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ASSOCIATION QUEBECOISE DE LUTTE CONTRE LA POLLUTION ATMOSPHERIQUE (AQLPA) / QUEBEC ASSOCIATION TO FIGHT AGAINST AIR POLLUTION

Interveners

# ARTICLES MENTIONED IN SECTION 41 OF DR. CARPENTER'S REPORT

## (CELLULAR AND ANIMAL STUDIES ON OF CANCER, GENOTOXICITY, NEUROTOXICITY AND OTHER HEALTH OUTCOMES FROM RF/MW RADIATION)

Referred to in **David O. CARPENTER**, *Expert Report*, Revised on May 14, 2012, C-SE-AQLPA-0072, SE-AQLPA-7, Doc. 1.1, parag. 41, 55.

Filed on May 15, 2012

## ARTICLES MENTIONED IN SECTION 41 OF DR. CARPENTER'S REPORT

### (CELLULAR AND ANIMAL STUDIES ON OF CANCER, GENOTOXICITY, NEUROTOXICITY AND OTHER HEALTH OUTCOMES FROM RF/MW RADIATION)

**40**. Many cellular and animal studies, of which the following are but a few, support conclusions of cancer, genotoxicity, neurotoxicity and other health outcomes from RF/MW radiation.

a. Sinha R. Chronic non-thermal exposure of modulated 2450 MHz microwave radiation alters thyroid hormones and behavior of male rats. Int. J. Radiation Biol. 84:6:505-513, 2008. This study concluded that the radiation was sufficient to alter the levels of thyroid hormone as well as emotional reactivity compared to controls.

b. Nittby H, Grafstrom G, Tian DP, Malmgren L, Brun A, Persson BRR, Salfor LG and Eberhardt J. Bioelectromagnetics 29: 219-232: 2008. This study showed cognitive impairment in rats after long-term exposure to PM MW radiation. This is study of rats shows that after 2 hours per week for 55 weeks there was impaired memory for objects in exposed as compared to sham animals.

c. Kimmel S et al. Electromagnetic radiation: Influences on honeybees (Apis mellifera). A significant difference between non-exposed and fully irradiated bees was the result of the influence of high-frequency PM RF/MW radiation.

d. Panagopoulos DJ et al. Bioeffects of mobile telephony radiation in relation to its intensity or distance from the antenna. Int. J Radiat Biol, 86;(5):345-357, 2010. The PM MW radiations at 900 and 1800 MHz decreased the reproductive capacity by cell death induction, with an increased bioactivity "window" at 10 $\mu W/cm^2$, and still evident down to 1 $\mu W/cm^2$.

e. Everaert J, Bauwens D. A possible effect of electromagnetic radiation from mobile phone base stations on the number of breeding house sparrow (passer domesticus). Electromagnetic Biology and Medicine, 26:63-72, 2007. Long-term exposure to higher-level low-intensity PM MW radiation negatively affects the abundance or behavior of House Sparrows in the wild.

f. Magras I, Xenos T. RF Radiation-Induced Changes in the Prenatal Development of Mice. Bioelectromagnetics 18:455-461, 1997. Near almost 100 TV and FM broadcast transmitters, with exposure levels between 0.168 $\mu W/cm^2$ and 1.053 $\mu W/cm^2$, found in the more exposed groups testicular damage and decreasing size of litters to irreversible infertility.

g. Balmori A. Electromagnetic pollution from phone masts. Effects on wildlife, Pathophysiology 2009. This large review of wildlife effects concludes, "pulsed telephony microwave radiation can produce effects on nervous, cardiovascular, immune and reproductive systems," including damage to the nervous system by altering EEG and changes to the blood-brain barrier, disruption of the circadian rhythms (sleep-wake) by interfering with the pineal gland and hormonal imbalances, changes in heart rate and blood pressure, impairment of health and immunity towards pathogens, weakness, exhaustion, growth problems, problems in building the nest or impaired fertility, embryonic development, hatching percentage, genetic and developmental problems, problems of locomotion, promotion of tumors and more.

*Int. J. Radiat. Biol.*, Vol. 84, No. 6, June 2008, pp. 505–513

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# Chronic non-thermal exposure of modulated 2450 MHz microwave radiation alters thyroid hormones and behavior of male rats

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**Abstract**

*Purpose:* The purpose of this investigation was to analyze the effects of leakage microwave (2450 MHz) irradiation on thyroid hormones and behavior of male rats.

*Materials and methods:* Experiments were carried out on two groups of male rats (exposure and control, respectively). Radio-immuno assay (RIA) methods were used for estimation of 3,5,3′-triiodothyronine ($T_3$), thyroxine ($T_4$) and thyrotrophin or thyroid stimulating hormone (TSH). The assessments of behavioral changes were performed in Open-Field (OF) and Elevated Plus-Maze (EPM) apparatuses.

*Results:* Following chronic microwave exposure, rats were found hyperactive and aggressive on the 16th and 21st days. Behavioral changes in OF were analyzed and found to be significantly changed from controls ($p < 0.05$) for immobilization, rearing and ambulation behavior. In EPM, rats showed increased activity with decreased time spent in the open arm and more time spent in the center on the 11th ($p < 0.05$), 16th ($p < 0.05$) and 21st day ($p < 0.01$) after irradiation. Changes in behavioral parameters are also correlated with the trend of changes, compared to control animals, in hormonal blood levels of T3 (decreased on the 16th day, $p < 0.05$ and 21st day, $p < 0.01$) and T4 (increased on the 21st day, $p < 0.05$).

*Conclusion:* Low energy microwave irradiation may be harmful as it is sufficient to alter the levels of thyroid hormones as well as the emotional reactivity of the irradiated compared to control animals.

**Keywords:** *2450 MHz microwave, behavioral changes, rat, thyroid hormones*

## Introduction

In the new era of development of science, microwaves are widely associated with numerous industrial, military, medical and domestic uses. Microwaves of 2450 MHz are currently widely used in microwave ovens that are utilized as a common kitchen appliance. A review of the literature suggested a significant interaction between the body of mammals and 2450 MHz microwaves (Adair et al. 1999). The direct non-thermal effects of microwave exposure and their interaction with the mammalian body have been a matter for discussion for a long time. Much of the literature does not suggest any significant interaction of low level microwave energy with biological systems (Sienkiewicz et al. 2000, Dubreuil et al. 2002, 2003, Cassel et al. 2004, Cosquer et al. 2005a, 2005b, 2005c). However recent research on alterations in neurological function due to low-level microwave exposure has indicated the need to consider possible mechanisms for these biological effects of microwave radiations (D'Andrea et al. 2003). The available literature indicates significant changes in different psycho-patho-physiological functions in exposed subjects even when the radiation power was quite low (in $\mu$W to mW range). It has also been reported that chronic exposure to leakage power microwave irradiation (low energy and non-thermal) is also harmful and has sufficient energy to alter different physiological variables (Bachmann et al. 2005, Hinrikus et al. 2005). Published data indicating changes in the permeability of the blood brain barrier due to cell damage by extremely low levels of microwave radiation suggest possible effects on the physiology of brain (Neubauer et al. 1990, Salford et al. 2003).

The thyroid gland is one of the most exposed and vital organs and may be a target for any type of electromagnetic radiation. It has been established that even a small change in thyroid hormone levels

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circulating in the blood are sufficient to alter the brain function of subjects (Bauer et al. 2002, Barnal 2005). Strong correlations between thyroid hormone levels and the functioning of the hypothalamus, through the hypothalamo-pituitary-thyroid axis, have already been evaluated (Lucia et al. 2001, Howdeshell 2002). The literature also suggests that cognition, learning and behavior of mammals are strongly associated with hypothalamic activity (Sinha 2006) and the physiology of thyroid gland (Wilson & Jefferson 1985, Bauer et al. 2002, Venero et al. 2005). However, the possible effects of chronic microwave exposure on thyroid hormones and their correlation with the behavior of subjects have not been well documented.

In the present work, chronic exposure to 1 KHz modulated 2450 MHz microwave radiation, an artificial system for leakage microwave radiation from a microwave oven used for cooking has been simulated in the laboratory. The aim of the present work is to understand and analyze the effects of chronic exposure to low energy 2450 MHz non-thermal microwave exposures on thyroid hormones and to analyze how these changes in thyroid hormones, if any, affect the reactiveness and emotional behavior of rats.

## Methods

### *Subjects*

Twenty male Charles Foster rats obtained from the animal house of Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India, of 4–5 weeks of age were analyzed for (i) thyroid hormone levels, and (ii) behavior. Each group of subjects was divided into two subgroups (each containing five rats), one of which was exposed to microwaves and one of which served as a control group. All rats were individually housed in polypropylene cages ($30 \times 20$ cm $\times$ 15 cm) with drinking water and commercial laboratory food pellets (Hindustan Liver Limited, Mumbai, Maharastra, India) *ad libitum*. The room was artificially illuminated with a 12 h light (L):12 h dark (D) (L cycle from 7:00 a.m. to 19:00 p.m. Indian Standard Time (IST)) cycle at $24 \pm 1^{\circ}$C. All procedures in this study have been conducted in compliance with the Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA) of India as well as with internal institutional policies and guidelines.

### *Microwave set-up and exposure pattern*

The microwave source M.S.G.-2114 (Integra Microwaves, Anaheim, CA, USA) with pyramidal horn antenna (throat dimension of $7.2 \times 3.4$ cm, and axial length of 10 cm) was used for the study. The flair angle ($\varphi$) of the antenna was $9.6^{\circ}$ in both the electric (E) and magnetic (H) plane. The required frequency was adjusted to 2450 MHz (in the S-Band) with 1 KHz square modulation. The maximum power output was measured as 19.8 mW using an 8481H power sensor and 836A power meter (Hewlett Packard Co., Palo Alto, CA, USA). For this microwave set-up, the 3 dB point for both H and E planes was calculated to use in the design of an animal holder for five rats. There were five animal holders (Figure 1). Distance of antenna from the top of the holder was 23 cm. Dimensions of the floor of the animal holder was $29.87 \times 25.3$ cm. Two of the holders had their long axis positioned parallel to E-field, whereas the other three holders had their long axis positioned parallel to the H-field of radiation.

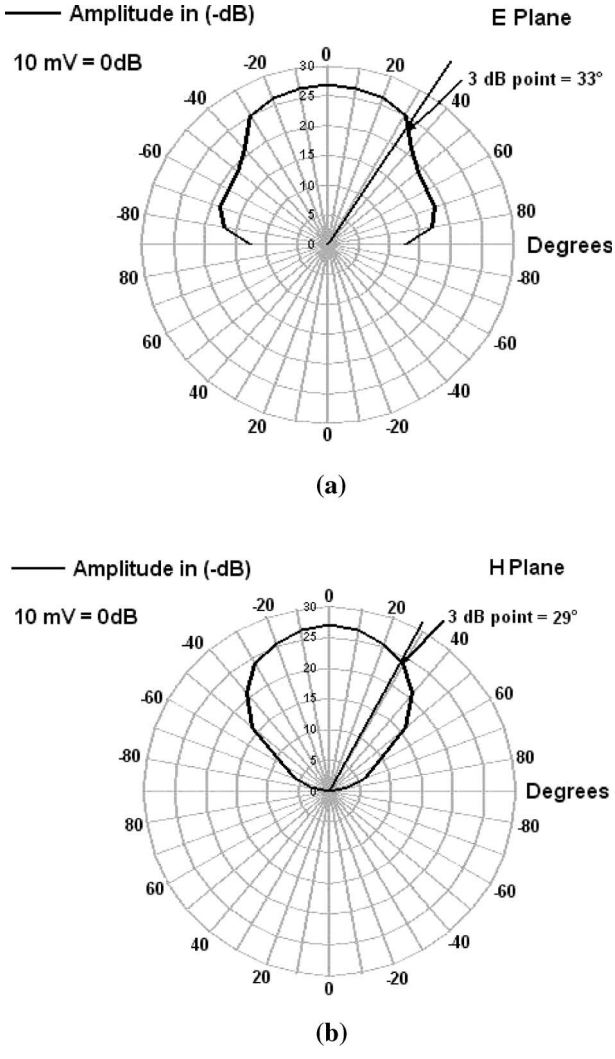


Figure 1. Radiation pattern of antenna in (a) E plane and (b) H plane for dimension designing of the animal holder.

The internal partitioning in the animal holder was made with pine wood due to its low dielectric constant and loss tangent. The outer surface was made up of Perspex. All external and internal walls, excluding the base of the animal holder were perforated at uniform distances to make the holder anechoic. However, rats were not restrained and had freedom to change their posture within the cage. The rats were rotated daily from one chamber to the next chamber in succession. The base of animal holder was 10 cm of Styrofoam. Radiation was given from the top of the holder. The phenomenon of polarization was studied theoretically and experimentally by previously described methods (Bachmann et al. 2005, Hinrikus et al. 2005). The power transmitted by the antenna was estimated to be 12.5 mW. The calculated power density (total power radiated per square unit of irradiated area) at the bottom of animal holder was 16.5 $\mu$W/cm$^2$. In the present study, the average specific absorption rate (SAR) (since the experiments were carried out on live and moving subjects) parallel to E plane was calculated by a simple empirical equation for average, whole body absorption (parallel to the electric field) in rats, developed by Gandhi et al. (1977). The SAR has also been calculated parallel to the H plane by the method given by Gandhi (1974). The average SAR calculated was 3.6 $\mu$W/gm parallel to the E plane and 0.98 $\mu$W/gm parallel to the H plane.

*Microwave exposure model*

All rats were divided initially into two groups and subjected to the microwave exposure set-up before hormonal and behavioral analyses. The microwave exposure set-up for the experiments is shown in Figure 2.

(a) *Chronic exposure group* ($n = 10$): To observe and analyze the chronic effects of low level 2450 MHz microwave radiations, experimental groups of rats were exposed with microwave exposure for 2 h from 08:00–10:00 a.m. (IST) daily up to 21 days.
(b) *Sham control group* ($n = 10$): To negate any other type of psychophysiological effects, separate groups of rats were also handled and processed parallel to the exposure group, but without microwave exposure. These groups of subjects were treated as sham control groups.

*Hormonal analysis*

Standard radio-immuno assay (RIA) methods, frequently used in clinical laboratories, were for estimation of 3,5,3′-triiodothyronine ($T_3$), thyroxine ($T_4$) and thyrotrophin or thyroid stimulating hormone (TSH). The RIA kits were obtained from Board of Radiation and Isotope Technology, Bhabha Atomic Research Center (BARC), Mumbai, Maharastra, India, for clinical analysis. The analyses were performed in the Department of Endocrinology, Institute of Medical Science, Banaras Hindu University, India.

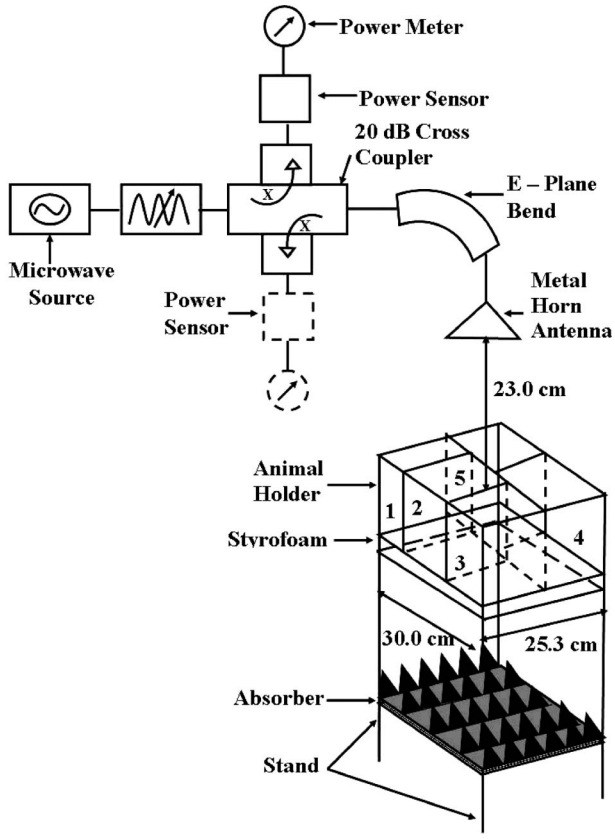


Figure 2. Schematic diagram of experimental set-up for microwave exposure. For the absorption of electromagnetic radiation, passing out from the animal holder, an absorber was placed below it. It was made up of Styrofoam and coated with carbon powder. The area of absorption was increased by fixing the carbon coated Styrofoam cones in sequential manner on the surface of absorber, facing toward the animal holder. x represents the directional couplers. Numbers 1–5 represent the identification numbers of cages in the animal holder.

With the help of local anesthesia (Xylocaine, obtained from Astra-idl Limited, Bangalore, Karnataka, India), Approximately 1 ml blood was taken from the tails of each rat on the 1st, 6th, 11th, 16th and 21st day immediately after irradiation or sham exposure. Each time, the same amount of blood was collected from a subject, put into test tubes and allowed to clot. The RIA estimation of hormones was performed on aspirated serum.

*Behavioral activity monitoring*

In the present study, the changes in behavior following acute immobilization stress in rats were

evaluated in a noise-free room by Open-Field (OF) and Elevated Plus-Maze (EPM) methods under blinded conditions. The OF experiments are mainly used to measure the fearfulness and reactiveness of the animals and the EPM is used to test the emotionality or anxiety of the subjects. The assessment of behavior was performed on the 1st, 6th, 11th, 16th and 21st day of the experiment. The methods of evaluation of behavioral alterations in different psychophysiological parameters using this behavioral testing equipment has been reported (Sarbadhikari et al. 1996, Sinha & Ray, 2004, Sinha 2006).

*Open-Field.* The field was a circular arena with an outer diameter of 84 cm; peripherally, there were 16 squares. The inner concentric circle of 56 cm diameter contained eight squares. A 100 W-frosted bulb was placed 1 m above the field in an otherwise dark room during the activity testing. The behavioral parameters of each rat were tested in the wake condition in the OF by placing the animal at the center of the apparatus for 3 min and observing: (i) *Immobilization*: Rats had eyes open, holding their heads against gravity but without any head, body or limb movements; (ii) *Grooming*: Rhythmic paw movements over the face and/or head for face washing and might include episodes of biting and cleaning of paws; (iii) *Rearing*: Standing still and upright on their hind limbs only; and (iv) *Ambulation*: When all four limbs of the animal were in one particular square (central or peripheral) of the open field.

*Elevated Plus-Maze.* The maze had two open arms ($50 \times 10$ cm), and at right angle to them, two closed arms ($50 \times 10 \times 40$ cm) with the top uncovered; an open central crossing ($10 \times 10$ cm) rose to a height of 50 cm. The behavioral parameters of each rat were tested for 5 min in wake condition in EPM by placing the rat at the end of an open arm and observing: (i) *Transfer Latency*: Time taken (in seconds) by the animal to move from the outer end of the open arm to either of two closed arms; (ii) *% Time in open arms*: The percentage of total testing time spent in the open arm; (iii) *% Time at central crossing*: The percentage of total testing time spent at the crossing of open and crossed arms; and (iv) *Number of crossing of the arms*: The number of times the animal crossed the center in going from one arm to any of the other three arms.

*Other parameters*

*Body temperature measurement.* Changes in core temperature of the subjects in this experiment is an important parameter to measure in order to see if the exposure pattern produces a thermal or non-thermal effect. The core temperature of each animal was recorded on the 1st, 6th, 11th, 16th and 21st days of experiment just after the removal of subjects from the animal holder for both the control and microwave-exposure groups of rats. The thermistor probe marked at 4 cm and connected to a 6-channel telethermometer was inserted into the rectum of the animal and kept static for 1 min to record the body temperature.

*Determination of edema and edematous swelling in the brain.* Animals were sacrificed after recording behavioral parameters and their brains were dissected. The wet and dry weights of the brain were noted and the percentage of water for each brain was calculated. Dry weights of each brain were determined after repeatedly drying the sample in an oven at 80°C until the weight remains constant. The percentage of edematous swelling was calculated by using the following formula (Sinha 2006):

$$\frac{\%\ \text{Water content in control animal} + \text{f}}{100 + \text{f}} = \frac{\%\ \text{Water content in experimental animal}}{100}$$

where f = % of swelling caused by edema.

*Statistical analysis*

All the statistical analyses were performed in the laboratory with the help of the software package (MS EXCEL-98) and also checked with manual calculation. The F-Test (Fisher test) and the one way Analysis of Variance (ANOVA-1) were performed to compare data for different parameters following microwave exposure with their respective controls.

## Results

Analyses of results showed that chronic exposure (2 h daily for 21days) of 1 KHz square wave modulated 2450 MHz microwave frequency produces insignificant changes in body temperature as analyzed on the 1st, 6th, 11th, 16th and 21st days with respect to the corresponding control group. This result reveals that at the very low power density (16.5 $\mu$W/cm$^2$) used in the present set-up, thermal effects were extremely unlikely. However, the present 2450 MHz microwave exposure protocol increased the brain water content by 2.1% ($70.54 \pm 0.22\%$ for exposure group and $68.47 \pm 0.29$ for control subjects), corresponding to a 6.97% increase in volume swelling in the brain due to chronic microwave exposure.

*Assessment of changes in hormones*

Statistical analyses of variations in $T_3$ level due to chronic microwave exposure compared to control subjects demonstrated an altered level of $T_3$ hormone. When chronically exposed to 1 KHz modulated 2450 MHz microwave frequencies, significantly decreased $T_3$ levels were observed on the 16th day ($F_{1,8} = 6.98$, $p < 0.05$) (subscript 1 and 8 represent the degree of freedom between samples and degree of freedom within samples, respectively) and on the 21st day ($F_{1,8} = 24.34$, $p < 0.01$) of exposure period (Figure 3a). At the same time, a significantly higher $T_4$ level ($F_{1,8} = 7.54$, $p < 0.05$) on the 21st day of microwave exposure was observed (Figure 3b). Statistically insignificant changes in TSH level were seen on all days of data recording during the chronic microwave exposure period.

