

C A N A D A

PROVINCE OF QUEBEC

DISTRICT OF MONTREAL

DOCKET No. R-3770-2011

RÉGIE DE L'ÉNERGIE / ENERGY BOARD

AUTHORIZATION OF AN INVESTMENT BY
HYDRO-QUEBEC DISTRIBUTION –
ADVANCED METERING PROJECT
PHASE 1

HYDRO-QUEBEC
As Electricity Distributor

Petitioner

-and-

STRATEGIES ENERGETIQUES (S.E.) /
ENERGY STRATEGIES (E.S.)

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

Intervenors

ARTICLES MENTIONED IN THE GRAPHS OF SECTION 63 OF DR. CARPENTER'S REPORT

(CLASSIFICATION OF SCIENTIFIC STUDIES
SHOWING BIOLOGICAL AND HEALTH EFFECTS
FROM EXPOSURES AT MUCH LOWER LEVELS THAN FCC LIMITS)

Referred to in **David O. CARPENTER**, *Expert Report*, Revised on May 14, 2012,
C-SE-AQLPA-0072, SE-AQLPA-7, Doc. 1.1, parag. 63.

Filed on June 4, 2012

Régie de l'énergie / Quebec Energy Board - Docket no. R-3770-2011

Authorization of an investment by Hydro-Quebec Distribution – Advanced Metering Project Phase 1

Exhibit SE-AQLPA-7 - Document 29

*Articles mentioned in the Graphs of Section 63 of Dr. Carpenter's Report
(Classification of scientific studies showing biological and health effects
from exposures at much lower levels than FCC limits)*

Attachment to the Expert Report of David O. Carpenter

Filed by Stratégies Énergétiques (S.É.) / Energy Strategies (E.S.) and the AQLPA

ARTICLES MENTIONED IN THE GRAPHS OF SECTION 63 OF DR. CARPENTER'S REPORT

**(CLASSIFICATION OF SCIENTIFIC STUDIES
SHOWING BIOLOGICAL AND HEALTH EFFECTS
FROM EXPOSURES AT MUCH LOWER LEVELS THAN FCC LIMITS)**

63. In the *BioInitiative Report*, we had recommended, as a precautionary or prudent measure, to limit RF exposure to a maximal power density of $1000 \mu\text{W}/\text{m}^2$ outside and $100 \mu\text{W}/\text{m}^2$ inside, even though there is no certainty of a complete absence of risk even under these limits, pending further research. These limits were therefore a reasonable assessment, given the possible risks already identified by current research.

Meeting these precautionary or prudent limits may be accomplished even as the existing standards remain unchanged for the moment. As further discussed, precautionary or prudent measures are, by definition, additional to existing standards as a means for managing scientific uncertainty or as interim measures during the process that could lead, in the future, in changing the standards.

These recommendations are the result of several scientific observations including those mentioned in the following graphs :

Cell Phone Frequency Studies Reporting Bioeffects and Adverse Health Effects Below FCC Safety Limits (Power Density)

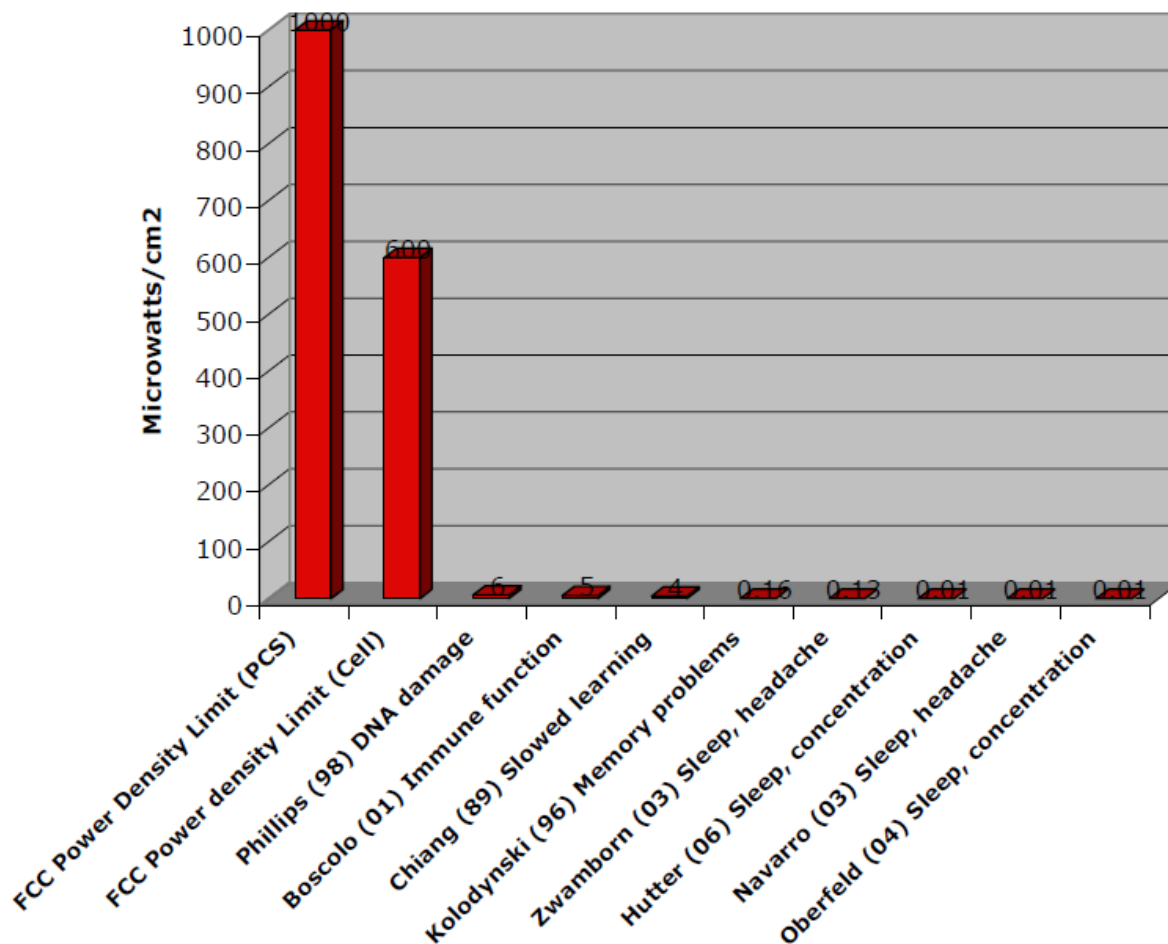


Exhibit SE-AQLPA-7 - Document 29

Articles mentioned in the Graphs of Section 63 of Dr. Carpenter's Report
 (Classification of scientific studies showing biological and health effects
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SAR Studies at Cell Phone Frequencies Reporting Bioeffects and Adverse Health Effects Below FCC Safety Limits

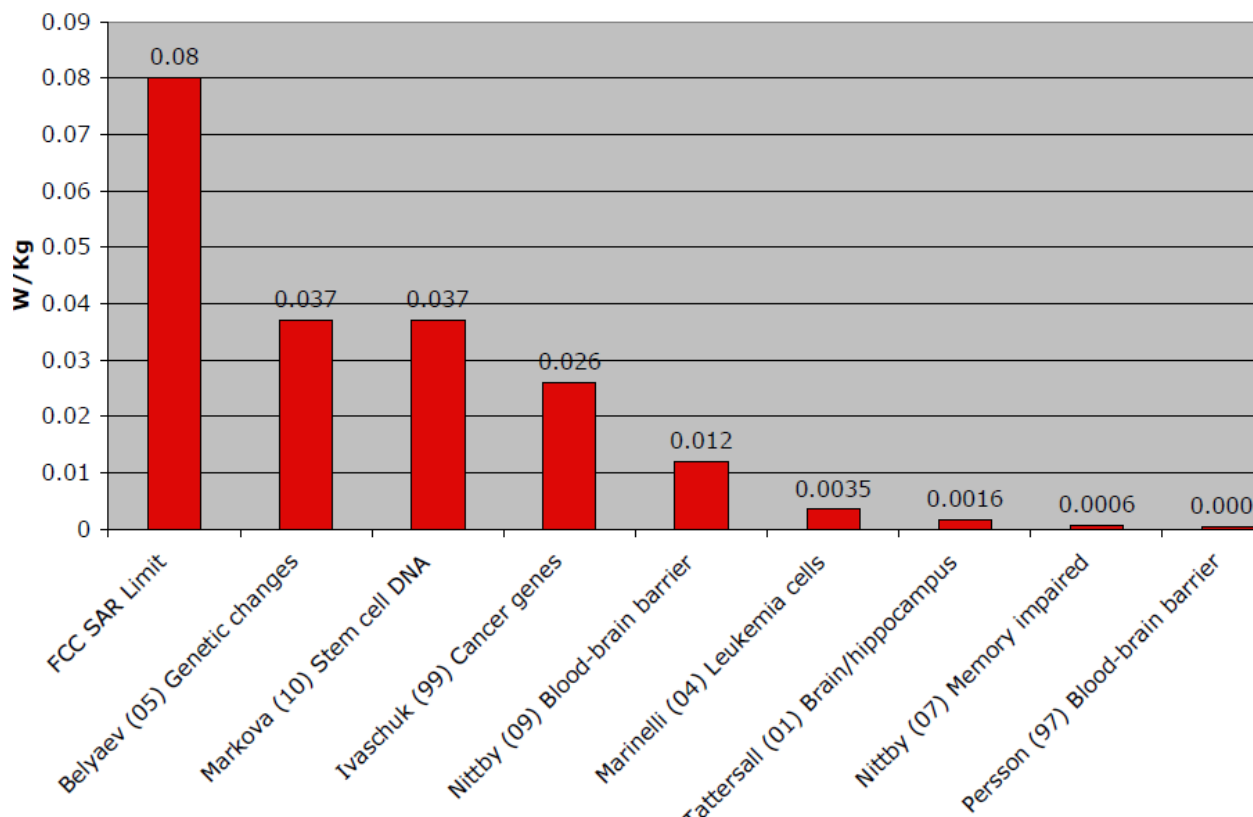


Exhibit SE-AQLPA-7 - Document 29

Articles mentioned in the Graphs of Section 63 of Dr. Carpenter's Report
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SAR Studies at Cell Phone Frequencies Reporting Bioeffects and Adverse Health Effects (Children absorb 45% more RF)

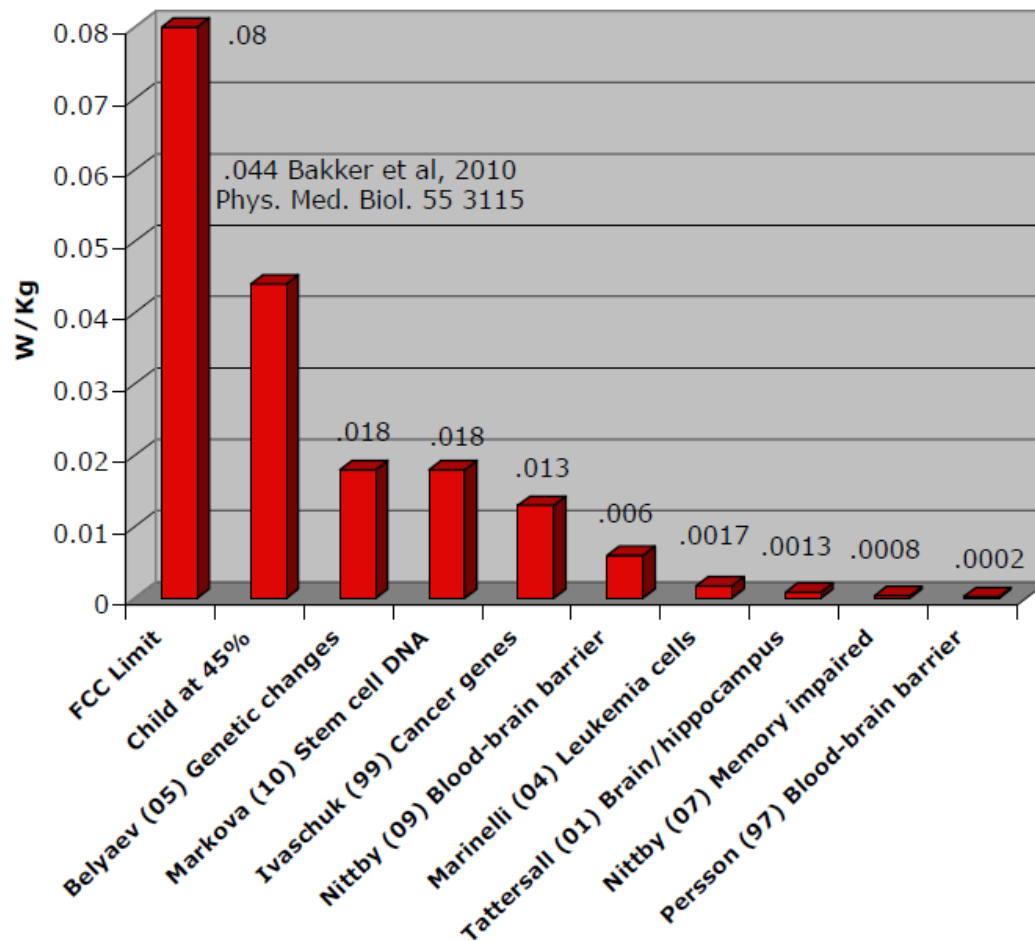


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Articles mentioned in the Graphs of Section 63 of Dr. Carpenter's Report
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Power Density: Radio frequency Non-ionizing Radiation

'... the possibility of harm from exposures [to low levels of radio frequency radiation] insufficient to cause important heating of tissues cannot yet be ruled out with confidence. Furthermore, the anxieties that some people feel when this uncertainty is ignored can in themselves affect their well-being.'

Sir William Stewart (Chairman)
*Mobile Phones and Health:
A report from the Independent Expert Group
on Mobile Phones,
(The Stewart Report, 2000)*

Power Density: Definition

Above 30 MHz, the usual unit of measurement is power density, though electric and magnetic fields can also be measured. It is usually expressed in milli- or microwatts per square centimetre (mW/cm² or µW/cm²), and is defined as the amount of power per unit area in a radiated microwave field or other type of electromagnetic field.

Introduction

Research from abroad, partially replicated (and in some instances expanded on) by scientists in English speaking countries, indicates many potential benefits in health, wellbeing and work productivity can be obtained from developing a more comprehensive understanding of potential EMF bio-effects. They also indicate ways in which present communications systems, and the electromagnetic nature of the microenvironments individuals occupy, can be improved to benefit all. It is suggested that the potential cost benefits of adopting improved EMF-hygiene protocols and developing new generations of technology that can actually improve biological functioning and human performance is immense, makes tremendous commercial sense and present enormous commercial opportunities.

As can be seen in the following table, both beneficial and detrimental biological effects are indicated at exposure levels far lower than those required for 'thermal effects', the traditional marker used to set many guidelines on presently 'acceptable' power density levels.

The Precautionary Principle/Approach?

'... [the] actions taken under the precautionary principle should be commensurate with anticipated risks of health detriment.'

Section 6.14
The Stewart Report, 2000

The need for additional impartial scientific research appears warranted to address such concerns for everyone's benefit and that of the planet...

**Power Density –
International Regulations and Possible Biological Effects**

Power Density	Reported Biological Effects / Comments	References
0.0000000000000001 $\mu\text{W}/\text{cm}^2$	Cosmic background at 1800 MHz approx. average	Powerwatch, International Guidance Levels, www.powerwatch.org.uk/gen/intguidance.asp
0.0000000001 $\mu\text{W}/\text{cm}^2$	Natural background level for all RF frequencies	Powerwatch, International Guidance Levels, www.powerwatch.org.uk/gen/intguidance.asp
0.0000000001 $\mu\text{W}/\text{cm}^2$	Threshold of human sensitivity	N.N. Kositsky, A.I. Nizhelska and G.V. Ponezha (2001), Influence of high-frequency electromagnetic radiation at non-thermal intensities on the human body (a review of work by Russian and Ukrainian researchers) <i>Translation by Patricia Ormsby, No Place To Hide</i> , 3(1) Supplement. www.emfacts.com/ussr_review.pdf
0.0000000001 – 0.00000001 $\mu\text{W}/\text{cm}^2$	Normalising effect on cell growth of isolated cells damaged by ionising radiation exposed for 7 minutes	L.S. Bundyuk, A.P. Kuz'menko, N.N. Ryabchenko and G.S. Litvinov (1994), Corrective action of millimeter waves on systems of various levels of hierarchy. <i>Physics of the Alive</i> , 2(1):12-25, cited by Kositsky et al 2001.
0.0000000002 $\mu\text{W}/\text{cm}^2$	Mobile phone handsets can work down to about this level	Powerwatch, International Guidance Levels, www.powerwatch.org.uk/gen/intguidance.asp
0.0000000001 $\mu\text{W}/\text{cm}^2$	Altered EEG in humans – a relaxation frequency of protein-bound water thought to occur between 100 - 1,000 MHz. Absorption and quantum effects may be the mechanistic basis for EEG changes noted in most subjects from 0.000000001 $\mu\text{W}/\text{cm}^2$ CW RF energy of 130-960 MHz.	W. Bise (1978), Low power radio-frequency and microwave effects on human electroencephalogram and behavior. <i>Physiological Chemistry and Physics</i> , 10(5):387-398. www.ncbi.nlm.nih.gov (abstract)
0.0000000027 $\mu\text{W}/\text{cm}^2$	Growth stimulation in <i>Vicia faba</i>	Brauer (1950), Experimental studies on the effect of meter waves of various field intensities on the growth of plants by division. <i>Chromosoma</i> 3:483-509.
0.000000001 $\mu\text{W}/\text{cm}^2$	Effects on immune system of mice exposed for 5 minutes per day for 5 days to 54-76 GHz at this level	L.S. Bundyuk, A.P. Kuz'menko, N.N. Ryabchenko and G.S. Litvinov (1994), Corrective action of millimeter waves on systems of various levels of hierarchy. <i>Physics of the Alive</i> , 2(1):12-25, cited by Kositsky et al 2001.
0.000000002 $\mu\text{W}/\text{cm}^2$	Stimulation of ovulation in chickens	P.A. Kondra, W.K. Smith, G.C. Hodgson, D.B. Bragg, J. Gavora, M.A.K. Hamid and R.J. Boulanger (1970), Growth and reproduction of chickens subjected to microwave radiation. <i>Canadian Journal of Animal Science</i> 50:639-644, cited by A. Firstenberg 2001.
<0.000001 $\mu\text{W}/\text{cm}^2$	Altered EEG in humans – temporary changes in brain waves and behaviour.	W. Bise (1978), Low power radio-frequency and microwave effects on human electroencephalogram and behavior. <i>Physiological Chemistry and Physics</i> , 10(5):387-398. www.ncbi.nlm.nih.gov (abstract)
0.000001 $\mu\text{W}/\text{cm}^2$	Burgerforum BRD proposal, sleeping areas (1999)	Powerwatch, International Guidance Levels, www.powerwatch.org.uk/gen/intguidance.asp
0.000005 $\mu\text{W}/\text{cm}^2$	Effect on cell growth rate in yeast <i>S. cerevisiae</i>	W. Grundler and F. Kaiser (1992), Experimental evidence for coherent excitations correlated with cell growth. <i>Nanobiology</i> 1:163-176
0.00001 $\mu\text{W}/\text{cm}^2$	Conditioned 'avoidance' reflex in rats	N.N. Kositsky, A.I. Nizhelska and G.V. Ponezha (2001), Influence of high-frequency electromagnetic radiation at non-thermal intensities on the human body (a review of work by Russian and Ukrainian researchers) <i>Translation by Patricia Ormsby, No Place To Hide</i> , 3(1) Supplement. www.emfacts.com/ussr_review.pdf
0.000027 $\mu\text{W}/\text{cm}^2$	Premature aging of pine needles	Selga, T. & Selga, M. (1996), Response of <i>Pinus sylvestris</i> L. needles to electromagnetic fields. Cytological and ultrastructural aspects. <i>The Science of the Total Environment</i> 180:65-73, Elsevier Science BV.
0.0001 $\mu\text{W}/\text{cm}^2$	Burgerforum BRD proposal, waking areas (1999)	Powerwatch, International Guidance Levels, www.powerwatch.org.uk/gen/intguidance.asp
0.0001 $\mu\text{W}/\text{cm}^2$	Salzburg GSM/3G inside houses (2002)	Powerwatch, International Guidance Levels, www.powerwatch.org.uk/gen/intguidance.asp

Power Density	Reported Biological Effects / Comments	References
0.001 $\mu\text{W}/\text{cm}^2$	100 Yards from a Cellular Phone	A. Firstenberg (2001), Radio Wave Packet, www.goodhealthinfo.net/radiation/radio_wave_packet.pdf .
0.001 $\mu\text{W}/\text{cm}^2$	Exposure Limit in New South Wales, Australia as at 2001	A. Firstenberg (2001)
0.001 $\mu\text{W}/\text{cm}^2$	Salzburg GSM/3G outside houses (2002)	Powerwatch, International Guidance Levels, www.powerwatch.org.uk/gen/intguidance.asp
0.002 $\mu\text{W}/\text{cm}^2$	Sleep disorders, abnormal blood pressure, nervousness, weakness, fatigue, limb pain, joint pain, digestive problems, fewer schoolchildren promoted – controlled study near a shortwave transmitter	Altpeter <i>et al.</i> (1995, 1997), Study on health effects of the shortwave transmitter station of Schwarzenburg, Berne, Switzerland, Study No. 55, Swiss Federal Office of Energy), cited by A. Firstenberg 2001.
0.0027 $\mu\text{W}/\text{cm}^2$	Growth inhibition in <i>Vicius fabus</i>	I. Brauer (1950), Experimental studies on the effect of meter waves of various field intensities on the growth of plants by division. <i>Chromosoma</i> 3:483-509, cited by A. Firstenberg 2001.
0.0027 to 0.065 $\mu\text{W}/\text{cm}^2$	Smaller tree growth rings	Balodis, V., <i>et al</i> (1996), Does the Skruna Radio Location Station diminish the radial growth of pine trees? <i>The Science of the Total Environment</i> 180:57-64.
0.0048 $\mu\text{W}/\text{cm}^2$	Median level, 15 US cities 1977 (mainly VHF & TV)	Powerwatch, International Guidance Levels, www.powerwatch.org.uk/gen/intguidance.asp
0.007 $\mu\text{W}/\text{cm}^2$	50 Feet from a Cordless Phone	A. Firstenberg (2001).
0.01 $\mu\text{W}/\text{cm}^2$	Human sensation	Kolbun and Sit'ko (1987), Sensory indications by the human body of EHF-range electromagnetic radiation. <i>Mechanisms of Biological Action of Electromagnetic Radiation: Proceedings of the Pushchino Symposium</i> , 27-31 Oct. 1987, cited by A. Firstenberg 2001.
0.01 $\mu\text{W}/\text{cm}^2$	EU-Parl, GD Wissenschaft, STOA GSM (2001), Public Exposure Guidelines at 1800 MHz	Powerwatch, International Guidance Levels, www.powerwatch.org.uk/gen/intguidance.asp
0.016 $\mu\text{W}/\text{cm}^2$	1 Mile from a Cellular Tower	A. Firstenberg (2001)
0.04 – 0.2 $\mu\text{W}/\text{cm}^2$	SAR-value of 80-400 $\mu\text{W}/\text{kg}$, 0.002 V/m at 947.5 MHz	O. Johansson (1995), 'E'ö'verkänslighet samt ö'verkänslighet mot mobiltelefoner: Resultat från en dubbel-blind provokationsstudie av metodstudiekaraktär' (=Electrohypersensitivity and sensitivity to mobile telephones: Results from a double-blind provocation study of pilot character', in Swedish), Enheten för Experimentell Dermatologi, Karolinska Institutet, Stockholm, Rapport nr. 2, 1995, ISSN 1400-6111
0.06 $\mu\text{W}/\text{cm}^2$	Altered EEG, disturbed carbohydrate metabolism, enlarged adrenals, altered adrenal hormone levels, structural changes in liver, spleen, testes, and brain – in white rats and rabbits	Dumanskij & Shandala (1974), The biologic action and hygienic significance of electromagnetic fields of super-high and ultrahigh frequencies in densely populated areas. <i>Biologic Effects and Health Hazards of Microwave Radiation, Proceedings of an International Symposium</i> , Warsaw, 15-18 Oct. 1973, P. Czerski <i>et al.</i> , eds., cited by A. Firstenberg 2001.
0.05 $\mu\text{W}/\text{cm}^2$	10 Feet from a Wireless Computer	A. Firstenberg (2001).
0.06 $\mu\text{W}/\text{cm}^2$	Slowing of the heart, change in EEG in rabbits	Serkyuk, reported in McRee 1980, cited by A. Firstenberg 2001.
0.1 $\mu\text{W}/\text{cm}^2$	Italy (single frequency), Public Exposure Guidelines at 1800 MHz	Powerwatch, International Guidance Levels, www.powerwatch.org.uk/gen/intguidance.asp
0.1 $\mu\text{W}/\text{cm}^2$	Salzburg 1998 (sum GSM), Public Exposure Guidelines at 1800 MHz	Powerwatch, International Guidance Levels, www.powerwatch.org.uk/gen/intguidance.asp
0.1 $\mu\text{W}/\text{cm}^2$ (0.001 W/Kg SAR)	EEG brain waves altered under exposure to cell phone signal	L. Von Klitzing (1995), 'Low-Frequency pulsed electromagnetic fields influence EEG of man.' <i>Physica Medica</i> , Vol. 11, No. 2, pps 77-80, April-June 1995, cited by C. Sage 2004.

Power Density	Reported Biological Effects / Comments	References
0.1 $\mu\text{W}/\text{cm}^2$	Increased in melatonin in cows on 1 st night of re-exposure after 3-30 MHz transmitter inoperational for 3 days – difference in salivary melatonin concentration statistically significant, indicating a 2-7-fold increase of melatonin concentration.	K.D.C. stark , T. Krebs, E. Altpeter, B. Manz, C. Griot and T. Abelin (1997), Absence of chronic effect of exposure to short-wave radio broadcast signal on salivary melatonin concentrations in dairy cattle. <i>Journal of Pineal Research</i> 22(4):171-176.
0.1 to 1.8 $\mu\text{W}/\text{cm}^2$	Decreased life span, impaired reproduction, structural and developmental abnormalities in duckweed plants	Magone, I. (1996), The effect of electromagnetic radiation from the Skrunda Radio Location Station on <i>Spirodela polyrhiza</i> (L.) Schleiden cultures. <i>The Science of the Total Environment</i> 180:75-80.
0.13 $\mu\text{W}/\text{cm}^2$	Decreased cell growth (human epithelial amnion cells)	Kwee & Raskmark (1997), Radiofrequency electromagnetic fields and cell proliferation. In <i>Proceedings of the Second World Congress for Electricity and Magnetism in Biology and Medicine</i> , June 8-12, 1997, Bologna, Italy, F. Bersani, ed.
0.16 $\mu\text{W}/\text{cm}^2$	Attention, memory and motor function of school children significantly affected in comparison to control groups. Reaction times slower and neuromuscular apparatus endurance also reduced.	A.A. Kolodynski and V.V. Kolodynska (1996), Motor and psychological functions of school children living in the area of the Skrunda radio location station in Latvia. <i>The Science of the Total Environment</i> , 180 (1):87-93.
0.168 $\mu\text{W}/\text{cm}^2$	Progressive decrease in number of newborns and irreversible infertility in mice after 5 generations exposure to radiation from 'antenna park'.	I.N. Magras and T.D. Zenos (1997), RF Radiation-Induced Changes in the Prenatal Development of Mice, <i>Bioelectromagnetics</i> , 18(6), pp. 455-461.
0.2 – 8 $\mu\text{W}/\text{cm}^2$	Two-fold increase in childhood leukaemia from AM-FM exposure from TV towers compared to areas with levels of 0.02 $\mu\text{W}/\text{cm}^2$	B. Hocking, I.R. Gordon and H.L. Grain (1996), Cancer incidence and mortality and proximity to TV towers. <i>Medical Journal of Australia</i> 165(11-12):599-600, cited by Sage 2004.
0.3 $\mu\text{W}/\text{cm}^2$	Impaired motor function, reaction time, memory and attention of schoolchildren, and altered sex ratio of children (fewer boys)	A.A. Kolodynski and V.V. Kolodynska (1996), Motor and psychological functions of school children living in the area of the Skrunda Radio Location Station in Latvia. <i>The Science of the Total Environment</i> 180:87-93.
0.6 $\mu\text{W}/\text{cm}^2$	Change in calcium ion efflux from brain tissue	S. K. Dutta et al, (1986). Microwave radiation-induced calcium ion flux from human neuroblastoma cells: dependence on depth of amplitude modulation and exposure time. <i>Biological Effects of Electropollution</i> , S. Dutta and R. Millis, eds., pp. 63-69. Philadelphia, PA: Information Ventures, cited by A. Firstenberg 2001.
0.6 $\mu\text{W}/\text{cm}^2$	Cardiac arrhythmias and sometimes cardiac arrest (frogs)	Frey, 1986. Evolution and results of biological research with low-intensity nonionizing radiation. <i>Modern Bioelectricity</i> , A.A. Marino, ed., pp. 785-837. New York, NY: Dekker.
0 – 4 $\mu\text{W}/\text{cm}^2$	Altered white blood cell activity in schoolchildren	H. Chiang et al., 1989. Health effects of environmental electromagnetic fields. <i>Journal of Bioelectricity</i> 8(1):127-131, cited by A. Firstenberg 2001
1.0 $\mu\text{W}/\text{cm}^2$	Headache, dizziness, irritability, fatigue, weakness, insomnia, chest pain, difficulty breathing, indigestion (humans—occupational exposure)	V. B. Simonenko et al., 1998. Influence of electromagnetic radiation in the radiofrequency range on the health condition of an organized collective. <i>Voenno-meditsinskiy zhurnal CCCXIX</i> (5):64-68, cited by A. Firstenberg (2001)
1.0 $\mu\text{W}/\text{cm}^2$	Stimulation of white cells in guinea pigs	M.G. Shandala and G.I. Vinogradov, 1978. Immunological effects of microwave action. <i>Gigiyena i Sanitariya</i> , no. 10:34-38, JPRS 72956, pp. 16-21, cited by A. Firstenberg (2001)
1 $\mu\text{W}/\text{cm}^2$	Change in immunological functions in NMRI mice after exposure to whole body microwave sinusoidal irradiation of 8.15-18 GHz (1 Hz within).	E.E. Fesenko, V.R. Makar, E.G. Novoselova and V.B. Sadovnikov (1999), Microwaves and cellular immunity. I. Effect of whole body microwave irradiation on tumor necrosis factor production in mouse cells. <i>Bioelectrochemistry and Bioenergetics</i> , 49(1):29-35.

Power Density	Reported Biological Effects / Comments	References
1 $\mu\text{W}/\text{cm}^2$	In vivo irradiation at 8.15-18 GHz (1 Hz within) increased cytotoxic activity of natural killer cells in rat spleen. For mice exposed 24-72 hours, activity of natural killer cells increased 130-150%. This level persisting within 24 hours after end of treatment. In vivo irradiation for 3.5 and 5 hours, and short exposure of splenic cells in vitro did not affect activities of natural killer cells.	E.E. Fesenko, E.G. Novoselova, N.V. Semiletova, T.A. Agafonova and V.B. Sadovnikov (1999), [Stimulation of murine natural killer cells by weak electromagnetic waves in the centimeter range]. <i>Biofizika</i> 44(4), pp.737-741, (Article in Russian), cited by A. Marino, Recent studies (1995-2000) on the biological effects of radiofrequency and cell phone radiation, www.niehs.nih.gov/emfrapid/extrmurabs/marino.html
1 $\mu\text{W}/\text{cm}^2$	Immune system response affected by a single 5-hour whole-body exposure to 8.15-18 GHz microwave radiation (with 1 Hz impulse frequency) that stimulated the immune potential of macrophages and T cells. Antioxidant treatment (through diet) was found to further enhance this effect.	E.G. Novoselova, E.E. Fesenko, V.R. Makar and V.B. Sadovnikov (1999), Microwaves and cellular immunity. II. Immunostimulating effects of microwaves and naturally occurring antioxidant nutrients. <i>Bioelectrochemistry and Bioenergetics</i> , 49(1):37-41.
1 $\mu\text{W}/\text{cm}^2$	Standards in the former USSR for permissible exposure levels to 30-300 MHz for 8-hour workday.	Yu.D. Dumanskiy and V.Ye. Prokhvatilo (1979), Electromagnetic field of industrial frequency as a factor in the environment and its hygienic regulation. <i>Gigiena i sanitariya</i> 5:72-74, cited by Kositsky et al 2001.
1 $\mu\text{W}/\text{cm}^2$	Wien (sum GSM)	Powerwatch, International Guidance Levels, www.powerwatch.org.uk/gen/intguidance.asp
1 $\mu\text{W}/\text{cm}^2$	Typical reading 100 metres from base station (0.2 to 6 V/m)	Powerwatch, International Guidance Levels, www.powerwatch.org.uk/gen/intguidance.asp
1.053 $\mu\text{W}/\text{cm}^2$	Irreversible infertility in mice after 3 generations exposure to radiation.	I.N. Magras and T.D. Zenos (1997), RF Radiation-Induced Changes in the Prenatal Development of Mice, <i>Bioelectromagnetics</i> , 18(6), pp. 455-461.
1.3 – 5.7 $\mu\text{W}/\text{cm}^2$	Exposure to AM RF caused two-fold increase in leukaemia in adults	H. Dolk, G. Shaddick, P. Walls, C. Grundy, B. Thakrar, I. Kleinschmidt and P. Elliott (1997), cited by Sage 2004. Cancer incidence near radio and television transmitters in Great Britain. <i>Am J Epidemiology</i> 145(1) P 1-9 Jan 1997.
2–10 $\mu\text{W}/\text{cm}^2$ Exposure Limit in Bulgaria, Hungary, Russia and Switzerland as at 2001, cited by A. Firstenberg 2001.		
2.0 $\mu\text{W}/\text{cm}^2$ (lower threshold not known)	'Microwave hearing'— buzzing, chirping, clicking, hissing, or high-pitched tones.	A.H. Frey (1963), Human response to very-low-frequency electromagnetic energy. <i>Nav. Res. Rev.</i> 1968:1-4. A.H. Frey (1971), Biological function as influenced by low power modulated RF energy. <i>IEEE Transactions on Microwave Theory and Techniques</i> , MTT-19(2):153-164. A.H. Frey and R. Messenger (1973), Human perception of illumination with pulsed ultrahigh-frequency electromagnetic energy. <i>Science</i> 181:356-358, cited by A. Firstenberg 2001.
2.0 $\mu\text{W}/\text{cm}^2$	'Microwave hearing'— buzzing, chirping, clicking, hissing, or high-pitched tones.	D.R. Justeson (1979), Behavioral and psychological effects of microwave radiation. <i>Bulletin of the New York Academy of Medicine</i> 55(11):1058-1078, cited by A. Firstenberg 2001.
2.0 $\mu\text{W}/\text{cm}^2$	'Microwave hearing'— buzzing, chirping, clicking, hissing, or high-pitched tones.	R.G. Olsen (1980), Evidence for microwave-induced acoustic resonances in biological material. <i>Bioelectromagnetics</i> 1:219, cited by A. Firstenberg 2001.
2.0 $\mu\text{W}/\text{cm}^2$	'Microwave hearing'— buzzing, chirping, clicking, hissing, or high-pitched tones.	C.W. Wieske (1963), 'Human Sensitivity to Electric Fields.' Proceedings of the First National Biomedical Sciences Instrumentation Symposium. (Vol. 1). Ed. Dr. Fred Alt. New York: Plenum Press, 1963, cited by A. Firstenberg 2001.
2.0 $\mu\text{W}/\text{cm}^2$	'Microwave hearing'— buzzing, chirping, clicking, hissing, or high-pitched tones.	J.C. Lin (1978), <i>Microwave Auditory Effects and Applications</i> . Springfield, IL: Charles C. Thomas, Publisher, Springfield, IL 1978, 221 pp, cited by A. Firstenberg 2001.
2.4 $\mu\text{W}/\text{cm}^2$	Belgium (Wallonia)	Powerwatch, International Guidance Levels, www.powerwatch.org.uk/gen/intguidance.asp
2.4 $\mu\text{W}/\text{cm}^2$	Interference caused to medical devices at least up to 1000 MHz (from digital mobile phones).	K.J. Clifford, K.H. Joyner, D.B. Stroud, M. Wood, B. Ward and C.H. Fernandez (1996), Mobile telephones interfere with medical electrical equipment. <i>Australas Phys Eng Sci Med</i> 1994 Mar. 17(1). P 23-7, cited by C. Sage 2004

Power Density	Reported Biological Effects / Comments	References
2.5 $\mu\text{W}/\text{cm}^2$	Breakdown of blood-brain barrier (digital cellular phone used to provide the radiation)	Salford <i>et al.</i> , (1997), Blood brain barrier permeability in rats exposed to electromagnetic fields from a GSM wireless communication transmitter. In: <i>Proceedings of the Second World Congress for Electricity and Magnetism in Biology and Medicine</i> , June 8-12, 1997, Bologna, Italy, F. Bersani, ed., cited by A. Firstenberg 2001.
2 – 4 $\mu\text{W}/\text{cm}^2$	Low power microwaves directly effect the operation of cellular ACh-related ion-channels that have vital roles in behavioural and physiological functions.	G. D'Inzeo, P. Bernardi, F. Eusebi, F. Grassi, C. Tamburello and B.M. Zani (1988), Microwave effects on acetylcholine-induced channels in cultured chick myotubes. <i>Bioelectromagnetics</i> 9(4):363-372.
4 $\mu\text{W}/\text{cm}^2$	Standards in the former USSR for permissible exposure levels to 3-30 MHz for 8-hour workday.	Yu.D. Dumanskiy and V.Ye. Prokhvatilo (1979), Electromagnetic field of industrial frequency as a factor in the environment and its hygienic regulation. <i>Gigiena i sanitariya</i> 5:72-74, cited by Kositsky et al 2001.
4 – 10 $\mu\text{W}/\text{cm}^2$	Lower memory function/visual reaction time in children slowed in tests	H. Chiang, G.D. Yao, Q.S. Fang, K.Q. Wang, D.Z. Lu and Y.K. Zhou (1989), Health effects of environmental electromagnetic fields. <i>Journal of Bioelectricity</i> , 8: 127-131, cited by Sage 2004.
5 $\mu\text{W}/\text{cm}^2$	Standards in the former USSR for permissible exposure levels to 0.3-300 GHz for 8-hour workday.	Yu.D. Dumanskiy and V.Ye. Prokhvatilo (1979), Electromagnetic field of industrial frequency as a factor in the environment and its hygienic regulation. <i>Gigiena i sanitariya</i> 5:72-74, cited by Kositsky et al 2001.
5.0 $\mu\text{W}/\text{cm}^2$	Study investigated immune systems of women exposed to 500 KHz-3 GHz fields from radio/television transmitters in their residential area for ≥ 2 years. Exposure levels of 4.3 ± 1.4 V/m (mean $\pm$ S.D.) measured on the balconies of the women's homes. Control group exposed to <1.8 V/m fields. Higher field exposure found to reduce cytotoxic activity in the women's peripheral blood without a dose-response effect.	P. Boscol, M.B. Di Sciascio, S. D'Ostilio, A. Del Signore, M. Reale, P. Conti, P. Bavazzano, R. Paganelli & M. Di Giocchino (2001), Effects of electromagnetic fields produced by radiotelevision broadcasting stations on the immune system of women. <i>Sci Total Environ</i> 273(1-3):1-10.
5.0 $\mu\text{W}/\text{cm}^2$	Leukaemia, skin melanoma and bladder cancer near TV and FM transmitter	H. Dolk, G. Shaddick, P. Walls, C. Grundy, B. Thakrar, I. Kleinschmidt and P. Elliott (1997), cited by Sage 2004. Cancer incidence near radio and television transmitters in Great Britain. <i>Am J Epidemiology</i> 145(1) P 1-9 Jan 1997.
5.0 $\mu\text{W}/\text{cm}^2$	Biochemical and histological changes in liver, heart, kidney, and brain tissue	V.S. Belokrinitskiy (1982), 'Hygienic evaluation of biological effects of nonionizing microwaves', <i>Gigiyena i Sanitariya</i> 6:32-34, JPRS 81865, pp. 1-5, cited by A. Firstenberg 2001.
5 – 10 $\mu\text{W}/\text{cm}^2$	Nervous system activity impaired	Dumanski and Shandala (1974), The Biological Action and Hygienic Significance of Electromagnetic Fields of Superhigh and Ultrahigh frequencies in Densely Populated Areas,' from <i>Biological Effects and Health Hazards of Microwave Radiation</i> . Proceedings of an International Symposium, Warsaw 15-18 October, 1973, Polish Medical Publishers, Warsaw, 1974, cited by Sage 2004.
7–10 $\mu\text{W}/\text{cm}^2$	Exposure Limit in People's Republic of China as at 2001,	Cited by A. Firstenberg 2001.
8 $\mu\text{W}/\text{cm}^2$	Association between increased incidences of childhood leukaemia and mortality through RF fields from TV transmitters in comparison to areas with lower power densities. Overall rate ratio of incidence was 1.58 (95% CI, 1.07-2.34). For mortality it was 2.32 (95% CI, 1.35-4.01). For childhood lymphatic leukaemia the rate ratio for incidence was 1.55 (95% CI, 1.00-2.41) and 2.74 (95% CI, 1.42-5.27) for mortality.	B. Hocking, I.R. Gordon, H.L. Grain and G.E. Hatfield (1996), Cancer incidence and mortality and proximity to TV towers. <i>Med J Aust</i> 165(11-12), pp. 601-605, 1996. (Published erratum appears in <i>Med J Aust</i> 166(2):80, 1997), cited by A. Marino, Recent studies (1995-2000) on the biological effects of radiofrequency and cell phone radiation, www.niehs.nih.gov/emfrapid/extrmurabs/marino.html

Power Density	Reported Biological Effects / Comments	References
8 $\mu\text{W}/\text{cm}^2$	Association between increased incidences of childhood leukaemia and mortality through RF fields from TV transmitters in comparison to areas with lower power densities. Overall rate ratio of incidence was 1.58 (95% CI, 1.07-2.34). For mortality it was 2.32 (95% CI, 1.35-4.01). For childhood lymphatic leukaemia the rate ratio for incidence was 1.55 (95% CI, 1.00-2.41) and 2.74 (95% CI, 1.42-5.27) for mortality.	B. Hocking, I.R. Gordon, H.L. Grain and G.E. Hatfield (1996), Cancer incidence and mortality and proximity to TV towers. <i>Med J Aust</i> 165(11-12), pp. 601-605, 1996. (Published erratum appears in <i>Med J Aust</i> 166(2):80, 1997), cited by A. Marino, Recent studies (1995-2000) on the biological effects of radiofrequency and cell phone radiation, www.niehs.nih.gov/emfrapid/extrmurabs/marino.html
9.5 $\mu\text{W}/\text{cm}^2$	Switzerland, Lichtenstein, Luxembourg	Powerwatch, International Guidance Levels, www.powerwatch.org.uk/gen/intguidance.asp
10 $\mu\text{W}/\text{cm}^2$	Russian Federation, People's Republic of China, Public Exposure Guidelines at 1800 MHz	Powerwatch, International Guidance Levels, www.powerwatch.org.uk/gen/intguidance.asp
10 $\mu\text{W}/\text{cm}^2$	Italy (sum of frequencies)	Powerwatch, International Guidance Levels, www.powerwatch.org.uk/gen/intguidance.asp
10 $\mu\text{W}/\text{cm}^2$	Maximum permitted exposure levels for base stations inside and outside of living, public and industrial areas for 300-2400 MHz frequencies (Russian Federation, since 1 June 2003)	Hygienic requirements for siting and exploitation of land mobile telecommunication systems, SanPiN 2.1.8/2.2.4.1190-03 (2003), Ministry of Health of Russian Federation / Russian Ministry of Health Protection, SanPiN (Sanitary and Epidemiological Norms). (Standard for siting and using 27-2400 MHz land mobile phone systems in the Russian Federation).
10 $\mu\text{W}/\text{cm}^2$	Impaired / reduced short-term memory function and significant differences in visual reaction time (1170 test subjects).	H. Chiang, G.D. Yao, Q.S. Fang, K.Q. Wang, D.Z. Lu and Y.K. Zhou (1989), Health effects of environmental electromagnetic fields. <i>J. Bioelectricity</i> 8(1):127-131.
10.0 $\mu\text{W}/\text{cm}^2$	Decreased size of litter, increased number of stillborns in mice	Il'Chevich (reported in McRee 1980), cited by A. Firstenberg 2001.
$\leq 10 \mu\text{W}/\text{cm}^2$ (max. mean exposure)	Sperm counts of Danish military personnel operating mobile ground-to-air missile units, which used several RFR emitting radar systems, were significantly lower than controls.	N.H. Hjollund, J.P. Bonde, J. Skotte (1997), Semen analysis of personnel operating military radar equipment. <i>Reprod Toxicol</i> 11(6):897, cited by www.energyfields.org/science/CWTI.RFR_studies_2.02.doc
10.0 $\mu\text{W}/\text{cm}^2$	Redistribution of metals in the blood, bones, brain, heart, liver, lungs, kidney, muscles, spleen and skin	O.I. Shutenko, I.P. Kozyarin and I.I. Shvayko (1981), Effects of superhigh frequency electromagnetic fields on animals of different ages. <i>Gigiyena i Sanitariya</i> , no. 10:35-38, JPRS 84221, pp. 85-90, cited by A. Firstenberg 2001.
10 $\mu\text{W}/\text{cm}^2$	Damaged mitochondria, nucleus of cells in hippocampus of brain	V.S. Belokrinskiy (1982), Destructive and reparative processes in hippocampus with long-term exposure to nonionizing microwave radiation. <i>Bulletin of Experimental Biology and Medicine</i> 93(3):89-92, cited by A. Firstenberg 2001.
10 $\mu\text{W}/\text{cm}^2$	Altered brain permeability	W.R. Adey (1982).
10 – 25 $\mu\text{W}/\text{cm}^2$	Changes registered in hippocampus of the brain	Belokrinskiy, 1982, cited by Sage 2004 'Destructive and reparative processes in hippocampus with long-term exposure to nonionizing radiation.' In U.S.S.R. Report, Effects of Nonionizing Microwave Radiation, No. 7, JPRS 81865, pp. 15-20.
10 – 100 $\mu\text{W}/\text{cm}^2$	RFR at low intensities (0.0027- 0.027 W/kg) induced behavioural and endocrine changes in rats. Decreases in blood concentrations of insulin and testosterone reported, though CW microwaves had no influence on insulin secretion. Inhibition of behaviour by microwaves may depend on strength, exposure time and inhibitory effects on nervous system. Activation correlated with decreases in serum concentrations of insulin and testosterone.	M.A. Navakatikian, L.A. Tomashevskaya (1994), Phasic behavioral and endocrine effects of microwaves of nonthermal intensity. In 'Biological Effects of Electric and Magnetic Fields, Volume 1,' D.O. Carpenter (ed) Academic Press, San Diego, CA, pp.333-342, cited by www.energyfields.org/science/CWTI.RFR_studies_2.02.doc

Power Density	Reported Biological Effects / Comments	References
20 $\mu\text{W}/\text{cm}^2$	Changes in brain wave patterns caused by microwave or radio frequency radiation	C.H. Dodge and Z.R. Glaser, 1977. 'Trends in non-ionizing electromagnetic radiation bio-effects research and related occupational health aspects', <i>Journal of Microwave Power</i> , 12, 4 (1977)), cited by P. Bentham (1991), VDU Terminal Sickness: Computer health risks and how to protect yourself, Green Print, London, ISBN 1 85425 043 4.
20 $\mu\text{W}/\text{cm}^2$	Pulsed RF radiation (900 MHz with 217 Hz pulse) slightly elevated cortisol serum level (cortisol is a hormone involved in stress reactions). The increase was transient, suggesting adaptation to the stimulus by the subject. No significant effects found for growth hormone, luteinizing hormone or melatonin under field exposure compared to control condition. The EEG sleep-data revealed no significant variations on exposure, although there was a trend for suppressed REM.	K. Mann, P. Wagner, G. Brunn, F.Hassan, C. Hiemke and J. Roschke (1998), Effects of pulsed high-frequency electromagnetic fields on the neuroendocrine system. <i>Neuroendocrinology</i> 67(2):139-144.
25 $\mu\text{W}/\text{cm}^2$ at 300 MHz-300 GHz.	Workers' exposure standard in Russia for 8-hour day (occupational standard introduced in 1986) The standard is based on the total amount of energy absorbed and permits exposures for shorter time periods, e.g. 100 $\mu\text{W}/\text{cm}^2$ for 2 hours, cited by C.W. Smith & S. Best (1989).	
27 $\mu\text{W}/\text{cm}^2$	Standards in the former USSR for permissible exposure levels to 0.3-3 MHz for 8-hour Workday.	Yu.D. Dumanskiy and V.Ye. Prokhvatilo (1979), Electromagnetic field of industrial frequency as a factor in the environment and its hygienic regulation. <i>Gigiena i sanitariya</i> 5:72-74, cited by Kositsky et al 2001.
30 $\mu\text{W}/\text{cm}^2$ (0.015 W/Kg SAR)	Elevation of PFC count (antibody producing cells) in immune system	B. Veyret, C. Bouthet, P. Deschaux, R. de Seze, M. Geffard, J. Joussot-Dubien, M. le Diraison, J.-M. Moreau and A. Caristan (1991), Antibody responses of mice exposed to low-power microwaves under combined, pulse and amplitude modulation,' <i>Bioelectromagnetics</i> 12: P 47-56), cited by Sage 2004.
30 $\mu\text{W}/\text{cm}^2$	Increased brain-amine levels	W.R. Adey (1982).
32.5 $\mu\text{W}/\text{cm}^2$	102 nd Floor, Empire State Building in New York	R. Tell & N. H. Hankin (1978), 'Measurements of Radio Frequency Field Intensity in Buildings with Close Proximity to Broadcast Systems', ORP/EAD 78-3, U.S. Environmental Protection Agency, Las Vegas.
50 $\mu\text{W}/\text{cm}^2$	Exposure Limit in Auckland, New Zealand as at 2001	A. Firstenberg (2001).
50 $\mu\text{W}/\text{cm}^2$	18% reduction in REM sleep, which is important to learning and memory functions	Mann <i>et al.</i> , 1996, cited by Sage 2004. Effects of pulsed high-frequency electromagnetic fields on human sleep. <i>Neuropsychobiology</i> 1996;33:41-7.
50 $\mu\text{W}/\text{cm}^2$	Decreased sperm counts	W.R. Adey (1982).
50 $\mu\text{W}/\text{cm}^2$	2.375 GHz exposure for 30 days resulted in decreased T-cell responses with suppressed phagocytosis noted in rats and guinea pigs.	M.G. Shandala, M.I. Rudnex, G.K. Vinogradov, N.G. Belonozhko and N.M. Gonchar (1977), Immunological and haematological effects of microwave radiation at low power densities. In: Proceedings of the International Union Radio Science Symposium on Biological Effects of Electromagnetic Waves, Airlie, V.A., p. 84, cited by Adey, 1982.
50 $\mu\text{W}/\text{cm}^2$	No differences noted in the awake EEG of healthy subjects exposed nearly 3.5 minutes to the 900 MHz radiation pulsed at 217 Hz with a pulse width of 580 microseconds when compared to effects of inactive GSM system.	J. Roschke and K. Mann (1997), No short-term effects of digital mobile radio telephone on the awake human electroencephalogram. <i>Bioelectromagnetics</i> 18(2), pp.172-176.
60 $\mu\text{W}/\text{cm}^2$	Disturbance of female cycles of test animals, reduced fertility, dystrophic changes in reproductive organs. Reduced weight and number of offspring; postnatal deaths of rat pups increased by factor of 2.5.	H.G. Nikitina and L.G. Andrienko (1989), Condition of reproductive functions in experimental animals under the influence of electromagnetic radiation of mm waves. <i>Fundamental and Applied aspects of Use of mm Electromagnetic Radation in Medicine, Proceedings of the 1st All-Union Symposium with International Participation</i> (10-13 May 1989, Kiev: VNK 'Otklik,' pp. 288-289, 1989, cited by Kositsky et al 2001.

