



Mobile Telecommunications and
Health Research Programme

The Effects of Radiofrequency Radiation on Brain Physiology and Function

Z Sienkiewicz

RUM 1

Project title:	The Effects of Radiofrequency Radiation on Brain Physiology and Function
Project reference:	RUM 1
Project Director:	Z Sienkiewicz
Project Monitor:	Mike Repacholi
Project start date:	1 February 2002
Project end date:	31 January 2005
Final report date:	28 April 2006
Date approved by Monitor:	19 November 2006
Date approved by Chairman:	22 December 2009

The effects of radiofrequency radiation on brain physiology and function

RUM 1

Z Sienkiewicz*

1. Executive Summary

There are concerns that the radiofrequency (RF) fields associated with mobile phones may cause adverse effects on the brain, and lead to detrimental changes in learning and memory. This project was conducted to study this possibility in animals and investigated the effects of exposure to a range of fields associated with mobile communications.

The project involved closely co-ordinated work between neuroscientists at Bristol University Research Centre for Neuroendocrinology, the Defence Science and Technology Laboratory at Porton Down, and the Radiation Protection Division of the Health Protection Agency (formerly the National Radiological Protection Board). The project searched for evidence at three different levels of cellular organisation and function that exposure to RF fields caused biological changes in the brain. This approach was only possible using animal models.

Following controlled, head-only exposure of adult male mice, gene and protein expression in selected areas of the brain was assayed using gene chip microarray and protein technologies, electrophysiological responses were explored using brain slice techniques, and effects on learned behaviour were investigated using learning and attention tasks. Effects were compared using three typical frequencies used for mobile communications (400, 900 and 2200 MHz corresponding to TETRA, GSM and 3G or UMTS signals), a range of exposures, and between single and repeated exposures. If changes were seen, say in the behaviour of the animals

exposed to a particular RF field, perhaps altering the way the animal solved a problem, then this approach would be able to see if corresponding changes occurred in the excitability of nerve cells in the brain associated with those behaviours, and then to see if these changes could be confirmed at the molecular level. This would give great creditability to any observed effect. A similar argument would apply if no effects were seen.

Overall, it was found that single or repeated exposures to RF fields at the frequencies used in the project did not appear to exert any lasting effects on brain physiology and function. While each of the approaches used in the project may have their own particular strengths and weaknesses, taken together they provide powerful and compelling evidence that exposure was not able to cause any important changes on the brain.

2. Aims and Objectives

The aims of the project were to address one of the principal areas for research recommended in the report published by the Independent Expert Group on Mobile Phones (IEGMP, 2000), namely to explore the effects of radiofrequency (RF) fields on brain function.

It has been hypothesized that low level RF fields produced by mobile phones may induce non-thermal processes that influence human brain physiology and function. Previous experimental studies have suggested that *in vitro* exposure to 700 MHz fields can affect both evoked and spontaneous electrical activity

*Radiation Protection Division, Health Protection Agency, Chilton, Didcot, Oxfordshire, OX11 0RO

in rat hippocampal slices, an effect which lasted beyond the end of the exposure period (Tattersall et al, 2001). Other experiments conducted on animals to investigate this claim have found changes in neurochemical measures in the hippocampus and surrounding cortex (see NRPB, 2004 for review) as well as changes in learned behaviours dependent on the integrity of the hippocampus (Lai et al, 1994; Wang and Lai, 2000; Lai, 2004). Thus it might be assumed that low level RF fields might induce robust changes in hippocampal physiology. However, other studies have not confirmed any field-dependent changes on behaviour (Cassel et al, 2004; Cobb et al, 2004; Cosquer et al, 2005; Dubreuil et al, 2002, 2003; Sienkiewicz et al, 2000) which challenge this assumption.

To help to resolve these uncertainties, the effects of exposure to RF fields were assessed on the functions of the hippocampus and cortex in adult, male mice. To achieve these aims and to avoid any potential problems associated with isolated observations using unique models, an integrated, multidisciplinary approach was adopted, and effects were assessed at different levels of biological organisation: on learned behaviour, using a spatial learning task and a serial reaction time task; on electrophysiological responses, using the hippocampal slice preparation; and on sub-cellular and cellular systems by investigating possible influences on gene and protein expression. In particular, the latter approach was able to exploit fully the exceptional power of gene chip profiling to critically assess the hypothesis that changes in gene expression occur in the mammalian brain following exposure to RF fields.

Another important aim of the project was to compare the effects of different frequencies and signals, and so three frequencies were used, with both pulsed and continuous wave signals. In addition, to increase the context and relevance of the results, a range of specific energy absorption rates (SARs) were used, as were both single and repeated acute exposures.

The overall aims and objectives of the project were to increase knowledge about the physiological and biochemical responses that may occur in the brain following exposure to specific RF signals typical of mobile phone systems, and thus help to formulate a more complete assessment of the potential biological impact of such systems and improve standards and guidelines for human exposure to RF fields.

3. Participants

The participants in this project came from three centres:

1) The Health Protection Agency, Radiation Protection Division (HPA RPD): Dr Anna Bottomley and Rachel Bartram carried out the behavioural studies and performed the exposures for the gene array studies, with assistance from Kevin Whitehill; Roger Blackwell designed and built the exposure systems, and with Darren Addison performed the physical dosimetry measurements; John Anderson constructed the exposure chambers; Dr Ian Scivill performed the theoretical dosimetry calculations; Dr Richard Haylock carried out the statistical analysis; and Dr Zenon Sienkiewicz provided supervision of these studies and the overall co-ordination of the project.

2) The Defence Science and Technology Laboratories (Dstl), Biomedical Sciences: Dr Patrick Harrison carried out the neurophysiological studies assisted by Adam Smith, Nicola Mifsud and Iain Scott; Georgina Underwood performed the exposures of the animals; and Dr John Tattersall provided supervision of these studies

3) The University of Bristol: the post-doctoral workers funded on the gene array studies were Dr Russell Hobson (February 2002-June 2004) and Dr Joanna Howarth (from August 2004 onwards). Yong Bok Lee, provided valuable technical and intellectual help with regard to the TaqMan and GeneSpring analysis. Prof James Uney provided supervision of these studies.

4. Achievements

4.1. Head-only exposure system for mice

A dedicated system was constructed that was capable of consistent, controlled exposures to RF fields at 400, 900 and 2200 MHz localised to the heads of up to four animals at one time. Fields could be either modulated (pulsed to represent some of the features of mobile phone signals) or an unmodulated carrier signal (continuous wave, CW).

The system consisted of an individual loop antenna for each animal fed via a power splitter from a narrow-band power amplifier for the frequency in use (Wessex Electronics) driven by a programmable signal source (Agilent E4432B, option UN8). The power to the antennas was measured using an Agilent E4418 power meter with 8481A power head. The design of antenna was developed by Chou et al (1999) who concluded that the rectangular loop shape was advantageous for coupling with small anatomical structures.

Careful matching of interconnecting cables and the use of isolators for each antenna minimised interactions and ensured that the power delivered to each antenna was similar within close limits. Separate antenna sets were used for each frequency, tuned for a return loss of >20dB (with a mouse in the animal holder). All parameters of the exposure system, were controlled by personal computer using custom software. The exposure mode and level was adjusted for each irradiation at predetermined values (designated as High, Medium or Low, and pulsed or CW) with minimal possibility for inadvertent exposure errors. The system maintained a sensibly constant drive to the antennas for the duration of the exposure and also recorded the exposure history to a file for later inspection.

The system was contained within a large metal-walled chamber. To allow simultaneous irradiation of four animals, this was partitioned into four equal sized compartments, lined on all surfaces by appropriate broad-band, graded absorber material. Each compartment was illuminated with a small light source, and masking noise provided by four externally

mounted fans that also provided ventilation within the chamber as a whole. A miniature video camera was positioned above each of the animals to allow them to be observed at all times.

Animals were held immobile using a custom-made acrylic holding tube and associated stand (Vet Tech Solutions, Congleton, UK). These holders were necessary to eliminate as far as possible even slight head movements which would affect the local SAR in the head. Using a system of stops and guides, the holders could be located precisely within each partition. Similarly the loop antennas in each compartment could be altered using a highly adjustable acrylic holder and locked into position. In this way the loop antennas could be reproducibly positioned against the same region of the head.

Mapping of the E-fields around the antennas and validation studies were undertaken to ensure the correct performance of the antennas.

All procedures involving animals were carried out in accordance with the Animals (Scientific Procedures) Act 1986 and with knowledge and approval of the relevant local ethical review committee at Dstl, Porton Down and HPA, Chilton. The exposure system was operated using a testing and development license granted under the Wireless Telegraphy Act 1949 and 1998.

