Whole body exposure with GSM 900 MHz affects spatial memory in mice

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Abstract

Extended work has been performed worldwide on the effects of mobile phone radiation upon rats' cognitive functions, however there is great controversy to the existence or not of deficits. The present work has been designed in order to test the effects of mobile phone radiation on spatial learning and memory in mice Mus musculus Balb/c using the Morris water maze (a hippocampal-dependent spatial memory task), since there is just one other study on mice with very low SAR level (0.05 W/kg) showing no effects. We have applied a 2 h daily dose of pulsed GSM 900 MHz radiation from commercially available mobile phone for 4 days at SAR values ranging from 0.41 to 0.98 W/kg. Statistical analysis revealed that during learning, exposed animals showed a deficit in transferring the acquired spatial information across training days (increased escape latency and distance swam, compared to the sham-exposed animals, on the first trial of training days 2–4). Moreover, during the memory probe-trial sham-exposed animals showed the expected preference for the target quadrant, while the exposed animals showed no preference, indicating that the exposed mice had deficits in consolidation and/or retrieval of the learned spatial information. Our results provide a basis for more thorough investigations considering reports on non-thermal effects of electromagnetic fields (EMFs).

Keywords: Electromagnetic fields; Morris water maze; Spatial memory

1. Introduction

The tremendous increase in the number of users of mobile phone technology in relation to possible health effects raised by several studies has forced a large number of scientists to get involved in the investigation of the biological and health effects [1]. Since the usual, without protective measures (hands free or blue tooth), use of the mobile phone (MP) takes place near the user’s head, the elucidation of the cellular, molecular and behavioral effects are of utmost importance, especially since the majority of life-time MP users will be the current teenagers. The key question therefore is, do living organisms in general react upon their exposure to man-made electromagnetic fields (EMFs) of non-ionizing electromagnetic radiation? To have this question answered extensive research is being performed in various laboratories as thoroughly presented in a recent review article [2]. The so far literature regarding the issue of risk assessment of EMFs reveals that the biological effects of EMF have been and are being investigated at different levels [3], starting downwards from the level of human population with epidemiological studies. At the immediate lower level of the individuals, human, animal and plant in vivo experiments are carried out. Consequently, at the level of organs, tissues and cells in vitro experiments are performed. At last but not least, at the sub-cellular level, biochemical, biophysical and molecular techniques are utilized:

Epidemiological–statistical studies have been successfully correlated exposure conditions to defects, primarily of brain tumors [4,5]. In some cases minor health symptoms have been reported [6], as well as behavioral problems in children exposed prenatally to mobile phone radiation [7]. Clinical studies in humans, mainly involving volunteers, have shown possible effects on sleeping conditions and memory function [8]. At the same, individual’s level, lab animal studies are very extensive and have been using sophisticated techniques including gene and protein expression studies, proteomics, development and reproduction following EMF exposure. Many animal models have been used, including
insects due to their enormous genetic/molecular background information, DNA fragmentation and induced cell death during oogenesis, along with a decrease in the offspring number, have been reported by our group [9,10]. Due to the fact that mobile phone use affects mainly the brain tissues, special attention has been given to behavioral studies of memory and learning in rodents, mostly in rats. There have been reports of impairment [11,12] or even improvement [13,14] of cognitive functions. One of the pioneer studies that demonstrated significant memory deficits in rats [16] explored their short term memory following exposure to EMFs by utilizing the Morris water maze [15]. Similarly, the same group performing behavioral studies on rats exposed to pulsed 2450 MHz microwaves (500 pulses/s, average power density 2 mW/cm² and average whole body SAR 1.2 W/kg) for 1 h just before each training session, revealed a deficit in their spatial “reference” memory [17]. On the contrary, other scientists repeating the previous experiments under the same conditions, but with minor methodological differences were unable to detect any effects on memory and learning [18]. Another work published the same year, exposing rats at similar exposure conditions and 2450 MHz frequency but using radial arm maze did not reveal any effects [19]. The same group, using a 12-arm maze apparatus bordered by 30 cm high opaque walls observed that EMF exposed rats behaved normally [20]. Therefore they concluded that microwave exposure under those conditions (2450 MHz, circularly polarized field) does not alter spatial working memory, when access to spatial cues was reduced. However, an earlier report had shown that microwaves affect specific cognitive aspects of behavior such as, attention, memory, learning, discrimination, time perception which may occur even at very low SAR levels [21].