*Assessment of changes in behavior*

*Changes in OF behavior.* Chronic microwave exposure with the present experimental design increases the mobilization activities in rats. Statistical analyses of results shows significantly higher mobility in microwave exposed rats on the 16th day ($F_{1,8} = 6.23$, $p < 0.05$) and 21st day ($F_{1,8} = 6.68$, $p < 0.01$) of the exposure period (Figure 4a), when compared with the control group. Similarly, rearing behavior was also analyzed to increase significantly on the 16th as well as on the 21st day ($F_{1,8} = 7.93$; 7.11, $p < 0.05$ respectively) of microwave exposure (Figure 4b). However, no statistical differences were seen in the grooming behavior of rats due to the effects of chronic microwave exposure compared to control rats. The analyses of results for peripheral ambulation in the OF suggest an increasing trend in this behavior with time of exposure. Significant increases in peripheral ambulation were recorded on the 16th day ($F_{1,8} = 7.52$, $p < 0.05$) that increased further on the 21st day ($F_{1,8} = 76.72$, $p < 0.01$) of the microwave exposure period (Figure 5a). Similar to peripheral ambulation, significantly higher central ambulation was seen on the 16th and 21st day ($F_{1,8} = 5.93$; 6.35, $p < 0.05$) of

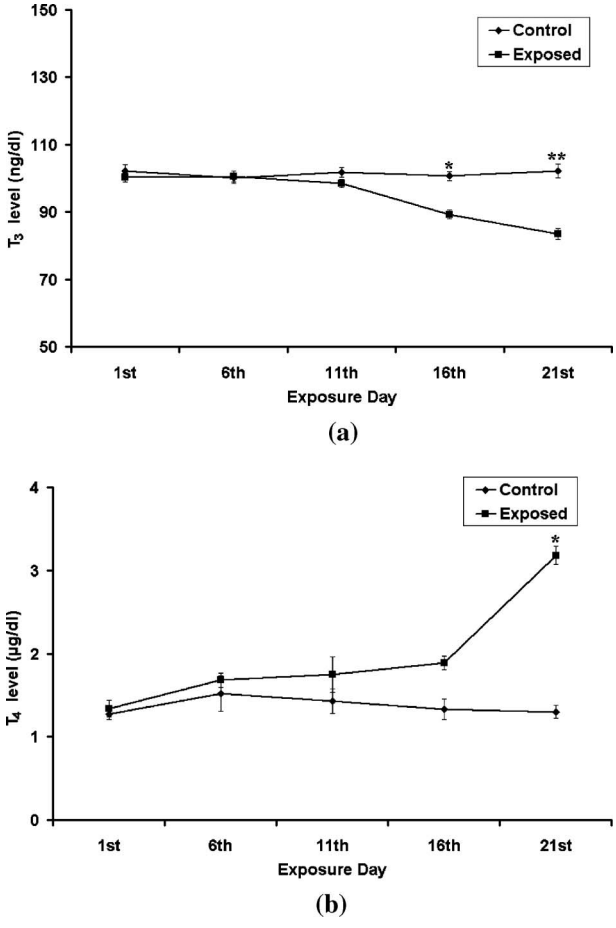


Figure 3. Analyses of changes in hormonal activities, (a) $T_3$ and (b) $T_4$ levels due to chronic microwave exposure group of rats with respect to the control group. Data are represented as mean ( $\pm$ SE) and compared $*p < 0.05$, $**p < 0.01$ to respective control group.

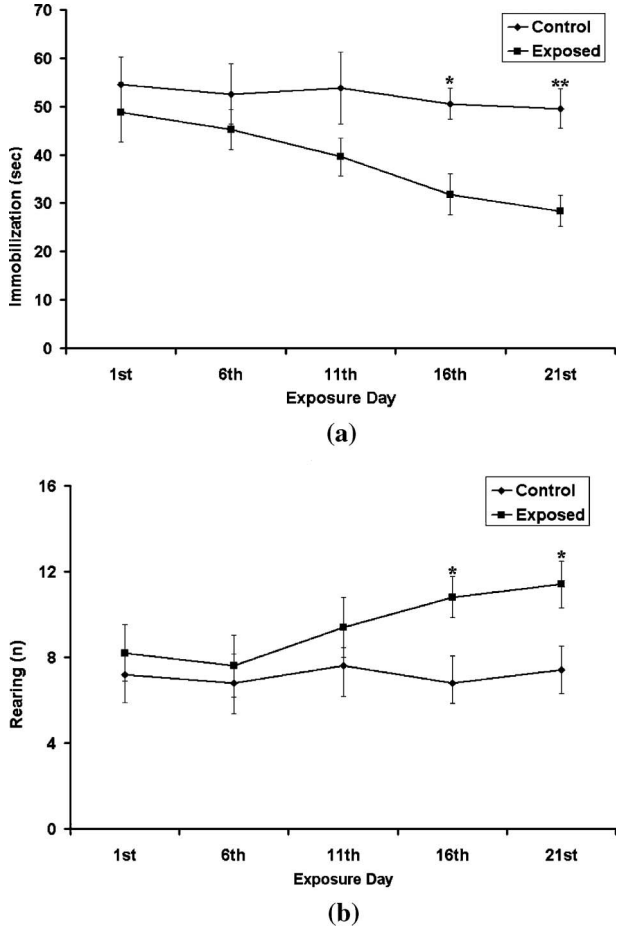


Figure 4. Analyses of changes in behavioral activity in OF apparatus, (a) immobilization and (b) rearing activities for chronic microwave exposure group of rats with respect to the control group. Data are represented as mean ( $\pm$ SE) and compared $*p < 0.05$, $**p < 0.01$ to respective control group.

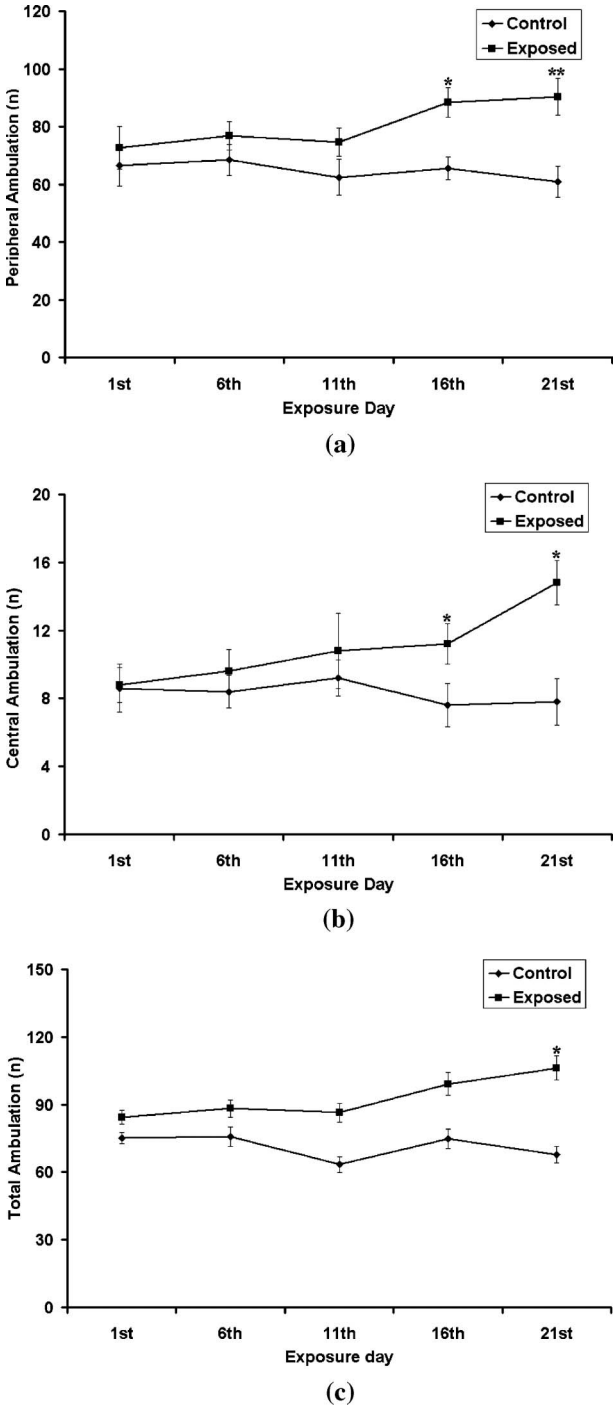


Figure 5. Analyses of changes in ambulation behavior in OF apparatus, (a) peripheral, (b) central and (c) total ambulation for chronic microwave exposure group of rats with respect to the control group. Data are represented as mean ($\pm$ SE) and compared $*p < 0.05$, $**p < 0.01$ to respective control group.

chronic microwave exposure in comparison to the control group of rats (Figure 5b). However, analyses of results for changes in total ambulation show a statistically significant increase only on the 21st day ($F_{1,8} = 8.27$, $p < 0.05$) of microwave exposure (Figure 5c).

*Changes in EPM behavior.* When changes in emotional behavior in EPM were analyzed, a significantly higher decrease ($F_{1,8} = 7.75$, $p < 0.05$) in transfer latency time was recorded on the 1st day of microwave exposure, while no changes were observed on other days (Figure 6a). The analyses of data for percentage time spent on an open arm reveals significantly decreased time on an open arm on the 11th and 16th day ($F_{1,8} = 6.89$, $p < 0.05$) and 21st day ($F_{1,8} = 42.23$, $p < 0.01$) of the exposure period (Figure 6b). The reverse was seen for the tome on center on the 11th, 16th and 21st days ($F_{1,8} = 9.21$, $p < 0.05$) of chronic microwave exposure (Figure 6c). No statistical differences were seen for the number of arms crossed between chronic microwave exposure rats and the control groups of rats.

## Discussion

Little is known about health risks from exposure to different sources of non-thermal microwave radiations. It has been established that chronic exposure to non-thermal microwave irradiations can affect sensitive vital organs but these effects depend on radiation intensity, frequency of exposure, modulation frequency and exposure duration (D'Andrea et al. 2003). To analyze the effects of low-level chronic exposure to microwave radiation, experiments are going on in different electromagnetic wave radiation laboratories simulating the power density and SAR of these effects in laboratory animals.

The present report assessed the effects of chronic exposure (2 h daily for 21 days) of 1 KHz square wave modulated 2450 MHz microwave frequency on thyroid hormones ($T_3$, $T_4$ and TSH). The exposure protocol did not produce any significant changes in core body temperature and the effects observed are assumed to be non-thermal in nature. Possible interactions between changes in thyroid hormone levels and the reactiveness and emotionality of the animal in OF and EPM testing apparatus have also been investigated. Despite numerous clinical data associating alterations in thyroid functions and mental and behavioral disorders of the subjects, the detailed mechanism(s) by which thyroid hormones can influence behavior and associated brain function remain obscure (Barykina et al. 2002). Except for reports on the acute effects (Navakatikian et al. 1990), no report is known to the author that demonstrates an effect of chronic exposure of 2450 MHz microwave on thyroid function and behavior of rats. The purpose of this study was to study and analyze the effects of leakage power (non-thermal) of household microwave appliances, which are operated on 2450 MHz frequency, on the thyroid gland and behavior of the subjects.

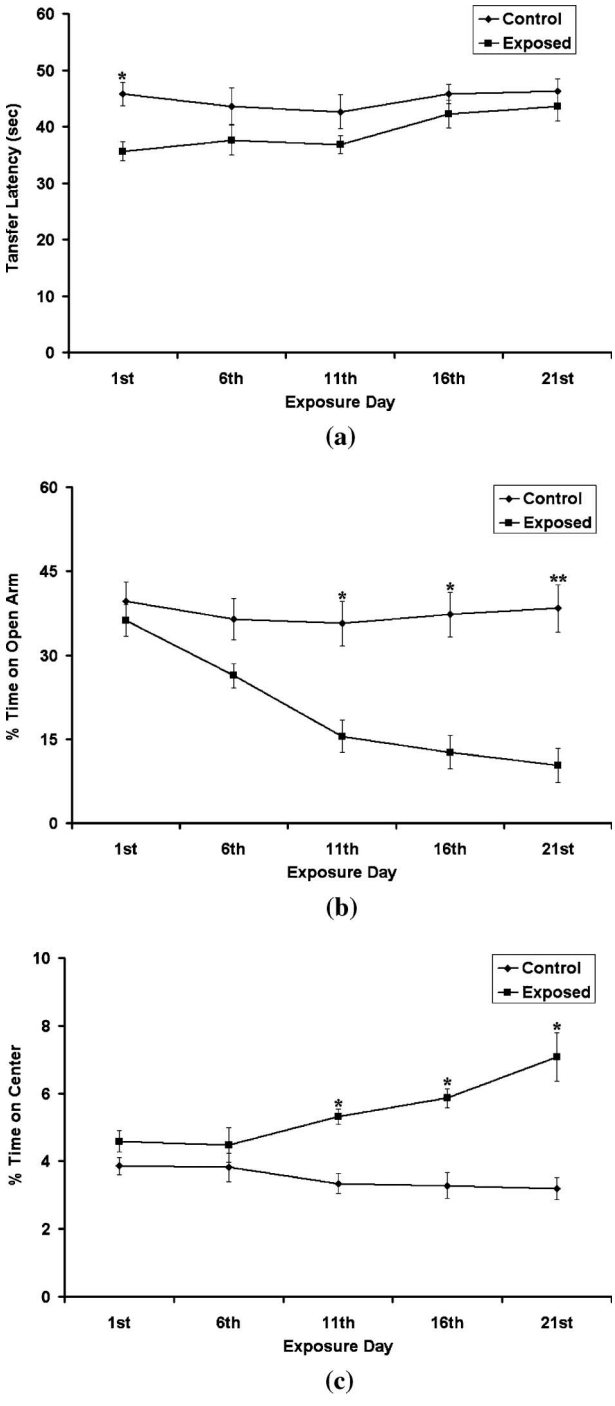


Figure 6. Analyses of changes in behavior in EPM apparatus (a) transfer latency, (b) percent time on open arm and (c) percent time on center for chronic microwave exposure group of rats with respect to the control group. Data are represented as mean ( $\pm$ SE) and compared $*p < 0.05$, $**p < 0.01$ to respective control group.

Experiments were performed on male rats (4–5 weeks) since thyroid hormones have a great influence on early development in mammals (Morreale de Escobar 2004). The thyroid hormone profile of rats in the present study showed decreased $T_3$, increased $T_4$ and normal TSH values. These changes in hormone levels are similar to the euthyroid state (Larsen & Ingbar 1999, Van den Berghe 2000). The decrease in $T_3$ level could be a protective mechanism to overcome metabolic changes likely to be produced by chronic exposure to microwave radiation. Elevated $T_4$ levels, however, are indicative of increased thyroid activity. In contrast to the above findings, rats were analyzed with having significantly greater mobility and ambulation on the 16th and 21st days of OF behavioral testing ($p < 0.05$ or better), which could also be due to a learning behavior of the subjects. However, in this manuscript, the learning effects have not been analyzed in detail. Furthermore, in EPM, rats showed increased activity with decreased time on open arm and were found to spend more time on the center as seen on the 11th, 16th and 21st days ($p < 0.05$ or less) of the experiment. However, the results of transfer latency indicated adaptation of the animals to the environment. Thus, these results suggest the possibility of a causal connection between effects on the thyroid system and behavior of the rats, exposed chronically to 2450 MHz microwave frequency for 21 days.

It has been demonstrated that deficiencies in thyroid hormones in rats are associated with mood disorders and enhance anxiety like behavior (Bauer & Whybrow 2001, Bauer et al. 2002, Venero et al. 2005). This intimate association between the thyroid system and behavior has been the impetus for exploring the effects of thyroid hormones in modulating affective illness and the role of hypothalamo-pituitrary-thyroid axis in the pathophysiology of mood disorder. Similar to the present finding, a reduction in $T_3$ has been suggested to induce increased anxiety-like behavior in the EPM, as animals spent less time in the open arm. Conversely, in this report $T_3$ is reported to depress the mobilization of the subjects contradicting our results observed in the OF test. In another publication, it has been observed that reduced concentrations of thyroid hormones did not alter locomotor activity in the OF test in Wistar rats (Gordon 1997, Barykina et al. 2002). It is remarkable that similar to the findings of this report, other authors also found a linear relationship between $T_4$ hormonal level and the reactiveness and mobility of rats (Fundaro 1986). However, a series of experiments and their published reports on the analysis of the effects of microwave radiations have suggested insignificant effects on the subjects and disagree with the present findings (Sienkiewicz et al. 2000, Dubreuil et al. 2002, 2003, Cassel et al. 2004, Cosquer et al. 2005a, 2005b, 2005c). Thus, the results of this work need to be confirmed by more experiments, different experimental protocols and different age groups of subjects.

The details of the mechanism associated with altered thyroid hormones and their interaction with animal behavior in OF and EPM apparatus, may also have been modified by the effects on the blood-brain due to chronic exposure to microwaves. In accordance with earlier published reports (Neubauer et al. 1990, Salford et al. 2003), the present findings indicated increased brain water content (6.97% increase in volume of brain) after 21 days of chronic microwave exposure suggesting increased blood-brain barrier permeability. It has been shown that a brain specific organic anion transporter is coupled with thyroid hormone transport at the blood-brain barrier (Koibuchi & Iwasaki 2006). This organic anion-transporting polypeptide-14 (OATP14), which is localized in the brain capillary endothelium, transports $T_4$ much more efficiently than $T_3$, and might be involved in the transport of $T_4$ through the blood-brain barrier through the choroid plexus. The blood-brain barrier is the route by which thyroid hormones are preferentially distributed throughout the brain, and this transporter will facilitate uptake of $T_4$ by astrocytes. Transfer of thyroid hormones through the choroid plexus achieves only a limited diffusion to the brain parenchyma after passage to the cerebrospinal fluid but would allow uptake of $T_4$ by tanycytes and subsequent $T_3$ generation in these cells (Barnal 2005). This differential transfer mechanism of thyroid hormones in the blood-brain barrier is suggested to influence hypothalamic activities (behavior regulating center of brain) to produce alterations in behavioral patterns of the subjects.

The detailed mechanism of extravasations of blood-brain barrier permeability in stressful events and the role of serotonin has already been discussed in detail (Sharma et al. 1998). It is hypothesized that serotonin plays a major role in mood modulation. This complex long track system that begins in the brain stem and extends through the midbrain into the limbic system and cortex modulates the activity of many of the brain regions related to the emotion and memory. The interdependence of this long track system with thyroid hormone metabolism has become better understood with the help of modern technology (Bauer et al. 2002). Although, alterations in serotonin synthesis are very well understood in prolonged and short term single exposure to psychophysiological stress (Sinha & Ray 2004, Sinha 2006), the mechanisms governing the relationships of serotonin at dissimilar brain locations with different thyroid hormones is hard to understand. It is also hard to understand how alterations in serotonin, or any other neurohormonal change, are responsible for modification of animal behavior, how these modifications can be quantified and which neurohormonal change is responsible for a particular type of behavioral change.

## Conclusion

In conclusion, it is suggested that low energy 2450 MHz microwave radiation can be harmful as it is sufficient to alter extravasations of blood-brain barrier permeability, changes in thyroid hormone metabolism and emotional reactiveness of the animals. It is hypothesized that alterations in animal behavior due to microwave exposure, as analyzed in OF and EPM apparatuses follows the changing patterns of $T_3$ and $T_4$ hormonal level, suggesting a significant interaction between behavior and thyroid functions. However, these hormones have different complex mechanisms for affecting different behavioral parameters. Since, thyroid hormones are also reported to interact with other neurohormones, the involvement of other neurotransmitters and hormonal systems in altered animal behavior following low energy, non-thermal chronic microwave exposure of 2450 MHz cannot be ruled out.

## Certificate of originality

This is to certify that the article submitted for publication in *International Journal of Radiation Biology* has not been published, nor is being considered for publication, elsewhere. All procedures in this study have been conducted in compliance with 'committee for purpose of control and supervision of experiments on animals (CPCSEA)', India, as well as with internal institutional policies and guidelines.

***Declaration of interest:*** The author reports no conflict of interest. The author alone is responsible for the content and writing of the paper.

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# 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. 

**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 $S = 52$ 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

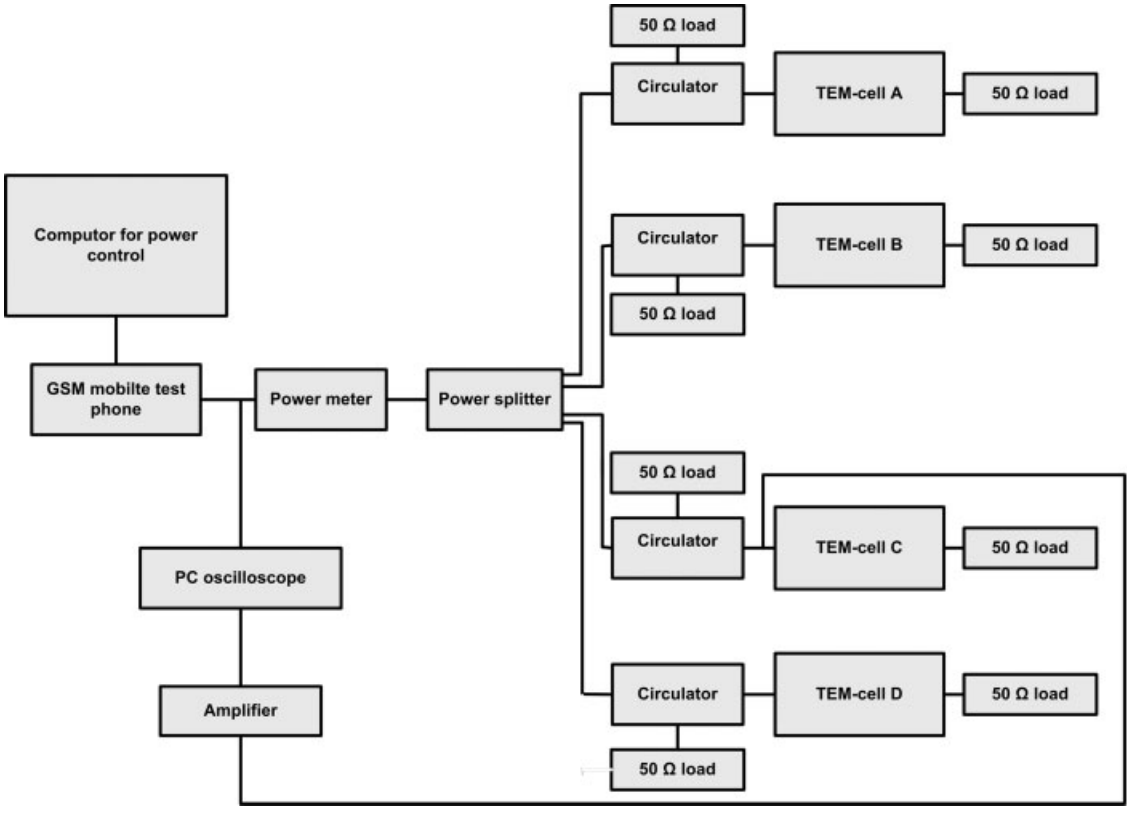


Fig. 2. Block diagram of the exposure set up. Four TEM-cells (A, B, C and D) are used. For sham exposure the same TEM-cells are used, but in this case they are not connected to the GSM mobile test phone and thus no RF EMFs are directed into the TEM-cells.

