To appear in: *Proceedings of the 15th International Symposium on Electromagnetic Compatibility*
Zurich, Switzerland (2003)

CELL MORTALITY IN MAGNETITE-PRODUCING BACTERIA EXPOSED TO GSM RADIATION

J. Dobson*, CG Cranfield*, J Al Maddan*, HG Wieser**
*Centre for Science & Technology in Medicine, Keele University, Thornburrow Drive, Hartshill, Stoke-on-Trent, ST4 7QB United Kingdom
**Neurologische Klinik, Universitätsspital Zürich, Frauenklinikstrasse 26, CH-8091, Zurich, Switzerland
(Principal contact: jdobson@keele.ac.uk)

Abstract: Ferromagnetic transduction models based on the presence of biogenic magnetite (Fe_3O_4) in the human brain have been proposed as a potential mechanism for mobile phone bioeffects. These models include ferromagnetic resonance effects in biogenic magnetite at mobile telecommunication frequencies [1] and magnetite-mediated mechanical activation of transmembrane ion channels [2]. We have tested these models experimentally for the first time using a bacterial analogue (*M. magnetotacticum*) which produces intracellular biogenic magnetite similar to that present in the human brain. Experimental evaluation revealed that exposure to mobile phone emissions resulted in a consistent and significantly higher proportion of cell death in exposed cultures vs. sham exposure ($p=0.037$). Separate examination of the RF (1.8 GHz) component using a controlled exposure system (REFLEX) did not reveal a significant effect. Though there appears to be a repeatable trend towards higher cell mortality in magnetite-producing bacteria exposed to mobile phone emissions, it is not yet clear that this would extrapolate to a deleterious health effect in humans.

1. Introduction

Mobile phones generate non-ionizing radiofrequency electromagnetic radiation generally at either 900MHz or 1.8GHz. However, in addition to the RF signal, there are lower frequency pulsed components associated with Time Division Multiple Access (TMDA) at 217Hz and 8.34Hz and a 2Hz Discontinuous Transmission (DTX) component produced when the user is connected but not speaking [3]. These low frequency components not only pulse the RF carrier wave, they also generate low frequency magnetic fields via batter current pulses.

The rapid proliferation of mobile phones has resulted in a public controversy surrounding possible health effects connected to their use. Though there are strict guidelines governing the power output of these devices, there have been many reports of non-thermal bioeffects from mobile phone-type exposure [e.g. 4,5]. Experimental evidence has been presented which supports both sides of the debate [4,5,6].

A major issue which is not often addressed in electromagnetic compatibility research, however, is that of a transduction mechanism. In order to properly assess the validity of any experimental results demonstrating mobile phone bioeffects, it is necessary to understand the mechanism by which these effects may occur. Though this area of research has been highlighted by the World Health Organization's EMF programme, there are few theoretical mechanisms which have been proposed or thoroughly evaluated experimentally [7].

Mechanisms based on ferromagnetic transduction via nanoscale biogenic magnetite have a sound biophysical basis but have not been thoroughly evaluated experimentally. Magnetite is a ferrimagnetic iron oxide which can couple strongly to external electromagnetic fields due to its permanent magnetic moment. This material was first discovered in human brain tissue in 1992 and has since been confirmed to be present as a naturally occurring iron phase in many regions of the brain with particularly high concentrations in the meninges (the outermost region of the brain closest to the mobile phone when in use) [8,9,10]. Transduction of mobile phone signals via biogenic magnetite can be accomplished in two ways: (i) mechanical activation/disruption of normal cellular processes due to low frequency battery current pulses [2]; (ii) local deposition of energy due to ferromagnetic resonance [1].

In the case of magnetic activation of ion channels, low frequency magnetic fields generated by battery current pulses at 2 Hz (DTX) exert a torque on nanoscale magnetite particles coupled to the cell membrane either directly or indirectly via cytoskeletal attachment. This torque produces a mechanical deformation of the membrane which activates mechanosensitive ion channels.

In the ferromagnetic resonance model, the RF signal is coupled to the magnetization vector causing it to resonate if the particle size and shape are consistent for resonance at the transmission frequency. This resonance excites vibrations in the magnetite crystal lattice which could disrupt normal cellular function depending on the location of the magnetite within the cell.