Another set of experiments with head-only exposure of rats, unlike the whole body exposure setup of the previous studies, provided no evidence that spatial and non-spatial memory can be affected by a 45-min exposure to 900 MHz GSM EMF, [22]. This study applied radial arm maze and object recognition task (ORT), at even higher SAR values of 1–3.5 W/kg.

On the other hand, in a recent report male Wistar rats were exposed to EMF deriving from 50 missed calls/day for 4 weeks of a GSM (900/1800 MHz) mobile phone in vibratory mode (no ring tone). It was found that exposed animals had significantly (∼3 times) higher mean latency to reach the target quadrant in the Morris water maze and spent significantly (∼2 times) less time in the target quadrant [23].

Exploring the cellular basis of the observed behavioral deficits, Leif Salford and collaborators have reported that a 2 h exposure of rats at GSM 915 MHz results in neuronal damage, 28 and 50 days later [24]. The same research group has shown also that the blood–brain barrier (BBB) is being disrupted following EMF irradiation [25,26], a finding that has been recently confirmed [27,28]. In order to test short term or long term effects, it has been shown in rats that whole body SAR values, as low as 0.6 and 60 mW/kg, significantly alter the performance during an episodic-like memory test after 55 weeks following just a 2 h exposure once a week [29].

Lastly, at the lower cellular/molecular level, several studies have been published, which are valuable in clarifying the actual primary damage created by EMFs. Thus, a decrease of excitatory synaptic activity and a reduced number of excitatory synapses in cultured hippocampus neurons following GSM 1800 radiation (15 min/day for 7 days) at a SAR value of 2.4 W/kg has been shown to occur [30]. Most recently effects on the endocytotic activity of murine melanoma cells induced by EMF have been reported [31].

Behavioral studies on the effects of microwave radiation on mice’ cognitive functions are very limited. In fact there is only one, published 9 years ago [32] in which animals were exposed within GTEM cells at GSM 900 MHz, but at very low SAR value of just 0.05 W/kg. No statistically significant deficits were resolved by 8-arm maze. Since no similar investigation is being published in mice so far using the Morris water maze task, the present work was designed in order to test the effects of mobile phone radiation on spatial learning and memory in Mus musculus Balb/c. Unlike the T-maze in which the animals have to make a binary decision (i.e. going left or right), in the Morris water maze successful performance requires continuous monitoring of the animal’s position in relation to extra maze cues: a process that involves “cognitive mapping”.

The exposure setup consisted of a commercially available mobile phone, as firstly introduced by our group in insects [9,10]. Free moving mice were exposed within their cages, as also followed in rats [23,33]. We exposed the mice to a daily dose of GSM 900 MHz in speaking mode, for four consecutive days and took advantage of the Morris water maze behavioral task, since spatial navigation is a complex cognitive function that depends on several neural and cognitive systems for successful completion [8]. In this task mice are placed for four consecutive days (training period) into a large circular pool of water from which they can escape onto a slightly submerged platform. Two hours after the last training exposure to the water maze, mice are allowed to swim for 60 s in the absence of the training platform (memory probe trial). Since normal mice learn very quickly to swim directly towards the platform, mainly in reference to the position of extra maze visual cues, we wanted to find out whether exposed mice would perform equally well in these learning and memory tasks.