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:

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

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 [Svendsen 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

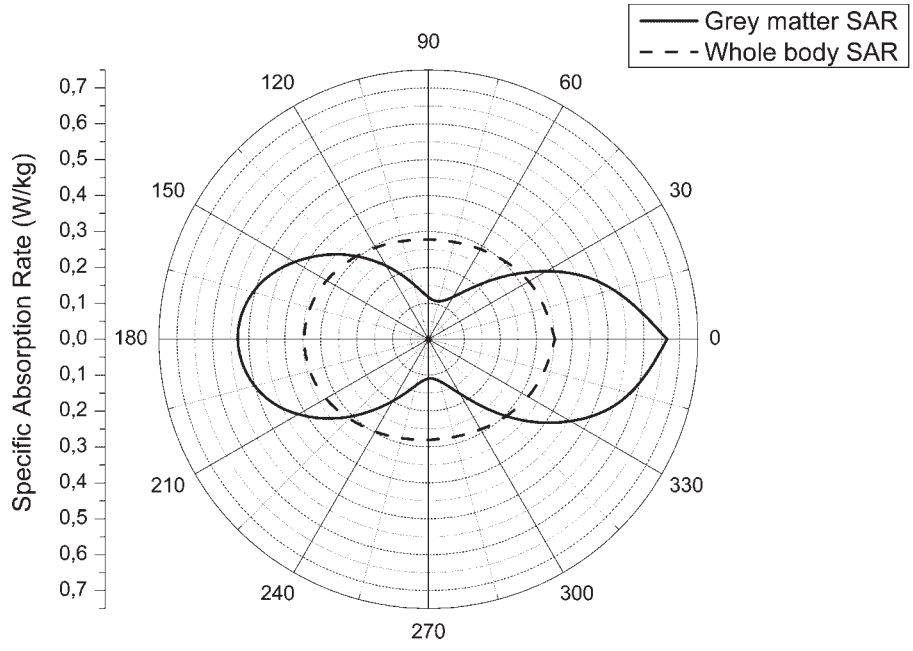


Fig. 3. FDTD calculation of SAR values in the brain grey matter and whole-body SAR (W/kg) in the rat at exposure to 900 MHz plane wave radiation, with a power density of 10 W/m$^2$, as a function of incident wave angle with respect to the long axis of the animal. The polarisation of the radiation is 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 $S = 33$ 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 ($S = 3.3$ 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 $545 \pm 24$ g and the females $304 \pm 23$ 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 $80 \times 80 \times 40$ 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 ($16 \times$ 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 (rearings), 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

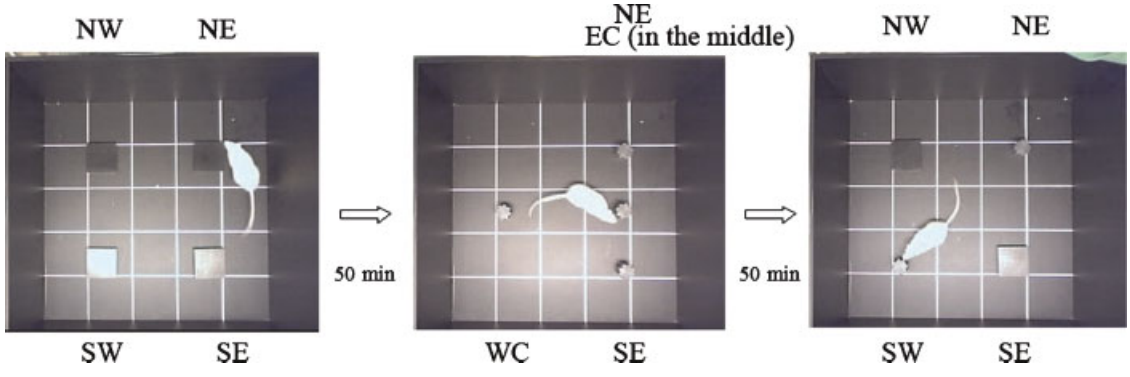


Fig. 4. Schematic drawing of the episodic-like memory test. The rats received two training trials and one test trial, each with 50 min inter-trial interval. On training trial 1 four black quadrants were arranged symmetrically one in each corner. On training trial 2 four grey cylinders were arranged in aT-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 the same location as in training trial 2 and 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

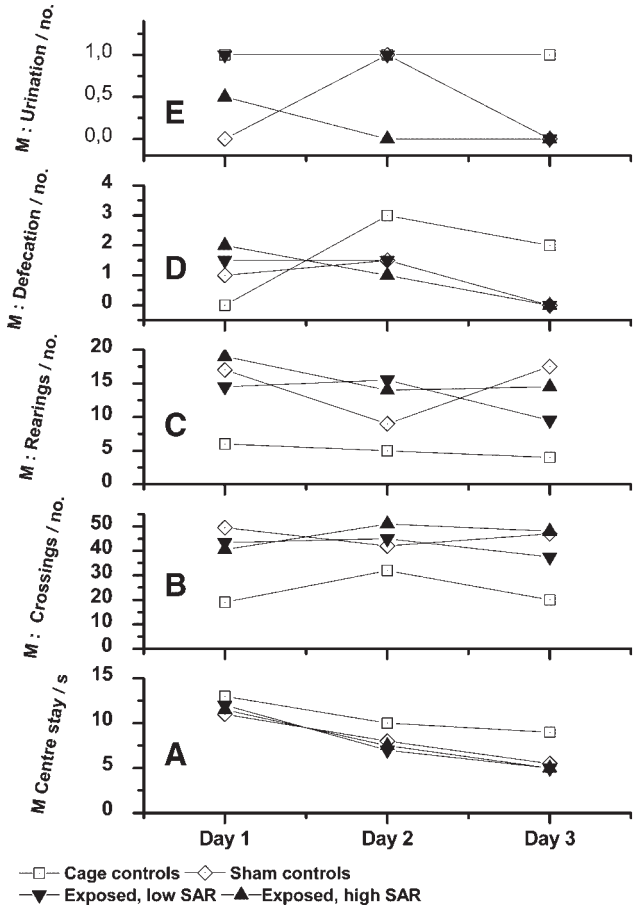


Fig. 5. Result from open-field test for males. **A**: Median value (M) of centre stay time measured in seconds. Median value (M) of number of (**B**) crossings; (**C**) rearings; (**D**) defecations and (**E**) urinations. No statistical significance was found between the GSM exposed rats compared to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

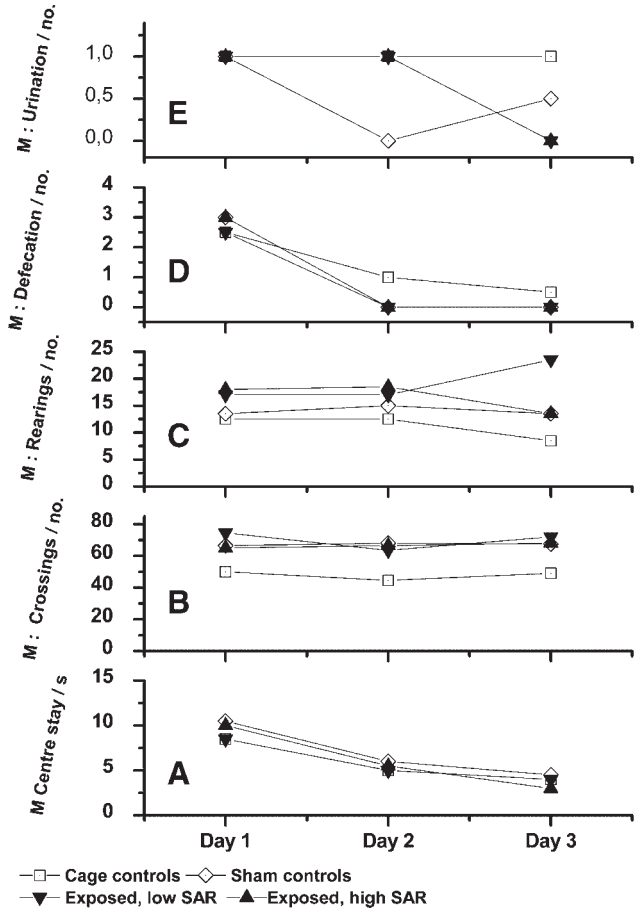


Fig. 6. Result from open-field test for females. **A**: Median value (M) of centre stay time measured in seconds. Median value (M) of number of (**B**) crossings; (**C**) rearings; (**D**) defecations and (**E**) urinations. No statistical significance was found between the GSM exposed rats compared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

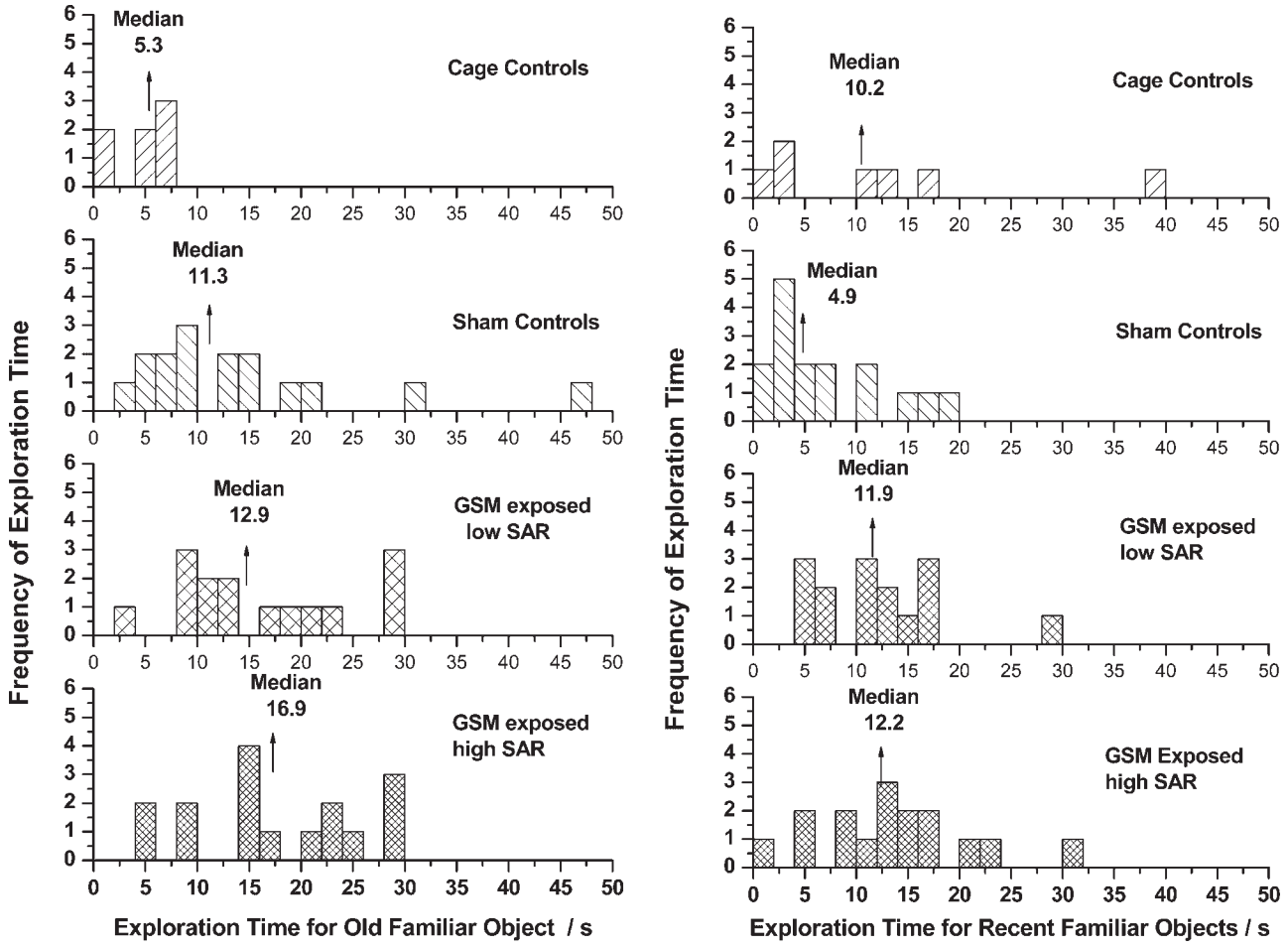


Fig. 7. Exploration time in the third trial of the episodic-like memory test. The results from each of the four groups (higher effect GSM exposed, lower effect GSM exposed, sham, cage control) are given for the old familiar objects and for the recent familiar objects. Median values for the exploration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

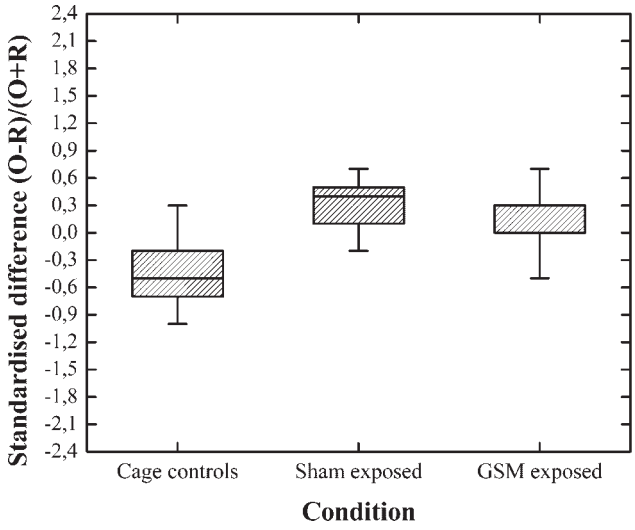


Fig. 8. Boxplot of the standardised difference $(O-R)/(O+R)$ for the exploration time of old familiar objects ($O$) versus the exploration time of the recent familiar objects ($R$). Median and inter-quartile range (IQR) are indicated in the boxes. The lines indicate 5–95% percentile ranges. GSM exposed 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 ($P < 0.001$).

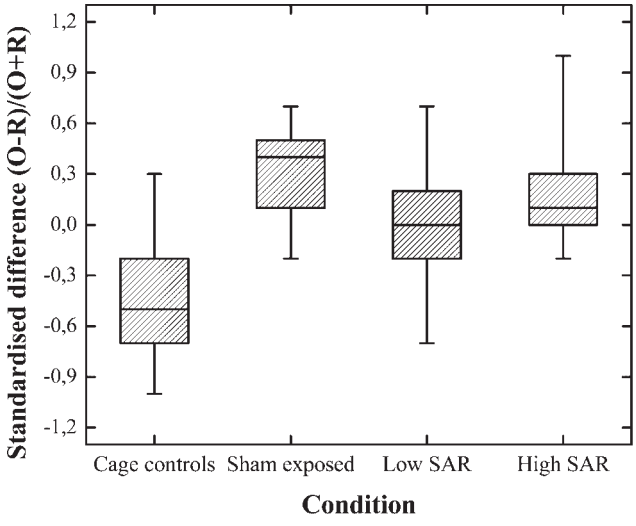


Fig. 9. As Figure 8, but high and low GSM exposure are shown separately. Both higher and lower GSM exposed rats spent a significantly shorter time exploring the old black familiar objects than the recent grey familiar objects when compared to sham exposed rats (Mann–Whitney $P = 0.05$ for higher GSM 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.

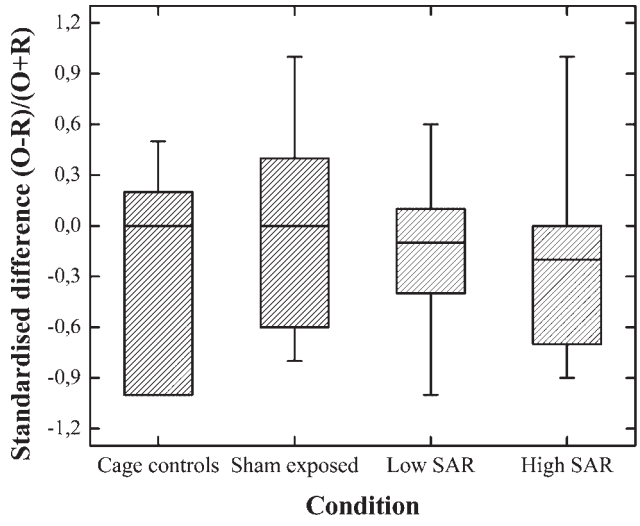


Fig. 10. As Figure 8, but the comparison is between displaced and stationary recent familiar objects in the episodic-like memory test. There was no statistically significant difference in exploration time between the displaced and stationary new familiar objects for the groups of sham exposed, GSM exposed 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

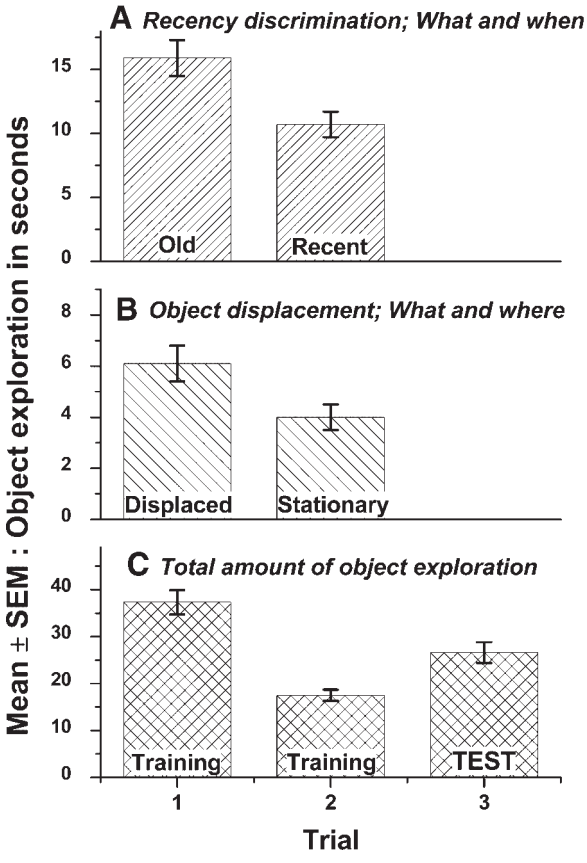


Fig. 11. All conditions except the cage controls are included in these observations. **A**: Mean time for exploration of the old familiar and recent familiar objects using the direct measurements from the test occasion, for males and females (paired *t*-test $P = 0.02$). **B**: Mean time for exploration 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.

## ACKNOWLEDGMENTS

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# Electromagnetic Radiation: Influences on Honeybees (*Apis mellifera)*

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## ABSTRACT

Focussing on the influences of non-ionizing radiation towards the behaviour of the honeybee (*Apis mellifera*), the here presented study reports partially significant results. Nowadays, there is a certain increase of radiation impact in today's environmental ecosystems, and the influence of higher frequencies on honey bees is analyzed by the workgroup "educational informatics" since 2001 (Stever & Kuhn 2001; Kuhn & Stever 2001; Kuhn & Stever 2002). In ecotoxicology, the honeybee (*Apis mellifera*) is of great importance as a tested species for agricultural chemicals, e. g. plant protection products and pesticides. In this case, significant variations in the behaviour of *Apis mellifera* under the influence of non-ionizing radiation were tested. The presented data set is based on earlier studies from 2005, which showed significant differences in returning, 39.7% of the non-irradiated bees came back compared to 7.3% of the irradiated ones.
Standard commercial DECT telephones were used as exposition source. Concerning possible variations in behaviour an experimental setup with irradiated and non-irradiated bee hives was assembled. The main emphasis of this study was the investigation on significant changes in the foraging flight under electromagnetic radiation influence.

**Keywords:** Honeybees, electromagnetic radiation, learning process, changing behaviour, ecotoxicology.

## 1. INTRODUCTION

This study focuses on the effects of an electromagnetic exposition caused by DECT Telephones on the behaviour of the honeybee. All researches and tests have been carried out at the Dienstleistungszentrum Ländlicher Raum (DLR), Fachzentrum Bienen und Imkerei, in Mayen during June/July 2006. There have been several scientific investigations throughout the past years concerning the electromagnetic radiation and its effects (Greenberg et al., 1981; Hartsgrove et al., 1987; Eulitz et al., 1998; Rothmana, 2000). In context of the increasing non-ionising radiation, this study focus on the effects of electromagnetic exposition on the behaviour of the honeybee. Especially towards crop pesticide testing, Apis mellifera is a confirmed test species in ecotoxicological researches. Furthermore the honeybee shows an effective learning behaviour, resulting in olfactory amenities and even forms, structures and faces and also in training abilities on certain plants (Vareschi & Kaissling, 1970; Hoefer & Lindauer, 1976; Dyer et al 2005). Apis mellifera is well suited as a bioindicator, because its brain anatomy as well as the learning regions of the bee brain are well known (Menzel & Müller, 1996; Zhang et al., 1999; Schwärzel & Müller, 2006) and the brain structure of the honeybee concerning associative learning is comparable to those of vertebrates (Bliss & Collinridge, 1993; Eichenbaum, 2004; Giurfa, 2003; Schwärzel &

Müller, 2006). Concerning the effects of electromagnetic radiation it might be possible to draw conclusions towards other organisms based on the results according to the monitoring of honeybees.