4.2. Theoretical and physical dosimetry

At the start of this project, an accurate voxel phantom of a mouse was not readily available. The nearest alternative was obtained from Prof Fujiwara and Dr Wang (Nagoya Institute of Technology, Japan). This phantom was originally derived from a MRI scan of a rat, and scaled to the dimensions of a mouse. It consisted of 18 distinct tissue types, including the brain. However, differences in anatomy between species mean that the calculations performed using this phantom can only be expected to provide an indication of the SAR induced in the brain of a mouse: physical dosimetry measurements were also considered necessary.



Figure 1 External view of the exposure chamber showing four exposure compartments.

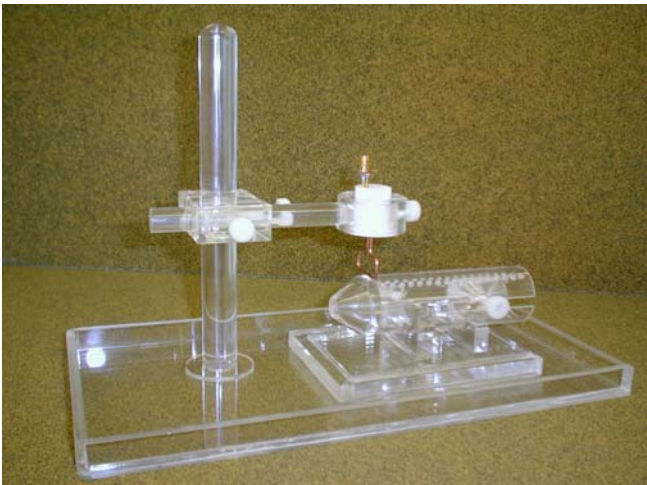


Figure 2 The animal holder and base tray, with the antenna and antenna support.

The SAR averaged over the whole brain induced by the loop antenna at 900 MHz was estimated using the finite difference time domain (FDTD) method (Taflove et al, 1995). The high 'Q' value of this antenna (Chou et al, 1999) meant that similar calculations could not be performed at the other frequencies.

Figure 3 shows a scale representation of the complete FDTD model: the acrylic animal holders could be neglected from this model because they were

constructed from materials which would not perturb the magnetic field. The FDTD mesh was specified with a uniform 1 mm cubic cell size, and was terminated with PML boundary conditions (Berenger, 1994). The 4-Cole-Cole dispersion model (Gabriel et al 1995, 1996a, 1996b, 1996c,) was used to calculate tissue electrical properties at 900 MHz. The antenna was positioned 5 mm above the head of the phantom, and transversely orientated with respect to the midline of the brain. The antenna was modelled as a 2 mm rectangular wire with perfect electrical conducting properties, and driven by a voltage source.

At 900 MHz, the SAR induced in the brain was calculated to be 15 W kg^{-1} per W.

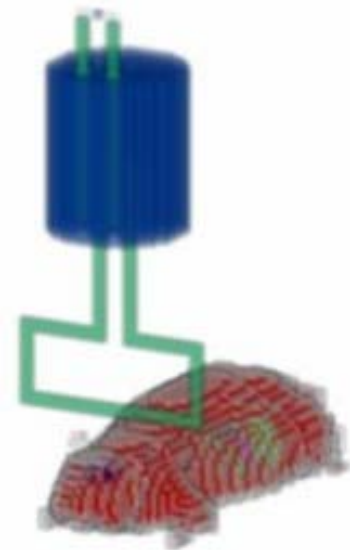


Figure 3 The FDTD model: view showing the loop antenna in position relative to the voxel phantom of the mouse. The PTFE block is also shown (in blue).

Physical dosimetry measurements were performed at each frequency using a non perturbing, fibre-optic temperature probe. Exposures using the highest possible power fed to one antenna were used. The absorbed energy per W of antenna power was calculated by measuring the initial temperature rise within the brain of exposed mouse cadavers.

The average SARs measured in the brain for each frequency used are given in Table 1. Overall, there was good agreement with the results of the

theoretical dosimetry calculations at 900 MHz: differences in absorbed energy were by less than 3 dB. The SARs shown in Table 1 were taken as the nominal values for exposure in all subsequent experiments.

Table 1 Average SARs (with SD) in $W\ kg^{-1}$ measured in the brain of a mouse corresponding to exposures designated as Low, Medium or High.

Exposure	400 MHz	900 MHz	2200 MHz
Low	2.6 (0.4)	3.6 (0.05)	2.05 (0.25)
Medium	13.0 (0.3)	17.9 (0.3)	10.3 (0.13)
High	26.0 (0.06)	35.7 (0.5)	20.5 (0.03)

4.3. Experimental studies

Adult, male C57BL/6J mice were used throughout the project, and common methods were followed regarding the husbandry and handling of the animals prior to any procedures in order to ensure good consistency between the centres involved in the project. In addition, standard protocols were used during the familiarization of the animals to the exposure system with all animals being gradually habituated to restraint stress over 10 days prior to any treatment (unless specifically excluded).

In these experiments, three frequencies were used, 400 MHz (representing TETRA), 900 MHz (representing GSM) and 2200 MHz (representing 3G or UMTS). Where practicable, three SARs were also used for each frequency, with both modulated and unmodulated (pulsed and CW) signals, and these values are given in Table 1. Sham exposed animals were treated the same way as those exposed to a field, but the RF signal was sent to a dummy load outside of the exposure chamber and was not connected to the loop antennas.

Acute exposure was defined as a single 1 h exposure to the field of interest (or sham exposure). Repeated exposures consisted of a daily 1 h exposure, 2 times per week for 12 weeks, to the same field of interest. A variant of the latter was used in the behavioural studies, and was termed intermittent repeated exposure, and here animals were sequentially exposed to pulsed and CW 900 MHz fields or were sham exposed.

4.3.1 Behavioural studies

Following the standard protocol to habituate the animals to the exposure system, adult male C57BL mice were exposed to pulsed 400, 900 or 2200 MHz signals or to equivalent unmodulated (CW) fields for 1 h using the loop antenna system. The power was adjusted to produce a range of SARs. The effects of single acute and repeated exposures on behaviour were investigated.

Integrity of spatial learning and working memory functions was assessed using a Morris water maze for mice. In this test, animals received daily blocks of four acquisition trials to find a submerged, non-visible platform located in a constant position in a circular swimming pool. The water was maintained at around 24 ° C and was made opaque. The pool itself was placed in a dedicated laboratory equipped with many distinct visual cues to aid spatial navigation. Animals were released from the poolside from each of the four cardinal compass points at random. They were given 60s to swim to the platform, and allowed to stay there for a further 30s. Animals that failed to find the platform within 60 s were placed on it by hand.

An image analysis system (Ethovision, Tracksys, UK) was used to record and score the behaviour of the animals. The main variables used to measure learning were swim path and latency to find the platform. After acquisition, a single probe trial without the presence of the platform was used to gauge overall accuracy of learning by measuring total time spent in the quadrant of the maze that previously contained the platform (quadrant analysis). The effects of RF fields were explored by exposing the animals immediately before the probe trial. Additional trials using visible platforms in constant positions or submerged platforms in random positions were used as controls. There were eight animals in each treatment group, and results were analyzed with ANOVA.

Overall, compared to the relevant sham exposed groups, all exposures had no significant effects on any measure of performance (in all cases, $p>0.05$).

Figure 4 shows the results of the mean acquisition trials for the animals used to investigate the effects of 900 MHz. All groups of animals show good increases in performance and displayed a typical asymptotic decrease in escape latency across the trials (note that treatments have not yet been applied).

The amount of time animals spend in each quadrant of the maze is another useful measure of performance: at first animals should not show any choice for a particular zone, but they should demonstrate a clear preference following acquisition. Figure 5a shows the performance of the groups of animals used to investigate the effects of 900 MHz. These results are expressed as times spent in each quadrant during the initial pretrial in the pool before the acquisition trials. No innate preferences for any particular quadrant of the pool before treatment can be seen.

Figure 5a shows the performance of the animals expressed as mean percentage of time spent in each quadrant of the pool during the probe trial, that is following treatment and without an escape platform being present. This analysis indicated a clear preference for the platform zone, that is the quadrant of the pool that used to contain the platform, and suggested that the memory of the location of the platform had not been changed by treatment. Thus the performance of the task was not affected by field modulation or SAR.

Similar results were seen for other measures of performance and for exposures at 400 and 2200 MHz. For example, the results of the study investigating 400 MHz are shown in Figure 6 with results collapsed across SAR to highlight any difference between modulation. Animals from all treatments spent more time in the quadrant where the platform was located during acquisition, suggesting there were no impairments in spatial memory.

