

Cognitive Impairment in Rats After Long-Term Exposure to GSM-900 Mobile Phone Radiation

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Considering the frequent use of mobile phones, we have directed attention to possible implications on cognitive functions. In this study we investigated in a rat model the long-term effects of protracted exposure to Global System for Mobile Communication-900 MHz (GSM-900) radiation. Out of a total of 56 rats, 32 were exposed for 2 h each week for 55 weeks to radio-frequency electromagnetic radiation at different SAR levels (0.6 and 60 mW/kg at the initiation of the experimental period) emitted by a (GSM-900) test phone. Sixteen animals were sham exposed and eight animals were cage controls, which never left the animal house. After this protracted exposure, GSM-900 exposed rats were compared to sham exposed controls. Effects on exploratory behaviour were evaluated in the open-field test, in which no difference was seen. Effects on cognitive functions were evaluated in the episodic-like memory test. In our study, GSM exposed rats had impaired memory for objects and their temporal order of presentation, compared to sham exposed controls ($P = 0.02$). Detecting the place in which an object was presented was not affected by GSM exposure. Our results suggest significantly reduced memory functions in rats after GSM microwave exposure ($P = 0.02$). Bioelectromagnetics 29:219–232, 2008. © 2007 Wiley-Liss, Inc.

Key words: microwaves; episodic-like memory test; memory; open-field-test; learning; exploratory behaviour; anxiety

INTRODUCTION

The worldwide use of Global System for Mobile Communication (GSM) mobile phones raises concerns about possible implications to human health. Since the introduction of the GSM network for mobile communication in 1992 in Western Europe, the use of this kind of phone has increased tremendously. Today one-third of the world's population relies on mobile phones for daily communication. For the foreseeable future, the use of mobile phones and related technologies will continue to increase [Stewart, 2000]. Keeping this vast and constantly increasing exposure of humans to mobile phones in mind, designating the use of mobile phones as the world's largest biological experiment ever [Salford et al., 2001] is indeed appropriate.

The close proximity of the mobile phone to the user's head leads to absorption of about 50% of the electromagnetic field (EMF) energy from the mobile in

the brain [Dimbylow and Mann, 1994]. The question of whether the deliberate and passive exposure to radio frequency (RF) EMF from mobile phones might affect cognitive functions is of great importance. Reports of impairment [Maier et al., 2004; Keetley et al., 2006] or improvement [Preece et al., 1999; Koivisto et al., 2000]

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of cognitive performances in humans are countered by findings that no changes occur [Haarala et al., 2003, 2007; Russo et al., 2006]. It is vital to realise that in these mentioned studies, the mobile phone exposure is within existing exposure guidelines from the International Commission on Non-Ionising Radiation Protection [ICNIRP, 1998] with a specific energy absorption rate (SAR) <2 W/kg for exposure to the head of humans. Exposure levels above these recommendations can cause a slight temperature increase, and it is shown that exposure to 10 W/kg results in impairment of cognitive functions [Mickley et al., 1994].

In rats, hippocampus is involved in aspects comparable to human declarative memory for facts, events and places [Hammond et al., 2004]. Lesions in hippocampus impair both spatial and non-spatial memory in rats. Interestingly, it has been shown that exposure to EMF below 100 mW/kg induces significant neuronal damage in the hippocampus, as well as the cortex and the basal ganglia of rats [Salford et al., 2003].

Hitherto, exposure to GSM microwaves has not been shown to affect memory performances of rodents. Dubreuil et al. [2003] concluded that exposure of rats to GSM 900 MHz microwaves, with SAR values of 1 and 3 W/kg, did not affect spatial and non-spatial memory functions. Sienkiewicz et al. [2000] demonstrated similar negative findings after GSM 900 MHz microwave exposure of mice, with whole-body SAR values of 0.05 W/kg. On the other hand, Xu et al. [2006] showed a selective decrease of excitatory synaptic activity and the number of excitatory synapses in cultured rat hippocampal neurons after exposure to GSM 1800 MHz microwaves with SAR values of 2.4 W/kg.

To evaluate whether long-term exposure to GSM mobile phones might give rise to changes in cognitive functions as well as morphological alterations, we exposed male and female rats to radiation from a genuine GSM mobile phone for 2 h once a week for a total of 55 weeks. The GSM microwaves had a frequency of 915 MHz and were pulsed at 217 Hz. With average whole-body SAR levels of 0.6 mW/kg and 60 mW/kg no thermal effects are induced [ICNIRP, 1998; Yamaguchi et al., 2003]. After this long-term exposure, animals were subjected to two cognitive tests, the open-field test and the episodic-like memory test, to evaluate the possible effects of mobile phone RF exposure. It has been shown in open-field tests that repeated exposure to the same environment decreases the rats' exploratory activity and anxiety. This is interpreted as habituation learning and is taken as an index of memory [Schildein et al., 2000]. In the episodic-like memory test the long-term memory for different objects, their spatial location and order of presentation are tested. In the present study we used a

modified version of the episodic-like memory test described by Dere et al. [2005]. All animals examined for cognitive functions were sacrificed by perfusion-fixation and the brains will be examined histopathologically for albumin leakage and neuronal damage and other markers of premature aging. The results are presently being analysed by our neuropathologist and will be published separately.

MATERIALS AND METHODS

GSM Exposure

TEM-cells (see Fig. 1) used for RF EMF exposure of the rats were designed by dimensional scaling from previously constructed cells at the National Bureau of Standards [Crawford, 1974]. These TEM-cells have previously been used for RF EMF exposure of rats, as described by Salford et al. [1992, 1993, 1994, 2001, 2003], Persson et al. [1997] and Belyaev et al. [2006]. The construction of the TEM-cell allows relatively homogeneous exposure of the animals [Malmgren, 1998]. A GSM mobile test phone with a programmable power output at the frequency of 900 MHz was



Fig. 1. TEM-cell used in our investigation. [The color figure for this article is available online at www.interscience.wiley.com.]

connected to four TEM-cells (see Fig. 2); no voice modulation was applied.

The TEM-cell is enclosed in a wooden box (inner dimensions of $15 \times 15 \times 15$ cm), that supports the outer conductor, made of brass net, and central conducting plate. The central plate separates the top and bottom of the outer conductor symmetrically. Eighteen holes (diameter 18 mm) in the side walls and top of the wooden box make ventilation possible. These holes are also used for examining the interior during exposure.

The rats were placed in plastic trays ($14 \times 14 \times 7$ cm) to avoid contact with the central plate and outer conductor. The bottom of the tray was covered with absorbing paper to collect urine and faeces. Each TEM-cell contained two plastic trays, one above and one below the centre septum. Thus, two rats can be kept in each TEM-cell simultaneously.