Though ferromagnetic transduction represents one of the most plausible mechanisms for bioeffects, the model has not been tested experimentally on cells. This is primarily due to the fact that magnetite-bearing cells from the human brain have been neither isolated nor cultured. Magnetic and electron microscopic analyses of human brain tissue have revealed that biogenic magnetite in human brain tissue is similar to that produced by the magnetotactic bacterium *M. magnetotacticum* [8,9]. Based on these findings, our group has been investigating the possibility of using *M. magnetotacticum* as a proxy for testing ferromagnetic transduction models [11]. The presence and characterization of mechanosensitive ion channels and biomineralized intracellular magnetite in *M. magnetotacticum* make it an excellent system for examining these models. Here we present the results of the first experimental evaluation of the ferromagnetic transduction models.

2. Methods

2.1 Bacterial Samples

M. magnetotacticum were cultured in ATCC revised magnetospirillum growth medium 1653 (MSGM) under anaerobic conditions at 30°C in airtight glass tubes. As it can be difficult to get *M. magnetotacticum* to produce magnetite in culture, before each experiment samples were checked to confirm that the bacteria were producing magnetite by observing their response to changes in magnetic fields under a light microscope. Magnetotactic bacteria are motile, gram-negative bacteria which use the Earth's magnetic flux lines for orientation during swimming [12]. Under microscopic observation, bacteria which are producing magnetite would reverse their swimming direction with a reversal of the magnetic field. Bacteria which were not producing magnetite were still motile, indicating viability, but did not respond to changes in the magnetic field. Bacterial samples also were observed using Transmission Electron Microscopy (TEM), in which the magnetite particles are clearly visible as electron-dense chains within the bacterium (Figure 1).

For experiments conducted using the REFLEX system (see section 2.3) a second bacterial strain - *Azoarcus spirillum* strain CC-26 - was used as an additional control. This strain is very similar to *M. magnetotacticum* but does not produce magnetite [13].

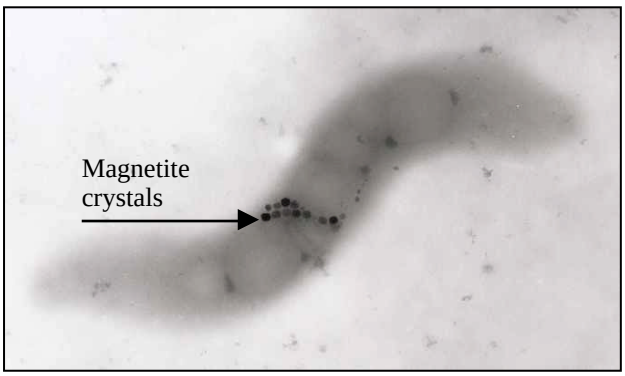

Figure 1: Transmission electron micrograph of the magnetotactic bacterium *M. magnetotacticum.* Opaque (electron-dense) particles are crystals of biogenic magnetite.

2.2 Mobile Phone Handset Experiments

For each experiment, 1 ml of equally concentrated magnetotactic bacterial cells was placed in individual sterile plastic tubes and divided into two groups – exposed and control. Each tube was placed on a custom-built plastic mount directly above the base of the mobile phone antenna (Figure 2). Samples were exposed to either 16 minutes of mobile phone emissions using a Motorola MR602 mobile phone with BT Cellnet, or 16 minutes of sham exposure. For sham exposures the mobile phone was left in position but switched off.

Custom-designed LabVIEW software running on a Macintosh G3 laptop computer produced a continuous tone through headphones connected to the computer's sound output port. One ear-piece of the headphones was placed over the microphone of the mobile phone. The tone was generated at two minutes on / two minutes off throughout the 16 min. exposure. This was done to create periods of discontinuous transmission signals (DTX – a 2Hz signal generated when the user is not speaking). Sham samples were placed in the same position on the plastic mount and exposed to the same tone generation sequence with the phone switched off.

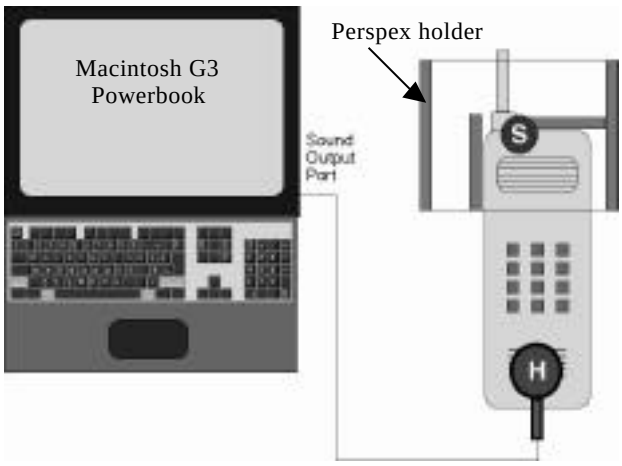

Figure 2: Schematic representation of the experimental set-up. **S** is the position of the sample. **H** is the position of the headphone speaker.