2. Materials and methods

2.1. Animals

A total of 24 Balb/c 50-day-old male mice were used (12 animals in the exposed group and 12 animals in the sham-exposed group). Animals were housed in groups of six in Plexiglas cages (267 mm × 207 mm × 140 mm), kept under
standard conditions (24 °C, 12:12 h light/dark cycle, lights on at 7:00 am) and received a standard laboratory diet and water ad libitum. All animal experimentations were carried out in agreement with ethical recommendation of the European Communities Council Directive of 24 November 1986 (86/609/EEC) and all efforts were made to minimize the number and suffering of the animals.

2.2. Conditions of EMF exposure

2.2.1. Field measurements

Before each set of experiments careful dosimetry was performed by measuring the mean power density of the radiation emitted by the mobile phone handset in the RF range at 900 MHz with the field meter, “RF Radiation Survey Meter, NARDA 8718, Narda Safety Test Solutions, Hauppauge, NY, USA”, with its probe placed inside the cage with the animals. In addition, we measured in the same way the mean electric and magnetic field intensities at the Extremely Low Frequency (ELF) range, with the field meter, “Holaday HI-3604, ELF Survey Meter, Holaday Industries, Inc., Eden Prairie, MN, USA”. The measured exposure values were in general below the established exposure limits [34]. We used commercially available digital mobile phone handsets, in order to analyze effects of real mobile telephony exposure conditions. Thus, instead of using simulations of digital mobile telephony signals with constant parameters (frequency, intensity etc.), or even “test mobile phones” programmed to emit mobile telephony signals with controllable power or frequency, we used real GSM signals which are never constant since there are continuous changes in their intensity (Fig. 1).

In this study, *M. musculus* Balb/c mice were exposed to EMF within their Plexiglas cages placing the mobile phone in the middle underneath the cage. Six mice were exposed each time applying “variable whole body exposure conditions” taking into account the multidimensional orientation of each mouse in relation to the near field EMF produced by the phone. Therefore there were two cages for each experimental group, exposed and sham exposed. The measured power density within the cages, where the mice were moving freely, was ranging between 0.05 and 0.2 mW/cm². In order to simulate the conditions of human voice and activate mobile phone EMF emission, a radio station was playing at moderate loudness of 60 db throughout the exposure time. The electrical field produced by the mobile phone was monitored by the Smart Fieldmeter, EMC Test Design, LLC, Newton, MA, USA, having dual band omni directional probe (900 and 1800 MHz) and the readings were between 23 and 36 V/m within the cage, depending on the sound level. The aim was to achieve similar exposing conditions to a user’s head when holding the mobile phone next to his/her ear. The specific absorption rate (SAR) for the brain tissue of the exposed mice can thus be approximately calculated according to the equation:

$$\text{SAR} = \frac{\sigma E^2}{\rho},$$

(1)
where $E$ is the root mean square value of the electrical field measured within the cages, $\sigma$ is the mean electrical conductivity of the tissues, and $\rho$ is the mass density \cite{34,35}. The SAR is a parameter widely used by most authors to compare the absorbed energy in different biological tissues. The parameters used for our mice were calculated \cite{36}, for young mice (50 days old), SAR values ranging from 0.41 to 0.98 W/kg were calculated using Eq. (1) by applying the measured electrical field density 23–36 V/m, adopting $\sigma = 0.8 S/m$ (average brain value) and mass density $\rho = 1040 \text{ kg/m}^3$. The dielectric properties of the mouse brain were estimated according to published parameters \cite{37,38}.

2.2. Exposure conditions

Mice (6 per experiment) were habituated for 1 h in the “exposure room” and then they were exposed or sham exposed within their home cages for 1 h just prior to each of the four daily training sessions. They were also kept exposed in between trials and in addition for 10 min at the end of each daily session in order to interfere with the consolidation of the learned spatial information. Finally they were exposed during the 2 h resting prior to the probe trial for the same purpose. Thus, irradiation was for the first 3 days, 1 h 55 min/day and 3 h 45 min for the fourth day making a total of 9 h and 30 min for the 4 days. Sham-exposed mice were kept under the same conditions as exposed mice, but without any radiation level as monitored by field meters. A turned-off mobile phone was placed underneath their cage and the same radio station was playing from an identical radio during the same time period as for the exposed animals. Real time graph showing the fluctuations in electrical field as detected by the Smartfieldmeter within the mice cage as a function of time, depending on the sound level was recorded by means of the WinDaq Data acquisition software, DATAQ Instruments, Inc., Akron, OH, USA (Fig. 1).