Power Density	Reported Biological Effects / Comments	References
60 $\mu\text{W}/\text{cm}^2$	Brain wave activation observed in human subjects exposed to 902.4 MHz mobile-phone radiation. Significant correlation on EEG recordings noted, particularly when the subjects eyes were closed. This was suggested to be a manifestation of cortex activation under mobile-phone EMF exposure.	N.N. Lebedeva, A.V. Sulimov, O.P. Sulimova, T.I. Kotrovskaya and T. Gailus (2000), Cellular phone electromagnetic field effects on bioelectric activity of human brain. <i>Crit Rev Biomed Eng</i> 28(1-2):323-337. Cited by www.energyfields.org/science/CWT1.RFR_studies_2.02.doc
65.9 $\mu\text{W}/\text{cm}^2$	50 th Floor, Sears Building in Chicago	R. A. Tell and N. N. Hankin (1978), "Measurements of radiofrequency field intensities in buildings with close proximity to broadcast stations," Environmental Protection Agency Technical Note, ORP/EAD 78-3, Aug. 1978 (NTIS Order No. PB 290 944/AS), cited by R.O. Becker & G. Selden (1985), <i>The Body Electric</i> , Quill, ISBN 0-688-06971-1.
67.4 $\mu\text{W}/\text{cm}^2$	47 th Floor, 1100 Milam Building in Houston	R. A. Tell and N. N. Hankin (1978), "Measurements of radiofrequency field intensities in buildings with close proximity to broadcast stations," Environmental Protection Agency Technical Note, ORP/EAD 78-3, Aug. 1978 (NTIS Order No. PB 290 944/AS), cited by R.O. Becker & G. Selden (1985).
1 - 97 $\mu\text{W}/\text{cm}^2$	Location specific values found inside tall U.S. buildings that housed or were near broadcast antennas.	R. A. Tell and N. N. Hankin (1978), "Measurements of radiofrequency field intensities in buildings with close proximity to broadcast stations," Environmental Protection Agency Technical Note, ORP/EAD 78-3, Aug. 1978 (NTIS Order No. PB 290 944/AS).
98.6 $\mu\text{W}/\text{cm}^2$	38 th Floor, One Biscayne Tower in Miami	R. A. Tell and N. N. Hankin (1978), "Measurements of radiofrequency field intensities in buildings with close proximity to broadcast stations," Environmental Protection Agency Technical Note, ORP/EAD 78-3, Aug. 1978 (NTIS Order No. PB 290 944/AS), cited by R.O. Becker & G. Selden (1985).
100 $\mu\text{W}/\text{cm}^2$ at 300 MHz-300 GHz for max. 2 hours.	Workers' exposure standard in Russia for 8-hour day (occupational standard introduced in 1986) Standard based on total amount of energy absorbed, cited by C.W. Smith & S. Best 1989.	
100 $\mu\text{W}/\text{cm}^2$	Maximum permitted exposure levels for MPEL for mobile stations (including cellular phones) for 300-2400 MHz frequencies (Russian Federation, since 1 June 2003)	Hygienic requirements for siting and exploitation of land mobile telecommunication systems, SanPiN 2.1.8/2.2.4.1190-03 (2003), Ministry of Health of Russian Federation / Russian Ministry of Health Protection, SanPiN (Sanitary and Epidemiological Norms). (Standard for siting and using 27-2400 MHz land mobile phone systems in the Russian Federation).
<100 $\mu\text{W}/\text{cm}^2$	54-900 MHz exposure of 95% U.S. urban population in 1979. In urban areas median exposure was 0.005 $\mu\text{W}/\text{cm}^2$.	EPA (Environmental Protection Agency) (1978), Population Exposure to VHF and UHF Broadcast Radiation in the United States, R.A. Tell and E.D. Mantiply, EPA Technical Report ORP/EAD 78-5. Cited in "An Assessment of Potential Health Effects from Exposure to PAVE PAWS Low-Level Phased-Array Radiofrequency Energy", Board on Radiation Effects Research (2005), http://books.nap.edu/openbook.php?record_id=11205&page=48
100 $\mu\text{W}/\text{cm}^2$	Changes registered in immune system function of male mice	Elekes et al., 1996. Effect on the immune system of mice exposed chronically to 50 Hz amplitude-modulated 2.45 GHz microwaves. <i>Bioelectromagnetics</i> Vol 17, Issue 3, pp.246-8, cited at www.cellphonesar.com/research/rf_radiation
100 $\mu\text{W}/\text{cm}^2$	26% drop in insulin registered	Navakatikian & Tomashevskaya, 1994. 'Phasic Behavioral and Endocrine Effects of Microwaves of Nonthermal Intensity,' by Carpenter DO and Ayrapetyan S, editors. <i>Biological Effects of Electric and Magnetic Fields</i> . Volume 1, published by Academic Press, cited at www.cellphonesar.com/research/rf_radiation
111.5 $\mu\text{W}/\text{cm}^2$	Belgium (ex Wallonia) Public Exposure Guidelines at 1800 MHz	Powerwatch, International Guidance Levels, www.powerwatch.org.uk/gen/intguidance.asp

Power Density	Reported Biological Effects / Comments	References
120 $\mu\text{W}/\text{cm}^2$	Pathological change noted in the blood brain barrier at 915 MHz	Salford, L.G., Brun, A., Perrson, B.R.R., and Eberhardt, J., 1993. 'Experimental studies of brain tumor development during exposure to continuous and pulsed 915 MHz radio frequency radiation,' in <i>Bioelectrochemistry and Bioenergetics</i> , Vol. 30: pp. 313-318.
180.3 $\mu\text{W}/\text{cm}^2$	Roof, Home Tower in San Diego	R. Tell & N. H. Hankin (1978), cited by R.O. Becker & G. Selden (1985).
200 $\mu\text{W}/\text{cm}^2$ Public exposure (average)	ICNIRP public guidance levels at 400 MHz (TETRA) and 28 V/m. ICNIRP (& UK) Standard Guidance is based on power levels averaged over 6 minutes. Reference level category 100 – 400 MHz, cited by A. Phillips (2002)	
200 $\mu\text{W}/\text{cm}^2$	Exposure Limit in Australia as at 2001, cited by A. Firstenberg 2001.	
200 – 1000 $\mu\text{W}/\text{cm}^2$	Exposure Limit in Canada, Germany, Japan, New Zealand and US as at 2001, cited by A. Firstenberg 2001.	
250-500 $\mu\text{W}/\text{cm}^2$	Decreased reproductive capacity and litter size , also premature cessation of reproductive function in mice exposed to microwaves for 4 hours a day for 48 weeks – details on carrier frequencies not given.	Z.V. Gordon, A.V. Rosein and M.S. Byskov (1974), 'Main directions and results of research conducted in USSR on the biologic effects of microwaves,' <i>Biologic Effects and Health Hazards of Microwave Radiation</i> , P. Czerski, ed., Polish Medical Publications, Warsaw, p. 22-35, cited by W.R. Adey, 1982.
$\leq 230 \mu\text{W}/\text{cm}^2$	Location specific values measured outside tall buildings in close proximity to broadcast antennas.	R. A. Tell and N. N. Hankin (1978), "Measurements of radiofrequency field intensities in buildings with close proximity to broadcast stations," <i>Environmental Protection Agency Technical Note, ORP/EAD 78-3</i> , Aug. 1978 (NTIS Order No. PB 290 944/AS).
0.2-360 $\mu\text{W}/\text{cm}^2$	Mortality rate of exposed chickens almost twice that of control colony.	C. Romero-Sierra and J.A. Tanner (1970), <i>Microwave Radiation and Egg Production in Chickens</i> . Proceedings of IMPI 5th Annual Microwave Symposium, Scheveningen, Holland. October 1970.
10-10,000 $\mu\text{W}/\text{cm}^2$	Deterioration noted in radiation sensitive Mimosa plant.	C. Romero-Sierra, J.A. Tanner, J. Bigu del Blanco (1973), <i>Interaction of Electromagnetic fields And Living Systems With Special Reference To Birds</i> , Control Systems Laboratory, Division of Mechanical Engineering / Division de Génie Mécanique, Canada, Report LTR-CS-113, presented to International Symposium on Biological Effects and Health Hazards of MW Radiation, World Health Organization, Warsaw, October 1973, 37 pp.
400 $\mu\text{W}/\text{cm}^2$	Standards in the former USSR for permissible exposure levels to 30-300 kHz for 8-hour workday.	Yu.D. Dumanskiy and V.Ye. Prokhvatilo (1979), <i>Electromagnetic field of industrial frequency as a factor in the environment and its hygienic regulation. Gigiena i sanitariya 5:72-74</i> , cited by Kositsky et al 2001.
>500 $\mu\text{W}/\text{cm}^2$	Autoimmune disease evoked , along with production of anti-liver and anti-brain antibodies.	M.G. Shandala, M.I. Rudnex, G.K. Vinogradov, N.G. Belonozhko and N.M. Gonchar (1977), <i>Immunological and haematological effects of microwave radiation at low power densities</i> . In: <i>Proceedings of the International Union Radio Science Symposium on Biological Effects of Electromagnetic Waves</i> , Airlie, V.A., p. 84, cited by Adey, 1982.
500 $\mu\text{W}/\text{cm}^2$	High Blood Pressure due to imbalances of Potassium and Sodium levels in the body , also significant shifts in carbon dioxide – rats exposed to 2,450 MHz for 7 hours a day for 3 months.	R.H. Lovely, A.W. Guy, R.B. Johnson, and M. Mathews (1978), <i>Alteration of behavioural and biochemical parameters during and consequent to 500 $\mu\text{W}/\text{cm}^2$ chronic 2450 MHz microwave exposure</i> , <i>Proceedings of the International Symposium on Electromagnetic Waves and Biology</i> , Ottawa, p. 34, cited by W.R. Adey (1982).
500 $\mu\text{W}/\text{cm}^2$	Peak level at residential locations near the Radio Location Station at Skrunda in Latvia which had 2 pulsed-radar systems operating at 152 to 162 MHz, at 1250 kW, with pulse duration of 0.8 msec, interpulse interval of 41 msec and pulse repetition rate of 24.5 Hz. The <u>average</u> intensity at these areas was <10 $\mu\text{W}/\text{cm}^2$.	A. Romancuks (1996), <i>Measurement of the intensity of the electromagnetic radiation from the Skrunda Radio Location Station. The Science of the total environment</i> , 180 (1):51-56. Cited in "An Assessment of Potential Health Effects from Exposure to PAVE PAWS Low-Level Phased-Array Radiofrequency Energy", Board on Radiation Effects Research (2005), http://books.nap.edu/openbook.php?record_id=11205&page=135

Power Density	Reported Biological Effects / Comments	References
900 $\mu\text{W}/\text{cm}^2$	ICNIRP (1998), WHO, Public Exposure Guidelines at 1800 MHz	Powerwatch, International Guidance Levels, www.powerwatch.org.uk/gen/intguidance.asp
1,000 $\mu\text{W}/\text{cm}^2$	FCC (USA) OET-65, Public Exposure Guidelines at 1800 MHz	Powerwatch, International Guidance Levels, www.powerwatch.org.uk/gen/intguidance.asp
<1,000 – 4,500 $\mu\text{W}/\text{cm}^2$ (0–45 mW/cm ²)	Beneficial effects noted in transient, reversible and dose-dependent alterations in rates of blastic transformation of unstimulated lymphocytes of hamsters after 15 minutes of irradiation of 2.45 GHz CW field for 5 day period.	A.T. Huang, M.E. Engle, J.A. Elder, J.B. Kinn and T.R. Ward (1997), The effect of microwave radiation (2450 MHz) on the morphology and chromosomes of lymphocytes, Radio Science, 12, Supplement 6, pp. 173-177, cited by W.R. Adey, 1982.
<1,000 $\mu\text{W}/\text{cm}^2$	Change in bioelectric activity of human muscles during deep stages of hypnosis after 10-20 second exposure at 57-78 GHz	S.I. Gerashchenko, O.I. Pisanko and Yu.N. (1991) Mus'kin., Some physiological reactions of organisms under the influence of EHF radiation. <i>Apparatniy kompleks 'Elektronika-KVCh' I yevo primeneniye v meditsine.</i> , L.G. Gassanova, ed. Moscow, 156 pp. NPO 'Saturn, Kiev, pp. 65-71, cited by Kositsky et al 2001.
600 $\mu\text{W}/\text{cm}^2$ at 900 MHz – FCC Exposure Limit in USA (FCC OET65:1997-01 based on NCRP report No.86)		
1000 $\mu\text{W}/\text{cm}^2$ at 1800 MHz – FCC Exposure Limit in uncontrolled environment in USA (FCC OET65:1997-01 based on NCRP report No.86)		
1,000 $\mu\text{W}/\text{cm}^2$	ODC activity increased up to 50% in human melanoma cells (450-500 MHz at 16 Hz).	W.R. Adey et al., Studies on ornithine decarboxylase (ODC), an enzyme essential for cell growth through DNA synthesis, cited by B.B. Levitt (1995), <i>Electromagnetic Fields: A Consumer's Guide to the Issues and How to Protect Ourselves</i> .
1000 $\mu\text{W}/\text{cm}^2$ Occupational exposure (average)	ICNIRP public guidance levels at 400 MHz (TETRA) and 28 V/m. ICNIRP (& UK) Standard Guidance is based on power levels averaged over 6 minutes.	Alasdair Phillips, Report 2213, Report regarding Microwave Emissions from the T-Mobile (UK) cellular telephone base station at James Stockdale Ltd, Ratten Row, Seamer, Nr Scarborough with respect to any possible adverse health effects. 24th July 2002
1000 $\mu\text{W}/\text{cm}^2$	At 2,450 MHz, maximum specific absorption rate (SAR) for energy of 2.0 W/kg occurs in outer 1.0cm of phantom head (dummy head used for testing).	H.N. Kritikos and H.P. Schwan (1972), Hot spots generated in conducting spheres by electromagnetic waves and biological implications. IEEE Transactions on Biomedical Engineering, 19 (1), 53-58. Cited by W.R. Adey (1982), 'Tissue Interactions with Nonionizing Electromagnetic Fields,' Physiological Reviews, 61(2), 435-51.
1000 $\mu\text{W}/\text{cm}^2$	At 918 MHz, energy absorption at centre of head is 0.45 W/kg.	W.R. Adey (1982), 'Tissue Interactions with Nonionizing Electromagnetic Fields,' Physiological Reviews, 61(2), 435-51.
2000 $\mu\text{W}/\text{cm}^2$	Australian Standard public exposure level – Australian Standard AS2772.1. (1990) Radiofrequency Radiation Part 1: Maximum Exposure Levels -- 100 kHz to 300 GHz. Sydney: Standards Australia.	
700-2,800 $\mu\text{W}/\text{cm}^2$	EEG changes resembling those induced by hallucinogenic drugs noted in rabbits exposed to 9.3 GHz radiation for 5 minutes. Change noted 10 minutes after exposure with decreased total integrated EEG lasting ≤ 15 minutes.	L. Goldstein and Z. Sisko (1974), A quantitative electroencephalographic study of the acute effects of X-band microwaves in rabbits. In: <i>Biological effects and health hazards of microwave radiation</i> (P. Czerski, Ed.), p. 128-133. Warsaw: Polish Medical Publishers. Cited by R.O. Becker and A.A. Marino (1982), <i>Electromagnetism & Life</i> , State University of New York Press, pp. 211, ISBN: 0873955609, www.ortho.lsuhs.edu/Faculty/Marino/EL/ELTOC.html
1,000-5,000 $\mu\text{W}/\text{cm}^2$ above 300MHz	The American National Standard Institution's voluntarily required limit for worker and public exposures as at 1989, cited by C.W. Smith and S. Best (1989).	
2,600 $\mu\text{W}/\text{cm}^2$	Maximum exposure in school with base station on roof. Maximum power density of 0.01 $\mu\text{W}/\text{cm}^2$ measured at two schools without nearby base stations.	A. Thansandote, G.B. Gajda and D.W. Lecuyer (1999), Radiofrequency radiation in five Vancouver schools: exposure standards not exceeded. Canadian Medical Association Journal, 161(10), pp. 1311-1312.
5,000 $\mu\text{W}/\text{cm}^2$	Increased bone marrow cellularity in mice exposed to a 2.88 GHz field (SAR 2.3 W/kg) for 80-400 hours – effect not noted at 10,000 $\mu\text{W}/\text{cm}^2$ indicating possible window effect.	H.A. Ragan and R.D. Philips (1978), Hematologic effects of mice exposed to pulsed and CW microwaves. In: Proc. Int. Union Radio Sci., Symp. On Biologic Effects of Electromagnetic Waves, Helsinki, p. 48, cited by W.R. Adey, 1982.

Power Density	Reported Biological Effects / Comments	References
5,500 $\mu\text{W}/\text{cm}^2$	Exposing developing chick embryos to 428 MHz radiation for >20 days caused lethal and/or teratogenic effects and delayed hatching.	K. Saito and K. Suzuki (1991), Lethal and teratogenic effects of long-term low-intensity radio frequency radiation at 428 MHz on developing chick embryo. <i>Teratology</i> , 43, pp. 609-614.
6400 $\mu\text{W}/\text{cm}^2$ Public exposure (peak)	ICNIRP public guidance levels at 400 MHz (TETRA) and 28 V/m. ICNIRP (& UK) Standard Guidance is based on power levels averaged over 6 minutes, cited by A. Philips 2002.	
2,640 $\mu\text{W}/\text{cm}^2$ at 400 MHz – General Public Exposure Limit in UK (NRPB, 1993) (TETRA operates at 400 MHz)		
3,300 $\mu\text{W}/\text{cm}^2$ at 900 MHz – old UK General Public Exposure Limit to June 2000). Now ICNIRP is used for 900 MHz		
31,000 $\pm$ 5,000 $\mu\text{W}/\text{cm}^2$ (Peak values)	Exposure of BALB/c mice to 42.2 GHz fields (with peak specific absorption rate (SAR) at skin of 622 ± 100 W/kg) for 30 minutes daily for 3 days found to ameliorate the immunosuppressive effects of cyclophosphamide (CPA) – a regularly used anticancer drug – by augmenting proliferation of splenocytes and altering activation and effector functions of CD4 ⁺ T cells.	V. Makar, M. Logani, I. Szabo, and M. Ziskin (2003), Effect of Millimeter Waves on Cyclophosphamide Induced Suppression of T Cell Functions, <i>Bioelectromagnetics</i> 24:356–365.
4,000 $\mu\text{W}/\text{cm}^2$	Threshold for neuroendocrine effects	W.R. Adey (1982).
5,000 $\mu\text{W}/\text{cm}^2$	FCC threshold in controlled environment.	Luxorion, Electromagnetic radiations and your health, www.astrosurf.com/luxorion/qs1-em-radiation
6400 $\mu\text{W}/\text{cm}^2$ Public exposure (peak)	ICNIRP (& UK) public guidance levels at 400 MHz (TETRA) and 28 V/m. based on power levels averaged over 6 minutes.	A. Philips (2002), Report 2213, Report regarding Microwave Emissions from the T-Mobile (UK) cellular telephone base station at James Stockdale Ltd, Ratten Row, Seamer, Nr Scarborough with respect to any possible adverse health effects.
<10,000 $\mu\text{W}/\text{cm}^2$	EHF EMR capable of changing functional condition of living organisms	O.I. Pisanko, V.I. Pyasetskiy and Yu.N. Mus'kin (1991), Questions of hygienic standardization of EHF radiation. <i>Apparatnyi kompleks 'Elektronika-KVCh' i yego primeneniye v meditsine</i> . L.G. Gassanova, ed. Moscow, 156 pp. NPO 'Saturn,' Kiev, pp. 18-24, cited by Kositsky et al., 2001.
10,000 $\mu\text{W}/\text{cm}^2$ at 1800 MHz	Old UK General Public Exposure Limit to June 2000). Now ICNIRP is used for 1800 MHz, cited by A. Firstenberg 2001.	
10,000 $\mu\text{W}/\text{cm}^2$	Exposure level recommended as safe by the NRPB for the frequency range 30 to 30,000 MHz (as at 1991) was 10 mW/cm ² or, 1 mW hour/cm ² , during any 1 hour period. The NRPB did not include considerations relevant to small children.	
10,000 $\mu\text{W}/\text{cm}^2$	Molecular and genetic effects (thermal)	W.R. Adey (1982).
10,000 $\mu\text{W}/\text{cm}^2$	Exposure to 2450 MHz radiation for 90 minutes produced activation of the hypothalamic-pituitary-adrenal axis and increased oestradiol in both virgin and pregnant rats, suggesting microwaves may greatly stress pregnant organisms.	H. Nakamura, T. Seto, H. Nagase, M. Yoshida, S. Dan and K. Ogino (1997), Effects of exposure to microwaves on cellular immunity and placental steroids in pregnant rats. <i>Occup Environ Med</i> 54(9), pp. 676-80, cited by A. Marino, www.niehs.nih.gov/emfrapid/extrmurabs/marino.html
10,000-30,000 $\mu\text{W}/\text{cm}^2$	Chickens exposed at pulse repetition rate of 8,000 pulses per sec and frequency of 16,000 Mc/s. Birds all exhibited a startled reaction at radiation onset, sustained extensor activity of wings and legs also noted. Similar findings obtained with pigeons and seagulls. <u>Note: ICNIRP levels are 200 $\mu\text{W}/\text{cm}^2$ at 400 MHz rising to 1000 $\mu\text{W}/\text{cm}^2$ at ≥ 2 GHz</u>	J.A. Tanner (1966), Effect of Microwave Radiation on Birds, <i>Nature</i> , pp. 636.
10,000 $\mu\text{W}/\text{cm}^2$	No detectable ocular damage to the eyes of rabbits and non-human primates after either single 8-hour exposure to 60 GHz CW radiation or five separate 4-hour exposures on consecutive days.	H.A. Kues, S.A. D'Anna, R. Osiander, W.R. Green and J.C. Monahan JC (1999), Absence of ocular effects after either single or repeated exposure to 10 mW/cm ² from a 60 GHz CW source. <i>Bioelectromagnetics</i> 20(8), pp.463-473.
1000 –10,000 $\mu\text{W}/\text{cm}^2$	Exposure limit in UK as at 2001.	A. Firstenberg (2001).

Power Density	Reported Biological Effects / Comments	References
10,000 $\mu\text{W}/\text{cm}^2$	US Occupational Safety and Health Administration's standard as at 1989	C.W. Smith & S. Best (1989), <i>Electromagnetic Man: Health & Hazard in the Electrical Environment</i> , J.M. Dent & Sons Ltd., London, ISBN 0-460-86044-5.
13,300 $\mu\text{W}/\text{cm}^2$ (Average Power Density)	Millimeter wave treatment (MMWT) is widely used in Eastern Europe. Among reported beneficial effects is suppression of melanoma growth. Tests on mice injected with B16 melanoma cells used 15-minute exposures (at 61.22 GHz). 5 daily exposures found to suppress subcutaneous tumour growth if started 5 days after inoculation; though if course started on day 1 or day 10 following inoculations they were ineffective.	A.A. Radzievsky, O.V. Gordienko, I. Szabo, S.I. Alekseev, and M.C. Ziskin (2004), <i>Millimeter Wave-Induced Suppression of B16 F10 Melanoma Growth in Mice: Involvement of Endogenous Opioids</i> , <i>Bioelectromagnetics</i> 25:466–473.
20,000-50,000 $\mu\text{W}/\text{cm}^2$	Exposed chickens respond with escape or avoidance reactions within seconds of radiation onset.	J. A. Tanner, C. Romero-Sierra and S. J. Davie (1967), <i>Non-thermal Effects of Microwave Radiation on Birds</i> , <i>Nature</i> 216, pp. 1139.
25,000 $\mu\text{W}/\text{cm}^2$	Young chicks became weak on entering pulsed 16 GHz fields. Some collapsed to cage floor (where field intensity shown was registered) until radiation switched off. Collapse time (5-20 seconds) varied with chicks' orientation in field. Induced panting continued briefly after field removed. Drowsiness also noted.	C. Romero-Sierra, J.A. Tanner, J. Bigu del Blanco (1973), <i>Interaction of Electromagnetic fields And Living Systems With Special Reference To Birds</i> , Control Systems Laboratory, Division of Mechanical Engineering / Division de Génie Mécanique, Canada, Report LTR-CS-113, presented to International Symposium on Biological Effects and Health Hazards of MW Radiation, World Health Organization, Warsaw, October 1973, 37 pp.
28,000 $\mu\text{W}/\text{cm}^2$	Teratogenic and tumour causing effects	W.R. Adey (1982), 'Tissue Interactions with Nonionizing Electromagnetic Fields,' <i>Physiological Reviews</i> , 61(2), 435-51.
32,000 $\mu\text{W}/\text{cm}^2$ Occupational exposure (peak)	ICNIRP public guidance levels at 400 MHz (TETRA) and 28 V/m. ICNIRP (& UK) Standard Guidance is based on power levels averaged over 6 minutes.	Alasdair Philips, Report 2213, Report regarding Microwave Emissions from the T-Mobile (UK) cellular telephone base station at James Stockdale Ltd, Ratten Row, Seamer, Nr Scarborough with respect to any possible adverse health effects. 24 th July 2002.
40,000-165,000 $\mu\text{W}/\text{cm}^2$	Dogs avoid exposure to 2800 MHz radiation at these intensities	S. Michaelson et al., (1958). The biological effects of microwave irradiation in the dog, <i>Proc. Second Tri-Serv. Conf. on Biological Effects of Microwave Energy</i> , Rome, New York, p.175, cited by A.S. Presman (1970), <i>Electromagnetic fields and life</i> , (Translated from Russian by F.L. Sinclair). Plenum Press, New York, ISBN 0-306-30395-7, 356pp.
45,000 $\mu\text{W}/\text{cm}^2$	Dorsally stimulated adult birds exhibited behaviour ranging from immobility to initiation of both flight and collapse. Contributory factors were found to be behaviour prior to exposure, area of bird radiated and bird's location.	C. Romero-Sierra, J.A. Tanner, J. Bigu del Blanco (1973), <i>Interaction of Electromagnetic fields And Living Systems With Special Reference To Birds</i> , Control Systems Laboratory, Division of Mechanical Engineering / Division de Génie Mécanique, Canada, Report LTR-CS-113, presented to International Symposium on Biological Effects and Health Hazards of MW Radiation, World Health Organization, Warsaw, October 1973, 37 pp.
46,000 $\mu\text{W}/\text{cm}^2$ (average field intensity 0.152m above floor)	Birds exposed to 9.3 GHz radiation pulsed at 416 pps with 2.3 μsec pulse-width. Collapse of wing and legs noted at start of irradiation. Birds align themselves to outside of field, with their outer side becoming paralysed. Some exhibit hyperactivity. Escape behaviour also noted.	J.A. Tanner, C. Romero-Sierra and S.J. Davie (1967), <i>Non-thermal Effects of Microwave Radiation on Birds</i> , <i>Nature</i> , 216, (5120), pp. 1139.
50,000 $\mu\text{W}/\text{cm}^2$	Almost total paralysis observed in chickens. After 10-20 seconds irradiation pigeons and seagulls showed increased signs of distress noted through defecation, vocalisation and initiation to flight.	J.A. Tanner and C. Romero-Sierra (1974), <i>Beneficial and harmful accelerated growth induced by the action of nonionizing radiation</i> , <i>Annals of New York Academy of Sciences</i> 238, pp. 171-175.
60,000 $\mu\text{W}/\text{cm}^2$	Significant differences noted in EEG patterns of birds when exposed to microwave field modulated sinusoidally at 4 Hz in comparison to non-irradiated situations.	F. Villa, C. Romero-Sierra and J.A. Tanner (1972), <i>Changes in EEG Patterns of Birds under Microwave Radiation</i> . NRC, DME Control Systems Laboratory Technical Report, LTR-CS-56, January 1972.

Power Density	Reported Biological Effects / Comments	References
60,000 $\mu\text{W}/\text{cm}^2$	Increased diffusion rate of aqueous solutions of electrolytes through membranes noted under 10 GHz, CW microwave radiation, being most pronounced when electric field vector oriented perpendicular to plane of the membrane.	J. Bigu del Blanco, C. Romero-Sierra, J.A. Tanner and M.L. Bigu (1973), Progress Report on the Investigation of the Effects of Microwave Radiation on the Diffusion Rate of Electrolytes through Membranes II. NRC, DME Control Systems LTR-CS-73.
80,000 $\mu\text{W}/\text{cm}^2$	Repeated 1 hour exposures (20-24 times) to 2.45 GHz fields caused lens opacities in the eyes of 1 of 11 rabbits tested – other studies do not find evidence of cumulative effects.	R.L. Carpenter, E.S. Ferri and G.J. Hagan (1974), 'Assessing microwaves as a hazard to the eye – progress and problems,' Biologic Effects and Health Hazards of Microwave Radiation, P. Czerski, ed., Polish Medical Publications, Warsaw, p. 178-185, cited by W.R. Adey 1982.
100,000 $\mu\text{W}/\text{cm}^2$	Repeated exposures caused lens opacities in the eyes of 4 of 10 rabbits tested – other studies do not find evidence of cumulative effects. (Lens opacities of rabbits used as model for human cataract induction)	R.L. Carpenter, E.S. Ferri and G.J. Hagan (1974), 'Assessing microwaves as a hazard to the eye – progress and problems,' Biologic Effects and Health Hazards of Microwave Radiation, P. Czerski, ed., Polish Medical Publications, Warsaw, p. 178-185, cited by W.R. Adey 1982.
120,000 $\mu\text{W}/\text{cm}^2$	Approximately two minutes exposure caused sudden wilting reaction in a Mimosa plant that died without reopening.	J. A. Tanner and C. Romero-Sierra (1974), Beneficial and harmful accelerated growth induced by the action of nonionizing radiation, Annals of New York Academy of Sciences 238, pp. 171-175.
120,000 $\mu\text{W}/\text{cm}^2$	Repeated exposures caused lens opacities in the eyes of 8 of 10 rabbits tested – other studies do not find evidence of cumulative effects.	R.L. Carpenter, E.S. Ferri and G.J. Hagan (1974), 'Assessing microwaves as a hazard to the eye – progress and problems,' Biologic Effects and Health Hazards of Microwave Radiation, P. Czerski, ed., Polish Medical Publications, Warsaw, p. 178-185, cited by W.R. Adey 1982.
140,000-190,000 $\mu\text{W}/\text{cm}^2$	5-10 minutes exposures at 140,000-190,000 $\mu\text{W}/\text{cm}^2$ caused plants' primary leaves to wilt. 10-30 minute exposures of most plants to 190,000 $\mu\text{W}/\text{cm}^2$ irradiation caused permanent wilting.	C. Romero-Sierra, J.A. Tanner, J. Bigu del Blanco (1973), Interaction of Electromagnetic fields And Living Systems with Special Reference To Birds, Control Systems Laboratory, Division of Mechanical Engineering / Division de Génie Mécanique, Canada, Report LTR-CS-113, presented to International Symposium on Biological Effects and Health Hazards of MW Radiation, World Health Organization, Warsaw, October 1973, 37 pp.

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DNA damage in Molt-4 T-lymphoblastoid cells exposed to cellular telephone radiofrequency fields in vitro

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Abstract

Molt-4 T-lymphoblastoid cells have been exposed to pulsed signals at cellular telephone frequencies of 813.5625 MHz (iDEN[®] signal) and 836.55 MHz (TDMA signal). These studies were performed at low SAR (average = 2.4 and 24 $\mu\text{W g}^{-1}$ for iDEN[®] and 2.6 and 26 $\mu\text{W g}^{-1}$ for TDMA) in studies designed to look for athermal RF effects. The alkaline comet, or single cell gel electrophoresis, assay was employed to measure DNA single-strand breaks in cell cultures exposed to the radiofrequency (RF) signal as compared to concurrent sham-exposed cultures. Tail moment and comet extent were calculated as indicators of DNA damage. Statistical differences in the distribution of values for tail moment and comet extent between exposed and control cell cultures were evaluated with the Kolmogorov–Smirnov distribution test. Data points for all experiments of each exposure condition were pooled and analyzed as single groups. It was found that: 1) exposure of cells to the iDEN[®] signal at an SAR of 2.4 $\mu\text{W g}^{-1}$ for 2 h or 21 h significantly decreased DNA damage; 2) exposure of cells to the TDMA signal at an SAR of 2.6 $\mu\text{W g}^{-1}$ for 2 h and 21 h significantly decreased DNA damage; 3) exposure of cells to the iDEN[®] signal at an SAR of 24 $\mu\text{W g}^{-1}$ for 2 h and 21 h significantly increased DNA damage; 4) exposure of cells to the TDMA signal at an SAR of 26 $\mu\text{W g}^{-1}$ for 2 h significantly decreased DNA damage. The data indicate a need to study the effects of exposure to RF signals on direct DNA damage and on the rate at which DNA damage is repaired. © 1998 Elsevier Science S.A.

Keywords: Radiofrequency radiation; Cellular telephones; Comet assay; Single cell gel electrophoresis; DNA damage; Single strand breaks

1. Introduction

There is growing concern about possible relationships between human pathology and exposure to electric and magnetic fields produced by power lines, electric appliances, and other devices, such as cellular telephones, radio towers, and radar apparatus. This concern has been driven by the results of numerous epidemiological studies, which have demonstrated an association between various disorders, including cancer and Alzheimer's disease, and either occupational or residential exposure to electromagnetic fields (EMFs) generated by high voltage power lines as well as by microwave generating devices [1–3]. Indeed, in an attempt to understand how EMF exposure may be linked with human disease, numerous in vitro studies have been performed and their results reported in the scientific

literature. Recently, Repacholi et al. [4] reported that *E μ -pim1* transgenic mice exposed to a pulse-modulated radiofrequency (RF) field similar to those used in digital mobile telecommunications exhibited a statistically significant 2.4-fold increase in lymphomas. However, there has been little insight into how electromagnetic signals couple with biological systems and, once such coupling has occurred, by what series of biochemical and molecular steps (i.e., a biological mechanism) pathology may result. This is not to say, however, that there is no information about effects produced in biological systems exposed to electromagnetic signals. Such exposures have produced, for instance, changes in gene transcription [5], enzyme activities [6–12], calcium status [13], and other key cellular parameters [reviewed in Ref. [14]]. Nonetheless, there is a lack of understanding of how, if at all, these alterations in cellular biochemistry may fit together and cooperate to change the course of cellular physiology.

Recently, there has been increased interest in the effect of exposure to various electromagnetic signals on the

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damage and repair of nuclear DNA. This attention is certainly justified from several perspectives. In a recent minireview, for instance, Cleaver stated, “DNA repair processes are now indispensable actors in any script for spontaneous and environmentally induced cancers” [15a,15b]. The bioelectromagnetics literature dealing with DNA damage and repair is relatively limited and deals more with damage than with repair. Furthermore, investigators studying the effects of exposure to electromagnetic signals on DNA damage have taken two different approaches, one looking at exposure-induced effects on chromosomal aberrations, sister chromatid exchange, or micronuclei formation, and the other measuring exposure-induced single- and double-strand breaks in nuclear DNA. Unfortunately, the results of studies reported to date have been conflicting, leaving us with an unclear picture of how exposure to ELF/EMF, RF, and microwave radiation may affect the integrity of a biological system’s genetic information. For instance, Cohen et al. [16,17], Khalil and Qassem [18], and Paile et al. [19] reported negative findings on the chromosomal aspects of EMF exposure. Additionally, Juutilainen and Liimatainen [20] reported that EMF exposure was negative in Ames’ Salmonella mutagenicity testing, while Frazier et al. [21] found no EMF-induced effect on the mutation rate of a genetic locus known to be responsive to various genotoxic mechanisms. On the other hand, others have reported chromosomal abnormalities in cells exposed to both ELF/EMF [22–24] and microwave radiation [25–27]. Most recently, Sarkar et al. [28] exposed mice to 2.45 GHz microwave radiation at a power density of 1 mW cm^{-2} for 2 h day⁻¹ for 120, 150 and 200 days. Isolated nuclear DNA from exposed and control animals was cleaved with the restriction enzyme *Hin* fl, electrophoresed, and hybridized with a synthetic oligonucleotide probe. It is intriguing that the DNA from brain and testes of exposed animals exhibited a distinctly different band pattern in the 7–8 kilobase range, although the mechanism underlying this chromosomal rearrangement is unknown.

Two groups have investigated DNA strand breaks using the comet, or single cell gel electrophoresis, assay. This technique is the most sensitive available for measuring DNA single-strand breaks, and can detect one break per 2×10^{10} daltons of DNA in lymphocytes [29]. Indeed, Singh et al. [30] have reported that the comet assay is more than twice as sensitive as other chromatid abnormality assays when assessing DNA damage produced by ionizing radiation. The assay is performed by embedding cells in agarose, lysing the cells, and then performing electrophoresis under alkaline or neutral conditions to detect and quantitate DNA single- or double-strand breaks, respectively. Lai and Singh [31,32] exposed rats to pulsed and continuous wave 2450 MHz radiation (SAR 1.2 W kg^{-1}). These investigators reported increased single- and double-strand DNA breaks in brain cells either immediately after a 2 h exposure or after a 4 h post-exposure

period, as compared to brain cells from sham-exposed, control rats.

We now report the results of comet assays performed to detect DNA single-strand breaks in Molt-4 T-lymphoblastoid cells exposed for short (2 and 3 h) and long (21 h) periods to pulsed signals at cellular telephone frequencies of 813.5625 MHz and 836.55 MHz. These studies were performed at low SAR (average = 2.4 and $24 \mu\text{W g}^{-1}$ and 2.6 and $26 \mu\text{W g}^{-1}$, respectively) in studies designed to look for athermal RFR effects.

2. Materials and methods

2.1. Cells

Molt-4 cells were the generous gift of Dr. Narendra Singh, University of Washington. These cells were chosen because of their sensitivity to agents which produce DNA damage (N.P. Singh, personal communication). Cells were maintained in RPMI-1640 tissue culture medium (Cellgro), which was supplemented with 10% fetal calf serum (Gemini Bioproducts), and kept in a Forma Model 3158 incubator in a humidified atmosphere at 37°/5% CO₂. Cells were seeded into 60 mm Petri dishes the day before experimentation and were at a cell density of approximately 1×10^6 cells ml⁻¹ at the time of experimentation. Medium depth in the dishes was 2.4 mm.

2.2. RF exposure

The system environmental control and physical arrangement was the same as that reported previously [33]. Two TEM cells (CS-110S, Instruments for Industry, Ronkonkoma, NY) were used and were housed in a single Napco Model 4300 water-jacketed incubator maintained at 37° in a humidified atmosphere of 5% CO₂ in air. The incubator was fitted with a heat exchanger and inline humidifier to precondition the atmosphere before its entry directly into the interior of the TEM cells. By this means, equilibration time has been reduced significantly. In exposure experiments, one TEM cell was powered (exposed) and one was unpowered (sham). In sham/sham experiments, both TEM cells were unpowered.

2.2.1. Exposure to North American Dual-Mode Cellular (NADC) fields with Time Domain Multiple Access (TDMA) modulation

This exposure apparatus has been described in detail [33], and was used without modification. Briefly, the output of a prototype NADC transmitter (provided by Motorola) was connected through a directional coupler to the TEM cell input. The TEM cell output was terminated with a high-quality 50 Ω resistive load. A power meter/chart recorder combination connected to the directional coupler provided constant monitoring of forward power.

For experiments at +10 dB, the transmitter output was attenuated to drive a power amplifier (Microwave Power Equipment PAS-53-0-800/1000). The amp output was passed through a directional coupler (Narda 3001-30) to the TEM cell input. Forward power was monitored and recorded as described above. In all cases, exposure timing was controlled by an electronic timer.

2.2.2. Exposure to iDEN® cellular phone fields

The iDEN® system utilizes time-domain multiplexing in which each second of time is broken down into a series of about 22 frames, each of 45 ms duration. Each frame is further divided into three 15 ms slots. Thus, the incident field at the head of a phone user is a series of RF carrier bursts, each 15 ms duration and repeating at 45 ms intervals. The iDEN® system signal also includes a brief amplitude training pulse at the leading edge of the slot. Normally this pulse is of approximately the same amplitude as the average power during the burst (slot-average power), but once every 200 frames (every 9 s), one training pulse is transmitted at approximately 10 times the slot average power. The peak power of this pulse in a 600 mW handheld radio is thus about 6 W, for a duration of a few hundred μ s. It was therefore necessary to utilize a linear amplifier with at least 10 dB headroom to accommodate the high-power training pulse without clipping.

The RF carrier was generated by a Motorola-supplied iDEN® transmitter (configured by Motorola to operate at a carrier frequency of 813.5625 MHz) driving a 200 W linear power amplifier (MPE PAS-53-0-800/1000) through an adjustable attenuator (Merrimac AU-45ASN) used to set power levels. The amplifier output was measured by a Narda 3001-30 directional coupler, HP 8482A power sensor, and an HP 435B power meter. The analog output from the power meter was connected to a chart recorder and was recorded at all times. The powered TEM cell was terminated with a high quality 50 Ω RF resistive load. Two power levels were used: the nominal exposure (450 mW slot-average input) and 10 dB higher (4.5 W). Before each series of experiments, the power at the TEM cell input was confirmed with an HP 8431B power sensor (with calibrated 30 W attenuator) and an HP 437B digital power meter. As stated above, the forward power was recorded throughout each experiment.

In conformity with our previous TDMA exposure protocols, the carrier was turned on and off at 20 min intervals by a Chronrol electronic timer. Total incubation times of 2, 3, and 21 h, therefore, represented total RF exposure periods of 1, 1.67, and 10.67 h, respectively.

2.3. Dosimetry

We used a CC-110s TEM cell (inside dimensions of 18 cm W $\times$ 18 cm D $\times$ 9 cm H, both above and below the septum) for RF exposures and placed the dishes on a 1.5 cm platform of styrene plastic that supported the 60 mm

Petri dish containing the cell culture within a region of reasonably uniform field ($\vec{E}$ normal to the dish). The platform was placed on the TEM cell septum. This decision was based on dosimetric assessments for this system performed by Prof. Dr. Niels Kuster and colleagues at the Swiss Federal Institute of Technology, Zurich, Switzerland [34]. The specific conditions used in this experiment were as follows: 1) 60 mm Petri dishes with 5 ml of medium, $\epsilon = 77$, and $\sigma = 1.8 \text{ mho m}^{-1}$; 2) 1 dish was placed centrally along the longitudinal axis on the septum in each TEM cell. These conditions were simulated using the MAFIA electromagnetic simulation tool (The Mafia Collaboration, User's Guide Mafia Version 3.x; CST GmbH, Lautenschlaegerstr. 38, D64289 Darmstadt, Germany). Calculations yielded the following slot average SAR values for exposure to the iDEN® signal at 0.8 mW cm⁻² (input power of 450 mW): 1) average SAR = $2.4 \mu\text{W g}^{-1}$; 2) standard deviation = $0.3 \mu\text{W g}^{-1}$. We have reported previously SAR values of $2.6 \mu\text{W g}^{-1}$ (average) and $1.9 \mu\text{W g}^{-1}$ (SD) for exposure to the TDMA signal at 0.9 mW cm⁻² (input power of 510 mW). For experiments performed at +10 dB, therefore, average SAR values were $24 \mu\text{W g}^{-1}$ for the iDEN® signal (input power of 4.5 W) and $26 \mu\text{W g}^{-1}$ for the TDMA signal (input power of 5.1 W). Importantly, there was no detectable rise in temperature at any power density used in these experiments. Media temperature was measured using a microprocessor-controlled thermometer developed in our laboratory. This instrument is based on a Vitek-type probe (BSD Medical Devices, Salt Lake City, UT), and can resolve temperature changes as small as 0.002°C.

2.4. Local static and 60 Hz magnetic fields

The local static field in the incubator used in this series of experiments was measured with a MAG-03 3-axis fluxgate magnetometer (Bartington Instruments, Oxford, England). Because of size limitations (i.e., size of the probe vs. size of the TEM cells), static field measurements were not made inside the TEM cells. Rather, measurements were made at nine locations on a square 10 cm grid on a shelf at the approximate center of the incubator. The magnitude of the local static field was $31 \pm 12 \mu\text{T}$ at an inclination angle of $-9 \pm 28^\circ$ relative to the horizontal. The ambient 60 Hz magnetic field was measured at each TEM cell location using a Monitor Industries 42B gaussmeter, and was found to be $0.13 \pm 0.02 \mu\text{T}_{\text{rms}}$ at the location of one Crawford cell (used for RF exposure) and $0.20 \pm 0.04 \mu\text{T}_{\text{rms}}$ at the location of the second Crawford cell (used for the sham exposure).

2.5. Comet assay

DNA single-strand breaks were measured with the alkaline comet, or single cell gel electrophoresis, assay, which

Table 1

Mean values for tail moment (TM) and comet extent (CE) from experiments in which Molt-4 populations, one placed in the upper TEM cell and the other in the lower TEM cell, were both sham exposed

Time ^a	TM (upper)	TM (lower)	<i>p</i> ^b	CE (upper)	CE (lower)	<i>p</i>
2	4.11 ± 0.42 ^c	4.29 ± 0.44	0.53	96.4 ± 2.1	96.7 ± 1.9	0.43
3	2.90 ± 0.41	2.61 ± 0.55	0.54	72.8 ± 2.0	78.0 ± 2.4	0.44
21	2.96 ± 0.42	3.09 ± 0.55	0.52	82.2 ± 1.8	81.4 ± 2.1	0.52

^aValues are the total incubation times in hours for cell cultures (see Section 2).

^bThe *p* value was derived from analysis of data using Kolmogorov–Smirnov distribution test.

^cValues given are the mean ± SE; the number of experiments pooled for each exposure condition were: 2 h, *n* = 5; 3 h, *n* = 3; 21 h, *n* = 3.

was performed using a modification of the technique reported by Singh et al. [29]. At the conclusion of each experiment, cells were collected by centrifugation in a microfuge (500 rpm, 5 min) at room temperature. Care was taken not to overspin the cells, since this resulted in increased DNA damage. The supernatant was discarded, and the pellet was suspended in 40 μl complete tissue culture medium. Slides were prepared by pipetting 120 μl agarose solution (0.5% 3:1 high resolution blend agarose, Amresco, Solon, OH) containing 5 mg ml⁻¹ proteinase K (Amresco, Solon, OH) onto fully frosted glass slides, which were covered immediately with a #1 coverglass. Slides were kept on ice for 30–60 s and the coverglass was then removed. Seventy five μl of cell suspension in agarose (10 μl suspended cell pellet + 200 μl agarose; mixed gently) was then pipetted onto the slide and again covered immediately with a coverglass. After 30–60 s on ice, the coverglass was removed, and a final 100 μl aliquot of agarose/proteinase K solution was pipetted onto the slide, which was covered with a coverglass. After 30–60 s on ice, the slides were immersed for 15 min in ice-cold lysis solution (2.5 M NaCl, 1% Na lauryl sarcosinate, 100 mM Na₂EDTA, 10 mM Tris (pH 10.0), plus

Triton X-100 added freshly each experiment to a final concentration of 1%). Slides were then transferred to a Coplin jar containing lysis solution at 37°, and incubation was continued for 2 h at this temperature. After lysis, slides were transferred to the horizontal slab of an electrophoresis unit and covered with electrophoresis buffer (300 mM NaOH, 0.1% 8-hydroxyquinoline, 2% dimethyl sulfoxide, 10 mM Na₄EDTA). After allowing 20 min for DNA to unwind, electrophoresis was performed at 10 V for 60 min. Buffer circulation was accomplished by stirring. After electrophoresis, slides were neutralized 3 × 30 min in 0.4 M Tris · HCl, pH 7.4. Subsequently, slides were dehydrated 3 × 30 min in absolute ethanol and air dried. All procedures were performed with either minimal direct lighting or red light.

2.6. Data acquisition and analysis

One slide at a time was stained with 50 μl 1 mM YOYO-1 (Molecular Bioprobes, Eugene, OR) and covered with a 24 × 50 mm coverglass. Fluorescently-stained DNA was detected with an Olympus BX40F3 fluorescent microscope equipped with a Dage SIT-68 camera and a Dage model DSP-200 image enhancer. Data was acquired for 50 randomly chosen comets per group with Komet 3.0 software (Integrated Laboratory Systems, Research Triangle Park, NC) and transferred to Excel spreadsheets for analysis. Two parameters were used to assess the extent of DNA damage in individual cells: 1) tail moment (TM), which is defined as tail length × tail intensity or percent migrated DNA; and 2) comet extent (CE) (i.e., comet length). Descriptive statistics (i.e., mean, standard error) were calculated to provide: 1) some characterization of the population of cells used for each experiment; and 2) an indication of the direction of change (i.e., increase or decrease) in cases where statistically significant differences in DNA damage were observed. Statistical differences in the distribution of TM and CE values in control vs. exposed groups

Table 2

Mean values for TM and CE for Molt-4 cells exposed to the iDEN[®] RFR signal (SAR of 2.4 μW g⁻¹; power density of 0.8 mW cm⁻²) vs. sham-exposed cells and the TDMA RFR signal (SAR of 2.6 μW g⁻¹; power density of 0.9 mW cm⁻²) vs. sham-exposed cells

	Time ^a	Control TM	Exposed TM	<i>p</i> ^b	Control CE	Exposed CE	<i>p</i>
iDEN [®]	2	6.24 ± 0.62 ^c	3.93 ± 0.33	< 0.0001 *	112.5 ± 1.7	105.3 ± 1.5	< 0.0001 *
	3	3.14 ± 0.36	3.41 ± 0.35	< 0.0001 *	97.9 ± 1.9	100.8 ± 1.6	< 0.0001 *
	21	4.22 ± 0.41	2.74 ± 0.37	< 0.0001 *	90.4 ± 1.7	88.3 ± 1.4	< 0.0001 *
TDMA	2	3.77 ± 0.38	3.50 ± 0.41	< 0.0001 *	90.3 ± 1.5	91.5 ± 1.5	< 0.0001 *
	3	2.91 ± 0.71	2.11 ± 0.50	0.68	84.5 ± 3.5	92.1 ± 2.3	0.001 *
	21	2.86 ± 0.26	1.68 ± 0.17	< 0.0001 *	75.7 ± 1.0	67.1 ± 0.9	< 0.0001 *

^{a,b}See Table 1.

^cValues given are the mean ± SE; the number of experiments pooled for each exposure condition were: iDEN[®]: 2 h, *n* = 5; 3 h, *n* = 6; 21 h, *n* = 6; TDMA: 2 h, *n* = 5; 3 h, *n* = 1; 21 h, *n* = 8.

Table 3

Mean values for TM and CE for Molt-4 cells exposed to the iDEN[®] RFR signal (SAR of 24 $\mu\text{W g}^{-1}$; power density of 8 mW cm^{-2}) vs. sham-exposed cells, and the TDMA RFR signal (SAR of 26 $\mu\text{W g}^{-1}$; power density of 9 mW cm^{-2}) vs. sham-exposed cells

	Time ^a	Control TM	Exposed TM	<i>p</i> ^b	Control CE	Exposed CE	<i>p</i>
iDEN [®]	2	6.09 $\pm$ 0.41 ^c	11.10 $\pm$ 0.58	< 0.0001 *	105.6 $\pm$ 1.3	121.0 $\pm$ 1.4	< 0.0001 *
	3	2.44 $\pm$ 0.38	1.93 $\pm$ 0.37	0.33	71.8 $\pm$ 1.4	69.3 $\pm$ 2.0	0.002 *
	21	4.31 $\pm$ 0.42	6.35 $\pm$ 0.60	< 0.0001 *	92.5 $\pm$ 1.9	93.6 $\pm$ 2.3	< 0.0001 *
TDMA	2	4.03 $\pm$ 0.41	3.44 $\pm$ 0.37	< 0.0001 *	109.4 $\pm$ 1.5	99.7 $\pm$ 1.5	< 0.0001 *
	3	3.49 $\pm$ 0.50	2.93 $\pm$ 0.50	0.65	106.7 $\pm$ 2.0	103.8 $\pm$ 1.7	0.18

^{a,b} See Table 1.

^c Values given are the mean $\pm$ SE; the number of experiments pooled for each exposure condition were: iDEN[®]: 2 h, *n* = 7; 3 h, *n* = 2; 21 h, *n* = 5; TDMA: 2 h, *n* = 6; 3 h, *n* = 2.

were determined using the Kolmogorov–Smirnov distribution test.

3. Results

Table 1 presents the results of our sham/sham experiments for total incubation times of 2, 3, and 21 h (RF exposure times of 1, 1.67, and 10.67 h, respectively; see Materials and Methods). These experiments were not performed as a continuous block, but were performed at random intervals during the entire course of this study. It is seen that for all conditions, the distribution of values for TM and for CE were not statistically different for cells incubated concurrently in the unpowered upper and lower TEM cells.

Table 2 presents the results of studies in which Molt-4 cells were exposed to either the iDEN[®] or the TDMA signal at SARs of 2.4 or 2.6 $\mu\text{W g}^{-1}$, respectively. Both signals induced a statistically significant shift in the distribution of TMs to lower values after incubation times of 2 and 21 h. On the other hand, after 3 h incubation time, the iDEN[®] signal produced a slight, although significant, shift in TMs upward. Although the TDMA signal appeared to shift TMs to higher values after 3 h total incubation time, this result is based on only a single experiment. CE values generally follow the same trends, although means are not shifted upward or downward to the same extent as seen with TM values.

Table 3 presents the results of studies in which Molt-4 cells were exposed to either the iDEN[®] or the TDMA signal at SARs of 24 or 26 $\mu\text{W g}^{-1}$, respectively. After total incubation times of 2 and 21 h, the iDEN[®] signal produced large and statistically significant shifts in the distribution of TMs to higher values. Interestingly, after 3 h total incubation time, the iDEN[®] signal shifted the distribution of TMs to lower values, although this result was not significant and based only on two experiments. In contrast, after 2 h total incubation time, the TDMA signal induced a statistically significant shift in the distribution of TMs to lower values. After 3 h total incubation time, TMs were again shifted to lower values although this result was not statistically significant and was based on only two

experiments. Here, as with the data of Table 2, CE values generally follow the same trends, although means are not shifted upward or downward to the same extent as seen with TM values. Regrettably, it was not possible to perform experiments at 9 mW cm^{-2} for 21 h total incubation time.

4. Discussion

In this study, we have assessed the damage produced in cells exposed in vitro to two different RF signals by employing the comet, or single cell gel electrophoresis, assay under alkaline conditions. We have analyzed two endpoints, tail moment (TM) and comet extent (or length; CE). The latter is the simplest parameter available to measure DNA damage and is that reported by Lai and Singh in their studies [31,32,35]. The concept of TM to assess DNA damage was introduced by Olive et al. [36], since this parameter increases linearly over a wider range of ionizing radiation doses than does CE. In fact, some consider TM to be a superior metric for assessing DNA damage, since TM incorporates a measure of both the smallest DNA fragment detectable (reflected in comet length) and the number of DNA fragments (represented by the amount of DNA in the tail). Indeed, our data indicate TM to be a more sensitive measure of DNA damage than CE, as judged by the greater magnitude of change demonstrated in TM as compared to CE for any given experiment or condition. It has been observed by others that there is an upper limit to CE for a given set of experimental conditions that is reached rapidly. Additional damage, therefore, increases the proportion of DNA in the tail, but it does not increase the CE. It is for this reason that we concentrate on RF signal-induced changes in TM in this discussion.

We have chosen to focus on the *distribution* of damage among the cells of a given population rather than on changes in group mean response, which can be altered easily by only a very few comets at a measurement extreme. Consequently, rather than analyze our data using analysis of variance as other investigators do, we have employed the Kolmogorov–Smirnov distribution test to assess differences between our two cell populations. Group

mean values have been calculated so that the direction in which changes in TM or CE distribution have occurred could be determined. Furthermore, we have pooled the data from all experiments at each specific exposure condition. This procedure increased the sensitivity with which we could detect shifts in the distribution of TM and CE values since group size was increased over that of the individual experiments, and has been employed by others to achieve the same purpose [37,38].

Our results are of interest for several reasons. First, the data indicate that two different RF signals were capable of interacting with a biological system *in vitro* and altering the extent to which damaged DNA could be observed. These results confirm, at least in concept, the reports by Lai and Singh [31,32] and by Sarkar et al. [28] of increased DNA damage in the organs of animals exposed *in vivo* to microwave radiation. Second, the iDEN[®] and TDMA RF signals produced generally similar decreases in DNA damage after exposure to SARs of 2.4 and 2.6 $\mu\text{W g}^{-1}$, respectively, for 2 h and 21 h total incubation time. In contrast, exposure of Molt-4 cells to the iDEN[®] signal at an SAR of 24 $\mu\text{W g}^{-1}$ produced a substantial increase in DNA damage after 2 h total incubation time, while exposure to the TDMA signal at an SAR of 26 $\mu\text{W g}^{-1}$ for 2 h total incubation time resulted in decreased DNA damage compared to control cell cultures. The decreased DNA damage in TDMA-exposed cell cultures is of particular interest, since it has been reported by us that long-term *in vivo* exposure of rats treated *in utero* with the chemical carcinogen, ethylnitrosourea, to the TDMA RF signal resulted in significantly fewer central nervous system tumors as compared to unexposed animals [39]. Indeed, even rats not treated with carcinogen but exposed to the TDMA signal demonstrated fewer spontaneous central nervous system tumors than control animals [39]. Furthermore, it appears that the apparent 'protective' effect of the TDMA signal may be related to the signal's modulation, since ethylnitrosourea-treated rats exposed to an FM (continuous wave) signal at the same frequency and power density and with the same exposure regimen demonstrated no difference in tumor incidence between control and exposed groups [40].

How is it that exposure of cells to the same signal under different conditions (i.e., of time and/or intensity) or to different RF signals can produce both increases and decreases in detectable DNA damage? We believe the key to interpreting such data lies in understanding the balance that exists between DNA damage and the repair of that damage. For instance, an overall increase in DNA damage may be caused by: 1) increased damage with no effect on repair mechanisms; 2) no effect on damage *per se*, but decreased capacity for repair; or 3) increased damage and decreased repair. Similarly, an overall decrease in DNA damage may be caused by: 1) decreased DNA damage with no effect on repair mechanisms; 2) no effect on damage *per se*, but increased capacity for repair; or 3)

decreased damage and increased repair. Furthermore, it must be borne in mind that our model system, Molt-4 cells, is unsynchronized and dynamic. Depending on the state of the cells at the start of each experiment (e.g., cell cycle distribution, growth rate), one response may be favored over another. Using the data of Table 2 as an example, we offer the following interpretation. After 2 h total incubation time, cells exposed to the iDEN[®] RF signal have, compared to unexposed control cells, either decreased DNA damage, increased damage repair capacity, or both. However, by 3 h total incubation time, either damage has increased so that the capacity of the repair systems have been exceeded, or the repair systems have become impaired or otherwise less active, or both of these. After 21 h total incubation time, we observe a situation similar to that seen at 2 h. A different situation is seen with the data of Table 3. After 2 or 21 h total incubation time, net DNA damage is greater in iDEN[®]-exposed as compared to control cultures because RF exposure has produced greater damage to DNA, or repair mechanisms have become less active, or both of these. Finally, we must stress that, because of the consistent results derived from our sham/sham exposure experiments, we believe our data to indicate a real effect of RF exposure on DNA damage detectable in the comet assay.

At this point, there are two key questions. First, is it possible for RF exposure to produce an increase in DNA damage directly (i.e., without affecting the rate of DNA repair)? Second, is it possible for RF exposure to alter, either by increasing or by decreasing, the rate at which DNA repair occurs? Each of these questions will be considered in turn.

There is continued study of the relationships between free radicals and human pathology. This is of interest in bioelectromagnetics research, since it has been proposed that electromagnetic signals may 'couple' to biological systems through effects on chemical reactions involving the formation of free radicals [41,42]. There is mounting evidence that reactive oxygen species (such as O_2^- , $\text{HO}^\cdot$, and H_2O_2) and reactive nitrogen species (such as $\text{NO}^\cdot$, $\text{NO}_2^\cdot$, and $\text{ONO}_2^\cdot$) contribute to human tumorigenesis through the production of genetic mutations that are associated with the initiation and progression of cancer and with changes in cell proliferation associated with chronic inflammation. Additionally, many neurological disorders may be derived from free radical-induced injury, simply because the high lipid content and high energy requirements of the brain make that organ especially sensitive to damage mediated by free radicals and oxidative stress. In this regard, it is of interest that long-term exposure to low-level extremely low frequency EMFs appears to be associated with increased incidences of cancer [43,44] and Alzheimer's disease in humans [45].

The production of free radicals is a natural consequence of aerobic metabolism and cellular biochemistry, and DNA damage produced by oxidation appears to be the most

significant endogenous damage [46]. It has been estimated that the 'hits' to DNA from endogenous oxidants are normally 10^5 per cell per day in the rat and 10^4 in the human [47,48]. Oxidative damage is repaired effectively, although not perfectly, and lesions that escape repair have a certain probability of producing mutations when the cell divides, a situation which may ultimately lead to disease. In proliferating cells, several mechanisms exist which allow damaged DNA to be repaired and the number of spontaneous or exogenously-induced genetic alterations to be minimized. These include: a) DNA excision repair pathways (repair single strand breaks, base damage, adduct formation); b) postreplication repair; c) repair of DNA double-strand breaks; and 4) delayed progress through the cell cycle, thus providing added time for DNA repair either prior to DNA replication (S phase of cell cycle) or to mitosis (M phase). As indicated above, the question is whether or not RF exposure or other EMF exposure can alter the rate at which DNA repair occurs. Unfortunately, this is an area that has not yet been investigated, although exposure to various electromagnetic signals has been reported to affect the activity of a variety of enzymes, such as protein kinases [6,49,50] acetylcholinesterase [51], and ornithine decarboxylase [7,9,52]. Additionally, studies from our lab have indicated that exposure of Molt-4 cells to a 1 G sinusoidal MF at 60 Hz decreased the activity of the repair enzyme, poly(ADP-ribose) polymerase, and increased the number of etoposide-treated cells that are destroyed by apoptosis (J.L. Phillips, unpublished data). Also, it is of interest that Lai and Singh [53] have recently demonstrated that free radicals may indeed play a part in RFR-induced DNA damage.