Following exposure, or sham exposure, cell mortality was assayed. Each 1ml sample was loaded with 1.5µl of each of the BacLight fluorescent viability kit components (Molecular Probes -3.34mM SYTO® 9 solution in DMSO and 20mM propidium iodide solution in DMSO) and placed on an electric rocker for 15 minutes. Samples were then loaded into a cuvette and analyzed in a Perkin-Elmer LS50B fluorescence spectrophotometer. Excitation was set at 470nm and the ratio of integrated intensities between 510-540nm and 620-650nm was determined (i.e. Green fluorescence/Red fluorescence). This gives a ratio of live (green) to dead (red) cells.

A one-tailed Student's t-test was performed on the control and exposed group for each experiment. In addition, a two-way ANalysis Of VAriance (ANOVA) was performed on the combined results.

2.3 Radiofrequency REFLEX Experiments

Experiments also were conducted using the REFLEX exposure system which provides very well controlled dosimetry along with continual monitoring of temperature conditions during the experiments [14] (Figure 3). This system consists of two 1.8GHz waveguides housed in a CO_2 incubator with continuous temperature monitoring. Each waveguide holds six petri dishes. The signal unit consists of an RF signal generator (R&S SMY 02B) modulated by an arbitrary function generator (Agilent 33120A) with a GSM frame generator which enables switching between DTX and non-DTX. RF exposure is controlled via a computer interfaced to the signal unit which excites one of the waveguides at random, creating blind experimental conditions.

Three millilitres of equally concentrated magnetotactic bacterial cells or CC-26 cells were placed in 12 individual tissue culture petri dishes (40mm x 12mm). Each culture dish was sealed with laboratory film to make them airtight. The samples were then labelled with either a "1" or a "2" and appropriately placed into the two waveguides, also labelled "1" and "2". The waveguides were housed inside a temperature-controlled incubator set at 30°C. The computer-controlled REFLEX exposure system was then pro-

grammed to produce a maximum 2 W/Kg dose of 1.800GHz RF radiation to one of the waveguides. This is done in a blind fashion so the experimenter does not know which waveguide has been excited until the assays are complete. Signals were pulsed at 217Hz and included an 8Hz component (every 26th pulse was blanked). These signals are similar to those signals produced by mobile phone devices.

Exposure duration was 30 minutes (with 34% DTX simulation) and exposure data were recorded onto the computer hard drive as an encrypted file. The file holds information on which waveguide is excited for each experiment as well as temperature monitoring data. The data were sent to IT'IS for decryption after the completion of each experiment.

Following exposure, or sham exposure, cell mortality was again assayed using the BacLight fluorescent viability kit. A one-tailed Student's t-test was performed on the control and exposed groups for each experiment.

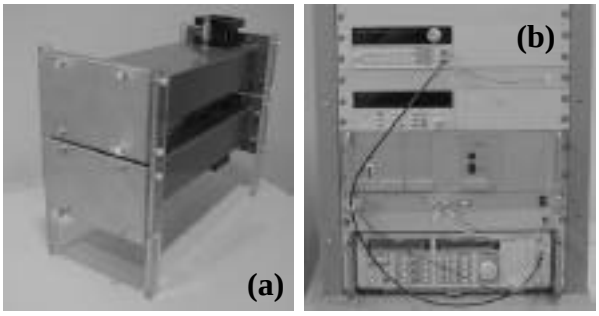

Figure 3: The REFLEX system: (a) two 1.8 GHz waveguides which fit into an incubator, and (b) the control electronics.

3. Results

3.1 Mobile Phone Experiments

The mobile handset exposure experiment was performed four times. In the first three experiments, the bacteria were confirmed to be producing magnetite by their response to magnetic fields under microscopic investigation. In the fourth experimental group, light microscopy investigation showed that the bacteria did not respond to changes in the magnetic field although they were motile – indicating they were viable but producing no magnetite.