2.3. Morris water maze (MWM) behavioral task

2.3.1. Apparatus

The water maze task was taking place in a circular pool (85 cm in diameter) filled with water maintained at 23 ± 1 °C placed in a suitably equipped room with constant temperature and humidity \cite{15}. The surface of a clear Plexiglas movable escape platform (8 cm × 10 cm) was submerged 1 cm below the water surface. The extra maze visual cues included signs on the walls, as well as parts of the video recording system and the stable position of the researchers.

2.3.2. Training and testing procedures

Investigators performing the behavioral experiments were not aware of the experimental group (exposed or sham exposed) the tested animals belonged to. Mice were brought into the MWM room 1 h before the trial for habituation during which, exposure or sham exposure was taking place. Mice were trained to find a submerged escape platform, located in a fixed position relative to the extra maze visual cues, during four consecutive daily sessions. Each session consisted of four trials. Four different starting positions, equally spaced around the perimeter of the pool, were used in a fixed order. Each animal was released in the water from the wall of the maze immediately after irradiation (for the exposed mice). Each trial had a maximum duration of 60 s and mice not finding the platform within these 60 s were placed on it. At the end of each trial the animals were allowed to remain on the platform for 20 s, and were then returned to their home cage and left there to rest for 15 min before the beginning of the next trial. Exposure or sham exposure was continued during the intervals between the trials. Two hours after the last training trial (the fourth trial of the fourth day) the animals were subjected to a memory probe trial during which the mice swam for 60 s in the absence of the training platform. All mice started from the same position, opposite to the target quadrant (the quadrant where the escape platform had been positioned).

2.3.3. Recording of behavior

Behavior in the Morris water maze experiments during the training and memory-testing procedures was digitally recorded at a frequency of 2–5 Hz using the Noldus Ethovision System (Ethovision 3.0, Noldus Information Technologies, Wageningen, The Netherlands). The following parameters were recorded for each training trial as well as for the probe trial: (a) the escape latency (in s), i.e. the time taken to escape on to the submerged platform, (b) the total distance swam (in cm) to escape on the submerged platform and (c) the mean velocity of swimming (in cm/s). For the training trials, these measures were averaged per mouse within each daily session in order to calculate the daily averages (mean values).

2.3.4. Statistical analysis

Statistical analysis was performed in a semi-blind manner: The investigators were aware only if animals belonged to the same experimental group but not to which of the two groups (exposed or sham exposed). Codes were broken only after the completion of the statistical analyses. Five measures during the acquisition of the task were analyzed statistically: for each training day (a) the mean escape latency, (b) the mean total distance swam and (c) the mean velocity of swimming (in cm/s). In addition, for the first trial of each training day we analyzed (a) the escape latency and (b) the total distance swam. Three measures during recall of the task were analyzed statistically: (a) the time spent in the target quadrant of the water maze vs. the time spent in the opposite one, (b) the percent of total distance moved in the target quadrant vs. in the opposite one and (c) the mean velocity of swimming (in cm/s). All behavioral data were analyzed using one way ANOVA with repeated measures, as appropriate. In cases of statistically significant interactions, post hoc tests were used. The level of statistical significance was set at 0.05. All
tests were performed with the SPSS software (Release 10.0.1, SPSS, USA).