In summary, our data indicate that exposure of Molt-4 T-lymphoblastoid cells in vitro to two different RF signals under athermal conditions altered the amount of DNA single-strand breaks detected by the alkaline comet assay. Depending on the signal and the time of exposure, DNA damage was observed to both increase and decrease. It is of interest to determine whether or not differences in the modulation of the TDMA and iDEN[®] signals have an effect on the direction of change. Indeed, Penafiel et al. [54] have reported recently that modulation of an 835 MHz RF signal played a role in determining the effect of RF exposure on ornithine decarboxylase activity in L929 murine fibroblasts. Furthermore, in order to more fully understand the underlying mechanism(s) responsible for these changes, it will be necessary in future studies to distinguish between RFR effects on DNA damage and RFR effects on DNA repair.

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Effects of electromagnetic fields produced by radiotelevision broadcasting stations on the immune system of women

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Abstract

The object of this study was to investigate the immune system of 19 women with a mean age of 35 years, for at least 2 years (mean = 13 years) exposed to electromagnetic fields (ELMFs) induced by radiotelevision broadcasting stations in their residential area. In September 1999, the ELMFs (with range 500 KHz–3 GHz) in the balconies of the homes of the women were (mean $\pm$ S.D.) 4.3 ± 1.4 V/m. Forty-seven women of similar age, smoking habits and atopy composed the control group, with a nearby resident ELMF exposure of < 1.8 V/m. Blood lead and urinary *trans-trans* muconic acid (a metabolite of benzene), markers of exposure to urban traffic, were higher in the control women. The ELMF exposed group showed a statistically significant reduction of blood NK CD16⁺CD56⁺, cytotoxic CD3⁺CD8⁺, B and NK activated CD3⁺HLA-DR⁺ and CD3⁺CD25⁺ lymphocytes. 'In vitro' production of IL-2 and interferon- γ (INF- γ) by peripheral blood mononuclear cells (PBMC) of the ELMF exposed group, incubated either with or without phytohaemagglutinin (PHA), was significantly lower; the 'in vitro' production of IL-2 was significantly correlated with blood CD16⁺CD56⁺ lymphocytes. The stimulation index (S.I.) of blastogenesis (ratio between cell proliferation with and without PHA) of PBMC of ELMF exposed women was lower than that of the control subjects. The S.I. of blastogenesis of the ELMF exposed group (but not blood NK lymphocytes and the 'in vitro' production of IL-2 and INF- γ by PBMC) was significantly correlated with the ELMF levels. Blood lead and

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urinary *trans-trans* muconic acid were barely correlated with immune parameters: the urinary metabolite of benzene of the control group was only correlated with CD16⁺-CD56⁺ cells indicating a slight effect of traffic on the immune system. In conclusion, this study demonstrates that high frequency ELMFs reduce cytotoxic activity in the peripheral blood of women without a dose-response effect. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Electromagnetic field; Immune system; Lymphocyte subpopulations; Cytokines; Traffic

1. Introduction

Several studies have reported that electromagnetic fields (ELMFs) modify the human genome and induce malignancies (Theriault, 1996). An increased incidence of cancer has been found in the area surrounding a radio and television transmitter in Great Britain (Dolk et al., 1997a). On the other hand, these results have not been confirmed by investigations performed on populations resident near other power transmitters in the same country (Dolk et al., 1997b). Although there is concern for the risk of cancer, until now, a clear relation between ELMF exposure and the incidence of neoplasms has not been demonstrated as the studies showed either contradictory results or the presence of confounding factors (Theriault, 1996; Knave, 2000). It is clear that more research on ELMF-exposed populations is needed to get satisfactory epidemiological study results or to demonstrate an involvement of ELMFs in mechanisms which may induce cancer.

Several studies report that the immune system exerts defence mechanisms against cancer: natural killer (NK) cell activity is reduced in patients with neoplastic diseases (Pross and Lotzova, 1993); cytotoxic activity against neoplastic cells is an important prognostic factor in patients with resected lung carcinoma (Fujisawa and Yamaguchi, 1997); modern therapy of cancer includes treatment with lymphokines such as interleukin (IL) 2 and interferon (INF) α and γ (Kamamura et al., 1998). For this reason, it may not be excluded that ELMFs may increase the incidence of cancer by impairing immune defences.

It was shown that ELMFs modify calcium fluxes in the membranes of immune cells of humans acting on the release of tromboxane B₂ and IL-1 (Conti et al., 1985). Moreover, peripheral blood mononuclear cells (PBMC) of humans exposed

‘in vitro’ to low frequency ELMFs showed changes in [³H]thymidine incorporation following ‘in vitro’ stimulation by mitogens (Conti et al., 1983, 1986). Recently, PBMC were exposed for 24, 48 and 72 h to an ELMF with repetition frequency of 50 Hz and field intensity of 1.5 mT (Di Gioacchino et al., 2000). The ELMF exposure influenced CD3⁺, CD4⁺, CD8⁺ and CD16⁺ surface marker expression and/or localisation. Moreover, DNA CD4⁺ expression strongly increased in the exposed cells.

‘In vitro’ cell proliferation of PBMC of subjects exposed to ELMFs produced by an Italian TV broadcasting station was different from that of the control group (Giuliani et al., 1996). Moreover, cytotoxicity tests showed a significant reduction of NK activity when PBMC of the exposed group were re-irradiated ‘in vitro’ by radiofrequencies (639.25 MHz, 12 V/m average, 50 Hz amplitude modulated or an ELMF of 50 Hz, 0.67 mT). We studied immune parameters of men and women employed in a museum who were exposed to an ELMF (range 0.2–3.6 μ T and 40–120 V/m) induced by 50 Hz electricity for 20 h a week (Boscolo et al., 2001). Men working in the museum showed, in relation to the control subjects, a statistically significant reduction of both number and percentage of NK CD16⁺-CD56⁺ and NK and B CD3⁺-CD25⁺ lymphocyte subsets; women showed a significant reduction in the percentage of CD3⁺-CD25⁺ lymphocytes and a slight reduction of CD16⁺-56⁺ lymphocytes. They also showed significantly lower levels of INF- γ in serum or produced ‘in vitro’ by PBMC, both spontaneously and stimulated by PHA, while they showed no significant changes of serum and ‘in vitro’ produced IL-4, as well as of blastogenesis of PBMC. These results were similar to the reduced NK and lymphokine activated activity found in subjects with poor lifestyle (Morimoto et al., 1999,

2001) or with a psychological behaviour characterised by difficulty in expressing feelings (De-waraja et al., 1997).

The purpose of this study was to investigate effects of ELMFs induced by radiotelevision broadcasting stations on the immune system of the ELMF exposed population.

2. Subjects and methods

Women were investigated because they remained in their homes on a hill near several radiotelevision broadcasting stations for a longer period (< 12 h a day) than men. This hill, San Silvestro, inhabited by approximately 2000 people, faces the Adriatic Sea 2 km away from Pescara, a town in Central Italy. During October 1997, the higher ELMF levels determined in San Silvestro by the Italian Istituto Superiore Prevenzione Sicurezza e Lavoro (ISPESL) and by the regional environmental protection agency (ARTA), were ranging from 11 to 40 V/m and from 0.5 to 4.0 W/m² (Vignati et al., 1997); in the same locality in September 1999, the ELMFs determined by ARTA ranged from 10 to 25 V/m. In the period 1997–1999, the values of ELMFs in the nearby towns of Pescara and Chieti (where the control group was resident), determined by ISPESL and ARTA, were < 1.8 V/m. The two towns (with a distance of 15 km) are enclosed between the Adriatic Sea and the Appennino Mountains. In this area, high levels of traffic are inside Pescara and Chieti and in high-speed motorways connecting these towns and those nearby. However, the toxic compounds produced by traffic and those emitted in lesser amounts by factories are almost uniformly distributed in the environment because of climatic and geographic conditions; the population show only slight differences in blood trace elements, including lead, mainly derived from tetraethyl and tetramethyl lead added to gasoline (Boscolo et al., 1993).

In September 1999, the values of ELMFs induced by the radiotelevision broadcasting stations were determined in the balconies and inside the homes of the women resident in San Silvestro recruited for this study (in addition to the values

above reported determined in the areas with high ELMF exposure). The following instruments (PMM, Milano, Italia) were utilised:

1. probes for electric fields BA-01 (range 500 KHz–3 GHz; f.s. 120 V/m);
2. probes for electric fields BA-05 (range 10 MHz–1 GHz; f.s. 30 V/m);
3. monitor 8051 with optic repeater OR-01;
4. cables with optic fibres to control the instruments at a distance (to avoid interference of the operators on ELMF levels); and
5. wood supports to insulate the probes.

Nineteen women with a mean age of 35 years (range 22–49 years) resident in San Silvestro at least from 1997 (mean period of residence = 13 years) were investigated. Most of them were housewives; six (31.5%) were smokers (less than 10 cigarettes a day); seven (36.8%) were atopics: six had suffered from seasonal asthma and/or rhinitis with serum IgE > 100 IU/ml (CAPFeia, Pharmacia, Uppsala, Sweden) and one of them, with 92 IU/ml of serum IgE, suffered from seasonal rhinitis with positive skin test to inhalant allergens.

The control group, in which blood lead, serum IgE and lymphocyte subsets were determined, was composed of 47 women with a mean age of 35 years (range 21–49 years). This group included another subset of 17 women with a mean age of 34 years (range 22–44 years), in which urinary *trans-trans* muconic acid and 'in vitro' lymphoproliferation and production of cytokines by PBMC was also measured. The control group of 47 subjects comprised 17 atopics (36.1%) and 14 smokers (29.8%); the control group of 17 subjects was also composed by six atopics (35.3%) and five smokers (29.4%). The diagnosis of atopy was made considering the levels of serum IgE, history of allergic disease and skin test to inhalant allergens. Therefore, the ELMF exposed and the control groups of 47 and 17 women showed similar age, atopy and smoking habit, while the control groups, resident in the above reported area of Pescara and Chieti, showed a higher exposure to traffic. The exposure to ELMFs produced by electric appliances in the homes (Mader and Peralta,

1992) of all the women recruited in this study was not considered.

Clinical assessment included physical examination and standard routine blood analyses. Pregnant women, those taking drugs or suffering from diseases were not recruited for the investigation. Atopic women in the active phase of allergic disease treated with systemic drugs (steroids and/or antihistamines) were also excluded.

Informed consent was obtained from the recruited subjects according to a procedure approved by the 'Ethic committee' of the University 'G. D'Annunzio' of Chieti. Blood and urine samples were collected in plastic cryovials (Nalgene, International PBI, Milano, Italy) at 08.00 h, with a standard procedure (Sabbioni et al., 1992) in order to determine blood lead and urinary *trans-trans* muconic acid (a metabolite of benzene), markers of exposure to traffic (Boscolo et al., 2000). Blood lead was determined by an atomic absorption spectrophotometer Perkin-Elmer 4100 ZL (Sabbioni et al., 1992). A further analytical quality control on 10% of samples was carried out in two different laboratories. Urinary *trans-trans* muconic acid was analysed by HPLC (Ducos et al., 1990, 1992).

Fluorescein isothiocyanate (FITC) and phycoerythrin (PE)-conjugated antibodies (Becton-Dickinson, San Jose, CA, USA) were used to determine lymphocyte subsets. The antibodies were CD4-CD45RO [to evaluate helper CD4⁺-CD45RO⁺ 'memory' and CD4⁺-CD45RO⁻ 'naive or virgin' lymphocytes (Male et al., 1996)], CD3-CD8, CD16-CD56 (NK cells), CD19 (B lymphocytes), CD3-HLADR and CD3-CD25 (both activated NK and B lymphocytes). Two-colour flow-cytometry analysis was performed by FACscan (Becton-Dickinson, San Jose, CA, USA) (Fleischer et al., 1988). Serum IgE was measured by ELISA (Brostoff et al., 1991).

For determining the production of cytokines, PBMC were incubated for 24 h at 37°C in 5% CO₂-humidified atmosphere in polypropylene tubes (Falcon, Italy) with or without 20 µg/ml of phytohaemoagglutinin (PHA) (Difco). At the end of the incubation period, cell-free supernatants were harvested and stored at -20°C until assay of IL-2, IL-4, IL-5 and INF-γ (Benfer-Scheller,

Key-Stone Laboratories, USA) with or without PHA by ELISA (Fridas et al., 1996).

The blastogenesis (proliferation) of PBMC was also determined 'in vitro' according to Conti et al. (1983, 1986). Blastogenesis was determined as the stimulation index (S.I.), which is the rate between [³H]thymidine incorporation by PBMC in the presence of PHA and without PHA, in the last 6 h of incubation after a 48-h incubation period.

Statistical analysis of the data was performed with Statistica, Release 4.5. Kolmogorov-Smirnov tests showed that most of the results concerning immune parameters did not conform to the normal distribution. Therefore, non-parametric methods were used for all simple descriptive statistics.

3. Results

ELMFs with range 500 KHz–3 GHz, determined during the mornings of September 1999 in the balconies of the homes of 17 out of the 19 investigated women resident in San Silvestro hill were (mean ± S.D.) 4.3 ± 1.4 V/m. ELMFs, within the same range of 500 KHz–3 GHz, determined inside the home, were approximately 30–50% lower than outside. ELMF values showed slight modifications during the 24-h. The levels of ELMFs of the homes of the recruited women were lower than those (ranging from 10 to 24 V/m reported in Section 2) determined in another area of San Silvestro. Only one ELMF exposed woman showed values of ELMF exposure (1.2 V/m outside her home) similar to that of the control group (< 1.8 V/m).

The values of blood lead and urinary *trans-trans* muconic acid, biomarkers, of exposure to traffic (Boscolo et al., 1999a, 2000) of the women of San Silvestro were lower than those of the control group resident in the nearby area of the towns of Pescara and Chieti (Table 1).

Serum IgE of the ELMF exposed women did not show statistically significant differences from those of the control subjects (Table 2).

ELMF exposed women showed a significant reduction of T 'virgin' CD4⁺-CD45RO⁻ lymphocytes and a higher ratio between 'memory' CD4⁺-

Table 1

Blood metals and urine *trans-trans* muconic acid of women exposed to electromagnetic fields^a

	Control			Exposed		
	No.	median	25th–75th percentiles	No.	Median	25th–75th percentiles
Blood lead ($\mu\text{g/l}$)	47	5.9	4.9–7.0	19	4.4	3.7–4.7*
Urinary <i>trans-trans</i> -muconic acid ($\mu\text{g/l}$)	17	42.0	19.1–107.5	19	15.0	9.5–32.5*
Urinary <i>trans-trans</i> -muconic acid ($\mu\text{g/g creatinine}$)	17	33.5	13.2–92.5	19	11.0	6.5–28.0**

^a Mann–Whitney *U*-test. Statistical significant difference: * $P < 0.05$; ** $P < 0.01$.

CD45RO⁺ and ‘virgin’ CD4⁺-CD45RO⁻ lymphocytes (Table 2). ELMF exposed women showed a reduction in both the number and percentage of total lymphocytes of blood NK CD16⁺-CD56⁺ lymphocytes, CD3⁻-CD8⁺ cells [CD8⁺ lymphocytes without the expression of the antigen T CD3⁺ and NK CD16⁺ cells (Male et al., 1996)] and of CD3⁻-CD25⁺ B and NK activated lymphocytes (Table 2 and Fig. 1). CD3⁻-HLA-DR⁺ B and NK activated lymphocytes of the ELMF exposed group were also significantly lower than

those of the control group when determined as the percentage of total lymphocytes (Table 2 and Fig. 1).

In ‘vitro’ production of di IL-2 and INF- γ by PBMC of the ELMF exposed women was significantly lower than that of the control group either without or with PHA in the incubation liquid (Table 3). On the other hand, there was no difference in the production of IL-4 and IL-5 by PBMC of the ELMF exposed and control women (Table 3).

Table 2

Serum IgE and blood lymphocyte subpopulations of women exposed to electromagnetic fields^a

Cells/ μl	Control (<i>n</i> 47)		Exposed (<i>n</i> 19)	
	median	25th–75th percentiles	median	25th–75th percentiles
Serum IgE (IU/ml)	25	10–79	41	14–121
Lymphocytes	1999	1780–2605	2050	1700–2545
CD3 ⁺	1421	1270–1811	1531	1214–1854
CD4 ⁺	884	711–1213	955	684–1152
CD4 ⁺ -CD45RO ⁻	323	260–477	264	211–347*
CD4 ⁺ -CD45RO ⁺	562	448–711	673	496–779
CD4 ⁺ -CD45RO ⁺ /CD4 ⁺ -CD45RO ⁻	1.50	1.01–2.02	2.36	1.99–3.18*
CD3 ⁺ -CD8 ⁺	520	442–726	562	467–674
CD3 ⁻ -CD8 ⁺	133	98–194	93	56–123**
CD16 ⁺ -CD56 ⁺	395	306–489	239	176–293**
CD19 ⁺	210	138–291	208	153–251
HLA-DR ⁺	482	392–601	432	361–561
CD3 ⁻ -HLA-DR ⁻	330	263–431	295	250–355
CD3 ⁺ -HLA-DR ⁺	124	92–176	131	89–220
CD25 ⁺	356	273–496	372	263–444
CD3 ⁻ -CD25 ⁻	95	77–126	68	31–92**
CD3 ⁺ -CD25 ⁺	248	195–351	287	239–386

^a Mann–Whitney *U*-test. Statistical significant difference: * $P < 0.05$; ** $P < 0.01$.

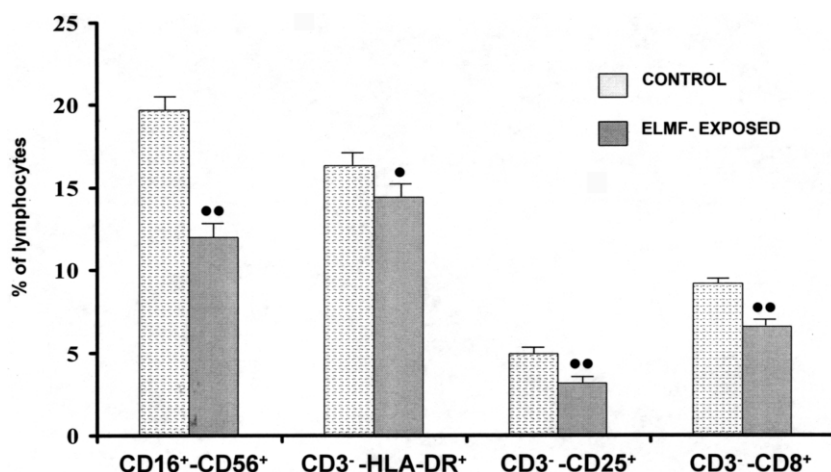


Fig. 1. Blood CD16⁺CD56⁺, CD3⁺HLA-DR⁺, CD3⁺CD25⁺ and CD3⁺CD8⁺ lymphocyte subsets (expressed as percentage of total lymphocytes) of women exposed to electromagnetic fields. Values are expressed as mean ± S.E. Mann-Whitney *U*-test (control women: *n* = 47; ELMF exposed women: *n* = 19). Statistical significant difference: **P* < 0.05; ***P* < 0.01.

The S.I. of blastogenesis (proliferation) of PBMC, determined using PHA, of the ELMF exposed group was significantly lower than that of the control group (Table 4). Notwithstanding this result, there was a significant correlation between the values of ELMFs determined from the balconies of 17 homes of the women resident in San Silvestro and their S.I. of blastogenesis. The curve which better interpolates data regarding the relation between V/m and S.I. is illustrated in Fig. 2: it is a logarithmic curve having the following equation: $y = 16.944x^{0.553}$ with $R^2 = 0.5519$. On the other hand, there was no significant correla-

tion between the values of ELMFs determined in the homes of the women resident in San Silvestro and their lymphocyte subsets or production of cytokines by their PBMC.

Blood lymphocytes CD16⁺CD56⁺ (reported as percentage of total lymphocytes) of all the examined women showed a positive linear correlation with the values of IL-2 produced 'in vitro' by PBMC either incubated in absence of PHA (*n* = 36, $R = 0.557$, $P < 0.001$) or with PHA (*n* = 36, $R = 0.416$, $P < 0.02$). Moreover, INF- γ produced 'in vitro' by PBMC with PHA was significantly correlated with the IL-2 produced 'in vitro' by

Table 3

'In vitro' production of cytokines by peripheral blood mononuclear cells (PBMC) incubated with or without phytohaemoagglutinin (PHA) of women exposed to electromagnetic fields^a

Cytokines (pg/ml)	Control (<i>n</i> 17)		Exposed (<i>n</i> 19)	
	median	25th–75th percentiles	median	25th–75th percentiles
IL-2 without PHA	17.70	5.50–38.70	1.57	1.29–2.50**
IL-2 with PHA	55.30	17.01–163.20	6.70	3.23–32.80*
IL-4 without PHA	1.70	1.29–3.01	2.11	1.5–2.59
IL-4 with PHA	3.30	1.60–5.80	3.31	2.35–5.40
IL-5 without PHA	1.28	0.85–1.98	1.90	1.10–3.27
IL-5 with PHA	12.50	6.95–18.10	11.40	6.20–37.25
INF- γ without PHA	0.81	0.49–1.11	n.d.	
INF- γ with PHA	27.50	8.30–51.50	7.60	1.03–15.65*

^a Mann-Whitney *U*-test. Statistical significant difference: **P* < 0.002; ***P* < 0.001; n.d. = non-determinable.

Table 4

Stimulation index (S.I.)^a of blastogenesis (proliferation) of peripheral blood mononuclear cells (PBMC) of women exposed to electromagnetic fields^b

	Control (n 17)		Exposed (n 19)	
	median	25th–75th percentiles	median	25th–75th percentiles
Simulation index (S.I.)	54.5	37.6–71.4	36.8	32.2–43.2*

^aS.I. is the rate between [³H]thymidine incorporation by PBMC in presence of phytohaemoagglutinin (PHA) and without PHA.

^bMann–Whitney U-test. Statistical significant difference: * $P < 0.05$.

PBMC both incubated in absence of PHA ($n = 36$, $R = 0.674$, $P < 0.0001$) and with PHA ($n = 36$, $R = 0.410$, $P < 0.02$).

Blood lead and urinary *trans-trans* muconic acid did not show a significant correlation with immune parameters with the exception of the correlation between urinary *trans-trans* muconic acid (reported as $\mu\text{g/g}$ creatinine) and $\text{CD}16^+\text{-CD}56^+$ lymphocytes ($n = 17$, $R = 0.627$, $P < 0.01$) and $\text{CD}4^+\text{-CD}45\text{RO}^-$ ‘virgin’ lymphocytes ($n = 17$, $R = 0.625$, $P < 0.01$) of the control group. For this reason, a correlation analysis between ELMF values of exposure and immune parameters (including $\text{CD}16^+\text{-CD}56^+$, $\text{CD}3^-\text{-HLA-DR}^+$, $\text{CD}3^-\text{-CD}25^+$, $\text{CD}3^-\text{-CD}8^+$ cells, S.I. of blastogenesis of PBMC and production of $\text{INF-}\gamma$ and IL-2 by

PBMC) was performed with adjustment for urinary *trans-trans* muconic acid: all these statistical analyses were not significant.

4. Discussion

The women exposed to ELMFs produced by radiotelevision broadcasting stations showed a reduction of NK $\text{CD}16^+\text{-CD}56^+$ and cytotoxic $\text{CD}3^-\text{-CD}8^+$ cells and of NK and B $\text{CD}3^-\text{-HLA-DR}^+$ and $\text{CD}3^-\text{-CD}25^+$ activated lymphocytes in the peripheral blood along with a significant reduction of IL-2 and $\text{INF-}\gamma$ produced ‘in vitro’ by PBMC. None of these immune parameters showed a dose–response effect with the ELMF values determined outside the homes of the investigated women.

Blood NK $\text{CD}16^+\text{-CD}56^+$ lymphocytes of all the examined subjects were significantly correlated with the values of IL-2 and $\text{INF-}\gamma$ produced ‘in vitro’ by PBMC. It is known that IL-2 and $\text{INF-}\gamma$, produced by activated T lymphocytes, exert a potent effect on macrophages and a synergistic action with that of other cytokines [e.g. $\text{TNF-}\alpha$ (Male et al., 1996)]. It is also known that $\text{INF-}\gamma$ produced by both T and NK lymphocytes, may activate NK lymphocytes with an ‘autocrine loop’ (Male et al., 1996). For this reason, lower levels of blood NK cells and of the ‘in vitro’ production of IL-2 and $\text{INF-}\gamma$ by PBMC of the ELMF exposed women are an expression of low cytotoxic activity in the peripheral blood.

The results of this study are in agreement with those of Giuliani et al. (1996), who showed altered cytotoxicity tests in subjects exposed to

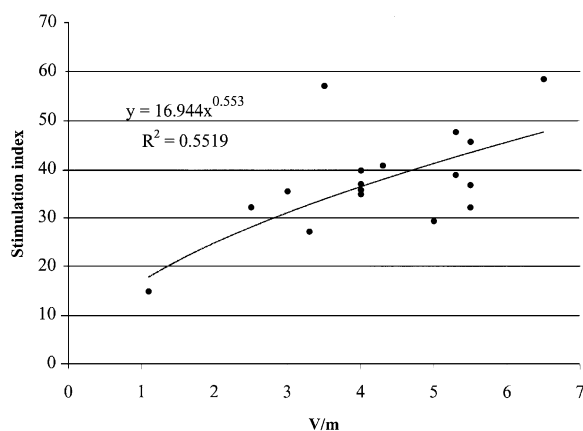


Fig. 2. Linear correlation between the values of electromagnetic fields produced by radiotelevision broadcasting stations on the balconies of the homes of women and their stimulation index (S.I.) of blastogenesis (proliferation) of peripheral blood mononuclear cells (PBMC). Pearson's correlation coefficient: $n = 17$, $R = 0.608$; $P < 0.01$.

ELMFs produced by a TV broadcasting station. This investigation also confirms the data of a previous study of our group on men and women employed in a museum exposed to an ELMF induced by 50 Hz electricity who showed low blood NK $CD16^+CD56^+$ and/or B and NK $CD3^-CD25^+$ lymphocytes and reduced production of $INF-\gamma$ by PBMC (Boscolo et al., 2001).

The results of this investigation are similar to those of Morimoto et al. (1999, 2001) and Dewaraja et al. (1997) who found low blood NK activity or a reduction of blood NK lymphocytes in subjects with poor lifestyle and with a psychological behaviour characterised by difficulty in expressing feelings. Therefore, we do not exclude that the effects of the ELMFs on the immune system may be, in part, mediated by those on nervous mechanisms which are closely connected with the immune ones (Male et al., 1996; Jankovic, 1992; Szentivay, 1997). With regard to this, the blood levels of both total and cytotoxic lymphocytes decreased following exposure to magnetic resonance imaging of the brain of volunteers, suggesting that the nervous system may regulate the trafficking of lymphocytes in peripheral blood (Reichard et al., 1996). It was also shown that the circadian biorhythm regulates not only the release and production of neurohormones, but also blood levels of lymphocyte subsets (Boscolo et al., 1999b).

Although blood cytotoxic activity may be modified by lifestyle (Morimoto et al., 1999, 2001), psychological behaviour (Dewaraja et al., 1997) and possibly occupational stress (Boscolo et al., 2001), the monitoring of blood cytotoxic activity and NK lymphocytes may be considered useful as a biomarker of exposure to ELMFs. A more interesting biomarker seems to be the $CD16^+CD56^+$ NK lymphocyte subpopulation, which is significantly correlated with the 'in vitro' production of IL-2 by PBMC.

Blood lead and urinary *trans-trans*-muconic acid, markers of exposure to traffic (Boscolo et al., 2000) of the control subjects, were higher than those of the ELMF exposed. These markers of traffic exposure were not correlated with parameters of cytotoxic activity, with the exception of the correlation between urinary *trans-trans*-muconic

acid and $CD16^+CD56^+$ lymphocytes of the control group. We could thus suggest that the difference in the values of $CD16^+CD56^+$ NK lymphocytes, between ELMF exposed and control women may rather depend on a higher effect of the toxic compounds produced by traffic on the control subjects. However, adjustment for urinary *trans-trans*-muconic acid did not show significant correlation between ELMF values and immune parameters (including $CD16^+CD56^+$ lymphocytes). For this reason, an effect of the exposure to traffic on the values of NK cells has to be considered as unimportant.

Urinary *trans-trans*-muconic acid was also positively correlated with $CD4^+CD45RO^-$ 'virgin or naïve' lymphocytes of the control group. Moreover, the control group showed higher blood levels of $CD4^+CD45RO^-$ lymphocytes, as well as of lead, than the ELMF exposed group. Sata et al. (1998) found a correlation between the percentage of 'naïve' lymphocytes and blood lead of male workers with mean blood lead of 19 $\mu\text{g}/\text{dl}$. These data suggest that the values of blood $CD4^+CD45RO^-$ and their ratio with $CD4^+CD45RO^+$ lymphocytes may depend on several factors, including lead and benzene produced by traffic.

ELMF exposed women showed a reduced stimulation of PHA on the proliferation of PBMC in relation to the control subjects with a higher exposure to traffic. We cannot exclude an enhancement of the proliferation of PBMC induced by the toxic compounds of traffic, as in the previous case. On the other hand, the S.I. of blastogenesis of the ELMF exposed women (although lower than that of the control subjects) was positively correlated with the ELMF levels determined outside their homes. Therefore, an effect of ELMFs in stimulating PBMC proliferation may also not be excluded.

A part of the immune parameters investigated in this study, plasma zinc and copper markers of metabolic activities (Prasad, 1988; Harbige, 1996), blood lead and urinary *trans-trans*-muconic acid were determined in women exposed to low and high frequency ELMFs and in control women considering the presence of atopy (Del Signore et al., 2000). Linear discriminant analysis applied to the above mentioned parameters showed no sig-

nificant difference between non-atopic groups exposed or not exposed to ELMFs, whereas there was a marked differentiation between atopic women non-exposed and atopic women ELMF exposed. This suggested that ELMFs have a greater influence on atopic women than on non-atopic ones.

We may conclude that exposure to ELMFs induces a modification of immune parameters in humans. ELMF exposure inhibits a Th1-like cytotoxic immune-response without a dose–response effect, while the enhancement of a Th2-like immune response is not demonstrated. Follow-up studies may be useful to explain the biological importance of the reported results.

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SHORT COMMUNICATION

**HEALTH EFFECTS OF ENVIRONMENTAL
ELECTROMAGNETIC FIELDS**

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ABSTRACT

We investigated the effects of exposure to environmental electromagnetic fields (EMFs) in 1170 subjects. Neutrophil phagocytosis was enhanced in the low-intensity exposure groups, but reduced significantly at relatively high intensities. Visual reaction time was prolonged and the scores of short-term memory tests were lower in some high-intensity exposure groups. EMFs may affect the central nervous and immune systems in man.

INTRODUCTION

The main environmental electromagnetic fields (EMFs) in China occur in the amplitude-modulated (AM) radio band (1), but various radar installations also result in exposure of the public to EMFs. The influence of low-level EMFs on health is a fundamental factor in formulating hygienic criteria to regulate and control EMF exposure. This study of subjects exposed to low-level EMFs was undertaken to provide data that would be pertinent to a consideration of exposure standards.

METHODS

Subjects living and working near radio antennae and radar installations were surveyed. The subjects exposed to microwaves were students at a radar college, third-year students at secondary schools located near radar installations, or soldiers whose duty included routine exposure to microwaves. The subjects exposed to AM EMFs were third-year students at secondary schools or kindergarten children. Subjects were admitted to the exposed cohort only if they had been exposed to the EMF for a minimum of one year. The control groups consisted of subjects of the same grade, age, sex and level of education who were not exposed to environmental EMFs. The exposed and control groups contained 1170 and 689 subjects, respectively.

The field intensities were measured with a Holaday HI-3004 and an isotropic broad frequency band probe. Power densities were measured with a Narda 8616 and an isotropic broad-band 8621 probe. If the power density was very low, the measurement was made using a horn antenna having a known gain, and a micro-wattmeter.

The subjects were evaluated using standardized written tests, and measurements of visual reaction time and white-blood-cell (WBC) phagocytosis. In the visual reaction time measurements, one of four lights (red, white, green or blue) appeared 20 times, and the subject's reaction time measured, and averaged over the 20 measurements. Short-term memory and concentration tests were administered to measure recognition, recall and memory. The total score for the test battery consisted of the sum of the scores of the individual tests. WBC phagocytosis index was measured in each subject using standard methods, because low-intensity EMFs have been reported to affect the metabolism and function of WBCs (2).

TABLE 1. Visual Reaction Time in Subjects Exposed to Microwaves. Numbers represent the mean (msec) $\pm$ standard deviation. Figures in parentheses are the numbers of subjects.

Group	0-4 $\mu\text{W}/\text{cm}^2$		10-15 $\mu\text{W}/\text{cm}^2$	13-42 $\mu\text{W}/\text{cm}^2$
	Boys**	Girls**	Male Soldiers	Male College Students
Exposed	610 $\pm$ 90 (109)	660 $\pm$ 80 (104)	628 $\pm$ 78 (28)	531 $\pm$ 91 (339)
Control	630 $\pm$ 90 (73)	660 $\pm$ 80 (84)	589 $\pm$ 80 (22)	520 $\pm$ 54 (197)
P	> 0.05	> 0.05	< 0.01	> 0.05***

** Boys and girls were from the third years of secondary schools.

*** $t = 1.76$

RESULTS

Following microwave exposure, the visual reaction time was delayed (or tended to be delayed) in the groups exposed to above 10 $\mu\text{W}/\text{cm}^2$ as compared with the corresponding control groups (Table 1). Groups exposed above 10 $\mu\text{W}/\text{cm}^2$ had lower scores in the memory function test than the corresponding control groups (Table 2). The scores of the boys and girls in the low-intensity group were higher than those of the control groups. Phagocytosis was significantly impaired at the highest exposure level, and significantly increased at the lowest exposure level (Table 3).

Following AM exposure, WBC phagocytosis increased or tended to increase in the group exposed to relatively low levels of AM, while the phagocytosis index decreased in the highest exposed group (22-23 V/m) (Table 4). The other parameters measured were not affected significantly by AM exposure.

DISCUSSION

Visual reaction time is a measure of the function of visual receptor and the central nervous system, and it is an indicator of the effect of environmental factors on human beings. Tests of memory and

TABLE 2. Memory Function Scores in Subjects Exposed to Microwaves. The numbers of subjects are the same as in Table 1.

Group	0-4 $\mu\text{W}/\text{cm}^2$		10-15 $\mu\text{W}/\text{cm}^2$	13-42 $\mu\text{W}/\text{cm}^2$
	Boys**	Girls**	Male Soldiers	Male College Students
Exposed	70.57 ± 18.31	71.17 ± 15.45	35.40 $\pm$ 23.66	80.30 $\pm$ 31.43
Control	65.69 ± 15.66	65.52 ± 13.37	54.86 $\pm$ 18.03	91.14 $\pm$ 25.65
P	< 0.05	< 0.01	< 0.01	< 0.01

TABLE 3. WBC Phagocytosis in Subjects Exposed to Microwaves. The numbers of subjects are the same as in Table 1. The ratio (average phagocytosis function of exposure group/phagocytosis function of control group) x 100 is shown.

PHAGOCYTOSIS INDEX			
0-4 $\mu\text{W}/\text{cm}^2$		10-15 $\mu\text{W}/\text{cm}^2$	13-42 $\mu\text{W}/\text{cm}^2$
Boys	Girls	Male Soldiers	Male College Students
123.0*	115.8*	99.3	87.2**

* P < 0.05

** P < 0.01

TABLE 4. WBC Phagocytosis in Subjects Exposed to AM (no. of exposed subjects/no. of controls). The ratio of the average values in the exposed and control groups x 100 is shown. The children were kindergarten students. The boys and girls were in the third year of secondary school.

PHAGOCYTOSIS INDEX					
3-4 (V/m)	4-11 (V/m)	10-18 (V/m)		22-23 (V/m)	
Children	Children	Boys	Girls	Boys	Girls
115.3 (42/27)	131.2** (44/29)	89.8 (78/74)	78.7 (49/71)	45.7** (63/55)	81.2* (85/66)

* P < 0.05

** P < 0.01

concentration are similarly useful because they also serve as an overall index of the function of the central nervous system. However, the test is limited due to the influence of cultural levels, subjective moods, consciousness and other environmental factors of the subjects. In contrast, WBC phagocytosis is an objective index of non-specific immune function. The results showed a bidirectional response to EMFs, in that phagocytosis was increased in the lower EMF exposure groups, but decreased in the groups exposed to stronger EMFs.

The data indicate that chronic exposure to EMFs are associated with significant changes in some physiological parameters.

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Motor and psychological functions of school children living in the area of the Skrunda Radio Location Station in Latvia

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Abstract

This paper presents the results of experiments on school children living in the area of the Skrunda Radio Location Station (RLS) in Latvia. Motor function, memory and attention significantly differed between the exposed and control groups. Children living in front of the RLS had less developed memory and attention, their reaction time was slower and their neuromuscular apparatus endurance was decreased.

Keywords: Electromagnetic field; Adolescent; Motor reaction; Memory; Attention

1. Introduction

An early warning military radio location station (RLS) has operated for more than 25 years in a populated region of Skrunda, Latvia. However, the study of chronic effects of electromagnetic radiation on the population at Skrunda has only recently started. Studies of motor and psychological development of children and adolescents that live close to the RLS may provide evidence of effects.

The Skrunda RLS is an pulse radar station that operates at frequencies of 154–162 MHz. The duration of pulses is 0.8 ms and time between pulses is 41 ms, i.e. the pulses occur at a fre-

quency of 24.4 Hz [3,7]. The electromagnetic field (EMF) effect of this frequency range on human motor and psychological function is insufficiently studied, however, there are already data available on the inhibiting effects of EMF [2,5,6,8]. The literature indicates that EMFs may influence human motor and psychological processes [5,9].

This report summarises the results of a study of the development of some motor and psychological functions of children who were born and constantly live under conditions of chronic EMF exposure in the Skrunda area.

2. Materials and methods

2.1. Subjects and groups

The studies were performed on 966 children (425 males and 541 females) aged 9–18 years. A

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total of 609 children were examined in the Kuldīga and Saldus regions within a 20-km radius of the Skrunda RLS. Of these, 224 pupils live in directly exposed areas. The Skrunda RLS is located in a valley, and the exposed population lives on the slope in the direction of the field of view of the station (westward). Exposure decreases with distance from the radar [3], and field intensities behind the radar are background levels. The control group of 357 pupils lived in the Preiļi region. Girls and boys were divided into five age groups with 2-year intervals (Table 1).

According to the Ministry of Environment Protection and Regional Development of Latvia, both of these regions have similar and low pollution levels (Table 2) [1]. Both are agricultural, without major point sources of pollution, with the exception of small boiler houses.

For the populations of children living in front of the radar and behind it, and for the control group, groups of similar age and sex were selected. We examined similar social groups of farming communities, and 95% of subjects lived on small farms. The tests were carried out in the spring, from April to May.

The studies were performed with a psychophysiological diagnostic system 'Polytest-8802'. The

'Polytest-8802' is a specialised computer that uses reaction to determine human functional state. In total, 11 tests were used for each child. A rest break was included and the test duration was 70 min. Each test included twenty measurements, which were used to obtain the arithmetical mean, standard deviation and standard error of the mean [4].

2.2. Tapping-test

To evaluate the functional state of the neuromuscular system, we used a tapping-test. The children examined had to press two keys with their right and left hands at maximum rate for 30 s. The rate of key pressing per second was registered for each hand separately.

2.3. Reaction time

Red light diodes were used to present light stimuli at the centre of the table and on the left and right sides. The visible diameter of the light was 2.5 mm and the stimulus duration was 40 ms [4]. Sound stimuli were given with stereo earphones (intensity, 60 dB; stimulus duration, 100 ms; frequency, 1 kHz). The interval between both light and sound stimuli was randomised (2.5–4 s). The children had to rapidly press and release keys

Table 1

Mean age $\pm$ S.E. (years) of five groups of children from the Skrunda area, further classified into unexposed and exposed, and the Preiļi region (control group)

Region	Age group (years)				
	9–10	11–12	13–14	15–16	17–18
Skrunda, unexposed					
Females	9.7 $\pm$ 0.1	11.5 $\pm$ 0.1	13.7 $\pm$ 0.1	15.3 $\pm$ 0.1	17.4 $\pm$ 0.1
<i>n</i>	31	73	49	41	23
Males	9.8 $\pm$ 0.1	11.4 $\pm$ 0.1	13.5 $\pm$ 0.1	15.3 $\pm$ 0.1	17.3 $\pm$ 0.1
<i>n</i>	30	64	35	31	8
Skrunda, exposed					
Females	9.6 $\pm$ 0.1	11.4 $\pm$ 0.1	13.7 $\pm$ 0.1	15.1 $\pm$ 0.1	17.6 $\pm$ 0.1
<i>n</i>	14	26	40	41	17
Males	9.6 $\pm$ 0.1	11.4 $\pm$ 0.1	13.4 $\pm$ 0.1	15.3 $\pm$ 0.1	17
<i>n</i>	19	20	22	24	1
Preiļi, control					
Females	9.7 $\pm$ 0.1	11.5 $\pm$ 0.1	13.7 $\pm$ 0.1	15.3 $\pm$ 0.1	17.4 $\pm$ 0.1
<i>n</i>	26	53	49	38	20
Males	9.4 $\pm$ 0.1	11.3 $\pm$ 0.1	13.5 $\pm$ 0.1	15.4 $\pm$ 0.1	17.5 $\pm$ 0.1
<i>n</i>	25	54	47	30	15

Table 2

Estimated rates of emissions (tons/km²) from all sources in Kuldīga and Preiļi districts in 1991

Region	Particles	SO ₂	NO _x	CO	Total
Kuldīga	790.1	1052.8	80.7	571.5	2496.2
Preiļi	1250.7	2011.4	352.3	1195.8	4818

after stimuli. Presentation of stimuli from the table centre or binaurally (RTB) required the child to press both buttons simultaneously. When the stimulus was presented from the side of the table (RTL), or in one earphone, the subject had to press the key on the side corresponding to the stimulus. In the cross variant of the test (RTC), the subject had press the key at the side opposite that of the stimulus. The response time, duration of press contact and the number of errors were registered.

2.4. Attention

The capacity for attention switching was tested according to a modified Shulte's procedure. The procedure used a double-coloured table of 64 squares. Each square consisted of two numbers: large black colour and small red (index), as well as a response button. At the beginning of the test the monitor displayed a number which the child was required to find among the black numbers. When the required black number was found, the child was required to press the appropriate key and memorise the associated red number. This new number was then searched for among the black numbers and the process was repeated 20 times.

The stability and capacity to focus attention were studied with an 'entangled lines' test. The test table was covered with entangled lines. Each line began with a number on the left side and finishes with another number on the right side. The child was required to rapidly follow each line visually and press the button at its finish. The time taken to follow every line and the number of errors were registered.

2.5. Memory

Memory was tested by the capacity to remember numbers. During one test, three numbers

were displayed on a monitor at 1-s intervals. The child then entered the three numbers on the keyboard. This was repeated seven times, progressively increasing the number of numbers displayed from three to nine. The volume of operative memory was determined according to the formula $V = A + (m/n)$ where A is the largest numbers of digits in the operation which was successfully reproduced by the child in all experiments, n is the number of experiments, m is the number of correctly reproduced number series. During the experiment, a series of numbers of increasing complexity was presented, beginning with three digits and ending with nine. White digits of 6×10 mm, on a dark-grey background were presented on the computer monitor.

2.6. Statistics

Statistically significant differences were evaluated by Student's criteria for quantitative variables.

3. Results

Preliminary data analysis showed that among grade 9 children, there were 16% fewer boys in Skrunda, and 25% fewer in the area exposed to the Skrunda RLS. This is uncommon in Latvia for Grade 9 school children and the reasons for these differences are not known. The rates of all motor reaction (tapping-test and reaction time) tests in boys were better than in girls, both in exposed and control groups, and hence male and female groups were treated separately.

The reaction time to both sound and visual stimuli in the children living around Skrunda was somewhat longer than in Preiļi children, for both girls and boys, although the differences are statistically significant only for younger groups (Fig. 1). The duration of retention of keys in the pressed

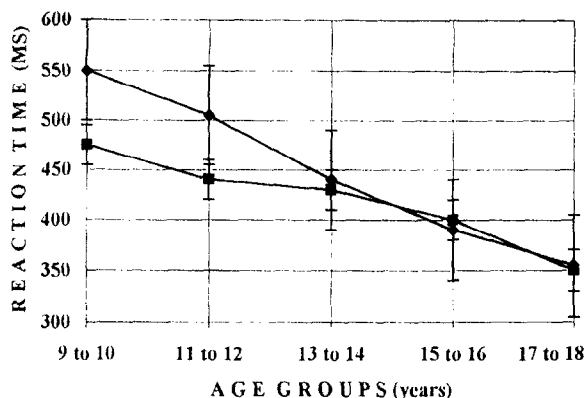


Fig. 1. Means $\pm$ standard errors of the cross response left hand reaction time (RT) to sound stimuli in children (female) in five age groups from Skrunda (♦) and Preiļi (■). For ages 9–10 and 11–12 years, the differences are significant at $P < 0.05$.

state was also significantly longer for all age groups in Skrunda, compared with Preiļi.

A similar tendency was also observed in memory and attention tests. In Skrunda, the memory and attention of children were worse than in Preiļi, but the differences were not significant. In the tapping test (Fig. 2), boys and girls from Preiļi performed better than those from Skrunda. Performance in this test improved with age.

More significant differences were observed when the Skrunda population was divided into

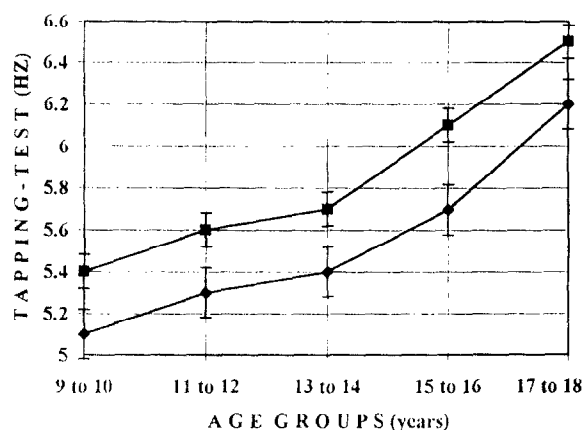


Fig. 2. Means $\pm$ standard errors of the tapping test frequencies of male children in five age groups from Skrunda (♦) and Preiļi (■), using the left hand. The differences for all age groups are significant at $P < 0.05$.

exposed and unexposed groups and compared with the Preiļi group. Memory (Fig. 3) and attention (Fig. 4) were considerably less ($P < 0.05$) in the children living in front of the Skrunda RLS. Motor reaction was also delayed in girls living in Skrunda, but not in boys (Fig. 5). For girls, the retention time of keys was almost always significantly longer for the Skrunda exposed group, compared with the unexposed group (Table 3). In males, however, only in some age groups for each test were the effects significant.

Every subject's address was registered, with the length of time they had lived in the given district. It was thus possible to determine the distance from the Skrunda RLS to the subjects' home. Generally, exposure decreases with distance from the RLS, but field intensities are extremely variable temporally and spatially due to factors such as local topography, operating regime, tree canopy, etc. [3]. For this reason, and the lack of large numbers of permanent field intensity recorders, it was not possible to measure the intensities at each home. There was a weak positive correlation (0.27, $P < 0.05$) between the distance from the RLS and rates achieved in the tapping-test and a negative correlation (-0.29 , $P < 0.01$) between the distance from the RLS and contact retention time in motor reaction. In this case, Pearson's coefficient was used.

4. Discussion

Measurements and calculations performed by the Air Material Command of the Royal Danish Air Force [7] demonstrated that the mean power density of the Skrunda RLS is insignificant. For example, at a distance of 3.7 km from the RLS the mean power density measured was only 3.205 mW/m^2 . However, the peak power density is 50 times higher (164.27 mW/m^2). It is more likely that the peak power density, rather than the mean value, is the factor which could cause effects on organisms. The literature lacks data on motor and psychological effects of weak pulsed radiofrequency fields of the range studied (154–162 MHz), with a pulse frequency of 24.4 Hz. This 24.4-Hz frequency coincides with that of

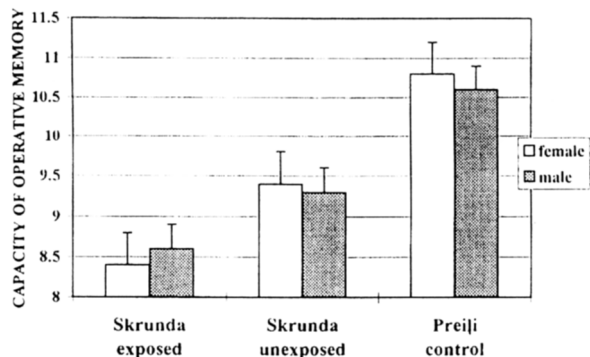


Fig. 3. Means $\pm$ standard errors of operative memory in male and female children aged 15–16 years, living in the Skrunda exposed and unexposed areas and the Preilji control area. The differences are significant ($P < 0.05$) between the Skrunda exposed and unexposed areas, as well as between the Skrunda unexposed and Preilji areas.

human electroencephalogram β -rhythms, and it is not excluded that low frequency pulses are one of the reasons for the observed alterations. Lyskov et al. [5] showed that low-frequency magnetic fields induced inhibition of motor and nervous process. Our earlier research [4] also demonstrated that a weak low frequency pulse EMF causes shifts in neurophysiological parameters, even after 15 min.

The weak correlations between the distance from the children's homes to the RLS, and the children's responses, are certainly consistent with the idea of an electromagnetic field effect. The

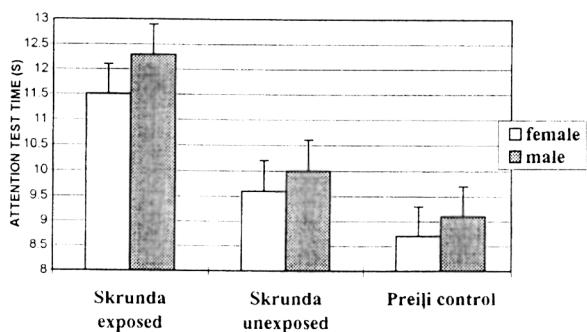


Fig. 4. Means $\pm$ standard errors of time of attention switching test duration in male and female children aged 15–16 years, living in the Skrunda exposed and unexposed areas and the Preilji control area. The differences are significant ($P < 0.05$) between the Skrunda exposed and unexposed areas, as well as between the Skrunda exposed and Preilji areas.

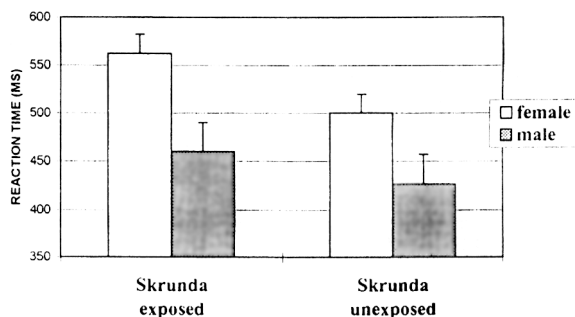


Fig. 5. Means $\pm$ standard errors of the cross response reaction time using the right hand to light stimuli in male and female children living in the Skrunda exposed and unexposed areas and the Preilji control area. The differences are significant ($P < 0.05$) only between girls living in the Skrunda exposed and unexposed areas.

exposure of each child cannot be monitored, due to spatially and temporally variable intensities, and the fact the subjects move out of and within the exposed zone. The children living in front of the Skrunda RLS have less developed memory and attention, slower reaction times and decreased endurance of neuromuscular apparatus. On the basis of the data obtained, one could propose the working hypothesis that the decreased endurance of neuromuscular apparatus, slower reaction time and less developed memory and attention are the results of chronic electromagnetic radiation effects. Evidence for a factor other than electromagnetic field having caused the observed results was not found, but its existence cannot be ruled out, for example, differences in the past experiences of children, local small pollution effects, differences in family behaviour, etc.

At present, we can only state that the children living in the exposed zone in front of the Skrunda RLS performed worse in the psychological tests given than the children living behind the RLS, and even worse again when compared with the control group.

The validity of a statement that the RFEM field at Skrunda has caused these differences can only be claimed with continuous and accurate assessment of dose, and close to exact standardisation of subjects. The measurement of dose is problematic, since the children move in and out

Table 3

Mean values $\pm$ S.E. of retention time of keys in the pressed state for right hands by male and female children from the Skrunda unexposed area and Skrunda exposed area, in reaction to sound stimuli presented biaurally and monaurally

Region	Age groups (years)				
	9–10	11–12	13–14	15–16	17–18
Biaural stimuli (RTB)					
<i>Female</i>					
Unexposed	214 $\pm$ 10	206 $\pm$ 8	164 $\pm$ 13	128 $\pm$ 8	126 $\pm$ 7
Exposed	250 $\pm$ 11	258 $\pm$ 12	228 $\pm$ 15	182 $\pm$ 14	167 $\pm$ 10
<i>P</i> -value	< 0.05	< 0.05	< 0.01	< 0.01	< 0.05
<i>Males</i>					
Unexposed	191 $\pm$ 9	182 $\pm$ 9	145 $\pm$ 11	105 $\pm$ 7	99 $\pm$ 6
Exposed	211 $\pm$ 10	223 $\pm$ 11	200 $\pm$ 8	156 $\pm$ 9	124 $\pm$ 10
<i>P</i> -value	NS	< 0.05	< 0.01	< 0.05	NS
Monoaural stimuli (RTL)					
<i>Female</i>					
Unexposed	224 $\pm$ 9	220 $\pm$ 10	221 $\pm$ 11	173 $\pm$ 10	192 $\pm$ 10
Exposed	265 $\pm$ 11	239 $\pm$ 11	249 $\pm$ 16	219 $\pm$ 15	224 $\pm$ 10
<i>P</i> -value	< 0.05	< 0.05	NS	< 0.05	< 0.05
<i>Male</i>					
Unexposed	212 $\pm$ 10	208 $\pm$ 9	202 $\pm$ 11	190 $\pm$ 12	110 $\pm$ 12
Exposed	243 $\pm$ 11	227 $\pm$ 10	210 $\pm$ 9	202 $\pm$ 11	133 $\pm$ 11
<i>P</i> -value	< 0.05	NS	NS	NS	NS
Cross variant of test (RTC)					
<i>Female</i>					
Unexposed	209 $\pm$ 11	197 $\pm$ 8	221 $\pm$ 11	199 $\pm$ 11	222 $\pm$ 10
Exposed	259 $\pm$ 14	238 $\pm$ 10	246 $\pm$ 11	237 $\pm$ 12	239 $\pm$ 11
<i>P</i> -value	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<i>Male</i>					
Unexposed	199 $\pm$ 9	175 $\pm$ 8	200 $\pm$ 11	170 $\pm$ 7	199 $\pm$ 10
Exposed	232 $\pm$ 10	207 $\pm$ 11	221 $\pm$ 8	191 $\pm$ 9	181 $\pm$ 9
<i>P</i> -value	< 0.05	NS	NS	NS	NS

RTL, key pressed at same side as stimulus; RTC, key pressed at opposite side as stimulus; NS, not significant.

of the radiation zone, and the temporal changes in intensity are high [3]. However, the results presented, especially the weak correlation between performance and distance to the RLS, certainly suggest that this path of research is worthwhile. Further work is continuing to increase the sample size and to attempt to arrive at estimations of dose.

Acknowledgements

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ORIGINAL ARTICLE

Subjective symptoms, sleeping problems, and cognitive performance in subjects living near mobile phone base stations

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Background: The erection of mobile telephone base stations in inhabited areas has raised concerns about possible health effects caused by emitted microwaves.

Methods: In a cross-sectional study of randomly selected inhabitants living in urban and rural areas for more than one year near to 10 selected base stations, 365 subjects were investigated. Several cognitive tests were performed, and wellbeing and sleep quality were assessed. Field strength of high-frequency electromagnetic fields (HF-EMF) was measured in the bedrooms of 336 households.