## 2. MATERIAL AND METHODS

*2.1 Physical aspects*

In this case, base stations of everyday used DECT telephones (Digital Enhanced Cordless Telecommunications) were fixed as radiation sources. Investigating on non-thermal influences of electromagnetic fields towards the learning behaviour of bees requires an exposition with an appropriate radiation frequency. The stations send out continually electromagnetic radiation with a frequency $f_S \approx 1900$ MHz and an average transmitting power $P_S$ of 10 mW. The peak power is 250 mW and the sending signal throughout a talk is frequency modulated and pulsed with a frequency $f_p$ of 100 Hz. For this study the base station is used as radiation source at a permanent standby mode reached with an average transmitting power of $P_S = 2.5$ mW. To analyze a possible effect of the radiation intensity, cubic radiation shields made of reed and clay were build around some of the DECT base stations (experimental group 2, EG2, refer to 2.2), which is completely permeable to the low-frequency pulse mentioned above, but enables a reduction of the high-frequency sending radiation about 50% (Moldan & Pauli, 2000). We also installed metal lattices (width 1x1 mm) between the exposed bee hives (experimental group) in order to avoid possible influences of the radiation on the non-exposed bee hives (control group, CG).

The stations were put at the bottom of a beehive, right under the honeycombs (Fig. 1).

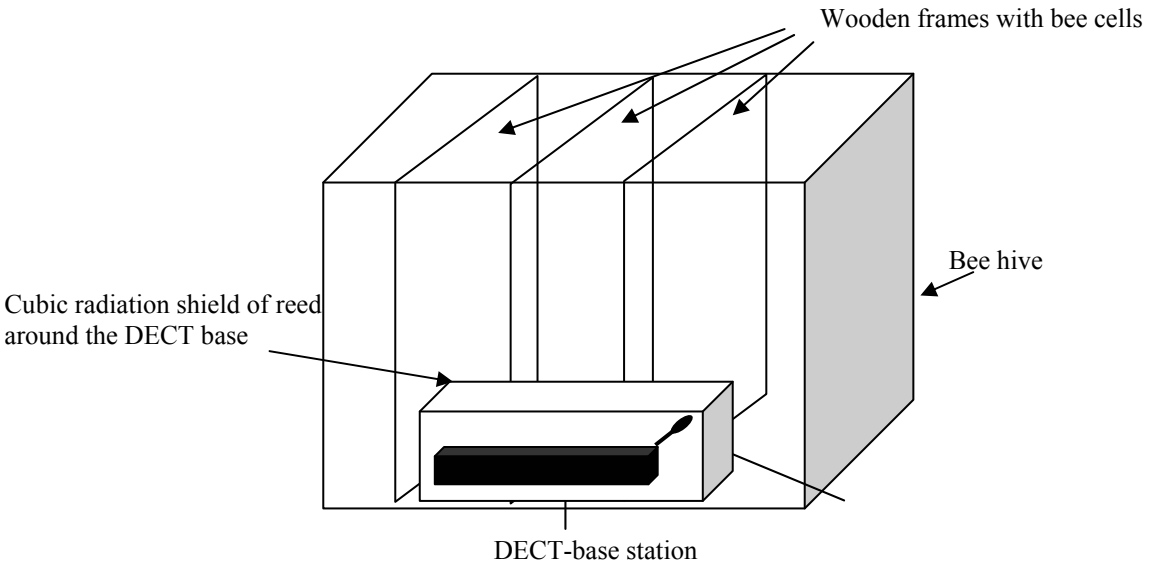


**Fig. 1:** Position of DECT base station within a bee hive

*2.2 test objects and method*

Overall, 16 Bee colonies of *Apis mellifera carnica* were used as test objects. With a permanent connection establishment between the wireless cells and the DECT base stations, the average sending power $P_s$ could be estimated. Five of eight exposed hives were under fully electromagnetic exposure (experimental group EG 1), while in three of the exposed colonies the radiation was shielded down to 50% (experimental group EG 2, see Fig. 1). The following figure shows the whole experimental set-up:

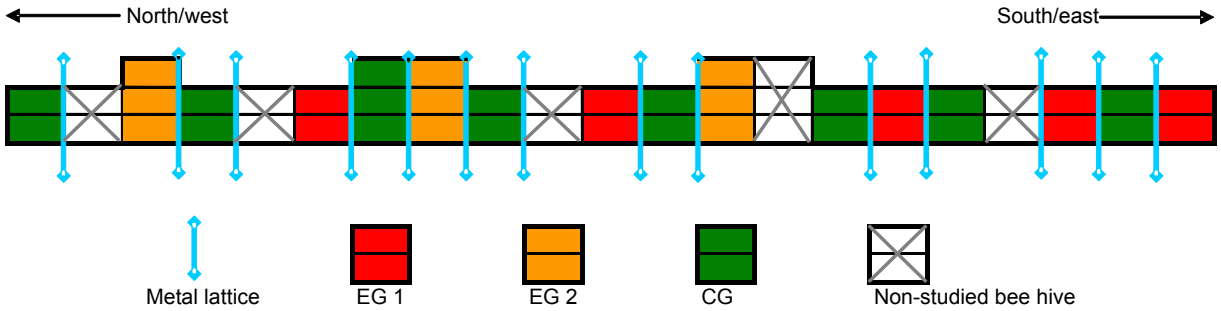


**Fig. 2**: Experimental set up

For one test run, 15 bees flying out of the hive were trapped with the help of plastic tubes at the hive entrance. All catched bees were short term paralyzed (using $CO_2$) and got marked with a marker dot on the thorax. At a distance of about 500 m to the hive all marked bees were set free simultaneously and got timed from that moment. Concerning the returning behaviour, in every test run irradiated bees were checked against non-exposed ones (EG 1 vs. CG; EG 1 vs. EG 2; EG 2 vs. CG). Time of flight for every single bee as well as certain aspects like weather, temperature and hive activity in common was reported. The returning bees were intercepted at the bee hive's entrance and the returning time was documented. The observation time lasted 45 minutes, bees that came back afterwards were disregarded in order to avoid possible mistakes for following test runs.

## 3. RESULTS

All results are based on collected data from June, 28.–29., and July, 9.-19., in 2006.

### *3.1 statistics*

52 paired comparisons had to be taken into consideration, 31 pairs of bee colonies "EG 1 vs. CG", 15 pairs "EG 2 vs. CG" and finally 6 pairs "EG 1 vs. EG 2". In 22 of the 31 tested pairs "EG 1 vs. CG" more of the non-exposed bees (CG) returned to their colonies. With the total amount of returned bees (non-exposed 293 = 63.0%, exposed 229 = 49.2%) the tendency of earlier researches (Stever et al., 2005) could be confirmed.

Overall, 482 (63%) bees of the CG, 203 (56.4%) bees of the EG 2 and 365 (54.1%) bees of the EG 1 returned to their hive. These differences between the groups were not significant (Kruskal-Wallis H test).

One of the main problems of the statistical analysis was to combine the amount of returning bees with their returning time in one single value ($tn_r$), which reflects the predominate circumstances and enables a comparison between different testing properties. The following term presents a possible solution to this problem:

$$tn_R = n_R * 46 - \Sigma\, t_R$$

In this term the amount of returning bees $n_R$ is multiplied by the maximum observation time + 1, then the sum of the returning time of each bee $t_R$, which were actually returned, is subtracted from this product. To standardize the $tn_R$-Index its term is related to the maximum value (for this study ($n_{Rmax}$ = 15 bees * [45min + 1]), the $tn_{max}$ is up to 690):

$$tn = tn_R * 100 / tn_{max}$$

It became obvious that in 29 of 31 tested pairs the *tn*-index was higher for non-exposed bees, with *tn*-index-mean ratio of 48.97 (SD 20.74) for non-exposed bees against a *tn*-index-mean ratio of 38.48 (SD 16.41) for exposed bees.
The comparison in pairs between bees of the EG 1 with bees of the CG is presented in Fig. 3:

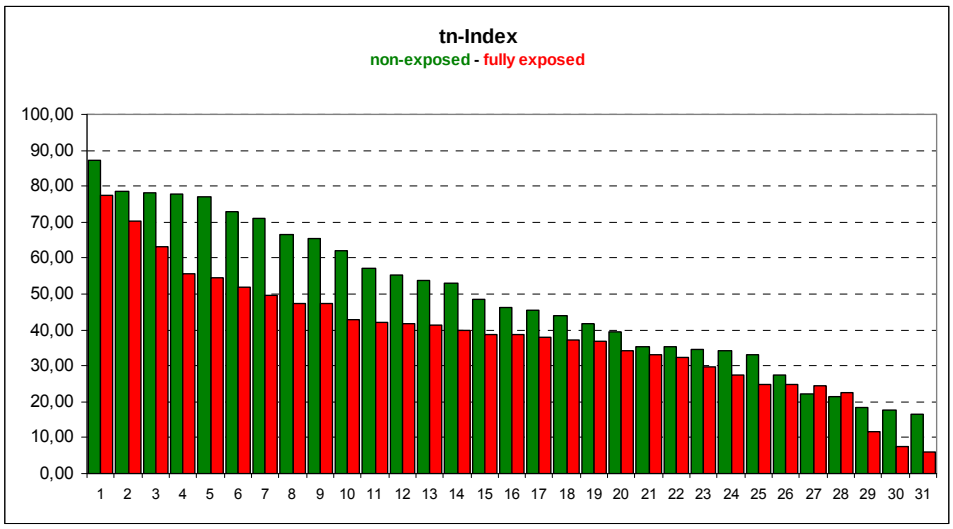


**Fig. 3:** *tn*-Index comparison CG (green) vs. EG 1 (red), decreasing ranks

*3.2 tn-index mean comparisons for all tested groups*
All deviations concerning the mean ratio for each compared group are tested for significant differences by conducting the t-test for independent variables.
Referring to the results of the t-test, mean differences between non-exposed and exposed honeybees (CG vs. EG 1) were significant ($p = 0.031$), whereas the other two tested pairs (CG vs. EG 2; EG 1 vs. EG 2) showed no significant differences.
Furthermore no correlations of uncontrollable factors like weather, temperature and flight frequency with the *tn*-Index were found, which shows that there is no influence of these uncontrollable factors concerning our results.

## 4. DISCUSSION

Obviously certain factors concerning the experimental set up are hard to control, but aspects such as homogenous bee colonies, the location of the tested hives and the interaction between studied bee colonies and disregarded neighbour colonies must be observed and controlled before starting a following study. Also the testing place should be selected as soon as possible, in order to allow the bees selecting a preferred region for collecting food.
The results of this study are much more heterogenic compared to our examination in 2005. But despite this aspect, still a significant difference between exposed and non-exposed bee colonies could be observed. A correlation between the independent factors weather, flight frequency and temperature on the *tn*-index could not be determined. A possible influence of the radiation intensity could not be proven by this study, because no significant differences between the group-pairs CG and EG 2 as well as EG 2 and EG 1 could be detected. Also, a clear distinction between the low-frequency pulse of the DECT base station and its high-frequency sending radiation could not be drawn, despite the fact that a significant difference between the non-exposed bees and the fully

irradiated ones can be counted as a result of the influence of high-frequency electromagnetic radiation.

A certain method to improve the experimental set up can be found in automating the testing intervals, e.g. by using a lock at the hive entrance for automatically collecting the bees. Finally, it would be also very important to measure the exact radiation intensity within the hives as well as the concrete character of the used radiation.

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# Bioeffects of mobile telephony radiation in relation to its intensity or distance from the antenna

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**Abstract**
*Purpose:* To examine the bioactivity of GSM 900 and 1800 (Global System for Mobile Telecommunications) radiations, in relation to the distance from the antenna or to the radiation-field intensities.
*Materials and methods:* *Drosophila melanogaster* adult insects were exposed to the radiation of a GSM 900/1800 mobile phone antenna at different distances ranging from 0 to 100 cm, and the effect on their reproductive capacity and cell death induction in the gonads by the use of TUNEL (Terminal deoxynucleotide transferase dUTP Nick End Labeling) assay, was studied.
*Results:* These radiations/fields decreased the reproductive capacity by cell death induction, at all the different distances tested. The effect diminished with the distance/decreasing intensities. An increased bioactivity 'window' was revealed at distances of 20–30 cm from the mobile phone antenna, (radiation intensity around 10 $\mu$W/cm$^2$) where the effect became highest, in relation to smaller or longer distances. The effect diminished considerably for distances longer than 40–50 cm and became not evident for distances longer than 1 m or radiation intensities smaller than 1 $\mu$W/cm$^2$.
*Conclusions:* GSM bioactivity is highest for intensities down to less than 10 $\mu$W/cm$^2$ and still evident until 1 $\mu$W/cm$^2$ exhibiting 'window' effects.

**Keywords:** *GSM, DCS, distances, intensity, cell death, electromagnetic fields, reproduction, bioactivity windows*

## Introduction

A number of biological effects from digital mobile telephony and radio frequency (RF)-microwave radiations, including changes in intracellular ionic concentrations, changes in the synthesis rate of different biomolecules, changes in cell proliferation rates, changes in the reproductive capacity of animals, changes in gene expression and even DNA damage and cell death, have already been reported and documented by many research groups (Bawin et al. 1975; 1978; Bawin and Adey 1976; Lai and Singh 1995, 1996, 1997; Magras and Xenos 1997; Kwee and Raskmark 1998; Velizarov et al. 1999; Salford et al. 2003; Xenos and Magras 2003; Panagopoulos et al. 2004, 2007a, 2007b; Aitken et al. 2005; Barteri et al. 2005; Belyaev et al. 2005; 2009; Caraglia et al. 2005; Diem et al. 2005; Markova et al. 2005; Nylund and Leszczynski 2006; Remondini et al. 2006; Eberhardt et al. 2008; Garaj-Vrhovac and Orescanin 2009; Lopez-Martin et al. 2009). At the same time, some epidemiological studies are starting to indicate a connection between the use of cellular mobile phones and certain types of cancer (Kundi 2004; Hardell et al. 2006, 2007, 2009; Hardell and Hansson Mild 2006; Hardell and Carlberg 2009; Khurana et al. 2009), as well as a connection between exposure to radiation from base stations and adverse health effects reported as 'microwave syndrome' (Navarro et al. 2003; Hutter et al. 2006; Blettner et al. 2009; Kundi and Hutter 2009; Viel et al. 2009).

Most of the experiments carried out in regards to the bioactivity of mobile telephony radiation were performed either by use of commercial mobile phone devices emitting real mobile telephony signals or by test mobile phones emitting idealized mobile telephony signals with constant and controllable parameters. Until now there were no experiments

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regarding the effects at different distances from mobile phone antennas corresponding to different intensities of the emitted radiation, neither experiments regarding the effects of mobile telephony base station antennas, except of statistical observations which have reported reduction of bird and insect populations around base station antennas (Balmori 2005; Everaert and Bauwens 2007).

Both systems of Digital Mobile Telephony Radiation established and commonly used in Europe, GSM 900 MHz (Global System for Mobile telecommunications), and GSM 1800 MHz, (also called DCS 1800 MHz – Digital Cellular System), except of their RF carrier signal, use a pulse repetition frequency of 217 Hz, plus other extremely low frequencies (ELF) necessary for the transmission of information (Tisal 1998; Hamnerius and Uddmar 2000; Hyland 2000; Clark 2001; Hillebrand 2002; Panagopoulos and Margaritis 2008). Thereby the signals of both systems combine RF and ELF frequencies. This combination of RF carrier and ELF pulsing frequencies is considered to play an important role in the bioactivity of this kind of radiation (Lin-Liu and Adey 1982; Penafiel et al. 1997).

Radiation from base station antennas is almost identical to that from mobile phones of the same system (GSM 900 or 1800), except that it is about 100 times more powerful, and uses a little higher carrier frequency. GSM 900 mobile phones emit between 890 and 915 MHz (uplink operation) while base stations emit between 935 and 960 MHz (downlink operation). The corresponding GSM (or DCS) 1800 spectrums are 1710–1785 MHz (uplink operation) and 1805–1880 MHz (downlink operation) (Tisal 1998; Hamnerius and Uddmar 2000; Hyland 2000; Clark 2001; Hillebrand 2002; Panagopoulos and Margaritis 2008). Thereby, effects produced by mobile phones at certain distances, could possibly be extrapolated to represent effects from base station antennas, of the same type of radiation, at about 100 times longer distances.

The difficulty in performing experiments with base station mobile telephony antennas is due to the fact of uncontrolled conditions in the open air that do not allow the use of sham-exposed animals, (exposed to identical other conditions like temperature, humidity, light etc.). In other words, there is no way to have a sham-exposed group of experimental animals under identical environmental conditions as the exposed ones, but without being exposed to the radiation at the same time. We thought that the only way to simulate the reality of the exposure by a base station antenna is to expose the animals at different distances from a mobile phone within the laboratory.

In order to study the bioactivity of mobile telephony signals at different intensities and distances from the antenna of a mobile phone handset, resembling effects from base station signals within residential areas, we used the same biological index as in previous experiments of ours, the reproductive capacity of the insect *Drosophila melanogaster*, defined by the number of $F_1$ (first filial generation) pupae derived during the three days of the insect's maximum oviposition, as this was found to be a reliable indicator for the bioactivity of electromagnetic fields (EMF) (Panagopoulos et al. 2000a, 2004, 2007a, 2007b; Panagopoulos and Margaritis 2002, 2003a).

Our previous experiments regarding a few minutes daily exposure of the same model animal to the near field of a mobile phone antenna have shown a large decrease in the reproductive capacity, affecting both sexes (Panagopoulos et al. 2004). Both systems of digital mobile telephony radiation GSM 900 MHz and GSM/DCS 1800 MHz were found to produce the same effects, but GSM 900 was found to be even more bioactive than 1800, mainly due to the higher intensity of GSM 900 antennas compared to GSM/DCS 1800 ones (Panagopoulos et al. 2007a). The decrease in the reproductive capacity was found to be due to induced cell death (DNA fragmentation) in the gonads, caused by both types of mobile telephony radiations (Panagopoulos et al. 2007b).

A widely used method for identifying cell death is TUNEL (Terminal deoxynucleotide transferase dUTP Nick End Labeling) assay. By use of this method, fluorescein dUTP is bound through the action of terminal transferase, onto fragmented genomic DNA which then becomes labelled by characteristic fluorescence. The label incorporated at the fragmented DNA is visualised by fluorescence microscopy (Gavrieli et al. 1992).

Each *Drosophila* ovary consists of 16–20 ovarioles. Each ovariole is an individual egg assembly line, with new egg chambers in the anterior moving toward the posterior as they develop, through 14 successive stages until the mature egg reaches the oviduct. The most anterior region is called the germarium. The most sensitive developmental stages during oogenesis for stress-induced cell death, are region 2 within the germarium and stages 7–8 just before the onset of vitellogenesis (Drummond-Barbosa and Spradling 2001; McCall 2004). Electromagnetic stress from mobile telephony radiations was found in our experiments to be extremely bioactive, inducing cell death to a high degree not only to the above two 'check points' (germarium and stages 7–8) but to all developmental stages of early and mid oogenesis and moreover to all types of egg chamber cells, i.e. nurse cells, follicle cells and the oocyte (OC) (Panagopoulos et al. 2007b).

In continuing our research on the biological impacts of the cellular mobile telephony radiation, the aim of the present study was to investigate the

dependence of GSM 900/1800 bioactivity on its intensity, within intensity levels that people are exposed to, from mobile phones and base station antennas as well. Finally, in the case that we would detect a decrease in the reproductive capacity at smaller intensities than in our previous experiments (Panagopoulos et al. 2004, 2007a, 2007b), our aim would be to confirm whether again the decrease is due to cell death induced by the radiation or not, by use of the TUNEL assay.

## Materials and methods

### *Drosophila culturing*

Wild-type strain Oregon R *Drosophila melanogaster* flies were cultured according to standard methods and kept in glass vials with standard food (Panagopoulos et al. 2004). Ovaries from exposed and sham-exposed flies were dissected into individual ovarioles at the sixth day after eclosion and then treated for TUNEL assay.

### *Exposure system*

As an exposure device we used a commercial cellular mobile phone itself, in order to analyse the effects of real mobile telephony signals. As in previous experiments (Panagopoulos et al. 2007a, 2007b), we used a dual band cellular mobile phone that could be connected to either 900 or 1800 networks simply by changing SIM ('Subscriber Identity Module') cards on the same handset. The highest Specific Absorption Rate (SAR), given by the manufacturer for human head, is 0.89 W/kg. The exposure procedure was the same as in earlier experiments of ours (Panagopoulos et al. 2007b). The handset was fully charged before each set of exposures. The experimenter spoke on the mobile phone's microphone during the exposures. Thereby, the emitted 900 or 1800 radiation during the exposures was 'modulated' by the human voice, ('speaking emissions').

Exposures and measurements of mobile phone emissions were performed at the same place where the mobile phone had full perception of both 900 and 1800 signals, as described before (Panagopoulos et al. 2007a). The measured mean power densities in contact and at different distances from the mobile phone antenna for 6 min of modulated emission, for GSM 900 MHz and for DCS 1800 MHz, are shown in Table I. As explained before (Panagopoulos et al. 2007a, 2007b), the GSM 900 MHz intensity at the same distance from the antenna and with the same handset was higher than the corresponding GSM/DCS 1800 MHz. Measurements at 900 and 1800 MHz were performed with a RF Radiation Survey Meter, NARDA 8718 (Hauppauge, NY, USA). Since both GSM 900 and 1800 signals use a pulse repetition frequency at 217 Hz plus other ELF pulses, we measured electric and magnetic field intensities in the ELF range, with a Holaday HI-3604 ELF Survey Meter (Eden Prairie, MN, USA). The measured values for the modulated ELF fields, excluding the ambient electric and magnetic fields of 50 Hz, for GSM 900 and 1800 at different distances from the antenna are also shown in Table I. All values shown in Table I are averaged over 10 separate measurements of each kind $\pm$ standard deviation (SD). These values are typical for digital mobile telephony handsets and they are all within the established current exposure criteria (International Commission for Non-Ionising Radiation Protection [ICNIRP] 1998).

The radiation and field measurements given in Table I show that although the ELF electric and magnetic field intensities fall within the background levels for distances longer than 50 cm from both GSM 900 and 1800 mobile phone antennas, the RF components of the signals are still evident for distances up to 100 cm.

