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Interaction of microwaves and a temporally incoherent magnetic field on spatial learning in the rat

Henry Lai*

Bioelectromagnetics Research Laboratory, Department of Bioengineering, University of Washington, Box 357962, Seattle, WA 98195-7962, USA

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Abstract

The effect of a temporally incoherent magnetic field ('noise') on microwave-induced spatial learning deficit in the rat was investigated. Rats were trained in six sessions to locate a submerged platform in a circular water maze. Four treatment groups of rats were studied: microwave-exposure (2450-MHz continuous-wave microwaves, power density 2 mW/cm^2, average whole-body specific absorption rate 1.2 W/kg), 'noise' exposure (60 mG), 'microwave+noise' exposure, and sham exposure. Animals were exposed to these conditions for 1 h immediately before each training session. One hour after the last training session, animals were tested in a 2-min probe trial in the maze during which the platform was removed. The time spent during the 2 min in the quadrant of the maze in which the platform had been located was scored. Results show that microwave-exposed rats had significant deficit in learning to locate the submerged platform when compared with the performance of the sham-exposed animals. Exposure to 'noise' alone did not significantly affect the performance of the animals (i.e., it was similar to that of the sham-exposed rats). However, simultaneous exposure to 'noise' significantly attenuated the microwave-induced spatial learning deficit (i.e. 'microwave+noise'-exposed rats learned significantly better than the microwave-exposed rats). During the probe trial, microwave-exposed animals spent significantly less time in the quadrant where the platform was located. However, response of the 'microwave+noise'-exposed animals was similar to that of the sham-exposed animals during the probe trial. Thus, simultaneous exposure to a temporally incoherent magnetic field blocks microwave-induced spatial learning and memory deficits in the rat.

Keywords: Microwaves; Temporally incoherent magnetic field; Spatial memory; Water maze

1. Introduction

Cellular phones and wireless communication devices emit microwaves, a form of electromagnetic field (EMF). Due to the close proximity to the body, users are exposed to relatively high level of the radiation. The specific absorption rate (SAR) in certain areas of the head of a user could reach a level up to 2.5 W/kg [1]. In previous studies, we found that central cholinergic activities, particular those in the frontal cortex and hippocampus, are affected in rats exposed to microwaves at a whole-body average SAR of 0.6 W/kg [2–5], and exposure to microwaves affected both short-term [6] and long-term [7] spatial learning and memory in the rat. We found that rats, after exposure to microwaves, had significant deficit performing in the Morris water maze, in which they had to learn to locate a submerged platform in a circular pool of opaque water using cues in the environment. This behavioral paradigm has been widely used to study spatial 'reference' memory of rodents. Relevance of spatial learning and memory of rodents to human health has been suggested [8,9], e.g., relating to aging, cognition, and development of neurodegenerative diseases.

Litovitz and his colleagues have proposed that cell membrane could detect EMFs in the environment. However, for a response to occur, an EMF has to be 'coherent',

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* Tel.: +1 206 543 1071; fax: +1 206 685 3925.
E-mail address: hlai@u.washington.edu (H. Lai).

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that is, its characteristic parameters, such as frequency, amplitude, waveform, and pattern have to remain constant over a certain period of time (>~10 s). Incoherent EMFs (i.e. those with changes in characteristic parameters over shorter periods of time) may be detected but do not trigger a biological response. Furthermore, superimposition of an incoherent EMF over a coherent EMF can make the sum field incoherent, thus blocking the biological effect of the coherent field. For details and theory of this EMF-detection model, readers are referred to the publications of Litovitz et al. [10,11]. Specifically, they have reported that a superimposed incoherent EMF ('noise') could inhibit the abnormalities in developing chick embryos [12] and changes in ornithine decarboxylase (ODC) activity [11,13,14] induced by a coherent 60-Hz magnetic field. They also found that a 'noise' superimposed on a microwave field blocked the microwave-induced increase in ODC activity in cultured cells [15]. Their hypothesis was further supported by results of the experiments of Raskmark and Kwee [16] showing that an incoherent magnetic field could mitigate the effect of a coherent 50-Hz magnetic field on proliferation of human epithelial amnionc cells, and of Lin and Goodman [17] that a superimposed incoherent magnetic field blocked the enhancement effect of a 60-Hz magnetic field on c-*myc* transcript levels in human leukemia cells.