The amount of radiation absorbed by a unit of mass of exposed tissue is indicated by the average value of the whole-body specific energy absorption rate (SAR value) [Malmgren, 1998]. By using the finite-difference time domain (FDTD) method [Martens et al., 1993] the SAR distribution within a rat brain phantom was found to vary <6 dB. These numerical

simulations also showed that an input power of 1 W would result in a whole-body SAR value of 1.67 W/kg in a small rat (<250 g) placed in the upper compartment of the TEM-cell, with the lower compartment kept empty. From a comparison of this computation with a FDTD computation of the whole-body SAR for a rat exposed to a plane wave (see below) it can be concluded that 1 W input power to the TEM cell corresponds to a power density $S = 52 \text{ W/m}^2$. The effective cross-sectional area of the TEM-cell appears to be 192 cm^2 compared to the geometrical cross section of 225 cm^2 . The reduction of the effective cross-sectional area can be attributed to inhomogeneous fields near the edges of the central septum.

When more than 1/3 of a TEM-cell compartment is occupied by the rat, or when both compartments are used simultaneously, the assumption that the animals do not perturb the electric field distribution in the cell significantly is no longer valid. Therefore, the average whole-body absorbed energy per rat was determined experimentally for rats of different weights placed in the upper, lower or both compartments of a TEM-cell. For a constant input power, the power reflected at the entrance and the power transmitted through the

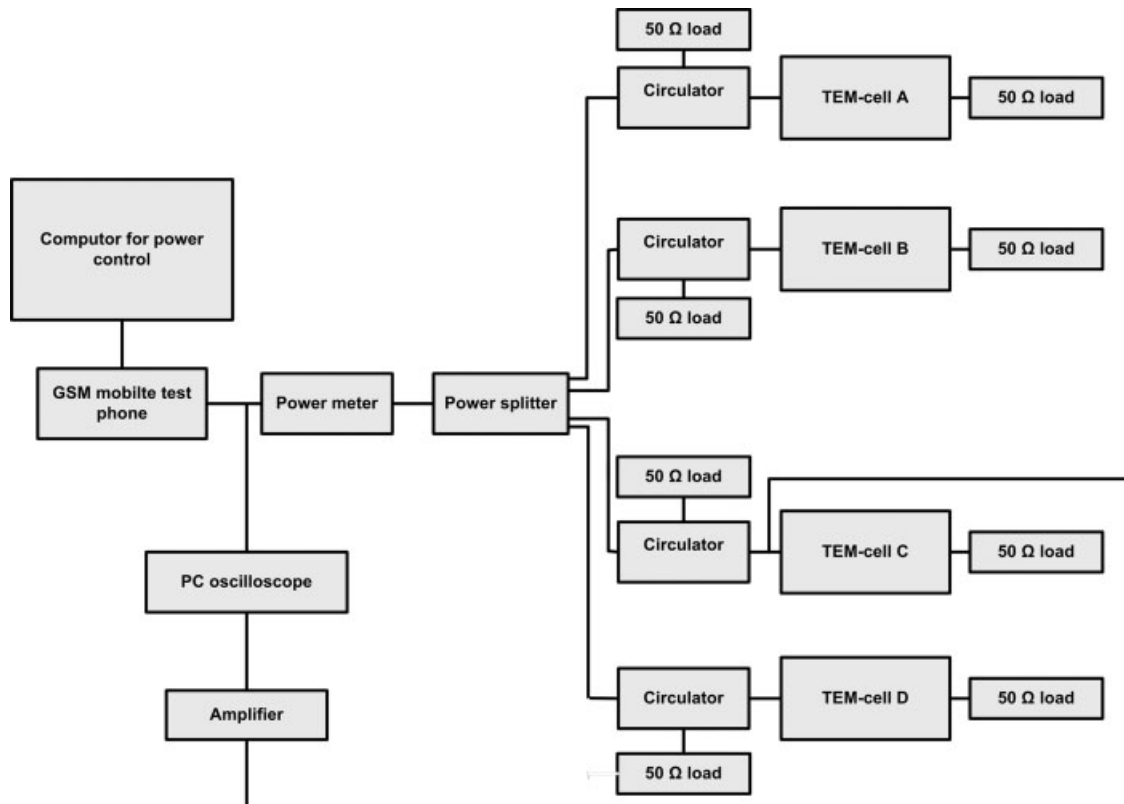


Fig. 2. Block diagram of the exposure setup. Four TEM-cells (A, B, C and D) are used. For sham exposure the same TEM-cells are used, but in this case they are not connected to the GSM mobile test phone and thus no RF EMFs are directed into the TEM-cells.

TEM-cell were measured at least five times for each experimental condition. For each measurement, the orientation of the rat with respect to the propagation direction of the microwave radiation was noted, since whole-body SAR and brain SAR vary with orientation. For the actual experimental situation with one rat in each compartment of the TEM-cell, the conversion factor K for SAR per unit of input power could be fitted to the data as:

$$K = (1.39 \pm 0.17) - (0.85 \pm 0.22) \cdot w \quad (1)$$

with w the sum of weights in kilograms of the two rats in the cell and the variance given as SEM.

To evaluate how orientation of the rat in the TEM-cell affects the SAR values (unpublished results), brain SAR and whole body SAR for 16 orientations of a 334 g rat phantom with respect to the incident radiation in the TEM-cell, were estimated in a FDTD-computation with the freely available FDTD program of Brooks Airforce Base (FDTD99) [LeBlanc et al., 2000]. In a simplified geometry, the rat is exposed by a plane wave with a power density of 10 W/m^2 in a far-field condition. The average SAR for the brain grey matter was 1.06 times the average whole-body SAR, with a standard deviation of 56% around the average value for the different orientations (Fig. 3).

The TEM-cells were placed in a temperature-controlled room under constant lighting conditions. The

temperature of the TEM-cells was kept constant by placing them on a ventilation table. All the animals, even the largest male rats, could move and turn around within the TEM-cells.

Animals

All animal procedures were performed according to the practices of the Swedish Board of Animal Research and were approved by the Animal Ethics Committee, Lund-Malmö. Fifty-six inbred male and female Fischer 344 rats (the rats were supplied by Scanbur AB, Stockholm, Sweden) were 4–6 months of age at the initiation of the EMF exposure. Male and female rats weighed approximately 350 and 200 g, respectively, as estimated in calibrations of rat weight as a function of age [Svendson and Hau, 1982]. The rats were housed in rat hutches, two in each cage, under standard conditions of 22°C room temperature, artificial daylight illumination and rodent chow and tap water ad libitum. Towards the end of the exposure period the male rats had grown in size and therefore were placed in rabbit hutches, two in each cage. The female rats were smaller and could still be kept in the rat hutches.