### *Exposure procedures*

In each single experiment, we separated the collected insects into thirteen groups: The first group (named '0') was exposed to GSM 900 or 1800 field with the mobile phone antenna in contact with the glass vial containing the flies. The second (named '1'), was exposed to GSM 900 or 1800 field, at 1 cm distance from the mobile phone antenna. The third group (named '10') was exposed to GSM 900 or 1800 field at 10 cm distance from the mobile phone antenna. The fourth group (named '20') was exposed to GSM 900 or 1800 field at 20 cm distance from the mobile phone antenna, etc, the 12th group (named '100') was exposed to GSM 900 or 1800 field at 100 cm distance from the mobile phone antenna. Finally, the 13th group (named 'SE') was the sham-exposed. Each group consisted of 10 male and 10 female insects as previously (Panagopoulos et al. 2004, 2007a).

In each experiment, we collected newly eclosed adult flies from the stock early in the afternoon, and separated them into the 13 different groups following the same methodology as in previous experiments (Panagopoulos et al. 2004).

We exposed the flies within the glass vials by placing the antenna of the mobile phone outside of the vials, parallel to the vial's axis. The total duration of exposure was 6 min per day in one dose and exposures were started on the first day of each experiment (day of eclosion). In each experiment, all the 12 exposed groups were simultaneously exposed

Table I. GSM 900 and 1800 radiation and field intensities ± SD, in the microwave and ELF regions, for different distances from a mobile phone antenna*.

| Distance from mobile phone antenna (cm) | GSM 900 radiation intensity at 900 MHz, ($mW/cm^2$) | GSM 900 electric field intensity at 217 Hz, (V/m) | GSM 900 magnetic field intensity at 217 Hz, (mG) | GSM 1800 radiation intensity at 1800 MHz, ($mW/cm^2$) | GSM 1800 electric field intensity at 217 Hz, (V/m) | GSM 1800 magnetic field intensity at 217 Hz, (mG) |
|---|---|---|---|---|---|---|
| 0 | 0.378 ± 0.059 | 19 ± 2.5 | 0.9 ± 0.15 | 0.252 ± 0.050 | 13 ± 2.1 | 0.6 ± 0.08 |
| 1 | 0.262 ± 0.046 | 12 ± 1.7 | 0.7 ± 0.13 | 0.065 ± 0.015 | 6 ± 0.8 | 0. 4 ± 0.07 |
| 10 | 0.062 ± 0.020 | 7 ± 0.8 | 0.3 ± 0.05 | 0.029 ± 0.005 | 2.7 ± 0.5 | 0. 2 ± 0.05 |
| 20 | 0.032 ± 0.008 | 2.8 ± 0.4 | 0.2 ± 0.04 | 0.011 ± 0.003 | 0.6 ± 0.12 | 0. 1 ± 0.02 |
| 30 | 0.010 ± 0.002 | 0.7 ± 0.09 | 0.1 ± 0.02 | 0.007 ± 0.001 | 0.3 ± 0.06 | 0.06 ± 0.01 |
| 40 | 0.006 ± 0.001 | 0.2 ± 0.03 | 0.05 ± 0.01 | 0.004 ± 0.0007 | 0.1 ± 0.04 | – |
| 50 | 0.004 ± 0.0006 | 0.1 ± 0.02 | – | 0.002 ± 0.0003 | – | – |
| 60 | 0.002 ± 0.0003 | – | – | 0.0016 ± 0.0002 | – | – |
| 70 | 0.0017 ± 0.0002 | – | – | 0.0013 ± 0.0002 | – | – |
| 80 | 0.0012 ± 0.0002 | – | – | 0.0011 ± 0.0002 | – | – |
| 90 | 0.0010 ± 0.0001 | – | – | 0.0005 ± 0.0001 | – | – |
| 100 | 0.0004 ± 0.0001 | – | – | 0.0002 ± 0.0001 | – | – |

*For distances longer than 30–50 cm from the mobile phone antenna, the ELF electric and magnetic field components of both GSM 900 and 1800 radiations, fall within the background of the stray 50 Hz fields within the lab.

during the 6-min exposure sessions. The exposures took place for five days in each experiment, as previously described (Panagopoulos et al. 2004). Then there was an additional 6-min exposure in the morning of the sixth day and one hour later, female insects from each group were dissected and prepared for TUNEL assay, as described before (Panagopoulos et al. 2007b). The daily exposure duration of 6 min, was chosen for reasons we have explained before (Panagopoulos et al. 2004, 2007a) and for keeping the same exposure conditions as in our previous experiments.

After each exposure, the corresponding sham-exposure took place. The SE group was 'exposed' for 6 min at zero distance from the mobile phone antenna, following exactly the same methodology (the experimenter spoke on the mobile phone, same voice, reading the same text) but the mobile phone was turned off. Before this we had already verified that sham-exposed groups at all the 12 different locations of exposure described above, did not differ significantly between them in their reproductive capacity and additionally did not differ significantly from a Control group (named 'C') which was never taken out of the culture room during the experiments and was not exposed or sham-exposed in any way (see Appendix). Comparison between SE and C groups in relation to the reproductive capacity and ovarian cell death on the same experimental animals was discussed also in a previous work of ours (Panagopoulos et al. 2007b).

In each experiment we kept the 10 males and the 10 females of each group, in separate vials for the first 48 h, for reasons we have explained before (Panagopoulos et al. 2004). After the first 48 h of each experiment, when both males and females of each group were sexually mature, they were put together (10 pairs) in another glass vial with fresh food. They were allowed to mate and lay eggs for the next 72 h, during which, the daily egg production of *Drosophila* is at its maximum (Panagopoulos et al. 2004).

After the last exposure in the morning of the sixth day from the beginning of each experiment, the flies were removed from the glass vials, and the ovaries of females were dissected and fixed for TUNEL assay. The vials were then maintained in the culture room for 6–8 additional days without further exposure, and then the number of $F_1$ pupae was counted in each group as in previous experiments (Panagopoulos et al. 2000a, 2004, 2007a). As explained in detail before (Panagopoulos et al. 2004), this number is a representative estimate of the insect's reproductive capacity.

The temperature during the exposures was monitored within the vials by a mercury thermometer with an accuracy of 0.05°C (Panagopoulos et al. 2004).

### *TUNEL assay*

To determine the ability of GSM and DCS radiation to induce cell death during early and mid oogenesis, we used the TUNEL assay, as follows: Ovaries were dissected in Ringer's solution and separated into individual ovarioles from which we took away egg chambers of stages 11–14. In egg chambers of stages 11–14 programmed cell death takes place normally in the nurse cells and follicle cells. Thereby we kept and treated ovarioles and individual egg chambers

from germarium up to stage 10. Samples were fixed in phosphate-buffered saline (PBS) solution containing 4% formaldehyde plus 0.1% Triton X-100 (Sigma Chemical Co., Munich, Germany) for 30 min and then rinsed three times and washed twice in PBS for 5 min each. Then samples were incubated with PBS containing 20 $\mu$g/ml proteinase K for 10 min and washed three times in PBS for 5 min each. In situ detection of fragmented genomic DNA was performed with Boehringer Mannheim kit (Boehringer Mannheim Corp., Indianapolis, IN, USA), containing fluorescein dUTP for 3 h at 37°C in the dark. Samples were then washed six times in PBS for 1 h and 30 min (total duration) in the dark and finally mounted in antifading mounting medium (90% glycerol containing 1.4-diazabicyclo (2.2.2) octane (Sigma Chemical Co.) to prevent from fading and viewed under a Nikon Eclipse TE 2000-S fluorescence microscope (Tokyo, Japan). The samples from different experimental groups were blindly observed under the fluorescence microscope (i.e., the observer did not know the origin of the sample) and the percentage of egg chambers with TUNEL positive signal was scored in each sample.

### *Statistical analysis*

The results on reproductive capacity and cell death induction were analysed statistically by single factor Analysis of Variance test which calculates the probability (*P*) that differences between groups are due to random variations. The smaller this probability is, the more significantly the groups differ between them (in their reproductive capacity or in the percentages of TUNEL positive egg chambers). In addition, linear (Pearson's) and non-parametric (Kendall's) correlation analysis were performed between reproductive capacity and radiation/field intensities in order to get an estimation of which parameter (RF radiation, ELF fields) might be more responsible for the effects (Weiss 1995; Maber 1999).

## Results

The average mean values of reproductive capacity (mean number of $F_1$ pupae per maternal insect) from eight separate identical experiments with GSM 900 and GSM/DCS 1800 exposures are listed in Table II and represented graphically in Figures 1 and 2.

The data show that GSM 900 mobile telephony radiation decreases reproductive capacity at distances from 0 cm up to 90 cm from the mobile phone antenna (corresponding intensities ranging from 378 $\mu$W/cm$^2$ down to 1 $\mu$W/cm$^2$ –Table I). Table II and Figure 1 show that the effect is at a maximum at 0 cm and at 30 cm from the antenna (corresponding to radiation intensities of 378 $\mu$W/cm$^2$ and 10 $\mu$W/cm$^2$, respectively) with an overall maximum at 30 cm. For distances longer than 30 cm from the mobile phone antenna, the effect decreases rapidly and becomes very small for distances longer than 50 cm, but it is still evident for distances up to 90 cm (intensities down to 1 $\mu$W/cm$^2$).

The data also show that GSM/DCS 1800 mobile telephony radiation decreases reproductive capacity at distances from 0 cm up to 80 cm from the mobile phone antenna (corresponding intensities ranging from 252 $\mu$W/cm$^2$ down to 1.1 $\mu$W/cm$^2$ –Table I). Table II and Figure 2 show that the effect is maximum at 0 cm and at 20 cm from the antenna, (corresponding to radiation intensities of 252 $\mu$W/cm$^2$ and 11 $\mu$W/cm$^2$, respectively) with overall

Table II. Effect of GSM 900 and 1800 radiation-fields on the reproductive capacity at different distances from the antenna.

| Groups-Distance from mobile phone antenna, (cm) | Average mean number of $F_1$ pupae per maternal fly ± SD, for GSM 900 MHz | Deviation from sham-exposed group | Average mean number of $F_1$ pupae per maternal fly ± SD, for GSM 1800 MHz | Deviation from sham-exposed group |
|---|---|---|---|---|
| 0 | 7.46 ± 0.73 | −46.14% | 9.10 ± 0.69 | −35.09% |
| 1 | 9.35 ± 0.62 | −32.49% | 11.35 ± 0.63 | −19.04% |
| 10 | 11.28 ± 0.81 | −18.56% | 11.93 ± 0.72 | −14.91% |
| 20 | 11.55 ± 0.79 | −16.61% | 8.33 ± 0.7 | −40.58% |
| 30 | 7.38 ± 0.65 | −46.71% | 12.77 ± 0.82 | −8.92% |
| 40 | 12.81 ± 0.97 | −7.51% | 13.52 ± 0.86 | −3.57% |
| 50 | 13.49 ± 0.82 | −2.60% | 13.72 ± 0.75 | −2.14% |
| 60 | 13.62 ± 0.83 | −1.66% | 13.81 ± 0.92 | −1.50% |
| 70 | 13.72 ± 0.92 | −0.94% | 13.79 ± 0.90 | −1.64% |
| 80 | 13.68 ± 0.80 | −1.23% | 13.85 ± 0.81 | −1.21% |
| 90 | 13.75 ± 0.95 | −0.72% | 14.03 ± 1.02 | +0.07% |
| 100 | 14.01 ± 1.01 | +1.16% | 14.05 ± 0.99 | +0.21% |
| SE | 13.85 ± 0.91 | | 14.02 ± 0.98 | |

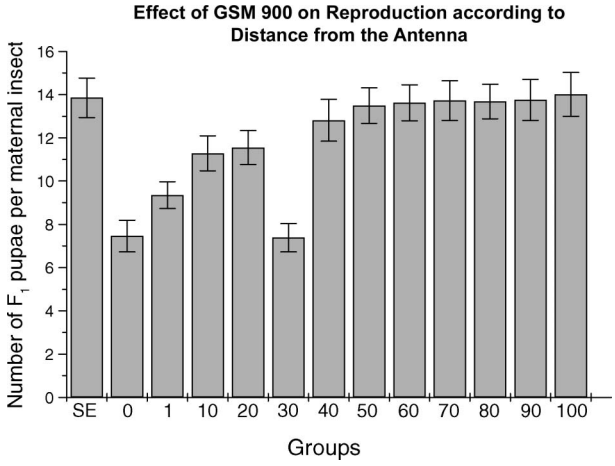


Figure 1. Reproductive capacity (mean number of $F_1$ pupae per maternal insect averaged over eight identical experiments) $\pm$ SD, in relation to the distance from a GSM 900 MHz mobile phone antenna (cm). The decrease in reproductive capacity is at a maximum at zero distance and at 30 cm distance from the antenna, corresponding to RF intensities 378 $\mu$W/cm$^2$ and 10 $\mu$W/cm$^2$ (see Table II).

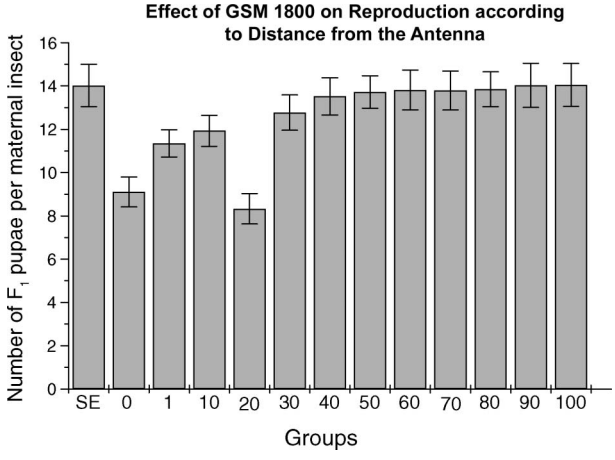


Figure 2. Reproductive capacity (mean number of $F_1$ pupae per maternal insect averaged over eight identical experiments) $\pm$ SD, in relation to the distance from a GSM/DCS 1800 MHz mobile phone antenna (cm). The decrease in reproductive capacity is at a maximum at zero distance and at 20 cm distance from the antenna, corresponding to RF intensities 252 $\mu$W/cm$^2$ and 11 $\mu$W/cm$^2$ (see Table II).

maximum at 20 cm. For distances longer than 20 cm from the mobile phone antenna, the effect decreases rapidly and becomes very small for distances longer than 40 cm, but it is still evident for distances up to 80 cm (intensities down to 1.1 $\mu$W/cm$^2$).

Thus, the effect of mobile telephony radiation on reproductive capacity is at a maximum at zero distance (intensities higher than 250 $\mu$W/cm$^2$) and then becomes maximum at a distance of 30 cm or 20 cm from the antenna for GSM 900 or 1800 MHz radiation, respectively. These distances of 30 cm and 20 cm, respectively, correspond to the same RF intensity around 10 $\mu$W/cm$^2$ and also to the same ELF electric field intensity of about 0.6–0.7 V/m (Table I).

The statistical analysis (single factor ANOVA test) shows that the probability that the reproductive capacity differs between groups, owing to random variations, is negligible both for GSM 900 and 1800 exposures, $P < 10^{-27}$ in both cases.

There were no temperature increases within the vials during the exposures, as shown by the sensitive Hg thermometer.

In Table III, the summarised data on cell death induction in the gonads of the female insects from three separate experiments are listed. These data are represented graphically in Figures 3 and 4. The percentages of TUNEL positive egg chambers in all groups were found to be very close to the corresponding decrease in the reproductive capacity of the same groups (Table III, Figures 3 and 4), verifying the results of earlier experiments of ours (Panagopoulos et al. 2007b). The maximum percentage of TUNEL positive egg chambers of exposed animals was found in the ovaries of female insects exposed at 0 and 20 cm distance from the antenna for GSM/DCS 1800 MHz (43.39% and 55.07%) and at 0 and 30 cm distance correspondingly for GSM 900 MHz (57.72% and 57.83%), in agreement with the corresponding maximum decreases in the reproductive capacity (Table III, Figures 3 and 4).

Figure 5a, shows an ovariole from a sham-exposed (SE) female insect, containing egg chambers from germarium to stage 8, all TUNEL negative. This was the typical picture in the vast majority of ovarioles and separate egg chambers from female insects of the sham-exposed groups. In the SE groups, only few egg chambers (including germaria), (less than 8%), were TUNEL positive (Table III, Figures 3 and 4), a result that is in full agreement with the rate of spontaneously degenerated egg chambers normally observed during *Drosophila* oogenesis (Nezis et al. 2000; Baum et al. 2005; Panagopoulos et al. 2007b).

Figure 5b shows an ovariole of an exposed female insect (group 50- GSM 900), which is TUNEL positive only at the two 'check points' germarium and stage 7 and TUNEL negative at all other developmental stages. This was a typical picture of ovarioles of exposed insects from the groups 40–90 for GSM 900 and 30–80 for GSM/DCS 1800.

Figure 5c, shows an ovariole of an exposed female insect (group 20- GSM1800), with a TUNEL positive signal at all developmental stages from germarium to 8 and in all the cell types of the egg chamber (nurse cells, follicle cells and the oocyte). This was a typical picture of ovarioles of exposed insects from the groups 0–30 for GSM 900 and 0–20 for GSM/DCS 1800.

Table III. Effect of GSM 900 and 1800 radiation-fields on ovarian cell death induction at different distances from the mobile phone antenna.

| Groups-Distance from mob. phone antenna (cm) | GSM 900 Sum ratio of TUNEL-positive to total number of egg-chambers from germarium to stage 10 + SD | Percentage of TUNEL-positive egg-chambers (%) | Deviation from sham-exposed groups (%) | GSM 1800 Sum ratio of TUNEL-positive to total number of egg-chambers from germarium to stage 10 + SD | Percentage of TUNEL-positive egg-chambers (%) | Deviation from sham-exposed groups (%) |
|---|---|---|---|---|---|---|
| 0 | 355/615 = 0.5772 ± 0.083 | 57.72 | +50.16 | 243/560 = 0.4339 ± 0.087 | 43.39 | +35.77 |
| 1 | 267/612 = 0.4363 ± 0.061 | 43.63 | +36.01 | 146/483 = 0.3023 ± 0.059 | 30.23 | +22.61 |
| 10 | 172/577 = 0.2981 ± 0.052 | 29.81 | +22.24 | 136/532 = 0.2556 ± 0.054 | 25.56 | +17.94 |
| 20 | 152/564 = 0.2695 ± 0.049 | 26.95 | +19.38 | 337/612 = 0.5507 ± 0.095 | 55.07 | +47.45 |
| 30 | 336/581 = 0.5783 ± 0.092 | 57.83 | +50.26 | 78/452 = 0.1726 ± 0.061 | 17.26 | +9.64 |
| 40 | 93/542 = 0.1716 ± 0.053 | 17.16 | +9.59 | 62/577 = 0.1075 ± 0.056 | 10.75 | +3.13 |
| 50 | 60/556 = 0.1079 ± 0.043 | 10.79 | +3.22 | 54/511 = 0.1057 ± 0.042 | 10.57 | +2.95 |
| 60 | 51/498 = 0.1024 ± 0.045 | 10.24 | +2.67 | 57/580 = 0.0983 ± 0.046 | 9.83 | +2.21 |
| 70 | 57/584 = 0.0976 ± 0.041 | 9.76 | +2.19 | 39/427 = 0.0913 ± 0.033 | 9.13 | +1.51 |
| 80 | 51/563 = 0.0906 ± 0.037 | 9.06 | +1.49 | 39/485 = 0.0804 ± 0.034 | 8.04 | +0.42 |
| 90 | 50/591 = 0.0846 ± 0.04 | 8.46 | +0.89 | 41/534 = 0.0768 ± 0.028 | 7.68 | +0.06 |
| 100 | 46/602 = 0.0764 ± 0.035 | 7.64 | +0.07 | 43/557 = 0.0772 ± 0.035 | 7.72 | +0.1 |
| SE | 47/621 = 0.0757 ± 0.038 | 7.57 | 0 | 48/630 = 0.0762 ± 0.034 | 7.62 | 0 |

Like in our earlier experiments (Panagopoulos et al. 2007b), although in the most egg-chambers where DNA fragmentation could be observed the TUNEL positive signal was most evident in the nurse cells, in many egg chambers of exposed animals and especially in the groups 0–30 for GSM 900 and 0–20 for GSM 1800 on which the bioactivity of the radiation was maximum, a TUNEL-positive signal was detected in all three kinds of egg chamber cells (Figure 5c).

In the SE groups, random DNA fragmentation was observed almost exclusively at the two developmental stages named check-points (germarium and stage 7–8) as also observed before (Panagopoulos et al. 2007b). Similarly, induced DNA fragmentation in the groups 40–100 for GSM 900 and 30–100 for GSM 1800 (Figure 5b), was observed mostly at the two check-points, (data not shown) and only in few cases at the other provitellogenic and vitellogenic stages, 1–6 and 9–10, correspondingly. In contrast, ovarian egg chambers of animals from the exposed groups 0–30 for GSM 900 and 0–20 for GSM 1800, were found to be TUNEL-positive to a high degree at all developmental stages from germarium to stage 10 (Figure 5c), (data not shown). In all cases (both in the SE and also in the exposed groups), the TUNEL-positive signal was observed predominantly and was most intense at the two check points, germarium and stages 7–8, as previously recorded (Panagopoulos et al. 2007b).

Statistical analysis (single factor analysis-of-variance test) shows that the probability that cell death induction differs between groups because of random variations, is $P < 10^{-10}$ both for GSM 900 MHz and 1800 MHz exposures.

The effect on the reproductive capacity, and the induced cell death in the ovaries of exposed female insects, diminishes considerably for distances longer than 40 cm from the mobile phone antenna and disappears for distances longer than 80–90 cm, corresponding to radiation intensities smaller than 1 $\mu$W/cm$^2$ (Tables I–III, Figures 1–4). For distances longer than 50 cm where the ELF components fall within the background of the stray 50 Hz fields, the decrease in reproductive capacity as well as the increase in cell death induction, in regards to the SE groups was very small falling within the standard deviation of the SE groups (Tables II and III, Figures 1–4).