In the present experiment, we investigated whether simultaneous exposure to a temporally incoherent magnetic field could attenuate the microwave-induced performance deficit in the water maze in the rat.

2. Methods and procedures

2.1. Animals

Male Sprague–Dawley rats (2–3 months old, 250–300 g) were purchased from B & K Laboratory, Bellevue, WA. They were housed in the same room in which they were exposed to EMF and adjacent to the room in which the water-maze testing was carried out. The area of housing was approximately 6 ft away from the Helmholtz coils used to generate the magnetic 'noise'. No significant change in magnetic field intensity was detected at this location when the coils were activated to generate a field of 60 mG. In addition, housing cages were covered by a Styrofoam cover, which muffled most of the sound in the laboratory.

The rooms were maintained on a 12-h light–dark cycle (light on between 7 a.m. and 7 p.m.) and at an ambient temperature of 22 °C. Animals were provided with Purina rat chow and water ad libitum during the experiment. A maximum of three rats were housed in one cage during an experiment. Animal use procedures of this research were approved by the Institutional Animal Care and Use Committee of the University of Washington.

2.2. Methods of microwave and incoherent magnetic field exposure

Rats were exposed to microwaves in a cylindrical waveguide exposure system designed by Guy et al. [18]. Briefly, the system consists of individual cylindrical waveguides connected through a power divider network to a continuous-wave microwave-power source (Hewlett Packard, HP-8616A signal generator). Waveguides were constructed of galvanized wire screen in which a circularly polarized TE_{11}-mode field configuration is exited. The tube contains a cylindrical plastic chamber (length 19.6 cm, diameter 17.6 cm, and a built-in floor with width 14.5 cm) to house a rat with enough space for it to move freely inside. The floor of the chamber is formed of glass rods, allowing waste to fall through plastic funnels into a collection container outside the waveguide.

For incoherent magnetic field exposure, a waveguide was placed between a set of Helmholtz coils, which were positioned across the area where the plastic animal-holding cage was located. Thus, an animal could be exposed simultaneously to microwaves and 'noise', or microwave or 'noise' alone. The Helmholtz coils were constructed on a frame made of 3/4 in. flexible copper tubing. Each coil was wound with 100 turns using gauge 18 magnet wire. The nominal resistance of the coil pair was 5.8 Ω. E-field shielding was provided by connecting the coil frame to electrical ground. The temporally incoherent magnetic field ('noise') was generated using a signal recorded in an audiocassette tape. The tape was played back in a continuous-play cassette player connected to a power amplifier (Hewlett Packard, HP-467A) whose output was in turn connected to the Helmholtz coil. The recorded 'noise' signal was provided by Dr. Miguel Penafiel of the Catholic University of America, Washington, DC. It was a band-limited, extremely low-frequency magnetic field which was randomly switched on and off for the duration of the exposure. The pass band of the 'noise' was specified to be nominally between 30 and 100 Hz. Details of generation of such a continuous 'noise' signal have been previously described [10]. The on/off action needed to produce the random on/off pattern was achieved using a computer-controlled switch. The switching pattern consisted of a repeating set of 660 alternating on and off intervals that cycled in approximately 11 min. The duration of these intervals was chosen at random from a set 11 time values which included 0.56 s, 1.67 s, and values in between separated by approximately 0.11 s. The selection was made so as to create a uniform distribution of on and off intervals within each 11-min cycle. All 'noise' exposures were carried out at an average magnetic field flux density of 60 mG that was monitored using an EMDEX meter (Enertech Consultants, Campbell, CA). The intensity of ambient AC magnetic fields (40–800 Hz) in the laboratory was 1.4 mG.

2.3. Exposure and water-maze running procedures

There were four treatment groups: microwave-exposed, ‘microwave+noise’-exposed, ‘noise’-exposed, and sham-exposed animals. There were eight animals in each treatment group. Rats in the microwave-exposed group were exposed in waveguides for 1 h to continuous-wave, circularly polarized, 2450-MHz microwaves at a spatially averaged power density of 2 mW/cm^2 (the average whole-body SAR was 1.2 W/kg [19]). ‘Microwave+noise’-exposed animals were exposed to microwaves and magnetic ‘noise’ (60 mG) simultaneously for 1 h in waveguides. A rat of the ‘noise’-exposed group was placed in a waveguide and exposed to the magnetic ‘noise’ (60 mG) for 1 h, with the waveguide not activated. For sham exposure, a rat was placed in a waveguide for 1 h with neither the microwave-waveguide nor the Helmholtz coils activated.