However it was found that the cage control animals were less active in the pool, and tended to have increased escape latencies compared to other groups (figures 4 and 5a). This reduced responsiveness was attributed to their decreased amount of handling caused by not being habituated to the exposure

system. In addition, these animals did not demonstrate a good acquisition of the task, since there were no significant differences for the times spent in each quadrant of the pool during the probe trial (figure 5 b; $p=0.088$).

An additional set of ANOVA analyses were carried out to examine if the differences in treatment could explain any of the variability between the eight different groups. However, no significant frequency or SAR effects could be identified.

To investigate the effects of exposures to 900 MHz fields over a longer time period, an experiment was performed to assess the effects of repeated exposures on behaviour. Following habituation to restraint, animals were exposed for 60 min, twice a week for 12 weeks to (High or Medium) CW or pulsed fields or were sham exposed; there was no cage control group. During their final week of exposures animals were trained over 4 days on the water maze task. As with the experiments using acute exposures, performance was assessed using latency to escape as well as swim pattern and swimming speed. As before, animals did not exhibit any innate preference for any quadrant of the maze and they quickly learned the task. The results indicated that there were no significant field-dependent effects on the indices of performance examined during the probe trial (in all cases, comparing between exposed and sham-exposed groups, $p>0.05$). Animals from all treatment groups spent more time in the quadrant where the platform was located during acquisition, suggesting there were no significant impairments in spatial memory.

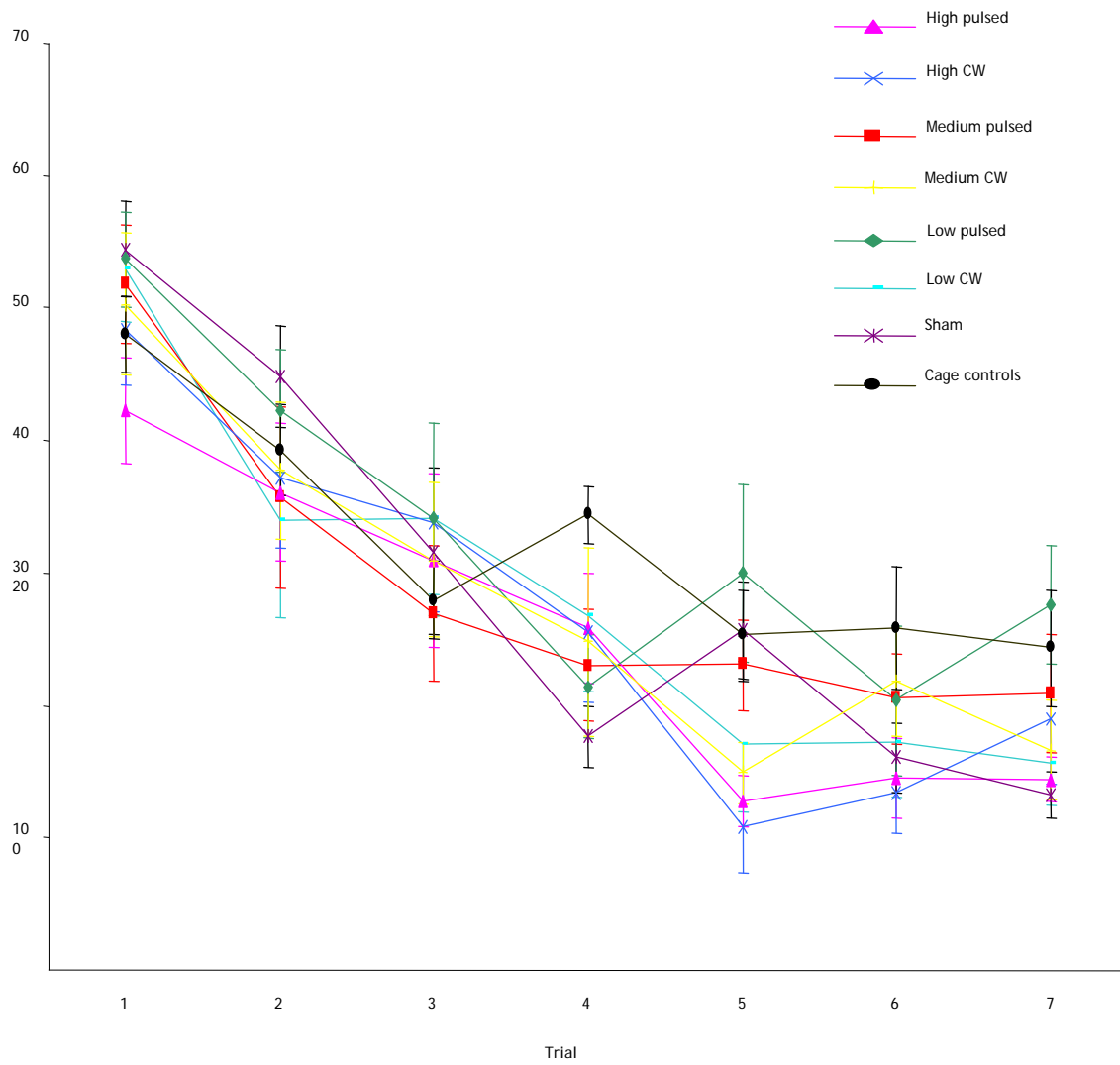


Figure 4 Acquisition trials in the water maze showing the mean escape latency (plus SEM) to locate the platform in the swimming pool. With the exception of the cage controls, the key indicates the treatment (SAR value and modulation) that would be applied following acquisition

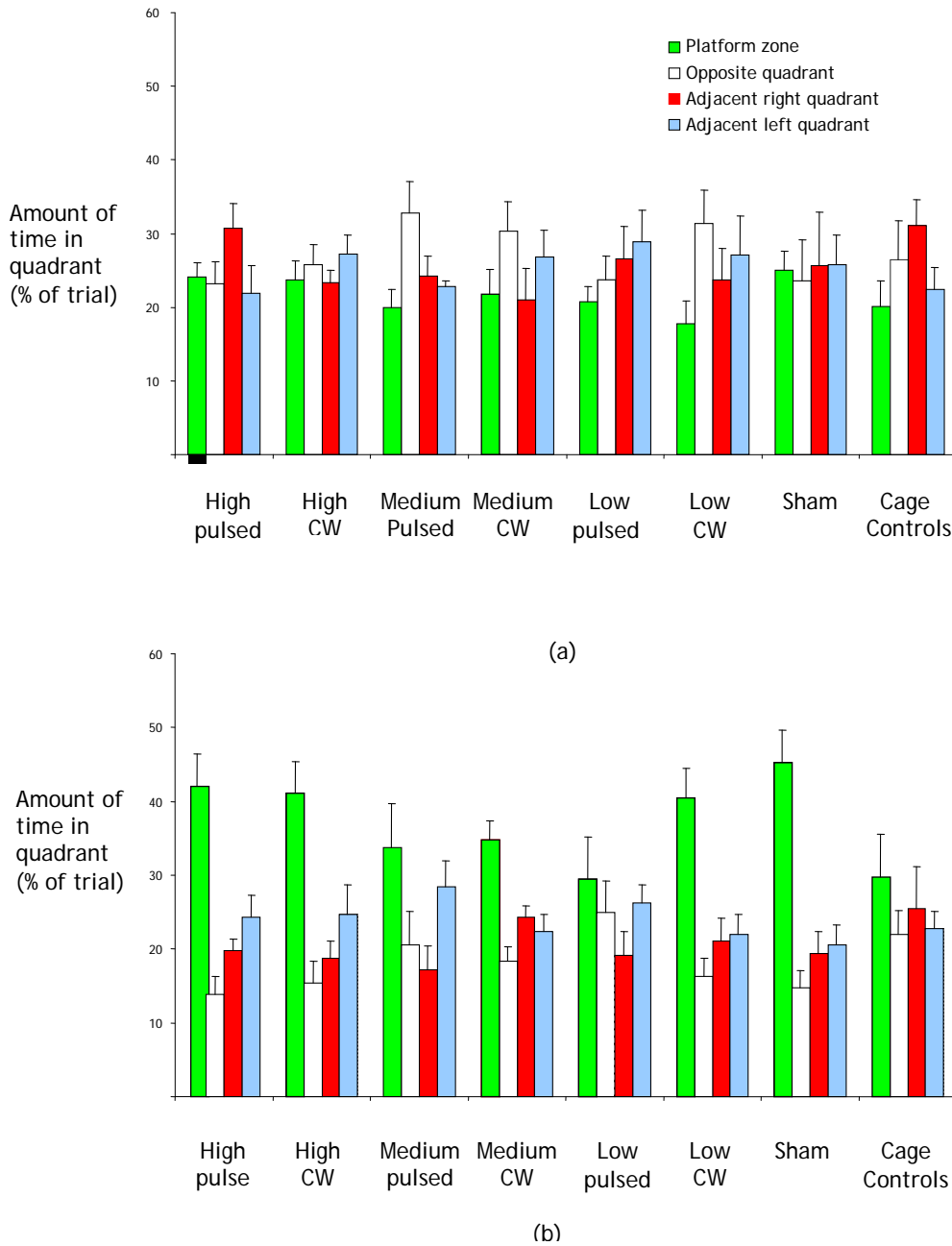


Figure 5 Performance in the water maze expressed as mean percentage of time (plus SEM) spent in each of the four quadrants of the pool (a) during the pretrial session before acquisition trials and (b) during the probe trial following acquisition and after treatment. High, Medium and Low indicate SAR values of 36, 18 and 3.6 W/kg¹.