Results: Total HF-EMF and exposure related to mobile telecommunication were far below recommended levels (max. 4.1 mW/m²). Distance from antennae was 24–600 m in the rural area and 20–250 m in the urban area. Average power density was slightly higher in the rural area (0.05 mW/m²) than in the urban area (0.02 mW/m²). Despite the influence of confounding variables, including fear of adverse effects from exposure to HF-EMF from the base station, there was a significant relation of some symptoms to measured power density; this was highest for headaches. Perceptual speed increased, while accuracy decreased insignificantly with increasing exposure levels. There was no significant effect on sleep quality.

Conclusion: Despite very low exposure to HF-EMF, effects on wellbeing and performance cannot be ruled out, as shown by recently obtained experimental results; however, mechanisms of action at these low levels are unknown.

Hand-held cellular telephones were introduced in the early 1980s. Due to the relatively high microwave exposure for users while they are on the telephone, the potential health effects of mobile phones have been studied in recent years. However, exposure to the much lower emissions from mobile phone base stations has been neglected. There have been only two observational pilot investigations,^{1,2} and one experimental study.³

The World Health Organisation (WHO)⁴ has recently recommended investigating the effects of exposure to emissions from mobile phone base stations to address public concerns.

It has often been argued that if there are detrimental long term effects from high-frequency electromagnetic fields (HF-EMF) as transmitted by mobile phone base stations, then such effects should have been found near powerful radio and television transmitters. This argument is invalid as: (1) there are very few studies on effects from radio and TV transmitters, ecological and cluster studies on cancer,^{5–10} and studies on sleep and other endpoints;^{11–12} (2) the results of these studies are compatible with the assumption of a moderately elevated risk; and (3) emissions from base stations differ substantially from those of other sources of HF-EMF.

There are numerous reports from physicians that base stations are associated with a number of health symptoms in neighbours. However, these symptoms might be due to fear about negative effects. Nevertheless there is evidence that long term, low level exposure to HF-EMF may result in a number of symptoms (for example, headaches, fatigue, sleep disorders, memory impairments),¹³ attributed as microwave sickness syndrome.¹⁴

This study investigated the relation between exposure from mobile telecommunication and other sources of HF-EMFs and the associations between exposure and symptoms.

METHODS

Selection of base stations

The study covers urban as well as rural areas in Austria. The city of Vienna was selected as the urban area while villages in Carinthia represented the rural areas. Two network providers were each asked to identify about five base stations within both regions that fulfilled the following requirements:

- The antenna must have been operating for at least two years
- There had been no protests by neighbours against the base station
- There was no other base station nearby (this could only be achieved in rural areas)
- Transmission was preferably only in the 900 MHz band.

Twenty one base stations were specified, from which 10 were selected for the study based on inspection of the local conditions (population density, other sources of exposure).

Selection of study area and participants

Data from the 10 selected antenna locations, including the antenna diagram, were provided by the network companies. In order to ensure a sufficient gradient of exposure, these data were used to define the study area around the selected base station. The investigation was carried out by trained students and a medical technical assistant in Carinthia and

Abbreviations: ANCOVA, analysis of covariance; BCCH, broadcast channel; CI, confidence interval; GSM, global system for mobile telecommunication; HF-EMF, high-frequency electromagnetic fields; MHz, megahertz; POR, prevalence odds ratio; SAR, specific (energy) absorption rate; SD, standard deviation; TDMA, time division multiple access; WHO, World Health Organisation

Table 1 Demographic characteristics of subjects by exposure category

	Exposure category (mW/m ²)			p value
	<0.1	0.1–0.5	>0.5	
Age	45 (SD 16)	40 (SD 14)	44 (SD 15)	0.390
Females	60%	58%	56%	0.829
Years of residence	19 (SD 16)	17 (SD 13)	20 (SD 16)	0.403
Hours at home	10 (SD 5)	10 (SD 4)	10 (SD 5)	0.413
Employed	56%	60%	61%	0.689
Urban residence	55%	42%	49%	0.171
Education > 12 y	42%	38%	40%	0.784
Mobile phone use	75%	77%	78%	0.866

p value from Kruskal–Wallis or χ^2 test.

Vienna. Based on power calculations, the projected number was 36 subjects for each of the 10 locations.

In Vienna, households were randomly selected from telephone register entries. Subjects were contacted by telephone. If after three attempts no contact could be achieved, the next entry in the telephone list was chosen. Subjects were told that the relationship between environmental factors and health would be investigated. They had to be older than 18 years, have been living in their present house for at least one year, and been staying there for a minimum of eight hours a day on average. Refusal was slightly above 40% and mainly due to time constraints. On acceptance of participation an appointment was made for a visit. In Carinthia the procedure was different because no clear relation of address to study area could be ensured (houses are not always numbered consecutively). Therefore a random selection of houses based on the site plan was performed. Investigators contacted subjects directly in their homes. In the case of acceptance, either an appointment for the investigation was made or it was carried out immediately. Rate of refusal was somewhat lower than in the urban area (32%). On contact, gender, age, and duration of residence in their present house (eligibility criteria) were registered. Non-participants were insignificantly more frequently males (47% v 41%) and significantly younger (40 v 44 years), and had a significantly shorter time living in their present house (13 v 16 years).

Data collection and measurements

All investigations were done in the homes of the subjects using a laptop computer. Performance tests as well as questionnaires were presented along with instructions on the screen. Handling was so simple that after a short introduction all subjects were able to fulfil the tasks without further assistance by the investigators. The investigation consisted of the following:

- Sociodemographic data, sources of EMF exposure within the household, regular use of mobile telephones.
- Evaluation of environmental quality, subjective scaling of the impact different environmental factors could have on the health of the subjects. Among the items listed were traffic noise, particulate matter, and mobile phone base station. Assumed impact was rated on a five point scale from 0 = not at all, to 4 = very strong impact.
- Subjective scaling of symptoms (Zerssen scale).¹⁵ Symptoms were rated on a four point scale from 0 = not at all, to 3 = strong. Symptoms of special interest were headaches, symptoms of exhaustion, and circulatory symptoms (see table 4). For analysis, ratings were dichotomised (0/1–3).
- Investigation of sleeping problems (Pittsburgh sleeping scale).¹⁶ Problems falling asleep and staying asleep were rated by the participants on a frequency scale ranging from never to more than 3 days a week. The global index is

Table 2 Exposure categories and results of analysis of covariance for tests of cognitive performance

Test	Exposure category (mW/m ²)			p value
	<0.1	0.1–0.5	>0.5	
Memory				
Immediate memory*	6.2 (1.4)	5.6 (1.4)	5.9 (1.5)	0.166
Short term memory (1 min)†	29.1 (4.3)	29.5 (4.1)	29.3 (3.9)	0.354
Short term memory (5 min)†	33.9 (2.9)	33.1 (3.1)	34.0 (1.9)	0.761
Short term memory (15 min)†	33.4 (2.9)	33.6 (2.4)	33.7 (2.0)	0.883
d' (1 min)‡	0.87 (0.48)	0.88 (0.42)	0.86 (0.41)	0.737
d' (5 min)‡	1.54 (0.39)	1.48 (0.62)	1.53 (0.32)	0.579
d' (15 min)‡	1.56 (0.39)	1.54 (0.32)	1.62 (0.27)	0.198
ln β (1 min)§	−0.34 (0.45)	−0.19 (0.32)	−0.29 (0.30)	0.235
ln β (5 min)§	−1.09 (0.58)	−1.11 (0.72)	−1.04 (0.54)	0.605
ln β (15 min)§	−1.36 (0.53)	−1.21 (0.52)	−1.47 (0.53)	0.095
Perceptual speed				
Speed score (sec)	4.3 (0.9)	4.0 (1.1)	3.8 (1.0)	0.061
Items solved (max. 8)	4.6 (2.4)	4.1 (2.3)	4.1 (2.5)	0.147
Choice reaction task				
Reaction time (msec)	582 (217)	511 (139)	585 (244)	0.485

Results expressed as mean (SD).

p values for exposure factor are shown.

*Highest number of correctly reproduced digits.

†Number of correctly identified items (sum of correct detections (from 20) and correct rejections (from 20 distraction items)).

‡d-prime from signal detection analysis.

§Natural logarithm of detection bias beta.

computed as the sum of seven sub-scales (see table 5) with each component scored 0 to 3 (higher score indicates greater problems).

- Cognitive performance.

- Memory tasks consisted of a short term memory test using 1–10 digit numbers that had to be reproduced immediately after presentation. The score was defined as the highest number of digits correctly reproduced. The assessment of medium term memory was based on 20 simple everyday objects in silhouette drawings presented together for 30 seconds on the screen. After 1, 5, and 15 minutes these items together with 20 distraction items (different for the three tests) were presented in random sequence, one at a time, and the subjects had to decide whether or not the picture was among those presented. Each response was followed by immediate feedback. After each test all objects were again presented for 15 seconds. The score was defined as the number of correct responses. In addition, *d*-prime and response bias (*beta*) from signal detection analysis were computed (*d*-prime is the normalised distance between the signal and noise answer distributions, the higher the *d*-prime, the less likely is confusion between target and distraction items; *beta* measures the bias to respond “yes” whether it is a target or distraction item).
- The choice reaction task consisted of a random sequence of squares of three different colours (red, green, and yellow) appearing at random locations on the screen. Subjects had to react as fast as possible by pressing a specified button for each colour. The score was defined as the average correct reaction time across 25 trials.
- Perceptual speed was tested by presenting two series of 10 letters (“meaningless words”) that differed at exactly one position. Eight of these double series were presented in random sequence. Subjects had to find the differing letter under time constraints (maximum 6 seconds) and place a cursor below it. These position varied between the 3rd and 7th letters. Score was defined as the average time to achieve the correct solution. In addition, the number of items solved within the time window was computed.

After completion of the questionnaires and tests, dates were arranged for exposure measurements. Measurements of high frequency EMFs were done by a specialist from a certified centre in Vienna (TGM). A biconic field probe (PBA 10200, ARC Seibersdorf) was used connected to a spectrum analyser (FSP, Rhode & Schwarz). Measurements were performed in the bedroom (this being typically the only place in the house where people consistently spend many hours a day). As exposure may vary at this location, in addition to the sum of power densities across all mobile phone frequencies, the maximum exposure from the base station was computed based on measurements of broadcast channels. Broadcast channels (BCCH) operate all the time at maximum power with all time slots occupied. Hence multiplication of measurements of BCCH by the ratio of the sum of the power of all channels to that of the BCCH results in maximum possible exposure level, while the sum of BCCH measurements gives the minimum. The former is the result of all channels operating at maximum power with all time slots occupied, while the latter occurs if no traffic channel is active.

Distance from the antenna was calculated based on the coordinates of the measurement location and the base station. It ranged between 24 m and 600 m in rural areas and between 20 m and 250 m in urban areas. The smaller

range in the latter was due to the vicinity of other base stations and the shadowing effect of high buildings.

Subjects

In total, 365 subjects were investigated (185 in Vienna and 180 in Carinthia). In some cases EMF measurements were not possible due to the absence of the inhabitants at the arranged date. Therefore, only data from 336 subjects could finally be evaluated.

Subjects were between 18 and 91 years of age (mean 44, SD 16 years). Fifty nine per cent were female. Average duration of residence in the house was 19 (SD 16) years, and subjects stayed for 10 (SD 5) hours a day in the immediate neighbourhood. Overall, six subjects occupied the place only after erection of the base station. All subjects slept normally at home.

Statistical analysis

Statistical evaluation of exposure from the base stations was done by analysis of covariance (ANCOVA) for components of the Pittsburgh Sleeping Scale and performance measurements, and by logistic regression analysis for subjective symptoms based on the following procedure. First the maximal power density estimates from base station frequencies were classified into three groups: ≤ 0.1 mW/m² (approximately up to median), 0.1–0.5 mW/m² (between median and 3rd quartile), and >0.5 mW/m². Originally it was planned to define four exposure categories based on quartiles. However, it turned out that the level of exposure was too low for the two lowest exposure categories to be meaningfully discriminated and consequently these categories were combined. Average exposure levels were 0.04 mW/m², 0.23 mW/m², and 1.3 mW/m², respectively. Exposure level, area (rural v urban), and interaction were included as fixed factors, age, sex, regular use of a mobile telephone, and the subjective rating of negative consequences of the base station on health were used as covariables. Normality was assessed by Kolmogorov–Smirnov tests using Lilliefors *p* values, homogeneity of variance by Levene’s tests. For all analyses the model with separate slopes was first tested. If none of the interactions with fix factors were significant at the 10% level, the model with homogenous slopes was computed. In addition, homogeneity of variance–covariance matrices of covariables and dependent variables across groups was tested by Box M tests. Unconditional logistic regression was performed using the same covariables. For all tests a *p* value below 0.05 was considered significant. No correction for multiple testing was applied.

RESULTS

Table 1 gives an overview of features of participants across exposure categories. Although none of the variables reached statistical significance, the somewhat higher proportion of subjects from the urban area in the lowest exposure category should be noted.

Exposure to high frequency EMFs was generally low and ranged from 0.0002 to 1.4 mW/m² for all frequencies between 80 MHz and 2 GHz; the greater portion of that exposure was from mobile telecommunications (geometric mean 73%), which was between 0.00001 and 1.4 mW/m². Maximum levels were between 0.00002 and 4.1 mW/m². Overall 5% of the estimated maximum exposure levels were above 1 mW/m². Average exposure levels were slightly higher in the rural area (0.05*/7.6 mW/m²) than in the urban area (0.02*/7.1 mW/m²).

Most subjects expressed no strong concerns about adverse health effects of the base station. In the urban and rural test areas, 65% and 61% respectively stated no concerns at all.

Table 3 Detailed results of analysis of covariance for speed score of perceptual speed as a dependent variable

Source of variation		df	MSQ	F value	p value
Covariates	Combined	4	54.980	19.721	0.000
	Concerns about base station	1	2.618	0.939	0.333
	Age	1	216.469	77.648	0.000
	Sex	1	0.028	0.010	0.920
Main effects	Use of mobile phone	1	0.803	0.288	0.592
	Combined	3	28.562	10.245	0.000
	Area (rural/urban)	1	69.948	25.090	0.000
	GSM exposure	2	7.869	2.823	0.061
Interaction		2	0.036	0.001	0.999

Factors and covariables are shown in the column "source of variation".
df, degrees of freedom; MSQ, mean sum of squares.

Table 2 gives an overview of results from ANCOVA on the different tests of cognitive performance for the exposure factor only; table 3 shows the full results for the test of perceptual speed. For perceptual speed a tendency for faster reaction in the higher exposure category was found. Omitting the three insignificant covariates from analysis resulted in a significant ($p=0.009$) main effect for exposure. Logistic

regression with the median chosen as a cut-off point was statistically significant. The estimated risk of a value below the median speed score relative to the lowest exposure category was 0.73 (95% CI 0.33 to 1.58) for the second and 0.42 (95% CI 0.18 to 0.98) for the third exposure categories. Accuracy of perceptual speed indicated by number of correct reactions showed the opposite effect, although not

Table 4 Relative risk estimates of subjective symptoms of primary interest for categories of exposure to microwaves from base stations in the bedroom against lowest exposure category

Symptom	Exposure category (mW/m ²)	% with symptom	Relative risk*	95% CI	p value
Headaches	≤ 0.1†	61	1.00		0.017
	0.1–0.5	66	1.36	0.62–2.99	
	> 0.5	79	3.06	1.22–7.67	
Vertigo	≤ 0.1†	17	1.00		0.306
	0.1–0.5	27	1.27	0.50–3.22	
	> 0.5	32	1.54	0.68–3.50	
Palpitations	≤ 0.1†	26	1.00		0.444
	0.1–0.5	32	1.06	0.45–2.47	
	> 0.5	38	1.37	0.61–3.11	
Tremor	≤ 0.1†	12	1.00		0.062
	0.1–0.5	9	0.68	0.19–2.41	
	> 0.5	26	2.37	0.96–5.87	
Hot flushes	≤ 0.1†	32	1.00		0.739
	0.1–0.5	26	0.90	0.39–2.09	
	> 0.5	26	0.87	0.37–2.01	
Sweating	≤ 0.1†	34	1.00		0.455
	0.1–0.5	38	1.05	0.47–2.32	
	> 0.5	40	1.35	0.61–2.97	
Cold hands or feet	≤ 0.1†	40	1.00		0.019
	0.1–0.5	46	1.03	0.40–2.63	
	> 0.5	62	2.57	1.16–5.67	
Loss of appetite	≤ 0.1†	13	1.00		0.069
	0.1–0.5	17	1.23	0.42–3.57	
	> 0.5	24	2.40	0.93–6.18	
Loss of energy	≤ 0.1†	63	1.00		0.886
	0.1–0.5	63	1.32	0.61–2.84	
	> 0.5	58	1.06	0.49–2.27	
Exhaustion	≤ 0.1†	44	1.00		0.098
	0.1–0.5	41	0.77	0.30–2.02	
	> 0.5	51	2.07	0.87–4.89	
Tiredness	≤ 0.1†	64	1.00		0.258
	0.1–0.5	89	1.97	0.64–6.10	
	> 0.5	88	1.92	0.62–5.96	
Difficulties to concentrate	≤ 0.1†	60	1.00		0.035
	0.1–0.5	64	1.32	0.61–2.86	
	> 0.5	76	2.55	1.07–6.08	
Feeling strained	≤ 0.1†	44	1.00		0.450
	0.1–0.5	51	1.67	0.76–3.65	
	> 0.5	40	0.74	0.33–1.63	
Urge for sleep	≤ 0.1†	47	1.00		0.630
	0.1–0.5	54	1.21	0.56–2.61	
	> 0.5	51	1.17	0.53–2.54	

p values for exposure factor are shown.

*Adjusted for age, sex, region, regular use of mobile telephone, and fear of adverse effects of the base station.

†Reference category.

Table 5 Results of analysis of covariance for components and global score of the Pittsburgh Sleep Quality Index and logistic regression for "poor sleepers" (global score >5)

Component	Exposure category (mW/m ²)			p value
	<0.1	0.1–0.5	>0.5	
Subjective sleep quality	0.71 (0.79)	0.60 (0.77)	1.00 (0.89)	0.240
Sleep latency	0.76 (0.93)	0.74 (0.95)	0.94 (0.98)	0.295
Sleep duration	1.06 (0.98)	1.14 (1.03)	1.21 (1.09)	0.504
Habitual sleep efficiency	0.54 (0.92)	0.70 (0.98)	0.74 (1.15)	0.061
Sleep disturbances	0.92 (0.58)	0.91 (0.66)	0.91 (0.62)	0.338
Daytime dysfunction	0.66 (0.75)	0.54 (0.70)	0.82 (0.90)	0.099
Sleep medication	0.10 (0.46)	0.17 (0.71)	0.21 (0.73)	0.216
Global score	4.74 (3.52)	4.78 (3.86)	5.87 (4.21)	0.282
Poor sleepers (%)	35%	31%	41%	0.225

Results expressed as mean (SD).
p values for exposure factor are shown.

to a significant extent. Hence there is some speed–accuracy trade-off.

For subjective symptoms of primary interest, effects of exposure from the base station are shown in table 4. Many symptoms were more frequent at higher exposure levels; headaches, cold hands or feet, and difficulties in concentrating, and to a lesser degree, tremor, loss of appetite, and feelings of exhaustion showed increased prevalence after correction for confounding factors.

Results for sleep quality are shown in table 5. Two subscales (sleep efficiency and daytime dysfunction) showed indications of poorer sleep at higher exposure categories. A highly significant effect of concerns about negative health implications of the base station was found for overall sleep quality (global score), with poorer quality in those concerned. As expected, age also had a significant influence. Without considering the influence of the subjects' concerns about the base station, the effect of exposure would have been statistically significant. Logistic regression analysis with the median score as a cut-off point showed no pronounced effect of exposure ($p = 0.131$).

DISCUSSION

Mobile phone base stations easily comply with current guidelines (for example, ICNIRP (International Commission on Non-Ionizing Radiation Protection) guidelines).¹⁷ Our measurements show that exposure of the public in the vicinity of base stations is indeed low. However, considering all HF-EMF exposures above 80 MHz, mobile telecommunication is responsible for an average of 73% of these exposures. This is consistent with representative measurements in Sweden¹⁸ and the UK.¹⁹

The present study was conducted to provide answers to intriguing methodological problems of the epidemiological investigation of base stations.

How is it possible to attribute effects to a specific source of HF-EMF? In study areas, exposure from other sources of HF-EMFs was from distant transmitters and therefore more or less constant. Effects from these exposures will therefore not confound the effects of base stations. As study areas were selected to guarantee a gradient of exposures from base stations, the only relevant contribution to the variance of HF-EMF exposure was from base stations (93% of variance).

Another problem is the time variation of exposure, depending on the number of connected calls (due to the TDMA (time division multiple access) mode of the GSM system). Of course the best approach would be a long term measurement of exposure, or to use personal "dosimeters". However, there are no such dosimeters available and long term measurements are not feasible due to economic restrictions as well as problems of compliance. A possible solution is to conduct a short term measurement at a location where subjects are assumed to spend considerable periods of time (we chose the bedroom), analyse the spectrum of exposure, and select the broadcast channels that are operating at constant maximum power. Based on these measurements a range of exposures can be computed. We analysed data based on broad categories so that this categorisation leads to almost equal allocation whether "average", minimum, or maximum exposure estimation is used. A broad categorisation was used because of other sources of variance of exposure (like movements of subjects) that cannot be accounted for.

A further problem is the dynamic development of telecommunication networks. For the present study, we selected base stations emitting with unchanged features for

Table 6 Results of analysis of covariance (ANCOVA) for global score of the Pittsburgh Sleep Quality Index as dependent variable

Source of variation		df	MSQ	F value	p value
Covariates	Combined	4	323.407	11.770	0.000
	Concerns about base station	1	482.088	17.545	0.000
	Age	1	661.076	24.059	0.000
	Sex	1	87.286	3.177	0.076
	Use of mobile phone	1	63.176	2.299	0.130
Main effects	Combined	3	42.571	1.549	0.202
	Area (rural/urban)	1	57.795	2.103	0.148
	GSM Exposure	2	34.959	1.272	0.282
Interaction		2	58.404	2.126	0.121

Factors and covariables are shown in the column "source of variation".
df, degrees of freedom; MSQ, mean sum of squares.

Main messages

- Exposure from mobile phone base stations is orders of magnitude below current guideline levels.
- Self-reported symptoms like headache and difficulties in concentrating show an association with microwave exposure from base stations, not attributable to subjects' fear of health effects from these sources.
- Other symptoms, like sleeping problems, seem to be more due to fear of adverse health effects than actual exposure.

at least two years. Furthermore, it was important that no other base station was nearby (which, however, could only be achieved in rural areas).

Because of the much higher exposure during telephoning compared to exposure from base stations, it is hardly conceivable that such small additional exposure could have an effect. However, these exposures have fundamentally different features. Exposure from the base station will be at low, but more or less constant levels for many hours a day, especially during the night. Comparing these levels is inappropriate if long term effects actually exist. If, for example, a subject is using a GSM mobile with a specific energy absorption rate (SAR) of 0.04 W/kg²⁰ for 10 minutes, this would be roughly equivalent to a 15 day exposure from a base station at an exposure level of 1 mW/m² if the principle of time-dose reciprocity is valid. However, it is not known whether this principle holds for exposure to HF-EMFs.

There is no a priori argument why the much lower levels from base stations should have no effect in the presence of widespread use of mobile telephones. Possible confounding by using a mobile has been considered in this study.

Generally, ratings were higher for most symptoms in subjects expressing concerns about health effects from the base station. Subjects who experience health problems might search for an explanation in their environment and blame the base station; another explanation would be that subjects with concerns are more anxious and also tend to give a more negative view of their body functions, or that some people generally give quite negative answers. Irrespective of these explanations there seem to be effects of exposure that occur independently of the fear of the subjects about the base station affecting their health. This is the case for headaches, cold hands or feet, and difficulties in concentrating, for example. These effects were robust with respect to additional potential confounders (for example, for headaches, inclusion of an indicator of socioeconomic status—years of education and type of occupation—slightly increased the risk estimator for exposure and decreased the p value from 0.017 to 0.016; inclusion of years of living in the present home and overall rating of environmental quality slightly increased the p value to 0.019; inclusion of hours staying at home did not change effect estimates at all). Interestingly these symptoms as well as some others that tended to be increased at higher exposure levels belong to those attributed to the microwave sickness syndrome. However, no clear relationship has been found for sleeping problems that are often mentioned in the public debate. The effect on sleep is dominated by concerns of the subjects of negative health effects of the base station. Many factors are known to influence sleep quality. Only a few could be considered in this study. Since some aspects of sleep quality, like sleep efficiency, showed a tendency for being affected by exposure, future studies should attempt to eliminate additional confounders.

Policy implications

- Despite very low emissions from mobile phone base stations, more research concerning the effects of radiofrequency radiation from base stations is indicated.
- As a precautionary measure, siting of base stations should be such as to minimise exposure of neighbours.

Concerning symptom reporting there are a number of personality factors for which an association has been established. Among these are state anxiety, depression, and negative affectivity. The main question concerning this range of factors is whether they might act as confounders. In discussions of the microwave sickness syndrome, depression has also been mentioned among the possible effects of exposure; confounding is therefore conceivable. Sleep quality, unspecific symptoms, depression, affectivity, and other personality characteristics are connected with each other in a network of relationships such that a clear understanding of the possible long term effects of exposure may only be determined by longitudinal studies.

No influence of the subjects' fear about negative effects of the base station was found for cognitive performance. There was a small but significant reduction of reaction time for perceptual speed at increased exposure levels. It is interesting to note that such facilitating effects have also been reported during short term experimental exposures^{20–22} and a study in teenagers using mobile phones.²¹ On the other hand, a study¹² in children chronically exposed to emissions from a radio tower reported increased reaction times and reduced performance in cognitive tasks. We found a reduction of reaction time in adults, but an insignificant decrease of accuracy. Recognition in the medium term memory task showed a reasonable and increasing differentiation between target and distraction items and a decreasing response bias over repeated tests, but there was no indication of an influence of exposure from the base station. Furthermore, cognitive performance varies with factors that have not been controlled or considered in this study. Indices of socioeconomic status, however, were tested and did not modify effect size of base station exposure.

The results of this study indicate that effects of very low but long lasting exposures to emissions from mobile telephone base stations on wellbeing and health cannot be ruled out. Whether the observed association with subjective symptoms after prolonged exposure leads to manifest illness remains to be studied.

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The Microwave Syndrome: A Preliminary Study in Spain

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ABSTRACT

A health survey was carried out in Murcia, Spain, in the vicinity of a Cellular Phone Base Station working in DCS-1800 MHz. This survey contained health items related to "microwave sickness" or "RF syndrome." The microwave power density was measured at the respondents' homes. Statistical analysis showed significant correlation between the declared severity of the symptoms and the measured power density. The separation of respondents into two different exposure groups also showed an increase of the declared severity in the group with the higher exposure.

Key Words: Public health; Cellular phone; Base stations; Microwave sickness.

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INTRODUCTION

The hypothesis that radiofrequency (RF) exposure might produce health damage has been analyzed mainly from several epidemiological studies. Insomnia, cancer, leukemia in children, and brain tumors are the clinical entities more frequently described (Dolk et al., 1997; Hocking et al., 1996; Maskarinec et al., 1994; Minder and Pfluger, 2001; Selvin et al., 1992). Moreover, the clinical consequences of being exposed to microwave radiation such as radar has been evaluated from military and occupational studies (Balode, 1996; Garaj-Vrhovac, 1999; Goldsmith, 1997; Johnson-Liakouris, 1998; Robinette et al., 1980).

A specific symptomatology, linked to radar exposure at low levels of RF, has been termed "microwave sickness" or "RF syndrome." (Johnson-Liakouris, 1998) With few exceptions, functional disturbances of the central nervous system have been typically described as a kind of radiowave sickness, neurasthenic or asthenic syndrome. Symptoms and signs include headache, fatigue, irritability, loss of appetite, sleepiness, difficulties in concentration or memory, depression, and emotional instability. This clinical syndrome is generally reversible if RF exposure is discontinued.

Another frequently described manifestation is a set of labile functional cardiovascular changes including bradycardia, arterial hypertension, or hypotension (Johnson-Liakouris, 1998). This form of neurocirculatory asthenia is also attributed to nervous system influence. More serious but less frequent neurologic or neuropsychiatric disturbances have occasionally been described as a diencephalic syndrome (Johnson-Liakouris, 1998). All these disturbances following low level exposures (of the order of microwatts/cm²) have been reported for many years from Eastern Europe. The exposures have been mainly low level and long term (Goldsmith, 1997; Johnson-Liakouris, 1998).

Also, several articles have found biological dysfunction at very low density of radiation without temperature elevation, favoring the hypothesis of nonthermal biological effects and pointing to the probability of clinical dysfunction below the actual standard of safety norms in the European Union (Arber and Lin, 1985; Baranski, 1972; Byus et al., 1988; Daniells et al., 1998; de Pomerai et al., 2000; D'Inzeo et al., 1988; Dutta et al., 1989; Kues et al., 1992; Lai and Singh, 1995–1997; Lai et al., 1984, 1989; Malyapa et al., 1998; Sanders et al., 1985; Sarkar et al., 1994; Stagg et al., 1997; Wachtel et al., 1975).

Low levels of RF are found around the GSM-DCS cellular phone Base Stations (BS), where antennas are usually located on the roofs or in the top of tall towers. GSM-DCS cellular phones use pulsed microwaves. These signals have a spectral similarity to radar signals. The spectral power distribution of pulsed signals includes low frequency harmonics. Typical pulse duration time ranges from 100 msec to 0.050 μ sec in radar, and 576.9 μ sec for each slot of GSM-DCS.

From this point of view, the hypothesis of a "microwave sickness" in the neighborhood of the GSM-DCS Base Stations is analyzed in this study. The present analysis tries to evaluate if there is some statistical justification to the complaints and related dysfunction locally associated with RF exposure from the GSM-DCS Base Stations, as has been found in previous studies (Santini et al., 2001, 2002a,b).

MATERIALS AND METHOD

A local team, specially trained for this work, delivered the questionnaires in La Ñora, a town of Murcia in Spain during January 2001. This was always introduced to

respondents as a part of a study to evaluate the impact on the area of the cellular phone Base Stations (GSM-DCS). In general, the people were quite prepared to cooperate (the ratio of returned to delivered was about 70%). The questionnaire was a Spanish language adaptation of the Santini publication (Santini et al., 2001). This was composed of 25 different items mainly concerning health information about the respondents.

The respondents scored and marked from 0 to 3 the presence of the suffered health dysfunction: 0 never, 1 sometimes, 2 often, 3 very often.

The asked symptoms were those described in earlier studies of the microwave syndrome: fatigue, irritability, headache, nausea, appetite loss, insomnia, depression, discomfort, difficulty in concentration, memory loss, skin alterations, visual dysfunction, auditory dysfunction, dizziness, gait difficulty, and cardiovascular alterations.

Questions included demographic data: address, sex, and age, distance to the antennas (distance in meters to the Base Station), exposure time in days/weeks, hours/days, and time from the beginning of the emissions. The questionnaire also collected information about proximity to power lines, and the use of personal computer and cellular phone.

More than 5% of the population of La Ñora (around 1900 habitants) answered the questionnaire. Questionnaires from people with a history of deep psychological or neurological disease were excluded. Finally, 101 surveys were considered valid.

The survey was supplemented with electric field measurements, conducted February 24, 2001, and March 10, 2001 (Saturday). Measurements were carried out from 11:00 hr to 19:00 hr each day, in the bedrooms of each respondent. More measurements were carried out in the streets during working days and weekends, to check the possible variability in time of the measurements. The measurements were individually added to the survey of each respondent.

A portable broadband (1 MHz–3 GHz) electric field meter (EFM) was used. The EFM was hand-oriented in order to measure the maximum field strength above the bed. The electric field in each room presented a standing wave pattern because of reflection of the waves from the walls and metallic structures such as windows and metallic furniture. Therefore the EFM was held around 1 m from the walls, 1.2 m above the ground, and was moved around a circle of 25 cm of radius, orienting the antenna to get the maximum electric field strength.

The EFM was calibrated in the anechoic chamber of the University of Valencia with a standard measurement set-up using a network analyzer HP-8510C.

To check the intensity of TV and radio channels, as well as the number of working channels of the GSM-DCS BS, measurements of the spectral power density were carried out with a probe antenna and a portable spectrum analyzer.

The TV and radio channels maintained their intensities during the measurements, but the cellular phone channels presented dramatic differences in amplitude from channel to channel, some of them going on and off the air at random times.

The probe was mounted on a linen phenolic tripod about 1.2 m above the ground. The location of the probe was the same on both days, on a hill next to the town, 20 m from the BS. With the spectrum analyzer we scanned the GSM and DCS bands, at the beginning of the journey, taking the average over a period of 6 min. The measurement of the spectrum was similar on both days, having a difference in the peak estimation (carriers of the channels) of about 1 dB.

RESULTS

The respondents were 47% male and 53% female, with a wide age range: 15–25 years (22%), 26–35 years (22%), 36–45 years (19%), 46–55 years (11%), 56–65 years (13%), and over 65 years (13%).

The exposure time, explained as the time spent in the vicinity of the BS, was more than 6 hr per day, 7 days a week, for 95% of the respondents. The bedroom was where the electric field was measured.

Concerning the attitude of the respondents about the use of cellular phone: 24% of them declared themselves to be active users of mobile GSM-DCS phone for more than 20 min per day.

The measurements were very low compared with European safety guidelines 1999/519/EC DOCE 30/7/99 (1999/519/EC:). Actually the levels were lower than $0.2 \mu\text{W}/\text{cm}^2$. The Spanish legislation established a maximum limit of $450 \mu\text{W}/\text{cm}^2$ at a single frequency (900 MHz), the same as the European safety guidelines 1999/519/EC DOCE 30/7/99. This is one of the characteristics of the present work: the low levels of RF exposures.

Table 1. Average severity of the reported symptoms in two groups having different exposure: higher exposure with average power density $0.11 \mu\text{W}/\text{cm}^2$ (distance < 150 mts), and lower exposure with average power density $0.01 \mu\text{W}/\text{cm}^2$ (distance > 250 mts).

			<i>P</i> value
Respondents	<i>N</i> = 54	<i>N</i> = 47	
Average power density $\mu\text{W}/\text{cm}^2$	0.11 ± 0.19	0.01 ± 0.04	< 0.001
Distance to BS	< 150 m 107 ± 57 m	> 250 m 284 ± 24 m	< 0.001
	Average value of reported severity	Average value of reported severity	
Fatigue	1.11 ± 1.13	0.74 ± 1.07	n.s.
Irritability	1.56 ± 1.08	1.04 ± 1.02	< 0.05
Headache	2.17 ± 0.86	1.53 ± 1.00	< 0.001
Nausea	0.93 ± 0.99	0.53 ± 0.88	< 0.05
Appetite loss	0.96 ± 1.03	0.55 ± 0.88	< 0.05
Discomfort	1.41 ± 1.11	0.87 ± 0.97	< 0.02
Gait difficulty	0.68 ± 0.93	0.94 ± 1.07	n.s.
ASTHENIC symptoms	8.81 ± 4.79	6.21 ± 5.33	< 0.02
Sleep disturbance	1.94 ± 0.92	1.28 ± 1.10	< 0.01
Depression	1.30 ± 1.19	0.74 ± 1.01	< 0.02
Difficulty in concentration	1.56 ± 1.14	1.00 ± 1.06	< 0.02
Memory loss	1.41 ± 1.05	1.04 ± 1.08	n.s.
Dizziness	1.26 ± 1.14	0.74 ± 1.05	< 0.05
DIENCEPHALIC symptoms	7.46 ± 3.90	4.81 ± 4.34	< 0.01
Skin alterations	0.72 ± 0.96	0.45 ± 0.93	n.s.
Visual dysfunction	1.11 ± 1.07	0.96 ± 1.12	n.s.
Auditory dysfunction	1.06 ± 1.12	0.81 ± 1.12	n.s.
SENSORIAL symptoms	2.89 ± 2.72	2.32 ± 2.45	n.s.
Cardiovascular alterations	0.76 ± 1.10	0.49 ± 0.93	n.s.

Table 2. Correlation coefficient between severity of the reported symptoms and the logarithm of the measured electric field.

	Correlation coefficient with power density	<i>p</i> value
ASTHENIC symptoms		
Fatigue	0.438	< 0.001
Irritability	0.515	< 0.001
Headache	0.413	< 0.001
Nausea	0.354	< 0.001
Appetite loss	0.485	< 0.001
Discomfort	0.544	< 0.001
Gait difficulty	0.127	n.s.
DIENCEPHALIC symptoms		
Sleep disturbance	0.413	< 0.001
Depression	0.400	< 0.001
Difficulty in concentration	0.469	< 0.001
Memory loss	0.340	< 0.001
Dizziness	0.357	< 0.001
SENSORIAL symptoms		
Skin alterations	0.358	< 0.001
Visual dysfunction	0.347	< 0.001
Auditory dysfunction	0.163	n.s.
CARDIOVASCULAR symptoms		
Cardiovascular alterations	0.290	< 0.01

Second column is the statistical significance (*p*) of the correlation coefficient.

We divided the surveys into two groups: One group with high exposure, averaging $0.11 \mu\text{W}/\text{cm}^2$, consisted of 47 respondents. These respondents declared themselves to be living less than 150 m from the BS. The second group, with an average exposure of $0.01 \mu\text{W}/\text{cm}^2$, were at a distance greater than 250 m.

Although both groups were obviously at different distances from the BS, there was still the risk of a distance perception that could influence the survey.

Table 1 shows the average declared severity in both groups.

A possible relationship between the declared severity of the symptom and the microwave power density was explored. A mathematical model with logarithmic dependence on the measured electric field (EFM) was used. The SPSS statistical package, with different regression methods, was used for this analysis. The results for the correlation coefficient and statistical significance are presented in Table 2. Correlation coefficients were grouped in four sections: asthenic, diencephalic, sensorial, and cardiovascular symptoms.

DISCUSSION

It is interesting to compare the severity of the reported symptoms between both groups of Table 1: more severe symptoms were reported in the first group. The first group (< 150 m from BS) was exposed to a mean EMF power density 10 times higher

than the second group (> 250 m from BS). Asthenic syndrome was 42% higher in the first group, diencephalic syndrome was 55% higher in the first group, sensorial alterations were 25% higher in the first group, and cardiovascular alterations 55% higher as well.

However, the use of mobile phones was 30% in the first group and 17% in the second group. Use of the personal computer was 16% in the first group and 1% in the second group. Therefore, these differences could bias the health response. The use of the mobile cellular phone implies a considerably higher exposure of the head to microwaves during the phone call, roughly 5 mW/cm^2 , 10,000 times higher than the maximum EMF exposure attributed to the BS. Moreover, the symptomatic response could be influenced by personal or human idiosyncrasy. The exposure to radiation from the computer screen occurs at extremely low frequencies and is under $0.3 \text{ } \mu\text{T}$, at normal distances. It is therefore not considered significant, but will be the subject of a future work.

Results from Table 2 indicate the correlation between severity of the reported symptoms and the logarithm of the measured electric field (EFM) with $p < 0.001$. We find that discomfort (0.544), irritability (0.515), and appetite loss (0.485) are the most relevant symptoms correlated with exposure intensity. Others symptoms, fatigue (0.438), headache (0.413), difficulty in concentrating (0.469), and sleep disturbances (0.413), also show a significant correlation with exposure intensity. However, other symptoms such as auditory dysfunction, gait difficulty, and cardiovascular, have a lower correlation coefficient, but significant $p < 0.01$.

However, the most interesting aspect of our results is the significance of the dependence between both variables: The declared severity of the symptom and the logarithm of the measured electric field. Another interesting observation is that four of the highly correlated symptoms (Table 2) such as headache, sleep disturbances, concentration difficulty, and irritability also show the most relevant differences between both groups and the highest values in the clinical scale, 2.17, 1.94, 1.56, and 1.56 respectively (Table 1).

The validity and interpretation of the results of Tables 1 and 2 must be analyzed in the proper context, by comparison with results from other researchers, or with our results from previous similar surveys. Actually there are no studies similar to the presented in this communication. However, our work shows a similarity in procedure and results with previous surveys on noise annoyance. Results for the correlation coefficients (Table 2) are similar to those obtained in previous social surveys on noise annoyance, where the maximum correlation coefficient was around 0.35 (Schultz, 1978).

If there is a casual relationship between severity of the symptoms and the measured electric field, it may be that the logarithmic approach is still too approximate, and a more elaborate model would be convenient. The logarithmic model is extended in the analysis of noise annoyance, since the devices used in noise measurements use logarithmic scales (dB_A). Moreover, the used measurement was a spatial-point, time-point, measurement. This would most likely be an improvement in correlation for EMF average levels during days or weeks. However, the existence of appropriate instrumentation is a limitation.

It is worth pointing out that noise is a recognized environmental pollutant, and the social surveys on noise annoyance address its subjective response. Although noise is perceived by the senses, the same is not true for the electromagnetic field. Therefore

biasing is less likely in the present study, and the results are probably more objective than in the surveys on noise annoyance.

Trying to find comparisons between our results and previous work, we can claim a strong similarity with the Lilienfeld study (Johnson-Liakouris, 1998), which showed a dose-response relationship between various neurological symptoms and microwave exposure. These symptoms were grouped under the name "microwave syndrome" or "radiofrequency radiation sickness."

The present results demonstrate a significant correlation between several symptoms of what is called microwave sickness and the microwave power density associated with the Base Station located on a hill at the edge of the town. The severity of the symptoms weakens for people who live far away, at a distance greater than 250 m from the main EMF source and at a power density lower than $0.1 \mu\text{W}/\text{cm}^2$.

As there is a significant difference between both groups in terms of the irradiated power density, a hypothetical relationship between the DCS emission and the severity of symptoms may exist.

There is a large and coherent body of evidence of biological mechanisms that support the conclusion of a plausible, logical, and causal relationship between RF exposure and neurological disease. Hence it is possible that cell sites are causing adverse health effects. Public health surveys of people living in the vicinity of cell site BSs should be carried out immediately, and continued over the next 2 decades. Prompt effects such as miscarriage, cardiac disruption, sleep disturbance, and chronic fatigue could well be early indicators of adverse health effects.

This is the first social survey concerning the microwave syndrome carried out in Spain, and is a preliminary study. Future surveys in another geographical locations are underway. More research and comparison of statistical results from different areas would be useful.

At present, the electromagnetic/microwave power density is not a recognized environmental pollutant. The reported results are obtained from one of the first social surveys on the health of the population living in the vicinity of a Base Station of GSM-DCS cellular phone.

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THE MICROWAVE SYNDROME – FURTHER ASPECTS OF A SPANISH STUDY

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Abstract

A health survey was carried out in La Ñora, Murcia, Spain, in the vicinity of two GSM 900/1800 MHz cellular phone base stations. The E-field (~ 400 MHz – 3 GHz) measured in the bedroom was divided in tertiles (0.02 – 0.04 / 0.05 – 0.22 / 0.25 – 1.29 V/m). Spectrum analysis revealed the main contribution and variation for the E-field from the GSM base station. The adjusted (sex, age, distance) logistic regression model showed statistically significant positive exposure-response associations between the E-field and the following variables: fatigue, irritability, headaches, nausea, loss of appetite, sleeping disorder, depressive tendency, feeling of discomfort, difficulty in concentration, loss of memory, visual disorder, dizziness and cardiovascular problems. The inclusion of the distance, which might be a proxy for the sometimes raised “concerns explanation”, did not alter the model substantially. These results support the first statistical analysis based on two groups (arithmetic mean 0.65 V/m versus 0.2 V/m) as well as the correlation coefficients between the E-field and the symptoms (Navarro et al, “The Microwave Syndrome: A preliminary Study in Spain”, *Electromagnetic Biology and Medicine*, Volume 22, Issue 2, (2003): 161 – 169). Based on the data of this study the advice would be to strive for levels not higher than 0.02 V/m for the sum total, which is equal to a power density of 0.0001 $\mu\text{W}/\text{cm}^2$ or 1 $\mu\text{W}/\text{m}^2$, which is the indoor exposure value for GSM base stations proposed on empirical evidence by the Public Health Office of the Government of Salzburg in 2002.

Introduction

The relationship between biological/health effects and electromagnetic exposure has been widely recognized from epidemiological and experimental studies. Even some institutional consensus has been reached and formal health risk assessments for exposure to ELF, extremely low frequency fields, e.g. from powerlines and electric appliances, have recently been scheduled. In 2002 the first IARC review on this topic classified ELF magnetic fields as “possibly human carcinogen” based on epidemiological studies of childhood leukemia [1]. In 2002 the California Department of Health judged ELF magnetic fields at least possibly related with leukemia in children and adults, brain tumors in adults, miscarriage and motor neuron disease [2].

With respect to radiofrequency (30 kHz – 300 MHz) and microwave exposure (300 MHz – 300 GHz) the scientific evidence from in vitro, in vivo and epidemiological studies shows a great spectrum of biological/health effects at low level exposures [3, 4, 5, 6, 7, 8, 9]. A specific symptomatology in humans linked to radiofrequency and microwaves, named “microwave sickness” or “radiofrequency syndrome” was described at low level exposure which include headache, fatigue, irritability, loss of appetite, sleeping disorders, difficulties in concentration or memory, and depression [10].

The growing use of mobile communication, GSM 900/1800, cordless telephones etc in the last decade has reintroduced concerns about whether some health risks could derive from microwave exposure, especially from mobile phones and their basestations. In contrast to the public debate on health risks from mobile phone base stations, only three epidemiological studies on this issue have been published until now. A study done in France

THE MICROWAVE SYNDROME - FURTHER ASPECTS OF A SPANISH STUDY

by Santini showed significant associations between symptoms fitting to the microwave sickness and the distance to mobile phone base stations [11]. It should be noted that the health related symptoms were most frequently reported at a distance of 50 – 100 m, which fits perfectly to the area with the highest microwave exposure in urban areas, where the main beam of the antennas usually hits the first houses. The second study done in Austria showed significant positive associations between the frequency selective measured electric field (GSM 900/1800) in the bedroom and cardiovascular symptoms, irrespective of the concerns of the people under study [12]. The third study was published by our group [13] where we measured the electric field via a broadband device in the bedroom of 97 participants in La Ñora, Murcia, Spain. The statistical analysis showed significantly higher symptom scores in 9 out of 16 symptoms in the group having an exposure of 0.65 V/m compared to the control group having an exposure of 0.2 V/m, both as an average mean. In the same paper we reported also significant correlation coefficients between the measured electric field and fourteen out of sixteen health related symptoms.

The aim of this paper is to present additional statistical tests like logistic regression of the La Ñora data set and a detailed investigation of the EMF spectrum in six bedrooms (8 participants) done on July 3rd, 2004.

Geographical Area and Time Schedule

The study was done in La Ñora, a town in the south-east of Spain, close to Murcia, with 1900 inhabitants, situated on the slope of a hill. For the mobile phone coverage of La Ñora two masts had been sited on two different positions near the top of the hill above the village. The start of the transmission of both stations is not clear. However for the GSM 900 base station the siting is not earlier than 1997/1998, for the GSM 1800 base station was sited in December 1999. The questionnaires have been distributed in October 2000 and collected in November 2000. Broad band measurements (~400 MHz – 3 GHz) in 97 bedrooms as well as some frequency selective measurements have been done in February and March 2001.

In July 2004 frequency selective measurements were done in 6 bedrooms of former study participants.

Questionnaire

We used a questionnaire, translated to Spanish, of the Santini publication [10] which refers to demographic data: Address, sex, age, distance to mobile phone basestations, exposure time (years, days per week, hours per day). The questionnaire also collected information about proximity to power lines < 100 m, proximity to transformer stations < 10 m, use of personal computers > 2 hours per day and the use of cellular phones > 20 minutes per day. Finally a symptom checklist allowed to know the frequency of 16 health related symptoms: 0 = never, 1 = sometimes, 2 = often, 3 = very often.

Many of the symptoms were those described as microwave/radiofrequency syndrom/sickness: Fatigue, irritability, headache, nausea, loss of appetite, sleeping disorders, depression, feeling of discomfort, difficulty in concentration, loss of memory, skin alterations, visual disorder, auditory disorder, dizziness, gait difficulty and cardiovascular alterations. The questionnaires were distributed in La Ñora in frequently used locations (hair dresser, pharmacy) in October/November 2000 and collected in November/December 2000. From 144 questionnaires returned, 97 measurements in the bedrooms were done in 2001. The difference of 47 subjects was due to the impossibility to read the name or adress in order to get the contact, no interest in the measurements, not at home at the scheduled measurement time or symptoms of the health questionnaire checked with an “x” instead of the proposed numbers “0”, “1”, “2”, or “3”. In 2004 the analysis of the La Ñora data set had been done with n=94 subjects having full information on exposure values from 2001, sex, age and symptoms except for one subject, where all informations were available except for the “skin disorder question” n=93.

Exposure Assessment

The exposure to mobile phone basestations was assessed in 2001 with a portable broad band measurement device (~ 400 MHz – 3 GHz) called LX-1435. The electric field meter had been calibrated with a network analyser HP-8510C inside the anechoic chamber of the University of Valencia, Spain. The electric field probe was held around 1 meter from the walls and 1.2 meters above the ground, to avoid reflection of the waves in the walls and metallic structures and moved around a circle of 25 centimeters´ radius, orientating the dipole antenna to get the maximum electric field strength above the bed.

The measurements were performed from 11:00 h to 19:00 h on February 24th, 2001, and on March 10th, 2001, in the respondents´ home. The bedroom was chosen because the pineal gland and its hormone melatonin is considered one of the target organs for EMF, having secretion peaks during the sleep.

To check the intensity of TV and radio channels (ultra short wave range), as well as the number and type of channels of the GSM 900/1800 base stations, measurements of the spectral power density were performed with a probe antenna and a portable spectrum analyser. The probe was mounted on a linen phenolic tripod about 1.2 meters above ground. Location of the probe was the same in both days, on a hill next to the town. With the spectrum analyser we scanned the GSM 900/1800 MHz bands, at the beginning of the journey, taking the average for a period of 6 minutes. The spectrum was similar in both days, with a difference in the peak estimation (carriers of the channels) of about 1 dB in radio and TV channels, GSM 900/1800 showed small differences, around 3 dB, associated to the working channels that were dependent on the traffic of cellular phones.

On July 3rd, 2004 from 11:00 to 19:00 h the spectrum of the electric field from 80 MHz – 2.5 GHz was measured in six bedrooms in La Ñora. The points of measurement were randomly selected from the study population which had been divided in three exposure groups (low, intermediate, high) with respect to the measured electric field in 2001. The aim was to check the exposure situation inside the houses in several places to validate the measurements of 2001 and to get the portion of radio, TV and GSM of the electromagnetic spectrum. A calibrated hand-held spectrum analyzer, FSH3 (100 kHz – 3 GHz) from the manufacturer Rhode & Schwarz, Germany and calibrated electric-field probes EFS 9218 (9 kHz – 300 MHz) and USLP 9143 (300 MHz – 5 GHz) from the manufacturer Schwarzbeck, Germany were used. A volume of about one m³ above the surface of the bed was examined holding the antennas in different polarization directions as well as different directions in order to pick up the highest signals. The spectrum analyzer was adjusted: detector: max peak, trace: max hold. In order to differentiate broadcast control channels (BCCH) from traffic channels (TCH) both GSM spectra (GSM 900/1800) had been analysed at the time of measurements. The traces were stored in the spectrum analyzer and analysed via FSH View Version 7.0 on the PC afterwards.

Results

From n=94 participants under study, 47 were female, 47 male. The age span was 14 to 81 years, with a median age of 39 years. In the questionnaire the distances to the next GSM 900/1800 base stations were given in six different categories.

Table 1: Distance to next GSM 900/1800 base stations

Distance	Frequency	Percent
< 10 m	7	7.4
10 – 50 m	6	6.4
50 – 100 m	9	9.6
100 – 200 m	30	31.9
200 – 300 m	14	14.9
> 300 m	28	29.8
Total	94	100.0

93 % reported to be exposed to the mobile phone base stations for more than one year. The time spent in the house of the study site, was more than 8 hours per day for at least 6 days in 94 % of the respondents.

17 % reported to be exposed to an electric transformer distance less than 10 m. 43 % reported to live closer than 100 m to a high voltage power line. 40 % reported that they live at a distance of less than 4 km from a radio / TV transmitter. Using a mobile phone for more than 20 minutes per day was reported by 29 %. Working on a personal computer more than two hours per day was reported by 14 % of the study participants.

TV and radio channels maintained constant intensity during the 2001 measurements, however the traffic channels of the mobile phone base stations (GSM 900/1800) showed typical fluctuations. Table 2 shows the measured broad band electric field in V/m and the corresponding power density in $\mu\text{W}/\text{cm}^2$ and $\mu\text{W}/\text{m}^2$ in the bedroom in 2001.

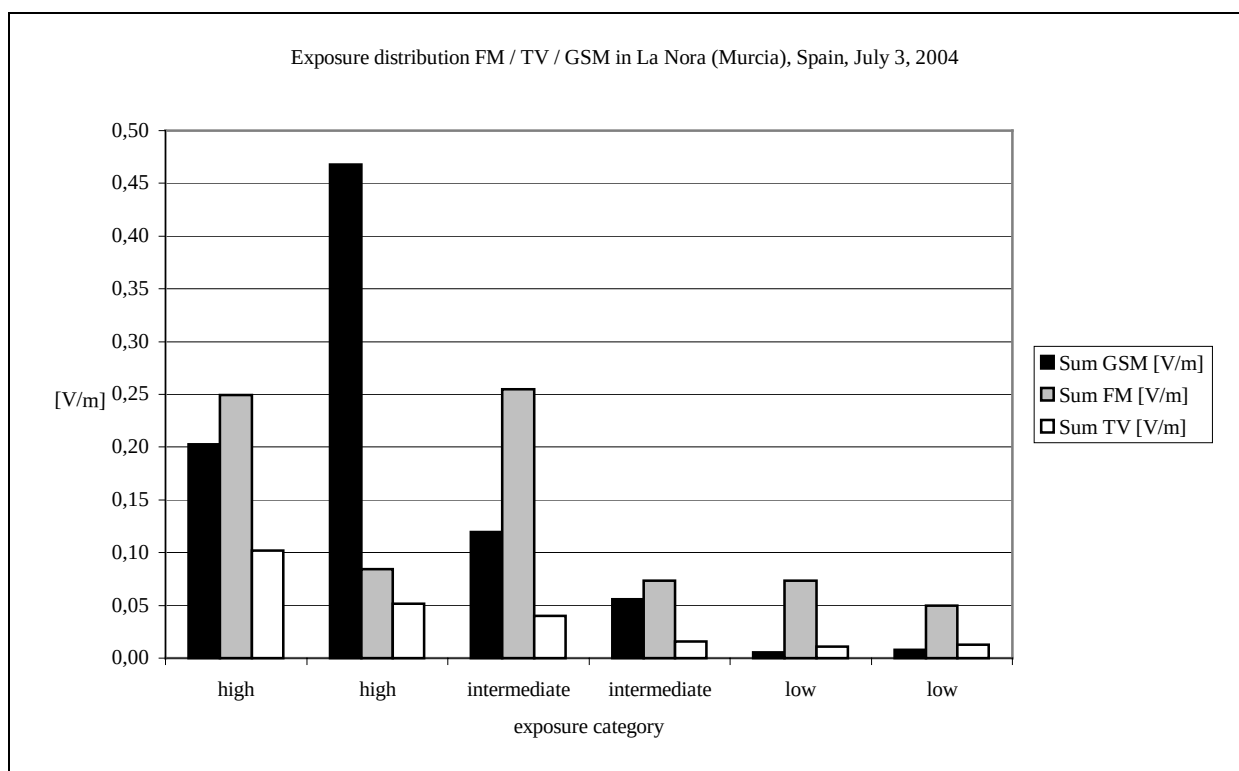
THE MICROWAVE SYNDROME - FURTHER ASPECTS OF A SPANISH STUDY

Table 2: Broad band measurement in the bedroom 2001

		E-field [V/m]	Power density [$\mu\text{W}/\text{cm}^2$]	Power density [$\mu\text{W}/\text{m}^2$]
n	valid	94	94	94
	missing	0	0	0
average		0.27	0.051615	516.15
median		0.11	0.003157	31.57
SD		0.35	0.107775	1077.75
Minimum		0.02	0.000088	0.88
Maximum		1.29	0.442028	4420.28

The frequency selective measurements done in 2004 in six bedrooms showed that the variance of the broad band signal is mostly due to differences in the strength of the GSM 900/1800 signal. Because the broad band measurements had an attenuation in the FM frequency range of 15 dB the contribution of the FM signals to the broad band results are of small influence. The TV signals showed also to be quite small in comparison to the GSM 900/1800 signal as well. In order to attribute the proportion of different signals to a health outcome a frequency selective exposure assessment on an individual level is preferred. Figure 1 shows the results of the frequency selective measurements of 2004.

