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Behavioural Brain Research 161 (2005) 331–334

BEHAVIOURAL BRAIN RESEARCH

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Short communication

Whole-body exposure to 2.45 GHz electromagnetic fields does not alter 12-arm radial-maze with reduced access to spatial cues in rats

Brigitte Cosquer [a], Niels Kuster [b], Jean-Christophe Cassel [a,*]

[a] *LN2C UMR 7521 Université Louis Pasteur-CNRS, Institut Fédératif de Recherche 37, GDR CNRS 2905, 12 rue Goethe, 67000 Strasbourg, France*
[b] *IT'IS Laboratories, Zeughausstrasse 43, 8004 Zürich, Switzerland*

Received 9 December 2004; received in revised form 8 February 2005; accepted 14 February 2005
Available online 23 March 2005

Abstract

Lai et al. [Lai H, Horita A, Guy AW. Microwave irradiation affects radial-arm maze performance in the rat. Bioelectromagnetics 1994;15(2):95–104] reported that exposure of rats to pulsed 2.45 GHz microwaves altered maze performance. Their maze was bordered by 20 cm high opaque walls. Using a maze test based on unrestrained access to spatial cues (no walls), we could not replicate this result [Cassel JC, Cosquer B, Galani R, Kuster N. Whole-body exposure to 2.45 GHz electromagnetic fields does not alter radial-maze performance in rats. Behav Brain Res 2004;155:37–43]. Here, we attempted another replication using a maze apparatus bordered by 30 cm high opaque walls. Performance of exposed rats was normal. These results show that microwave exposure as used herein does not alter spatial working memory, when access to spatial cues is reduced.

Keywords: High frequency; Microwaves; Spatial memory; Working memory

Research on health effects of electromagnetic fields (EMF) has grown exponentially with the expansion of mobile phone communication. Based on clinical and experimental data, it was claimed that EMFs might produce a variety of adverse in vivo effects (e.g. headaches, sleep disturbances) and alter cognitive function (e.g. [4,9,16]). In animals, one significant study on cognitive effects of EMFs (0.1 MHz–300 GHz) was published by Lai et al. [14]. It showed that whole-body exposure to pulsed 2.45 GHz microwaves (500 pulses/s, specific absorption rate 0.6 W/kg) for 45 min produced spatial working memory perturbations (e.g. [8]). This and other studies (e.g. [10,18]) contributed to sustain the debate on whether EMFs may be dangerous or not for health. In a recent experiment, we tried to replicate the Lai et al. [14] study using a radial maze in which rats had unrestrained access to distal cues present in the testing room (there were no side walls on the arms and only transparent borders around the central platform [1]). In this study, we could not confirm the findings reported by Lai et al. [14]. Cobb et al. [2] came to an identical conclusion. In their study, Lai et al. [14] used a radial maze, which was bordered all around by 20 cm high opaque walls, whereby rats only had limited access to the spatial cues of their distal environment. As this major difference between both studies might have influenced the way by which rats were dealing with the task in terms of navigation processes, it might also be another potential reason for our replication failure. Cobb et al. [2] used a maze that was bordered by 20 cm high opaque walls, but their rats were always injected with vehicle or drugs before exposure. This manipulation might have been a stressful event that could interfere with wave effects or even performance. In the present study, we have run an additional experiment respecting the same conditions as in our previous experiment [1], except that opaque walls were placed all around the arms and the central platform. Furthermore, rats were not injected before exposure.

All procedures involving animals and their care were conducted in conformity with institutional guidelines (NIH publication no. 80–23, revised 1996) and all efforts were made

* Corresponding author. Tel.: +33 390 241 952; fax: +33 390 241 958.
E-mail address: jean-christophe.cassel@psycho-ulp.u-strasbg.fr (J.-C. Cassel).

0166-4328/$ – see front matter
doi:10.1016/j.bbr.2005.02.026

both to use as few subjects as possible regarding statistical constraints, and to minimize suffering throughout the experiments. We used 48 male Sprague–Dawley rats (R. Janvier, Le Genest St-Isle, France) aged about 3 months (270–320 g). At arrival at the laboratory, the rats were placed one per cage (42 cm × 26 cm × 15 cm) with food and water ad libitum. The colony and the testing rooms were on a 12-h light/12-h dark cycle (lights on at 7:00) under controlled temperature (22 °C). The rats were allowed to habituate to the laboratory conditions for 10 days. Subsequently, they were placed under a food-restricted diet, which enabled to progressively reduce the body weight to 90% of its initial value (over 10 days). The body weight was maintained at this level over the 10-day testing period.