Table 1: Results of the BacLight viability assay for four experimental runs where N_C = number of control (sham) samples and N_E = number of experimental samples. Mean is the mean fluorescence ratio as described in the methods section. P is the probability based on Student's t-test for experimental runs 1-4 and on two-way analysis of variance [ANOVA] of the combined data from runs 1-3 (magnetite producing bacteria).

Experimental Run	N_C/N_E	Mean (Sham)	Mean (Exposed)	P
1	5/5	2.144	2.022	0.089
2	5/5	10.03	9.773	0.110
3	10/10	10.66	10.27	0.061
4 (non-magnetic)	10/10	11.97	12.62	0.223
ANOVA	20/20			**0.037**

In the three experiments on magnetite-producing bacteria, each experiment resulted in a higher proportion of dead cells in the samples exposed to mobile phone emissions vs. the sham cultures (Table 1). For the experiment on non-magnetic *M. magnetotacticum*, cell mortality was higher in the sham group, though this was not significant ($p = 0.22$). Though each of the three experiments on magnetite-producing cultures showed a consistently higher cell mortality with exposure, individually the results were not significant at the 95% confidence level. Two of the experiments on magnetite-producing bacterial cultures were significant at the 90% confidence level and a combined two-way ANOVA on the three experiments with magnetic bacteria was significant ($p = 0.037$; Table 1).

3.2 REFLEX Experiments

Results of the five REFLEX experiments conducted on the non-magnetite-producing CC-26 bacterial strain did not show any consistent bioeffects due to RF exposure (Table 2). Cell mortality was not consistently higher in either group and none of the experiments reached statistical significance.

Nine initial REFLEX experiments conducted on *M. magnetotacticum* produced more ambiguous results. In eight of the nine experiments, cell mortality rates were higher in the exposed cultures than in the sham cultures – significantly so in two of the eight (Table 3). In one of the nine experiments, mortality was significantly higher in the sham cultures.

These experiments have since been repeated using a new BacLight kit and no significant trends were observed (Table 4).

Table 2: Results of the first nine experimental runs using the REFLEX system. N = 6 sham and 6 exposed for each experimental run. Mean is the mean fluorescence ratio. P is the probability from a one-tailed Student's t-test. Significant results are in bold.

Experimental Run	Mean (Sham)	Mean (Exposed)	P
1	7.57	7.56	0.48
2	5.24	5.09	0.18
3	6.45	6.33	0.31
4	6.19	5.88	**0.04**
5	6.37	5.95	**0.02**
6	2.92	3.08	**<0.01**
7	5.58	5.37	0.24
8	3.15	3.00	0.15
9	3.77	3.76	0.41

Table 3: Results of the five REFLEX experimental runs on the CC-26 non-magnetic bacteria. N = 6 sham and 6 exposed for each experimental run. Mean is the mean fluorescence ratio. P is the probability from a one-tailed Student's t-test.

Experimental Run	Mean (Sham)	Mean (Exposed)	P
1	18.28	18.76	0.12
2	33.07	32.76	0.26
3	24.01	24.98	0.25
4	12.10	12.27	0.29
5	16.90	17.08	0.36

Table 4: Results of the five REFLEX experimental runs using the new BacLight kit. N = 6 sham and 6 exposed for each experimental run. Mean is the mean fluorescence ratio. P is the probability from a one-tailed Student's t-test.

Experimental Run	Mean (Sham)	Mean (Exposed)	P
1	18.33	18.33	0.49
2	21.75	22.65	0.07
3	21.13	21.31	0.33
4	16.05	16.03	0.48
5	18.14	18.31	0.11

4. Discussion & Conclusions

The results of the four mobile phone handset experiments show a consistent increase in cell mortality with exposure. Though individual results were not significant at 95%, a two-way ANOVA showed that the combined results were significant. This provides an indication that ferromagnetic transduction of mobile phone emissions has resulted in increased mortality in magnetite-producing cultures of *M. magnetotacticum*. That the effects is due to ferromagnetic transduction is somewhat supported by the results on non-magnetic cultures, though there is not enough data to strongly support this.

Although the final REFLEX experiments using a new BacLight kit did not confirm the trend observed in the first nine, it is difficult to imagine how the assay could have been systematically affecting the cultures when waveguide excitation is random. Though only two of the first nine results were significant, eight of the nine showed the same trend (higher mortality in the exposed cultures). A binomial probability test on these first experiments (k=8, N=9) shows p=0.02. However, a binomial analysis performed on only those results which were significant (k=2, N=3) shows p=0.375.