Figure 1: Exposure distribution (GSM 900/1800, FM, TV) in six bedrooms 2004



For the logistic regression model we divided the broad band measured electric field in three exposure categories: Low exposure 0.02 – 0.04 V/m (1 – 4 $\mu\text{W}/\text{m}^2$), intermediate exposure 0.05 – 0.22 V/m (6 – 128 $\mu\text{W}/\text{m}^2$) and high exposure 0.25 – 1.29 V/m (165 – 4400 $\mu\text{W}/\text{m}^2$). We calculated a raw model to derive the odds ratio (OR) and the corresponding 95%-confidence interval (95%-CI) as well as the probability value (p-value) for all 16 health related symptoms for the intermediate and the high exposure category – using the low exposure category as the reference. In the second model we controlled for sex and age. In the third model we controlled for sex, age and distance to the next mobile phone base station reported by the study participants. The distance was added in order to see if there is any significant contribution to the model (which still includes the measured electric field, sex and age) from this variable. If one assumes the reversed distance as a proxy for concerns from the antennas, the reversed distance might show up as a variable with a certain amount of explanation of the model. In two out

of 16 symptoms the reversed distance showed a significant contribution to the model in addition to the sex and age adjusted model. The variables being “sleeping disorders” with OR 1.47 (95%-CI 1.01 – 2.15) and “dizziness” with OR 1.71 (95%-CI 1.17 – 2.51). In comparison with the explanation of the measured E-field, the contribution is very small. See table 5, where the symptom “sleeping disorder” was associated with the measured E-field OR 10.39 (95%-CI 2.43 – 44.42) and OR 10.61 (95%-CI 2.88 – 39.19) and “dizziness” OR 2.98 (95%-CI 0.62 – 14.20) and OR 8.36 (95%-CI 1.95 – 35.82). A relevant influence of the reversed distance would result in a substantially alteration of the odds ratios associated with the E-field, which is not the case.

We also calculated logistic regression models including other variables like living closer than 100 m to high voltage power lines or 10 m to a transformer, living closer than 4 km to a radio / TV station, use of a computer > 2h/day or a cell phone > 20 minutes/day. For some of the above mentioned variables we found a significant contribution to the explanation of the model (data not shown) for few of the symptom variables which did not alter the overall associations of the models presented in this paper. For future studies we advice that the exposure to high voltage power lines and transformers as well as to radio / TV stations should be measured on an individual level in order to reduce exposure misclassification.

In 13 out of the 16 health related symptoms significant exposure-response relationships and very high and significant odds ratios for the measured electric fields were found which is one of the main findings of this study. An other important finding is that 10 out of 16 symptoms showed significantly elevated OR between the reference exposure category (0.02 – 0.04 V/m) and even the intermediate exposure category (0.05 – 0.22 V/m). In order to derive guideline values for the protection of public health from electromagnetic fields from mobile phone base stations GSM 900/1800 MHz, one should take into account that epidemiological studies usually underestimate individual risks, as well as the uncertainty with respect to the reference exposure category, which could be at a sufficiently low level but that is not known in this study and an open question in this issue as well. In order to take this arguments into account a provisional reference level of about 0,02 V/m for the sum total of electric fields from mobile phone base stations GSM 900/1800 MHz is recommended and is in line with the level recommended in 2002 by the Public Health Office of the Government of Salzburg, based on empirical evidence.

Table 3 shows the raw logistic regression model. Table 4 shows the sex and age adjusted model. Table 5 shows the sex, age and distance adjusted model.

Table 3: Raw Model

Health Outcome	0.05 – 0.22 V/m (6 – 128 $\mu\text{W}/\text{m}^2$)			0.25 – 1.29 V/m (165 – 4400 $\mu\text{W}/\text{m}^2$)			p for the trend
	OR	95%-CI	p	OR	95%-CI	p	
Fatigue	23.46	2.77 – 198.82	0.0038	33.88	4.16 – 276.04	0.0010	0.0044
Irritability	3.71	1.19 – 11.55	0.0234	10.73	3.48 – 33.13	0.0000	0.0002
Headaches	7.46	2.10 – 26.55	0.0019	6.56	2.14 – 20.05	0.0010	0.0005
Nausea	7.62	0.83 – 69.89	0.0726	14.67	1.77 – 121.49	0.0128	0.0382
Loss of Appetite	5.82	0.61 – 55.61	0.1263	24.00	2.94 – 195.94	0.0030	0.0028
Sleeping Disorder	7.67	2.36 – 24.86	0.0007	6.64	2.30 – 19.20	0.0005	0.0003
Depressive Tendency	32.00	3.79 – 270.21	0.0015	42.66	5.23 – 348.33	0.0005	0.0021
Feeling of Discomfort	4.80	1.41 – 16.33	0.0121	12.21	3.72 – 40.12	0.0000	0.0002
Difficulty in Concentration	8.46	2.31 – 31.00	0.0013	18.12	5.05 – 64.99	0.0000	0.0000
Loss of Memory	1.65	0.53 – 5.14	0.3844	4.69	1.65 – 13.32	0.0037	0.0108
Skin Disorder	4.50	0.82 – 24.55	0.0825	5.19	1.08 – 26.21	0.0463	0.1278
Visual Disorder	1.65	0.53 – 5.14	0.3844	3.31	1.17 – 9.32	0.0236	0.0707
Hearing Disorder	2.72	0.87 – 8.52	0.0852	1.10	0.35 – 3.47	0.8702	0.1534
Dizziness	5.29	1.26 – 22.25	0.0229	9.44	2.43 – 36.77	0.0012	0.0053
Gait Difficulties	0.74	0.21 – 2.62	0.6454	1.08	0.36 – 3.25	0.8886	0.8321
Cardiovascular Problems	9.60	1.07 – 85.72	0.0429	14.67	1.77 – 121.49	0.0128	0.0442

THE MICROWAVE SYNDROME - FURTHER ASPECTS OF A SPANISH STUDY

Table 4: Age and Sex adjusted model

Health Outcome	0.05 – 0.22 V/m (6 – 128 $\mu\text{W}/\text{m}^2$)			0.25 – 1.29 V/m (165 – 4400 $\mu\text{W}/\text{m}^2$)			p for the trend
	OR	95%-CI	p	OR	95%-CI	p	
Fatigue	25.79	2.94 – 225.85	0.0033	37.72	4.42 – 321.49	0.0009	0.0040
Irritability	3.36	1.06 – 10.66	0.0395	9.60	3.05 – 30.26	0.0001	0.0006
Headaches	8.06	2.14 – 30.31	0.0020	7.29	2.22 – 23.94	0.0011	0.0007
Nausea	7.53	0.80 – 70.75	0.0774	14.33	1.68 – 122.55	0.0150	0.0445
Loss of Appetite	6.03	0.60 – 60.19	0.1260	25.84	2.98 – 223.80	0.0032	0.0031
Sleeping Disorder	13.982	3.50 – 55.85	0.0002	12.39	3.47 – 44.26	0.0001	0.0001
Depressive Tendency	44.87	4.85 – 414.69	0.0008	64.28	7.05 – 586.27	0.0002	0.0011
Feeling of Discomfort	4.34	1.25 – 15.03	0.0207	10.97	3.27 – 36.77	0.0001	0.0005
Difficulty in Concentration	9.40	2.44 – 36.21	0.0011	20.55	5.35 – 79.00	0.0000	0.0001
Loss of Memory	2.40	0.70 – 8.26	0.1642	7.91	2.37 – 26.35	0.0008	0.0027
Skin Disorder	6.25	1.05 – 37.13	0.0437	7.67	1.36 – 43.44	0.0212	0.0647
Visual Disorder	2.57	0.74 – 9.08	0.1380	5.88	1.75 – 19.74	0.0041	0.0158
Hearing Disorder	4.45	1.23 – 16.13	0.0231	1.75	0.49 – 6.24	0.3859	0.0643
Dizziness	5.37	1.24 – 23.16	0.0243	9.70	2.39 – 39.33	0.0015	0.0063
Gait Difficulties	1.09	0.28 – 4.24	0.8970	1.86	0.54 – 6.41	0.3235	0.5629
Cardiovascular Problems	12.56	1.32 – 118.99	0.0274	20.43	2.26 – 184.95	0.0073	0.0267

Table 5: Age, Sex and distance adjusted model

Health Outcome	0.05 – 0.22 V/m (6 – 128 $\mu\text{W}/\text{m}^2$)			0.25 – 1.29 V/m (165 – 4400 $\mu\text{W}/\text{m}^2$)			p for the trend
	OR	95%-CI	p	OR	95%-CI	p	
Fatigue	28.53	3.03 – 268.78	0.0034	40.11	4.56 – 352.44	0.0009	0.0039
Irritability	3.12	0.91 – 10.68	0.0704	9.22	2.86 – 29.67	0.0002	0.0009
Headaches	5.99	1.50 – 23.93	0.0113	6.10	1.80 – 20.65	0.0037	0.0050
Nausea	5.92	0.60 – 58.68	0.1288	12.80	1.48 – 110.64	0.0205	0.0499
Loss of Appetite	6.66	0.62 – 71.52	0.1175	27.53	3.07 – 247.03	0.0031	0.0030
Sleeping Disorder	10.39	2.43 – 44.42	0.0016	10.61	2.88 – 39.19	0.0004	0.0008
Depressive Tendency	39.41	4.02 – 386.40	0.0016	59.39	6.41 – 550.11	0.0003	0.0016
Feeling of Discomfort	4.29	1.14 – 16.15	0.0314	10.90	3.16 – 37.56	0.0002	0.0007
Difficulty in Concentration	8.27	2.01 – 34.01	0.0034	19.17	4.91 – 74.77	0.0000	0.0001
Loss of Memory	2.35	0.62 – 8.89	0.2090	7.81	2.27 – 26.82	0.0011	0.0031
Skin Disorder	7.04	1.06 – 46.62	0.0429	8.22	1.39 – 48.51	0.0201	0.0628
Visual Disorder	2.48	0.65 – 9.44	0.1830	5.75	1.68 – 19.75	0.0054	0.0186
Hearing Disorder	3.89	0.99 – 15.21	0.0510	1.63	0.45 – 5.95	0.4572	0.1285
Dizziness	2.98	0.62 – 14.20	0.1712	8.36	1.95 – 35.82	0.0042	0.0117
Gait Difficulties	1.32	0.30 – 5.84	0.7114	2.07	0.57 – 7.50	0.2690	0.5211
Cardiovascular Problems	9.42	0.93 – 95.07	0.0572	17.87	1.96 – 162.76	0.0105	0.0333

Summary

Frequency selective measurements done in July 2004 (n=6) showed that the main contribution and the main variability of the broad band signal measured in February and March 2001 is due to GSM 900/1800 signals (n=97). This is further supported by the fact that the dipol antenna used in 2001 is quite insensitive to frequencies below 400 MHz which is related to FM (80 – 110 MHz) and that the TV channels were quite weak in comparison to the GSM signals. However we would prefer to have frequency selective personal exposure values for all important signals of the electromagnetic spectrum in future studies.

For the logistic regression we divided the broad band measurements of the electric field in three exposure groups, the low exposure group served as the reference category. We calculated the odds ratios and 95% CI for the raw model, an age and sex adjusted model and an age, sex and distance adjusted model. All models showed statistical significant associations between the measured electric field (~ 400 MHz – 3 GHz) and 13 out of 16 health related symptoms. The strongest five associations found are depressive tendency, fatigue, sleeping disorder, difficulty in concentration and cardiovascular problems. The symptoms associated are in line with the symptoms reported in the literature as “Microwave Syndrom”. The odds ratios are quite high having small p-values. Some kind of selection bias cannot be ruled out, because of the way the questionnaires were distributed, but that would affect more or less all cases and therefore affect the odds ratios not substantially. The introduction of the reversed distance to the nearest base station, which might serve as a surrogate for the sometimes claimed “concerns explanation” for health related symptoms attributed to mobile phone base stations, did not alter the odds ratios substantially and the OR associated with the measured electric fields remained at their high level. It should be noted that the findings of this study might be of great importance for Public Health and should be taken seriously. Further epidemiological studies are warranted but do not preclude measures to reduce microwave exposures from GSM 900/1800 base stations now. Based on the data of this study the advice would be to strive for levels not higher than 0.02 V/m for the sum total, which is equal to a power density of 0.0001 $\mu\text{W}/\text{cm}^2$ or 1 $\mu\text{W}/\text{m}^2$, which is the indoor exposure value for GSM base stations proposed on empirical evidence by the Public Health Office of the Government of Salzburg in 2002 [14].

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THE MICROWAVE SYNDROME - FURTHER ASPECTS OF A SPANISH STUDY

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915 MHz Microwaves and 50 Hz Magnetic Field Affect Chromatin Conformation and 53BP1 Foci in Human Lymphocytes From Hypersensitive and Healthy Persons

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We used exposure to microwaves from a global system for mobile communication (GSM) mobile phone (915 MHz, specific absorption rate (SAR) 37 mW/kg) and power frequency magnetic field (50 Hz, 15 μ T peak value) to investigate the response of lymphocytes from healthy subjects and from persons reporting hypersensitivity to electromagnetic field (EMF). The hypersensitive and healthy donors were matched by gender and age and the data were analyzed blind to treatment condition. The changes in chromatin conformation were measured with the method of anomalous viscosity time dependencies (AVTD). 53BP1 protein, which has been shown to colocalize in foci with DNA double strand breaks (DSBs), was analyzed by immunostaining in situ. Exposure at room temperature to either 915 MHz or 50 Hz resulted in significant condensation of chromatin, shown as AVTD changes, which was similar to the effect of heat shock at 41 °C. No significant differences in responses between normal and hypersensitive subjects were detected. Neither 915 MHz nor 50 Hz exposure induced 53BP1 foci. On the contrary, a distinct decrease in background level of 53BP1 signaling was observed upon these exposures as well as after heat shock treatments. This decrease correlated with the AVTD data and may indicate decrease in accessibility of 53BP1 to antibodies because of stress-induced chromatin condensation. Apoptosis was determined by morphological changes and by apoptotic fragmentation of DNA as analyzed by pulsed-field gel electrophoresis (PFGE). No apoptosis was induced by exposure to 50 Hz and 915 MHz microwaves. In conclusion, 50 Hz magnetic field and 915 MHz microwaves under specified conditions of exposure induced comparable responses in lymphocytes from healthy and hypersensitive donors that were similar but not identical to stress response induced by heat shock. Bioelectromagnetics 26:173–184, 2005. © 2005 Wiley-Liss, Inc.

Key words: DNA DSBs; hypersensitivity; stress response; apoptosis

INTRODUCTION

Several investigations have shown that weak electromagnetic field (EMF) can affect biological systems [Goodman et al., 1995; Adey, 1999]. Within the extremely low frequency (ELF) range, exposure at 50 or 60 Hz is of major concern because electrical appliances and power lines emit such fields. ELF has recently been classified as a possible carcinogen by IARC [International Agency for Research on Cancer, 2002]. Concern about occupational and residential exposure to the microwave frequency transmitted by mobile phones is growing. In recent papers, it was

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reported that increased incidence of brain tumors correlated with exposure to mobile phones microwaves [Hardell et al., 2001, 2003]. Independent confirmation of brain tumors has recently been published [Lonn et al., 2004]. There is evidence that weak EMF can result in DNA damage and changes in permeability of brain–blood barrier [Lai and Singh, 1997; Persson et al., 1997].

There are a number of publications indicating that EMF can produce stress response [Lin et al., 1997; Junkersdorf et al., 2000; de Pomerai et al., 2000] and apoptosis [Simko et al., 1998; Mangiacasale et al., 2001; Olsson et al., 2001]. “Null” effects regarding all of the above-mentioned endpoints were also reported, however, accumulating experimental evidence suggest that EMF effects occur only in specific frequency and amplitude “windows” depending on several physical parameters [Adey, 1999; Binhi, 2002]. Therefore, both positive and “null” effects should be expected depending on exposure parameters.

Several biological variables are also of importance for effects of ELF and microwaves [Belyaev et al., 1999a, 2000]. In particular, the same exposure can result in apoptosis in some cells lines but does not induce apoptosis in others [Mangiacasale et al., 2001]. Recent evidence has indicated that mobile phone microwaves activate a variety of cellular signal transduction pathways, among them the hsp27/p38MAPK stress response pathway [Leszczynski et al., 2002].

The effects of ELF fields have been observed within relatively narrow “frequency windows” [Smith et al., 1987; Blackman et al., 1988, 1994; Belyaev et al., 1994; Prato et al., 1995] and “amplitude windows” [Blackman et al., 1982, 1994; Liboff et al., 1987; Lednev, 1991; Prato et al., 1995]. It has been found by Blackman et al. [1985] that the ambient static magnetic fields (SMF) can significantly influence the effects of ELF fields. The importance of SMF for the ELF effects has been confirmed in several studies [Lednev, 1991; Belyaev et al., 1994; Blackman et al., 1994; Fitzsimmons et al., 1994; Prato et al., 1995]. Therefore, the effects of weak ELF are usually observed only under specific combinations of frequency, amplitude, and SMF.

It was shown in our previous investigations, that weak EMF affected the conformation of chromatin in cells of different types including human lymphocytes [Belyaev et al., 1999a, 2000; Belyaev and Alipov, 2001a; Olsson et al., 2001]. In particular, temporal condensation of chromatin similar to condensation during apoptosis or stress response has been observed in human lymphocytes in response to 8 Hz magnetic field [Belyaev et al., 1999a; Belyaev and Alipov, 2001a]. Our present study was designed to test the possible effects of magnetic field at 50 Hz and microwaves on stress response and apoptosis in human lymphocytes.

Lai and Singh [1997] studied the effects of EMF in brain cells with the comet assay. The authors interpreted the data as showing induction of DNA breaks, but another interpretation based on changes in chromatin conformation indicative for stress response is also possible [Belyaev et al., 1999b]. New technology has recently become available to study double strand breaks (DSBs) based on analysis of DSB-colocalizing proteins. Several proteins involved in DNA repair and DNA damage signaling, such as the tumor suppressor p53 binding protein 1 (53BP1), have been shown to produce foci in response to DNA damage [Schultz et al., 2000]. In order to analyze whether EMF induced DNA damage, we performed immunofluorescence analysis of the 53BP1 protein *in situ*.

There is a growing concern about so-called hypersensitivity to electricity. These hypersensitive people experience several symptoms in the proximity to different sources of EMF. The symptoms are not specific and there is no known pathophysiological marker or diagnostic test for this illness [Hillert et al., 1999]. The causal relationship between EMF and symptoms reported by the afflicted individuals has not been confirmed in double-blind provocation studies [Bergqvist and Vogel, 1997; Flodin et al., 2000], however, individual responses to specific frequencies in a wide frequency range from 0.1 Hz to 5 MHz has been observed [Rea et al., 1991]. These data are in line with results of *in vitro* studies showing individual variability in response of human lymphocytes to EMF in specific frequency windows [Belyaev and Alipov, 2001a].

Here, human lymphocytes both from healthy and hypersensitive subjects were exposed to 50 Hz magnetic field under conditions which affect chromatin conformation [Belyaev et al., 2001c; Sarimov et al., *in preparation*], as well as to microwaves that previously have been shown to affect the brain–blood barrier in rats [Persson et al., 1997]. The specific research goals included (1) comparison of the effects of weak EMF on lymphocytes from healthy donors and subjects with hypersensitivity to EMF; (2) investigation of DNA breaks and apoptosis in EMF-exposed lymphocytes.

MATERIALS AND METHODS

Donors and Blood Samples

Blood samples from seven healthy donors and seven patients reporting hypersensitivity to EMF, but otherwise healthy, were obtained at the Department of Occupational and Environmental Health, Stockholm County Council, Sweden. The group of hypersensitive persons was selected based on self-reported hypersensitivity to EMF and characterized with regard to

TABLE 1. Details for Hypersensitive Subjects and Matched Control Healthy Persons

Subject	Gender	Age	Occupation	Duration of hypersensitivity (year)
1*	Female	34	Sick leave	1
2	Female	35	Secretary	—
3	Female	56	Art teacher	10
5	Female	55	Secretary	—
70*	Male	36	Unemployed	9
75	Male	31	Student	—
87	Male	46	Musician	1
88	Male	45	Physician	—
81*	Female	41	Sick leave	7
82	Female	46	Physician	—
91	Female	57	Controller	11
92	Female	58	Nurse	—
95	Female	44	Salesman	1
97	Female	43	Nurse	—

An asterisk designates cases of pronounced hypersensitivity.

symptom profile, triggering factors, time relation and avoidance behavior [Hillert et al., 1999]. The group reporting hypersensitivity to EMF consisted of two men and five women, 36–57 years old (Table 1). Control healthy subjects were matched by age (± 5 years) and gender (Table 1). All patients and controls were non-smokers and none was on any regular medication. Two persons reporting hypersensitivity were on sick leave, one was unemployed and four were working full or part time. All control persons were working. All patients reported symptoms to be triggered by electrical equipment that were not sources of light and all but two hypersensitive reported that symptoms were triggered by mobile phones. The two subjects that did not report this gave the comment that they had no experience of this exposure since they avoided mobile phones.

In all pairs of patient and matched control, the patient scored higher than the matched control in the questionnaire on symptoms (29 symptoms scored for frequency and severity, maximum scoring 232) [Hillert et al., 1999]. The mean score was 89 for patients and 12 for controls. In four patients neurovegetative symptoms (fatigue, headache, and difficulties concentrating) dominated over the skin symptoms (heat, burning sensation, tingling, and redness). In all but one hypersensitive patient the symptoms were always experienced within 24 h after exposure to a triggering factor, in most cases within 1 h. All patients reported that they tried to avoid triggering factors. Those three patients who tried as much as possible to avoid activated electrical equipment were classified as pronounced cases (Table 1). In all these three cases neurovegetative symptoms were most pronounced. Two of these patients lived in cottages in rural areas without electricity. Fresh blood samples from persons reporting hypersensitivity and

matched controls were coded and all data were analyzed in blind. Ethical permission was obtained from the Ethic Committee of the Karolinska Institutet, Stockholm, Sweden.

Chemicals

Reagent grade chemicals were obtained from Sigma (St. Louis, MO, USA) and Merck (Darmstadt, Germany). Pulsed-field gel electrophoresis (PFGE) grade agarose, λ ladder and λ digest pulse markers and low melting agarose were purchased from BioRad (Richmond, CA, USA).

Cells

Lymphocytes were isolated from peripheral blood by density gradient centrifugation in Ficoll-Paque (Pharmacia LKB, Sweden) according to the manufacturer's instructions. The cells were transferred to basal medium (BM); RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 12.5 IU/ml penicillin, 12.5 μ g/ml streptomycin (ICN Pharmaceuticals, Inc., Costa Mesa, CA, USA) at 5% CO₂ and 37 °C in a humidified incubator. Adherent monocytes were removed by overnight incubation of the cell suspension in culture flasks (Becton Dickinson & Co., Franklin Lakes, NJ, USA) at the cell density of 3×10^6 cells/ml in the volume of 10–40 ml. After this incubation, the cells in suspension were collected by centrifugation. The cell density was adjusted to approximately 2×10^6 cells/ml in fresh BM and the lymphocytes were preincubated for 2 h before exposure. The viability of cells was always above 95% as measured with trypan blue exclusion assay.

Cell Exposure

Coded samples from hypersensitive subject and matched control subject were simultaneously exposed in seven independent experiments. All exposures were performed at room temperature, 22–23 °C, in 14 ml round-bottom tubes (Falcon), 2.5 ml of cell suspension per tube, 2×10^6 cells/ml. The background ELF during exposures was not more 100 nT (rms) as measured with a three-dimensional microTeslameter (Field Dosimeter 3, Combinova, Sweden). Duration of all exposures was 2 h. In preliminary experiments, temperature was measured every 10 min during exposure in the 915 MHz/50 Hz-exposed samples. These measurements were performed at different locations across the exposed samples using a thermocouple with a precision of 0.1 °C. No changes in temperature were induced during 915 MHz/50 Hz exposures as compared to sham exposed and control samples.

Extremely low frequency. The 50 Hz exposure unit has been previously described [Alipov et al., 1994].

Briefly, it is based on three pairs of Helmholtz circular coils, which produce relatively homogenous (5% variation) magnetic field inside the working volume. The sinusoidal signal is supplied by a function generator and controlled by means of an oscilloscope and a frequency meter. The intensities of static and alternating magnetic fields were controlled in two ways: by direct measurements using a magnetometer (Sam3, Dowty Electronics Ltd., Cannock, UK) and one-dimensional microteslameter (G79, NPO Mikroprovod, Russia) and by measuring the current and subsequent calculation of the magnetic fields. Cells were exposed to a vertical 50 Hz magnetic field at the amplitude of 15 μ T. Vertical SMF was 43.5 μ T and the horizontal SMF was “nulled” by the vertical Helmholtz coils. Under these conditions of exposure, 50 Hz has been shown to induce changes in chromatin conformation as measured with AVTD [Belyaev et al., 2001c; Sarimov et al., in preparation].

GSM microwaves. Exposure of lymphocytes to microwaves was performed using a GSM900 test-mobile phone, which produced a GSM signal with controlled frequency and power level as observed under exposure from mobile phones. The output of the phone was connected by coaxial cable to a transverse electromagnetic transmission line cell (TEM cell) that has previously been described [Salford et al., 2003; Sarimov et al., 2004]. In principle, this is a spliced coaxial cable with a central electrode and an outer shield electrode with the unique characteristic of having both linear amplitude and phase response versus frequency. The fields of various waveforms including continuous waves and pulsed (or modulated) fields can be accurately generated in the TEM cell.

The construction of the TEM cell allows relatively homogeneous exposure of samples. There are 124 different channels/frequencies, which are used in GSM900 mobile communication. They differ by 0.2 MHz in the frequency range between 890 and 915 MHz. Frequency is supplied randomly to mobile phone users. The test-mobile phone was programmed to use a preset frequency. We used the channel 124 with the frequency of 915 MHz. The signal included standard GSM modulations. No voice modulation was applied. The test phone was programmed to regulate output power in pulses in the range of 0.02–2 W (13–33 dBm). This power was kept constant at 33 dBm during exposure as monitored on-line using a power meter (Bird model 43). During all exposures, two samples were exposed at one time, specific absorption rate (SAR) was 37 mW/kg and discontinuous transmission mode (DTX) was off. The SAR value was determined by measurements and calculations. Both the incident and the reflected power

at the input and the transmitted power through the TEM cell were measured for an input power of 1 W to the TEM cell. The absorbed power was then calculated and the SAR value was determined. Distribution of 915 MHz SAR in our exposure system has been recently described [Sarimov et al., 2004]. Even at peak values, the SAR was well below detectable heating.

Sham exposure, heat shock, and irradiation. Simultaneously with exposure to 915 MHz/50 Hz, the control cells were kept at room temperature, 22–23 °C, under the same conditions as the exposed cells. During all post exposure incubations, control and exposed cells were at 5% CO₂ and 37 °C in a humidified incubator. In four separate experiments, sham exposures were performed in both the ELF unit and the GSM unit with power off and compared with control cells. The sham exposed cells were kept under the same conditions as the exposed ones, including samples at room temperature. During GSM sham exposure, the cells were inside the GSM unit with power off. During ELF sham exposure, the coils were reconnected to produce anti-parallel magnetic fields of the same values as for real exposure. These magnetic fields compensated each other. No significant differences were observed between control and sham exposed cells using techniques described below. Heat shock, 41 °C for 2 h in a water bath, was used as a positive control for stress response. As a positive control for 53BP1 foci formation and apoptosis, the cells were irradiated with ¹³⁷Cs γ rays, 3 Gy, using a Gammacell 1000 (Atomic Energy of Canada Ltd., Ottawa, Canada) source. The dose rate was 10.6 Gy/min.

Apoptosis

Morphological changes associated with apoptosis, e.g., chromatin condensation, membrane blebbing and appearance of apoptotic bodies, were visualized by simultaneous staining of cells with fluorescent dyes acridine orange and propidium iodide and viewed by a fluorescence microscope (Nikon Eclipse E6000) as previously described [Belyaev et al., 2001b]. One hundred cells were scored as normal, apoptotic, or necrotic cells in each of three slides prepared for each treatment condition. The total number of counted cells was 300 per each treatment condition. All samples were analyzed 24 and 48 h after exposure by observers blind to exposure conditions.

Apoptotic fragmentation of DNA was analyzed by PFGE as described previously [Belyaev and Harms-Ringdahl, 2002]. Briefly, after two washes with PBS the cells were mixed with 1% low melting point agarose (Sigma). Agarose blocks were prepared in triplicate using 100 μ l plug molds. The cells were lysed by incubation of agarose blocks in lysis solution (0.25M

Na₂EDTA, 2% w/v sarcosyl, 10 mM Tris-base, pH 7.4) at 37 °C for 48 h. After three washes with Tris-EDTA (TE) buffer (10 mM Tris, 1 mM EDTA, pH 8.0), PFGE was run at 14 °C in 0.5× TBE buffer using a CHEF-DR II apparatus (BioRad) as follows: 1.5% agarose gel, 190 V, run time was 7 h and pulse time was ramping from 1 to 30 s. After electrophoresis, the gels were stained with 0.5 µg/ml ethidium bromide and images were acquired at appropriate saturation using a CCD camera (Gel Doc 1000, Bio-Rad, Hercules, CA). The images were analyzed using Quantiscan (Biosoft) software. Measurements of integrated optical density (IOD) were performed on the images. Calibration curves were obtained for each PFGE using three to five different concentrations of the λ digest pulse marker. The data obtained showed a linear dependence of IOD on the amount of loaded DNA (not shown), which meant that the DNA fragmentation could be quantitatively compared. The percentage of apoptotic DNA fragmentation was calculated assuming that genome of human lymphocyte contains 6 pg DNA.

AVTD Measurements

The conformation of chromatin was studied by the method of anomalous viscosity time dependencies (AVTD). Cell lysis was performed as has been previously described [Belyaev et al., 1999b]. Briefly, lymphocytes were lysed in polyallomer centrifuge tubes (14 mm, Beckman) by addition of 3.1 ml lysis solution (0.25M Na₂EDTA, 2% w/v sarcosyl, 10 mM Tris-base, pH 7.4) to 0.1 ml of cell suspension. The lysates were prepared in triplicate and kept at 23 °C for 5 h in darkness before AVTD measurements. The AVTD were measured in lysates as described using an AVTD-analyzer (Archer-Aquarius, Ltd., Moscow, Russia) [Belyaev et al., 1999b]. The AVTDs were measured at the shear rate of 5.6 s⁻¹ and shear stress of 0.007 N/m². For each treatment condition, the AVTD was measured three times. Two parameters were measured: normalized maximum relative viscosity (NRV), and the relative time (Rt) when the maximum relative viscosity was observed [Belyaev et al., 1999b].

Immunostaining and Foci Analysis

Anti-53BP1 antibodies were a gift by Dr. T. Halazonetis, The Wistar Institute, University of Pennsylvania, Pennsylvania, PA. The antibodies recognize the C-terminal domain of the protein that corresponds to the breast cancer susceptibility gene-1 carboxyl terminus (BRCT) domain. The immunostaining was performed according to Schultz et al. [2000] with some modifications. Immediately after exposure the cells were placed on ice to inhibit eventual repair of DNA damage. Cytospin preparations were fixed in 4% para-

ormaldehyde at room temperature for 10 min, washed once with PBS, permeabilized with 0.2% Triton X-100 for 5 min at room temperature, washed three times for 5 min in PBS, stained with primary antibodies for 1 h, followed by three washes in PBS, incubated with secondary antibodies conjugated with FITC or Texas red, washed three times and mounted with 80% glycerol solution in PBS containing 2.5% 1,4 diazabicyclo-(2.2.2.) octane. Bisbenzimidazole (Hoechst 33258) was added at a concentration of 0.4 µg/ml to the secondary antibody for DNA staining. The images were recorded from three to five randomly selected fields of vision on a DAS microscope Leitz DM RB with a Hamamatsu dual mode cooled CCD camera C4880. One hundred cells were analyzed for each treatment condition.

Statistical Analysis

The data were statistically analyzed using Kolmogorov–Smirnov test. The data that fulfilled the normal distribution were further analyzed with Student's *t*-test. Otherwise, the data were compared by the Mann–Whitney *U*-test or by the Wilcoxon signed ranks test. A correlation analysis was performed using Spearman rank order correlation test. Results were considered as significantly different at $P < .05$.

RESULTS

Apoptosis

Measurements of apoptotic DNA fragmentation and morphological analysis of apoptosis and was performed 24 and 48 h postexposure (Fig. 1). Analysis of data pooled from 50 Hz and from 915 MHz exposures and also from heat shock (41 °C) treatment showed no significant induction of apoptosis in cells from normal or hypersensitive donors. Furthermore, analysis of data pooled from normal and hypersensitive donors showed no statistically significant effects from EMF treatments. Significant apoptotic response was seen after irradiation with 3 Gy (not shown). The data from PFGE and morphology studies were comparable (Fig. 1). The only exception was in cells from donor 3 (Table 1), where PFGE provided significant increase in apoptotic DNA fragmentation after exposure of cells to 50 Hz ($P < .05$, Mann–Whitney *U*-test). This donor was hypersensitive, but not a case of pronounced hypersensitivity. The control levels of apoptosis varied between normal and hypersensitive donors and no difference in the levels of apoptosis between groups has been found (Table 2).

Chromatin Conformation

Effects of exposure were analyzed as data pooled from all subjects, normal and hypersensitive, for each of the treatment conditions. The analysis showed

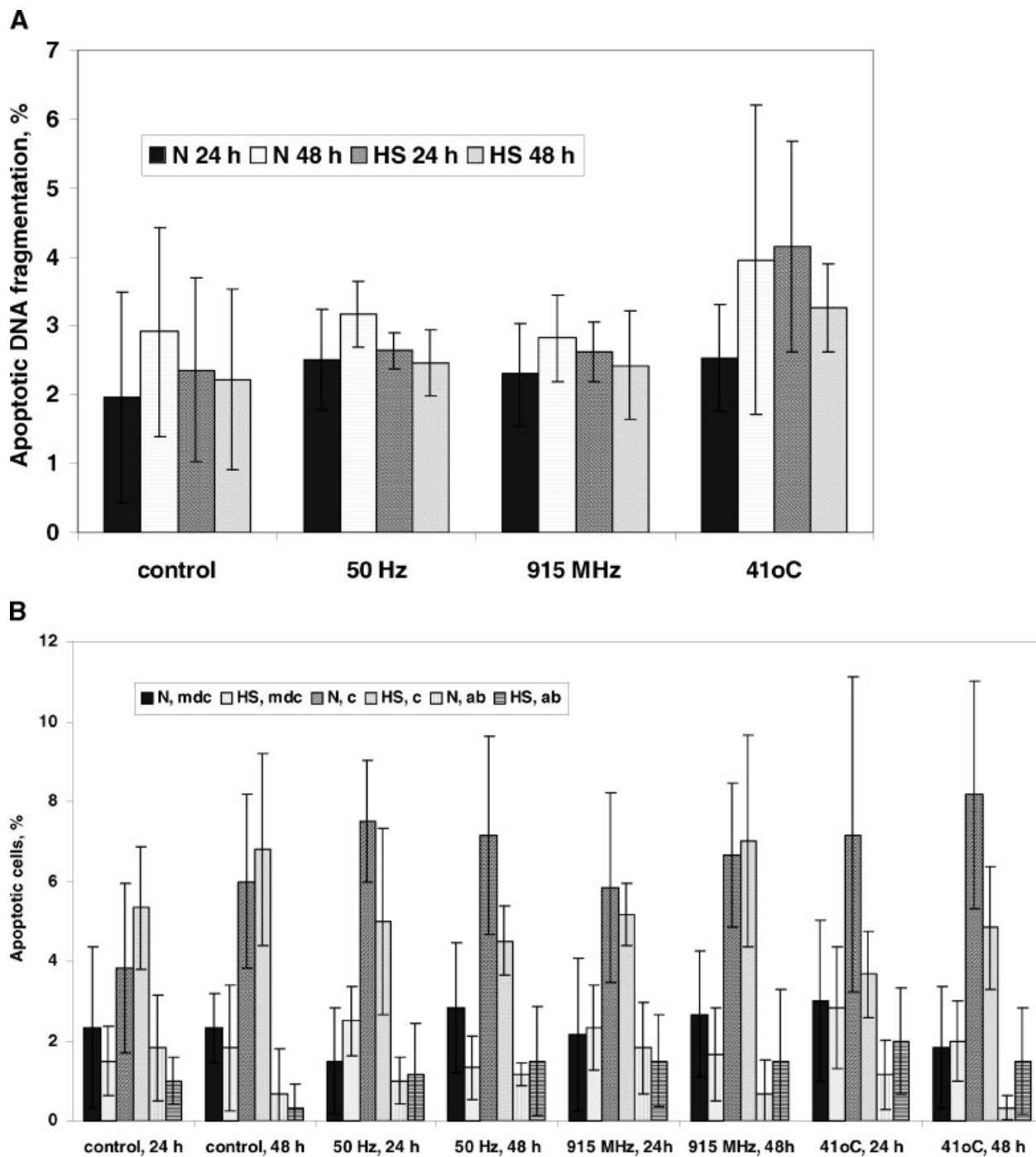


Fig. 1. The histogram shows percentages of apoptotic DNA fragmentation as measured with pulsed-field gel electrophoresis (PFGE) (A) and cells with apoptotic morphological changes (B) after exposure of cells from matched hypersensitive donors (HS) and normal controls (N). Cells were exposed to 915 MHz, 50 Hz, and heat shock (41 °C) for 2 h. Measurements were performed 24 and 48 h following exposures. Percentages for fragmented DNA, membrane-damaged cells (mdc), cells with apoptotic bodies (ab), and cells with condensed chromatin (c) are shown as mean and standard deviations of cell measurements from each of the seven matched-pairs of hypersensitive—normal donors.

TABLE 2. Percentage of Fragmented DNA (Apoptosis) as Measured by PFGE in Unexposed Lymphocytes From Hypersensitive and Control Donors 24 h Postexposure

Matched donors	Hypersensitive	Control	Wilcoxon signed ranks test, <i>P</i>
1*-2	1.8	2.6	.55
3-5	1.4	2.1	
70*-75	5.2	4.4	
87-88	1.6	0.2	
81*-82	1.8	1.9	
91-92	2.0	2.5	
95-97	2.7	0.0	
Mean	2.4	2.0	
SD	1.3	1.5	

An asterisk designates cases of pronounced hypersensitivity.

statistically significant condensation of chromatin in the pooled cells immediately after exposure to 50 Hz and 915 MHz ($P < .015$ and $P < .004$, respectively, Wilcoxon signed ranks test). Based on analysis of these pooled data, 50 Hz induced condensation almost disappeared 2 h postexposure ($P = .059$, Wilcoxon signed ranks test). At this time, the 915 MHz effect was still statistically significant ($P < .02$, Wilcoxon signed ranks test).

Exposure to 50 Hz and 915 MHz resulted in significant changes in chromatin conformation in cells from three and six donors, respectively (Table 3). This

TABLE 3. Changes in Chromatin Conformation in Response to Exposure

Subject	50 Hz		915 MHz	
	NRV	<i>P</i>	NRV	<i>P</i>
1*	0.74 ± 0.06	.02**	0.87 ± 0.04	.03**
2	1.07 ± 0.07	.34	0.98 ± 0.06	.79
3*	0.81 ± 0.09	.1	0.73 ± 0.06	.02**
5	0.81 ± 0.08	.09	0.67 ± 0.09	.03**
70*	0.91 ± 0.04	.09	0.86 ± 0.02	.003**
75	0.99 ± 0.03	.7	0.94 ± 0.05	.32
87*	0.8 ± 0.1	.18	0.81 ± 0.08	.11
88	0.97 ± 0.02	.15	0.87 ± 0.08	.19
81*	0.76 ± 0.09	.05**	0.62 ± 0.04	.001**
82	0.93 ± 0.12	.62	0.98 ± 0.19	.93
91*	0.85 ± 0.09	.21	0.93 ± 0.13	.64
92	0.72 ± 0.08	.03**	0.72 ± 0.09	.03**
95*	1.25 ± 0.43	.53	1.17 ± 0.11	.17
97	0.82 ± 0.10	.17	0.78 ± 0.07	.07

Lymphocytes from hypersensitive subjects and matched control healthy persons were exposed to 50 Hz/915 MHz and NRV was analyzed by the AVTD assay immediately after exposure. Mean and standard error of NRV measured as ratio of three EMF-exposed divided by three measurements of corresponding controls is shown along with *P*-values for effects as analyzed by the Student's *t*-test. An asterisk designates hypersensitive subjects. Double asterisks designate statistically significant effects.

effect was observed both in cells from normal and hypersensitive subjects, two and four cases, respectively (Table 3). The effect of 50 Hz and 915 MHz exposure was similar to stress response induced by heat shock at 41 °C. Two hours after the treatment ended the changes tended to disappear in cells from normal subjects, but not in cells from hypersensitive subjects (Table 3, Fig. 2).

No effects on Rt were observed (not shown). Decrease in NRV was not statistically significant when analyzed across each of the two groups. No statistically significant differences in effects on chromatin conformation were seen between the hypersensitive group and the group of matched controls as measured either immediately or 2 h after exposures/heat shock (Wilcoxon signed ranks test).

The effects reported here on chromatin condensation did not correlate significantly with the age of the persons under investigation, correlation coefficients between the age and the AVTD parameters being in the range from 0.19 to 0.45 (Spearman rank order correlation test).

Interesting to note, that both 50 Hz and 915 MHz effects were stronger in cells of pronounced hypersensitive donors (1, 70, 81) as compared with cells of matched donors (2, 75, 82) (Tables 1 and 3). However, these differences were statistically insignificant when pooled data across ages and gender for these subgroups, three controls versus three pronounced hypersensitive subjects, were compared.

Immunostaining

Our 53BP1 foci analysis included a positive control with γ rays at the dose of 3 Gy. Maximal number of foci was observed 15–30 min after irradiation (Fig. 3). Afterwards, the number of foci decreased, but even 2 h postirradiation, significant increase of foci was observed (Fig. 3). In contrast, neither cells from control subjects nor cells from hypersensitive subjects responded to 50 Hz or 915 MHz by induction of 53BP1 foci (Fig. 4). Rather, we observed a distinct EMF induced reduction in the level of 53BP1 foci both in cells from control and hypersensitive subjects (Fig. 5). Similar reductions in 53BP1 foci were observed in lymphocytes from control (Fig. 5A) and hypersensitive subjects (Fig. 5B). Under identical conditions of treatments, the amounts of foci were not significantly different between cells from matched controls and hypersensitive subjects as analyzed with the Wilcoxon signed ranks test. Therefore, the data from all experiments with cells from control and hypersensitive subjects were pooled. Statistical analysis of these pooled data has shown that both 50 Hz and 915 MHz exposures significantly reduced 53BP1 foci in human lymphocytes ($P < .04$ and $P < .006$,

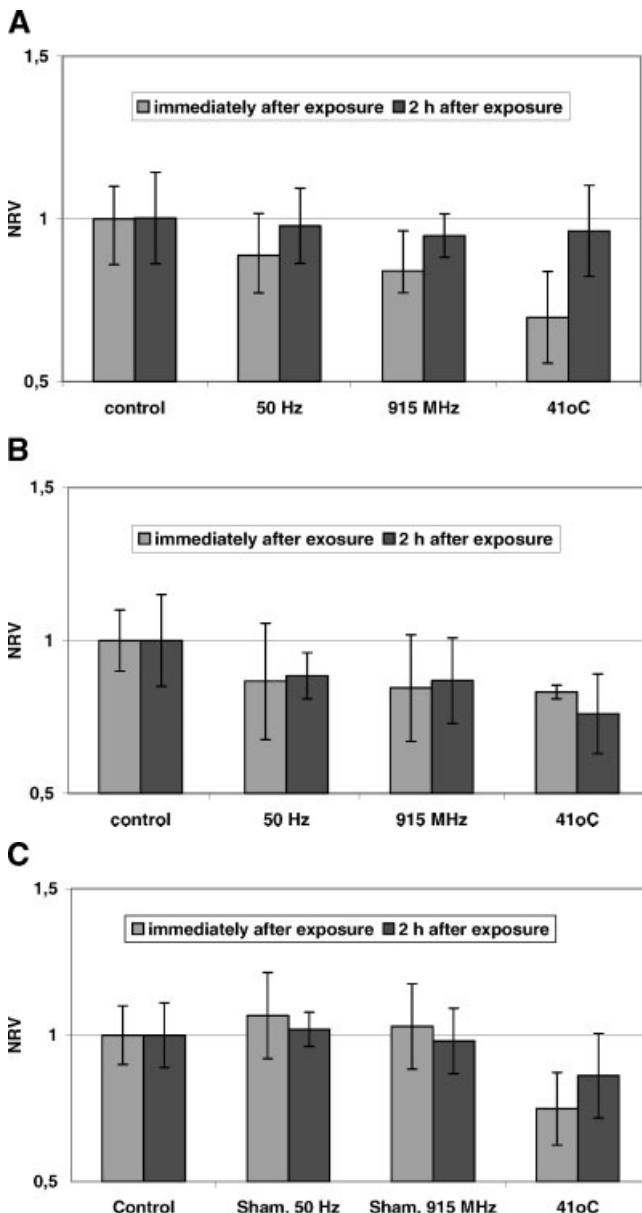


Fig. 2. Normalized maximum relative viscosity in lysates of human lymphocytes from normal controls (A) and hypersensitive subjects (B) as measured with the anomalous viscosity time dependencies (AVTD) method. The cells were exposed 2 h to 915 MHz, 50 Hz, and heat shock (41 °C) as described in the Materials and Methods section. The measurements were performed immediately after exposure (left column) and 2 h later (right column). The mean value based on experiments with cells of seven different donors and standard deviation is shown in each point. In panel C, mean values from four experiments with sham exposures of cells from healthy subjects are shown.

respectively, Mann–Whitney *U*-test). Even stronger reduction was observed after heat shock ($P < .001$, Mann–Whitney *U*-test). No statistically significant age related correlations of effects were found (Spearman rank order correlation test).

DISCUSSION

It was shown in our previous investigations that weak ELF magnetic fields and microwaves affected conformation of chromatin in *E. coli* cells, rat thymocytes, human lymphocytes, and SPD8/V79 Chinese hamster cells under specific conditions of exposure [Belyaev et al., 1994, 1999a, 2000; Belyaev and Alipov, 2001a; Olsson et al., 2001].

Chromatin conformation changes are non specific cellular responses and may be induced by diverse stimuli such as temperature, DNA intercalators, inhibitors of DNA-topoisomerases, electromagnetic fields and ionizing radiation [Belyaev et al., 1999b, 2000, 2001b; Belyaev and Alipov, 2001a]. Usually, in human lymphocytes, the NRV decreased transiently after exposure to weak ELF field in contrast to an increase in the NRV, which was observed immediately after genotoxic impacts such as irradiation [Belyaev et al., 1999b, 2001b]. Several experimental observations have suggested that the increase in the NRV is caused by relaxation of DNA domains. Single cell gel electrophoresis and halo assay confirmed this suggestion [Belyaev et al., 1999b, 2001b]. On the other hand, the decrease in the NRV can depend on chromatin condensation as well as DNA fragmentation [Belyaev et al., 2001b]. Our 53BP1 foci analysis has indicated that no DSBs were produced in response to 915 MHz/50 Hz.

Therefore, the decrease in the NRV in response to these exposures was likely caused by chromatin condensation. However, this condensation was somewhat different from the one induced by ethidium bromide (EtBr) because no decrease in *Rt* was observed here, contrary to EtBr induced condensation as described previously [Belyaev et al., 1999b]. A decrease in NRV was also induced by mild heat shock at 41 °C. These AVTD data correlate with literature data showing condensation of chromatin in mammalian cells in response to mild heat shock [Plehn-Dujowich et al., 2000].

The accepted markers of stress response such as induction of heat shock proteins should be tested to verify molecular mechanisms for the observed effects of 915 MHz/50 Hz. No heating was observed in samples exposed to 915 MHz/50 Hz. Therefore, the effects could not be attributed to heating induced by the exposure systems used. The cells were treated at room temperature, which is more than 10 °C below their normal growth temperature, so it is unlikely that a heat-induced, normal stress-response would occur.

Several proteins involved in DNA repair form distinct nuclear foci after DNA breakage. These include the Rad51 protein [Haaf et al., 1995], the BRCA1 protein [Scully et al., 1997], Mre11-Rad50-Nbs1 complex

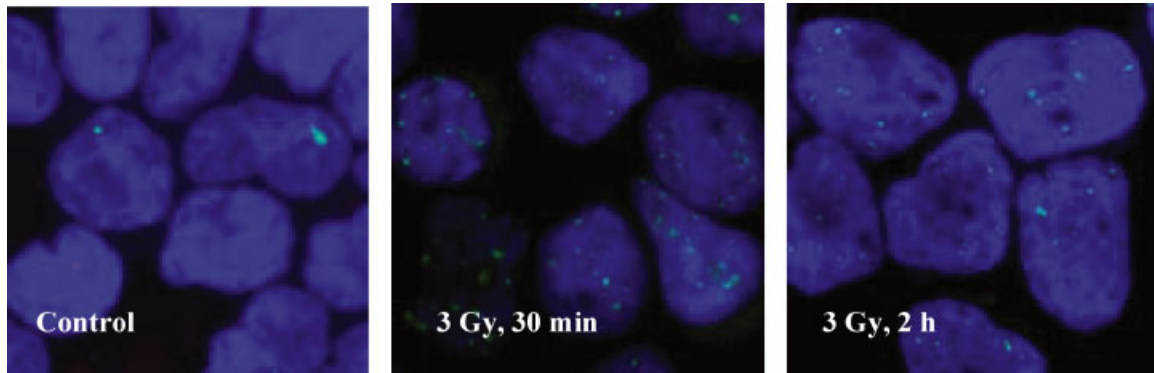


Fig. 3. Panels show 53BP1 foci (stained in green with FITC) 30 min after 3 Gy γ -irradiation of human lymphocytes (counterstained in blue with Hoechst 33258) and 53BP1 foci remaining 2 h after irradiation, as measured by immunostaining with antibody to 53BP1 protein. The images were recorded using a DAS microscope Leitz DMRB at the magnification of 700. [The color figure for this article is available online at www.interscience.wiley.com.]

[Maser et al., 1997; Nelms et al., 1998], CDKN1A [Jakob et al., 2000], γ -H2AX [Rogakou et al., 1999], and 53BP1 [Schultz et al., 2000]. An increasing body of data provides evidence that 53BP1 protein, along with γ -H2AX and mre11-Rad50-nbs1 complex colocalize with DSB in so-called foci in response to genotoxic insults [Maser et al., 1997; Schultz et al., 2000; Sedelnikova et al., 2002]. Analysis of foci formation is a more sensitive assay as compared to other available techniques to measure DSBs such as PFGE or neutral comet assay. Using this sensitive technique we did not find any genotoxic effects of 915 MHz and 50 Hz under the specific conditions of exposure applied here. No apoptotic responses were detected in cells exposed either to 915 MHz or 50 Hz, even though relatively sensitive tools were used [Belyaev et al., 2001b; Olsson et al., 2001; Belyaev and Harms-Ringdahl, 2002]. In particular, statistically significant induction of 50 kb apoptotic DNA fragmentation was consistently detected in lymphocytes following γ -irradiation at low doses

of 5, 10, and 20 cGy [Belyaev and Harms-Ringdahl, 2002]. We assumed that the exposures to 915 MHz and 50 Hz under conditions employed here did not affect significantly apoptosis in human lymphocytes.

Effects of microwaves have been shown to depend on several biological and physical parameters such as frequency, flux density, cell density, and presence of divalent ions and radical scavengers during exposure [Blackman et al., 1989; Adey, 1999; Belyaev et al., 2000]. The ELF effects are also dependent on variety of physical and biological parameters [Smith et al., 1987; Blackman et al., 1988, 1994; Belyaev et al., 1994, 1999a; Prato et al., 1995]. Moreover, exposure of human lymphocytes or V79 SPD8 cells to magnetic field at 8 Hz (21 μ T rms) resulted in DNA fragmentation [Belyaev et al., 2001c; Olsson et al., 2001]. Therefore, the absence of genotoxic effects under the specific parameters of exposure used in this study does not support a conclusion on absence of genotoxic effects of ELF and microwaves in general.

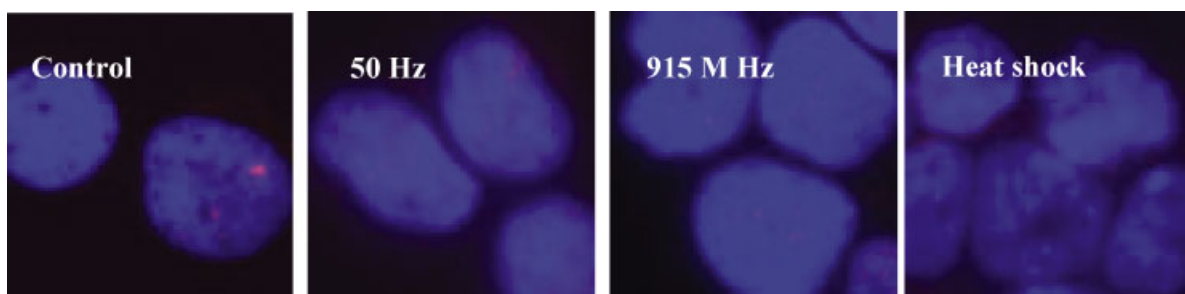


Fig. 4. Panels show typical images of human lymphocytes (counterstained in blue with Hoechst 33258) with 53BP1 foci (stained in red with Texas red) as revealed by immunostaining of 53BP1 protein. Foci were seen in control cells. Fewer foci were observed in cells immediately after 2 h exposure to 50 Hz, 915 MHz, and heat shock, 41 $^{\circ}$ C during 2 h. [The color figure for this article is available online at www.interscience.wiley.com.]

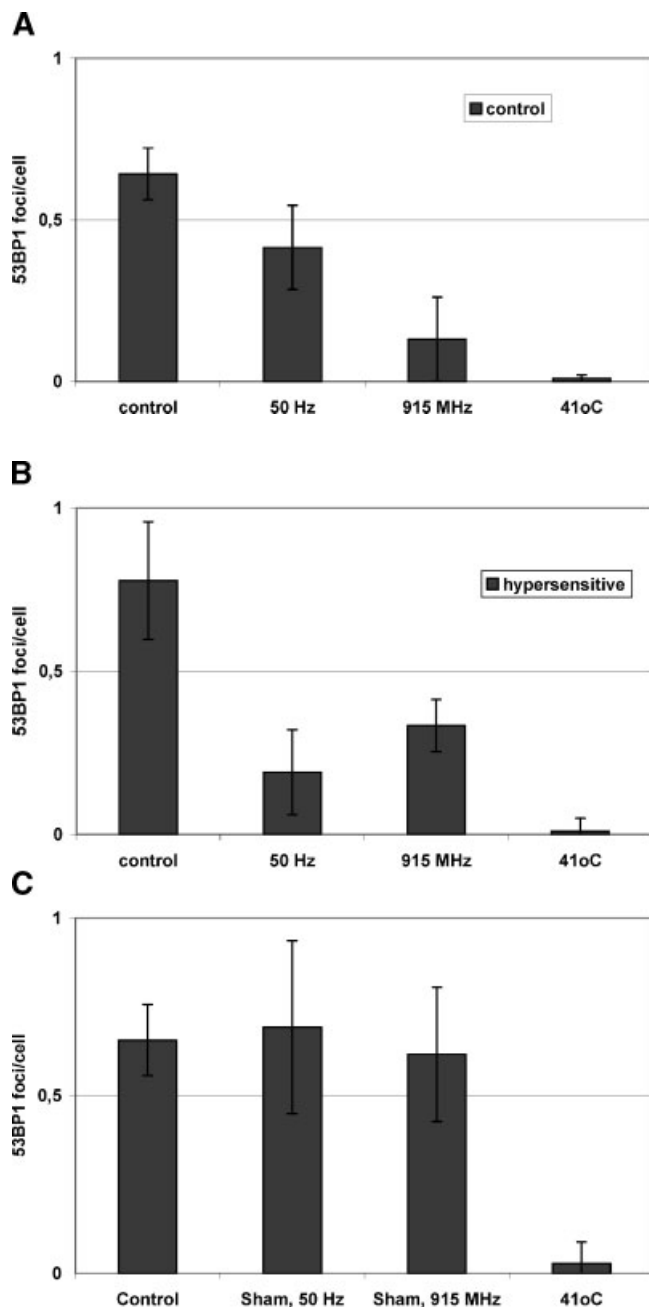


Fig. 5. 53BP1 foci in human lymphocytes of matched controls (A) and hypersensitive (B) subjects before and after exposure to 50 Hz, 915 MHz and heat shock, 41 °C, as measured by immunostaining with antibody to 53BP1 protein following 2 h treatment. Mean values for amounts of foci per cell from three independent experiments and standard errors of mean are shown. No 53BP1 foci were induced by exposures and heat shock. Instead, reduced background level was seen after all treatments. The effects of 915 MHz, 50 Hz and heat shock were statistically significant (Mann–Whitney *U*-test) as analyzed the pooled data. In panel C, mean values from four experiments with sham exposures of cells from healthy subjects are shown.