The exposure system was the same as the one used by Lai et al. [14] and has been described in detail, including dosimetry, in our previous publications [1,3], see also [7]. To achieve a dominant orientation of the animal head towards the incident wave, a light source (60 W) was placed near the waveguide opposite to the input source, as described in a previous article [3].

The radial arm maze was the same as in our previous experiment [1], except that 30 cm high opaque gray polyvinyl chloride sidewalls were bordering the maze (see Fig. 1). All training and testing procedures were identical to those described in [1]. A trial lasted at most 10 min. The radial maze and the exposure system were in a large room, separated by an opaque curtain that delimited a 380 cm × 360 m (maze) and a 280 cm × 360 cm area (exposure system). All rats were brought to the exposure/testing room in pairs, 15 min before exposure. Naïve rats were kept in their cage during exposure or sham-exposure of their counterparts. Data were collected and analysed as described previously [1]. Multiple comparisons used a Newman–Keuls test [19]. In order to compare the performance of the rats used in the present study with those of our previous one [1], we computed an average score over the 10 trials and compared the data using a group × study design.

Data are shown in Fig. 2. ANOVA of the number of errors (Fig. 2A) showed no significant group effect ($F(2,45) < 1.0$), a significant day effect ($F(9,405) = 4.8$, $p < 0.001$), and no significant group × day interaction ($F(18,405) < 1.0$). The significant day effect was due to overall performance, which improved over days, although not all comparisons yielded significant differences (e.g. overall performance on days 7–10 was significantly better than on days 1 and 2). ANOVA of the number of arms visited before the first error was committed (Fig. 2B) showed no significant group effect ($F(2,45) < 1.0$), a significant day effect ($F(9,405) = 10.2$, $p < 0.001$), and no significant group × day interaction ($F(18,405) < 1.0$). The significant day effect was due to overall performance, which improved over days, although not all comparisons yielded significant differences. When the number of errors as defined by Lai et al. was analysed (Fig. 2C), i.e. the number of errors in the 12 first visits, ANOVA also failed to show significant group ($F(2,45) < 1.0$) and interaction ($F(18,405) < 1.0$) effects, but there was again a significant overall day effect ($F(9,405) = 5.5$, $p < 0.001$), which reflected performance improvement over trials.

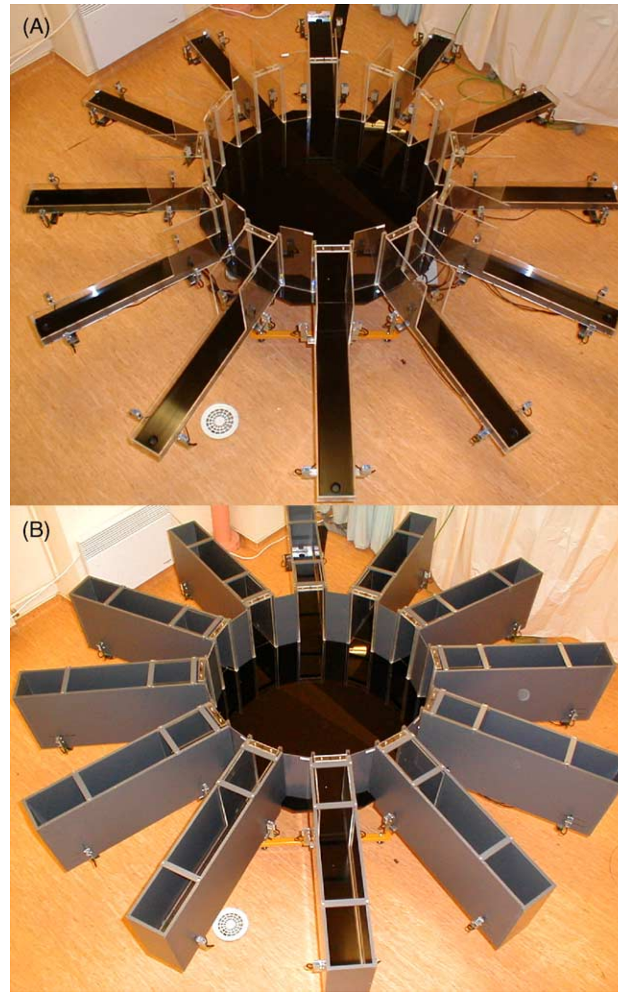

Fig. 1. Photograph of the radial maze used in our previous studies (A) [1], and the maze used in the present one (B). In the present study, there were 30 cm high opaque walls all around the maze. Photographs by Dr. R. Galani.