The twenty-eight male and twenty-eight female rats were divided into four groups with an equal number of male and female rats in each group. Each animal was given a number and the division into groups was

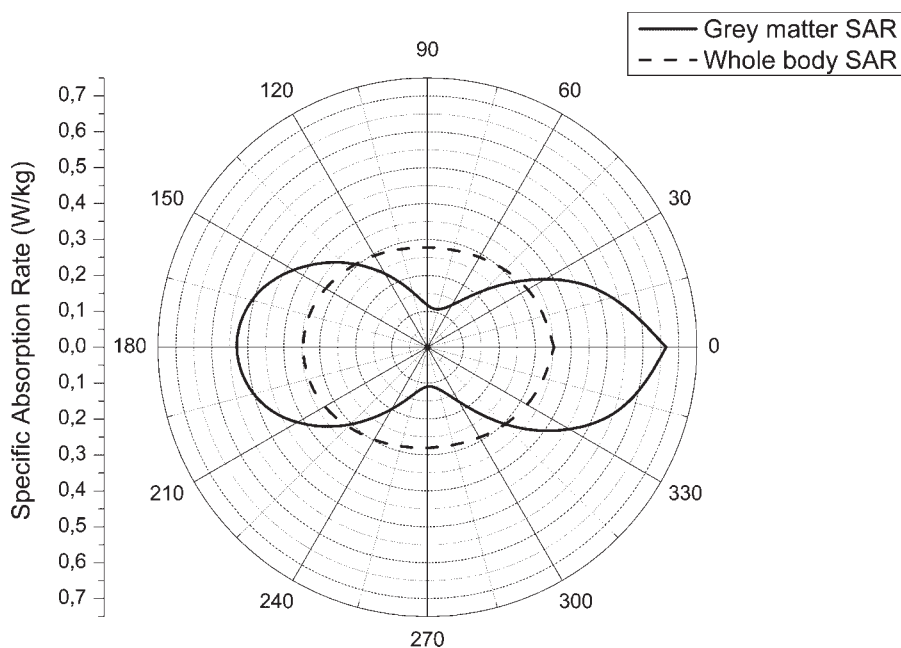


Fig. 3. FDTD calculation of SAR values in the brain grey matter and whole-body SAR (W/kg) in the rat at exposure to 900 MHz plane wave radiation, with a power density of 10 W/m^2 , as a function of incident wave angle with respect to the long axis of the animal. The polarisation of the radiation is identical to the situation in the TEM-cell. An angle of 0 degree is defined as the head of the rat pointing in the direction of the plane wave.

randomised with reference to these numbers. Sixteen animals were sham exposed. Sixteen animals were exposed to lower power level of GSM, with a peak output power (during a pulse) from the GSM mobile telephone fed into each of the TEM-cells of 5 mW (corresponding to a time averaged power density of $S = 33 \text{ mW/m}^2$), generating average SAR values of 0.50 mW/kg for males (range 0.62 mW/kg) and 0.66 mW/kg for females (range 0.37 mW/kg) (the range is defined as the difference between the maximum and minimum SAR values according to expression 1; see discussion on dosimetry above), with an average SAR value of 0.6 mW/kg for males and females together. Sixteen animals were exposed to higher power level of GSM, with a peak output power from the GSM mobile telephone fed into each of the TEM-cells of 0.5 W ($S = 3.3 \text{ W/m}^2$), generating average SAR values of 50 mW/kg for males (range 62 mW/kg) and 66 mW/kg for females (range 37 mW/kg), with an average SAR value of 60 mW/kg for males and females together. Eight animals were cage controls, which never left the animal house. These SAR values are valid at the initiation of the experimental period.

At the end of the experimental periods, the males were weighing $545 \pm 24 \text{ g}$ and the females $304 \pm 23 \text{ g}$. The groups of sham, GSM exposed and cage control animals did not significantly differ in weight. Due to the increase in weight, the SAR for the males dropped to 59% of the initial value and for the females to 84% (see expression 1 above) to average SAR values of 0.29 mW/kg for males (range 0.55 mW/kg) and 0.55 mW/kg for females (range 0.53 mW/kg) at the lower power level of GSM; and 29 mW/kg for males (range 55 mW/kg) and 55 mW/kg for females (range 53 mW/kg) at the higher power level of GSM. This generated average SAR values of 40 and 0.4 mW/kg for males and females together at the higher and lower GSM exposure levels, respectively.

For each exposure the rats were assigned different TEM-cells quasi-randomly according to a rolling timetable. The duration of the GSM-900 exposure as well as the sham exposure was 2 h at one occasion weekly for 55 weeks. Exposure was scheduled on Mondays (males) and Tuesdays (females) each week. Behavioural tests were performed during a period from 3 to 7 weeks after the last EMF or sham exposure. Thus, long-standing behavioural effects could be evaluated and confusion due to acute stress avoided.

Animals that were subjected to GSM EMF and sham exposure in TEM-cells were handled once a week in connection with exposure when the animals were transported from the animal house to the experimental laboratory. Three to four weeks after completed exposure behavioural tests were initiated. No a priori

habituation tests were carried out. Two animals died of unknown reason before the initiation, one male cage control and one male exposed to lower effect GSM. The remaining 44 rats were now 17–19 months of age.

Test Equipment

The open-field test equipment used was an $80 \times 80 \times 40 \text{ cm}$ black box made of plywood with an open roof. The floor and the walls were covered with black self-adhesive plastic. The black background contrasted well with the white-coloured rats, simplifying tracking. The open roof allowed the rats to use landmarks in the room to facilitate navigation. The floor was divided by white lines into 25 equally sized quadrants ($16 \times 16 \text{ cm}$). The inside of the box was cleaned with a napkin wetted with 70% ethyl alcohol as required, but at least once a day.

The behavioural tests were performed in a sound-attenuated room with the two observing scientists standing around the open-field. The observing scientists were positioned in the same way during each test occasion and remained silent and stationary during the test procedure. A fluorescent tube radiating 400–500 lx was placed 1.5 m above the centre of the open-field. Next to the tube was a video camera for documenting the behaviour of the rats.

Open-Field Test

Open-field tests were performed on 3 consecutive days on each rat at intervals of 24 h, males starting 3 weeks after the final day of GSM exposure and females starting 4 weeks after the final day of GSM exposure. The animals were tested in numerical order according to the numbers they had been given at the initiation of exposure. Since the EMF exposure had been randomised with reference to these numbers, the experiments were blind with respect to the exposure condition. All males were tested one week and all females the other week as a practical result of the numerical order used for the test randomisation. Each test session lasted 2 min. One rat at a time was carried from its housing room into the arena and returned after the test session, thus minimising the stress component on rat behaviour. The animal was placed in a dark box in the centre of the open-field for 30 s, after which behaviour was observed as the dark box was removed and the animal was free to move. The time spent in the centre of the open-field before the rat moves further on (centre-stay time) is an indication of general anxiety, the time being shorter in less anxious animals. Also, the number of defecations and urinations is connected to anxiety. The number of crossed squares (crossings) shows the locomotor activity and the number of times

the rat lifts its fore paws (rearing), is regarded as a general exploratory behaviour.

Episodic-Like Memory Test

Each rat was allowed to rest for 14 days after the open-field tests before performing the episodic-like memory test. This rest was also necessary for practical reasons, since the scientists performed tests on the other animals during this period. The episodic-like memory tests were all run blind with respect to the exposure condition.

The episodic-like memory test is a modified version of the episodic-like memory task for mice described by Dere et al. [2005]. It tests the recollection of a unique past experience in terms of what happened, and where and when it happened. Two different kinds of objects (in quadruplicate) were encountered, blocks with a plain surface made of black PVC and cylinders with a grooved surface made of grey PVC. The different characteristics ensure that the rats are able to distinguish the objects. However, material preference due to olfactory cues is avoided by using PVC for both objects.