A novel result of this study is the reduction in the number of 53BP1 foci in response to heat shock, ELF and microwaves. These immunostaining data correlated with the AVTD results providing evidence that 915 MHz and 50 Hz under specific conditions of exposure induced a stress-like response. We hypothesize that chromatin condensation, which was detected by the AVTD technique, reduced the availability of DNA breaks to 53BP1 antibody. In addition, chromatin condensation might also block accessibility of DNA for nucleases and DNA repair enzymes. Our data suggest that 53BP1 foci along with AVTD measurements may provide a new tool to analyze stress response.

Comparison of pooled data obtained with 50 Hz and 915 MHz did not show significant differences in effects between groups of controls and hypersensitive subjects. This result might be explained by the heterogeneity in groups of hypersensitive and control persons. Even if there was such a difference, it would be masked by the large individual variation between donors, which was observed in both control and hypersensitive groups. Whether this individual response has a genetic component remains to be elucidated. An additional problem may be the lack of objective criteria for selection of a study group consisting of persons that are truly hypersensitive to EMF, although this has yet to be proven. Any observation associated with reported hypersensitivity may also be a result of other factors associated with long suffering of ill health regardless of the background. In the hypersensitive group, the subjects who were classified as “pronounced hypersensitive,” showed the stronger response to both 915 MHz and 50 Hz as compared with matched control subjects. However, these data are based only on three cases and may be considered only as a possible trend before new investigations with extended groups will be performed. Another trend was a prolonged change in chromatin conformation in cells of hypersensitive subjects (Fig. 2B). The observed trends for effects of longer duration in lymphocytes from hypersensitive subjects and for generally stronger effects in lymphocytes from pronounced hypersensitive subjects deserve further investigations.

CONCLUSION

Weak 50 Hz magnetic field and nonthermal microwaves from mobile phone induced stress-like responses in human lymphocytes similar to heat shock. These effects included changes in chromatin conformation and reduced level of 53BP1 foci. No significant difference in response was observed between lymphocytes from hypersensitive and healthy subjects.

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Exposure of Nerve Growth Factor-Treated PC12 Rat Pheochromocytoma Cells to a Modulated Radiofrequency Field at 836.55 MHz: Effects on *c-jun* and *c-fos* Expression

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Rat PC12 pheochromocytoma cells have been treated with nerve growth factor and then exposed to athermal levels of a packet-modulated radiofrequency field at 836.55 MHz. This signal was produced by a prototype time-domain multiple-access (TDMA) transmitter that conforms to the North American digital cellular telephone standard. Three slot average power densities were used: 0.09, 0.9, and 9 mW/cm². Exposures were for 20, 40, and 60 min and included an intermittent exposure regimen (20 min on/20 min off), resulting in total incubation times of 20, 60, and 100 min, respectively. Concurrent controls were sham exposed. After extracting total cellular RNA, Northern blot analysis was used to assess the expression of the immediate early genes, *c-fos* and *c-jun*, in all cell populations. No change in *c-fos* transcript levels were detected after 20 min exposure at each field intensity (20 min was the only time period at which *c-fos* message could be detected consistently). Transcript levels for *c-jun* were altered only after 20 min exposure to 9 mW/cm² (average 38% decrease). *Bioelectromagnetics* 18:223–229, 1997. © 1997 Wiley-Liss, Inc.†

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INTRODUCTION

There is presently great interest in the potential effects of exposure to electromagnetic fields on human health and development. Concerns encompass a large portion of the electromagnetic spectrum, ranging from extremely low frequency (ELF), which is associated with electrical power transmission, distribution, and use (50–60 Hz), to the much higher frequencies, which are associated with radiofrequency and microwave radiation. Interest has been piqued and research in the area has been driven by a number of epidemiology studies that have indicated an association between long-term exposure to ELF magnetic fields (MFs) and increased rates of cancer in children and adults [see, e.g., Feychting and Ahlbom, 1993; Savitz, 1993; Floderus et al., 1994]. Still other studies, often conflicting in their results, have sought associations between exposure to radiofrequency radiation (RFR) and microwave radiation (MW) and certain adverse outcomes, such as teratogenic and other reproductive effects and cancer [Brown-Woodman et al., 1988; Bernhardt, 1992; Kun-

jilwar and Behari, 1993]. Interest is rapidly growing in the possible biological effects of RFR associated with portable and mobile communications.

In all of these areas, however, the same questions are asked: Is there a firm association or a causal relationship between exposure to electromagnetic field-generating or electromagnetic radiation-generating devices and adverse effects on human health and development? If so, then by what biophysical and biological mechanisms do ELF MFs or RFR/MW produce their ultimate effects? Although there are numerous reports of ELF MF-induced effects on key cellular activities, such as enzyme activities

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[Litovitz et al., 1991; Dacha et al., 1993; Nossol et al., 1993] and gene expression [for review, see Phillips, 1993], there are far fewer reports dealing with cellular actions of RFR/MW. This would appear to be due in part to the erroneous assumption that RFR/MW-induced effects occur only as a result of heat generation. Indeed, reviews of work in this area often emphasize the relationship of "RFR/MW"-induced effects and effects produced by increased temperature [Saunders et al., 1991; Bernhardt, 1992]. Nonetheless, there are reports in the literature describing athermal or isothermal effects produced in cell systems by RFR/MW exposure [Terada et al., 1994]. These include effects on enzyme systems [Byus et al., 1984, 1988; Krause et al., 1991; Miura et al., 1993], neoplastic transformation of normal cells [Balcer-Kubiczek and Harrison, 1989, 1991], chromosome damage [Garaj-Vrhovac et al., 1990a,b, 1991, 1992; Fucic et al., 1992; Maes et al., 1993; Haider et al., 1994], cell proliferation [Cleary et al., 1990a,b; Czerska et al., 1992], and in vitro fertilization [Cleary et al., 1989].

We have reported previously that exposure of CCRF-CEM T-lymphoblastoid cells to a 100 μ T sinusoidal MF at 60 Hz altered the transcription of the immediate response genes, *c-jun*, *c-fos*, and *c-myc*, and the gene encoding protein kinase C (β -form) [Phillips et al., 1992]. Here, we have employed a rat cell line of neural origin, PC12 pheochromocytoma cells, which have been treated with nerve growth factor (NGF). NGF is required for the survival and development of neuronal populations in both the central and peripheral nervous systems [Levi-Montalcini, 1987], and it influences the phenotypic characteristics of both adult and embryonic neurons in vivo and in vitro. PC12 cells respond to NGF at several levels, including activation of the protooncogene product Trk, tyrosine kinase activity, increased serine/threonine kinase activities, cessation of cell division, acquisition of a sodium-based action potential mechanism, expression of genes encoding neuronal-specific proteins, and neurite outgrowth [Halegoua et al., 1991]. We report the results of experiments to determine whether or not exposure of NGF-treated PC12 cells to an athermal modulated radiofrequency field at 836.55 MHz altered the expression of the genes encoding *c-jun* and *c-fos*.

MATERIALS AND METHODS

Cells

PC12 cells were the generous gift of Dr. Steven Sabol [Sabol and Higuchi, 1990]. This clone of PC12 cells is highly responsive to NGF and was selected for this reason. Cells were maintained in monolayer cultures in DME tissue culture medium (Cellgro) sup-

plemented with 5% fetal calf serum (Gemini Bioproducts), 5% horse serum (Gemini Bioproducts), and 2 mM glutamine/1 mM sodium pyruvate (Cellgro) and were kept in a Forma model 3158 incubator in a humidified atmosphere at 37 °C/5% CO₂. Cells were seeded onto 100 mm Petri dishes (Falcon Primaria) and were approximately 80% confluent at the time of experimentation. Medium depth in the dishes was 3 mm. Just prior to RFR exposure, cell cultures were treated with 50 ng/ml NGF (Promega Corporation).

RFR Exposure

Our exposure apparatus and exposure regimen were designed to meet two criteria: 1) Users of cellular phones may experience fields around 1 mW/cm² at the surface of the head, and power levels in this range have shown consistent effects on biological systems [for review, see Adey, 1990]. 2) Chronic intermittent exposures both simulate on/off epochs of phone usage and mimic the required repeated treatment schedule demonstrated for classic tumor-promoting agents [Diamond et al., 1974; Peterson et al., 1977; Cain et al., 1993]. Consequently, RFR exposures were performed in TEM-mode transmission-line cells (model CC-110s; Instruments for Industry, Ronkokoma, NY) of square cross section at a frequency of 836.55 MHz.

The pulsed carrier was active for 6.67 ms during a 20 ms frame, producing a 33% duty cycle. The carrier "bursts" consist of compressed digital information encoded in $\pi/4$ differential quadrature phase-shift keying. Each bit is represented as a phase rotation of the RF carrier of either ± 45 or $\pm 135^\circ$ from the phase state of the previous bit. The power densities used were 0.09, 0.9, and 9 mW/cm² with the carrier on (slot average) for time-average power densities of 0.03, 0.3, and 3 mW/cm², based on measured (time-averaged) input powers of 17 mW, 0.17 and 1.7 W, and the dimensions of the TEM cell. $\vec{E}$ was normal to the Petri dishes. Two TEM cells, one powered and the other for sham exposure, were placed in the same water-jacketed incubator (model 4300; Napco) maintained at 37 °C/5% CO₂. The transmitter was switched on/off at 20 min intervals for total exposures of 20, 40, and 60 min (20, 60, and 100 min total incubation times).

The exposure system was constructed around a time-domain multiple-access (TDMA) transmitter that was specially configured by Motorola Corp. (Plantation, FL) for external carrier control and automatic power-up initialization. The RF output from the transmitter passed through an in-line Wattmeter (model 4381; Bird) to a 1:4 splitter (model ZFSC-4; Mini Circuits). One splitter port was utilized for monitoring the RF power levels, and the other three output ports were available for connection to three TEM cells (only two

TEM cells were used for this study). Connections to the TEM cells were by RG-58 coaxial cables of equal length, and each TEM cell was terminated in 50 Ω . A programmable timer controlled the exposure timing (model CD2S; Chronrol). The RF power monitor port was connected through a Narda 3020A bidirectional coupler and a Narda 766-20 attenuator to a diode detector. The forward power port of the directional coupler was connected to a power meter (model 431B RF; Hewlett Packard) through a thermistor mount (model 8478B; Hewlett Packard). The analog output of the power meter, in turn, was connected to a chart recorder (model 023; Perkin-Elmer), which ran continuously. The diode detector provided a demodulated signal, which was low-pass filtered and then fed to a locally built loss-of-signal detector. This unit provided a reset to the transmitter in the event of RF output failure. The entire system was powered from the facility's uninterruptible power supply, which would continue to supply power in the event of external grid power failure. No failures occurred during these experiments.

For each experiment, RF power at the TEM cell input was measured by using a Hewlett Packard model 437B power meter and model 8481B power sensor and matched attenuator.

Dosimetry

We elected to use a TEM cell for RFR exposures and to place the dishes on the septum (E normal to the dish) in most experiments and on the septum and chamber bottom in some experiments. This decision was based on early dosimetric assessments for this system performed by Prof. Dr. Niels Kuster and colleagues at the Swiss Federal Institute of Technology, Zurich, Switzerland. Dr. Kuster's initial calculations indicated that this arrangement produced the most uniform specific absorption rate (SAR) distribution within the dish [personal communication]. These dosimetric studies were later expanded by Dr. Kuster and his colleagues and have been accepted for publication [Burkhardt et al., 1996]. Herein, we present only the SAR values resulting from these recent calculations. In addition, the increased variation in SAR distribution within the TEM cell discovered by Dr. Kuster is discussed below and is presented in more detail in Burkhardt et al. [1996].

The specific conditions used in this experiment were as follows: 1) 100 mm Petri dishes with 20 ml of medium, $\epsilon = 77$, and $\sigma = 1.8$ mho/m; 2) in most experiments, two stacks of two dishes each placed centrally on the septum in each TEM cell; and 3) in a few experiments, dishes placed on both the septum and the bottom of the TEM cells in an identical fashion. These conditions were simulated by using the MAFIA electromagnetic simulation tool (The Mafia Collaboration,

User's Guide Mafia Version 3.x; CST GmbH, Darmstadt, Germany). Calculations yielded slot average SAR values for exposure to 0.9 mW/cm² (input power of 0.51 W) as follows: 1) average SAR = 2.6 μ W/g; 2) standard deviation = 1.9 μ W/g; 3) single dish minimum = 0.5 μ W/g (for dishes farthest from the septum); and 4) single dish maximum = 4.6 μ W/g (for dishes closest to the septum).

Importantly, there was no detectable rise in temperature at any power density used in these experiments. Media temperature was measured by using a locally built microprocessor-controlled thermometer. This instrument is based on a Vitek-type probe (BSD Medical Devices, Salt Lake City, UT) and can resolve temperature changes as small as 0.002 $^{\circ}$ C.

Local Static and 60 Hz Magnetic Fields

The local static field in the incubator used in this series of experiments was measured with a MAG-03 three-axis flux-gate magnetometer (Bartington Instruments Ltd., Oxford, United Kingdom). Because of size limitations (i.e., size of the probe vs. size of the TEM cells), static field measurements were not made inside the TEM cells. Rather, measurements were made at nine locations on a square 10 cm grid on a shelf at the approximate center of the incubator. The magnitude of the local static field was 31 ± 12 μ T at an inclination angle of $9 \pm 28^{\circ}$ relative to the horizontal. The ambient 60 Hz magnetic field was measured at each TEM cell location by using a Monitor Industries 42B gaussmeter and was found to be 0.13 ± 0.02 μ T_{rms} at the location of one Crawford cell (used for RFR exposure) and 0.20 ± 0.04 μ T_{rms} at the location of the second Crawford cell (used for the sham exposure).

Northern Blot Analysis

Following exposure/sham exposure, dishes were removed randomly for each exposure condition (e.g., 20 min exposure for *c-fos* analysis or 20 min exposure for *c-jun* analysis); consequently, data points for each exposure condition were derived from dishes located at all possible positions in the TEM cell. RNA was isolated with Ultraspec reagent (Biotecx Labs) according to the manufacturer's directions and then size fractionated on 0.28 M formaldehyde/1% agarose gels. RNA was transferred to MSI Magna nylon membranes by positive pressure (PosiBlot, Stratagene) and then UV cross linked for 5 min at 0.6 mW/cm². Hybridizations were performed in modified Church's buffer (United States Biochemical), which contained 7% sodium dodecyl sulfate (SDS), 1% casein, 1 mM EDTA, and 0.25 M sodium phosphate, at 68 $^{\circ}$ C for 18–24 h by using cDNA probes that had been labeled with α -[³²P]dCTP (DuPont/NEN; specific activity

TABLE 1. Effect of RFR Exposure on the Expression of *c-fos* in NGF-Treated PC12 Cells

	Ratio E/C ^a			
	Sham/Sham	0.09 mW/cm ²	0.9 mW/cm ²	9 mW/cm ²
	1.15	1.25	1.18	1.01
	1.05	1.10	1.10	0.95
	1.03	0.99	1.00	0.89
	0.90	0.95	0.97	0.87
	0.80	0.85	0.90	0.58
Mean $\pm$ SD	0.99 $\pm$ 0.14	1.03 $\pm$ 0.15	1.03 $\pm$ 0.11	0.86 $\pm$ 0.17

^aThe ratio E/C is defined as the ratio of [*c-fos* dpm/GPDH dpm]_{exposed}/[*c-fos* dpm/GPDH dpm]_{control}. These data were derived from Northern blot analyses as described in Materials and Methods. The results from 5 separate experiments are presented for each exposure condition. Exposure time for each experiment was 20 min.

3,000 Ci/mmol) by a random primer method (Prime-A-Gene, Promega Corporation). cDNA probes were obtained from the American Type Culture Collection and were *pfos-1* (*c-fos*), *pJAC.1* (*c-jun*), and *pHcGAP* (glyceraldehyde-3-phosphate dehydrogenase; GPDH). GPDH was used as the internal standard, because we have not found changes in GPDH expression with any EMF exposure [Phillips, unpublished data]. Blots were washed three times for 10 min at 65 °C in a solution containing 20 mM sodium phosphate, pH 7.4, 1 mM EDTA, and 1% SDS.

Data were generated and evaluated with an Ambis 4000 radioanalytic imager and accompanying software. The *c-fos*, *c-jun*, and GPDH cDNA probes recognized mRNAs of 2.2 kb, 2.7 and 3.2 kb, and 1.4 kb, respectively. For each individual experiment, variations in GPDH band intensity were generally ± 15 –20%, indicating even gel loading, transfer, and hybridization. Results were first expressed as the ratio of *c-fos* or *c-jun* dpm/GPDH dpm for each exposed and control sample and then as the ratio of exposed to control values (ratio E/C). All analyses were carried out without knowledge of sample identity.

RESULTS

Table 1 presents the results of studies to determine whether or not exposure of NGF-treated PC12 cells to a modulated RF field alters the expression of *c-fos*. For this work, it was possible to measure levels of *c-fos* mRNA only after the first 20 min exposure period (*c-fos* expression peaks 15–30 min after treatment with NGF and returns to baseline levels by 60 min poststimulation; data not shown). After the second 20 min exposure period, representing 40 min total RFR exposure and 60 min total time in the incubator, *c-fos* mRNA levels had returned to baseline (or were close to baseline) levels and were no longer detectable. Table 1 shows that RFR exposures at slot average intensities of 0.09, 0.9, and 9 mW/cm² did

not alter the levels of *c-fos* mRNA from levels observed in sham/sham experiments.

The dynamics of *c-jun* expression are different from those observed for *c-fos* (see above). In our hands, *c-jun* expression peaked 30–45 min after NGF stimulation and then returned to baseline levels by 120 min poststimulation (data not shown). Table 2 presents the results of studies to determine whether or not exposure of NGF-treated PC12 cells to a modulated RF field alters the expression of *c-jun*. The only significant change in *c-jun* transcript levels was produced after 20 min exposure to 9 mW/cm² (an average 39% decrease).

DISCUSSION

Exposure of NGF-treated PC12 cells to a modulated RF field at slot-average intensities of 0.09, 0.9, and 9 mW/cm² has been found to produce no alterations in the expression of the early response gene *c-fos*. In addition, RFR exposure did not alter the expression of another early response gene, *c-jun*, with the exception of an average 39% decrease in transcript level after 20 min exposure to 9 mW/cm². The lack of effect of RFR exposure on *c-fos* expression is in contrast to other studies from this laboratory [Phillips et al., 1992], in which a 30 min exposure of CCRF-CEM T-lymphoblastoid cells to a 100 μ T sinusoidal MF at 60 Hz produced an average 2.5-fold increase in *c-fos* transcription and *c-fos* transcript level. In recent studies, however, we have found that exposure of NGF-treated PC12 cells to 60 Hz sinusoidal MFs of 12.5, 25, 50, and 100 μ T also produced no change in expression of *c-fos* [J.L. Phillips, unpublished data].

It is possible that the lack of effect of RFR exposure on *c-fos* expression results from using a system that is already maximally stimulated, because, at levels greater than 12.5 ng/ml, NGF produces maximal *c-fos* transcript levels (data not shown). Consequently, it would be of interest to look for field-induced effects in PC12 cells

TABLE 2. Effect of RFR Exposure on the Expression of *c-jun* in NGF-treated PC12 Cells

	Ratio E/C ^a		
	20 min ^b	40 min	60 min
Sham/Sham	1.18	1.12	1.25
	1.10	1.02	1.08
	1.03	0.95	1.02
	0.97	0.90	1.00
	0.90	0.82	0.98
Mean $\pm$ SD	1.04 $\pm$ 0.11	0.96 $\pm$ 0.11	1.07 $\pm$ 0.11
0.09 mW/cm ²	1.07	1.20	1.25
	1.05	1.10	1.05
	1.00	1.05	1.00
	0.90	1.02	1.00
	0.85	1.00	0.97
Mean $\pm$ SD	0.97 $\pm$ 0.10	1.07 $\pm$ 0.08	1.05 $\pm$ 0.11
0.9 mW/cm ²	1.27	1.30	1.18
	1.18	1.23	1.13
	1.15	1.08	0.98
	0.99	0.98	0.95
	0.80	0.60	0.86
Mean $\pm$ SD	1.08 $\pm$ 0.18	1.04 $\pm$ 0.27	1.02 $\pm$ 0.13
9 mW/cm ²	0.78	1.15	1.16
	0.63	1.00	1.14
	0.60	1.00	1.08
	0.55	0.90	0.95
	0.50	0.70	0.95
Mean $\pm$ SD	0.61 $\pm$ 0.11 ^c	0.95 $\pm$ 0.17	1.06 $\pm$ 0.10

^aThe ratio E/C is defined as the ratio of [*c-jun* dpm/GPDH dpm]_{exposed}/[*c-jun* dpm/GPDH dpm]_{control}. These data were derived from Northern blot analyses as described in Materials and Methods. The results from 5 separate experiments are presented for each exposure condition.

^bCells were exposed to RFR or sham exposed for the times given, as described in Materials and Methods.

^c*P* < 0.05 (ANOVA).

treated with suboptimal doses of NGF. Indeed, the ability of electromagnetic signals to act synergistically with suboptimal doses of chemical agents in eliciting biological effects has been reported by others [Stuchly et al., 1992; Cain et al., 1993]. It is interesting, however, that *c-fos* transcription can be increased 2.5- to 3.5-fold over the level observed in cells treated with an optimal dose of NGF if they are treated simultaneously with forskolin (10 μ M) and the phorbol ester, tetradecanoylphorbol acetate (TPA; 10 μ M) [J.L. Phillips, unpublished data]. Hence, we can conclude that RFR exposure, as it was employed in our studies, did not directly activate either adenylate cyclase (as done by forskolin) and/or protein kinase C (as done by TPA).

The decrease in *c-jun* transcript level in cells exposed at 9 mW/cm² for 20 min is similar to the decrease in *c-jun* transcription reported by us for CCRF-CEM cells exposed to a 100 μ T sinusoidal MF at 60 Hz for 30 min [Phillips et al., 1993b]. We have also observed a similar decrease (average 42%) in *c-jun* expression

in NGF-treated PC12 cells exposed to a 100 μ T sinusoidal MF at 60 Hz [J.L. Phillips, unpublished data]. The apparent consistency of exposure to an electromagnetic signal and a decrease in *c-jun* expression is of interest, especially because it might allow determination of a biological mechanism leading to decreased gene transcription. In this light, we have observed the following changes in CCRF-CEM cells exposed to a 100 μ T sinusoidal MF at 60 Hz, all of which are consistent with decreased *c-jun* transcription: decreased IP3 levels; decreased tyrosine kinase activity, increased levels of GTP·ras p21, decreased protein kinase C activity, and decreased DNA-binding activity of the transcription factor AP1 [Phillips et al., 1993a]. It would be of great interest to determine which very early event is sensitive to the imposition of an exogenous electromagnetic signal and could initiate the necessary series of events leading from transduction of that signal to changes in gene transcription.

We chose to study the genes *c-fos* and *c-jun*, because their transcription, which normally is low, may be stimulated by various agents. Indeed, stimulation is a rapid event (see Results), and it leads to the synthesis of c-FOS and c-JUN proteins, which, as parts of the dimeric protein AP1, regulate the subsequent transcription of a variety of genes, including *c-jun* itself [Morgan and Curran, 1991]. Because many of the reported effects attributed to EMF exposure would seem to involve changes in gene transcription, we thought it appropriate to start at the "beginning" and study two important transcriptional regulators. This approach has the drawback that *c-fos* and *c-jun* encode only two of a larger number of transcriptional regulators. It is possible, therefore, that, if EMF exposure does in fact alter gene transcription, then such an effect may involve regulatory proteins other than those that are part of the AP1 family.

Three items deserve further attention. First, in the real world, there is very real concern about intermittency of exposure, and our 20 min on/20 min off duty cycle was intended to provide some degree of intermittency to the exposures employed in our experiments. From our data, we are able to conclude that the imposition of on/off periods in the exposure regimen did not alter the cell's responsiveness to NGF. The patterns of expression for *c-fos* and *c-jun* were the same in control and exposed cell populations (see Results), even though the exposed cells were subjected to up to three on/off events. Even in cell populations exposed to 9 mW/cm², *c-jun* expression returns to control levels after 60 min exposure (three on/off periods, 100 min total incubation time). Therefore, multiple "hits" of RFR exposure did not alter the dynamics of *c-fos* and *c-jun* expression induced by treatment of PC12 cells with NGF.

Second, the SARs in this experiment were chosen to allow study of athermal field effects and are well below values representing actual cellular telephone use (~ 0.6 mW/g) [Q. Balzano, personal communication]. This decision was based on other studies (cited above) that have demonstrated biological effects of athermal RFR and microwave exposures that are relevant to studies of altered immediate early gene expression [see, e.g., Byus et al., 1984; Balcer-Kubiczek and Harrison, 1989, 1991]. An additional concern here is the variability of SAR with location of the tissue culture dish in the TEM cell. Our data, however, were derived from sampling dishes at several locations in the TEM cell. For instance, *c-fos* data were derived from dishes exposed on the septum and on the chamber bottom. The consistency of our data indicates that there is no apparent effect of RFR exposure on gene expression over the range of SAR values obtainable in our exposure setup.

Third, as stated above, the PC12 cells in this study were stimulated with an optimal dose of NGF. Consequently, it is realistic to have expected only three outcomes as a result of RFR exposure: 1) no change in transcript levels, 2) decreased transcript levels, and 3) a prolongation of that time period over which transcript levels remained at maximal levels (i.e., overexpression of the gene through a change in the dynamics of gene expression). The final two possibilities have potential consequences to human health. Increased expression of *c-fos* and *c-jun* are induced by, and serve as a protective response to, UV light and reactive oxygen species [Devary et al., 1991, 1992; Xanthoudakis et al., 1992; Shah et al., 1993]. Any agent capable of inhibiting oxidant-induced increases in *c-fos* and *c-jun* expression may decrease the magnitude of any protective response to these agents. In addition, overexpression of these genes can lead to cellular transformation [Castellazzi et al., 1991; Suzuki et al., 1991; Miao and Curran, 1994].

In this light, it is important to keep in proper perspective studies that have examined EMF-induced effects on gene transcription. All such studies to date that report increases in gene expression observed either small increases that occur over a short period of time (i.e., 20 min) [Broude et al., 1994; Karabakhtsian et al., 1994], or two- to threefold increases that follow normal expression patterns [Phillips et al., 1992]. In all cases, however, these changes in gene expression have not been associated with changes in cell proliferation or differentiation. Therefore, it remains to be demonstrated what significance, if any, the reported EMF-induced changes in gene expression have. Furthermore, it is of consequence that this study found only one significant change in the expression of *c-fos* and *c-jun* in cultured cells exposed to a modulated RF field: a 39% decrease in *c-jun* expression after 20 min exposure

to 9 mW/cm² that returned to control levels after 40 and 60 min exposure. This change in *c-jun* expression, as stated previously (see Discussion), is of interest only in that it may lead to a determination of the mechanism by which RFR exposure can alter gene expression. Such a transient change in *c-jun* expression is probably of no physiologic consequence.

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Increased blood–brain barrier permeability in mammalian brain 7 days after exposure to the radiation from a GSM-900 mobile phone

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Abstract

Microwaves were for the first time produced by humans in 1886 when radio waves were broadcasted and received. Until then microwaves had only existed as a part of the cosmic background radiation since the birth of universe. By the following utilization of microwaves in telegraph communication, radars, television and above all, in the modern mobile phone technology, mankind is today exposed to microwaves at a level up to 10^{20} times the original background radiation since the birth of universe.

Our group has earlier shown that the electromagnetic radiation emitted by mobile phones alters the permeability of the blood–brain barrier (BBB), resulting in albumin extravasation immediately and 14 days after 2 h of exposure.

In the background section of this report, we present a thorough review of the literature on the demonstrated effects (or lack of effects) of microwave exposure upon the BBB.

Furthermore, we have continued our own studies by investigating the effects of GSM mobile phone radiation upon the blood–brain barrier permeability of rats 7 days after one occasion of 2 h of exposure. Forty-eight rats were exposed in TEM-cells for 2 h at non-thermal specific absorption rates (SARs) of 0 mW/kg, 0.12 mW/kg, 1.2 mW/kg, 12 mW/kg and 120 mW/kg. Albumin extravasation over the BBB, neuronal albumin uptake and neuronal damage were assessed.

Albumin extravasation was enhanced in the mobile phone exposed rats as compared to sham controls after this 7-day recovery period (Fisher's exact probability test, $p = 0.04$ and Kruskal–Wallis, $p = 0.012$), at the SAR-value of 12 mW/kg (Mann–Whitney, $p = 0.007$) and with a trend of increased albumin extravasation also at the SAR-values of 0.12 mW/kg and 120 mW/kg. There was a low, but significant correlation between the exposure level (SAR-value) and occurrence of focal albumin extravasation ($r_s = 0.33$; $p = 0.04$).

The present findings are in agreement with our earlier studies where we have seen increased BBB permeability immediately and 14 days after exposure. We here discuss the present findings as well as the previous results of altered BBB permeability from our and other laboratories. © 2009 Elsevier Ireland Ltd. All rights reserved.

Keywords: Albumin; Blood–brain barrier; Mobile phone; Rat

1. Introduction: radiofrequency radiation and the blood–brain barrier

Today about half of the world's population owns the microwave-producing mobile phones. An even larger number is exposed to the radiation emitted from these devices through “passive mobile phoning” [1]. Life-long exposure to the microwaves (MWs) from mobile phones, with start already at a young age, is becoming increasingly common

Abbreviations: BBB, blood–brain barrier; CNS, central nervous system; CW, continuous wave; EMF, electromagnetic field; GSM, global system for mobile communication; ICNIRP, International Commission of Non-ionizing Radiation Protection; MRI, magnetic resonance imaging; RF, radio frequency; SAR, specific absorption rate; TEM-cell, transverse electromagnetic transmission line chamber.

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among the new generations of mobile phone users. The question is: to what extent are living organisms affected by these radio frequency (RF) fields?

The mobile phones are held in close proximity to the head, or within a metre of the head when hands-free sets are used. The emitted microwaves have been shown to have many effects upon the mammalian brain; e.g. alterations of cognitive functions [2,3], changes of neurotransmitter levels such as decrease of cholinergic activity [4], gene expression alterations in cerebellum [5], cortex and hippocampus [6], and impact upon the brain EEG activity [7]. Also, the human brain EEG beta rhythms energies were increased by exposure to 450 MHz MWs modulated at different low frequencies [8]. Recent epidemiological studies also indicate that long-term exposure increases the risk of not only for benign vestibular schwannoma (previously named acoustic neurinoma) [9], but also malignant glioblastoma multiforme [10] for mobile phone use longer than 10 years and with cumulative exposure from mobile phones exceeding 2000 h.

It has been shown that electromagnetic fields (EMFs) increase the permeability of the blood–brain barrier (BBB) (for reference see [11]). The BBB is a hydrophobic barrier, formed by vascular endothelial cells of the capillaries in the brain, with tight junctions between these endothelial cells. It protects the mammalian brain from potentially harmful compounds in the blood. Also, perivascular structures such as astrocytes and pericytes as well as a bi-layered basal membrane help maintaining the BBB.

The current recommendations for limits of exposure to the general public for EMF radiation [12] are set in order to avoid thermal effects upon the brain parenchyma.

In our previous studies we have seen that non-thermal RF fields cause significantly increased leakage of the rats' own albumin through the BBB of exposed rats sacrificed immediately after the exposure, as compared to sham exposed control animals [11,13–18]. Two hours of exposure to the radiation from a global system for mobile communications (GSM) phone at 915 MHz, at non-thermal specific absorption rates (SAR) values of 0.12 mW/kg, 12 mW/kg and 120 mW/kg, gives rise to focal albumin extravasation and albumin uptake into neurons also 14 days after exposure, but not after 28 days [19]. Significant neuronal damage is present 28 days [19] and 50 days after exposure [20], but not after 14 days [19]. Also, in experiments from other laboratories, BBB permeability is increased in connection to mobile phone exposure [21–23] and other kinds of EMF such as magnetic resonance imaging (MRI) exposure [24–26]. In other studies, no such BBB alterations have been demonstrated in connection to mobile phone exposure [27–29] or other kinds of EMF exposure [30,31].

1.1. The blood–brain barrier

An intact BBB is necessary for the protection of the mammalian brain from potentially harmful substances circulating in the blood. In the normal brain, the passage of compounds over the BBB is highly restricted and homeostasis within

the sensitive environment of the brain parenchyma can be maintained.

The BBB is formed by the vascular endothelial cells of the capillaries of the brain and the glial cells wrapped around them. The tight junctions, that seal the endothelial cells together, limit paracellular leakage of molecules. A bi-layered basal membrane supports the abluminal side of the endothelial cells. The glial astrocytes, surrounding the surface of the basal membrane cells, are important for the maintenance, functional regulation and repair of the BBB. The protrusions of the astrocytes, called end feet, cover the basal membrane on the outer endothelial surface and thus form a second barrier to hydrophilic molecules and connect the endothelium to the neurons. Twenty-five per cent of the abluminal membrane of the capillary surface is covered by pericytes [32], which are a type of macrophages. Seemingly, they are in the position to significantly contribute to the central nervous system (CNS) immune mechanisms [33].

The physiological properties of the CNS microvasculature are different from those of peripheral organs. The numbers of pinocytotic vesicles for nutrient transport through the endothelial cytoplasm are low and there are no fenestrations. Also, there is a fivefold higher number of mitochondria in the BBB endothelial cells as compared to muscular endothelial cells [34].

In a functioning BBB, the membrane properties control the bidirectional exchange between the general circulation and the CNS. Water, most lipid-soluble molecules, oxygen and carbon dioxide can diffuse from the blood to the nerve cells. The barrier is slightly permeable to ions such as sodium, potassium and chloride, but large molecules, such as proteins and most water-soluble chemicals only pass poorly. However, when this barrier is damaged, in conditions such as tumours, infarcts or infections, also the normally excluded molecules can pass through, possibly bringing toxic molecules out into the brain tissue. The selective permeability is disrupted temporally in cases of epileptic seizures [35,36] and severe hypertension [37]. The result of this can be cerebral oedema, increased intracranial pressure and irreversible brain damage. Also, toxic substances from the blood circulation now reach out to the neurons. Even transient openings of the BBB can lead to permanent tissue damage [37].

1.2. The earliest studies on the effects of microwave exposure

The first studies on the MW effects upon the BBB were reported in the 1970s, when the radiation from radars and MW ovens were considered to be possible health threats. Increased leakage of fluorescein after 30 min of pulsed and CW exposure [38] and passage of ^{14}C -mannitol, inulin and dextran at very low energy levels [39] were reported. The permeation of mannitol was found to be a definite function of exposure parameters such as power density, pulse width, and the number of pulses per second. Also, the BBB permeability depended on the time between the EMF exposure and the

sacrifice of the animals, with more pronounced effects seen in the animals sacrificed earlier after the EMF exposure. In attempts to replicate the findings of Oscar and Hawkins [39], however, these results were not found [40,41]. Similar lack of MW induced BBB effects, was reported by Ward et al. [42] after exposure of rats to CWs at 2450 MHz; Ward and Ali [43] after exposure at 1.7 GHz; and Gruenau et al. [44] after exposure to pulsed or CW waves at 1.8 GHz (including totally 31 rats). On the other hand, Albert and Kerns [45] observed EMF-induced BBB permeability after exposure at 2450 MHz CWs, with an increase in the number of pinocytotic vesicles among the irradiated animals, but after a recovery time of 1–2 h, the permeation was hardly detectable anymore. For details concerning the EMF exposure parameters in these studies, see [11].

1.3. MRI exposure

MRI entails a concurrent exposure to a high-intensity static field, a RF field and a time-varying magnetic field. In connection to the introduction of the MRI technique, the effects of exposure to these kinds of fields upon the BBB permeability were investigated.

As mentioned above, Shivers et al. [24] observed that the EMF exposure of the type emitted during a MRI procedure resulted in a temporarily increased BBB permeability in the brains of rats. Through transendothelial channels, a vesicle-mediated transport of horseradish peroxidase (HRP) took place. Replications of the initial findings by Shivers et al. [24] were made by Garber et al. [46], whereas Adzamlı et al. [30] and Preston et al. [31] could not repeat the findings.

After some years, quantitative support of the findings by Shivers et al. [24] was presented by the same group [25,26]. In rats exposed to the MRI, the BBB permeability to DTPA (diethylenetriaminepentaacetic acid) increased. A suggested mechanism explaining the increased permeability was a stimulation of endocytosis, made possible through the time-varying magnetic fields.

Also our studies supported the findings of the Shiver–Prato group; seeing that BBB permeability to albumin was increased after exposure to MRI radiation [13]. The most significant effect was observed after exposure to the RF part of the MRI.

1.4. Studies on mobile phone exposure

The mobile phone induced effects upon the BBB permeability is a topic of importance for the whole society today. We have previously found an increased BBB permeability immediately after 2 h of mobile phone exposure [14], and also after 14 days [19] and 50 days [20].

Repetitions of our findings of increased BBB permeability after mobile phone exposure have been made [47,21,22]. Four hours of GSM-900 MHz exposure at brain power densities ranging from 0.3 to 7.5 W/kg resulted in significantly increased albumin extravasation both at the SAR-value of

7.5 W/kg, which is a thermal effect, but also at 0.3 W/kg and 1.3 W/kg [47] (statistical evaluations discussed by Salford et al. [1]). Albumin extravasation was also seen in rats exposed for 2 h to GSM-900 MHz at non-thermal SAR-values of 0.12, 0.5 and 2 W/kg using fluorescein-labelled proteins [21,22]. At SAR of 2 W/kg a marked BBB permeabilization was observed, but also at the lower SAR-value of 0.5 W/kg, permeabilization was present around intracranial blood vessels. However, the extravasation at 0.5 W/kg was seen at a lesser extent as compared to that seen at 2 W/kg. Subgroups of the rats included in these studies were sympathectomised, which means that they were in a chronic inflammation-prone state with increased BBB opening due to changes in the structures of the blood vessels. Interestingly, the sympathectomised rats exposed to GSM radiation had a remarkable increase of the BBB leakage as compared to their sympathectomised sham controls. From these findings it seems likely that an already disrupted BBB is more sensitive to the RF fields than an intact BBB.

In another study, the uptake of rhodamine–ferritin complex through the BBB was investigated [23]. In this study, increased BBB permeability was clearly seen at exposure levels of 2 W/kg and durations of 30–120 min. When the rats were pre-treated with colchicine, the EMF-induced rhodamine–ferritin uptake was however blocked. Colchicine is well-known for its inhibition of microtubular function. It was concluded that the microtubules seemed to play an important role for the BBB opening.

Lack of EMF-induced BBB alterations has also been reported [27–29,48]. In a small study including only 12 EMF exposed animals, no albumin extravasation was seen, neither after 2 nor 4 weeks of 1 h of daily exposure (average whole-body exposure at 0.25 W/kg) [27]. In a study including 40 animals, Kuribayashi et al. [28] concluded no BBB alterations was seen after 90 min of daily EMF exposure for 1–2 weeks at SAR-values of 2 or 6 W/kg. Finnie et al. [29] exposed mice for 1 h daily. However, only the SAR-value of 4 W/kg, which is above the ICNIRP limit [12], was included. In a further study by Finnie et al. [48] 207 mice were exposed for 104 weeks at SAR-values of 0.25–4 W/kg, however without any observable effects upon the BBB permeability. The same group also reported that the immature BBB was insensitive to mobile phone exposure, seen after GSM-900 radiation exposure of pregnant mice from day 1 to day 19 of gestation (SAR of 4 W/kg, exposure for 60 min daily). No increased albumin extravasation was seen in the new-born mice immediately after parturition [49] and the same lack of GSM-900 radiation effects upon the BBB permeability was reported for young rats by Kumlin et al. [50], however, in this case only 12 out of totally 48 exposed rats were analyzed histopathologically. The remaining rats were subject to memory tests, where an improved learning and memory was seen in the EMF exposed rats as compared to the sham controls. Notably, in all these studies, the SAR-values for exposure are relatively high; never including the low SAR-values below 200 mW/kg.

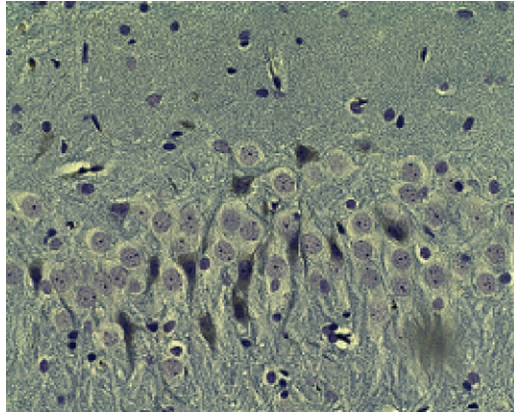


Fig. 1. Albumin neuronal uptake and early neuronopathy in the hippocampal pyramidal cell row among normal neurons. Albumin: cresyl violet, $\times 20$.

In more recent years, *in vitro* models have been increasingly applied to investigate the BBB; in one of these, it was shown that EMFs at 1.8 GHz increase the permeability to sucrose [51]. After modifications of the BBB model to one with higher tightness, however, the same group could not replicate their initial findings [52]. With application of the EMF of the kind emitted from 3G mobile phones, the same group further concluded that their *in vitro* BBB model also did not alter its tightness or transport behaviour in connection to this type of exposure [53].

1.5. Neuronal damage in connection to mobile phone exposure

In our previous studies of animals surviving a longer period after the exposure, we have evaluated the occurrence of neuronal damage extensively [19,20]. This neuronal damage is seen as condensed dark neurons. Dark neurons have been proposed to have three main characteristics [54]: (i) irregular cellular outlines, (ii) increased chromatin density in the nucleus and cytoplasm and (iii) intensely and homogeneously stained nucleus. Twenty-eight days after 2 h of mobile phone exposure, the neuronal damage was significantly increased in the exposed rats as compared to the sham exposed controls [19]. Also 50 days after the same kind of mobile phone exposure, there was an increased occurrence of neuronal damage [20].

In our studies, normal neurons have been shown to have increased uptake of albumin [19] (Fig. 1). Also, in dark neurons this neuronal albumin uptake can be seen (Fig. 2). In our previous studies, damaged neurons were seen in all locations, intermingled with normal neurons especially in the cortex, hippocampus and basal ganglia. The damaged neurons were often shrunken and dark staining, homogenized with loss of discernable internal cell structures (Fig. 3). Some damaged neurons showed microvacuoles in the cytoplasm (Fig. 4). These vacuoles are a sign of severe neuronopathy, indicating an active pathological process. There was no evidence of haemorrhages or glial reaction.

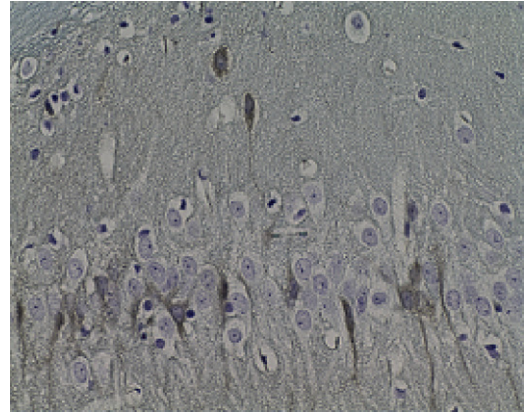


Fig. 2. Shrunken homogenized dark neurons with brownish discoloration due to uptake of albumin, interspersed among normal neurons in the hippocampal pyramidal cell row. Albumin: cresyl violet, $\times 20$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

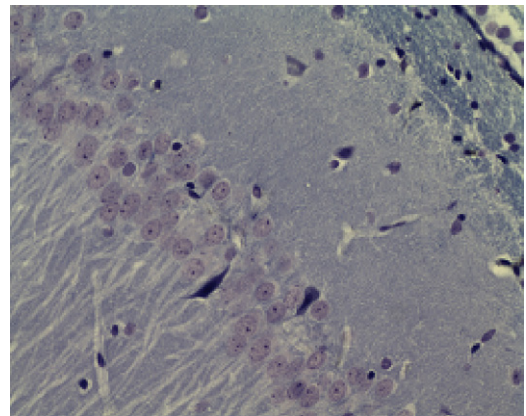


Fig. 3. Two dark neurons in the hippocampal pyramidal cell row. Albumin: cresyl violet, $\times 20$.

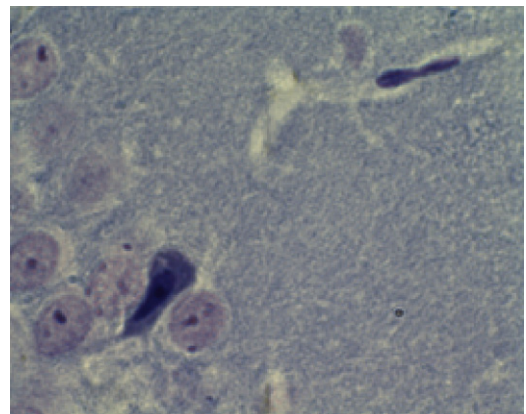


Fig. 4. Dark neuron in the hippocampal pyramidal cell row, with homogenized nucleus and cytoplasm with a vacuole. Higher magnification of part of the figure. Albumin: cresyl violet, $\times 40$.

Dark neurons are reported in clinical and experimental neuropathology from living tissues, but not in autopsy material unless the post-mortem period is short. This could indicate that the formation of dark neurons is an active process that requires living neurons and that these cells must be reasonably intact [55]. This could be in accordance with our findings from the 50-days survival animals, where apoptosis could not be detected in any of the RF EMF exposed brains with application of Caspase-3 [56].

Dark neurons occur not only after GSM exposure [19,20] but also in connection to experimental ischemia [57], hypoglycemia [58] and epilepsy [59]. Possibly, dark neurons could be artefacts, having a pressure-derived mechanical origin, as has been shown for cortical biopsies (this is less likely considering the atraumatic method of dissection used here including fixation before handling and in view of the deep location of the dark neurons). However, dark neurons also appear as a result of other, and not fully clarified, mechanisms, as seen in the case of GSM exposure, ischemia, hypoglycemia and epilepsy. A pharmacologic origin, such as depolarization related to tissue glutamate release in injury, could explain the pathogenetic mechanism for dark neurons in these cases, rather than the pressure-derived mechanical origin. Indeed, the formation of dark neurons can be prevented using pharmacologic forms of glutamate antagonism [55]. In the case of our studies, our technique for the resection of the rat brains is chosen to avoid mechanical pressure.

Findings of dark neurons in connection to mobile phone exposure have been reported by Ihan et al. [60] (GSM exposure of rats for 7 days, 1 h daily). Also, an increase of oxidative damage was seen in the exposed rats as a significant increase in malondialdehyde (MDA) (an index for lipid peroxidation), nitric oxide (NO) levels, brain xanthine oxidase (XO) and adenosine deaminase (ADA) activities, as compared to the controls. With treatment of the anti-oxidant *Ginkgo biloba*, the EMF induced increments of XO, ADA, MDA and NO were prevented. The anti-oxidant activity of *G. biloba* is attributed to its flavinoid glycosides, which are the active compounds in the leaves. The action of these flavinoids is to destroy free radicals, such as NO and lipid peroxide radicals. Also the formation of dark neurons was reported to be prevented when the rats had been treated with *G. biloba*. Other attempts to repeat our findings of dark neurons after mobile phone exposure have been performed in a collaborative effort with Bernard Veyret's group in Bordeaux [61]. In this study, the situation 14 days and 50 days after 2 h of GSM-900 radiation exposure at average brain SAR-values of 0.14 W/kg and 2.0 W/kg was evaluated. No increased amount of dark neurons was reported.

It has been suggested that BBB leakage is the major reason for nerve cell injury, such as dark neurons in stroke-prone spontaneously hypertensive rats [62]. Albumin leaks into the brain and neuronal degeneration is seen in areas with BBB disruption in several circumstances: after intracarotid infusion of hyperosmolar solutions in rats [63]; in the stroke prone hypertensive rat [65]; in acute hypertension by aor-

tic compression in rats [37]. The linkage between albumin extravasation over the BBB and neural damage might be a potentiating effect of albumin upon the glutamate-mediated neurotoxicity [64]. Indeed, both albumin- and glutamate-induced lesions have the same histopathological appearance with invasion of macrophages and absence of neuronal cell bodies and axons in the lesion areas [65]. The glutamate itself can also increase the BBB opening [66], leading to further albumin extravasation out into the brain parenchyma. From our previous findings of albumin extravasation 14 days after exposure [19] and dark neurons not until after 28 days [19] and 50 days [20], it could be hypothesized that albumin extravasation into the brain parenchyma, is the first observable effect of the mobile phone exposure. The albumin leakage precedes and possibly could be the cause of, the damage to the neurons seen as the dark neurons later on. In this connection, the findings of [37] that transient openings of the BBB can result in permanent tissue damage, can also be mentioned. Hypertensive opening of the BBB resulted in albumin extravasation after 2 h, but the effects remained, although to a lesser extent, also after 7 days. Many neurons with cytoplasmatic immunoreactivity for albumin appeared shrunken. Seven days after the BBB opening, there was a neuronal loss in these areas and a vigorous glial reaction, indicating that some neurons were irreversibly damaged [37].

2. Aims of the present study

In the present study we have continued to investigate the effects of EMFs upon the rat brain, now with focus on what happens 7 days after GSM exposure at 915 MHz for 2 h at non-thermal energy levels of 0.12 mW/kg, 1.2 mW/kg, 12 mW/kg and 120 mW/kg. The main questions to be answered were: whether the same increase of the BBB permeability is seen 7 days after exposure as that showed previously immediately after exposure and after 14 days, and whether different exposure levels result in a different response.

In order to compare to our previous findings, we have used the same exposure system, GSM signal, animal model and histopathological methods as in our previous studies.

3. Materials and methods

3.1. GSM exposure

The animals were exposed to RF EMFs in the same transverse electromagnetic transmission line cell (TEM-cells) as previously described and used by [1,2,5,13–19]. The TEM-cells were designed by dimensional scaling from previously constructed cells at the National Bureau of Standards [67]. TEM-cells are known to generate uniform EMFs for standard measurements.

A genuine GSM mobile phone, operating at the 900 MHz frequency band, with programmable power output, was con-

nected via a coaxial cable to the TEM-cells. Through a power splitter, the power was divided into equal parts fed into the four TEM-cells used (TEM-cell A, B, C and D). No voice modulation was applied. Each of the four TEM-cells is connected to a 50 Ω dummy load, into which the output is terminated. By using these TEM-cells, the pulse modulated exposure fields can be accurately generated without the distortion that is typically introduced when conventional antennas are used to establish impulse test fields. Thus, a relatively homogeneous exposure of the animals is allowed [68].

The TEM-cell is enclosed in a wooden box (inner dimensions of 15 cm $\times$ 15 cm $\times$ 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 sidewalls and top of the wooden box make ventilation possible. Air is circulated through the holes of the TEM-cells using four fans, each placed next to the outer walls of its respective TEM-cell. The holes are also used for examination of the interior during exposure. For a further description of the TEM-cell, see [68].

The rats were placed in plastic trays (14 cm $\times$ 14 cm $\times$ 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 could be kept in each TEM-cell simultaneously. All the animals could move and turn around within the TEM-cells.

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

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

with w the sum of weights in kilograms of the 2 rats in the cell and the variance given as SEM. For a more detailed description, see [2].

Whole-body SAR and brain SAR vary with orientation. In our present set-up, the average of 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, as estimated by us previously [19].

3.2. 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.

Forty-eight inbred male and female Fischer 344 rats (the rats were supplied by Scanbur AB, Stockholm, Sweden) were 2–3 months of age at the initiation of the EMF exposure. Male and female rats weighed 225 g $\pm$ 56 g (standard deviation) and 233 g $\pm$ 60 g (standard deviation) respectively.

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*.

The 48 rats were divided into four exposure groups, each group consisting of 8 rats, and one sham exposed group with 16 animals.

The peak power output from the GSM mobile phone fed into the TEM-cells was 1 mW, 10 mW, 100 mW and 1000 mW per cell respectively for a period of 2 h. This resulted in average whole-body SAR of 0.12 mW/kg, 1.2 mW/kg, 12 mW/kg and 120 mW/kg for the four different exposure groups.

All animals were kept in the animal facilities for a recovery period of 7 days after exposure. At the end of this period they were anaesthetized and sacrificed by perfusion-fixation with 4% formaldehyde.

3.3. Histopathology and methods

The brains were fixed *in situ* through saline perfusion through the ascending aorta for 3 min followed by 4% formaldehyde for 10 min and immersion in 4% formaldehyde for 24 h. They were then removed from the skulls by a non-traumatic technique (resection of bone structures at the skull base, followed by a midline incision from the foramen magnum to the nose) and immersion fixed in 4% formaldehyde for more than 24 h. Whole coronal sections of the brains were dehydrated and embedded in paraffin, sectioned at 5 μ m with a microtome and stained for RNA/DNA with cresyl violet to visualize damaged neurons. Albumin was demonstrated with the IgG fraction of rabbit anti-rat albumin (Dakopatts, Helsingborg, Sweden) diluted 1:8,000. This reveals albumin as brownish spotty or more diffuse discolorations. Biotinylated swine anti-rabbit IgG was used as a secondary antibody. Then avidin, peroxidase conjugated, was coupled to the biotin and visualized with DAB (diaminobenzidine).

All brains were examined histopathologically by our neuropathologist (A.B.). All microscopic analyses were performed blind to the test situation.

Regarding albumin extravasation, the number of immunopositive extravasates (foci) were recorded under a microscope. None or occasional minor leakage was rated as normal, whereas one larger or several leakages were regarded as pathological. Immunopositive sites were, however, disregarded when localized in the hypothalamus, above the median eminence and laterally including the lateral hypothalamic nuclei, in the immediate vicinity of the third ventricle and just beneath the pial membrane. These structures are well known for their insufficient BBB. Also the presence and distribution of albumin uptake into neurons was judged semi-quantitatively.

Regarding neuronal damage, this were judged semi-quantitatively as no or occasional (score 0), moderate (score 1) or abundant occurrence (score 2) of dark neurons.

3.4. Statistics

As an initial discriminative test, the occurrence of an effect of exposure (score 1 or higher for albumin foci; score 0.5 or higher for neuronal albumin uptake and dark neurons) was tested against sham exposed controls using Fisher's exact probability test.

The Kruskal–Wallis one-way analysis of variance by ranks was used for a simultaneous statistical test of the score distributions for the five conditions of sham or EMF exposure. When 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 and sham exposed animals.

The occurrence of covariates such as gender, the position of the rat in the TEM-cell (upper/lower compartment) and the TEM-cell used (TEM-cell A, B, C or D) was evaluated using linear regression analysis.

Spearman's non-parametric correlation analysis was used for evaluation of correlation between exposure level, albumin foci, neuronal albumin and dark neurons.

4. Results

In exposed animals there were albumin positive foci around capillaries in the white and grey matter (Fig. 5). The albumin had diffused into the neuropil between the cell bodies, surrounding the neurons, which either contained no albumin or contained albumin in some foci. Scattered neurons were albumin positive. Regarding the dark neurons, cresyl violet staining showed that these were scattered and sometimes grouped within the brain parenchyma.

The occurrence of albumin outside brain vessels was characterized as albumin foci around vessels. After the 7 days recovery time, albumin foci were found significantly more often among exposed rats (25%) than among sham exposed

rats (0%) (Fisher's exact probability test, $p = 0.04$). There was a low, but significant correlation between the exposure level (SAR-value) and the occurrence of albumin foci (Spearman analysis, $r_s = 0.33$; $p = 0.04$). Taking the level of exposure and quantification of neuropathological effects into account it could be concluded from a simultaneous non-parametric comparison of all 5 exposure levels with the Kruskal–Wallis test, that the distribution of albumin foci differed significantly (Kruskal–Wallis, $p = 0.012$).

Pair-wise comparisons between the different exposure levels and sham exposed animals revealed statistically significant differences for SAR of 12 mW/kg (Mann–Whitney, $p = 0.007$), whereas a trend of increased albumin extravasation could be seen for 0.12 mW/kg (Mann–Whitney, $p = 0.1$) and 120 mW/kg (Mann–Whitney, $p = 0.1$).

Also, the occurrence of neuronal albumin was evaluated. A simultaneous analysis for all exposure levels revealed a significant difference between the five groups (Kruskal–Wallis, $p = 0.03$, however Fisher's exact probability, $p = \text{ns}$). A pair-wise comparison revealed that albumin uptake occurred more frequently at 1.2 mW/kg as compared to sham exposed (Mann–Whitney, $p = 0.02$). No difference was found for the occurrence of neuronal damage (Kruskal–Wallis, $p = \text{ns}$; Fisher's exact probability test, $p = \text{ns}$).

Linear regression analysis did not reveal any influence of gender, position of the animals in the TEM-cell (upper/lower compartment) or the TEM-cell used (TEM-cell A, B, C or D) on the frequency of albumin foci, neuronal albumin or occurrence of dark neurons.

