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Acute Exposure to Pulsed 2450-MHz Microwaves Affects Water-Maze Performance of Rats

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Rats were trained in six sessions to locate a submerged platform in a circular water maze. They were exposed to pulsed 2450-MHz microwaves (pulse width 2 μs, 500 pulses/s, average power density 2 mW/cm^2, average whole body specific absorption rate 1.2 W/kg) for 1 h in a circular waveguide system immediately before each training session. One hour after the last training session, they were tested in a probe trial during which the platform was removed and the time spent in the quadrant of the maze in which the platform had been located during the 1-min trial was scored. Three groups of animals, microwave-exposed, sham-exposed, and cage control, were studied. Microwave-exposed rats were slower than sham-exposed and cage control rats in learning to locate the platform. However, there was no significant difference in swim speed among the three groups of animals, indicating that the difference in learning was not due to a change in motor functions or motivation. During the probe trial, microwave-exposed animals spent significantly less time in the quadrant that had contained the platform, and their swim patterns were different from those of the sham-exposed and cage control animals. The latter observation indicates that microwave-exposed rats used a different strategy in learning the location of the platform. These results show that acute exposure to pulsed microwaves caused a deficit in spatial "reference" memory in the rat. Bioelectromagnetics 21:52–56, 2000. © 2000 Wiley-Liss, Inc.

Key words: acute exposure; rats; spatial reference memory; learning; behavior

INTRODUCTION

Effects of radiofrequency electromagnetic radiation (RFR) exposure on the central nervous system in laboratory animals, as indexed by neurochemical and behavioral end points, have been reported [Lai, 1994]. Owing to the relevance of spatial learning of rodents to human health [Anger, 1991; Gallagher and Nicoll, 1993], we studied rats' performance in the radial-arm maze after microwave exposure [Lai et al., 1994]. In that experiment, microwave-exposed rats showed retarded learning, indicating a deficit in spatial cognitive function. In the present research, the effect of acute microwave exposure on spatial learning and memory functions was further studied via the Morris water maze, in which rats learn to locate a submerged platform in a circular pool of opaque water by using cues in the environment. This behavioral paradigm has been widely used to study spatial "reference" memory of rodents.

METHODS AND PROCEDURES

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 microwaves and adjacent to the room in which the water-maze-testing was carried out. The rooms were maintained on a 12-h light-dark cycle (light on between 7 h and 19 h) and with 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.

Method of Microwave Exposure

The construction of the 2450-MHz cylindrical waveguide exposure system has been described in

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detail by Guy et al. [1979]. The system consists of individual cylindrical waveguides connected through a power divider network to a pulsed microwave-power source (Applied Microwave, Andover, MA; model PG5KB). Waveguides were constructed of galvanized wire screen in which a circularly polarized TE_{11}-mode field configuration is exited. The tube contains a plastic chamber 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. Animals were subjected simultaneously to microwave or sham exposure in an experiment. Microwave-exposed rats were exposed to pulsed (2 µs pulses, 500 pulses/s), 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) [Chou et al., 1984]. During sham exposures, animals were placed in similar waveguides for the same period of time as the microwave-exposed animals, except that the waveguides were not activated.

In addition to the microwave-exposed ($N = 11$) and sham-exposed ($N = 11$) animals, a group of cage control ($N = 12$) rats was also included in this experiment. These controls were housed in cages in the laboratory and subjected to the same maze-running procedure as were the animals of the other two groups. However, they were not subjected to the experimental or sham exposure procedures.

Water-Maze Running Experimental Procedure

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 PM and 3 PM. As many as eight rats were run sequentially in a session by staggering training 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 exposed to microwaves or sham-exposed for 1 h in the waveguides. Cage-control animals were taken directly from their home cages and subjected to the maze training procedure. Each animal 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 (sixth) 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 1 min.