The rats had been familiarised with the test environment in connection with the previous open-field tests 2 weeks earlier. The objects were placed allowing enough space for the animals to move unhindered along the walls of the open-field. At the centre of the box is a free space, where the animal is placed at the initiation of the test. This reminds the rat of the test situation in the open-field test. The order in which the animals were tested was randomised with reference to the exposure condition in the same way as for the open-field test. The observing scientists were blind to the exposure situation.

Each animal received two training trials and one test trial. There was a delay of 50 min between each trial. The exploration time allocated for each trial was 6 min. On the first training trial four black blocks known as old familiar objects were placed symmetrically one in each corner of the open-field (see Fig. 4). On the second training trial four grey cylinders known as recent familiar objects were placed in a T-shaped configuration. On the test trial, two old familiar objects were placed in the same locations as in the first training trial, one in the northwest corner and one in the southeast corner. Two recent familiar objects were placed one in the northeast corner and one in the southwest corner. Thus, one recent familiar object was displaced, whereas the other recent familiar object was stationary. The time spent exploring the old versus the new familiar objects is measured in the test trial. According to previous studies [Dere et al., 2005] normal rats will spend more time exploring the old familiar objects than the recent familiar objects. Thereby, the memory for objects, their placement and their temporal order of presentation can be assessed. The episodic-like memory test requires an assessment of the relative recency of two remembered objects, the old familiar one and the recent familiar one [Hannesson et al., 2004]. In addition, normal rats also have an ability to discriminate based on the novelty of an object location [Ennaceur et al., 1997]. Therefore the normal behaviour is to spend more time exploring the displaced new familiar object than the stationary new familiar object.

Data Collection

For the open-field test the centre-stay time was measured using stopwatches at the instant of the test

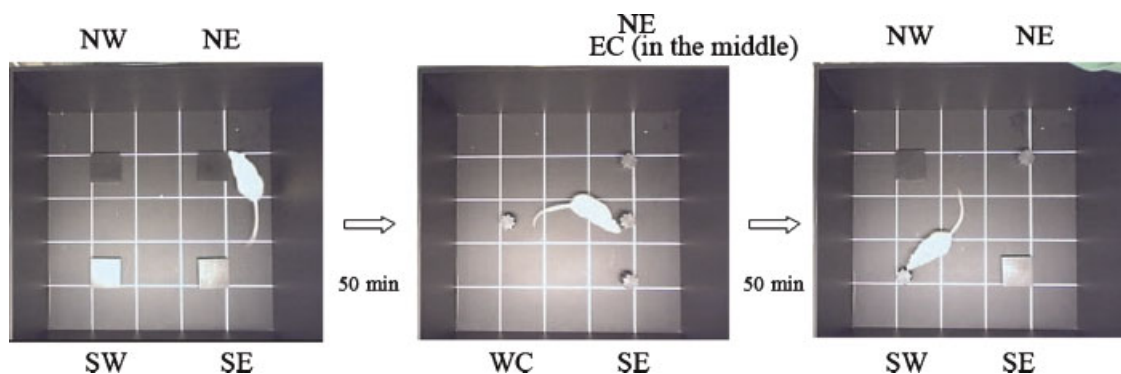


Fig. 4. Schematic drawing of the episodic-like memory test. The rats received two training trials and one test trial, each with 50 min inter-trial interval. On training trial 1 four black quadrants were arranged symmetrically one in each corner. On training trial 2 four grey cylinders were arranged in a T-shaped configuration. During the test trial two old familiar objects were placed as in training trial 1, whereas one recent familiar object was placed in a novel location. Object locations: EC, east centre; NE, northeast; NW, northwest; SE, southeast; SW, southwest; WC, west centre.

occasion. Also, the number of crossings, rearings, defecations and urinations was recorded. For the episodic-like memory test the cumulative exploration time was measured using stopwatches at the instant of the test occasion. Exploration of an object in the episodic-like memory test was operationally defined as active investigation or physical contact between the object and the rat's paws, snout or vibrissae. The rat was considered to be actively investigating an object when it had approached it within a distance corresponding to the length of its vibrissae and simultaneously looked at the approached object. During the first and second training trial, the total time spent exploring the four objects was measured. During the test trial, the total time spent exploring the two black old familiar objects and the total time spent exploring the two grey recent familiar objects was measured.

In the test trial, one recent familiar object was placed in a novel location, whereas the three other objects remained in the same locations they were presented during the training trials. The separate exploration time for each of the four objects from the test trial is of interest. To measure this, recordings from the video camera were used after completion of the test sessions. With these measurements investigations could be made to find out whether the rats had been able to memorize where the objects were localized during the two training trials. Measurements of the exploration time for each of the four objects could not be taken directly at the test occasion since it would have required more observing scientists. This could give rise to unnecessary distress among the animals. The correlation between the exploration time measured from the video recordings and the exploration time measured directly at the test occasion was $r = 0.9$. The discrepancy between direct observations and video recordings can be explained by the fact that it is easier to directly observe the exact position of the rat relative to the objects when making direct observations. Thus, the measurements made directly at the test occasion should be deemed of highest significance for evaluating the rats' behaviour.

Statistic Evaluations

For the open-field test, the Kruskal–Wallis one-way analysis of variance by ranks was used for simultaneous statistical test of the score distributions for the different GSM exposed animals, the sham exposed animals and the cage controls. Centre-stay time, number of crossings, rearings, defecations and urinations were separately tested. If the null hypothesis could be rejected, the non-parametric Mann–Whitney U -test for independent samples was

used to compare each of the groups of GSM exposed, sham exposed and cage control animals to each other. To separately investigate the contributing effects of sex, day of exposure and exposure or non-exposure condition, respectively, multiple regression analysis was performed.

For the episodic-like memory test within-group differences of the time spent exploring old familiar and recent familiar objects across the three trials were analysed by Kruskal–Wallis one-way analysis of variance followed by the Mann–Whitney U -test, using the same procedure as described for evaluation of the open-field test. For the third test trial, the standardised difference between old familiar object exploration time (O) and recent familiar object exploration time (R) was used for comparison, the standardised difference being defined as $(O - R)/(O + R)$. Comparing the exploration time in our set-up of the episodic-like memory test to that described by Dere et al. [2005] we used Student's t -test.

RESULTS

Open-Field Test

Multiple regression analysis revealed that the different behavioural parameters were influenced by sex, day of testing and being a cage control instead of a sham or GSM exposed animal, but not by GSM exposure. Generally, the habituation learning developed on consecutive days. Females showed a more pronounced habituation than males. Cage controls had less developed habituation learning. This was concluded after evaluating centre-stay time, numbers of crossings, rearings, defecations and urinations separately (see Figs. 5 and 6).

The centre-stay time decreased on consecutive days ($P < 0.0001$), but males stayed longer in the centre than females ($P < 0.0001$) (see Figs. 5A and 6A). Since the centre-stay time represents the freezing behaviour of an animal encountered to a new environment, it is an index of anxiety. Thus, we found that anxiety decreases on consecutive days, when the animals have become more used to the open-field; however, males are more anxious than females.

The number of crossings (see Figs. 5B and 6B) indicates the general locomotor behaviour. Multiple regression analysis showed that females performed more crossings than males ($P < 0.0001$) and cage controls performed fewer crossings than sham and GSM exposed animals ($P < 0.0001$).