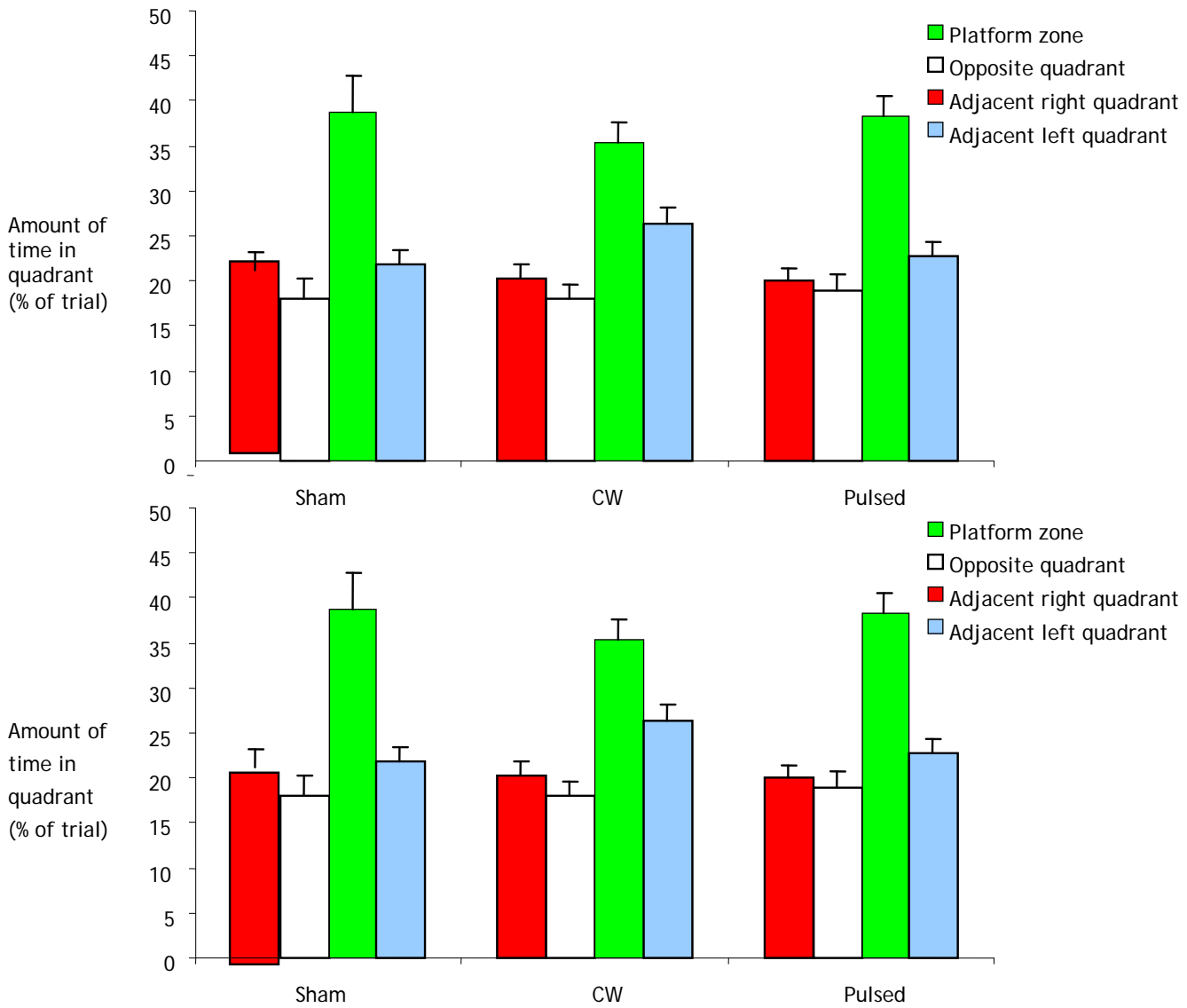


Figure 6 Effects of exposure to 400 MHz fields on performance in the water maze expressed as mean percentage of time spent in each quadrant (plus SEM) during the probe trial. All groups of animals spent significantly longer in the platform zone than elsewhere irrespective of treatment, suggesting no field-related effects on spatial memory.

Finally, the effects of intermittent, repeated exposure to RF fields were studied on the performance of a 5-choice serial reaction time task (Carli et al, 1983). This visual attention task was chosen in order to model reported effects of RF fields on human reaction time. Here, animals were required to attend to an array of visual stimuli within an operant chamber (Paul Frey, Cambridge, UK) and were only reinforced with food for correctly performing the appropriate response to the correct cue. Response times were measured by a personal computer which also controlled all aspects of the experiment. A group of 11 animals was habituated to restraint, and trained to perform the task over several months. Once performance was stabilized, animals were exposed to either pulsed or CW fields two or three times a week for 11 weeks. In this longitudinal design, animals acted as their own controls, and sham exposures were used once a week to check for stability of performance and to investigate any carry over effects. The behaviour of these animals proved very stable, and no significant field-dependent changes in any measure of performance, including effects of response times (latency), were observed (Table 2).

In summary, no consistent changes in behaviour were observed with either task following acute or repeated exposure to RF fields. In addition, there were no differences between pulsed or unmodulated fields for any of the three frequencies examined. Changes in the behaviour of a group of cage control animals were observed, and these were ascribed to differences in handling associated with the lack of habituation to the exposure system.

3.3.2 Electrophysiological studies

Following the standard protocols, adult male C57BL mice (30-45g) were exposed to pulsed 400, 900 or 2200 MHz signals or to equivalent unmodulated (CW) fields for 1 h using the loop

antenna system. The power was adjusted to produce a SAR of around 20 W kg^{-1} (400 and 2200 MHz) or 36 W kg^{-1} (900 MHz). To investigate the effects of repeated exposures, mice were exposed to pulsed 900 MHz fields for 1 h per day, 2 days per week for 12 weeks, with a SAR in the brain of 18 W kg^{-1} .

Immediately following exposure, the mice were anaesthetised with halothane and decapitated, and parasagittal slices of brain tissue (400 μm thick) containing the hippocampus were prepared. The slices were maintained at $31.0 \pm 0.1^\circ \text{C}$ and perfused with artificial cerebrospinal fluid. Extracellular field potentials were recorded in CA1 *stratum pyramidale* using glass microelectrodes filled with 2 M NaCl. Responses were evoked every 30s by a concentric bipolar stainless steel stimulating electrode placed in *stratum radiatum*. Long term potentiation (LTP) was induced by theta burst stimulation (5 trains of 4 pulses at 100 Hz separated by 200 ms, repeated twice with an interburst interval of 10 s) (Morgan and Teyler, 2001). One slice was tested from each animal. The investigators recording and analysing the slice data were blind to the exposure conditions for each animal.

Figure 7 shows stimulus-response relationships for the field excitatory postsynaptic potential (fEPSP) and the population spike (PS) in slices from animals acutely exposed to 400 MHz (TETRA), 900 MHz (GSM) and 2200 MHz (UMTS) fields. Graphs comparing the sham-exposed groups are also shown, to give an indication of the variability between unexposed groups. No differences between exposed and sham groups were apparent, except in the case of the group exposed to a pulsed 400 MHz field, in which the PS amplitude did not rise as steeply with increasing stimulus intensity as it did in the sham or unmodulated exposure groups. There was, however, no difference in the threshold for the response.

Table 2 Results of the 5-choice serial reaction time task showing five of the measured outcomes of behaviour. All animals (n =11) received all experimental treatments and served as their own controls. The sequence of exposure to 900 MHz fields was random, with the animals receiving at least one sham exposure per week to maintain a baseline performance and one field exposure. All exposures were 1 h in duration and a 10 minute rest period was allowed between finishing exposure and starting the task. Each animal received 13 sham exposures and 17 randomized field exposures. Low, Medium and High correspond to SAR values of 3.6, 18 and 36 W kg⁻¹; pulsed and CW correspond to modulated and unmodulated fields. Values shown are mean and (SEM). Any differences between treatment groups were not significant (in all cases, p>0.05).