3. Results

3.1. Learning of the Morris water maze

All mice, irrespective of being exposed or sham exposed, appeared to swim normally and with a similar swimming speed (20.3 cm/s for the sham-exposed group and 19.3 cm/s for the exposed group) and showed no difficulty in mounting the hidden platform provided. Statistical analyses on the mean latency and mean distance swam to locate the hidden platform during acquisition of the task showed only a significant effect of day on both the mean latency ($F_{(3,23)} = 3.639$, $p = 0.017$; Fig. 2A) and mean distance ($F_{(3,23)} = 3.918$, $p = 0.012$; Fig. 2B), since mice decreased their mean latency and mean distance swam between the first and the second day of training. No group effect or significant group × day interaction has been observed in either latency or distance, indicating that both groups of animals performed equally well in learning the Morris water maze. However, when we statistically analyzed the escape latency and the total distance swam during the first trial of each training day a different pattern was identified: A significant group effect has been observed in both the escape latency ($F_{(3,23)} = 4.972$, $p = 0.036$; Fig. 2C) and the distance moved ($F_{(3,23)} = 6.109$, $p = 0.022$; Fig. 2D), with exposed animals showing both higher latency and larger distance moved during the first trial of the second, third and fourth training day compared to the sham-exposed animals.

3.2. Memory trial of the Morris water maze

In the probe trial, during the fourth day, statistical analysis of the data revealed a significant quadrant × group interaction on both time spent ($F_{(1,23)} = 4.699$, $p = 0.041$; Fig. 3A) and percent of total distance covered ($F_{(1,23)} = 4.371$, $p = 0.048$; Fig. 3B and C) in each quadrant. Further analysis revealed that only sham-exposed animals showed a clear preference for the target quadrant (for the time spent, post hoc $p = 0.006$; for the distance, post hoc $p = 0.004$; Fig. 3), whereas exposed animals showed no quadrant preference (for the time spent, post hoc $p = 0.241$; for the distance, post hoc $p = 0.143$; Fig. 3). Finally, when swim speed was examined, no difference was found between the two experimental groups (17 cm/s for the sham-exposed group and 18.2 cm/s for the exposed group). Respective videos reveal very impressively what is being pointed in the figures: exposed animals seem to swim around without being able to retrieve the information learned during the past 4 days of training regarding the position of the submerged platform. In contrast, sham-exposed mice exhibit a clear preference for the quadrant in which the platform was located during training, showing that they have con-
solidated the learned information and they can effectively retrieve it.

4. Discussion

In these experiments we investigated the effect of a commercially available mobile phone pulsed radiation at 900 MHz on the spatial learning and memory of Balb/c mice using the Morris water maze [15] in which the animals were required to find a submerged platform in the circular pool after 4 days of training by creating a “reference map” (reference memory) [39]. Although the overall learning performance of both groups was normal, a more detailed analysis of their behavior during the first trial of each training day revealed that the animals exposed to the near field of a commercially available mobile phone could not transfer the learned information across training days. Nevertheless, these animals were able to acquire the spatial information regarding the position of the escape platform and effectively locate it in the subsequent trials of each training day, i.e. when the time intervals were short (15 min). Moreover, the data from the memory probe trial (2 h after the last training trial) support the notion that mice of the exposed group had difficulty in memory consolidation and/or retrieval of the stored information of the position of the hidden platform, since these animals showed no preference for the target quadrant.

To our knowledge this is the first time that a clear-cut effect on spatial learning and memory deficiency is demonstrated for mice following exposure to non-ionizing electromagnetic radiation emitted by a mobile phone. A previous report in mice failed to reveal any deficits using the 8-arm maze behavioral task [32], possibly due to very low SAR value applied (just 0.05 W/kg and also less daily exposure, 45 min/day but for 10 days). It is possible that the daily exposure to radiation, just prior to the Morris water maze trial, is very crucial in disturbing the mice’ memory consolidation and retrieval process. Reports on rats are controversial and have not been replicated successfully so far. A number of studies have used a range of SAR values, from 0.02 up to 4 W/kg in order to induce and detect memory deficits. In the vast majority of the studies the TEM cells (Transversal Electromagnetic Mode cells) were used to expose the animals at a given power density from an RF generator. Using pulsed 2450 MHz microwaves at 2 mW/cm² power density, they have reported similar to our learning and memory deficit in rats with the Morris water maze [17]. Later investigators have failed to demonstrate memory deficits in rats exposed mainly to repeated low level radiation at 2450 MHz for 45 min prior to a 12-arm maze behavioral test [18,19,21,22]. On the other hand, in a recent report [23] using similar to ours setup protocol (free moving rodents within the cage) exposed male Wistar rats, 10–12 weeks old (which are developmentally comparable to human teenagers) to 50 missed calls/day for 4 weeks using a GSM (900/1800 MHz) mobile phone in vibratory mode (no ring tone). After the experimental period, the animals were tested for spatial memory performance using the Morris water maze as well. Exposed animals had significantly (~3 times) higher mean latency to reach the target quad-