Our present results were also compared with those of our former study [1]. ANOVA of the number of errors failed to show significant group ($F(2,74) = 1.6$) and study effects ($F(1,74) = 3.56$, $p = 0.06$), as well as a significant interaction between both factors ($F(2,74) < 1.0$). Additional consideration of the trial factor did not change the conclusions of the analysis. The almost significant study effect was due to the fact that the overall number of errors found in the present study tended to be larger than in our former study [1]. ANOVA of both other variables, i.e. the number of arms visited before the first error and the number of errors in the 12 first visits yielded similar conclusions ($F(1,74)$ or $F(2,74) < 1.0$ in all cases, except for the interaction on the latter variable, $F(2,74) = 1.2$).

Lai et al. reported that whole-body exposure to 2.45 GHz microwaves altered memory performance in a radial maze [14], and confirmed their conclusion in a Morris water maze

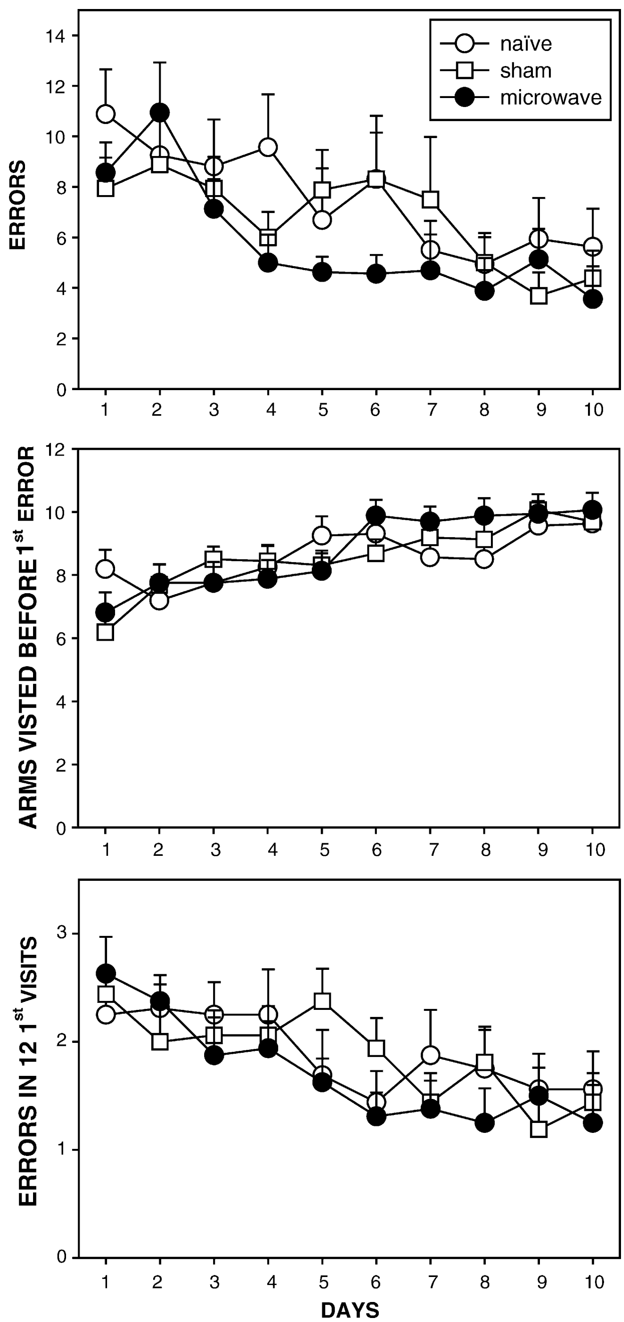

Fig. 2. Mean ± S.E.M. number of errors (top), of arms visited before the first error (middle) and of errors made in the 12 first visits (bottom) over a 10-day period of 12-arm radial-maze testing. Rats were naïve ($n = 16$), sham-exposed for 45 min ($n = 16$; placed in wave guide but not exposed to microwaves), or exposed to pulsed microwaves ($n = 16$) before being tested. Signal characteristics were: 2.45 GHz, 2 μs pulses, 500 pps, 0.6 W/kg SAR.