Further on, regarding the number of rearings (see Figs. 5C and 6C), cage controls performed fewer rearings than sham and GSM exposed animals

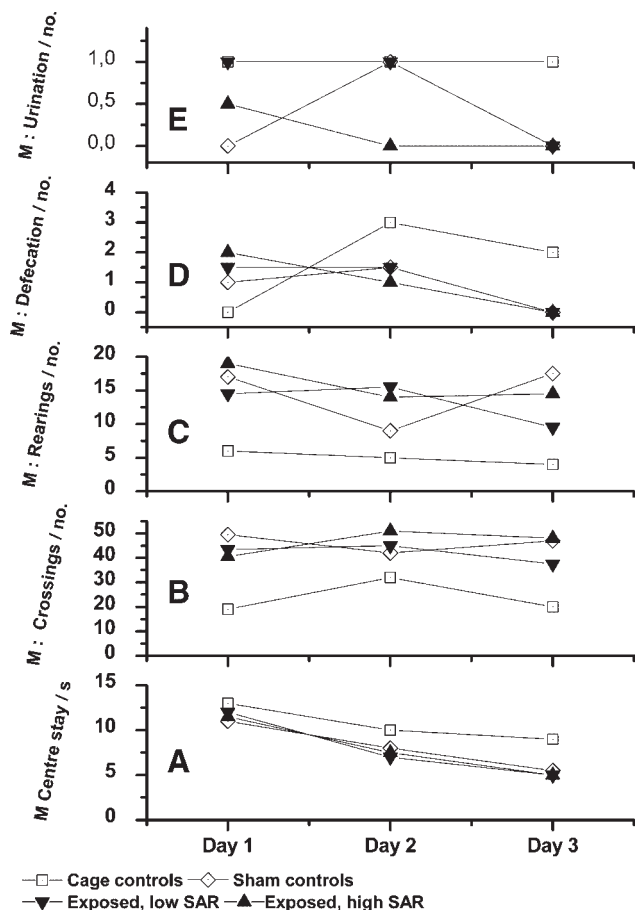


Fig. 5. Result from open-field test for males. **A**: Median value (M) of centre stay time measured in seconds. Median value (M) of number of **(B)** crossings; **(C)** rearings; **(D)** defecations and **(E)** urinations. No statistical significance was found between the GSM exposed rats compared to the sham exposed animals.

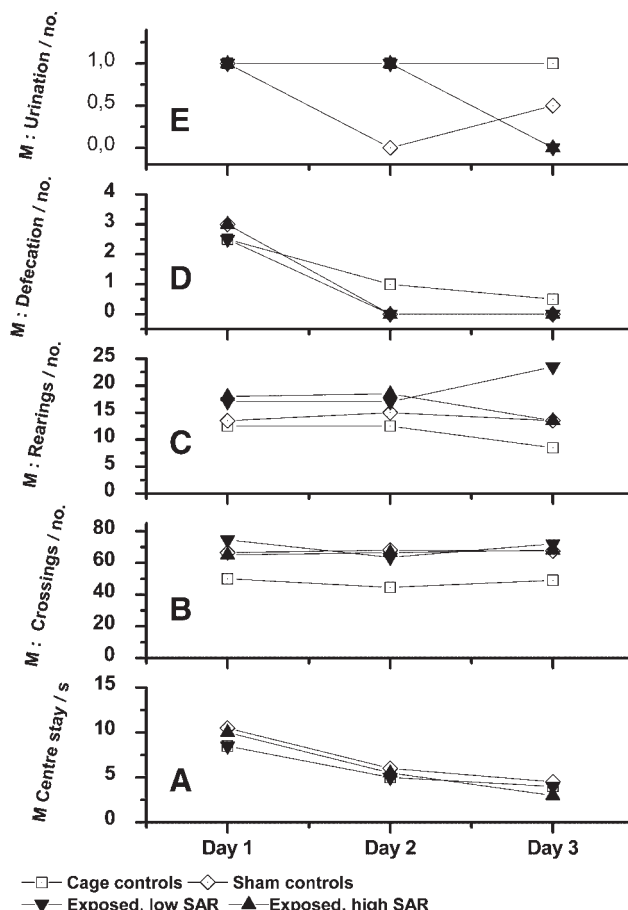


Fig. 6. Result from open-field test for females. **A**: Median value (M) of centre stay time measured in seconds. Median value (M) of number of **(B)** crossings; **(C)** rearings; **(D)** defecations and **(E)** urinations. No statistical significance was found between the GSM exposed rats compared to the sham exposed animals.

($P < 0.0001$), and females performed more rearings than males ($P = 0.006$). The number of rearings is an index of exploratory behaviour.

Defecation and urination indicates the anxiety of the animals. Both decreased on consecutive days ($P < 0.0001$) (see Figs. 5D,E and 6D,E), which is a natural reaction when the animals have become more used to the open-field test environment. However, urination decreased less for cage controls than for sham and GSM exposed animals ($P = 0.002$). This indicates a higher degree of anxiety in the inexperienced cage controls compared to the other animals. Also, the urination decreased less for males than for females ($P = 0.025$).

Episodic-Like Memory Test

Regarding the influence of sex on the performance, no statistically significant differences were observed (Mann–Whitney $P = 0.67$). Therefore, for

the remainder of the statistical analyses females and males were analysed together.

In Figure 7 the time spent exploring the old familiar objects and the recent familiar objects is shown. The standardised difference between the time spent exploring old familiar objects and recent familiar objects during the third test trial differed for the four groups (Kruskal–Wallis $P = 0.001$).

The GSM exposed rats spent a significantly shorter time than sham rats exploring old familiar objects relative to recent familiar objects (Mann–Whitney $P = 0.02$ for exposed animals versus sham; $P = 0.05$ for higher GSM exposed versus sham; $P = 0.05$ for lower GSM exposed versus sham) (see Figs. 8 and 9). No statistically significant difference was seen between the higher GSM or lower GSM exposed animals (Mann–Whitney $P = 0.19$).

The cage controls spent a significantly shorter time exploring the old familiar objects than