The water maze was a plastic circular pool (diameter: 246 cm; height: 39 cm; wall thickness: 1 mm) filled with water (22 °C) to a depth of 27 cm. The water was made opaque by addition of powdered milk. A Plexglas platform (15×20 cm) was placed at the center of one quadrant (designated as the N–E quadrant) of the maze and submerged 5 cm below the surface of the water. Each rat was given two training sessions daily separated by 4 h on three consecutive days. Maze training was carried out between 9 a.m. and 3 p.m. As many as four rats were run sequentially in a session by staggering exposure times between two rats by 10 min. The sequence in which the rats were run was the same over the six sessions.

In each training session, an animal was first exposed to microwaves, ‘microwave+noise’, or ‘noise’ or sham-exposed for 1 h in a waveguide. It was then released into the water from the wall of the maze at arbitrarily defined east, south, west, and north points. Therefore, there were four trials per training session per animal. The sequence of points of release into the water followed a random order, but included one release from each of the east, south, west, and north points in each training session. The animal was allowed to swim to the platform. If it could not locate the platform within 1 min, it would be picked up and placed on the platform. After landing or being placed on the platform, it was allowed to stay there for 30 s before another trial or was removed from the maze after the fourth trial. Performance in the maze was videotaped via a closed-circuit television system for detailed analysis later. In addition, 1 h after the last (6th) training session, each animal was given a ‘probe trial’, in which the platform was removed from the maze and the animal was released from the south point and allowed to swim in the maze for 2 min.

2.4. Data analysis

From the video recording, escape time (i.e. the time between release in the water to landing on the platform) was measured by a stopwatch. Trials with no successful ‘escape’ were given a score of 60 s. The average escape time of the four trials (released at east, south, west, and north points of the maze) in each training session of each rat was used in data analysis. For the probe trial, time spend in the quadrant of the maze where the platform was previously located (N–E) was scored. These analyses were conducted by an experimenter unaware of the treatment conditions of the rats being scored. Escape time data from training sessions were analyzed by the ANOVA for mean (treatment, training trial) and interaction (Treatment × Trial) effects. Individual response curves were analyzed by the trend analysis and compared by the method of Krauth [20] using the Mann–Whitney *U* test to compare performance between treatment groups. Data from the probe trial were analyzed using the one-way ANOVA. Difference between treatment groups was compared by the Newman–Keuls test. A difference at $P \leq 05$ was considered statistically significant.

3. Results

Data on escape time are shown in Fig. 1. ANOVA shows significant treatment [$F(3,28)=7.90$, $P<.005$] and training trial [$F(5,140)=34.55$, $P<.005$] effect and a significant Treatment × Trial interaction effect [$F(15,140)=3.26$, $P<.025$]. Animals progressively learned the location of the platform with training. A significant decrease in escape time with training sessions was observed in all four groups of animals [trend analysis for trial effect: sham, $F(5,35)=29.85$, $P<.005$; noise, $F(5,35)=9.52$, $P<.005$; microwave, $F(5,35)=6.93$, $P<.005$; microwave+noise, $F(5,35)=5.72$, $P<.005$]. How-

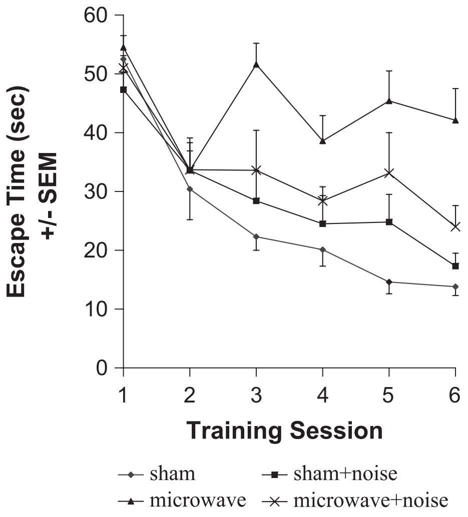

Fig. 1. Average ‘escape time’ (i.e. time to reach the platform after release into the water) during the six training sessions of microwave, sham, ‘sham+noise’ and ‘microwave+noise’-exposed rats. *n*=8 in each group.