[18]. Since then, several studies also focused on possible adverse effects of whole-body or head-only exposure to 900 MHz GSM EMFs on spatial working memory in mice [17] and rats [5,6], no such effects could be detected. In another recent article, Yamaguchi et al. [20] showed that 1439 MHz signals did not alter food-rewarded T-maze alternation in the absence of thermal effects. The discrepancy between the outcome of the studies using 900 MHz GSM EMFs, and the data reported by Lai et al. [14] could be due to differences in either the microwave characteristics (900 versus 2450 MHz) or the task (8-arm versus 12-arm radial maze). In a recent study, however, we found that a 45-min exposure to 2.45 GHz microwaves did not alter performance assessed in a 12-arm radial maze [1]. In this study, the strain, sex and weight of the rats, the radial maze testing protocol, the microwave characteristics (500 pps, pulse width = 2 μs, average whole body SAR = 0.6 W/kg), the exposure duration (45 min), and the exposure system were comparable to those reported by Lai et al. [14]. There was nevertheless an important difference between both studies; in the replication attempt by Cassel et al. [1], there were no opaque borders on the maze and rats could see all distal cues from inside the maze while being tested. Conversely, in the study by Lai [10], the maze was bordered by 20 cm high opaque walls. This difference, which has been discussed in our recent article [1], might have influenced the way by which rats dealt with the task (higher load on spatial memory in the absence of opaque walls bordering the maze against possible use of alternative non-spatial strategies in the presence of opaque borders), and thus could be one of the most obvious explanations for our failure to replicate Lai et al.'s findings. However, right before the publication of our previous article [1], Cobb et al. [2] failed to replicate the original report by Lai et al. [14]; these authors also injected their rats with various substances before the exposure onset and used a protocol based on restricted access to distal spatial cues. With the present experiment, we confirm that limited access to spatial cues is not the factor that enabled microwave-induced memory deficits to be detected in a 12-arm radial maze test, whether present or not, the opaque walls surrounding the maze did not significantly influence performance (see Table 1).

This additional failure to replicate the original experiment by Lai et al. [14] is of importance as, in a series of different experiments, e.g. [10–13,15], these authors have fed the controversy about (and challenged) the innocuousness of signals used in mobile phone communications. Based on the outcome of our previous [1] and on present studies, but also on the results reported by Cobb et al. [2], it seems that the balance now tends towards the innocuousness of the microwave signals initially used by Lai et al. [14], at least as long as spatial working-memory performance is concerned. The data reported by Cobb et al. [2], our former conclusion [1] and our present results, however, should, in no case, be considered an invitation to close part of a debate on health effects of EMfs. One has to keep in mind that our results are valid only within the limits of a particular model. Given the current extent and the still enormous extension potential of the mobile phone market, given the variety of microwave signals used (e.g. 880–960, 1439, 1710–1785, 1805–1880, 1900–2200 MHz), given the fact that even a very small risk may still have a large impact on public health due to the world-wide dimension of the population of mobile phone users, the need for further research on possible health effects of EMFs may be of current concern.

Table 1
Average ± S.E.M. number of errors, arms visited before the first error, and errors in the 12 first visits over 10 radial-maze trials in naïve, sham-exposed and microwave-exposed rats

	Study	
	Present (limited access to distal cues)	Previous (access to distal cues not limited)
Number of errors		
Naïve	7.55 ± 0.94	6.08 ± 0.63
Sham	6.75 ± 0.59	5.32 ± 0.67
Microwave	5.81 ± 0.52	4.96 ± 0.52
Number of arms visited before the first error		
Naïve	8.66 ± 0.40	8.15 ± 0.36
Sham	8.59 ± 0.23	8.84 ± 0.30
Microwave	8.71 ± 0.25	8.75 ± 0.22
Number of errors in the 12 first visits		
Naïve	1.89 ± 0.21	1.96 ± 0.14
Sham	1.88 ± 0.14	1.63 ± 0.14
Microwave	1.71 ± 0.11	1.67 ± 0.13

Performance in the present study (limited access to distal cues, 30 cm high opaque walls all around the maze) was compared to performance observed in our previous study [1], in which each group comprised 12 rats. The only methodological difference between both studies was the presence or absence of 30 cm high opaque walls bordering the maze.

Acknowledgements

The authors acknowledge Catherine Krieger and Olivier Bildstein for excellent technical assistance, Walter Wolf (CNRS) for construction of the 12-arm radial maze, Emmanuel Gamelin (CNRS) and Simon Rakotonirina (ULP) for their help in equipping the maze with opaque borders, as well as Sébastien Geiger, who developed both the hardware and the software for automated data acquisition. Dr. Rodrigue Galani is acknowledged for his photographs of the testing apparatuses. Within the Perform-B programme, this work was funded by the MMF and the GSM A. Administrative contribution of ULP and CNRS is also recognized.

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