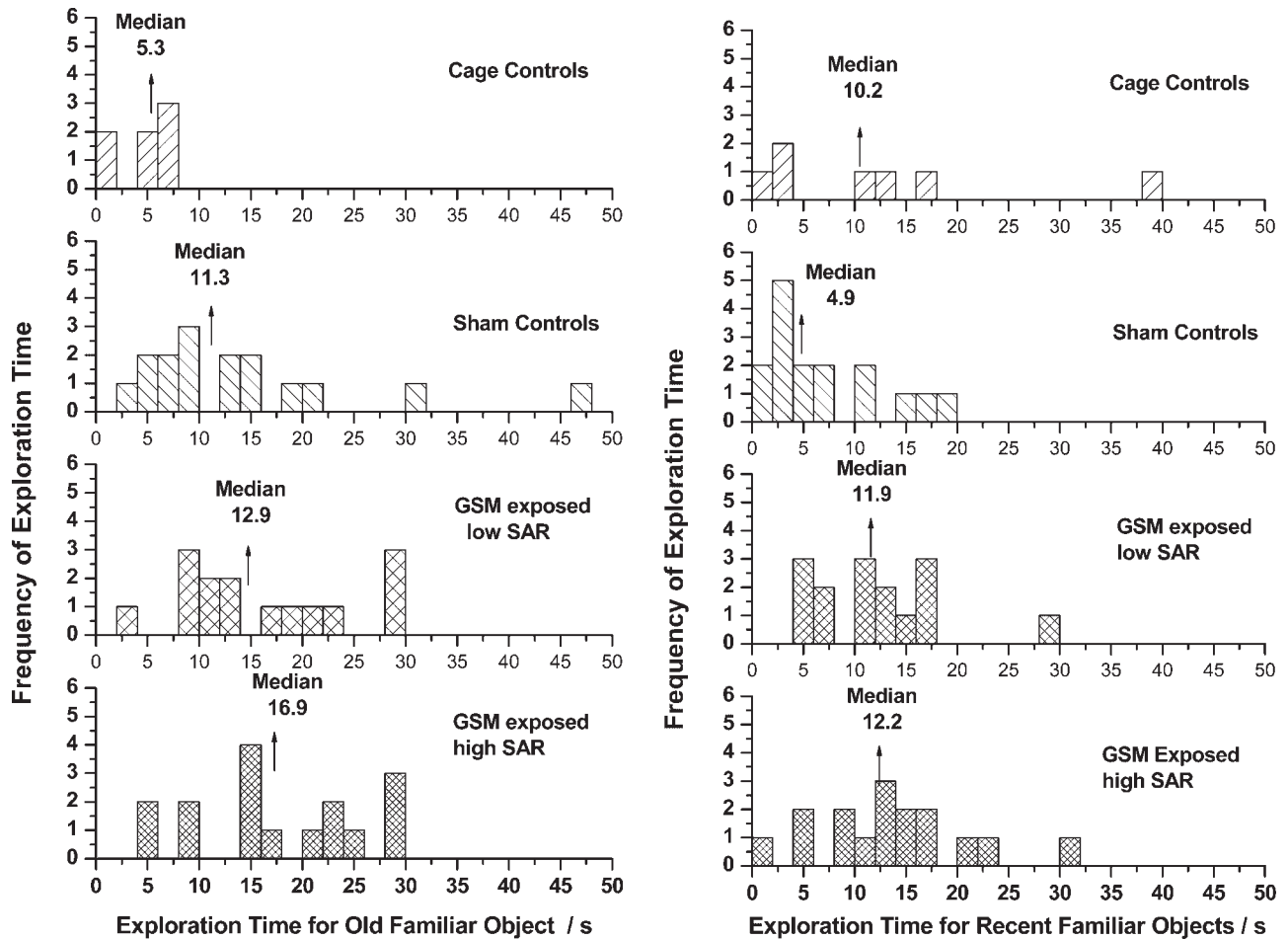


Fig. 7. Exploration time in the third trial of the episodic-like memory test. The results from each of the four groups (higher effect GSM exposed, lower effect GSM exposed, sham, cage control) are given for the old familiar objects and for the recent familiar objects. Median values for the exploration time of each group are also indicated in the figure.

the recent familiar objects when compared to the sham as well as the GSM exposed animals, (Mann–Whitney $P < 0.001$ for cage controls versus sham exposed animals; $P = 0.006$ for cage controls versus lower GSM exposed animals; $P = 0.005$ for cage controls versus higher GSM exposed animals).

The recollection of the place in which a unique experience occurred was not influenced by any of the experimental conditions: The Kruskal–Wallis statistic did not reveal any difference in exploration time between the displaced and stationary new familiar objects for the groups of sham exposed, GSM exposed and cage controls (see Fig. 10).

Our measurements confirmed the exploratory behaviour and memory patterns described by Dere et al. [2005]. In the following comparisons the cage controls were excluded. There was a preference for exploring the old familiar objects when compared to the recent familiar objects, indicating a memory for what

and when (paired t -test $P = 0.02$ for males and females) (see Fig. 11A). Also, displaced objects were examined more carefully than stationary objects (paired t -test $P = 0.005$ for males and females) (see Fig. 11B). This is evidence of memory for what and where. Comparison of exploration time for each of the two old familiar objects showed no preference for either of the objects relative to the other, as expected. The only aspect of object exploration in which our findings deviated from those observed by Dere et al. [2005] is the change of total time spent exploring the objects during each session. We found that the exploration time was longest during training trial one, followed by a decrease of exploration time during training trial two and an intermediate exploration time during the test trial (paired t -test $P < 0.001$; see Fig. 11C). Contrary to our findings, Dere et al. [2005] observed an increase of exploration time with each consecutive session.

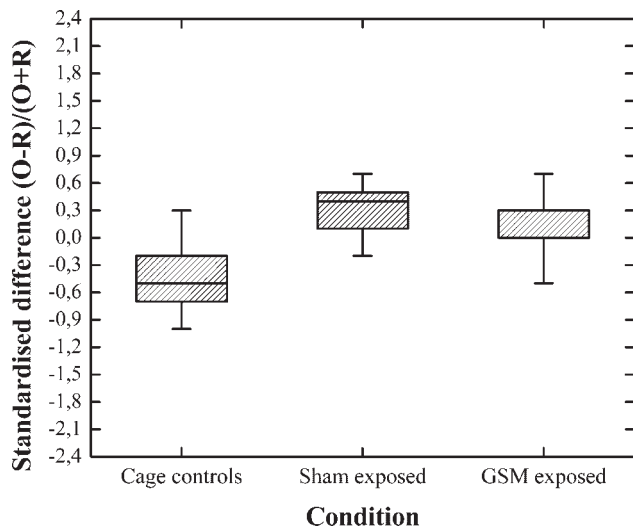


Fig. 8. Boxplot of the standardised difference $(O - R)/(O + R)$ for the exploration time of old familiar objects (O) versus the exploration time of the recent familiar objects (R). Median and interquartile range (IQR) are indicated in the boxes. The lines indicate 5–95% percentile ranges. GSM exposed rats spent shorter time exploring the old black familiar objects than the new grey familiar objects when compared to sham exposed rats (Mann–Whitney $P=0.02$; multiple regression $P=0.03$). Cage controls spent shorter time exploring the old familiar black objects than the new grey familiar objects when compared to sham exposed rats ($P < 0.001$).