Table 1
Average time spent during the probe trials in the quadrant where the platform was located during training sessions

Treatment	Mean time spent in previously platformed quadrant (s)±S.E.M.
Sham	39.1±3.6
Noise	40.8±3.6
Microwave	26.3±1.6
Microwave+noise	41.1±3.4

n=8 for each treatment group.

ever, comparison of the response curves of the various groups shows that microwave exposure significantly affected the animals in learning the location of the platform. They took significantly longer time to locate and land on the platform (sham vs. microwave, $P<.001$), as compared by the method of Krauth [20] using the Mann–Whitney U test. ‘Noise’ exposure alone did not significantly affect learning (sham vs. ‘noise’, $P=.172$). Simultaneous ‘noise’ exposure with microwaves significantly attenuated the effect of microwaves (microwave vs. ‘microwave+noise’, $P=.025$). However, there was also a significant difference in learning between the ‘microwave+noise’ and the sham-exposed animals ($P=.016$).

Results of the probe trials are shown in Table 1. It contains the ‘time spent in the previously platformed quadrant’ during the 2-min probe trial of the four groups of animals. One-way ANOVA of the data shows a significant treatment effect [$F(3,28)=3.186$, $P<.05$]. Newman–Keuls test shows that the microwave-exposed rats spent significantly less time ($P<.05$) in the quadrant than the other three treatment groups (sham, ‘noise’ and ‘microwaves+noise’). There is no significant difference among the responses of these three groups.

4. Discussion

Data from the present experiment show that acute exposure (1 h) of rats to a continuous-wave 2450-MHz microwaves, at an average whole-body SAR of 1.2 W/kg, significantly affected their rate of learning to locate a submerged platform in a water maze, which indicates that ‘reference’ memory was affected by microwave exposure. This behavioral deficit was further confirmed by the results of the probe trials. The data also show that simultaneous exposure to a temporally incoherent magnetic ‘noise’ could attenuate the effect of microwaves on spatial learning. These data support the hypothesis of Litovitz that ‘cellular response to EMFs occurs through a detection process involving temporal sensing’ [15]. Superpositioning the incoherent magnetic field could have attenuated the sensing and thus biological response to the microwave radiation. A magnetic ‘noise’ of flux density of 60 mG was used in this study because this intensity has been shown in another study in our laboratory to be able to block microwave-induced DNA damage in rat brain cells (unpublished results).

In the water maze, rats form spatial reference mapping (i.e. using the relative position of various different environmental cues as guides) to locate the position of the platform [21,22]. Thus, microwave-exposed rats are deficient in forming a ‘reference’ spatial map based on environmental cues. The neuroanatomical and neurochemical processes associated with water-maze performance are well studied. Cholinergic innervations to the cerebral cortex and hippocampus play important roles in spatial learning [23–25] and learning and memory in the water maze [26–29]. Deficit in water maze performance could be caused by a decrease in cholinergic activity in the brain. Thus, our previous findings [2–5] that microwave exposure decreased cholinergic activities in the frontal cortex and hippocampus of the rat may explain the spatial learning deficit observed in animals after microwave exposure. It would be interesting to investigate whether a temporally incoherent magnetic ‘noise’ could block the effects of microwaves on central cholinergic activities.

Another possible mechanism of interaction is the endogenous opioid systems in the brain. We have previously reported that microwaves activate endogenous opioids in the brain of the rat that in turn leads to a decrease in cholinergic activities in the frontal cortex and hippocampus [5,30–32]. Furthermore, microwave-induced deficit in radial-arm maze learning in the rat could be blocked by pretreating animals with naltrexone, but not by the peripheral opiate antagonist naloxone bromide [6]. Even though we do not have any information regarding the neural mechanisms of microwave-induced water-maze deficit, central cholinergic and opioid systems are involved in water-maze performance [33,34]. It is possible that the ‘incoherent’ magnetic field could neutralize the effect of microwaves via its action on the cholinergic and opioid systems, since effects of extremely low-frequency magnetic fields on the cholinergic [35–37] and opioids [38–42] systems have been reported.

However, because temporally incoherent EMF has been shown to block a variety of seemingly unrelated biological effects of coherent EMF, including chick embryo development [12], ornithine decarboxylase activity [11,13,14], cell proliferation [16], c-*myc* transcription [17], DNA damage (Lai and Singh, unpublished results), and behavior (this study), it is likely that the interaction occurs at a more basic biological level, such as the cell membrane as proposed by Litovitz et al. [10,11].

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