	Sham exposed	Low pulsed	Medium pulsed	High pulsed	Low CW	Medium CW	High CW
% correct responses	93.3 (0.5)	94.7 (0.7)	94.3 (0.7)	92.7 (1.2)	94.4 (1.3)	93.1 (0.9)	94.5 (0.9)
Correct response latency (s)	0.79 (0.02)	0.74 (0.03)	0.77 (0.03)	0.78 (0.02)	0.84 (0.04)	0.8 (0.04)	0.79 (0.05)
Reward retrieval latency (s)	2.1 (0.09)	2.3 (0.5)	2.5 (0.3)	2.0 (0.2)	3.0 (0.6)	2.5 (0.3)	2.2 (0.2)
Number of panel pushes	81.5 (3.4)	87.9 (6.5)	74.1 (4.7)	95.7 (6.4)	59.2 (6.7)	69.2 (6.0)	52.4 (8.1)
Number of perseverative responses	57.5 (3.2)	49.8 (6.1)	51 (4.6)	45.2 (5.2)	63 (8.2)	60 (6.3)	65.6 (9.1)

Plots of the relationship between fEPSP slope and PS amplitude are shown in Figure 8. This relationship, known as E-S coupling, gives an indication of the ability of a given level of synaptic depolarisation to induce the postsynaptic cell to fire an action potential. No differences between sham and exposed groups were observed, and the apparent change in the PS stimulus-response relationship for the pulsed 400 MHz group was not reflected in the E-S coupling.

LTP is widely regarded as a cellular basis for learning and memory. This is seen as a prolonged (>1 h) increase in the amplitude of the evoked response following the application of a high frequency stimulation pattern (theta burst). Figure 9 shows the induction of LTP in hippocampal slices from animals exposed to 400 and 900 MHz fields. Similar results were obtained in experiments with 2200 MHz fields and with animals chronically exposed to 900MHz fields (data not shown). No differences between exposed and sham groups were apparent, and there were no significant differences in the magnitude of LTP, measured at 1 h after theta burst stimulation (ANOVA and t-test).

In summary, no consistent changes in the electrophysiological responses of the hippocampus

were observed following exposure to RF fields. Nevertheless, the stimulus-response relationship for the PS appeared to be depressed in the group exposed to the 400 MHz signal (although the threshold for the response was unchanged). However, no such difference was apparent in the fEPSP stimulus response relationship for this group, nor in E-S coupling. This suggests that the difference observed in the PS stimulus-response coupling was due to experimental variation and did not reflect a real change in hippocampal function. Further confirmation was provided by experiments on LTP, which provided no evidence for changes in cellular memory processes.

The time between the end of exposure to the RF fields and the commencement of electrophysiological recordings was approximately 2 h. It is possible that any acute effects of the RF exposure on electrophysiological properties could have decayed during the time; however, the results of these experiments indicate that there were no lasting effects of either acute or repeated exposures on electrical signaling in the hippocampus. So overall, it is concluded that previous exposure to RF fields does not appear to engender any lasting changes in neuronal excitability in the hippocampus.

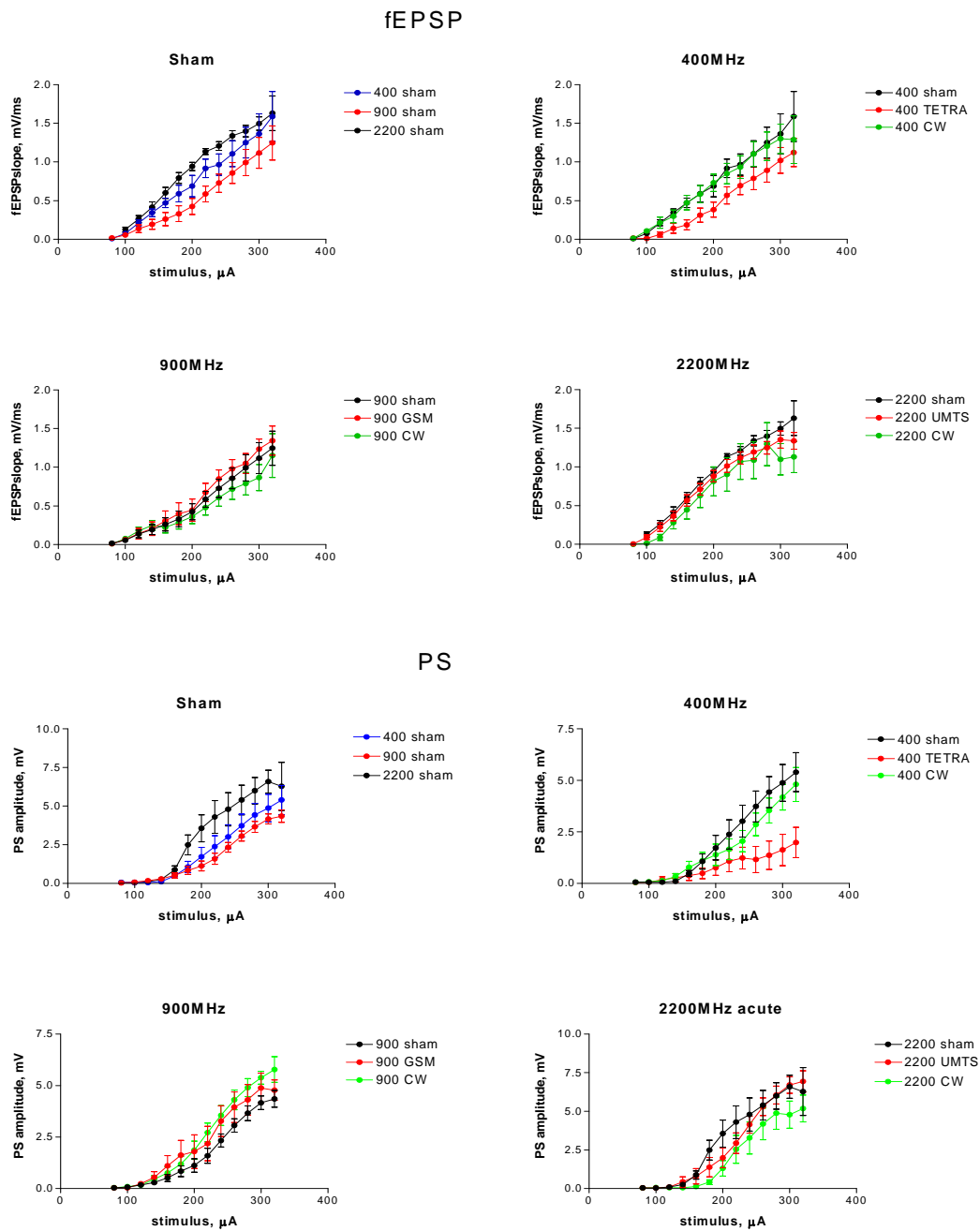


Figure 7 Constant current pulses (70 μ s duration) were delivered at increasing intensities to characterise stimulus-response relationships for the field excitatory postsynaptic potential (fEPSP, top panels) and population spike (PS, bottom panels). Data points show mean \pm SEM for 8 to 13 slices.

The effects of radiofrequency radiation on brain physiology and function

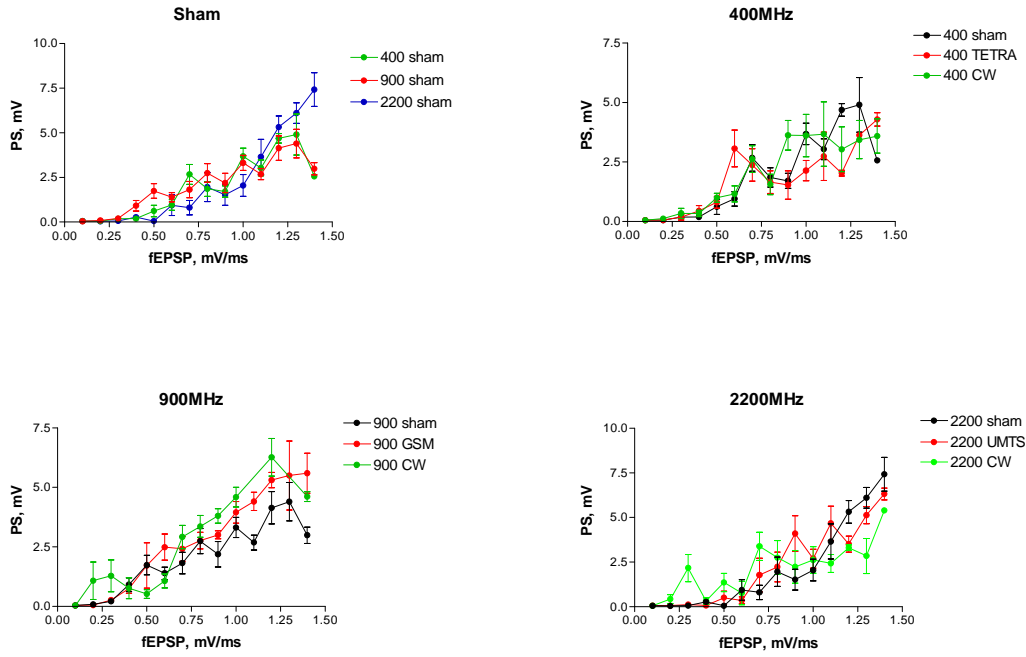


Figure 8 Plots of E-S coupling derived from the stimulus response data shown in Figure 1. Data points show mean ± SEM for 8 to 13 slices.