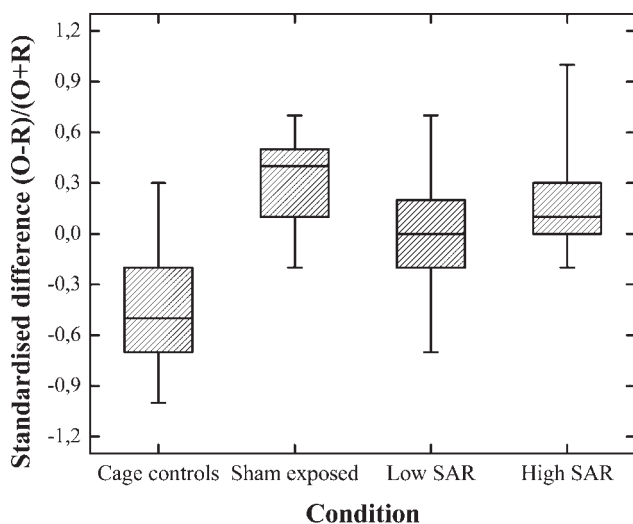


Fig. 9. As Figure 8, but high and low GSM exposure are shown separately. Both higher and lower GSM exposed rats spent a significantly shorter time exploring the old black familiar objects than the recent grey familiar objects when compared to sham exposed rats (Mann–Whitney $P=0.05$ for higher GSM versus sham; $P=0.05$ for lower GSM vs. sham). Even though Mann–Whitney test did not show any statistically significant difference between higher and lower GSM exposed animals, multiple regression reveals that lower GSM exposure influences the performance to a larger extent in the test than higher GSM exposure.

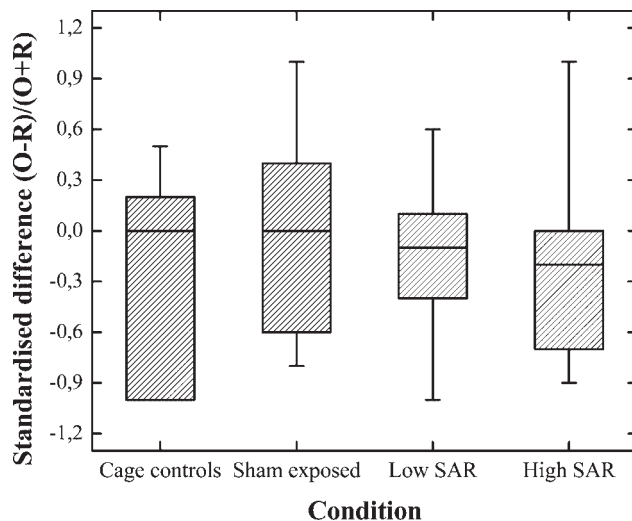


Fig. 10. As Figure 8, but the comparison is between displaced and stationary recent familiar objects in the episodic-like memory test. There was no statistically significant difference in exploration time between the displaced and stationary new familiar objects for the groups of sham exposed, GSM exposed and cage controls (Kruskal–Wallis statistics not significant).

In our statistical evaluation of the third test trial we compared the time spent exploring the old familiar versus the recent familiar objects. However, one recent familiar object was displaced compared to the location on which it was placed during the training session. According to our findings above (see Fig. 11) and those discussed by Dere et al. [2005] this would increase the rats' interest for the displaced recent familiar object relative to the other, stationary recent familiar object. Since both old familiar objects are stationary, the interest in exploring these objects is not affected by their location. Thus, most likely the difference between the exploration time for the old familiar objects and the recent familiar objects would have been even more obvious if both recent familiar objects had been stationary.

DISCUSSION

The present study provides evidence of alterations of memory functions after long-term exposure to mobile phones. Long-term exposure to GSM-900 microwaves with whole-body SAR values of 0.6 and 60 mW/kg, significantly altered the performance of rats during the episodic-like memory test. These SAR values are far below the thermal limit of 2 W/kg for exposure to the head for thermal effects on humans, according to ICNIRP [1998]. The GSM-exposed animals showed a significant impairment in episodic-like memory ($P = 0.02$) when compared to that of sham

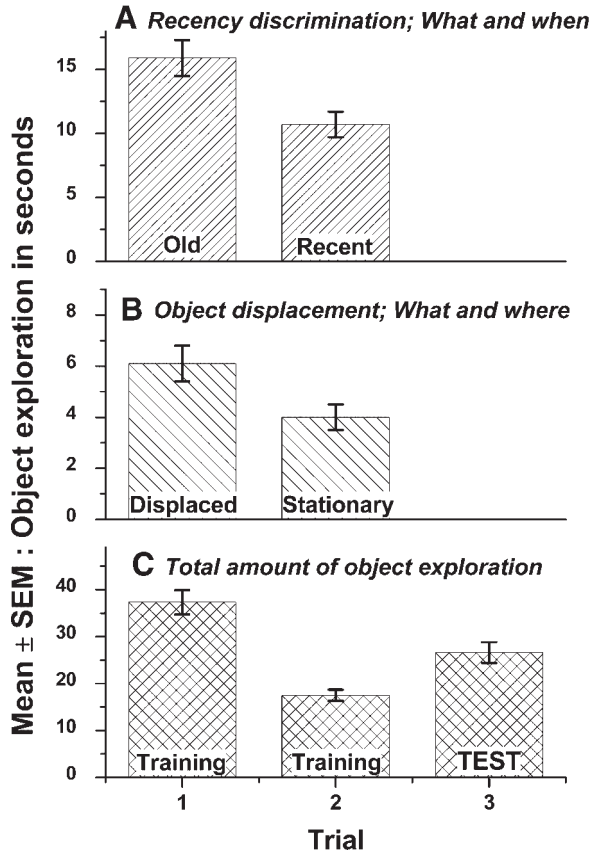


Fig. 11. All conditions except the cage controls are included in these observations. **A:** Mean time for exploration of the old familiar and recent familiar objects using the direct measurements from the test occasion, for males and females (paired *t*-test $P = 0.02$). **B:** Mean time for exploration of the displaced and stationary recent familiar objects, for males and females (paired *t*-test $P = 0.005$). **C:** Total amount of object exploration time during each of the trials, for males and females (paired *t*-test $P = < 0.001$).

exposed animals. The memory tests were performed during a period of 3–7 weeks after the last exposure occasion. Thus, the observed impairment following GSM exposure cannot be attributed to acute stress. Rather, this might constitute evidence of long-lasting effects of GSM microwaves on memory. To our knowledge, no other studies have investigated the effects upon memory of such long-term exposure to mobile phone radiation.

In the episodic-like memory test, normal rats will spend more time exploring the old familiar objects than the recent familiar objects [Kart-Teke et al., 2006]. Deviations from this behaviour, as we have seen in GSM exposed rats, indicate impaired memory for objects and their temporal order of presentation. In sham exposed animals no such deviations occurred. It should be noted that sham exposed animals have been treated exactly the same as the GSM exposed animals,

except that they were not exposed to GSM radiation. Furthermore, performance in the episodic-like memory test cannot be explained by influence of time elapsed between the training trials and the test trials. The reason for this is that it has been shown that rats have a capacity to express memory for at least 2 h after the learning procedure [Hannesson et al., 2004]. The total time from initiation to completion of the present episodic-like memory test falls within the terms of these references.

When comparing the differences between the lower and higher GSM exposed rats regarding performance in the episodic-like memory test, we observed no statistically significant difference ($P = 0.19$). This might be attributed to a power density dependency, where the biological effects do not necessarily increase with higher exposure levels [Blackman et al. 1989].

In other studies, exposure to GSM microwaves has not been shown to affect the memory performance of rodents [Sienkiewicz et al., 2000; Dubreuil et al., 2003]. However, in these studies the animals have not been exposed during a long-term period of more than 1 year, as is the case in our present study.