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 stop watch. Trials with no successful escape were given a score of 60 s. The average escape time of the four trials in each training session of each rat was used in data analysis. The swimming pattern of each trial was also traced on transparencies. The distance swum was measured from the tracings, scaled to the dimension of the pool. Swim speed (cm/s) was calculated for each rat for each training trial by dividing the distance swum by the time of escape. 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 done by an experimenter unaware of the treatment conditions of the rats being scored. Escape time and swimming speed data from training sessions were analyzed by a repeated measurement analysis of variance (ANOVA); response curves were compared by the method of Krauth [1980]. The difference between groups in the probe trials was compared by the Newman–Keuls test. A difference at $p \leq .05$ was considered statistically significant.

RESULTS

Results of escape time during the six training sessions are shown in Figure 1. Data analysis showed a significant session effect ($F[5, 155] = 47.51$, $p < .001$, i.e., a significant decrease in escape time with training), treatment effect ($F[2, 31] = 6.66$, $p < .005$), but no significant "treatment × session" interaction effect ($F[10, 155] = 1.26$, $p > .05$). Thus there was a significant difference in performance among the three groups of animals during training. By the method of Krauth, the mean escape time over the training

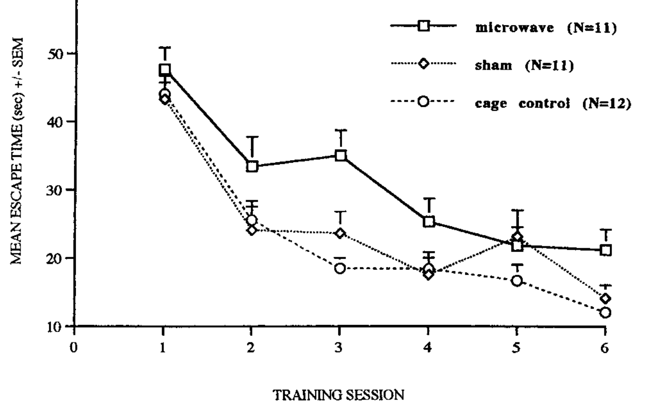

Fig. 1. Average escape time, i.e., time to reach the platform after release into the water, during the six training sessions of microwave, sham-exposed and cage control rats. N is the number of animals studied in each group.

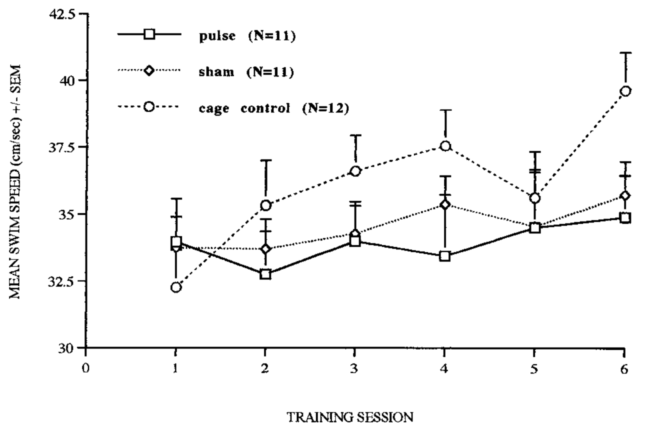

Fig. 2. Average swim speed (cm/s) of the three groups of experimental animals during training sessions.

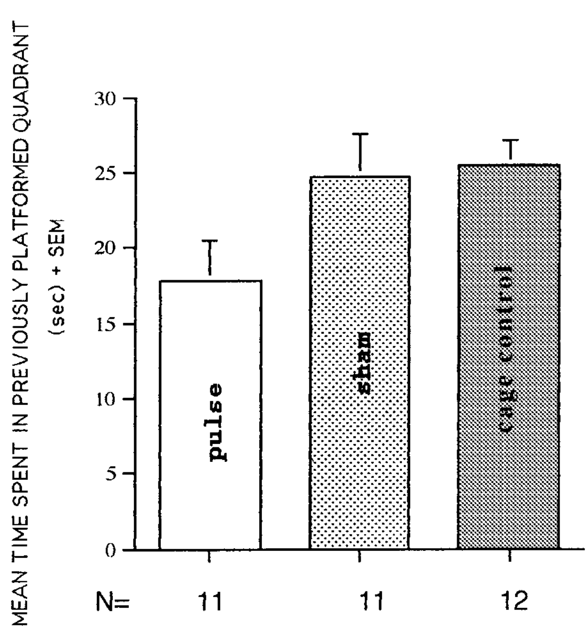

Fig. 3. Average time spent during the probe trials in the quadrant where the platform was located during training sessions.