5. Discussion

The present study provides evidence that GSM exposure results in disruption of the BBB permeability, with remaining, observable effects 7 days after the exposure occasion. Only non-thermal SAR-levels, below the limits of allowed exposure for humans [12] are considered. This finding of increased albumin extravasation after 7 days (Kruskal–Wallis, $p = 0.012$ with all animals included in the analysis, which is also in agreement with the Fisher's exact probability test, $p = 0.04$) is in line with our earlier findings of albumin leakage both immediately following 2 h of GSM exposure [16] and 14 days [19] after 2 h of GSM exposure. Also, the increased occurrence of neuronal albumin 7 days after the exposure is in line with the findings 14 days after exposure [19].

In our previous study, where the animals have been sacrificed immediately after the EMF exposure, we have seen albumin extravasation only at the most in 50% of the identically exposed animals, although all animals are inbred Fischer 344 rats [16]. Among the rats exposed to the pulse modulated EMFs at 915 MHz, 35% showed albumin extravasation. Also in the sham exposed animals, albumin leakage was present (in 17% of the animals). When the animals have survived 7 days after the EMF exposure, albumin extravasation is seen in a lesser proportion (25% of the exposed

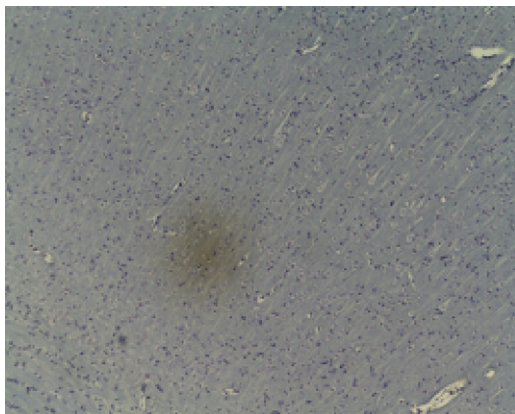


Fig. 5. Focal leakage of albumin shown in brown in the cortex. Albumin: cresyl violet, $\times 10$. GSM-900 EMF exposure at 12 mW/kg. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

animals) and in none of the sham controls. This could be due to a rapid diffusion of extravasated albumin down to, and beyond concentrations possible to demonstrate immunohistochemically. Numerous routes of clearance of extravasated molecules out from the brain tissue are present in the living brain and compounds can also become sequestered intracellularly, become protein bound or metabolized. After 14 days, albumin extravasation is seen in a somewhat larger proportion of the EMF exposed animals (29% of the exposed animals) and none of the sham controls. Thus, a secondary BBB opening might have started at some time point after the initial opening, leading to a vicious circle of albumin leakage.

The mechanism for the passage of albumin over the BBB is not clear. Extravasation might occur through paracellular routes, including alterations of tight junctions between the vascular endothelial cells, or transcellular routes with induction of pinocytosis or transcytosis, formation of transendothelial channels or disruption of the endothelial cell membrane. In connection to EMF exposure, amplified vesicle-mediated transport across the microvessel endothelium occurs, including also transendothelial channels, but no passage through disrupted inter-endothelial tight junctions [24].

One remarkable observation is that exposure at very low whole-body average power densities gives rise to a pronounced albumin leakage. In the present study, significant effects could be seen already at 12 mW/kg, although the different animal groups included a relatively small number of animals. Most certainly, the trends seen for exposure levels of 0.12 mW/kg and 120 mW/kg would have reached statistical significance if more animals had been included in the different exposure groups.

The phenomenon with increased BBB permeability already at very low energy levels might represent a U-curve response. In our other studies, we have seen that the rats in several of the groups with different SAR-levels of EMF exposure have a significant BBB opening [16,19]. The U-response curve occurs also in connection with other kinds of MW exposure, where cerebral vessel permeability after an initial rise decreased with increasing MW power [39].

Further investigation of BBB permeability in connection to EMF exposure is important not only in order to reduce the potentially harmful effects, but also to use possible beneficial effects [69]. The transport of drugs over the BBB might be regulated, so that targets within the brain can be reached. For example, steering of BBB passage of the antiretroviral agent saquinavir has been accomplished in an *in vitro* model of the human BBB, where a frequency of 915 MHz generated the highest BBB permeability [69]. This could be extremely important in order to reduce the HIV replication in the brain of HIV-infected individuals.

6. In conclusion

The time between EMF exposure and sacrifice of the animals is of great importance for the detection of albumin foci.

Seven days after 2 h of GSM mobile phone exposure, there is still an increased permeability of the BBB of exposed rats. This is in concordance with earlier findings of albumin extravasation out into the brain parenchyma immediately and 14 days after 2 h of mobile phone exposure.

7. General conclusion

Taken together, it can be concluded that in a number of studies MW effects upon the BBB permeability have been observed. Increased permeability can be seen both immediately after exposure, but also 7 days after the exposure, as reported in this primary report, and after 14 days. It seems that the effects of the MW radiation might result in persistent changes, such as those seen in our own studies with neuronal damage both 28 and 50 days after 2 h of mobile phone exposure. In a future perspective, with increasing number of active mobile phone users, passive mobile phoning, radiation emitted from base stations and also MWs emitted from other communication sources, effects of low non-thermal levels of exposure must be considered further. The effects seen in the rat studies give some clues about what might possibly happen in the human brain, with a BBB very similar to that of rats. While awaiting latency periods long enough for adequate epidemiological interpretations, further studies on both animals and cells are of utmost importance.

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Exposure to 900 MHz Electromagnetic Field Induces an Unbalance Between Pro-Apoptotic and Pro-Survival Signals in T-Lymphoblastoid Leukemia CCRF-CEM Cells

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It has been recently established that low-frequency electromagnetic field (EMFs) exposure induces biological changes and could be associated with increased incidence of cancer, while the issue remains unresolved as to whether high-frequency EMFs can have hazardous effect on health. Epidemiological studies on association between childhood cancers, particularly leukemia and brain cancer, and exposure to low- and high-frequency EMF suggested an etiological role of EMFs in inducing adverse health effects. To investigate whether exposure to high-frequency EMFs could affect in vitro cell survival, we cultured acute T-lymphoblastoid leukemia cells (CCRF-CEM) in the presence of unmodulated 900 MHz EMF, generated by a transverse electromagnetic (TEM) cell, at various exposure times. We evaluated the effects of high-frequency EMF on cell growth rate and apoptosis induction, by cell viability (MTT) test, FACS analysis and DNA ladder, and we investigated pro-apoptotic and pro-survival signaling pathways possibly involved as a function of exposure time by Western blot analysis. At short exposure times (2–12 h), unmodulated 900 MHz EMF induced DNA breaks and early activation of both p53-dependent and -independent apoptotic pathways while longer continuous exposure (24–48 h) determined silencing of pro-apoptotic signals and activation of genes involved in both intracellular (Bcl-2) and extracellular (Ras and Akt1) pro-survival signaling. Overall our results indicate that exposure to 900 MHz continuous wave, after inducing an early self-defense response triggered by DNA damage, could confer to the survivor CCRF-CEM cells a further advantage to survive and proliferate. *J. Cell. Physiol.* 198: 324–332, 2004. © 2003 Wiley-Liss, Inc.

Epidemiological studies on association between childhood cancers, particularly leukemia and brain cancer, and exposure to low- and high-frequency electromagnetic field (EMF) suggested an etiological role of EMFs in inducing adverse health effects (Wertheimer and Leeper, 1979; Savitz et al., 1988, 1995; Ahlbom et al., 1993, 2000; Theriault et al., 1994; Gurney et al., 1996; Preston-Martin et al., 1996). Although it has been well established that low-frequency EMFs exposure induces biological changes, including effects at both cytoplasmic membrane (Bersani et al., 1997) and nuclear levels (Jin et al., 1997) and an increase in the transcription level of specific genes (see for review, Goodman and Blank, 2002), the issue remains unresolved as to whether high-frequency EMFs can have hazardous effect on health. Few recent data suggested an effect of high-frequency EMF on cell proliferation (Kwee and Raskmark, 1999;

Velizarov et al., 1999) as well as on activation of *c-jun* and *c-fos* oncogenes transcription (Rao and Henderson, 1996; Ivaschuk et al., 1997; Goswami et al., 1999). In vivo studies on blood lymphocytes of workers exposed to

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microwaves radiation reported a considerable micro-nucleus incidence, a widely recognized hallmark of apoptosis, and significant increase in chromosomal aberrations similar to those observed on workers exposed to chemical pollutants (Garaj-Vhrovac et al., 1990; Maes et al., 1993, 1995). Furthermore, microwave radiation caused single- and double-strand DNA breaks in brain cells of rat both in vitro and in vivo (Lai and Singh, 1995, 1996; Adey, 1997) either immediately or 4 h after EMF radiation as well as in T-lymphoblastoid cells (Phillips et al., 1998) suggesting that high-frequency EMFs might act as DNA damage agent at cellular level.

Acute T-lymphoblastic leukemia (T-ALL) is responsible for 80% of childhood acute leukemia with a peak incidence occurring between 3 and 7 years of age. T-ALL also occurs in adults, where it comprises 20% of all adult leukemia. Some toxins such as benzene, some chemotherapeutic agents and radiation are thought to contribute to the induction of leukemia. Moreover, abnormalities in chromosomes may also play a role in the development of acute leukemia and it has been shown to be associated with aggressive nature of childhood T-ALL (O'Connor et al., 1991; Hoelzer et al., 2002). Both epidemiological and experimental data suggest that leukemic cells could be particularly susceptible to high-frequency EMFs exposure. Therefore, we investigated the effects of unmodulated 900 MHz EMF on the survival chance of a T-lymphoblastoid leukemia cell line (CCRF-CEM), as a function of exposure time.

Normal cell growth rate is the result of a balance between pro-apoptotic and pro-survival signals. Any unbalance in this equilibrium predisposes to transformation and, when the cell self-defense mechanisms fail, to cancer. Multiple interactions between cell cycle control proteins, mainly pRb/p105 and its related proteins, and pro-apoptotic p53-dependent and -independent pathways have been shown (Hsieh et al., 1999). *pRb/p105* is a tumor suppressor gene (Cinti and Giordano, 2000) recognized to be a central component of signaling pathways that negatively control cell proliferation. pRb/p105 mediates growth suppression functions by binding E2F transcription factors thus leading to the inhibition of E2Fs transactivation activity. E2F1 is the prototype member of E2F family and its target genes are involved not only in S-phase progression but also in G1-arrest and apoptosis. E2F1 has been shown to induce p53-dependent and -independent apoptosis since it can control accumulation of p53 (Hiebert et al., 1995; Kowalik et al., 1998), transcription of p73 (Irwin et al., 2000; Stiewe and Putzer, 2000; La Sala et al., 2003), and transcription of Apaf-1, a key element of apoptosome (Moroni et al., 2001). The p53 tumor suppressor gene encodes a sequence specific transcription factor, which has anti-proliferative and pro-apoptotic effects and is stimulated in response to a variety of stress signals. p53 protein directly stimulates the expression of p21/WAF1, an inhibitor of cyclin-dependent kinases (CDKs), and bax, the best characterized mediators of p53-induced apoptosis (Carr, 2000). p53 is frequently mutated in a variety of tumors and previous studies have described two heterozygous p53 missense mutations (Cheng and Haas, 1990; Cinti et al., 2000) in CCRF-CEM cells, that do not impair its ability to bind DNA, as well as its transactivating activity (Park et al., 1994; Cinti et al., 2000).

Moreover, cell survival is determined also by extracellular signals whose transduction depends on the activation of intracellular pathways. Ras protein is a GTPase protein bound to plasma membrane and is activated by a variety of external signals such as UV irradiations, osmotic stresses and others (Macaluso et al., 2002). Ras-GTP activates a cascade of serine/threonine protein kinases leading to transcriptional activation of many genes involved in cell cycle progression (Gille and Downward, 1999). Ras has been shown to protect cells from apoptosis through activation of Akt1 via PI3-kinase, which provides a universal survival signal (Kennedy et al., 1997; Downward, 1998; Macaluso et al., 2002). Activated Akt1 (Akt1-P) promotes the decrease of transcription of pro-apoptotic genes and is implicated in the negative regulation of pRb/p105 functions (Khwaja et al., 1997; Du and Montminy, 1998). Activated Ras and/or Akt1 protect cells from apoptosis by preventing cytochrome c release and apoptosome formation, a common event in many forms of apoptosis (Rytomaa et al., 2000).

Here we show that short time exposure to unmodulated 900 MHz EMF induced in CCRF-CEM cells DNA breaks and early activation of both p53-dependent and -independent apoptotic pathways while at longer time of exposure we found an increase in DNA synthesis (S-phase) and the activation of pro-survival Ras pathways. These results strongly suggest that high-frequency EMFs affect cellular systems by inducing both genotoxic damage and changes in gene expression levels.

MATERIALS AND METHODS

Cell exposure system

To guarantee the best field homogeneity throughout the culture medium so that all the cells receive the same dose inside the culture flasks, we designed a transverse electromagnetic (TEM) cell (Fig. 1) able to be fed by frequencies greater than 800 MHz with characteristic impedance of 50 Ω . The unmodulated 900 MHz electromagnetic field was generated by a TEM cell placed inside an incubator NAPCO 9500-IR with infrared temperature control maintained at culture conditions 37°C, 95% humidity, and 5% CO₂. The TEM cell is a specially constructed copper box of 19, 25 × 20 × 50 cm with 3 mm thickness in order to obtain about 50 Ω impedance, which is more suitable for the petri dishes. Its geometry and field propagation has been well described in literature (Stuchly and Stuchly, 1996). The box contains the "strip line," a flat copper septum which divides the inner

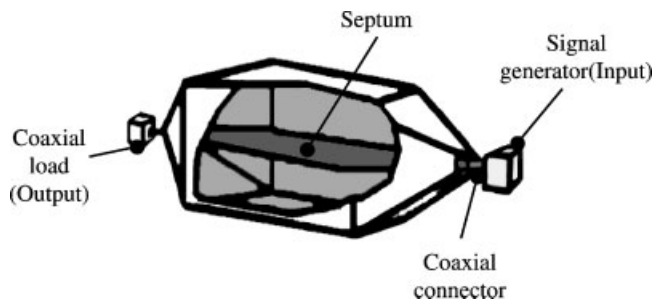


Fig. 1. Sketch of transverse electromagnetic (TEM) cell. TEM cell is a 3 mm copper box of 19, 25 × 20 × 50 cm with a tapered horizontal copper septum which divides the inner space into two parts.

room into two equal parts. The tapering at the ends of septum, allows the mechanical matching between the strip line and the input and output 50 Ω standard N coaxial connectors. The cell is terminated into a 50 Ω load through a simple tuneable impedance matching section. The transformer-impedance adapter inserted between the TEM cell and the final load minimizes steady-state waves.

To allow gaseous exchanges between the incubator and the cell culture, one side of TEM wall was made of a removable 2.5 mm size copper mesh. The cultures were exposed to an EMF produced by a RF system sketched as a block diagram in Figure 2. In detail, we used a HP 8620C sweep oscillator as a signal generator covering the range 0.01–2.4 GHz via the HP86222B plug-in, a HP 796D directional coupler to feed a PM 1038 scalar meter equipped with CRT display necessary to optimise and continuously check the input return loss (RL) of the TEM cell. Ancillary equipment was a universal counter HP5316A and a power meter HP431A. Properly adjusted, the system measured 27 dB of RL at the 900 MHz frequency with and without the petri dish inside the TEM cell. This was the necessary condition favoring the best uniformity of the electric field with no differences higher than 2dB.

Microwave exposure

Nine hundred megacycles per second field was applied as continuous waves (CW) with 1 mW power input generating an electric field, inside the TEM cell and perpendicular to the septum plane, of 2.96 V/m and a magnetic field of 8 mA/m at a power density of 23.68 mW/m² resulting in a specific absorption rate (SAR) value of 3.5 mW/kg in the cell cultures. Fifty hertz electric and magnetic field generated by the incubator did not exceed 0.8 V/m and 0.16 μ T, respectively all along the exposure times. The exposure times were 2, 4, 12, 24, 48 h.

Cell culture position

Five petri dishes of 10 cm diameter containing the cells cultured in 20 ml of medium were placed in the TEM cell at 1 cm distance above and below the septum.

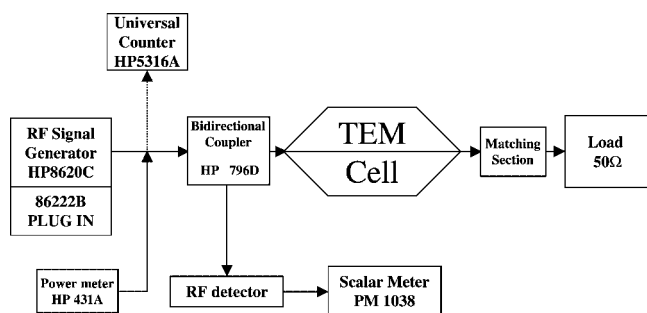


Fig. 2. Experimental cell-exposure arrangement. The electromagnetic signal is generated by signal generator, HP 8620C sweep oscillator covering the range 0.01–2.4 GHz via the HP86222B plug-in, which produce a field inside the TEM cell through the HP 796D directional coupler. The signal was output to the 50 Ω final load by a transformer-impedance adapter. In order to optimize and continuously check the input return loss (RL) inside the TEM cell a RF detector and PM 1038 scalar meter were connected. Universal counter HP5316A and a power meter HP431A were used as ancillary equipments.

The cultures did not exceed more than one third of the volume between the septum and the outer wall.

Administered field check

Spectrum analyzer equipped with a magnetic loop EMCO (1 cm diameter) or with electrospherical probe (3 cm diameter) were used in order to measure the magnetic and electric field generated by the system within the TEM cell. Detected values matched the theoretically expected values within the systematic instrumental range of errors.

Temperature control

In order to detect whether the administered power determined any thermal increment in the culture medium, a continuous temperature monitoring was performed in the medium and inside the petri dishes along the exposure time. To monitor the temperature, isolated thermo-couples and conventional alcohol thermometer were used. Thermo-probes were positioned inside the petri dishes placed in the upper plate of the TEM cell, inside and outside the TEM cell. During our performed experiments we detected no more than 0.15°C of temperature difference so that the observed EMF effects were independent from thermal phenomena.

Sham exposure

In order to check the effect of the complete equipment on the culture cells, CCRF-CEM cells were cultured for 2, 24, and 48 h inside and outside the TEM cell without electromagnetic field emission (generator switched off). At the end of the sham exposure, the cell viability test (MTT) and FACS analysis were carried out on cells inside TEM cell and compared to those outside the TEM (control).

Cell culture

Human CCRF-CEM obtained from the American Type Culture Collection (ATCC, Manassas, VA), were cultured in total volume of 20 ml RPMI-1640 medium supplemented with 10% FCS in petri dishes of 10 cm diameter at a density of 1×10^5 cells/ml and exposed to unmodulated electromagnetic fields of 900 MHz for 2, 4, 12, 24, and 48 h. Briefly, for each experiment, exponentially growing cells from a 175 cm² flask were scattered in the petri dishes and five petri dishes were located inside the TEM cell and the same quantity of cells was used as a control in the same incubator outside the TEM cell. The exposed and unexposed cells were consecutively picked up finely up to 48 h. As a further control system, the cells not exposed to fields were incubated in a different incubator from that containing the TEM cell. To have well-controlled biological experimental system, we performed the experiments at following condition: the diameter of the petri dishes were half of the strip line size, and the dishes were placed in the volume formed by the strip line and one half size of both upper and bottom distance of the septum from walls. The amount of medium was always of 20 ml/dish. The cells, which initially float in the medium, line down on the bottom of the petri disk in a few minutes. For this reason the attenuation of field at the bottom of the petri dish used can be ignored since it has been calculated to be less than

2% (Burkhardt et al., 1996). The medium was always pre-heated at 37°C in order to avoid the temperature raise time and the stress to the cells. All the experiments were performed comparing 900 MHz EMF-exposed with unexposed cells and, to avoid the variability inherent to the used assays, all tests were performed for fifteen independent experimental exposures. Any measure was performed soon after the end of the various exposure periods.

Cell viability test (MTT)

The MTT was performed following manufacturer's instructions (Cell Proliferation Kit I-MTT, Roche, Mannheim, Germany). Hundred microliter/well of exposed and non-exposed cell suspension at 2, 4, 12, 24, and 48 h were aliquoted in a microtiter plate (tissue culture grade, 96-wells, flat bottom). Cells were incubated with MTT (3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyl-tetrazolium bromide, final concentration 0.5 mg/ml) for 4 h at 37°C. After this incubation period, purple formazan salts crystals were formed by a NADP/NADPH dependent process, from metabolically active cells. These salt crystals are insoluble in aqueous solution, but may be solubilized by adding the solubilization solution (0.01 M HCl, 10% SDS) and incubating the plates overnight in humidified atmosphere (37°C, 5% CO₂). The solubilized formazan product was spectrophotometrically (550–690 nm) quantified using an ELISA plate reader. The number of viable cells is directly correlated to the amount of purple formazan crystals formed.

Statistical analysis

Each control and exposed sample at 2, 4, 12, 24, and 48 h were aliquoted in twelve wells of a microtiter plate. The relative absorbance (550–690 nm) of each well was spectrophotometrically quantified and the mean values and standard deviation (SD) were calculated.

For each experimental setting, the cell viability index was calculated by making a ratio between the mean absorbance (Abs) values of the examined sample and a reference value (Abs value of cell suspension at 0 h). Statistical significance of the differences between controls and exposed cells was evaluated by Student's *t*-test.

Flow cytometry (FACS) analysis

In order to determine the percentage of cell population in different cell cycle phases, the cells, cultured for 2, 24, and 48 h, with or without electromagnetic field, were fixed in 70% ethyl alcohol at 4°C for 30 min. The nuclei were stained with 25 µg/ml of propidium iodide and incubated with 1 mg/ml of RNases for 1 h at 37°C. The nuclear DNA content which discriminate the cell cycle phases was determined using flow cytometry using Becton–Dickinson FACScan.

DNA ladder

A large quantities of CCRF-CEM cells (10⁷), for each samples, was pelleted, washed in phosphate-buffer saline (PBS) and gently suspended in 500 µl of lysis buffer (1× PBS, 1% Nonidet P40, 0.5 ng/ml Proteinase K). During lysis, samples were kept on ice for 1 h. After incubation, the lysates were centrifuged at 14,000 rpm for 15 min. The supernatants were treated with RNase A (100 µg/ml) at 37°C for 30 min. Four microliters of 6× gel loading solution (Sigma Chemical, St. Louis, MO)

were added to 20 µl of each mixture and applied on 2% agarose gel. Gel electrophoresis (3 V/cm) was proceeded in 1× TBE buffer, then the gel was stained with ethidium bromide (1 µg/ml) water bath.

Western blot analysis

Whole cell lysates were prepared by re-suspending pelleted cells in lysis buffer (50 mM HEPES pH 7.5, 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1 mM EGTA, 1.5 mM MgCl₂, 100 mM NaF, 10 mM disodium pyrophosphate, 10 g/ml aprotinin, 10 g/ml leupeptin). Forty grams of proteins were denatured by boiling in 2× sample buffer (100 mM Tris HCl pH 6.8, 200 mM dithiothreitol, 2% SDS, 0.1% bromophenol blue, 10% glycerol) and size fractionated by electrophoresis in SDS/polyacrilamide gel and transferred onto a nitrocellulose membrane (BioRad, Hercules, CA). After saturation with 3% fat-free-milk and 2% BSA solution, the membranes were incubated with the following monoclonal antibodies: anti-human-bax (2D2) (Kamiya Biomedical Company, Seattle, WA), anti-Bcl2 (100/D5) (Kamiya Biomedical Company), anti-p53 (Ab6) (Calbiochem, Darmstadt, Germany), anti-p21 (4D10) (Medac Diagnostika, Wedel, Germany), anti-E2F1 (KH95) (Santa Cruz Biotechnology, Santa Cruz, CA), anti-H-Ras (F235) (Santa Cruz Biotechnology). For pRb/p105, p73 Akt1 and phospho-Akt expression analysis the membranes were incubated polyclonal antibodies: anti-pRb antibody (C15), anti-p73 (H-79), anti-Akt1 (D-17) (Santa Cruz Biotechnology) and anti-phospho-Akt (Ser473) (New England BioLabs, Beverly, MA). To normalize Western blot analysis the anti-actin antibody (Sigma) was used. After three washings with PBS-Tween-20, the membranes were incubated with secondary anti-mouse, anti-goat, or anti-rabbit IgGs, coupled with horseradish peroxidase (Amersham, Life Science, Buckinghamshire, UK). Signal was detected using the ECL system (Amersham, Life Science, UK).

RESULTS

Effects of EMF on cell viability, cell growth rate, and apoptosis

To investigate the possible effect of electromagnetic fields on cellular viability, we evaluated the amount of metabolically active exposed and unexposed cells with MTT test. This test is especially useful for quantifying viable cells, because the incorporation of formazan dye by metabolic active cells induce their cleavage. The results of this analysis showed a statistically significant decrease ($P < 0.01$) in the total viable CCRF-CEM cells number after 24 and 48 h of exposure to 900 MHz EMF with respect to the control cells. No significant difference in cellular viability was observed between exposed and unexposed cells for shorter exposure times (2, 4, and 12 h) (Fig. 3A). Data from sham exposure, obtained by comparing cells cultured outside the TEM cell equipment or inside but with the generator turned off do not cause any alteration of viable cell number (Fig. 3B).

To assess whether the difference between control and exposed cells in cellular viability and activity depends on deregulation of cell cycle phases and/or induction of an apoptotic response, we performed FACS analysis. As it is shown in Table 1, exposure to EMFs induced a statistically significant increase of the percentage of cells

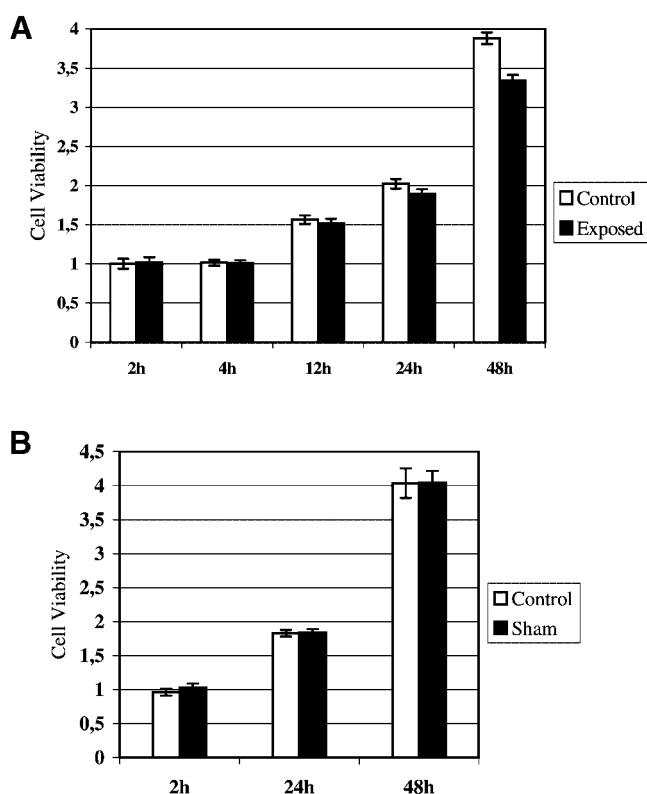


Fig. 3. **A:** Proliferation index of 900 MHz EMF exposed T-lymphoblastoid leukaemia (CCRF-CEM) cells (exp) and unexposed cells (ctrl). The proliferation index of the cells was calculated by making a ratio between the absorbance (Abs) value of the examined samples and a reference value (Abs value of cell suspension at 0 h). **B:** Proliferation index of CCRF-CEM cells cultured for 2, 24, and 48 h inside (sham) and outside the TEM cell (ctrl) without electromagnetic field emission (generator switched off). Statistical significance of the differences was evaluated by Student's *t*-test.

undergoing apoptosis after 2 h (exposed cells 18.07% vs. 3.89% control cells; $P < 0.01$). This pro-apoptotic effect of EMFs gradually decreased after 24 and 48 h of exposure notwithstanding the fact that differences between exposed and unexposed cells were still statistically significant (24 h: exposed 7.98% vs. 4.03% control, $P < 0.01$, 48 h: exposed 3.38% vs. 1.37% control; $P < 0.05$). On the other hand, statistically significant differences in the distribution of cell cycle phases were detected only after 48 h. The percentage of cells, which started DNA synthesis (S-phase) increased (39.63% in the exposed cells vs. 22.6% in the control, $P < 0.01$) while the cells

which underwent growth arrest (G_0/G_1 phase) decreased (26.68% in the exposed cells vs. 40.06% in the controls, $P < 0.01$).

To confirm the flow cytometry data, which indicated a significant apoptosis induction, we performed a DNA ladder assay. Genomic DNAs extracted from exposed and control cells after 2, 24, and 48 h of culture showed a typical oligonucleosomal DNA ladder after 2 and 24 h of electromagnetic field CCRF-CEM cells exposure. No DNA ladder was detected after 48 h of EMF exposure (Fig. 4). This first series of experiments indicate that exposure to 900 MHz EMF determines a DNA damage inducing early programmed cell death in a fraction of CCRF-CEM cells. However, after longer exposure times, the surviving cells show greater viability due to higher DNA synthesis rate.

EMF biological effects on genes expression level

To investigate the activation of which genes could explain the observed effects, we evaluated the expression level of the most representative pro-apoptotic and cell cycle regulator genes in CCRF-CEM cell line at the end of various 900 MHz EMF exposure times. Data are shown in Figure 5 where we report the results of Western blot analysis for both control and exposed cells. p53 expression level was higher in exposed cells compared to the control at 2, 4, and 12 h and gradually decreased to reach basal expression level at 24 and 48 h. p21/WAF1 showed a similar trend but the increase of its level was shifted by 2 h with respect to p53 and maintained at steady level for the next 12 and 24 h to decrease at 48 h. As regard to the expression level of pro-apoptotic gene *bax*, we found a strong over-expression at 2 and 4 h followed by its gradual down-regulation. On the other hand, the Bcl-2 pro-survival oncoprotein, which antagonizes *bax* function, increased its expression starting from 4 h and kept steady high levels for 48 h.

The expression level of pRb/p105, which negatively controls cell proliferation, progressively increased starting from 2 h, with highest expression at 48 h, in electromagnetic field exposed cells with respect to control cells. E2F1 level increased after 2 h exposure to 900 MHz EMF and was maintained higher until 12 h while a down-regulation of E2F1 expression started at 24 h. On the other hand, in control cells E2F1 expression level was always lower with respect to exposed cells. As regard the p73 expression level, a pattern similar to that detected for p53 has been observed. In fact its level was higher in exposed cells compared to the control at 2, 4, and 12 h and gradually decreased at 24 and 48 h.

TABLE 1. FACS analysis of T-lymphoblastoid leukemia (CCRF-CEM) cells unexposed (control) and exposed to 900 MHz electromagnetic field at 2, 24, and 48 h*

Time (h)		G_0/G_1	S	G_2/M	Apoptosis
2	Control	45.75	35.55	13.42	3.89
	900 MHz EMF exposure	38.5	32.38	9.13	<u>18.07</u> ($P < 0.01$)
24	Control	56.31	21.95	13.79	4.03
	900 MHz EMF exposure	54.82	22.15	11.09	<u>7.98</u> ($P < 0.05$)
48	Control	40.05	22.60	33.97	1.37
	900 MHz EMF exposure	<u>26.68</u> ($P < 0.01$)	<u>39.63</u> ($P < 0.01$)	27.62	<u>3.38</u> ($P < 0.05$)

*The values represent the percentage of cells in the different cell cycle phases. The underlined values are statistically significant (Student's *t*-test).

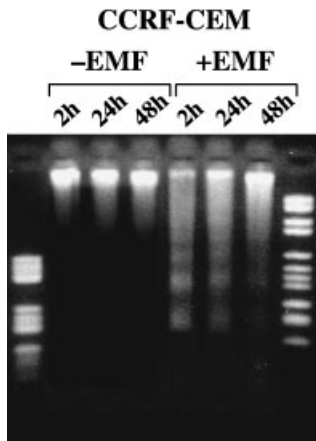


Fig. 4. DNA ladder of unexposed (–EMF) and exposed (+EMF) CCRF-CEM cells after 2, 24, and 48 h of culture.

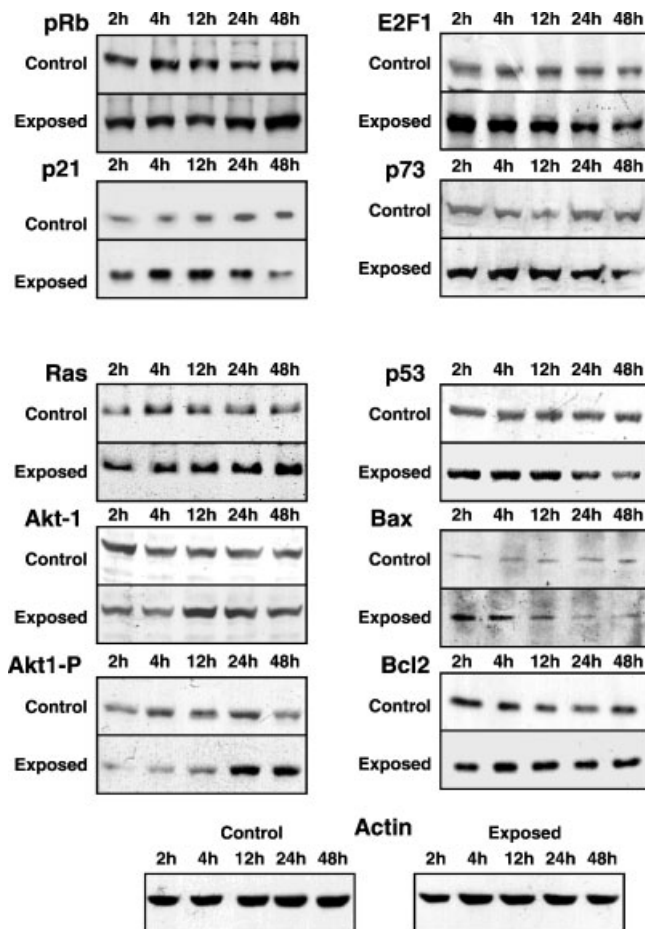


Fig. 5. Western blot analysis of genes involved in cell cycle control (pRb/p105, p21/Waf1), p53-dependent (p53, bax, bcl2), and p53-independent (E2F1, p73) apoptosis and in pro-survival signal (Ras, Akt1, Akt1-P). Forty micrograms of whole cell lysate at the various times after 900 MHz EMF exposure (exposed) and of unexposed cells (control) were electrophoretic fractionated. Western blots were normalized by using α -actin antibody.

These data indicate that the E2F1-p73 dependent pro-apoptotic pathway is also triggered at first and thereafter silenced.

To evaluate the effect of 900 MHz EMF on the expression of proteins involved in extra-cellular pro-survival signaling, we investigated the expression of the two proto-oncogenes, Ras and Akt1. Our data evidenced a quick (2 h) and progressive increment of Ras protein until 48 h in the exposed with respect to the control cells, which showed a lower expression level for all the time. On the other hand, the expression level of the Akt1 inactive form increased 12 h after EMF exposure and was kept high until 48 h while an increase of the phosphorylated-Akt1 active form (Akt1-P) was evident at 24 h and maintained high until 48 h only in exposed cells. These results show that at longer exposure times, when both p53-dependent and -independent pathways are no more effective, pro-survival signals are maximally operative.

DISCUSSION

High-frequency EMFs are a very important part of the electromagnetic spectrum and the mean level of environment emission has progressively increased in developed countries. A positive correlation between high-frequency EMFs exposure and tumorigenesis is suggested by epidemiological studies carried out in highly exposed subjects (Milham, 1985; Szmigielski et al., 1988; Goldsmith, 1995; Szmigielski, 1996; Michelozzi et al., 2002). These results are supported by a few *in vitro* and *in vivo* data showing that high-frequency EMFs can induce DNA breaks in cells (Sarkar et al., 1994; Lai and Singh, 1995, 1996; Adey, 1997; Malyapa et al., 1998; Phillips et al., 1998), chromosome aberration (Garaj-Vhrovac et al., 1990; Maes et al., 1993, 1995), changes in cell proliferation (Kwee and Raskmark, 1999; Velizarov et al., 1999) as well as activation of oncogenes transcription (Ivaschuk et al., 1997; Goswami et al., 1999).

Despite the fact that these *in vivo* and *in vitro* experiments suggest a possible damaging effect of electromagnetic field, other *in vivo* and *in vitro* studies suggest opposite points of view.

One of the controversial aspects is whether the biological changes can be induced by high-frequency EMFs through thermal or a-thermal phenomena. Some studies suggested the possibility that EMFs could affect biological systems by transferring thermal energy to particles of biological material, which possess own average thermal kinetic energy, depositing enough energy to alter some structure significantly (Moulder et al., 1999). On the other hand, other studies have established, both *in vitro* and *in vivo*, that these biochemical and molecular effects on cells may be independent from thermal phenomena produced by field exposure (Litovitz et al., 1990; Adey, 1993; Astumian et al., 1995; Barnes, 1996). The fact that EMF can produce thermal phenomena does depend on the grade of power density field administered. For this reason, in order to avoid thermal effect on our experimental setting we exposed the cells to unmodulated 900 MHz EMF with low power density field.

Studies on genotoxic potential effects of high-frequency EMFs have been done in cell culture and

animals. While some studies have not revealed significant genotoxicity (Leonard et al., 1983; Lloyd et al., 1986; Saunders et al., 1988), other studies have reported positive effects (Garaj-Vhrovac et al., 1990; Maes et al., 1993, 1995; Lai and Singh, 1995, 1996; Adey, 1997; Phillips et al., 1998). These divergent results mostly derive from non-homogeneous experimental design. In fact, *in vitro* and *in vivo* high-frequency EMF studies often differ in experimental conditions with respect to time and type of exposure (modulated or unmodulated field and power) as well as most epidemiological studies lack of systematic exposure measurements for individuals (Moulder et al., 1999).

We investigated the effects of unmodulated 900 MHz at low power density EMF to affect cellular systems by inducing both genotoxic damage and changes in gene expression levels.

We first assessed that, in our experimental conditions, unmodulated 900 MHz EMF with 1 mW input power produced the 3.5 mW/kg specific absorption value (SAR) for each cell without any relevant thermal effect. On the other hand, epidemiological and experimental data suggest that leukemic cells could be particularly susceptible to high-frequency EMFs exposure. Therefore, we investigated the effects of unmodulated 900 MHz EMF on the survival chance of human CCRF-CEM cell line, as a function of exposure time. Normal cell growth rate is the result of a balance between pro-apoptotic and pro-survival signals and any unbalance in this equilibrium predisposes to cellular transformation and, when the cell self-defense mechanisms fail, to cancer. Consequently, we focused our analysis on cellular viability and signal pathways involved in cell cycle control and apoptosis. A significant decrease in CCRF-CEM cell viability became detectable after 24–48 h of continuous 900 MHz EMF exposure. This decreased viability was the mere result of the effect of unmodulated electromagnetic field on cultured cells and was not dependent on TEM cell itself since the sham exposure did not evidence any changes in cell viability index in the absence of electromagnetic field. However, already from the first 2 h of 900 MHz exposure, DNA ladder and FACS analysis evidenced the presence of numerous apoptotic cells. During apoptosis, cellular endonucleases cleave genomic DNA between nucleosomes, producing fragments whose lengths vary by multiples of 180–200 bp (Arends and Wyllie, 1991; Enari et al., 1998). When genomic DNA extracted from apoptotic cells is resolved using agarose gel electrophoresis, these DNA fragments appear as a nucleosomal ladder, a widely recognized hallmark of apoptosis (Compton, 1992). As apoptosis is normally a protective mechanism removing DNA damaged cells, our data suggest that unmodulated EMF might act as a genotoxic agent quickly inducing DNA damage.

DNA fragmentation represents a cell signal for activation of growth arrest and pro-apoptotic genes. It is well known that the apoptotic machinery is engaged only when survival signals are withdrawn and death signals are amplified. To characterize the expression profiles of genes involved in both growth arrest and p53-dependent and -independent apoptosis, we performed Western blot analysis in CCRF-CEM cells exposed to 900 MHz EMF and compared the results with the controls at various exposure times. It is well known that there are multiple

interactions between the retinoblastoma family protein pathway, whose main function is to control G₁ to S-phase progression, and p53-dependent and -independent pro-apoptotic pathways, which guard against genomic instability by inducing both arrest of the cell cycle and apoptosis (Hsieh et al., 1999). Moreover, it has been recently shown that E2F1, the main target pRb/p105 growth suppressive function, plays a dual role by directly influencing p53-dependent and -independent apoptosis execution (Johnson and Schneider-Broussard, 1998; Hsieh et al., 1999). Namely, E2F1 has been shown to induce accumulation of p53 (Hiebert et al., 1995; Kowalik et al., 1998) and transcription of p73 (Irwin et al., 2000; Stiewe and Putzer, 2000; La Sala et al., 2003). In our experimental model, an effect on growth arrest was reflected by the up-regulation of both pRb/p105 and p21/WAF1 while the induction of an apoptotic response was evidenced by the early activation of p53, bax, E2F1 and p73. Activation of the apoptotic signals was maintained for the first 24 h when it prevailed over survival signals in accordance to what observed by DNA ladder and FACS analysis.

In the face of accumulating genomic damage, the cell might also undergo an activation event that provides an increased but inappropriate level of survival signaling, which allows it to evade subsequent attempts to auto-destruction. Actually, following 900 MHz EMF exposure we observed an activation of bcl-2 pro-survival oncoprotein, which antagonizes bax pro-apoptotic protein, as well as of both Ras and its down-stream partner, Akt1, delayed with respect to the pro-apoptotic effect. The ability of high-frequency EMFs to induce Ras activation suggests that they actually work as a stress signal. Our data suggest that the activation of proto-oncogenes such as Ras and Akt1 may provide an increase in survival potential sufficient to speed up-replication of cells with carcinogenic damage (Chin et al., 1999; Datta et al., 1999). In CCRF-CEM Ras-dependent pro-survival signals counteracted the pro-apoptotic ones only after continuous 24–48 h exposure to 900 MHz EMF as it is evidenced by the prevalence of S-phase cells observed by FACS analysis. It is known that Ras protects cells from apoptosis by activating Akt1 via PI3-kinase. Activated Akt1 (Akt1-P) is a key component of cell survival since it promotes a decrease in pro-apoptotic genes transcription and is implicated in the negative regulation of pRb/p105 functions (Kennedy et al., 1997; Downward, 1998; Du and Montminy, 1998; Rytomaa et al., 2000; Macaluso et al., 2002). We found that phosphorylated Akt1 is up-regulated after 24 h in concomitance with p53 and bax down-regulation. Collectively these data indicate that the cells initially respond to genotoxic damage induced by EMF exposure by inducing cell cycle arrest and apoptosis while at longer exposure times the activation of the p53-dependent and -independent pro-apoptotic pathway is no more effective. Therefore, notwithstanding the fact that continuous exposure of tumor cells to 900 MHz EMF induces a reduction in the number of viable cells, the survivor cells show an increase in DNA synthesis rate sustained by an activation of pro-survival signals and a consequent silencing of pro-apoptotic ones. Prevented programmed cell death due to constant signals promoting survival is critical for tumor progression and leads to metastasis formation (Chin et al., 1999;

Wong et al., 2001). Therefore, overall our data strongly support the hypothesis that high frequency EMFs exposure leads cancer cells to acquire a greater survival chance, a phenomenon linked to tumor aggressiveness.

Experiments are in progress to verify whether exposure to high frequency EMFs can promote as well the tumor transformation of normal cells.

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Research report

Effects of low intensity radiofrequency electromagnetic fields on electrical activity in rat hippocampal slices

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Abstract

Slices of rat hippocampus were exposed to 700 MHz continuous wave radiofrequency (RF) fields (25.2–71.0 V m⁻¹, 5–15 min exposure) in a stripline waveguide. At low field intensities, the predominant effect on the electrically evoked field potential in CA1 was a potentiation of the amplitude of the population spike by up to 20%, but higher intensity fields could produce either increases or decreases of up to 120 and 80%, respectively, in the amplitude of the population spike. To eliminate the possibility of RF-induced artefacts due to the metal stimulating electrode, the effect of RF exposure on spontaneous epileptiform activity induced in CA3 by 4-aminopyridine (50–100 μM) was investigated. Exposure to RF fields (50.0 V m⁻¹) reduced or abolished epileptiform bursting in 36% of slices tested. The maximum field intensity used in these experiments, 71.0 V m⁻¹, was calculated to produce a specific absorption rate (SAR) of between 0.0016 and 0.0044 W kg⁻¹ in the slices. Measurements with a Luxtron fibreoptic probe confirmed that there was no detectable temperature change (±0.1°C) during a 15 min exposure to this field intensity. Furthermore, imposed temperature changes of up to 1°C failed to mimic the effects of RF exposure. These results suggest that low-intensity RF fields can modulate the excitability of hippocampal tissue *in vitro* in the absence of gross thermal effects. The changes in excitability may be consistent with reported behavioural effects of RF fields. Crown copyright © 2001 Published by Elsevier Science B.V. All rights reserved.

Theme: Disorders of the Nervous System

Topic: Neurotoxicity

Keywords: Radiofrequency fields; Microwaves; Hippocampus; Seizures; Brain slices

1. Introduction

Current safety guidelines for human exposure to radiofrequency (RF) electromagnetic fields are based on the well-understood heating effects produced by the fields [1,23]. In contrast, the effects on biological systems of exposure to RF fields at levels that do not produce thermal changes are largely unknown. The possibility that RF

fields may produce subtle, ‘non-thermal’ effects in biological tissues is becoming an increasingly important issue, since it is not certain that exposure guidelines based on gross thermal effects will predict fully the hazards associated with exposure to short, high amplitude pulses of RF. The rapidly growing use of pulsed waveforms in applications such as telecommunications and radar therefore raises important questions of safety.

A number of studies have suggested that low frequency electromagnetic fields may interact with cognitive processes. It has been reported that exposure to weak extremely low frequency (ELF, 1–100 Hz) magnetic fields can influence brain electrical activity (EEG) in humans and animals [10–12,34]. ELF magnetic fields have also been

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reported to interact with learning and memory processes [25,27,33,34]. For example, exposure of rats to 60 Hz magnetic fields has been reported to disrupt spatial learning in a radial arm maze task [32], an effect which has been confirmed by studies on mice exposed to 50 Hz magnetic fields [40]. More recently, Lai et al. [30] have reported that exposure to a 60 Hz magnetic field affects the performance of rats in the Morris water maze. These behaviours have a substantial hippocampal involvement [9] and studies in vitro have suggested that ELF fields may influence neuronal activity in this brain structure. A series of in vitro studies by Bawin et al. [5–8] reported that low frequency (1–60 Hz) sinusoidal electric and magnetic fields could indeed produce effects on excitability in rat hippocampal slices. These effects included increases or decreases in the amplitude of the evoked population spike in CA1, disruption of rhythmic slow activity induced by carbachol and potentiation or depression of penicillin-induced epileptiform activity. The effects of endogenous and applied electric fields on the hippocampus and other structures have been reviewed by Jeffreys [24].

Other studies have suggested that much higher frequency fields in the RF range may also affect cognitive processes. Exposure of rats to pulsed RF radiation at a frequency of 2.45 GHz has been reported to disrupt spatial learning in a radial arm maze task [32]. Since the radial arm maze task is widely accepted to have a substantial hippocampal involvement, these studies suggest that RF fields may affect hippocampal function. This is supported by a report that exposure to pulsed microwaves can change electrical activity in the hippocampus of rabbits, but not in other brain areas [22]. A study by Preece et al. [37] found that radiation emitted by simulated analogue and GSM digital mobile phones produced a significant decrease in choice reaction time in human subjects. Similar results have been observed in a more recent study by a different group [28], and Freude and co-workers [16,18] have reported evidence that electromagnetic fields emitted by GSM phones depressed slow brain potentials in humans performing a visual monitoring task.

Despite this evidence from animal and human studies, there have been no reported studies to assess the effects of RF fields on neuronal activity in slices of brain tissue in vitro. In the experiments reported here, we have attempted to determine whether unmodulated weak RF electromagnetic fields can affect electrical activity directly in rat hippocampal slices. The hippocampal slice preparation is the most useful model of cerebral organisation for in vitro investigations of the cellular mechanisms underlying learning and memory and is therefore a potentially powerful tool for the study of interactions of RF fields with memory processes. The results of this study suggest that RF fields can indeed affect electrical activity in hippocampal tissue. The effects occurred at low field intensities and were not associated with detectable temperature increases.

2. Materials and methods

Male Porton Strain Wistar-derived rats (120–150 g) were anaesthetised with halothane and killed by decapitation. The brain was removed and 400 μm thick parasagittal slices were cut from a block of tissue containing the hippocampus using a Vibratome. The slices were stored at room temperature in a holding chamber containing ACSF artificial cerebrospinal fluid (NaCl 125 mM; KCl 3 mM; MgCl_2 1 mM; NaH_2PO_4 1.25 mM; NaHCO_3 26 mM; CaCl_2 2 mM; glucose 11 mM; pH 7.4, gassed with 95% O_2 and 5% CO_2).

Experiments were performed in a Haas type interface chamber (Medical Systems Base Unit with BSC-HT Top) which was adapted to allow the top unit to function separately from the base unit on which it normally sits. This enabled the top unit to be placed in the exposure area of the waveguide exposure apparatus described below. The tissue holder had dimensions of 64 mm wide by 110 mm long by 16 mm thick, with a 6 mm deep well in which the tissue was placed. The permittivity of the tissue holder was 1.6 and its loss factor was <0.01 (measured by Microwave Consultants). The brain slice was placed on a thin nylon mesh through which it was perfused from below with ACSF at a flow rate of 1 ml min^{-1} . The depth of the bathing solution beneath the slice was no more than 400 μm . Warmed and humidified gas (95% O_2 –5% CO_2) was passed over the top of the slice. The temperature was tightly controlled at $34.0 \pm 0.1^\circ\text{C}$ by feedback from a bead thermistor placed 1 mm downstream from the slice. Slices were allowed to equilibrate for at least 30 min in the recording chamber before exposure to RF fields.

Extracellular field potential responses were recorded in stratum pyramidale or stratum radiatum of the CA1 or CA3 regions using glass microelectrodes filled with 2 M NaCl (5–10 M Ω resistance). Contact was made with the solution in the recording electrode using a chlorided silver wire. The reference electrode (Ag–AgCl) was located below the nylon mesh supporting the slice. The recordings were displayed on a digital oscilloscope and digitised at 10 kHz with a CED 1401 computer interface (Cambridge Electronic Design) for analysis using the programmes SIGAVG, CHART and SPIKE2 (Cambridge Electronic Design). Afferent pathways were stimulated with a concentric bipolar stainless steel electrode placed in stratum radiatum. Constant current stimuli (pulse duration 70 μs , amplitude 100–300 μA) were delivered at intervals of 10–30 s to avoid the development of long-term potentiation or depression [43]. In some experiments, epileptiform activity was induced by perfusion with 50–100 μM 4-aminopyridine (4-AP, Sigma).

Exposures to RF fields were performed using a calibrated parallel plate waveguide apparatus (Fig. 1) [45]. In this system, the E field was normal to the surface of the recording bath and the stimulating and recording electrodes

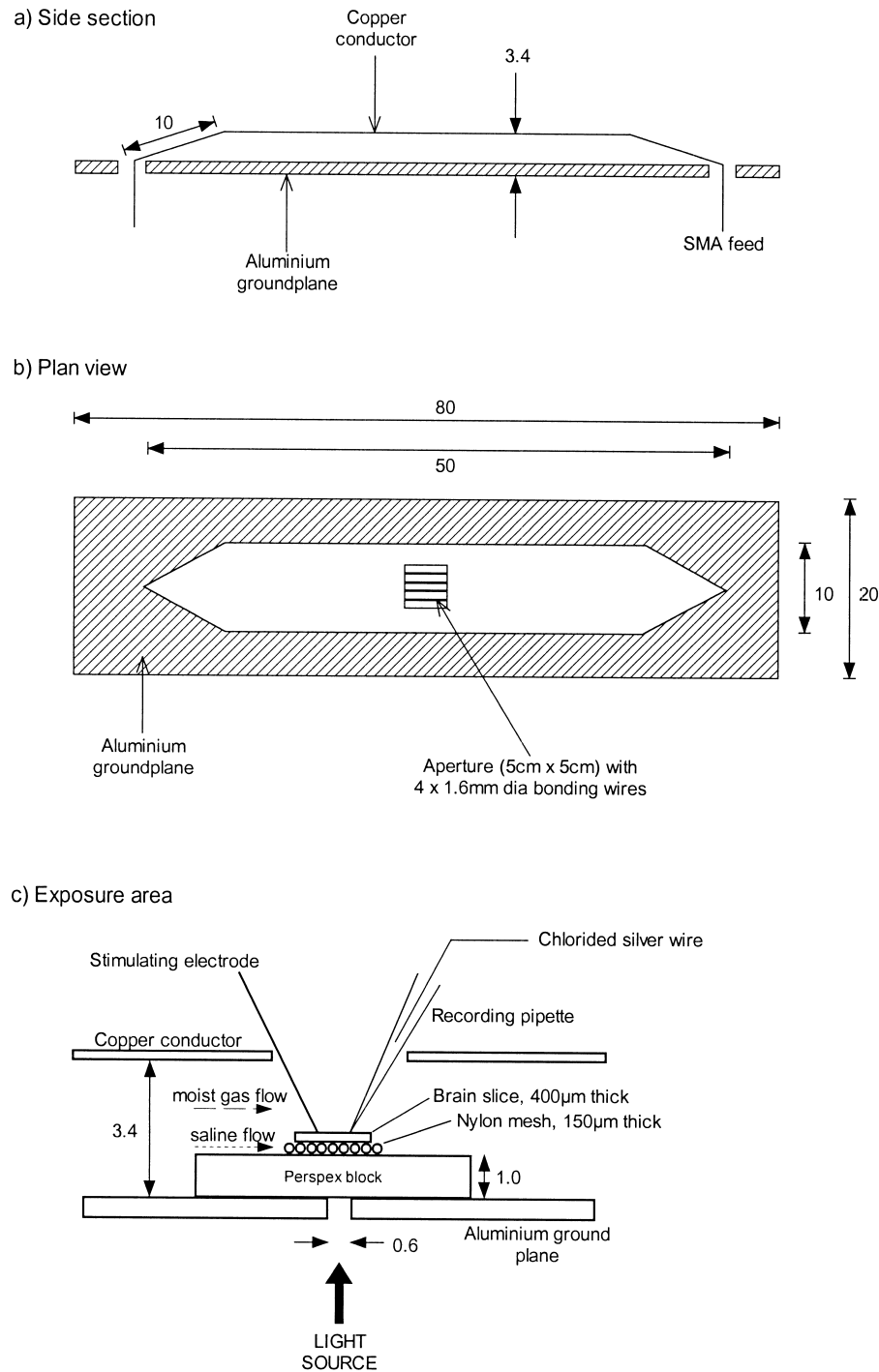


Fig. 1. Diagrams of the waveguide exposure system. Side, plan and cross-sectional views showing the arrangement of the tissue chamber and electrodes. Dimensions are in cm unless otherwise stated.

were placed at an angle of approximately 45° to the E field. RF signals were produced by an HP8648C signal generator (Hewlett-Packard) supplying a power of up to 0.126 W at 700 MHz. The frequency of 700 MHz was chosen because it is close to the 750 MHz frequency which had previously

been found to produce effects on heat-shock gene expression in the nematode *Caenorhabditis elegans* [15].

The electric field was monitored by a square law probe placed close to the sample. The electric field (E , $V\ m^{-1}$) in the waveguide was related to the input power (P_i , W) by

the simple equation $E=200\sqrt{P_i}$ [4,45]. Measurements were made at different locations in the waveguide with 10 and 15 mm monopole antennae using a calibrated HP8753A network analyser controlled by an HP 9000 series computer. These showed that the field varied by less than ± 1 dB across the exposure area where the slices were placed. Recordings were made from slices placed in several positions and orientations in the apparatus, with no difference in the observed results. The bath temperature, recorded with a thermistor with a resolution of 0.1°C , did not increase during exposure. The recording system was shielded by a Faraday cage lined with radiation-absorbent material (RAM) to reduce internal reflections and interference by external EM fields. Sham exposures were carried out by attaching a dummy load to the generator output, in place of the waveguide.