As is apparent from the episodic-like memory test, the performance of the cage controls is significantly reduced compared to that of the sham rats. In fact, the cage controls have an even more pronounced reduction in performance than the GSM exposed rats. This is not surprising and can be attributed to two main reasons. Firstly, the cage controls have led their lives in a less enriched environment. Secondly, the cage controls are less experienced and thus more prone to stress-induced reduction of memory functions.

It is well known that animals brought up in an enriched environment have much better memory functions [Gardner et al., 1975]. An enriched environment provides more stimuli in quantity and diversity than the standard cages [Moncek et al., 2004]. During the 1-year period of exposure sessions, the sham and GSM exposed animals have been moved and experienced different environments and human contact. The cage controls, on the other hand, have never left the animal house and thus have not had the opportunity to experience the same environmental enrichment. Moncek et al. [2004] point out that enriched environments might be considered to be more natural settings compared to standard cages and that this is important to consider in studies in which rats are kept under standard conditions.

Rats, such as the cage controls of our present study which are brought up in a less enriched environment, habituate less effectively to repeated handling and have a higher level of stress-hormones in connection with the handling [Moncek et al., 2004]. This leads to stress-induced reduction of memory functions. One brain

structure of major importance in this aspect is hippocampus, which is considered to play a crucial role in memory performance in tests such as the episodic-like memory test. Stress impairs hippocampus-dependent object-recognition memory [Kim and Diamond, 2002]. Long-term potentiation (LTP) is a long-lasting activity-dependent change in the strength of synaptic transmissions and is of crucial importance for memory storage. Kim and Diamond [2002] point out that stress impairs LTP for at least 48 h in rats and that this impairment is present in hippocampus. A key in this stress-induced reduction of hippocampal memory function is assumed to be the activity exerted by amygdala on hippocampus. It is important to point out that the cage controls are not considered to be unhealthy compared to the sham and GSM exposed animals, they are just in a less favourable situation at the time of the episodic-like memory test. In agreement with this, the sham and GSM exposed animals also perform much better than the cage controls.

Contrary to the episodic-like memory test, the open-field test revealed no effects of GSM exposure. Instead, differences between the performance of males and females were evident. Generally, males appeared to be more anxious, with longer centre-stay time and higher amount of urinations. Furthermore, the females had a higher degree of exploratory behaviour as seen by the number of rearings. Also, the females had a more pronounced locomotor behaviour than the males, revealed by the number of crossings. Our findings here are in accordance with previous conclusions [Andrews, 1996], stating that females are more active than males.

The rats in our study were young at the initiation of GSM exposure, comparable to human teenagers. At the time of the memory tests, the animals had reached an age comparable to late human middle age. It has been speculated that brain capacity might be reduced in the long run after protracted mobile phone exposure [Salford et al., 2003] due to neuronal damage. Our findings might be evidence of this phenomenon.

The underlying mechanisms for the changes of memory functions we observed are not clear. It is known that in rats, hippocampus is involved in aspects comparable to human declarative memory [Hammond et al., 2004; Kart-Teke et al., 2006]. We have previously also observed that GSM-900 MHz short-term exposure disrupts the integrity of the blood-brain barrier (BBB), which leads to extravasation of endogenous albumin from the blood vessels into the brain tissue [Persson et al., 1997; Salford et al., 2003]. The BBB regulates the transport of substances between the blood and the brain in mammals. Disruption of the BBB leads to a reduced protection of the brain from harmful substances, such as albumin, which has previously been found to be taken

up by not only astrocytes but also neurons. Further cellular and biochemical modifications correlated to GSM exposure have been observed. Xu et al. [2006] showed a selective decrease of excitatory synaptic activity and of the number of excitatory synapses in cultured rat hippocampal neurons after exposure to 1800 MHz GSM microwaves (SAR value 2.4 W/kg).

From these previous observations, it seems possible that the reduced memory functions we observed are correlated to hippocampal alterations induced by mobile phone exposure. Furthermore, hippocampal tissue also seems to be sensitive to other kinds of radio frequencies. Lai et al. [1994] found that the performance of rats in the radial-arm maze was reduced after exposure to 2450 MHz microwaves (SAR value 0.6 W/kg). This test has a well-recognized hippocampal involvement. Alterations in opioid neurotransmitter properties were suggested as a plausible explanation. However, these findings could not be replicated by Cassel et al. [2004] or Cobb et al. [2004].

Our observations of reduced performance in the episodic-like memory test after GSM exposure might also be explained by alterations of the temporal order memory, which is needed to discriminate the relative recency of events. Cortical areas associated with this function are the perirhinal cortex in the medial temporal lobe where recognition memory is situated, and the prefrontal cortex, where high order memory functions such as the temporal order memory are situated. Also, interactions between these cortical sites are important for temporal order memory to function properly, as stated by Hannesson et al. [2004].

The histopathological examinations of the brains of rats participating in the present study, especially from the hippocampal region, are presently under way and will be published separately. Albumin antibodies are applied to reveal albumin as brownish spotty or diffuse discolorations. Cresyl violet is used to detect dark neurons. Furthermore, studies of hypothetically premature aging are performed using different markers for brain aging, including gliosis with GFAP (glial fibrillary acidic protein), staining pigment in neurons with Sudan Black B, a histological staining method for lipofuscin to demonstrate the neuronal content of this wear and tear product. Possibly, an increase of lipofuscin might be caused by EMF discrete damage to membranes or organelles, the indestructible residues of which would be deposited in the neuronal lysosomal vacuome as peroxidized membrane lipids, heavy metals and other components. With the silver method by Gallyas, we will look for signs of cytoskeletal and neuritic neuronal changes of the type seen in human aging, possibly precipitated in rats by cellular stress caused by EM fields on organelles and membranes.

Also, a possible reduction of synaptic density will be studied with immunostaining to synaptophysin.

Albumin extravasation and the amount of dark neurons have been analysed so far. Results from these analyses indicate that no albumin extravasation or increase in the amount of dark neurons can be seen after 55 weeks of exposure to the GSM radiation of our present study compared to sham exposed animals. However, we do not know whether there might have been an observable albumin leakage during the earlier stages of the whole-year exposure period, comparable to the albumin leakage we have observed in previous studies [Salford et al., 1992, 1993, 1994, 2003; Persson et al., 1997; Eberhardt et al., 2007]. It is likely that albumin leakage at an initial stage of the more than 1 year long experimental period might have been absorbed after some time, leaving behind a damage expressed, for example, as accelerated aging. Furthermore, it can be hypothesised that if an accelerated ageing process is present, this could explain some of our findings of altered memory functions. However, all these studies have to be concluded and statistical evaluations performed before final conclusions can be drawn. The results might shed further light upon the mechanisms underlying the cognitive changes observed.

CONCLUSIONS

Our observations follow long-term mobile phone exposure lasting more than a year. Obviously, further investigations into this area are necessary. Our observations are evidence of what happens to rats, not humans, after mobile phone exposure. Differences in brain size as well as functional and anatomical organization demand caution regarding straightforward interpretations [Stewart, 2000]. Yet, the behaviour of rats is regarded as a good model for human function. Keeping the frequent and widespread use of mobile phones in mind, possible cognitive implications are indeed an important issue for the whole society.

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