sessions of microwave-exposed animals was found to be significantly longer than that of the sham-exposed animals ($\chi^2 = 4.55$, df = 1, $p < .05$). We observed that in training sessions the microwave-exposed rats tended to spend much more time attempting to climb up the wall or swimming along the wall of the pool. There was no significant difference in escape time during the training sessions between the sham-exposed and cage control animals.

Figure 2 shows the mean swim speeds of the three groups of animals during the training sessions. Analysis of variance of the data showed a significant treatment effect (F[2, 31] = 46.3, $p < .005$) and a treatment by session interaction effect (F[10, 155] = 2.04, $p < .05$), but no significant session effect (F[5, 155] = 1.39, $p > .05$). However, there was no significant difference between microwave- and sham-exposed groups ($\chi^2 = 0.18$, df = 1, $p < .05$) and between microwave-exposed and cage controls ($\chi^2 = 0.38$, df = 1, $p < .05$) as compared by the method of Krauth [1980].

Results of the probe trial are presented in Figure 3, which shows the average duration of time (in seconds) for the three groups of rats during the 1-min probe-trial period in the quadrant in which the platform was located during training sessions. Analysis of the data by the one-way ANOVA showed no significant treatment effect (F[2, 31] = 3.163, $p > .05$). However, comparing results of individual groups with the Newman–Keuls test showed that microwave-exposed rats spent significantly less time in the quadrant than the sham-exposed ($p < .05$) and cage-control ($p < .01$) rats.

Figure 4 shows all the tracings of swim pattern of microwave- (N = 11, Figure 4a) and sham-exposed (N = 11, Figure 4b) animals during the probe trials. Swimming patterns of microwave-exposed rats were different from those of the sham-exposed rats. The microwave-exposed animals did less searching for the "missing" platform in the N–E quadrant and swam more over other area of the maze.

DISCUSSION

Data from this experiment show that acute exposure (1 h) of rats to pulsed, circular polarized, 2450-MHz microwaves, at an average whole body SAR of 1.2 W/kg, significantly affected the rate of learning to locate a submerged platform in a water-