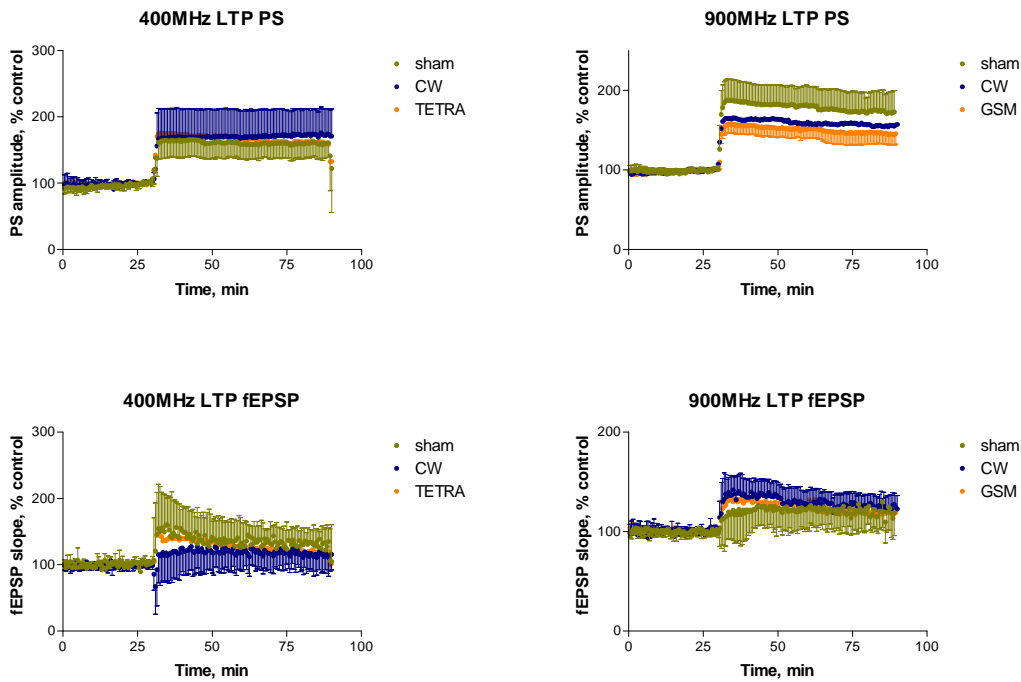


Figure 9 Plots of PS amplitude (top panels) and fEPSP slope (bottom panels) during induction of long term potentiation (LTP) in brain slices from animals exposed to 400MHz (left panels) or 900MHz (right panels) fields. LTP was induced at 30 minutes using theta burst stimulation. Data points show mean ± 95% confidence intervals for 8 slices in each group (omitted on some groups for clarity).

4.3.3 Gene and protein expression studies

This project exploited the exceptional power of gene chip profiling to critically assess the possibility that changes in gene expression may occur in the brain following exposure to RF fields. It was envisaged that changes in the expression profiles of individual genes would be found following the statistical analysis. To ensure such results were not false positives, however, Taqman (RT-PCR) and/ or western blot analysis were carried out. Furthermore, to ensure that heating effects were not causing changes in gene expression, the levels of heat shock genes were also monitored.

At first, to ensure that optimal yields of RNA could be extracted from very small quantities of mouse brain tissue three different RNA extraction methods were assessed. Experiments using the identical brain areas (and weights) to be used in the main study showed that guanadine isothiocyanate extraction followed by a column based purification method resulted in the highest yields from the very small amounts of brain used. We also chose to use a liquid preservation method, which stabilizes the RNA within tissue immediately after extraction. Further experiments showed that the use of this preservative did not alter the RNA yield following extraction

Next, preliminary gene array experiments were conducted to investigate whether the exposure procedure itself (which necessitates a period of restraint stress for the animals) resulted in changes in gene expression. This was a blind study and compared: cage controls (non stressed group); an acute stress group with no recovery, an acute stress with four days recovery; and a 10 day habituated group (n=4, in each treatment group). The results showed that there was no difference in the expression of genes in the hippocampus when single stressed groups were compared to habituated. However, the expression profiles of less than twenty genes (whose expression and function may be associated with the regulation of brain stress circuits) were altered when control animals were compared to acute stressed and habituated.

Since, a very small number of genes are altered following the exposure procedure it was concluded that there should be no difficulty in observing changes in hippocampal gene expression following exposure to RF fields.

Brain samples from the hippocampus, cerebellum, frontal and lateral cortex and thalamus were collected following exposure of animals and stored at -80 C. Acute exposures were performed with 400, 900, or 2200 MHz fields, and repeated exposures were performed using 900 MHz alone.

Following sample collection, RNA was extracted from the hippocampal samples (n=3 in each treatment group) and hybridised to Affymetrix mouse 430 2.0 gene chips to profile the genes expressed in control and experimental groups. The mouse arrays contain over 39,000 mouse gene transcripts, including over 34,000 well-substantiated mouse genes. To ensure that variation in hybridization efficiency does skew the interpretation of the results the Affymetrix chip contains 22 independent measures of the hybridization process for each of the 39,000. These internal controls are a marker (or 'flag') of the reliability of the individual gene-mRNA interaction and hence allow the subsequent statistical analysis to be conducted reliably. It should be noted that all samples were coded as the study was conducted blind.

The results were statistically analysed by ANOVA and Student-Newman-Keuls Post-Hoc test using GeneSpring software. When this analysis was conducted under normal filtering conditions (flags present in 75% of samples) no significant changes in gene expression were found when intra- and inter-group comparisons were made. Less stringent testing, again filtering by flags but varying the need for flags to be present (e.g. so flags were present in 10% of samples) again did not yield any statistical changes in gene expression. Only when we filtered by fold-change (in the absence of flags) or when we altered group size by excluding some samples (a process which is more prone to yielding false positives) were a small number of genes potentially involved in cell cycle control identified.

For example, we found that there was a statistically significant fall ($P < 0.005$) in the expression of the Rbm-5 gene in groups exposed to pulsed 900 MHz fields at 18 and 36 W kg⁻¹. Rbm-5 (identified originally as LUCA-15, and subsequently as H37) (gene bank accession number BE446879) is a known modulator of apoptosis, an RNA binding protein, and a putative tumour suppressor. There is a considerable amount of data implicating Rbm5 deregulation in tumour formation. A recent study determined that RBM5 RNA was down-regulated in 82% of primary non-small-cell lung carcinoma specimens examined compared to normal adjacent tissue, and in many lung cancer cell lines and in RAS transformed Rat-1 cells. There is also a 27-fold reduction of RBM5 RNA in vestibular schwannomas, as detected by gene chip array analysis. It was also reported that full-length RBM5 mRNA is up-regulated following induced overexpression of the oncogenes Her-2 in both MCF-7 breast cancer cells and CaOv-3 ovarian cancer cells (4.5 and 3-fold, respectively), and demonstrates an 88% expression correlation with Her-2 in human primary breast cancer. Clearly a change in the expression of this gene following exposure to RF fields would be of great interest.

However, as already stated when analysing 39,000 gene sequences false positives commonly arise and hence a further analysis of RBM-5 expression using RT-PCR TaqMan analysis was undertaken. The results of this analysis (figure 10) show that there was no change in the expression of RBM-5 following exposure of hippocampal tissue to 900 MHz fields. A number of changes in gene expression were detected when the data was filtered by fold change and we chose to measure the expression of GSK-3beta and Pten as they are implicated in the regulation of the cell-cycle. Western blot analysis using antibodies against GSK-3beta and Pten showed that there was no reproducible change in protein levels in hippocampal or (lateral) cortical tissue exposed to 900MHz fields (figure 11).

In summary, no changes in gene expression in the hippocampus following exposure to RF fields were detected when the statistical analysis was carried out under normal stringency.

When the analysis was conducted using filtering likely to yield an increase in false positive some changes were seen. However, these changes in gene expression were not substantiated by Taqman and Western blot analysis.