The results of Pearson's linear correlation analysis show a slightly stronger linear relationship between reproductive capacity and ELF electric field intensity (linear correlation coefficient, $r \cong -0.72$, $P < 0.01$ for GSM 900 and $r \cong -0.65$, $P < 0.03$ for GSM/DCS 1800), than between reproductive capacity and RF radiation intensity ($r \cong -0.70$, $P < 0.02$ and $r \cong -0.63$, $P < 0.03$, respectively), both for GSM 900 and 1800 exposures. Since our results show that the dependence of reproductive capacity and cell death induction on RF and ELF intensities is non-linear (Figures 1–4), we applied also Kendall's non-parametric correlation analysis for a better estimation of the non-linear correlation between the variables. This correlation analysis in contrast to the previous one, showed a slightly stronger relationship between reproductive capacity and RF radiation intensity (correlation coefficient, $r \cong -0.85$, $P < 0.001$ for GSM 900 and $r \cong -0.88$, $P < 0.001$ for GSM/DCS 1800), than between reproductive capacity and ELF electric field intensity $r \cong -0.79$, $P = 0.001$

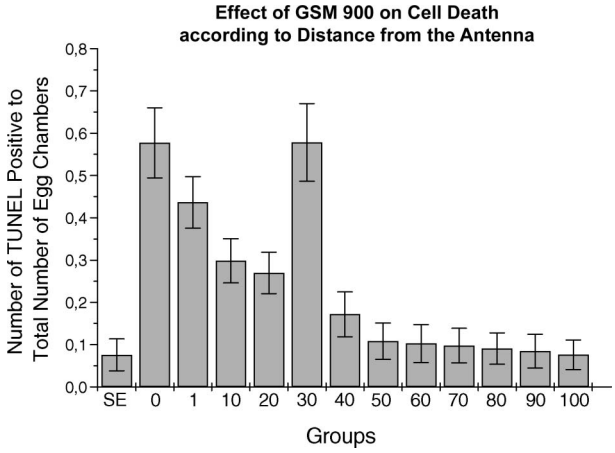


Figure 3. Mean ratio of ovarian cell death (number of TUNEL-positive to total number of egg-chambers, averaged over three identical experiments) ± SD, in relation to the distance from a GSM 900 MHz mobile phone antenna (cm). The increase in cell death induction is at a maximum at zero distance and at 30 cm distance from the antenna, corresponding to RF intensities 378 $\mu$W/cm$^2$ and 10 $\mu$W/cm$^2$ (see Tables I and III).

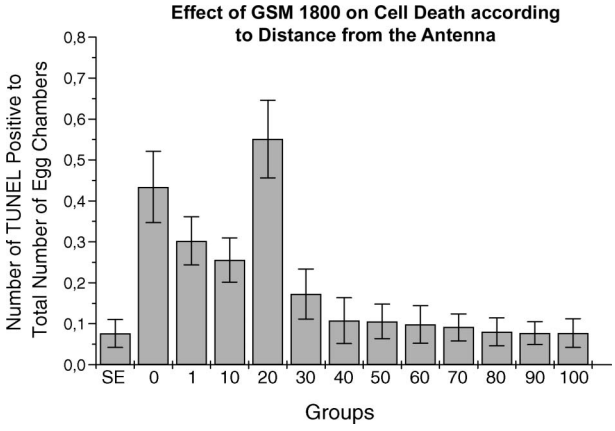


Figure 4. Mean ratio of ovarian cell death (number of TUNEL-positive to total number of egg-chambers, averaged over three identical experiments) ± SD, in relation to the distance from a GSM/DCS 1800 MHz mobile phone antenna (cm). The increase in cell death induction is at a maximum at zero distance and at 20 cm distance from the antenna, corresponding to RF intensities 252 $\mu$W/cm$^2$ and 11 $\mu$W/cm$^2$ (see Tables I and III).

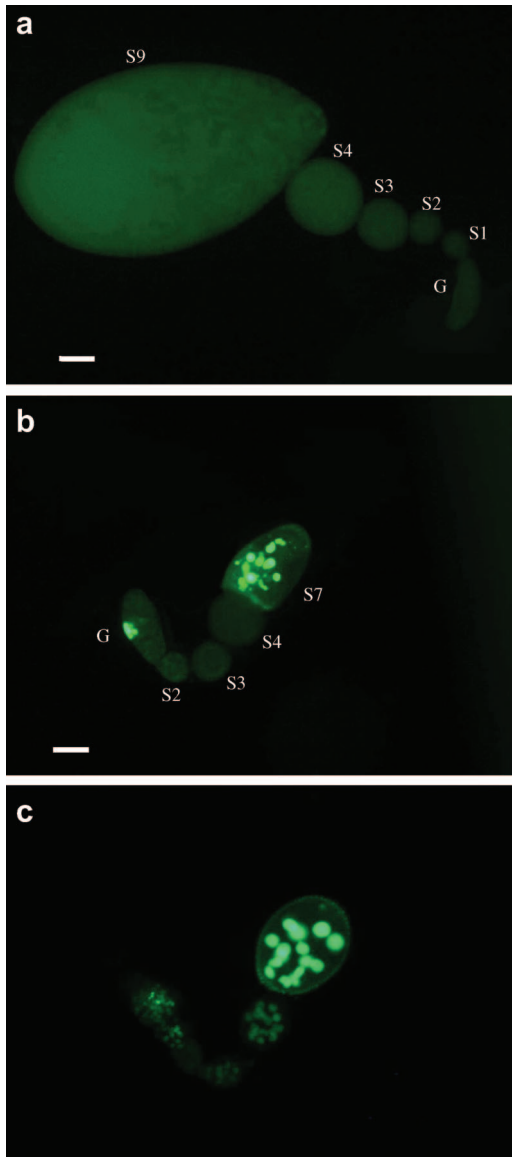


Figure 5. (a) Typical TUNEL-negative fluorescent picture of an ovariole of a sham-exposed female insect, containing egg chambers from germarium to stage 9. Bar: 10 $\mu$m. (b) Ovariole of an exposed insect (group GSM 900, 50 cm) with TUNEL-positive signal only at the two check points, germarium plus stage 7 egg chamber and TUNEL-negative intermediate stages. Bar: 10 $\mu$m. (c) Ovariole of exposed female insect (group GSM 1800, 20 cm) with fragmented DNA at all stages from germarium to stage 8 and in all kinds of egg chamber cells. NC, nurse cells; FC, follicle cells; OC, oocyte. Bar: 10 $\mu$m.

and $r \cong -0.78$, $P = 0.001$), both for GSM 900 and 1800 exposures. We note that the *P*-values (the probabilities that the corresponding *r*-values are due to random variation in the data points) in the case of Kendall's non-parametric correlation are smaller than the corresponding ones in Pearson's linear correlation, suggesting that non-parametric correlation analysis is perhaps more appropriate in the case of our (non-linear) results. The correlation analysis between reproductive capacity and distance from the antenna, gave the same values as between reproductive capacity and RF intensity and the correlation between reproductive capacity and ELF magnetic field was found to be even weaker than with ELF electric field.

## Discussion and conclusion

The effect of mobile telephony radiation on the reproductive capacity and the corresponding induced cell death in the ovaries of the exposed female insects, is very intense for distances up to 30 cm

from the mobile phone antenna, then diminishes considerably for distances longer than 40 – 50 cm from the mobile phone antenna where the ELF components fall within the background, but it is still evident for distances up to 100 cm (radiation intensities down to 1 $\mu$W/cm$^2$). This fact suggests that this kind of radiation is bioactive for intensities higher than 1 $\mu$W/cm$^2$.

The statistical analysis (single-factor Analysis of Variance) shows that the groups differ between them in reproductive capacity and cell death induction because of the GSM 900/1800 exposures at the different distances-intensities. The reason that the $P$ value is much smaller in the case of reproductive capacity ($P < 10^{-27}$) than in cell death induction ($P < 10^{-10}$), is only that the number of experiments for cell death induction was smaller.

The fact that for distances longer than 50 cm where the ELF components fall within the background, the bioactivity of the radiation although is still evident decreases considerably and falls within the standard deviation of the SE group, might suggest that the ELF components of digital mobile telephony signals, play a crucial role in their bioactivity, alone or in conjunction with the RF carrier wave. This is in agreement with the mechanism that we have proposed for the action of EMF on living organisms, according to which, lower frequency fields are more bioactive than higher frequency ones (Panagopoulos et al. 2000b, 2002; Panagopoulos and Margaritis 2003b). According to this mechanism, ELF electric fields of the order of $10^{-3}$ V/m, are able to disrupt cell function by irregular gating of electrosensitive ion channels on the cell membranes. As shown in Table I, the ELF components of both GSM 900 and 1800 fields appear to possess sufficient intensity for this, for distances up to 50 cm from the antenna of a mobile phone (or about 50 m from a corresponding base station antenna).

It is interesting that the decrease in the reproductive capacity was found to be maximum not only within the near field of the mobile phone antenna (0–5.2 cm from the antenna for GSM 900 and 0–2.6 cm for GSM 1800) (Panagopoulos and Margaritis 2010), where the intensity of the radiation is maximum, but also within the far field, at 20–30 cm distance from the mobile phone antenna. Thus, in the present experiments, we have discovered the existence of increased bioactivity 'windows' for both GSM 900 and 1800 radiations. These 'bioactivity windows' appear at distances 20 or 30 cm from the GSM 1800 or 900 mobile phone antenna respectively, where the radiation intensity is in both cases close to 10 $\mu$W/cm$^2$ and the ELF electric field intensity 0.6–0.7 V/m. At these distances, the bioeffect becomes even more intense than at zero distance from a mobile phone antenna where the RF intensity is higher than 250 $\mu$W/cm$^2$, and the ELF electric filed intensity higher than 13 V/m (Table I). Another series of experiments is now necessary, aiming to reveal the nature of these bioactivity 'windows', (i.e., whether they depend on the intensity of the radiation/fields, or on any other parameter like for example the wavelength of the radiation which happens to be close to the distance where the 'window' appears) (Panagopoulos and Margaritis 2010).

The distance of 20–30 cm from a mobile phone antenna where the bioactivity 'windows' are observed, corresponds to a distance of about 20–30 m from a base station antenna (Panagopoulos and Margaritis 2008). Since mobile telephony base station antennas are usually located within residential areas, at distances 20–30 m from such antennas there are often houses and workplaces where people are exposed for up to 24 h per day. Therefore, our present findings show that mobile telephony radiation can be very bioactive at intensity levels encountered at residential and working areas around base station antennas.

We do not know which constituent of the real mobile telephony signal, (i.e., the RF carrier, the ELF pulse repetition frequencies, or the combination of both), is more responsible for the bioactivity of the signal or for the existence of the 'windows' found in our experiments. Real mobile telephony signals are always RF carrier signals pulsed at ELF in order to be able to transmit information. Furthermore, real mobile telephony signals are never constant in intensity or frequency. Therefore, we consider that performing experiments with idealised continuous signals corresponding to the RF carrier alone or to the ELF constituents alone would not represent reality.

Non-parametric Correlation analysis showed a slightly more increased relationship with the RF intensity than with ELF electric field intensity, while Linear Correlation analysis gave an opposite result. A possible conclusion from the Correlation analysis is that both RF and ELF parameters of the mobile telephony radiations are responsible for the effects, but since non-parametric correlation analysis might be more appropriate because of the non-linearity of our data, perhaps RF is slightly more responsible than ELF. Although the correlation analysis between reproductive capacity and distance from the antenna gave the same values as between reproductive capacity and RF intensity, distance is only indirectly related to the phenomenon. The effect of the distance depends basically on the fact that the RF and ELF intensities change with the distance. Nevertheless, other possibilities like effect of the radiation wavelength, wave interference, or effect of the differences between near and far field zone of the antenna cannot be excluded and will be investigated

and discussed in a separate series of experiments together with the nature of the observed bioactivity 'windows' (Panagopoulos and Margaritis 2010).

Although windows of increased bioactivity of RF radiations have been recorded over many years (Bawin et al. 1975, 1978; Bawin and Adey 1976; Blackman et al. 1980, 1989; Goodman et al. 1995), there is still no widely accepted explanation for their existence.

We do not know whether the bioactivity 'windows' found in our present experiments are related exclusively with the certain organism we used as experimental animal, or they would appear for other organisms too. More experiments with different experimental animals exposed at different distances from a mobile phone antenna are necessary to answer this question. Since the effect of cell death induction was observed in all three different kinds of female reproductive cells (nurse cells, follicle cells and the oocyte) and since most cellular functions are identical in both insect and mammal cells, we consider that it is possible for the above 'windows' of increased bioactivity to exist for other organisms and humans as well. The bioactivity 'windows' found in our present experiments could possibly correlate with recent results of another experimental group reporting that GSM radiation caused increased permeability of the blood-brain barrier in rat nerve cells and the strongest effect was produced by the lowest SAR values which correspond to the weakest radiation intensity (Eberhardt et al. 2008).

Our present experiments verify our earlier results (Panagopoulos et al. 2007b) that the reduction in reproductive capacity caused by digital mobile telephony radiation is due to induced cell death in the gonads. Furthermore, our present results show that induced cell death is the reason for the reduction in reproductive capacity also at longer distances from the antenna (or at lower intensities) than in our earlier experiments.

Our results show that exposure of living organisms to mobile telephony radiation is highly bioactive and able to induce cell death at intensities higher than few $\mu$W/cm$^2$ and this bioactivity is still evident for intensities down to 1 $\mu$W/cm$^2$ (corresponding to distances up to 100 cm from a mobile phone, or up to about 100 m from a base station antenna). Effects were not observed at intensities lower than 1 $\mu$W/cm$^2$ in the specific biological system that we studied. Therefore, our present results might suggest that public exposure should be restricted at intensities below this value.

As in our earlier experiments (Panagopoulos et al. 2007b), although egg chambers during early and mid oogenesis in *Drosophila* were not reported before to exhibit either stress-induced by other stress factors than EMF, or physiological degeneration, at other stages except germarium and stages 7–8 (Drummond-Barbosa and Spradling 2001; Nezis et al. 2000, 2002; McCall 2004), mobile telephony radiation was found to induce cell death at all provitellogenic and vitellogenic stages 1–10 and the germarium. Additionally again cell death could be observed in all the cell types of the egg chamber, i.e., not only in nurse cells and follicle cells on which it was already known to be induced by other stress factors than EMF (Cavaliere et al. 1998; Foley and Cooley 1998; Drummond-Barbosa and Spradling 2001; Nezis et al. 2000, 2002; McCall 2004), but also in the oocyte (Figure 5c). A possible explanation for these phenomena as given by us before (Panagopoulos et al. 2007b) is based on the fact that the electromagnetic stress induced in the ovarian cells by the GSM 900 and 1800 fields is a new and probably more intense type of external stress, against which ovarian cells do not have adequate defence mechanisms like they do in the case of other kinds of external stresses like poor nutrition, heat or chemical stress.

The fact that electromagnetic stress induces DNA fragmentation in the oocyte (except of the nurse and follicle cells which anyway degenerate physiologically at stages 11–14) shows that the action of the electromagnetic stress is genotoxic and not just a shift of the physiological apoptotic stages in time as someone could possibly think as an alternative explanation. Besides, if it was just a shift of physiological apoptosis towards earlier stages, it would seem more likely for the organism to eliminate the defective egg chambers in the existing check points, germarium and stages 7–8, since this is the reason for the existence of the check points.

It is again important to emphasize that induced DNA fragmentation in the oocyte which undergoes meiosis during the last stages of oogenesis may result in heritable mutations upon DNA damage induction and repair, if not in cell death (Panagopoulos et al. 2007b).

Although we cannot simply extrapolate, we consider that similar effects on humans are possible for two reasons. First, insects are found to be more resistant than mammals, at least to ionising radiation (Abrahamson et al. 1973; Koval et al. 1977). Second, our results are in agreement with similar reported effects on mammals (although of course under different experimental conditions) (Lai and Singh 1995, 1996; Salford et al. 2003; Aitken et al. 2005). It is also possible that induced cell death on a number of brain cells can explain symptoms like headaches, fatigue, sleep disturbances etc., reported as 'microwave syndrome' (Navarro et al. 2003; Hutter et al. 2006).

In conclusion, we consider that our results imply the very cautious use of mobile phones at distances not shorter than 40 cm from the user's head and a reconsideration of the current exposure criteria in order to restrict public exposure from base station antennas to intensities not higher than 1 $\mu$W/cm$^2$. According to the present study, even some of the lowest national current corresponding exposure limits might not be safe enough, like for example, the Chinese limit for public exposure (40 $\mu$W/cm$^2$) or the corresponding limit of Russia, Italy and Poland (10 $\mu$W/cm$^2$) (International EMF Project). In contrast, the recent decision of Liechtenstein to reduce its national exposure limit from 9.5 $\mu$W/cm$^2$ (6 V/m) to 0.095 $\mu$W/cm$^2$ (0.6 V/m) (http://worldradio.ch/wrs/news/wrsnews/liechtenstein-to-vote-on-mobile-phone-masts.shtml?15942) seems to be in agreement with the results of the present study, moreover including a safety factor of more than 10 times a lower limit than 1 $\mu$W/cm$^2$.

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## Appendix
## Sham-exposure data

Reproductive capacity of sham-exposed at different distances from the antenna and control groups.

| SE/C groups: Distance from mobile phone antenna (cm) | Average mean number of $F_1$ pupae per maternal fly $\pm$ SD |
|---|---|
| SE (0) | 13.73 $\pm$ 0.91 |
| SE (1) | 13.43 $\pm$ 1.52 |
| SE (10) | 14.07 $\pm$ 0.57 |
| SE (20) | 13.53 $\pm$ 0.80 |
| SE (30) | 14.03 $\pm$ 1.43 |
| SE (40) | 13.4 $\pm$ 1.67 |
| SE (50) | 13.13 $\pm$ 1.25 |
| SE (60) | 13.7 $\pm$ 1.01 |
| SE (70) | 14.17 $\pm$ 1.06 |
| SE (80) | 13.33 $\pm$ 1.27 |
| SE (90) | 13.67 $\pm$ 1.33 |
| SE (100) | 14.1 $\pm$ 1.28 |
| C | 14.18 $\pm$ 1.12 |

Average reproductive capacity (mean number of $F_1$ pupae per maternal fly) from three separate experiments $\pm$ SD for SE groups at the 12 different exposure distances from the mobile phone antenna and C groups. Single factor Analysis of Variance test showed that the reproductive capacity did not differ significantly between the 12 SE groups ($P > 0.99$), meaning that the differences between the 12 SE groups have more than 99% probability to be due to random variations.

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# A Possible Effect of Electromagnetic Radiation from Mobile Phone Base Stations on the Number of Breeding House Sparrows (*Passer domesticus*)

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*A possible effect of long-term exposure to low-intensity electromagnetic radiation from mobile phone (GSM) base stations on the number of House Sparrows during the breeding season was studied in six residential districts in Belgium. We sampled 150 point locations within the 6 areas to examine small-scale geographic variation in the number of House Sparrow males and the strength of electromagnetic radiation from base stations. Spatial variation in the number of House Sparrow males was negatively and highly significantly related to the strength of electric fields from both the 900 and 1800 MHz downlink frequency bands and from the sum of these bands ($Chi^2$-tests and AIC-criteria, $P < 0.001$). This negative relationship was highly similar within each of the six study areas, despite differences among areas in both the number of birds and radiation levels. Thus, our data show that fewer House Sparrow males were seen at locations with relatively high electric field strength values of GSM base stations and therefore support the notion that long-term exposure to higher levels of radiation negatively affects the abundance or behavior of House Sparrows in the wild.*

**Keywords** Antenna; Bird; Electromagnetic radiation; GSM base station; Non thermal effect.

## Introduction

Mobile phones, also called cellular phones or handies, are now an integral part of modern life. The widespread use of mobile phones has been accompanied by the installation of an increasing number of base station antennas on masts and buildings. GSM base stations emit electromagnetic fields at high frequencies in the 900 and 1800 MHz range (=downlink frequency bands), pulse modulated in low frequencies (Hyland, 2000). In recent years, increased public awareness and scientific research have questioned to what extent the non thermal exposure to low-intensity electromagnetic fields may affect the health, reproduction, well-being, and behavior

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of humans and other organisms. There is an active and, as yet, unsettled controversy about current safety standards. Some researchers and national committees advised more stringent safety standards, based on experimental data with reported biological effects from (chronic) non thermal exposures (Belyaev, 2005a,b; Hyland, 2000).

There are studies showing frequency-specific biological effects and studies demonstrating that a high frequency signal modulated at certain low frequencies, or a signal that is pulsed, has more harmful effects than an unmodulated, steady carrier. These so-called "window effects" greatly complicate any attempt to understand the relationship between electromagnetic radiation and health (Adey, 1981; Belyaev, 2005a; Hyland, 2000; Lai, 2005).

Public and scientific concern were also raised by results of some epidemiologic studies that examined the effects of long-term exposure on humans living near mobile phone base stations. A growing number of studies point to the existence of effects, ranging from changes in cognitive performance and sleep disturbances to serious illness and disablement, with even higher cancer rates (Abdel-Rassoul et al., 2006; Bortkiewicz et al., 2004; Eger et al., 2004; Hutter et al., 2006; Navarro et al., 2003; Santini et al., 2002; Wolf and Wolf, 2004).

Short-term laboratory experiments used mice, rats, chickens, and other birds as study models to better understand the possible implications of electromagnetic fields on organismal functioning. In many studies, however, "mobile communication-like" signals were investigated that in fact were different from the real exposures in such aspects as intensity, carrier frequency, modulation, polarisation, duration, and intermittence (Belyaev, 2005a,b; Lai, 2005).

Studies of the effects of exposure to electromagnetic fields on populations of wild birds can provide further insights into the potential impacts on animal and human health (Fernie and Reynolds, 2005). Birds are candidates for being good biological indicators for low-intensity electromagnetic radiation: they have thin skulls, their feathers can act as dielectric receptors of microwave radiation, many species use magnetic navigation, they are very mobile, and possible psychosomatic effects are absent (Balmori, 2005; Bigu-del-Blanco and Romero-Sierra, 1975a,b). Field studies of wild populations can also reveal possible effects of long-term exposure to radiation from GSM base stations. In addition, species like the House Sparrow (*Passer domesticus*) are especially of interest because a large proportion of the birds use higher breeding height locations like roof spaces (Wotton et al., 2002) where potentially higher levels of base station radiation are present.