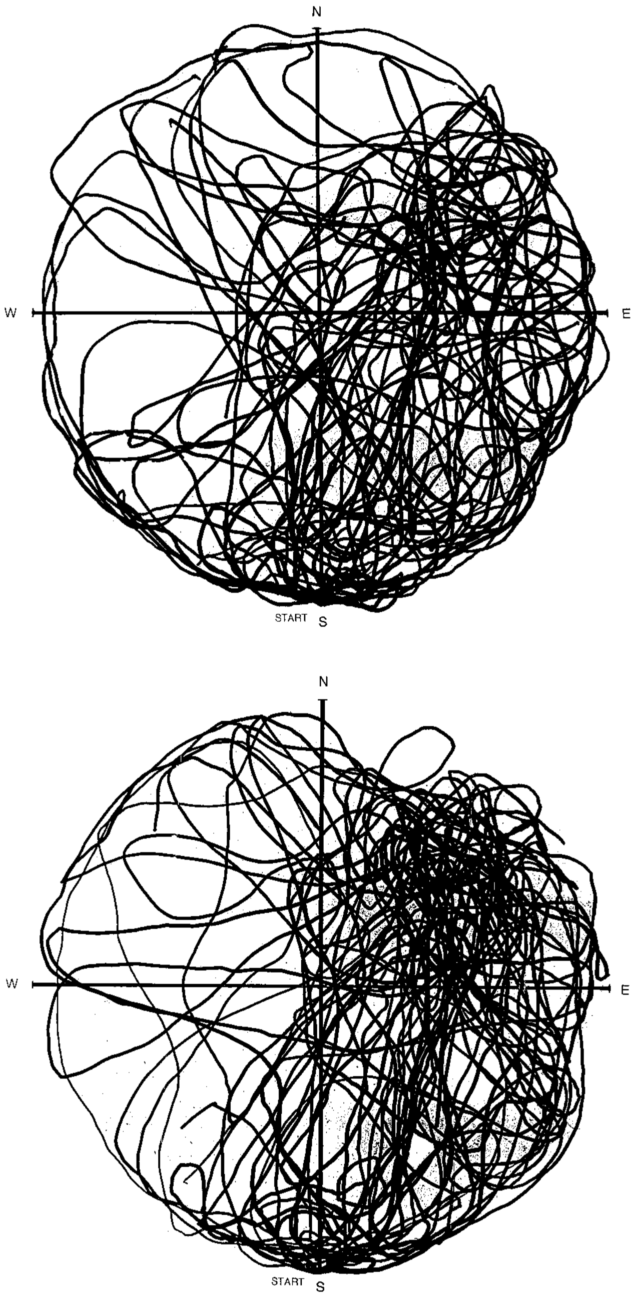

Fig. 4. Cumulative swim patterns during the probe trials of microwave-exposed rats (N = 11, top) and sham-exposed rats (N = 11, bottom). The platform was located at the center of the north–east (N–E) quadrant during training sessions. Rats were released at the south (S) point.

maze, which may indicate that "reference" memory was affected by microwave exposure. Although microwave-exposed rats displayed some knowledge of the location of the platform during the learning trials, their "spatial map" was significantly less precise so that their escape time and distance to land on the submerged platform were longer than those of the sham-exposed and cage controls. However, the swimming speed of the rats was not significantly affected by microwave-exposure. This indicates that the difference in learning may not be due to a change in motor activity or difference in motivation.

Microwave-exposed rats showed a swimming pattern during the probe trials that differed from that of controls. They showed less of a tendency to search for the "missing" platform. In the water maze, rats can use different behavioral strategies to escape to the hidden platform [Noonan et al. 1996; Chapillon and Roullet, 1996]. Spatial reference mapping or "place" learning (i.e., using the relative position of various different ambient cues as guides) perhaps is the most important and efficient. Apparently, this strategy was used by the control animals to locate the submerged platform. From the difference in swim patterns, it is very likely that microwave-exposed rats used a different strategy in learning to locate the submerged platform. Instead of forming a "reference" spatial map based on environmental cues, they might have learned to use a certain fixed pattern of action (known as "praxis learning"), or to use one particular cue in the environment (known as "cue learning") to locate the platform during the training trials. Both "praxis" and "cue" learning are generally not considered true "spatial" learning, and they are less complex and flexible than "place" learning. Disturbance to the central nervous system by microwave exposure may lessen an animal's learning capability and the animal has to resort to simpler learning strategies.

The mechanism by which pulsed microwave could affect spatial reference learning is not known. Since pulsed microwaves were used in this study, a possibility is that the behavioral effect observed was caused by the auditory effect of the pulsed radiation [Chou et al., 1982]. According to Chou et al. [1985], the threshold of hearing in the circular waveguide is 0.9 to 1.8 mJ/kg per pulse. For the 1.2 W/kg whole body SAR used in the present study, the specific absorption (SA) for the pulses is 2.4 mJ/kg, which is definitely above the hearing threshold. However, neuroanatomical and neurochemical processes associated with water maze performance are well studied. Cholinergic innervations to the cerebral cortex and hippocampus play different and important roles in learning and memory in the water maze [Sutherland et al., 1982; Decker et al., 1988; Gallagher and Pelleymounter, 1988; Brandeis et al., 1990]. Deficit in water maze performance could be caused by a decrease in cholinergic activity in the brain. We [Lai et al., 1988, 1989, 1994] have found that acute microwave exposure decreased cholinergic activities in the frontal cortex and hippocampus of the rat, and both cholinergic and endogenous opioid neurotransmitter systems in the brain are involved in the pulsed microwave-induced spatial learning deficit in the radial-arm maze. It has been shown that central cholinergic pathways, especially those of the frontal cortex and hippocampus, are involved in "place" learning [Whishaw et al., 1985, 1987, 1989]. Decreases in frontal cortical and hippocampal cholinergic activity may be responsible for the water maze learning deficit seen after acute exposure to pulsed microwaves.