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Fig. 3. Memory probe trial—performed 2 h after the last training trial—of the hidden version of the Morris water maze. (A) Time spent and (B) percent of total distance swam in the target and opposite quadrants during the probe trial. Bar graphs depict mean ± SEM. Note that only sham-exposed animals showed a preference for the target quadrant. (C) Representative paths followed by sham-exposed and exposed mice during the probe trial. Quadrant × group interaction: §p < 0.05.
rant and spent significantly (∼2 times) less time in the target quadrant than sham exposed indicating a deficit in spatial memory.

As in other recent reports we have chosen to perform our experiments in animals allowed to move freely in their home cages during exposure to radiation [23,33], in order to minimize possible confounding effects of restraint stress. It has been suggested that stress affects learning and memory [40]. In fact EMF is considered as an environmental stress factor. Exposure conditions were carefully selected in order to simulate as close as possible commercially available mobile phone use (duration and variable signal strength). Electromagnetic fields with changing parameters are found to be more bioactive than fields with constant parameters [41,42,43] probably because it is more difficult for living organisms to get adapted to them. Experiments with constant GSM or DCS signals have been performed, in order to ensure reproducible exposure setup but they do not simulate actual conditions.

International guidelines limit the local SAR to a maximum of 2 [34] or 1.6 W/kg [35]. Since the maximum SAR calculated in our experiments is 0.98 W/kg and since this SAR value is not expected to affect the mice’s body temperature [34] the observed effects in our experiments can be considered non-thermal. Furthermore, we selected the age of the experimental animals (50-day-old M. musculus Balb/c) as in other report [23] to correspond approximately to that of late adolescence in humans, a population in which mobile phone use is particularly prevalent. Similar exposure conditions as ours have been used by other groups [44] who exposed rats with commercially available mobile phone operating at a maximum power of 0.607 W. They found by mRNA analysis an effect on injury associated proteins leading to cellular damage to the rat brain. Since it is well known that performance in the Morris water maze is dependent on the hippocampus, it is plausible to assume that irradiation in our experiments affected this brain area. This may be supported by the observation that apoptotic cells have been detected in the hippocampus of rats after a 2 h exposure setup and duration it remains to be replicated by other similar studies and other strains since the evaluation of effects on spatial memory demands the application of different behavioral tasks and mouse strains [58]. The previous controversial reports on rats cannot be taken directly in comparison with this work since there are a lot of differences concerning the setup, the duration, the animal model and the behavioral test.

5. Conclusions

These results clearly demonstrate that exposure of mice to EMF deriving from commercially available mobile phone at SAR values within the ICNIRP guidelines for 2 h prior to the daily Morris water maze trial, affects the spatial learning and memory function in Balb/c mice. Our findings on mice are very similar to the published work using rats on a similar MWM task [17]. As shown, radiation exposure interferes with the consolidation and/or retrieval of spatial information. Being the first study of this kind, in terms of animal model, exposure setup and duration it remains to be replicated by other similar studies and other strains since the evaluation of effects on spatial memory demands the application of different behavioral tasks and mouse strains [58]. The previous controversial reports on rats cannot be taken directly in comparison with this work since there are a lot of differences concerning the setup, the duration, the animal model and the behavioral test.

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