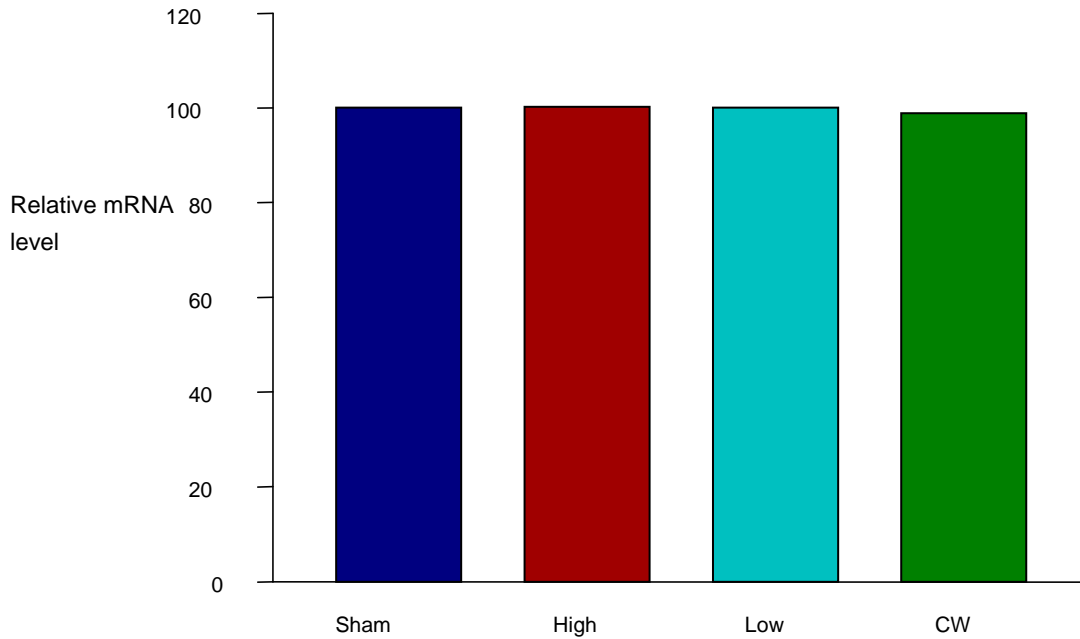


Figure 10 Taqman analysis of RBM5 mRNA expression in the hippocampus (relative to GAPDH) following exposure to pulsed 900 MHz fields at 36 W kg^{-1} (High) and 3.6 W kg^{-1} (Low) and unmodulated fields at 18 W kg^{-1} (CW). The results show no changes in expression occur in any of the groups analyzed.

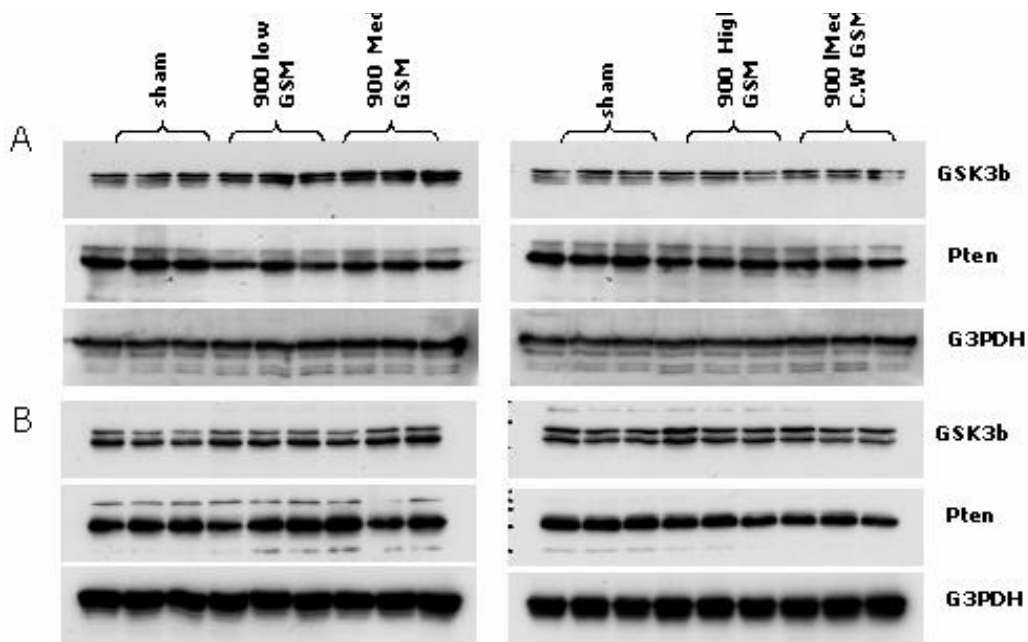


Figure 11 Western blot analysis of GSK3B and Pten expression in the hippocampus (A) and cerebellum (B) following exposure to either pulsed 900 MHz fields at 36 W kg^{-1} (High GSM) 18 W kg^{-1} (Med GSM) and 3.6 W kg^{-1} (Low GSM) unmodulated fields at 18 W kg^{-1} (Med CW) or sham exposure (sham). No consistent increase or decrease in protein levels are seen across the groups.

4. Analysis of Objectives Met

Overall, this project can be seen to have met its aims and objectives. To begin with, it was necessary to design and produce an exposure system capable of exposing the head (brain) of a mouse to a variety of RF fields in a controlled and highly reproducible manner. This was fully realized, and the loop antenna system was able to expose up to four animals at one time to either 400, 900 or 2200 MHz over a large dynamic range of SARs. Further the exposure system was very robust and performed very reliably during the course of the project. A phantom mouse was sourced for the theoretical dosimetry calculations, and the results with this model gave good agreement with physical measurements. Using this equipment, the planned experimental studies were successfully undertaken: behavioural studies looked at the effects of acute and repeated exposure on performance of tasks dependent on the integrity of the hippocampus; *in vitro* electrophysiological experiments used slices of hippocampal tissues from animals exposed *in vivo*; and molecular biology studies investigated whether exposures resulted in changes in gene and protein expression in the hippocampus.

From a scientific point of view, there were no significant problems encountered by the project throughout its duration. The main problem experienced was related to changes or losses in members of staff allocated to the project in all three centres. This resulted in the need for an extension to the length of the project being granted to enable the gene expression studies to be finished. The other memorable problem was of a technical nature, and related to obtaining purchasing the components for the exposure system, in that one piece of equipment did not perform to its published specifications and this resulted in a delay in producing the system while a replacement was sourced and obtained. Otherwise the project remained very much on schedule and to budget. Particular credit should go to Dr Anna Bottomley for not only running the behavioural studies very proficiently but for organizing the day-

to-day exposure schedules for both the behavioural and gene expression studies: it was her dedication that ensured much of the success of this project.

The objectives of this study were to determine whether exposure of C57BL mice to RF fields associated with mobile telephones could influence brain physiology and function. These objectives were fully met, and the results provided no evidence that exposure to various RF fields across a range of SARs resulted in consistent changes in learned behaviour, hippocampal electrophysiology or in gene expression.

The loop antenna exposure system and many of the results have been presented at one national conference on health (held in Warwick, September 2003) and three international conferences on electromagnetic fields (held in Washington DC, June 2004; Dublin, June 2005; and Glasgow, September 2005) and it is intended to submit the combined results of the project to a peer-reviewed journal.

5. Interpretation

The results obtained in this project provided no evidence that exposure to the various RF fields used resulted in any consistent changes in physiology or functions of the hippocampus. Such a result is consistent with the majority of similar studies in this area using both animals and humans. Here, the effects of RF fields on learned behaviours, hippocampal electrophysiology and on gene and protein expression were investigated in order to assess effects on the brain. Each of these particular models has its own particular strengths and weaknesses, but taken together they provide powerful and compelling evidence that RF fields were not able to engender significant biological effects using either single or repeated exposures.

This approach, to seek concordant changes in hippocampal function using three well established methods, and so avoid potential criticisms about the need for independent replication of any particular result, would appear to be a unique strength of the project, and one that has not, to the best of our knowledge, been attempted before. In particular, the project exploited the exceptional power of gene chip profiling to assess the possibility that changes may occur in the brain following exposure to RF fields. It was envisaged that changes in the expression profiles of individual genes would be found following the statistical analysis. To ensure such results were not false positives, however, Taqman or western blot analysis were carried out. Furthermore, to ensure that heating effects caused by exposure to RF fields were not causing changes in gene expression, the levels of heat shock genes were also monitored. If field-dependent changes in electrophysiology or in behaviour were to have been reported then there would had to have been concomitant changes in gene and protein expression.