Here we report results of a preliminary study that explored putative effects of electromagnetic radiation emitted by mobile phone base stations on the number of House Sparrows during the breeding season. Specifically, we examined small-scale geographic variation within each of six study areas in both the number of birds and the strength of electromagnetic radiation. If electromagnetic fields from GSM base stations have adverse effects on bird populations, this should result in a decreasing number of House Sparrows with increasing levels of radiation.

## Materials and Methods

### *Data Collection*

We determined, during the spring of 2006, the number of House Sparrow males and the strength of electromagnetic radiation from mobile phone (GSM) base

stations at 150 locations that were distributed over 6 residential areas in the region of Gent–Sint-Niklaas (province of East Flanders, Belgium). The study areas were similar in overall appearance, with abundant hedges, bushes, and other vegetation between the houses, and with one or more GSM base stations nearby.

The 150 study locations were selected in advance as points on a map (ArcGIS). All locations were situated along small roads within the residential areas and were at variable distances from the nearest GSM base station (mean = 352 m, range = 91−903 m, about 90% at 100−600 m). The number of locations, and study dates, within each area were: Lokeren–Eksaarde ($N = 19$; April 9), Lokeren–Spoele ($N = 27$, April 15), Lokeren–Bergendries ($N = 17$, April 17), Sint Niklaas–Clementwijk ($N = 25$, April 20), Gent–Wondelgem ($N = 38$, April 25), and Gent–Mariakerke ($N = 24$, April 26).

At each location, a point count of five minutes (see "point transect count" in Bibby et al., 2000; Hustings et al., 1985) was made of the number House Sparrow males that were singing or otherwise visible within a distance of ca. 30 m. Sightings of birds were done with binoculars (Swarovski EL 10 × 42). Counts were restricted to the morning hours (7–11 h), when male House Sparrows are most active (Hustings et al., 1985; Van Dijk, 2004), on days with favorable weather conditions (no rain, little wind, sunny, normal temperatures).

Simultaneously, we measured the maximum value (peak hold) of the electric field strength (in V/m) from the downlink frequencies of GSM 900 MHz (925–960 MHz) and GSM 1800 MHz (1805–1880 MHz) base station antennas. Measurements at each location were made during two minutes for each frequency band. The electric field strength was measured using a portable calibrated high-frequency spectrum analyzer (Aaronia Spectran HF-6080; typ. accuracy ± 3 dB) with calibrated EMC directional antenna (HyperLOG 6080; logarithmic-periodic). To measure the maximum radiation values, the EMC antenna was turned around in all directions.

Additional antennas for the new UMTS-system are now being installed on several existing base stations in Belgium. Therefore, at several locations within each study area, the electric field strength from the downlink frequencies of UMTS antennas (2110–2170 MHz) was also checked, but no significant signals were found. Consequently, the UMTS variable was not taken into account for further analysis.

### *Data Analyses*

The sum ($E_{gsm}$) of the measured GSM 900 MHz ($E_{gsm900}$) and 1800 MHz ($E_{gsm1800}$) electric field strength values was calculated using the formula: $E_{gsm} = \sqrt{E^2_{gsm900} + E^2_{gsm1800}}$ (Electronic Communications Committee, 2003). Prior to all analyses, the electric field strength variables were logarithmically transformed to achieve normality of their frequency distributions.

We explored relations between the number of House Sparrow males (dependent variable) and each of the three electric field strength variables. As the dependent variable consists of count data and is hence discontinuous, standard regression (or correlation) techniques are inappropriate. Instead, we used Poisson regressions (i.e., generalized linear models) with a log link function to examine putative relationships. Preliminary analyses indicated that significant variation among the six study areas was present for all variables (ANOVA, $P < 0.001$). Therefore we included "area" as a categorical factor in all models and considered it to be a proxy for all unknown,

and hence unmeasured, variables causing among area variation in the number of House Sparrows (e.g., habitat characteristics, food availability, temporal differences among censuses). Statistical analyses were done with S-PLUS v. 6.2.

## Results

The number of House Sparrow males varied between zero and four at the different locations (Table 1). The measured electric field strengths were seldom higher than 1 V/m, and most often well below that value (Table 1).

To explore the putative effects of area, electric field strength, and their interaction on the number of House Sparrows, we performed separate analyses for each of the three radiation variables. As no significant interaction effect between area and electric field strength was detected in any of the three analyses ($Chi^2$-tests and AIC-criteria, $P > 0.20$), we excluded the interaction term from further treatments. The final regression models were highly similar for the three electric strength variables. They revealed significant variation among study areas ($Chi^2$-tests, $P < 0.001$), and a highly significant negative effect of electric field strength on the number of House Sparrow males ($Chi^2$-tests and AIC-criteria, $P < 0.001$; Fig. 1). Estimates of the scaled deviance (1.06–1.14) were very close to 1, and examination of the regression residuals revealed no clear patterns or deviations from normality. These observations indicate an adequate fit of the models to the data.

**Table 1**

Summary statistics (mean, 95% confidence interval, range) of the number of House Sparrow males and electric field strength variables in the six study areas. Means and confidence limits of the radiation variables were calculated after back-transformation of the logarithmically transformed data; the confidence intervals are therefore asymmetrical around the mean

| Study area | | Number of House Sparrow males | $E_{gsm900}$ (V/m) | $E_{gsm1800}$ (V/m) | $E_{gsm}$ (V/m) |
|---|---|---|---|---|---|
| 1: Lokeren–Eksaarde | mean | 1.5 | 0.153 | 0.075 | 0.193 |
| | 95% CI | 0.8–2.2 | 0.108–0.216 | 0.046–0.123 | 0.139–0.270 |
| | Min–Max | 0–4 | 0.036–0.494 | 0.015–0.333 | 0.052–0.505 |
| 2: Lokeren–Spoele | mean | 1.9 | 0.084 | 0.083 | 0.130 |
| | 95% CI | 1.5–2.3 | 0.059–0.120 | 0.058–0.120 | 0.091–0.183 |
| | Min–Max | 0–4 | 0.008–0.327 | 0.013–0.394 | 0.016–0.412 |
| 3: Lokeren–Bergendries | mean | 0.8 | 0.245 | 0.017 | 0.247 |
| | 95% CI | 0.3–1.3 | 0.186–0.323 | 0.009–0.031 | 0.187–0.327 |
| | Min–Max | 0–3 | 0.052–0.537 | 0.004–0.125 | 0.052–0.551 |
| 4: Sint Niklaas–Clementwijk | mean | 1.0 | 0.130 | 0.056 | 0.148 |
| | 95% CI | 0.6–1.4 | 0.098–0.173 | 0.039–0.082 | 0.111–0.197 |
| | Min–Max | 0–3 | 0.019–0.412 | 0.009–0.231 | 0.021–0.469 |
| 5: Gent–Wondelgem | mean | 1.3 | 0.109 | 0.040 | 0.121 |
| | 95% CI | 0.9–1.6 | 0.079–0.151 | 0.030–0.054 | 0.089–0.165 |
| | Min–Max | 0–4 | 0.016–1.006 | 0.009–0.321 | 0.022–1.056 |
| 6: Gent–Mariakerke | mean | 0.8 | 0.043 | 0.080 | 0.160 |
| | 95% CI | 0.3–1.2 | 0.024–0.078 | 0.049–0.130 | 0.107–0.240 |
| | Min–Max | 0–4 | 0.006–1.022 | 0.017–0.824 | 0.040–1.023 |

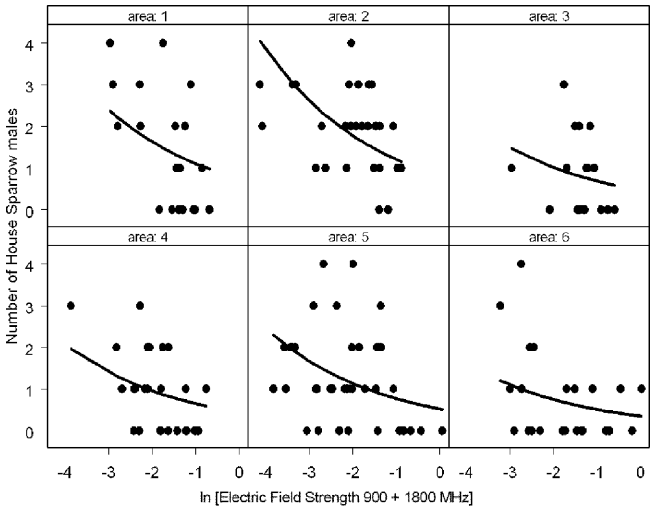


**Figure 1.** Scatterplots of the observed number of House Sparrow males as a function of the sum (Egsm) of GSM 900 MHz and GSM 1800 MHz electric field strength values (logarithmic scale) at the different locations within each of the six study areas. Regression lines were obtained by Poisson regressions and incorporated the effects of area and radiation intensity (see text).

We further explored the separate effects of electromagnetic radiation at the two frequencies by modeling the number of House Sparrow males as a function of area, electric field strength at 900 MHz, electric field strength at 1800 MHz, and their interactions. The final model retained included highly significant effects of area and the two electric field strengths ($Chi^2$-tests and AIC-criteria, $P < 0.001$) and a marginally significant interaction effect between both field strengths ($Chi^2$-test, $P = 0.02$). This strongly suggests that the electromagnetic radiations at both frequencies have complex additive effects on the number of House Sparrow males.

Overall, analyses indicated that the strength of all three radiation variables decreased with increasing distance to the nearest base station (F-tests, $P < 0.001$). We therefore examined whether the negative relation between the number of birds and strength of radiation was induced by variation among sampling locations in the distance to GSM base stations. Upon adding distance to the nearest base station as an additional factor to the regression models that included area and electric field strength, distance did not account for a significant portion of the residual variation ($Chi^2$-tests and AIC-criteria, $P > 0.50$). Conversely, when we forced distance as the first factor into the regression equations, both area and radiation strength were subsequently selected as highly significant factors ($Chi^2$-tests and AIC-criteria, $P < 0.001$).

## Discussion

Our results indicate that spatial variation among sampling locations in the number of House Sparrow males was negatively related to the strength of electric fields emitted by GSM base stations. Importantly, this relation was highly similar among

the six study areas, as evidenced by the non significant interaction effects between area and electric field strength, despite differences among areas in both the number of birds and radiation levels. Moreover, the negative association was detected for electric field strengths from both the 900 and 1800 MHz frequency bands and from the sum of these frequency bands. Our analyses also revealed that the negative relation between the number of birds and strength of radiation was not a simple consequence of differences among sampling locations in distances to the nearest GSM base station. This can probably be attributed to variations in the orientation, position, and number of antennas and to the shielding effects and multiple reflections from structures like buildings and trees, which affect local levels of exposure to electromagnetic radiation. Thus, our data show that fewer House Sparrow males were seen at locations with relatively high electric field strength values of GSM base stations and therefore support the notion that long-term exposure to higher levels of radiation negatively affects the abundance or behaviour of House Sparrows in the wild.

Nevertheless, our study should be considered as preliminary for several reasons. First, sampling locations were each visited only once, such that counts of the number of House Sparrow males and measurements of electric field strength are subject to some variation and estimation error. However, it is most likely that these errors are randomly distributed among locations. We also note that a single visit during the peak of the breeding season (April–May) is considered to be adequate to locate House Sparrow breeding territories (Hustings et al., 1985; Van Dijk, 2004). Second, because of the short study period, we ignore whether differences in bird counts reflect variation in abundance of breeding birds or in short-term behavioural responses like the tendency to sing. However, a decrease in singing intensity will result in a decrease of reproductive success and ultimately a decline of population size. Third, only the radiation from GSM base station antennas was measured. Probably, the distribution of possible other significant electromagnetic signals will be random, but due to the lack of measurements in other frequency bands (except for UMTS), this remains an object of further study. Fourth, as with all descriptive field studies, we cannot provide evidence for a causal relationship between radiation levels and the number of birds. Nevertheless, the fact that we found a highly similar pattern in each of the six study areas strengthens the possibility that the relationship is not a spurious one.

There are several unpublished and anecdotal reports about birds and mobile phone base stations, but we know of only one other published study that examined the effects of electromagnetic radiation from mobile phone base stations on wild bird populations. Balmori (2005) found a significantly lower number of White Stork (*Ciconia ciconia*) fledglings in nests exposed to relatively high electromagnetic radiation ($2.36 \pm 0.82$ V/m) than in nests receiving lower levels of radiation ($0.53 \pm 0.82$ V/m). Together with observations on aberrant behaviours of the adult birds, these results suggest that electromagnetic radiation interferes with reproduction in this wild population.

What could be the underlying mechanisms of the (putative) negative effects of radiation from GSM base stations on wild bird populations? Because all measured electric field strength values were far below what is required to produce heating as low as 0.5°C (i.e., 10 mW/cm$^2$ or ca. 194 V/m; Bernhardt, 1992), the effects should be considered as non thermal at very low intensities.

Non thermal effects of microwaves on birds were reported already 40 years ago (Tanner, 1966; Tanner et al., 1967). Most studies indicate that exposure of birds to

electromagnetic fields generally changes, but not always consistently in effect or in direction, their behaviour, reproductive success, growth, development, physiology, endocrinology, and oxidative stress (Fernie and Reynolds, 2005; Grigor'ev et al., 2003; Wasserman et al., 1984). Of special relevance within the context of our research are laboratory studies that demonstrate negative effects of electromagnetic radiation from mobile phones on the development and survival of bird embryo's (Farrel et al., 1997; Grigoriew, 2003; Youbicier-Simo and Bastide, 1999).

Bird feathers are known to act as dielectric receptors of high frequency electromagnetic fields and some experiments indicate that audiofrequency pulse-modulated high frequency fields may induce piezoelectric effects in the feathers (Bigu-del-Blanco and Romero-Sierra, 1975a,b). These results are important in view of the fundamental role that feathers play in the life of birds and in the influence of environmental factors on bird behavior. Experiments also indicated that microwave radiation can have the same averse effects on birds in flight as those observed in caged birds (Romero-Sierra et al., 1969).

Several bird species also use magnetic navigation (Liboff and Jenrow, 2000; Muheim et al., 2006) and can become disorientated when exposed to weak ($< 1/50$ of geomagnetic field strength) high frequency magnetic fields (Ritz et al., 2004; Thalau et al., 2005). The available evidence concerning magnetoreception suggests that birds use a radical pair mechanism for a chemical compass, and a mechanism based on magnetite particles (Mouritsen and Ritz, 2005; Wiltschko and Wiltschko, 2005). Magnetite is an excellent absorber of microwave radiation at frequencies between 0.5 and 10.0 GHz through the process of ferromagnetic resonance (Kirschvink, 1996), so that interaction with electromagnetic fields from mobile phone base stations might be possible.

In an experiment with Zebra Finches (*Taenopygia guttata*) that were temporary (10 min) stimulated with a pulsed electromagnetic field similar to the signal produced by mobile phones with carrier frequency 900 MHz, significant non thermal changes in the amount of neural activity by more than half of the brain cells were detected (Beasond and Semm, 2002). The effect did not appear tot be limited to magnetic sensory cells, but occurred in any part of the brain. The authors postulate that similar neural responses to different frequencies point toward a common mechanism of low frequency modulation, perhaps at the cell membrane. Such a stimulus might mimic a natural mechanism involved in cell communication. Although the peak electric field strength used in that experiment ($0.1\,\mathrm{mW/cm^2}$ = approx. $19\,\mathrm{V/m}$; Beasond and Semm, 2002) was higher than the values measured in our study, results from other studies indicate that a long-term exposure at low intensities can produce the same effects as a short-term exposure at higher intensity (Belyaev, 2005a; D'Andrea et al., 1986a,b; Lai, 2005). This suggests that the non thermal effects of relatively weak electromagnetic radiation from mobile phone base stations can accumulate over time and have significant implications, as detected by several pilot epidemiological studies on humans (see Introduction).

Radiation from GSM base stations may also affect the local abundance of insects or other invertebrates and thereby indirectly influence the number of House Sparrows. Although adult House Sparrows are mainly seed-eaters, they need insects and other invertebrates to feed their young, such that it is likely that they will prefer areas with high abundance of invertebrates at the beginning of the breeding period. Several researchers have postulated that the lack of invertebrates might be an important factor in the reported decline of House Sparrow populations in

urban areas (Summers-Smith, 2003; Wotton et al., 2002). Short-term exposure of pulsed mobile phone radiation with carrier frequency 900 MHz resulted in a 50–60% decrease of the reproductive capacity of insects (Panagopoulos et al., 2004). Similar results were also found with microwave radiation at other frequencies (Atli and Unlu, 2006; Bol'shakov et al., 2001).

The results of our study suggest that long-term exposure to low-intensity (pulsed) electromagnetic radiation from GSM base stations may have significant effects on populations of wild birds. The exact mechanisms of these effects are as yet poorly understood. Given the potential importance that such effects may have on aspects of biodiversity and human health, more detailed studies in both the laboratory and the field are urgently needed to corroborate our results and to uncover the underpinning mechanistic relationships.

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# Electromagnetic pollution from phone masts. Effects on wildlife

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**Abstract**

A review on the impact of radiofrequency radiation from wireless telecommunications on wildlife is presented. Electromagnetic radiation is a form of environmental pollution which may hurt wildlife. Phone masts located in their living areas are irradiating continuously some species that could suffer long-term effects, like reduction of their natural defenses, deterioration of their health, problems in reproduction and reduction of their useful territory through habitat deterioration. Electromagnetic radiation can exert an aversive behavioral response in rats, bats and birds such as sparrows. Therefore microwave and radiofrequency pollution constitutes a potential cause for the decline of animal populations and deterioration of health of plants living near phone masts. To measure these effects urgent specific studies are necessary.


*Keywords:* Effects on wildlife; Effects on birds; Electromagnetic radiation; Mammals; Microwaves; Mobile telecommunications; Non-thermal effects; Phone masts; Radiofrequencies

## 1. Introduction

Life has evolved under the influence of two omnipresent forces: gravity and electromagnetism. It should be expected that both play important roles in the functional activities of organisms [1]. Before the 1990's radiofrequencies were mainly from a few radio and television transmitters, located in remote areas and/or very high places. Since the introduction of wireless telecommunication in the 1990's the rollout of phone networks has caused a massive increase in electromagnetic pollution in cities and the countryside [2,3].

Multiple sources of mobile communication result in chronic exposure of a significant part of the wildlife (and man) to microwaves at non-thermal levels [4]. In recent years, wildlife has been chronically exposed to microwaves and RFR (Radiofrequency radiation) signals from various sources, including GSM and UMTS/3G wireless phones and base stations, WLAN (Wireless Local Area Networks), WPAN (Wireless Personal Area Networks such as Bluetooth), and DECT (Digital Enhanced (former European) Cordless Telecommunications) that are erected indiscriminately without studies of environmental impact measuring long-term effects. These exposures are characterized by low intensities, varieties of signals, and long-term durations. The greater portion of this exposure is from mobile telecommunications (geometric mean in Vienna: 73% [5]). In Germany the GSM cellular phone tower radiation is the dominating high frequency source in residential areas [6]. Also GSM is the dominating high frequency source in the wilderness of Spain (personal observation).

Numerous experimental data have provided strong evidence of athermal microwave effects and have also indicated several regularities in these effects: dependence of frequency within specific frequency windows of "resonance-type"; dependence on modulation and polarization; dependence on intensity within specific intensity windows, including super-low power density comparable with intensities from base stations/masts [4,7–9]. Some studies have demonstrated different microwave effects depending on wavelength in the range of mm, cm or m [10,11]. Duration of exposure may be as important as power density. Biological effects resulting from electromagnetic field radiation might depend on dose, which indicates long-term accumulative effects [3,9,12]. Modulated and pulsed radiofrequencies seem to be more effective in producing effects [4,9]. Pulsed waves (in blasts), as well as certain low frequency modulations exert greater

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biological activity [11,13–15]. This observation is important because cell phone radiation is pulsed microwave radiation modulated at low frequencies [8,9].

Most of the attention on possible biological effects of electromagnetic radiation from phone masts has been focused on human health [5,16–21]. The effects of electromagnetic pollution on wildlife, have scarcely been studied [22–25].

The objective of this review is to detail advances in knowledge of radiofrequencies and microwave effects on wildlife. Future research may help provide a better understanding of electromagnetic field (EMF) effects on wildlife and plants and their conservation.

## 2. Effects on exposed wildlife

### 2.1. Effects on birds

#### 2.1.1. Effects of phone mast microwaves on white stork

In monitoring a white stork (*Ciconia ciconia*) population in Valladolid (Spain) in vicinity of Cellular Phone Base Stations, the total productivity in nests located within 200 m of antennae, was $0.86 \pm 0.16$. For those located further than 300 m, the result was practically doubled, with an average of $1.6 \pm 0.14$. Very significant differences among total productivity were found ($U = 240$; $P = 0.001$, Mann–Whitney test). Twelve nests (40%) located within 200 m of antennae never had chicks, while only one (3.3%) located further than 300 m had no chicks. The electric field intensity was higher on nests within 200 m ($2.36 \pm 0.82$ V/m) than nests further than 300 m ($0.53 \pm 0.82$ V/m). In nesting sites located within 100 m of one or several cellsite antennae with the main beam of radiation impacting directly (Electric field intensity >2 V/m) many young died from unknown causes. Couples frequently fought over nest construction sticks and failed to advance the construction of the nests. Some nests were never completed and the storks remained passively in front of cellsite antennae. These results indicate the possibility that microwaves are interfering with the reproduction of white stork [23]. (Fig. 1)

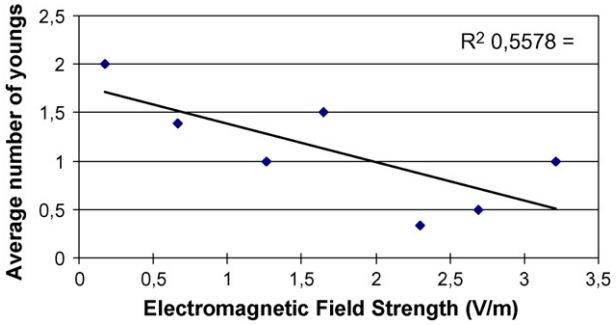


Fig. 1. Average number of youngs and electric field intensity (V/m) in 60 nests of white storks (*Ciconia ciconia*) (Hallberg, Ö with data of Balmori, 2005 [23]).