REFERENCES

Anger WK. 1991. Animal test systems to study behavioral dysfunctions of neurodegenerative disorders. Neurotoxicol 12:403–413.

Brandeis R, Dachir S, Sapir M, Levy A, Fisher A. 1990. Reversal of age-related cognitive impairments by an M1 cholinergic agonist, AF102B. Pharmac Biochem Behav 36:89–95.

Chapillon P, Roullet P. 1996. Use of proximal and distal cues in place navigation by mice changes during ontogeny. Devel Psychobiol 29:529–545.

Chou CK, Guy AW, Galambos R. 1982. Auditory perception of radiofrequency electromagnetic fields. J Acoust Soc Am 71: 1321–1334.

Chou CK, Guy AW, Johnson RB. 1984. SAR in rats exposed in 2450-MHz circularly polarized waveguide. Bioelectromagnetics 5:389–398.

Chou CK, Yee KC, Guy AW. 1985. Auditory response in rats exposed to 2,450 MHz electromagnetic fields in a circularly polarized waveguide. Bioelectromagnetics 6: 323–326.

Decker MW, Pelleymounter MA, Gallagher M. 1988. Effects of training on a spatial memory task on high affinity choline uptake in hippocampus and cortex in young adult and aged rats. J Neurosci 8:90–100.

Gallagher M, Pelleymounter MA. 1988. Spatial learning deficits in old rats: A model for memory decline in the aged. Neurobiology 9:549–556.

Gallagher M, Nicolle MM. 1993. Animal models of normal aging: Relationship between cognitive decline and markers in hippocampal circuitry. Behav Brain Res 57:155–162.

Guy AW, Wallace J, McDougall JA. 1979. Circular polarized 2450-MHz waveguide system for chronic exposure of small animals to microwaves. Radio Sci 14(6S):63–74.

Krauth J. 1980. Nonparametric analysis of response curves. J Neurosci Meth 2:239–252.

Lai H. 1994. Neurological effects of microwave irradiation. In: Lin JC, editor. Advances in Electromagnetic Fields in Living Systems, Vol. 1, New York: Plenum Press, pp 27–80.

Lai H, Horita A, Guy AW. 1988. Acute low-level microwave exposure and central cholinergic activity: Studies of irradiation parameters. Bioelectromagnetics 9:355–362.

Lai H, Carino MA, Horita A, Guy AW. 1989. Low level microwave irradiation and central cholinergic activity: A dose-response study. Bioelectromagnetics 10:203–208.

Lai H, Horita A, Guy AW. 1994. Microwave irradiation affects radial-arm maze performance in the rat. Bioelectromagnetics 15:95–104.

Noonan M, Penque M, Axelrod S. 1996. Septal lesions impair rats' Morris test performance but facilitate left-right response differentiation. Physiol Behav 60:895–900.

Sutherland RJ, Kolb B, Whishaw Q. 1982. Spatial mapping: Definitive disruption by hippocampal or medial frontal cortical damage in the rat. Neurosci Lett 31:271–276.

Whishaw IQ. 1985. Cholinergic receptor blockade in the rat impairs locale but not taxon strategies for place navigation in a swimming pool. Behav Neurosci 99:979–1005.

Whishaw IQ. 1989. Dissociating performance and learning deficits on spatial navigation tasks in rats subjected to cholinergic muscarinic blockade. Brain Res Bull 23:347–358.

Whishaw IQ, Tomie JA. 1987. Cholinergic receptor blockade produces impairments in a sensorimotor subsystem for place navigation in the rat: Evidence from sensory, motor, and acquisition tests in a swimming pool. Behav Neurosci 101:603–616.