Taken together, the results of this study as a whole are inconsistent. The mobile phone experiments appear to indicate that there is an effect on cell mortality in magnetite-producing bacteria, however, this is not confirmed by the REFLEX experiments. The major difference in these two exposures (handset & REFLEX) is that in the REFLEX system, the low frequency magnetic field (battery current) pulses are absent. Though the system simulates DTX and TDMA, the cultures in the waveguides are still only exposed to RF radiation pulsed with these frame structures – not with a pulsed magnetic field as is the case in a real handset. This may be an indication that the low frequency battery current components are very important in the examination of mobile phone bioeffects and could be responsible for effects observed in the handset experiments.

It should be stressed that it is not clear whether the effects observed in the handset experiments would extrapolate to a deleterious health concern in humans. This point is particularly important in light of the fact that the distribution and particle characteristics of biogenic magnetite *in vivo* in the human brain are not well constrained. We are currently in the process of further assessing these models and examining the effects of RF and low frequency components of mobile phone emissions separately.

5. Acknowledgements

This work was supported by the ETH/Sunrise Telecommunications Research Co-operation for Sustainable Mobile Communication. We would like to thank Dr. Quentin Pankhurst, Dr. John Thompson, Prof. Niels Kuster, Dr. Jurgen Schuderer and Dr. Kate Bowers for technical assistance and helpful discussions. We also thank Dr. Nabuo Kato for supplying the CC-26 strain.

6. References

[1] Kirschvink, JL (1996) Microwave absorption by magnetite: A possible mechanism for coupling non-thermal levels of radiation to biological systems. *Bioelectromagnetics* 17: 187-194.

[2] Dobson, J and TG St. Pierre (1996) Application of the Ferromagnetic Transduction Model to D.C. and Pulsed Magnetic Fields: Effects on Epileptogenic Tissue and Implications for Cellular Phone Safety. *Biochem. Biophys. Res. Commun.,* **227**: 718-723.

[3] Hyland, G. Physics and biology of mobile telephony. *The Lancet* **356**, 1833-1836 (2000).

[4] Repacholi, MH, A Basten, V Gebski, D Noonan, J Finnie, AW Harris (1997) Lymphomas in Em-Pim1 transgenic mice exposed to pulsed 900 Mhz electromagnetic fields. *Rad. Res.* **147**: 631-640.

[5] De Pomerai, D, C Daniells, H David, J Allan, I Duce, M Mutwakil, D Thomas, P Sewell, J Tattersall, D Jones, P Candido (2000) Non-thermal heat-shock response to microwaves. *Nature* **405**: 417 - 418.

[6] Utteridge, TD, V Gebski, et al. (2002) Long-term exposure of Eμ-Pim1 transgenic mice to 898.4 MHz microwaves does not increase lymphoma incidence. *Radiat Res.* **258**: 357-364.

[7] Repacholi, MH (1998) Low-level exposure to radiofrequency electromagnetic fields: Health effects and research needs. *Bioelectromagnetics* **19**: 1-19.

[8] Kirschvink, JL, A Kobayashi-Kirschvink, BJ Woodford (1992) Magnetite biomineralization in the human brain. *Proc. Natl. Acad. Sci., USA* **89**: 7683-7687.

[9] Schultheiss-Grassi, PP, R Wessiken, J Dobson (1999) TEM observation of biogenic magnetite extracted from the human hippocampus. *Biochim. Biophys. Acta* **1426**: 212-216.

[10] Dunn, JR, M Fuller, J Zoeger, J Dobson, F Heller, E Caine, BM Moskowitz (1995) Magnetic material in the human hippocampus. *Brain Res. Bull.* **36**: 149-153.

[11] Stewart, Z, B Martinac, J Dobson (2000) Evidence for mechanosensitive transmembrane ion channels of small conductance in magnetotactic bacteria. *Electro- and Magnetobiol.* **19**: 81-89.

[12] Blakemore, RP (1975) Magnetotactic bacteria. *Science* **190**: 377-379.

[13] Shinoda, Y, Y Sakai, M Ue, A Hiraishi, N Kato (2000) Isolation and characterization of a new denitrifying spirillum capable of anaerobic degradation of phenol. *Appl. Env. Microbiol.* **66**: 1286-1291.

[14] The REFLEX exposure system was designed at the Institute for Information Technology in Society, Zurich, Switzerland. Information on the specifics of the design can be found at http://www.it is.ethz.ch/results/results1-03.html.