#### 2.1.2. Effects of phone mast microwaves on house sparrows

A possible effect of long-term exposure to low-intensity electromagnetic radiation from mobile phone (GSM) base stations on the number of house sparrows during the breeding season was studied in Belgium. The study was carried out sampling 150 point locations within six areas to examine small-scale geographic variation in the number of house sparrow males and the strength of electromagnetic radiation from base stations. Spatial variation in the number of house sparrow males was negative and highly significantly related to the strength of electric fields from both the 900 and 1800 MHz downlink frequency bands and from the sum of these bands (Chi-square-tests and AIC-criteria, $P < 0.001$). This negative relationship was highly similar within each of the six study areas, despite differences among areas in both the number of birds and radiation levels. Fewer house sparrow males were seen at locations with relatively high electric field strength values of GSM base stations and therefore support the notion that long-term exposure to higher levels of radiation negatively affects the abundance or behavior of house sparrows in the wild [24].

In another study with point transect sampling performed at 30 points visited 40 times in Valladolid (Spain) between 2002 and 2006, counting the sparrows and measuring the mean electric field strength (radiofrequencies and microwaves: 1 MHz to 3 GHz range). Significant declines ($P = 0.0037$) were observed in mean bird density over time, and significantly low bird density was observed in areas with high electric field strength. The logarithmic regression of the mean bird density vs. field strength groups (considering field strength in 0.1 V/m increments) was $R = -0.87$; $P = 0.0001$ According to this calculation, no sparrows would be expected to be found in an area with field strength >4 V/m [25]. (Fig. 2)

In the United Kingdom a decline of several species of urban birds, especially sparrows, has recently happened [26]. The sparrow population in England has decreased in the last 30 years from 24 million to less than 14. The more abrupt decline, with 75% descent has taken place from 1994 to 2002. In 2002, the house sparrow was added to the Red List of U.K. endangered species [27]. This coincides with the rollout of mobile telephony and the

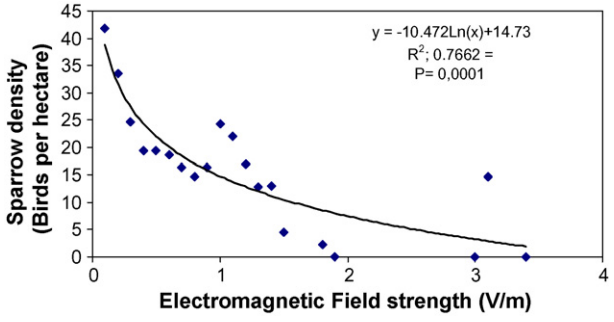


Fig. 2. Mean sparrow density as a function of electric field strength grouped in 0.1 V/m. (Balmori and Hallberg, 2007 [25]).

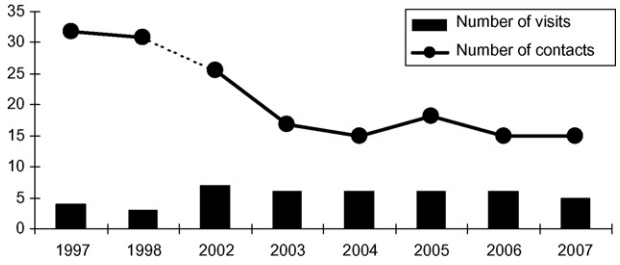


Fig. 3. Annual number of contacts (Mean) for 14 species studied in "Campo Grande" urban park (lack the information of the years 1999–2001).

possible relationship of both circumstances should be investigated.

In Brussels, many sparrows have disappeared recently [28]; similar declines have been reported in Dublin [29]. Van der Poel (cited in Ref. [27]) suggested that sparrows might be declining in Dutch urban centres also.

### 2.1.3. *Effects on the bird community at an urban park*

Microwaves may be affecting bird populations in places with high electromagnetic pollution. Since several antennas were installed in proximities of "Campo Grande" urban park (Valladolid, Spain) the bird population has decreased and a reduction of the species and breeding couples has occurred. Between 1997 and 2007, of 14 species, 3 species have disappeared, 4 are in decline and 7 stay stable (Balmori, unpublished data) (Fig. 3). In this time the air pollution (SO2, NO2, CO and Benzene) has diminished.

During the research some areas called "silence areas" contaminated with high microwave radiation (>2 V/m), where previously different couples usually bred and later disappeared, have been found. Several anomalies in magpies (*Pica pica*) were detected: plumage deterioration, locomotive problems (limps and deformations in the paws), partial albinism and melanism, especially in flanks [30]. Recently cities have increased cases of partial albinism and melanism in birds (*Passer domesticus*, *Turdus merula* and *P. pica*) (personal observation).

### 2.1.4. *Possible physiological mechanisms of the effects found in birds*

Current scientific evidence indicates that prolonged exposure to EMFs, at levels that can be encountered in the environment, may affect immune system function by affecting biological processes [3,31,32]. A stressed immune system may increase the susceptibility of a bird to infectious diseases, bacteria, viruses, and parasites [33].

The plumage of the birds exposed to microwaves looked, in general, discolorated and lack of shine. This not only occurred in ornamental birds; such as peacocks, but also in wild birds; such as, tits, great tits, house sparrows, etc (personal observation). We must mention that plumage deterioration is the first sign of weakening or illnesses in birds since damaged feathers are a sure sign of stress.

Physiological conditions during exposure minimize microwave effects. Radical scavengers/antioxidants might be involved in effects of microwaves [4].

Microwaves used in cellphones produce an athermal response in several types of neurons of the birds nervous system [34]. Several studies addressed behavior and teratology in young birds exposed to electromagnetic fields [23,25,35–37]. Most studies indicate that electromagnetic field exposure of birds generally changes, but not always consistently in effect or in direction, their behavior, reproductive success, growth and development, physiology and endocrinology, and oxidative stress [37]. These results can be explained by electromagnetic fields affecting the birds' response to the photoperiod as indicated by altered melatonin levels [38].

Prolonged mobile phone exposure may have negative effects on sperm motility characteristics and male fertility as has been demonstrated in many studies made in man and rats [39–46]. EMF and microwaves can affect reproductive success in birds [23,25,35,36,47]. EMF exposure affected reproductive success of kestrels (*Falco sparverius*), increasing fertility, egg size, embryonic development and fledging success but reducing hatching success [35,36].

The radiofrequency and microwaves from mobile telephony can cause genotoxic effects [48–55]. Increases in cytological abnormalities imply long-term detrimental effects since chromosomal damage is a mechanism relevant to causation of birth defects and cancer [55].

Long-term continuous, or daily repeated EMF exposure can induce cellular stress responses at non-thermal power levels that lead to an accumulation of DNA errors and to inhibition of cell apoptosis and cause increased permeability of blood–brain barrier due to stabilization of endothelial cell stress fibers. Repeated occurrence of these events over a long period of time (years) could become a health hazard due to a possible accumulation of brain tissue damage. These findings have important implications with regards to potential dangers from prolonged and repeated exposure to non-ionizing radiation [56,57].

Pulsed magnetic fields can have a significant influence on the development and incidence of abnormalities in chicken embryos. In five of six laboratories, exposed embryos exhibited more structural anomalies than controls. If the data from all six laboratories are pooled, the difference for the incidence of abnormalities in exposed embryos and controls is highly significant [58]. Malformations in the nervous system and heart, and delayed embryo growth are observed. The embryo is most sensitive to exposure in the first 24 h of incubation [58]. An increase in the mortality [59] and appearance of morphological abnormalities, especially of the neural tube [13,60,61] has been recorded in chicken embryos exposed to pulsed magnetic fields, with different susceptibility among individuals probably for genetic reasons. A statistically significant high mortality rate of chicken embryos subjected to radiation from a cellphone, compared to the control group exists [62,63]. In another study eggs exposed to a magnetic

field intensity of 0.07 T showed embryonic mortality during their incubation was higher. The negative effect of the magnetic field was manifested also by a lower weight of the hatched chicken [64]. Bioelectric fields have long been suspected to play a causal role in embryonic development. Alteration of the electrical field may disrupt the chemical gradient and signals received by embryo cells. It appears that in some manner, cells sense their position in an electrical field and respond appropriately. The disruption of this field alters their response. Endogenous current patterns are often correlated with specific morphogenetic events [65].

Available data suggests dependencies of genotype, gender, physiological and individual factors on athermal microwave effects [4,9]. Genomic differences can influence cellular responses to GSM Microwaves. Data analysis has highlighted a wide inter-individual variability in response, which was replicated in further experiments [4]. It is possible that each species and each individual, show different susceptibility to radiation, since vulnerability depends on genetic tendency, and physiologic and neurological state of the irradiated organism [15,35–37,61,66–68]. Different susceptibility of each species has also been proven in wild birds exposed to electromagnetic fields from high-voltage power lines [47].

## 2.2. Effects on mammals

### 2.2.1. Alarm and aversion behavior

Rats spent more time in the halves of shuttle boxes that were shielded from 1.2 GHz. Microwaves irradiation. The average power density was about 0.6 mW/cm$^2$. Data revealed that rats avoided the pulsed energy, but not the continuous energy, and less than 0.4 mW/cm$^2$ average power density was needed to produce aversion [69]. Navakatikian & Tomashevskaya [70] described a complex series of experiments in which they observed disruption of rat behavior (active avoidance) from radiofrequency radiation. Behavioral disruption was observed at a power density as low as 0.1 mW/cm$^2$ (0.027 W/kg). Mice in an experimental group exposed to microwave radiation expressed visible individual panic reaction, disorientation and a greater degree of anxiety. In the sham exposed group these deviations of behavior were not seen and all animals show collective defense reaction [71]. Microwave radiation at 1.5 GHz pulsing 16 ms. At 0.3 mW/cm$^2$ power density, in sessions of 30 min/day over one month produced anxiety and alarm in rabbits [72].

Electromagnetic radiation can exert an aversive behavioral response in bats. Bat activity is significantly reduced in habitats exposed to an electromagnetic field strength greater than 2 V/m [73]. During a study in a free-tailed bat colony (*Tadarida teniotis*) the number of bats decreased when several phone masts were placed 80 m from the colony [74].

### 2.2.2. Deterioration of health

Animals exposed to electromagnetic fields can suffer a deterioration of health and changes in behavior [75,76].

There was proof of frequent death in domestic animals; such as, hamsters and guinea pigs, living near mobile telecommunication base stations (personal observation).

The mice in an experimental group exposed to microwave radiation showed less weight gain compared to control, after two months. The amount of food used was similar in both groups [71]. A link between electromagnetic field exposure and higher levels of oxidative stress appears to be a major contributor to aging, neurodegenerative diseases, immune system disorders, and cancer in mammals [33].

The effects from GSM base transceiver station (BTS) frequency of 945 MHz on oxidative stress in rats were investigated. When EMF at a power density of 3.67 W/m$^2$, below current exposure limits, were applied, MDA (malondialdehyde) level was found to increase and GSH (reduced glutathione) concentration was found to decrease significantly ($P < 0.0001$). Additionally, there was a less significant ($P = 0.0190$) increase in SOD (superoxide dismutase) activity under EM exposure [77].

### 2.2.3. Problems in reproduction

In the town of Casavieja (Ávila, Spain) a telephony antenna was installed that had been in operation for about 5 years. Then some farmers began blaming the antenna for miscarriages in many pigs, 50–100 m from the antenna (on the outskirts of the town). Finally the topic became so bad that the town council decided to disassemble the antenna. It was removed in the spring 2005. From this moment onwards the problems stopped (C. Lumbreras personal communication).

A Greek study reports a progressive drop in the number of rodent births exposed to radiofrequencies. The mice exposed to 0.168 μW/cm$^2$ become sterile after five generations, while those exposed to 1.053 μW/cm$^2$ became sterile after only three generations [22].

In pregnant rats exposed to 27.12 MHz continuous waves at 100 μW/cm$^2$ during different periods of pregnancy, half the pregnancies miscarried before the twentieth day of gestation, compared to only a 6% miscarriage rate in unexposed controls, and 38% of the viable foetuses had incomplete cranial ossification, compared to less than 6% of the controls. Findings included a considerable increase in the percentage of total reabsorptions (post-implantation losses consequent to RF radiation exposure in the first post-implantation stage). Reduced body weight in the exposed dams reflected a negative influence on their health. It seems that the irradiation time plays an important role in inducing specific effects consequent to radiofrequency radiation exposure [78]. There was also a change in the sex ratio, with more males born to rats that had been irradiated from the time of conception [2]. Moorhouse and Macdonald [79] find a substantial decline in female Water Vole numbers in the radio-collared population, apparently resulting from a male skew in the sex ratios of offspring born to this population. Recruits to the *radio-tracked* population were skewed heavily in favour of males (43:13). This suggests that radio-collaring of females caused male-skewed sex ratios.

Mobile phone exposure may have negative effects on sperm motility characteristics and male fertility in rats [46]. Other studies find a decrease of fertility, increase of deaths after birth and dystrophic changes in their reproductive organs [11]. Intermittent exposure showed a stronger effect than continuous exposure [4]. Brief, intermittent exposure to low-frequency EM fields during the critical prenatal period for neurobehavioral sex differentiation can demasculinize male scent marking behavior and increase accessory sex organ weights in adulthood [80].

In humans, magnetic field exposures above 2.0 mG were positively associated with miscarriage risk [81]. Exposure of pregnant women to mobile phone significantly increased foetal and neonatal heart rate, and significantly decreased the cardiac output [82].

#### 2.2.4. Nervous system

Microwaves may affect the blood brain barrier which lets toxic substances pass through from the blood to the brain [83]. Adang et al. [84] examined the effect of microwave exposure to a GSM-like frequency of 970 MHz pulsed waves on the memory in rats by means of an object recognition task. The rats that have been exposed for 2 months show normal exploratory behavior. The animals that have been exposed for 15 months show derogatory behavior. They do not make the distinction between a familiar and an unfamiliar object. In the area that received radiation directly from "Location Skrunda Radio Station" (Latvia), exposed children had less developed memory and attention, their reaction time was slower and neuromuscular apparatus endurance was decreased [85]. Exposure to cell phones prenatally and, to a lesser degree, postnatally was associated with behavioral difficulties such as emotional and hyperactivity problems around 7 years of age [86]. Electromagnetic radiation caused modification of sleep and alteration of cerebral electric response (EEG) [87–89]. Microwave radiation from phone masts may cause aggressiveness in people and animals (personal observation).

### 2.3. Effects on amphibians

Disappearance of amphibians and other organisms is part of the global biodiversity crisis. An associated phenomenon is the appearance of large numbers of deformed amphibians. The problem has become more prevalent, with deformity rates up to 25% in some populations, which is significantly higher than previous decades [90]. Balmori [91] proposed that electromagnetic pollution (in the microwave and radiofrequency range) is a possible cause for deformations and decline of some wild amphibian populations.

Two species of amphibians were exposed to magnetic fields at various stages of development. A brief treatment of early amphibian embryos produced several types of abnormalities [92]. Exposure to a pulsed electromagnetic field produced abnormal limb regeneration in adult Newts [93]. Frog tadpoles (*Rana temporaria*) developed under electromagnetic field (50 Hz, 260 A/m) have increased mortality. Exposed tadpoles developed more slowly and less synchronously than control tadpoles and remain at the early stages for longer. Tadpoles developed allergies and EMF caused changes in blood counts [94].

In a current study exposing eggs and tadpoles ($n = 70$) of common frog (*R. temporaria*) for two months, from the phase of eggs until an advanced phase of tadpole, to four telephone base stations located 140 m away: with GSM system 948.0–959.8 MHz; DCS system: 1830.2–1854.8; 1855.2–1879.8 MHz. and UMTS system: 1905–1910; 1950–1965; 2140–2155 MHz. (electric field intensity: 1.847–2.254 V/m). A low coordination of movements, an asynchronous growth, with big and small tadpoles, and a high mortality (90%) was observed. The control group ($n = 70$), under the same conditions but inside a Faraday cage (metallic shielding component: EMC-reinforcement fabrics 97442 Marburg Technic), the coordination of movements was normal, the development was synchronously and the mortality rate was only 4.2% [95].

### 2.4. Effects on insects

The microwaves may affect the insects. Insects are the basis and key species of ecosystems and they are especially sensitive to electromagnetic radiation that poses a threat to nature [96].

Carpenter and Livstone [97] irradiated pupae of Tenebrio molitor with 10 GHz microwaves at 80 mW for 20–30 min and 20 mW for 120 min obtained a rise in the proportion of insects with abnormalities or dead. In another study exposing fruit flies (*Drosophila melanogaster*) to mobile phone radiation, elevated stress protein levels (Hsp70) was obtained, which usually means that cells are exposed to adverse environmental conditions ('non-thermal shock') [98]. Panagopoulos et al. [99] exposed fruit flies (*D. melanogaster*) to radiation from a mobile phone (900 MHz) during the 2–5 first days of adulthood. The reproductive capacity of the species reduced by 50–60% in modulated radiation conditions (emission while talking on the phone) and 15–20% with radiation nomodulated (with the phone silent). The results of this study indicate that this radiation affects the gonadal development of insects in an athermal way. The authors concluded that radio frequencies, specifically GSM, are highly bioactive and provoke significant changes in physiological functions of living organisms. Panagopoulos et al. [100] compare the biological activity between the two systems GSM 900 MHz and DCS 1800 MHz in the reproductive capacity of fruit flies. Both types of radiation were found to decrease significantly and non-thermally the insect's reproductive capacity, but GSM 900 MHz seems to be even more bioactive than DCS 1800 MHz. The difference seems to be dependent mostly on field intensity and less on carrier frequency.

A study in South Africa finds a strong correlation between decrease in ant and beetle diversity with the

electromagnetic radiation exposure (D. MacFadyen, personal communication.). A decrease of insects and arachnids near base stations was detected and corroborated by engineers and antenna's maintenance staff [101]. In houses near antennas an absence of flies, even in summer, was found.

In a recent study carried out with bees in Germany, only a few bees irradiated with DECT radiation returned to the beehive and they needed more time. The honeycomb weight was lower in irradiated bees [102]. In recent years a "colony collapse disorder" is occurring that some authors relate with pesticides and with increasing electromagnetic pollution [96].

The disappearance of insects could have an influence on bird's weakening caused by a lack of food, especially at the first stages in a young bird's life.

### 2.5. *Effects on trees and plants*

The microwaves may affect vegetables. In the area that received radiation directly from "Location Skrunda Radio Station" (Latvia), pines (*Pinus sylvestris*) experienced a lower growth radio. This did not occur beyond the area of impact of electromagnetic waves. A statistically significant negative correlation between increase tree growth and intensity of electromagnetic field was found, and was confirmed that the beginning of this growth decline coincided in time with the start of radar emissions. Authors evaluated other possible environmental factors which might have intervened, but none had noticeable effects [103]. In another study investigating cell ultrastructure of pine needles irradiated by the same radar, there was an increase of resin production, and was interpreted as an effect of stress caused by radiation, which would explain the aging and declining growth and viability of trees subjected to pulsed microwaves. They also found a low germination of seeds of pine trees more exposed [104]. The effects of Latvian radar was also felt by aquatic plants. *Spirodela polyrrhiza* exposed to a power density between 0.1 and 1.8 $\mu W/cm^2$ had lower longevity, problems in reproduction and morphological and developmental abnormalities compared with a control group who grew up far from the radar [105].

Chlorophylls were quantitatively studied in leaves of black locust (*Robinia pseudoacacia* L.) seedlings exposed to high frequency electromagnetic fields of 400 MHz. It was revealed that the ratio of the two main types of chlorophyll was decreasing logarithmically to the increase of daily exposure time [106].

Exposed tomato plants (*Lycopersicon esculentum*) to low level (900 MHz, 5 V/m) electromagnetic fields for a short period (10 min) measured changes in abundance of three specific mRNA after exposure, strongly suggesting that they are the direct consequence of application of radio-frequency fields and their similarities to wound responses suggests that this radiation is perceived by plants as an injurious stimulus [107]. Non-thermal exposure to radiofrequency fields induced oxidative stress in duckweed (*Lemna minor*) as well as unespecific stress responses, especially of antioxidative enzymes [108].

For some years progressive deterioration of trees near phone masts have been observed in Valladolid (Spain). Trees located inside the main lobe (beam), look sad and feeble, possibly slow growth and a high susceptibility to illnesses and plagues. In places we have measured higher electric field intensity levels of radiation (>2 V/m) the trees show a more notable deterioration [109]. The tops of trees are dried up where the main beams are directed to, and they seem to be most vulnerable if they have their roots close to water. The trees don't grow above the height of the other ones and, those that stand out far above, have dried tops (Hargreaves, personal communication and personal observation). White and black poplars (*Populus sp*.) and willows (*Salix sp*.) are more sensitive. There may be a special sensitivity of this family exists or it could be due to their ecological characteristics forcing them to live near water, and thus electric conductivity. Other species as *Platanus sp*. and *Lygustrum japonicum,* are more resistant (personal observation). Schorpp [110] presents abundant pictures and explanations of what happens to irradiated trees.

## 3. Conclusions

This literature review shows that pulsed telephony microwave radiation can produce effects especially on nervous, cardiovascular, immune and reproductive systems [111]:

- Damage to the nervous system by altering electroencephalogram, changes in neural response or changes of the blood–brain barrier.
- Disruption of circadian rhythms (sleep–wake) by interfering with the pineal gland and hormonal imbalances.
- Changes in heart rate and blood pressure.
- Impairment of health and immunity towards pathogens, weakness, exhaustion, deterioration of plumage and growth problems.
- Problems in building the nest or impaired fertility, number of eggs, embryonic development, hatching percentage and survival of chickens.
- Genetic and developmental problems: problems of locomotion, partial albinism and melanism or promotion of tumors.

In the light of current knowledge there is enough evidence of serious effects from this technology to wildlife. For this reason precautionary measures should be developed, alongside environmental impact assessments prior to installation, and a ban on installation of phone masts in protected natural areas and in places where endangered species are present. Surveys should take place to objectively assess the severity of effects.

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