Nevertheless, it is acknowledged that a few changes were observed in this project. For example, the stimulus-response relationship for the population spike appeared to be depressed in the group of animals exposed to 400 MHz (but not other frequencies). However, the threshold for the response was unchanged, and no such difference was apparent in other pertinent measures of excitability. This suggested that the observed difference was probably due to experimental variation and did not reflect a real change in hippocampal function. Further confirmation was provided by experiments on long-term potentiation which provided no evidence for changes in long-lasting cellular processes. With a large and complex project of this nature, some false positive results are to be expected, and these and other isolated changes were not considered to form a consistent pattern of response or to produce any significant consequence of exposure.

6. Future Priorities

These results clearly indicate that single and repeated exposures to RF fields at the frequencies used in the project did not appear to have any lasting effects on brain physiology and function in adult mice. When the project was started, there were concerns that low level exposure to RF fields could have serious and dramatic consequences on brain function. Although some controversies remain, more recent work has tended to negate these concerns, as more studies have not reported convincing evidence to indicate that RF fields associated with mobile phones have detectable effects on the brain or behaviour in animals or man. If it is appropriate to generalize the results of the present project beyond the specific tasks, frequencies and exposures used, then it is possible to argue that further work on brain function in adult animals may not be required, except perhaps for obtaining independent replication of the present results.

The present project did not, however, address the possibility of effects on developing brains. Children and juveniles have been suggested to be more vulnerable to the effects of RF fields for a number of reasons (IEGMP, 2000) although the evidence remains equivocal (Sienkiewicz et al, 2005). Future studies could investigate this possibility by applying the methodology developed here to immature animals. Further evidence could be obtained by determining the effects of RF exposure on neurogenesis and neuronal development in the hippocampal formation of exposed mice. In vitro models of neuronal development could also help to address this question, for example, by using organotypic slice cultures of brain tissue.

7. Publications

Blackwell RP, Addison D, Bottomley AL and Sienkiewicz ZJ (2004). A novel head-only RF exposure system for mice. *Bioelectromagnetics Society Abstracts* 27, 277.

Bottomley AL, Bartram R, Blackwell RP, Haylock RGE and Sienkiewicz ZJ (2004). Effects of head-only exposure to radiofrequency fields on learned behaviour in mice. *Bioelectromagnetics Society Abstracts* 27, 260.

Bottomley A, Jones N, Haylock RGE, Saunders RD, Kuster N and Sienkiewicz ZJ (2005). Effects of exposure to RF fields on spatial learning processes in mice. *Abstracts, Joint BEMS and EBFA Conference, Dublin, June 2005, 292-293*

Saunders RD, Sienkiewicz ZJ, Bottomley AB, Jones NS, Cox R, Uney JB and Tattersall JEHT (2003). Studies on the possible effects of radiofrequency fields on brain physiology and function in mice. *Abstracts, Health Protection Agency Inaugural Conference, University of Warwick, September 2003, 184.*

Smith AJ, Underwood G, Harrison PK, and Tattersall JEHT (2004). Electrophysiological properties of hippocampal slices from mice exposed to 900MHz GSM fields. *Bioelectromagnetics Society Abstracts, 26, 257.*

Smith AJ, Harrison PK, Underwood G, Mifsud NCD and Tattersall JEHT (2005). Electrophysiological properties of hippocampal slices from mice exposed to electromagnetic fields. *Abstracts, Joint BEMS and EBFA Conference, Dublin June 2005, 507-508.*

Tattersall JEHT, Smith AJ, Harrison PK, Underwood G, Uney JB, Hobson RJ, Bottomley AL, Bartram R and Sienkiewicz ZJ (2004). The effects of radiofrequency radiation on brain physiology and function: a study in mice. *Bioelectromagnetics Society Abstracts* 26, 32.

Tattersall JEHT, Smith AJ, Harrison PK, Underwood G and Mifsud NCD (2005). Electrophysiological properties of hippocampal slices from mice exposed to radiofrequency fields. *E-M Technologies (RF-THz): Applications, Safety and Human Interaction. IoP/IPEM/IEE Workshop. Glasgow, September 2005.*

8. Financial summary

	Total project spend	VAT	Total spend including VAT	Original anticipated costs
HPA (NRPB)	114,904	20,108	135,012	170,018
Dstl	151,975	26,596	178,571	176,997
Bristol	243,647	42,638	286,285	243,647
Total	510,526	89,342	599,868	590,662

9. References

- Berenger J-P (1994). A perfectly matched layer for the absorption of electromagnetic waves. *J Comput Phys*, 114, 185-200.
- Cassel JC, Cosquer B, Galani R and Kuster N (2004). Whole-body exposure to 2.45 GHz electromagnetic fields does not alter radial-maze performance in rats. *Behav Brain Res*, 155, 37-43.
- Carli M, Robbins TW, Evenden JL, and Everitt BJ (1983). Effects of lesions to ascending noradrenergic neurones on performance of a 5-choice serial reaction task in rats; implications for theories of dorsal noradrenergic bundle function based on selective attention and arousal. *Behav Brain Res*, 9, 361-80.
- Chou CK, Chan KW, McDougall JA and Guy AW (1999). Development of a rat head exposure system for simulating human exposure to RF fields from handheld wireless telephones. *Bioelectromagnetics*, 20, 75-92.
- Cosquer B, Kuster N and Cassel JC (2005). Whole-body exposure to 2.45 GHz electromagnetic fields does not alter 12-arm radial-maze with reduced access to spatial cues in rats. *Behav Brain Res*, 161, 331-4.
- Cobb BL, Jauchem JR and Adair ER (2004). Radial arm maze performance of rats following repeated low level microwave radiation exposure. *Bioelectromagnetics*, 25, 49-57.
- Dimbylow PJ (2002). Fine resolution calculations of SAR in the Human Body for frequencies up to 3 GHz," *Phys Med Biol*. 47, 2835-2846.
- Dubreuil D, Jay T and Edeline JM (2002). Does head-only exposure to GSM-900 electromagnetic fields affect the performance of rats in spatial learning tasks? *Behav Brain Res*, 129, 203-10.
- Dubreuil D, Jay T and Edeline JM (2003). Head-only exposure to GSM 900-MHz electromagnetic fields does not alter rat's memory in spatial and non-spatial tasks. *Behav Brain Res*, 145, 51-61.
- Gabriel C (1995). *Compilation of the dielectric properties of body tissues at RF and microwave frequencies*. Report prepared for the NRPB by Microwave Consultants Ltd.
- Gabriel C, Gabriel S and Corthout (1996a). The dielectric properties of biological tissues: 1. Literature Survey. *Phys Med Biol*, 41, 2231-2249.
- Gabriel S, Lau R W and Gabriel C (1996b). The dielectric properties of biological tissues: 2. Measurements in the frequency range 10 Hz to 20 GHz. *Phys Med Biol*, 41, 2251-2269.
- Gabriel S, Lau R W and Gabriel C (1996c). The dielectric properties of biological tissues: 3. Parametric models for the dielectric spectrum of tissues. *Phys Med Biol*, 41, 2271-2293.
- Lai H (2004). Interaction of microwaves and a temporally incoherent magnetic field on spatial learning in the rat. *Physiol Behav*, 82, 785-9.
- IEGMP (2000). Mobile Phones and Health. Independent Expert Group on Mobile Phones, Chilton. ISBN 0-85951-450-1.
- NRPB (2004). Review of the Scientific Evidence for Limiting Exposure to Electromagnetic Fields (0-300 GHz). *Doc NRPB* 15 No3, 1-195
- Morgan, SL and Teyler TJ (2001). Electrical stimuli patterned after the theta-rhythm induce multiple forms of LTP. *J Neurophysiol*, 86, 1289-1296.
- Sienkiewicz ZJ, Blackwell RP, Haylock RG, Saunders RD and Cobb BL (2000). Low-level exposure to pulsed 900 MHz microwave radiation does not cause deficits in the performance of a spatial learning task in mice. *Bioelectromagnetics*, 21, 151-8.
- Sienkiewicz Z, Jones N and Bottomley A (2005). Neurobehavioural effects of electromagnetic fields. *Bioelectromagnetics*, Suppl 7, S116-26.
- Taflove A (1995). *Computational Electromagnetics - The Finite Difference Time Domain Method*. London, Artech.
- Tattersall JEH, Scott IR, Wood SJ, Nettell JJ, Bevir MK, Wang Z, Somasiri NP and Chen X (2001). Effects of low intensity radiofrequency electromagnetic fields on electrical activity in rat hippocampal slices. *Brain Res*, 904, 43-53.

MTHR Scientific Co-ordination Team
www.mthr.org.uk

c/o Health Protection Agency
Centre for Radiation Chemical and
Environmental Hazards
Chilton, Didcot, Oxfordshire OX11 0RQ