology in China between July 2010 and June 2011.

Materials

Animals

A total of 20 clean, male, Wistar rats, aged 8 weeks and weighing 250–300 g, were supplied by the Division of Laboratory Animal, Tongji Medical College, Huazhong University of Science and Technology, China (Animal license No. SCXK (E) 2010-007). The rats were housed in transparent Makrolon cages (47 cm × 31 cm × 26 cm) in a temperature-controlled room (25°C). They were maintained on a 12-hour light/dark cycle with free access to rodent chow and water. All procedures were conducted in accordance with the *Guidance Suggestions for the Care and Use of Laboratory Animals*, formulated by the Ministry of Science and Technology of China[27].

Instruments

A ZH-3000 eight-arm maze video analysis system (Huaibei Zhenghua Biological Apparatus Facilities Co., Ltd., Huaibei, Anhui Province, China) was used to track and record the movement of the rats. The maze consisted of a central platform (46.5 cm in diameter) and eight equally spaced arms (47.5 cm × 14.5 cm × 22.5 cm) (supplementary Figure 1 online). In the exposure system, the antenna connecting to the microwave power generator (Tianhua Zhongwei Technology, Beijing, China) could generate 916 MHz continuous electromagnetic waves, which are in the mobile phone frequency. The electromagnetic field power density around the antenna was measured by a radiation measure meter (China Institute for Radiation Protection, Xi'an, Shaanxi Province, China).

Methods

Training and selection of the rats

Rats were placed in the maze covered with food-reward (sunflower seeds with sesame oil) wells located 2 cm from the extremity of arms 1, 2, 4, and 7, and no food in arms 3, 5, 6, and 8. They were allowed to freely explore the maze for 10 minutes per day over 2 days to become familiar with the positions of the food. The rats were food-deprived such that they were 85% of their free-feeding weight on the day before the test. At the start of a trial, each rat was placed in the central area for 15 seconds with all of the doors closed. Then, the eight doors were opened simultaneously and the animal was allowed to run completely into any of the arms. The variables recorded were the time taken to eat all food (completion time), the number of entries into an arm with food (correct choice), and the number of entries into an arm without food (total error choice). The total error variable included the number of entries into an arm that never contained food (reference memory error) and the number of entries into an arm where food had been previously eaten (working memory error). A test ended when all the food was eaten or 5 minutes had expired. Each rat was tested twice a day with a 10 hours of intertrial interval. The maze was cleaned with 70% alcohol to minimize olfactory cues between rats. The rats that made no more than one reference memory error and no working memory errors in five successive tests were considered to have memorized the maze, and were included in the subsequent experiments.

Electrode array implantation

One rat from the exposed group and one from the control group were anesthetized and fixed on a stereotaxic apparatus (Shanghai Softmaze Information Technology Co., Ltd., Shanghai, China). In reference to a rat brain stereotaxic map[28], a 4 × 4 electrode array (Cyberkinetics Neurotechnology Systems Inc., Foxborough, MA, USA) was implanted into the rat hippocampal CA1 region at a depth of 2.8 mm to 3.2 mm and coordinates 3.6 mm posterior to and 1.9 mm lateral to the Bregma zero point. The electrode tip was secured with dental cement[29-31].

Electromagnetic field exposure

The experiments were conducted after the rats recovered from the surgery. The exposed rat was exposed to an electromagnetic field for 6 hours on Monday through Friday of every week. The electromagnetic field power density near the center of the cage housing this rat was adjusted to 10 W/m^2, which is close to that of mobile phone antennae. The control rat was housed under the same conditions as the exposed rat, but not exposed to an electromagnetic field.

Eight-arm radial maze experiment

The eight-arm radial maze testing was the same as the previously described training and selection phase. A camera 100 cm above the eight arms enabled the movements of rats to be tracked and recorded by a computerized analysis program. Both groups engaged in an eight-arm radial maze experiment on the Saturday of each week of the experiment, and no testing or electromagnetic field exposure was conducted on any Sunday. The experiment stopped when no obvious changes in behavior occurred in 3 consecutive weeks. The performance of rats in the maze was assessed by averaging the completion time and error rate of the rats in the exposure and control groups.

Recording neuronal activities

Hippocampus neuronal activities were detected by the implanted 16-channel 4 × 4 electrode array with its tip connecting to a Cerebus™ Data Acquisition System (Cyberkinetics Neurotechnology Systems Inc., Foxborough, MA, USA) through a flat cable. The signals were recorded with a 2 kHz sampling frequency while rats explored the eight-arm radial maze.

The data collected from multiple neurons were imported into MATLAB software (MathWorks, Natick, MA, USA). Abnormal discharges because of external interference were removed based on the video recording. Data from channels 4, 8, 12 and 15 were rejected because the connecting lines broke during the experiment. Spikes per second were obtained by averaging the spike number of all neurons during the recording.

Statistical analysis

Data were analyzed by SPSS statistical software (SPSS, Chicago, IL, USA) with the assumptions of a normal distribution and equal variance. An independent samples *t*-test was used to compare behavior between the exposed and control rats. Neuronal activities were analyzed using repeated-measures analysis of variance, followed by Student-Newman-Keuls *post-hoc* test.

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Author contributions: Dongmei Hao and Lei Yang designed the study, analyzed the data, and wrote the manuscript. Shuicai Wu acquired the funding and provided technical support. Su Chen and Yonghao Tian participated in the study design, conducted the experiment, and collected the data.

Conflicts of interest: None declared.

Ethical approval: This study was conducted with permission from the Animal Care and Research Committee of South-Central University for Nationalities, Wuhan, China.

Supplementary information: Supplementary data associated with this article can be found in the online version by visiting www.nrronline.org.

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