2.1. Computer modelling of the exposure system

Finite difference time domain (FDTD) modelling was performed using a package called XFDTD developed by REMCOM (USA). The whole waveguide space was meshed in a $2\times 2\times 2$ mm grid, generating a total $401\times 101\times 24=972\,024$ grids. The voltage standing wave ratio (VSWR) was calculated as 1.84, which agreed well with the measured value of 1.90, and the standing wave component of the field had a distance between antinodes of approximately 207 mm, considerably greater than the longest dimension of the tissue holder. FDTD confirmed the empirical formula obtained from experimental measurements: a 1 W input power produced an E field of 205.9 V m^{-1} , close to the 200 V m^{-1} predicted by the formula. When the tissue holder was placed in the waveguide, the electric field produced by a 1 W input decreased to 164.5 V m^{-1} inside the tissue holder, and increased to 252.0 V m^{-1} in the air just above the holder. The effect of the saline was also modelled, assuming a relative permittivity of 80 and $\sigma=1.5\text{ S m}^{-1}$. Due to the limitation of memory, the sub-gridding function of XFDTD could not be activated on the PC used, so the thickness of the saline layer was increased to fill one 2 mm grid, an overestimate. When this was done, the electric field values in and above the tissue holder were both reduced by approximately 20 V m^{-1} .

Using these field values, the volume around the tissue slice was remodelled using a $200\times 200\times 200\text{ }\mu\text{m}$ grid. The tissue properties used were: $\epsilon_r=41.5$, $\sigma=0.86\text{ S m}^{-1}$, $\rho=1000\text{ kg m}^{-3}$ (Sphere Benchmark Phantom: Protocol, Microwave Consultants). Due to the very small size of the tissue slice, the average-specific absorption rate (SAR) over a 1 or 10 g volume cannot be obtained; the program can only calculate the SAR distribution (corresponding to $|E|$) and the peak SAR values. The peak SAR value obtained with the maximum 0.126 W input power used in the electrophysiological experiments was very low, only

0.0044 W kg^{-1} . This is because the electric field strength is low inside this high permittivity material, 2.26 V m^{-1} .

2.2. Calculation of specific absorption rate

The specific absorption rate (SAR) of electromagnetic energy into the slice was determined by calculating the relationship between the external electric field (E_0) and the electric field induced in the slice (E_i). Since the slice is small enough to cause insignificant perturbations to the magnetic field, the long wavelength approximation may be used [13]. In this situation, the electric field E_i in the slice is a combination of the field induced by the field E_0 of the travelling plane wave and the field induced electromagnetically by the corresponding time varying magnetic field H_0 .

Considering the slice as a thin disc of radius a and thickness $2h$, there are three basic orientations of the slice, H_0 parallel to the disc and E_0 perpendicular, E_0 parallel and H_0 perpendicular, and both E_0 and H_0 parallel. In these experiments, the first orientation applies. For the E field perpendicular to the slice, E_i is approximately uniform and independent of the disc thickness:

$$\frac{E_i}{E_0} = \frac{\sigma_0}{\sigma_c},$$

where $\sigma_0 = -i\omega\epsilon$ and $\sigma_c = \sigma - i\omega\epsilon_0$ in which σ is the conductivity of the tissue, i is the complex number $\sqrt{-1}$, ω is the frequency in radians ($=2\pi f$) and ϵ_0 is the permittivity of free space. $\epsilon = \epsilon_r\epsilon_0$, where ϵ_r is the permittivity of the tissue relative to that of free space.

For the electric field induced by the magnetic field parallel to the slice, E_i varies linearly across the slice (apart from minor edge effects) and is independent of the disc radius:

$$\frac{E_i}{E_0} = \frac{ih\omega}{c}$$

where c is the velocity of light in free space.

The tissue properties used were the same as in the FDTD modelling: $\epsilon_r=41.5$, $\sigma=0.86\text{ S m}^{-1}$, $\rho=1000\text{ kg m}^{-3}$. With $\epsilon_0=8.8\times 10^{-12}$, this gives $\sigma_c/\sigma_0=41.5+22.2i$. For a disc of radius 0.5 cm and thickness $400\text{ }\mu\text{m}$, the induced field factors are $|E_i/E_0|=0.019$ for the electric field contribution and $|E_i/E_0|=0.0022$ for the magnetic field.

The SAR was then calculated as equal to $\sigma|E_i|^2/\rho$; this gives a value of 0.0019 W kg^{-1} for $E_0=71\text{ V m}^{-1}$, the highest external field intensity to which the slices were exposed. This agrees well with the value of 0.0044 W kg^{-1} obtained from FDTD modelling.

To explore the dependence of the SAR estimation on the dielectric properties used, the calculation was repeated using a different set of tissue properties, those for grey matter given by Gabriel and Gabriel [21]. These were $\sigma=0.977\text{ S m}^{-1}$ at 700 MHz, $\epsilon_r=51.60$ and $\rho=1030\text{ kg}$

m^{-3} . Using these values, the SAR was calculated to be slightly lower than for the previous dielectric values, 0.0016 W kg^{-1} for $E_0 = 71 \text{ V m}^{-1}$.

The orientation in these experiments, with the slice perpendicular to the external electric field, gives the minimum induced electric field and therefore SAR. In other orientations, these quantities would be larger: in particular, if the external electric field E_0 was parallel to the disc, the induced field E_i would be $0.5 E_0$ and for even thinner discs it would approach E_0 .

2.3. Statistics

Data are expressed as $\text{mean} \pm \text{S.D.}$, unless stated otherwise. Statistical analyses were performed using GraphPad InStat version 3.00 and GraphPad Prism version 2.00 (GraphPad Software, San Diego, CA, USA, www.graphpad.com). A difference between means was considered significant if the probability of it being due to random variation, P , was less than 0.05.

3. Results

3.1. Evoked field potentials

In the first series of experiments, each slice was subjected to three RF exposures of 5, 10 and 15 min duration, respectively, with a 5 min recovery period between successive exposures. The E field intensity was 71.0 V m^{-1} . A change in response was defined as a sustained ($>2 \text{ min}$) increase or decrease of greater than twice the standard deviation of the response during the 5 min preceding the exposure. The first exposure period (Fig. 2) was found to produce more variable effects than the subsequent exposures. During the first exposure period, five out of 12 slices exposed to 700 MHz RF fields showed an increase of between 20 and 120% in the amplitude of the CA1 population spike (PS), while four showed a decrease of between 20 and 80%. The mean change for all 12 slices was $+8.7 \pm 61.1\%$ ($\text{mean} \pm \text{S.D.}$); the mean change was not significantly different from sham-exposed control slices ($+7.6 \pm 11.3\%$, $n=6$), but the RF exposure

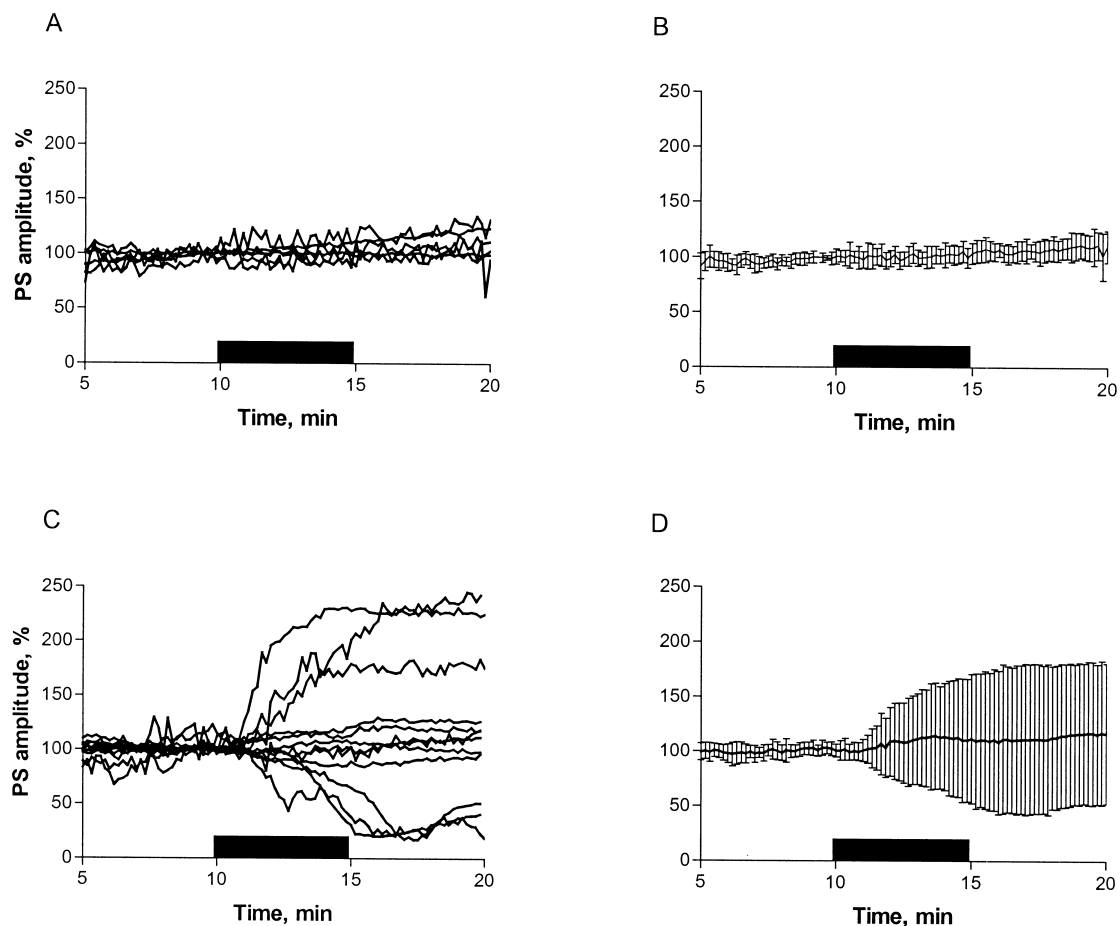


Fig. 2. Effects of exposure to 700 MHz fields on the population spike (PS) amplitude in hippocampal slices. (A) Data for six slices which were sham-exposed for 5 min (solid bar). (B) Mean $\pm$ S.D. for the six slices shown in (A). (C) Data for 12 slices exposed to a field intensity of 71 V m^{-1} for 5 min (solid bar). (D) Mean $\pm$ S.D. for the 12 slices in (C).

produced a very significant increase in the variance of the data, compared with pre-exposure levels (F -test, $P < 0.0001$) and with sham-exposed controls ($P = 0.0009$).

The second exposure produced an increase in the PS amplitude of 27% in one slice out of 10 tested and decreases of between 30 and 90% in eight others (mean change $-48.6 \pm 36.8\%$). These changes were statistically significant compared to sham-exposed controls, both in terms of the variance (F -test, $P = 0.0089$) and the mean (t -test with Welch's correction for unequal variance, $P = 0.0009$). The third exposure had no effect on one slice but induced decreases of between 27 and 78% in the PS of the other six tested (mean change $-48.0 \pm 36.2\%$). Again, the changes in mean and variance were statistically significant ($P = 0.0063$ and $P = 0.0107$, respectively). In all but one case, the decreases in PS amplitude reversed fully or partially after the exposure was terminated; in contrast, the increases in PS amplitude persisted in four out of the five slices showing this effect. No effects were seen in six sham-exposed control slices (Fig. 2).

The effects of RF fields on the field excitatory post-synaptic potential (fEPSP) slope were smaller than on the PS amplitude. The first exposure produced an increase of approximately 10% in two out of the 11 slices tested, a decrease in one slice, an increase followed by a decrease in another preparation and had no effect in the remaining seven slices. During the second exposure, an increase was observed in one slice, a decrease in two and no effect in a further six slices tested. The third exposure resulted in a decrease in two slices and no effect in the remaining three preparations tested.

Two series of experiments were conducted to determine the relationship between the 700 MHz RF field intensity and the effects on hippocampal field potentials. In the first, five slices were exposed for 5 min periods to different RF field intensities, with a 5 min gap between successive exposures. The E field intensities ranged from 20 to 71 V m^{-1} and the order of exposures was varied randomly between experiments. The higher field intensities produced either increases or decreases in the amplitude of the population spike, resulting in an increased variance between slices (Fig. 3). Due to the variability of the response to RF between slices, however, none of the field intensities produced a statistically significant effect in terms of the mean change, although clear effects were apparent in the individual slices.

In a second series of experiments, 12 slices were exposed for consecutive 5 min periods to 700 MHz RF fields. The field intensity was increased for each successive exposure and there was no gap between the exposures. This cumulative exposure produced more consistent effects, shown in Fig. 4. A small increase in PS amplitude was apparent in five slices at 25.2 and 31.7 V m^{-1} , there was a small decrease in five slices at 39.9 V m^{-1} and a larger decrease in six slices at 50.2 V m^{-1} , but none of these changes was statistically significant. There was,

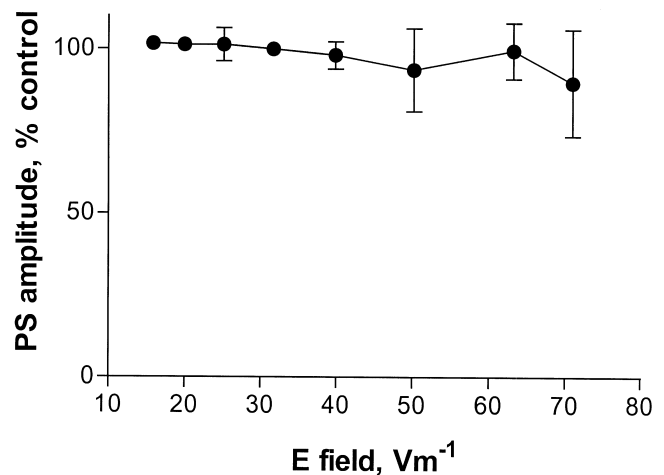


Fig. 3. The effect of a 5 min exposure to 700 MHz RF at different field intensities on PS amplitude. Each point is the mean $\pm$ S.E.M. for five slices. Slices were exposed to a number of different intensities in random order, with a 10 min interval between exposures. The variance of the PS amplitude was significantly greater at field intensities of 50.2 V m^{-1} and greater (F -test, $P < 0.05$).

however, a significant increase in variance at 50.2 V m^{-1} (F -test, $P < 0.05$). Significant decreases in PS amplitude were produced by 63.2 and 71.0 V m^{-1} fields (Kruskal–Wallis test followed by Dunn's multiple comparison test, $P < 0.05$). These effects were apparent in 10 out of the 12 slices; full or partial recovery was observed in all but one of the slices after the exposure was terminated.

3.2. Epileptiform activity

In the experiments on evoked field potentials, a metal stimulating electrode was used to elicit the responses. The presence of this electrode in the RF field raises the possibility that the observed changes in the evoked response may have been caused, at least in part, by an interaction between the applied field and the metal electrode. To investigate this possibility, we tested the effects of exposure to RF fields on epileptiform bursting induced by 4-aminopyridine, a drug which blocks voltage-dependent potassium channels [3]. Since no electrical stimulation was required in these experiments, only the glass microelectrode filled with 2 M NaCl was present in the field.

Perfusion with 4-aminopyridine (50–100 μM) induced spontaneous synchronised bursts of activity in the CA3 region which occurred at a frequency of 0.1–0.3 per second. Slices were exposed in the waveguide to 700 MHz RF fields at increasing field intensities up to 71.0 V m^{-1} for 5 min at each intensity. In four out of the 11 slices tested, exposure to the highest field intensity produced a transient increase in the frequency of the bursting, accompanied by a decrease in the amplitude of the bursts; this was followed by a lasting decrease in frequency of

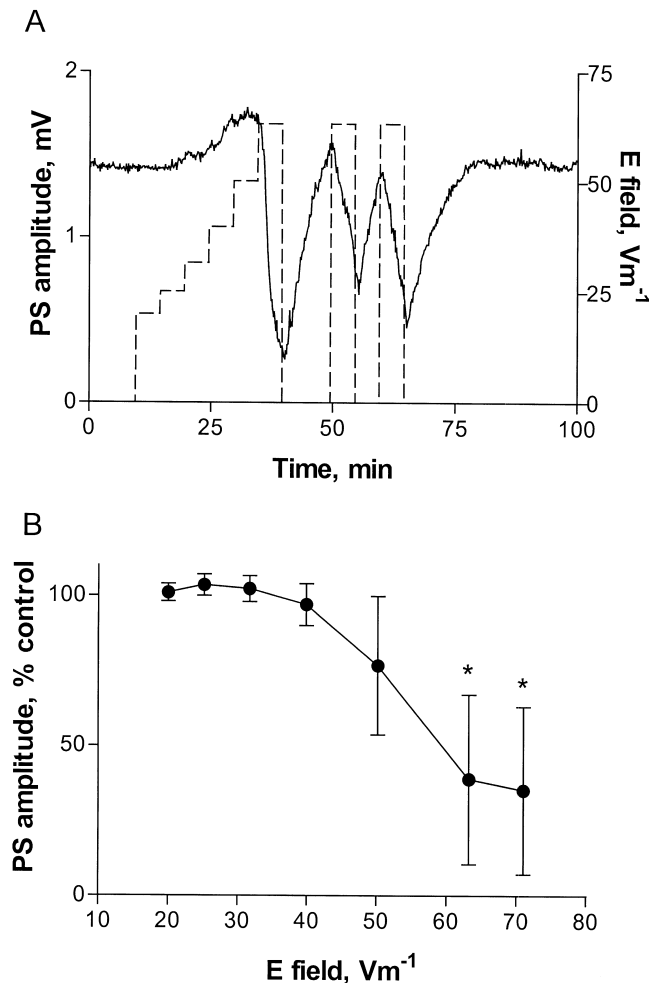


Fig. 4. (A) PS amplitude (solid line, left axis) in a hippocampal slice exposed to a 700 MHz field at increasingly greater intensities, with 5 min at each field intensity (broken line, right axis). Exposure to fields greater than 25 V m⁻¹ resulted in an increase of the PS amplitude from the pre-exposure level; in contrast fields greater than 50 V m⁻¹ caused a decrease in amplitude which recovered to pre-exposure level when the field was turned off. Two subsequent exposures to the highest field intensity, 63.2 V m⁻¹, produced decreases in PS amplitude which also recovered when the field was switched off. (B) Summary data for 12 slices exposed to a 700 MHz field at increasingly greater intensities, as shown in (A). Each point shows the mean $\pm$ 95% confidence interval for 12 slices during 5 min exposure periods. Asterisks indicate statistically significant changes from pre-exposure values: $*=P<0.05$ (Kruskal–Wallis test followed by Dunn’s multiple comparison test).

bursting which recovered slowly when the field was turned off (Fig. 5). No effect was seen in six sham-exposed slices.

3.3. Temperature measurements

In order to confirm that the RF exposures did not produce thermal changes, in some experiments a non-perturbing Luxtron fibreoptic probe was placed next to the slice during exposure. This measured the temperature of the solution immediately adjacent to the slice with an

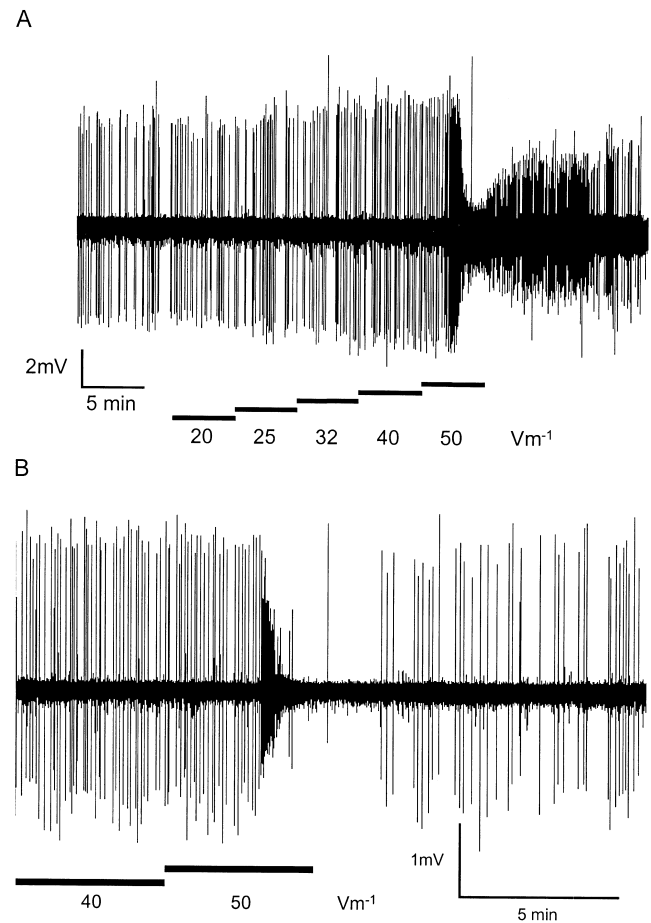


Fig. 5. Effects of exposure to 700 MHz fields on epileptiform activity induced by 4-aminopyridine (4-AP) in hippocampal slices. (A) Epileptiform activity in a slice exposed to 700 MHz fields at increasing intensities (indicated by the solid bars). Exposure to the highest field intensity, 50.2 V m⁻¹, resulted in a transient increase in the frequency of electrical discharges, followed by a cessation of epileptiform activity which recovered slowly when the field was switched off. (B) Epileptiform activity in a different slice shown on a faster time base. Once again, exposure to a 700 MHz field at 50.2 V m⁻¹ resulted in a transient increase in the frequency of discharges, followed by abolition of bursting which reappeared after the end of the exposure period.

accuracy of $\pm 0.1^\circ\text{C}$ [14]. No detectable increase in the temperature was observed during a 15 min exposure to 700 MHz RF fields at the maximum intensity used.

3.4. Imposed temperature increases

To investigate further the possibility that the observed changes were due to heating effects during exposure to RF, evoked field potentials were recorded while the temperature of the perfusing solution was raised from 34 to 35°C. This imposed temperature change, which took place over approximately 5 min, did not produce significant changes in the PS amplitude or fEPSP slope in any of the four slices tested.

4. Discussion

The results of this study show that acute exposure to 700 MHz electromagnetic fields produced significant changes in evoked and spontaneous electrical activity in hippocampal slices in the absence of detectable increases in temperature. At low field intensities (25.2 and 31.7 V m⁻¹), the predominant effect was a potentiation of the amplitude of the evoked population spike by up to 20%, but higher intensity fields (up to 71.0 V m⁻¹) could produce either increases or decreases of up to 80% in the amplitude of the population spike. Changes in the field EPSP were smaller and were observed in fewer slices than changes in PS amplitude.

It is conceivable that the change in the evoked response may have been due, at least partially, to an artefact caused by the presence in the RF field of the metallic stimulating electrode used to evoke the responses. However, such an artefact would be expected to be of the same polarity at all field intensities, and it is not clear how this could produce a potentiation of the PS at low field intensities and a depression at the highest intensity. Furthermore, the decreases in PS amplitude reversed fully or partially in the majority of slices, whereas the increases in amplitude usually persisted after termination of the RF exposure. This suggests that two different processes may have been occurring during exposure to RF fields.

In the experiments on spontaneous epileptiform activity, no stimulating electrode was present during exposure to the RF field. Nevertheless, changes were observed during exposure to RF fields in approximately 36% of the slices tested, a similar proportion to that seen in the experiments on evoked responses. Furthermore, this effect became apparent at a field intensity of 50 V m⁻¹, which was similar to the threshold field intensity to produce changes in the evoked field potential. Thus, the presence or absence of the stimulating electrode appeared to have no influence on the field intensity required to produce an effect.

It could be argued that the glass recording pipette, filled with 2 M NaCl solution, may have been a further source of artefact in these experiments, but this seems unlikely. Firstly, no change in DC offset across the pipette was observed when the RF field was turned on, nor was there a change in pipette resistance. Secondly, it made no difference to the response to the RF field whether the chlorided silver wire used to make contact with the solution in the recording pipette extended into the waveguide or was kept outside by lengthening the pipette. Thirdly, the epileptiform activity recovered only slowly after the RF field was switched off, and did not return to pre-exposure levels for at least 10 min. Finally, epileptiform activity induced by 4-AP is known to originate in a large population of cells in the CA3 subfield, so it is very unlikely that a current induced in the recording pipette could be large enough to affect the activity of the entire CA3 area. The amplitude of an effective induced current would be high enough to

generate a measurable DC offset across the recording pipette.

Glass microelectrodes do not distort electromagnetic fields to any measurable degree in biological tissues [17,26]. Seaman and Wachtel [39] found that less than 1 mV of DC potential and less than 10 pA of current was generated in glass microelectrodes filled with 2.5 M KCl (2–10 MΩ resistance) during exposure to 1.5 or 2.45 GHz fields in a stripline waveguide system, to produce an SAR of up to 70 mW g⁻¹. Since this current is several orders of magnitude lower than that required to evoke electrical activity in a brain slice, it is extremely unlikely that interactions of the RF field with the recording electrode could have caused the observed effects. A similar absence of electrode artefacts was found by Seaman et al. with an open-ended coaxial exposure device [38].

The observed changes during exposure to RF fields did not appear to be due to gross heating effects. The maximum field intensity used in these experiments was 71.0 V m⁻¹, which was calculated to produce an SAR between 0.0016 and 0.0044 W kg⁻¹. This is considerably lower than the minimum SAR that would be expected to produce measurable temperature rises. For example, the NRPB and ICNIRP exposure standards, which are based on the thermal mechanisms of interaction between RF fields and biological tissues, are derived from an SAR of 10 W kg⁻¹. This is more than three orders of magnitude greater than the largest SAR in the current experiments.

As expected, no rise in temperature could be measured during exposures of up to 15 min duration, either with the feedback bead thermistor used to control the chamber temperature or with a Luxtron fibreoptic probe placed immediately next to the slice. It has been suggested that exposure to low intensity RF fields may produce so-called ‘micro-heating’ effects, in which microscopic ‘hot spots’ are produced in tissues [17,36], but this seems unlikely at the intensities used in the experiments reported here. Such an effect would presumably require resonance or focussing of the applied field, which is unlikely to occur with wavelengths much greater than the dimensions of the slice, as is the case in the present experiments. Furthermore, imposed temperature changes of up to 1°C did not mimic the effects of RF fields.

If RF fields are able to affect the excitability of neurones in the hippocampus *in vitro*, it might be expected that they could also produce behavioural changes *in vivo*. In particular, the hippocampus is important in spatial learning and memory processes [35]. Some studies have indeed suggested that electromagnetic fields can affect behavioural responses which have a hippocampal involvement. Lai et al. [32] reported that exposure to pulsed 2450 MHz microwaves (2 μs pulses, 500 pps, 1 mW cm⁻², average whole body SAR 0.6 W kg⁻¹) retarded the learning of a radial arm maze task by rats, indicating a deficit in spatial ‘working memory’ function. The behavioural deficit was reversed by pretreatment with physostigmine or naltrex-

one, but not by naloxone. A previous study by these authors [31] reported that the same exposure reduced choline uptake in the hippocampus and frontal cortex of rats. These effects appeared similar to restraint stress and were blocked by naloxone and naltrexone. There have been few attempts to replicate these results using RF fields, but Sienkiewicz et al. [41] reported that exposure of mice to a 50 Hz magnetic field reduced the rate of acquisition of a radial arm maze task but did not affect the overall accuracy. These results agreed with those of an earlier study by Lai [29] on rats exposed to a 60 Hz magnetic field.

A study by Preece et al. [37] reported a decrease in choice reaction time in human volunteers exposed to 915 MHz RF fields from a simulated mobile phone. Analogue (i.e., continuous wave) fields produced a larger effect than a simulated digital field (217 Hz modulation with a 12.5% duty cycle). There was no effect on 14 other tasks associated with working or secondary memory and attention. The authors proposed that RF fields may have affected the angular gyrus, which was directly under the antenna, and suggested that the effect could have been due to mild heating of this area of the brain. They did not, however, rule out the possibility of an effect which was not related to heating, and the changes in neuronal excitability observed in brain slices in the present experiments would be consistent with the effect reported in the human study. A more recent study has reported similar effects on human brain function and cognition [28] and a significant decrease in reaction time in a forced-choice, duration-discrimination task has also been reported in human subjects exposed to a 50 Hz magnetic field [46], which would not be expected to produce heating effects.

During *in vivo* exposures at RF frequencies, most of the applied electromagnetic field will be absorbed by superficial tissues, such as skin, cranial muscles and the skull, and the amount of power reaching the brain will be greatly attenuated by this absorption process. Although the fields induced in deeper structures, such as the hippocampus, will be very much smaller than the external field, it is possible that the SAR in the hippocampus could approach that induced in the present experiments in brain slices, at least in rats and mice. The results of the current study are therefore consistent with the behavioural effects reported by Lai et al. [32], although these are as yet not widely accepted. Due to the much larger size of the head and brain in humans, the proportion of the applied RF field reaching the hippocampus would be considerably smaller than in rats and mice; however, there may be a significant field in more superficial structures, such as the cortex, which would be consistent with the results of Preece et al. [37] and Koivisto et al. [28].

Although there has been no previously published work on the effects of RF fields on brain slices, Bawin et al. [7] have reported studies in brain slices exposed to ELF fields; they found that sinusoidal electric fields (20–70 mV peak-

to-peak in the bath, approximately 25% smaller in the tissue) could affect the excitability of rat hippocampal slices in ways which appeared strikingly similar to those found with RF fields in the present work. A brief exposure (5–30 s) to 5 or 60 Hz fields was found to produce an increase in the population spike amplitude in CA1 which lasted for longer than 10 min. The 60 Hz fields (but not 5 Hz) could also induce a short-term (1–6 min) depression or transient excitation (15–30 s). With a longer period of exposure (3 min), 5 Hz fields produced a long-lasting potentiation, whereas 60 Hz fields always produced a progressive depression which persisted for a few minutes after the end of the exposure. The effects were independent of the phase of the sine wave at which the population spike was evoked and were also independent of the orientation of the slice in the electric field, suggesting that the sinusoidal fields were not directly evoking cellular activity. In a subsequent study [6], ELF (1–100 Hz) magnetic fields were reported to disrupt rhythmic slow activity induced by carbachol in rat hippocampal slices.

Since these effects of electric fields appear similar to the changes produced by exposure to RF in the present study, it is conceivable that the effects of RF fields could be mediated by the induction of weak electric fields in the tissue. In the experiments on evoked field potentials, the amplitude of the population spike was affected by exposure to RF fields to a much greater degree than was the slope of the fEPSP. Similar effects can be produced by weak ($\sim 4 \text{ V m}^{-1}$) DC electric fields in hippocampal slices [24]. Furthermore, the electric fields generated around hippocampal pyramidal cells during synchronised activity may be sufficiently large to excite adjacent cells; this has been proposed as a potential mechanism for the propagation of epileptiform activity in the hippocampus. It is possible that perturbation of these electric fields could contribute to the inhibition of epileptiform activity during exposure to RF fields. Such an interaction would, however, depend on a mechanism for converting the radiofrequency field into a much lower frequency signal which would be able to influence neuronal excitability. As yet, there is little evidence for such a mechanism and further studies will be needed to test this possibility [42]. Interestingly, a recent study [47] has reported that exposure to DC magnetic fields (2–3 mT for 20 min) can produce decreases and increases in population spike amplitude in hippocampal slices similar to those found in our experiments.

A range of mechanisms has been suggested that would enable RF fields to interact with biological systems (for reviews, see Refs. [2,44]). Many of these are speculative theoretical mechanisms, which have not been widely accepted and require experimental confirmation. Some of the postulated mechanisms require amplitude or pulse modulation of the carrier frequency, which is clearly not applicable to the experiments reported here. Others have addressed resonant interactions with molecules and parts of molecules that would be expected in the millimetre wave

and far-infrared regions of the electromagnetic spectrum [19,20], at much higher frequencies than that used in our experiments. We believe that the experiments reported here do not provide sufficient evidence to support a specific mechanism of interaction; however, the effects we have described appear to be sufficiently robust to enable further studies to investigate this question in greater detail.

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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. © 2007 Wiley-Liss, Inc.

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

INTRODUCTION

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

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

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

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

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

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

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

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

MATERIALS AND METHODS

GSM Exposure

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



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

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

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

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

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

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

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

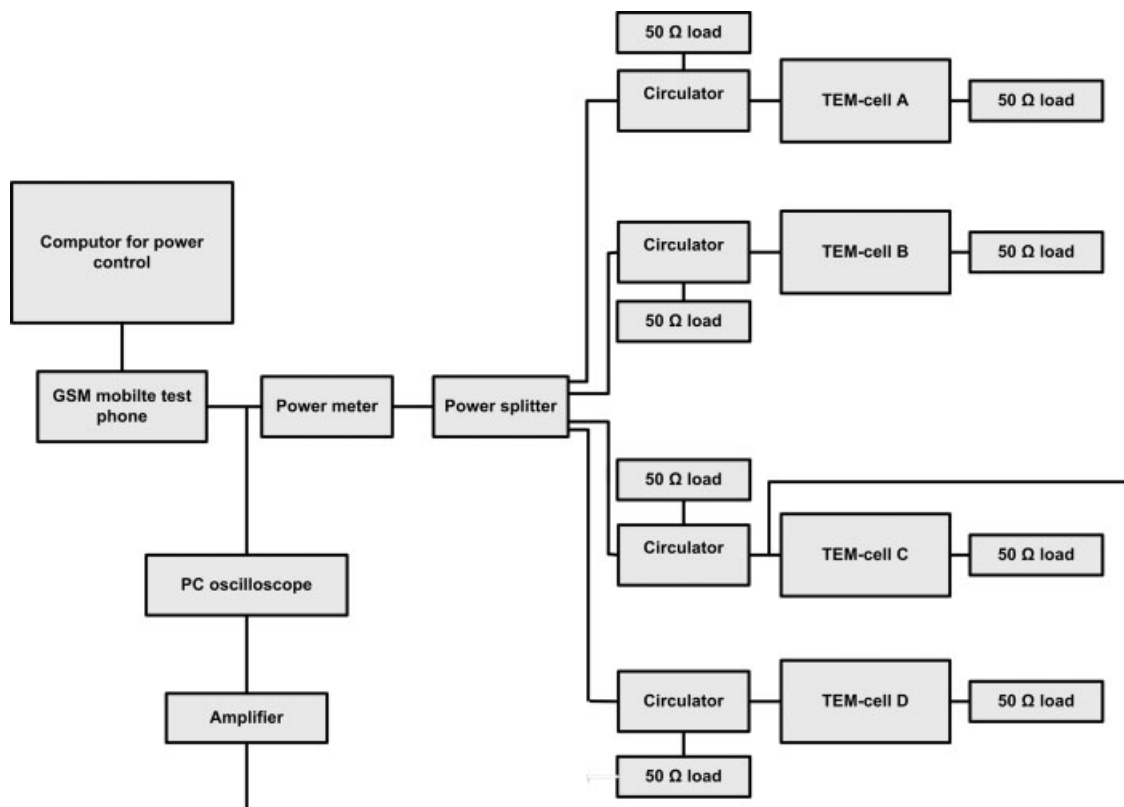


Fig. 2. Block diagram of the exposure setup. Four TEM-cells (A, B, C and D) are used. For sham exposure the same TEM-cells are used, but in this case they are not connected to the GSM mobile test phone and thus no RF EMFs are directed into the TEM-cells.

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

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

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

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

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

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

Animals

All animal procedures were performed according to the practices of the Swedish Board of Animal Research and were approved by the Animal Ethics Committee, Lund-Malmö. Fifty-six inbred male and female Fischer 344 rats (the rats were supplied by Scanbur AB, Stockholm, Sweden) were 4–6 months of age at the initiation of the EMF exposure. Male and female rats weighed approximately 350 and 200 g, respectively, as estimated in calibrations of rat weight as a function of age [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

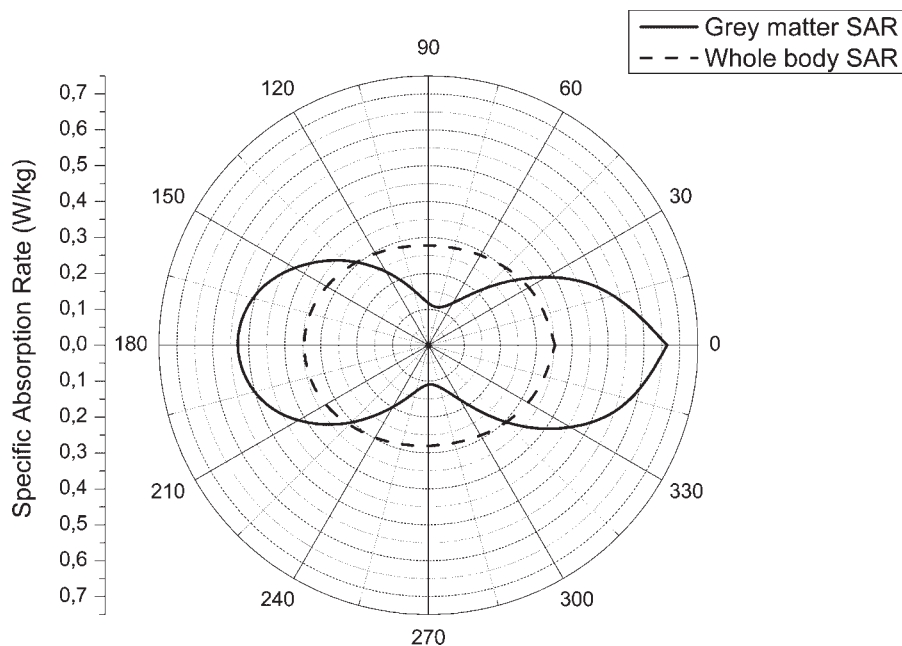


Fig. 3. FDTD calculation of SAR values in the brain grey matter and whole-body SAR (W/kg) in the rat at exposure to 900 MHz plane wave radiation, with a power density of 10 W/m^2 , as a function of incident wave angle with respect to the long axis of the animal. The polarisation of the radiation is identical 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 $S = 33 \text{ mW/m}^2$), generating average SAR values of 0.50 mW/kg for males (range 0.62 mW/kg) and 0.66 mW/kg for females (range 0.37 mW/kg) (the range is defined as the difference between the maximum and minimum SAR values according to expression 1; see discussion on dosimetry above), with an average SAR value of 0.6 mW/kg for males and females together. Sixteen animals were exposed to higher power level of GSM, with a peak output power from the GSM mobile telephone fed into each of the TEM-cells of 0.5 W ($S = 3.3 \text{ W/m}^2$), generating average SAR values of 50 mW/kg for males (range 62 mW/kg) and 66 mW/kg for females (range 37 mW/kg), with an average SAR value of 60 mW/kg for males and females together. Eight animals were cage controls, which never left the animal house. These SAR values are valid at the initiation of the experimental period.

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

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

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

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

Test Equipment

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

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

Open-Field Test

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

the rat lifts its fore paws (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

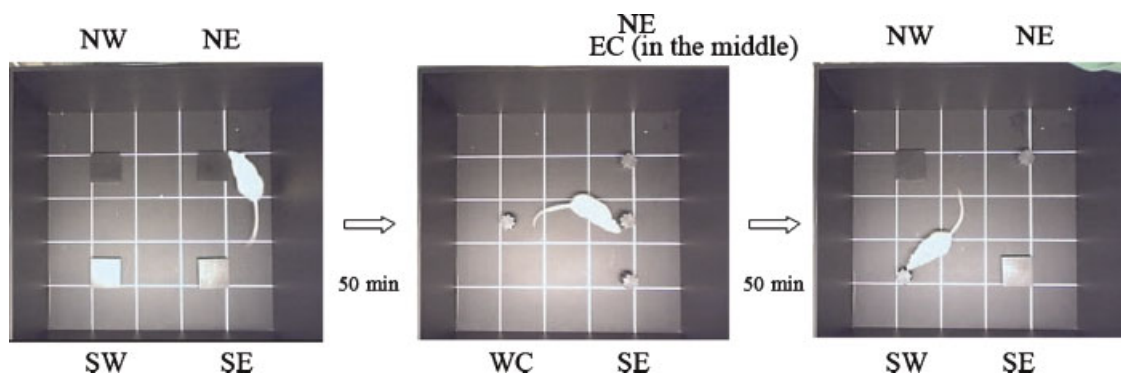


Fig. 4. Schematic drawing of the episodic-like memory test. The rats received two training trials and one test trial, each with 50 min inter-trial interval. On training trial 1 four black quadrants were arranged symmetrically one in each corner. On training trial 2 four grey cylinders were arranged in a T-shaped configuration. During the test trial two old familiar objects were placed as in training trial 1, whereas one recent familiar object was placed in 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

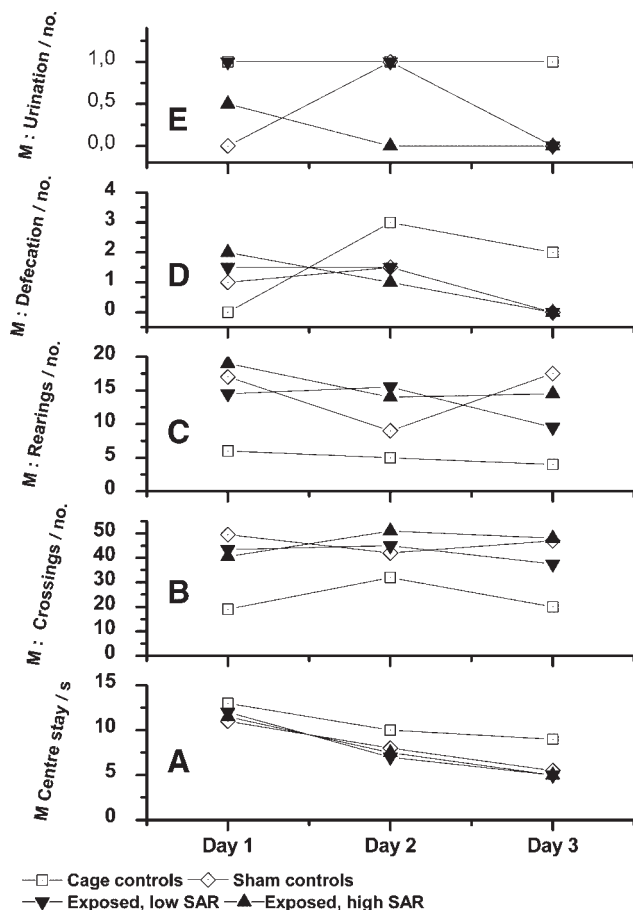


Fig. 5. Result from open-field test for males. **A**: Median value (M) of centre stay time measured in seconds. Median value (M) of number of **(B)** crossings; **(C)** rearings; **(D)** defecations and **(E)** urinations. No statistical significance was found between the GSM exposed rats compared to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

($P < 0.0001$), 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

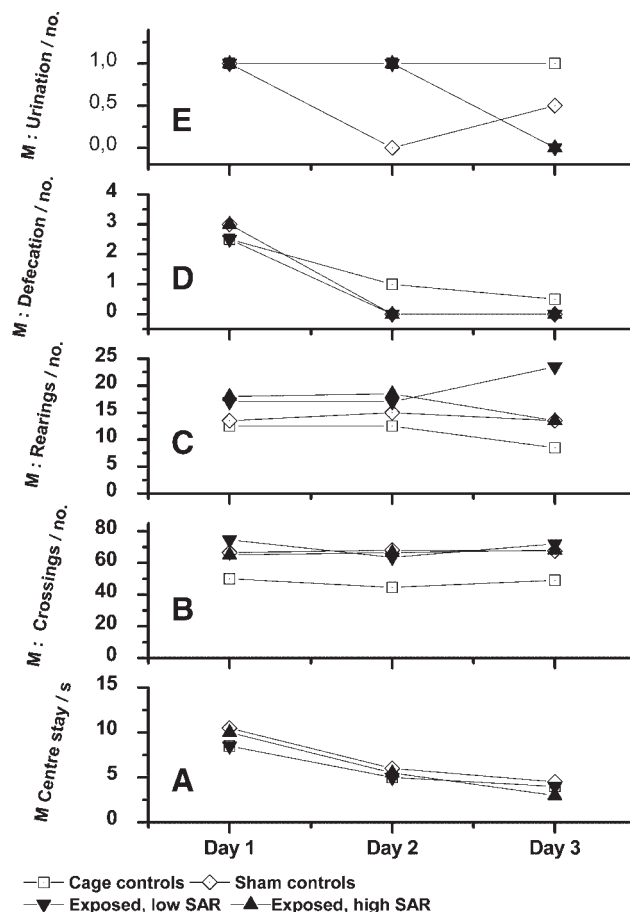


Fig. 6. Result from open-field test for females. **A**: Median value (M) of centre stay time measured in seconds. Median value (M) of number of **(B)** crossings; **(C)** rearings; **(D)** defecations and **(E)** urinations. No statistical significance was found between the GSM exposed rats compared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

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

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

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

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

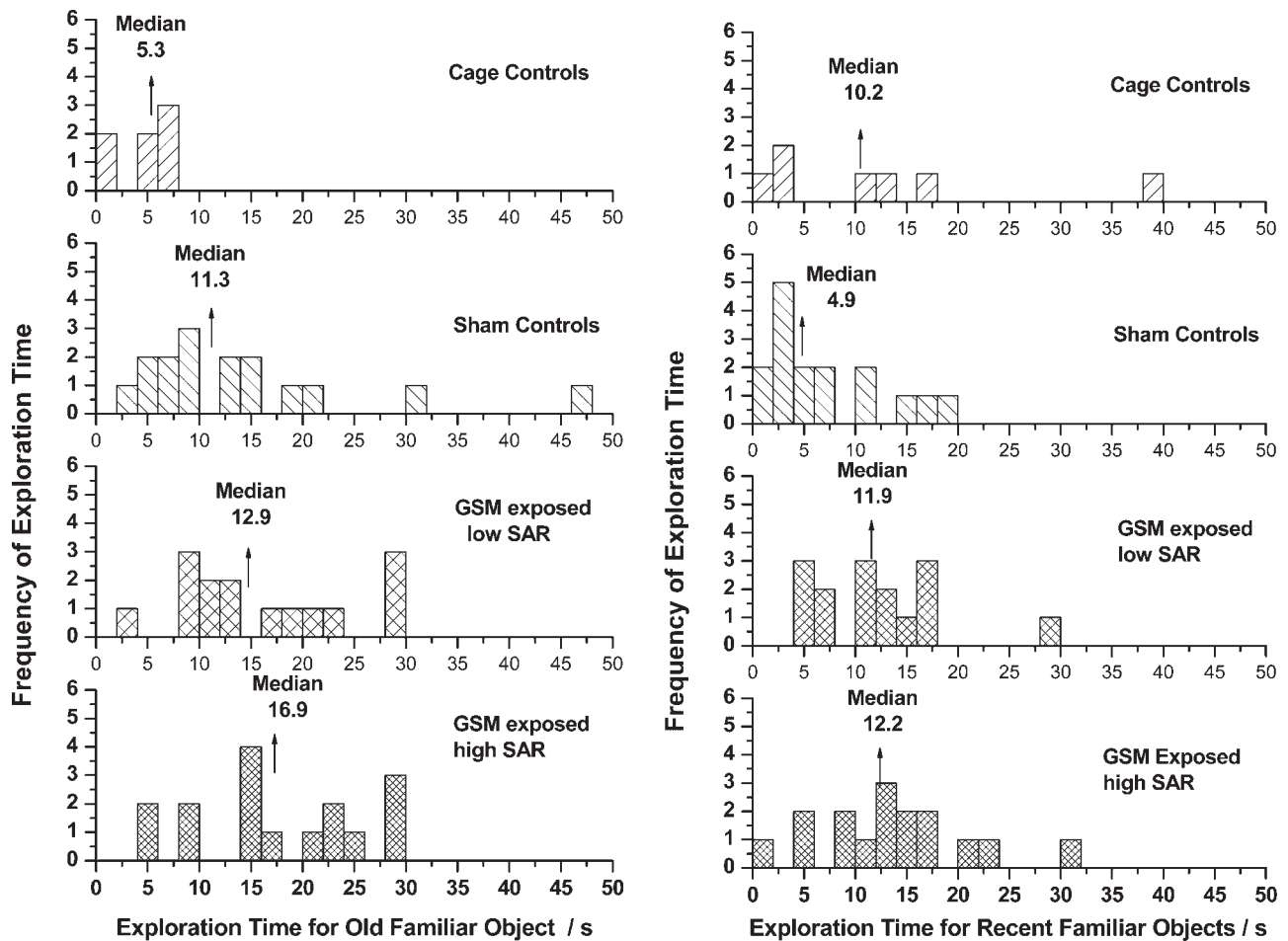


Fig. 7. Exploration time in the third trial of the episodic-like memory test. The results from each of the four groups (higher effect GSM exposed, lower effect GSM exposed, sham, cage control) are given for the old familiar objects and for the recent familiar objects. Median values for the exploration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

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

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

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

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

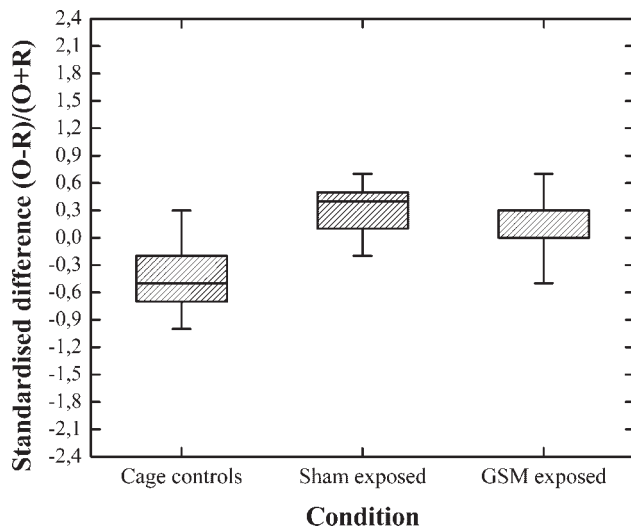


Fig. 8. Boxplot of the standardised difference $(O - R)/(O + R)$ for the exploration time of old familiar objects (O) versus the exploration time of the recent familiar objects (R). Median and interquartile range (IQR) are indicated in the boxes. The lines indicate 5–95% percentile ranges. GSM exposed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

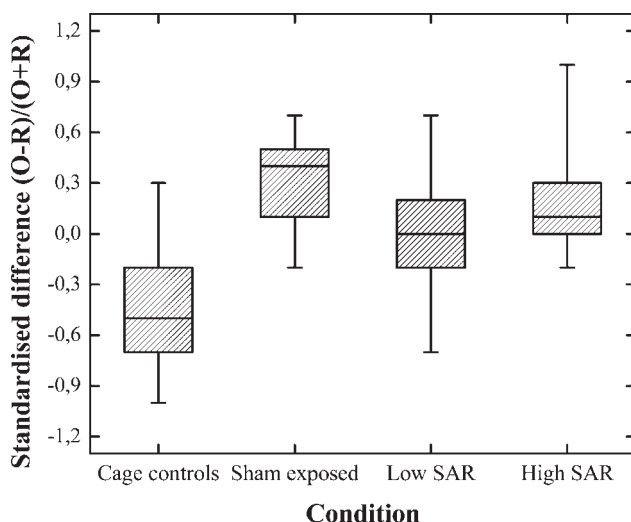


Fig. 9. As Figure 8, but high and low GSM exposure are shown separately. Both higher and lower GSM exposed rats spent a significantly shorter time exploring the old black familiar objects than the recent grey familiar objects when compared to sham exposed rats (Mann–Whitney $P = 0.05$ for higher GSM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

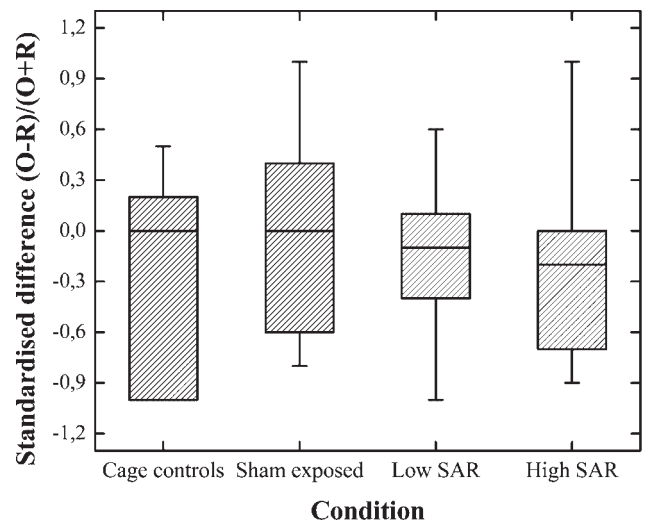


Fig. 10. As Figure 8, but the comparison is between displaced and stationary recent familiar objects in the episodic-like memory test. There was no statistically significant difference in exploration time between the displaced and stationary new familiar objects for the groups of sham exposed, GSM exposed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

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

DISCUSSION

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

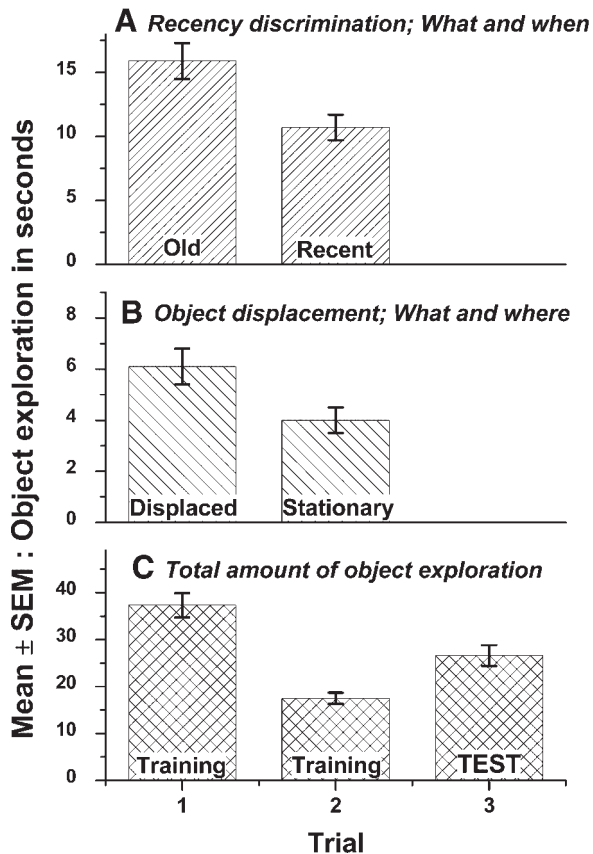


Fig. 11. All conditions except the cage controls are included in these observations. **A:** Mean time for exploration of the old familiar and recent familiar objects using the direct measurements from the test occasion, for males and females (paired *t*-test $P = 0.02$). **B:** Mean time for exploration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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

CONCLUSIONS

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

ACKNOWLEDGMENTS

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T8

Blood-brain barrier permeability in rats exposed to electromagnetic fields used in wireless communication

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Biological effects of *radio frequency* electromagnetic fields (EMF) on the blood-brain barrier (BBB) have been studied in Fischer 344 rats of both sexes. The rats were not anaesthetised during the exposure. All animals were sacrificed by perfusion–fixation of the brains under chloralhydrate anaesthesia after the exposure. The brains were perfused with saline for 3–4 minutes, and thereafter perfusion fixed with 4% formaldehyde for 5–6 minutes. Whole coronal sections of the brains were dehydrated and embedded in paraffin and sectioned at 5 μm . Albumin and fibrinogen were demonstrated immunohistochemically and classified as normal versus pathological leakage. In the present investigation we exposed male and female Fischer 344 rats in a Transverse Electromagnetic Transmission line chamber to microwaves of 915 MHz as continuous wave (CW) and pulse-modulated with different pulse power and at various time intervals. The CW-pulse power varied from 0.001 W to 10 W and the exposure time from 2 min to 960 min. In each experiment we exposed 4–6 rats with 2–4 controls randomly placed in excited and non-excited TEM-cells respectively. We have in total investigated 630 exposed rats at various modulation frequencies and 372 controls. The frequency of pathological rats is significantly increased ($p < 0.0001$) from 62/372 (ratio: 0.17 ± 0.02) for control rats to 244/630 (ratio: 0.39 ± 0.03) in all exposed rats. Grouping the exposed animals according to the level of specific absorbed energy (J/kg) give significant difference in all levels above 1.5 J/kg. The exposure was 915 MHz microwaves either pulse modulated (PW) at 217 Hz with 0.57 ms pulse width, at 50 Hz with 6.6 ms pulse width or continuous wave (CW). The frequency of pathological rats (0.17) among controls in the various groups is not significantly different. The frequency of pathological rats was 170/481 (0.35 ± 0.03) among rats exposed to pulse modulated (PW) and 74/149 (0.50 ± 0.07) among rats exposed to continuous wave exposure (CW). These results are both highly significantly different to their corresponding controls ($p < 0.0001$) and the frequency of pathological rats after exposure to pulsed radiation (PW) is significantly less ($p < 0.002$) than after exposure to continuous radiation (CW).

1. Introduction

The mammalian brain is protected from potentially harmful compounds in the blood by the so called blood-brain barrier (BBB). It is a selectively permeable, hydrophobic barrier that is readily crossed by small, lipid-soluble molecules [19,24]. Certain lipid-insoluble molecules such as glucose also readily crosses the cell layers constituting the barrier through carrier proteins that have a high affinity with specific molecules.

Although knowledge of the barrier's adaptive role is far from complete, there is a growing consensus that it serves not only to restrict entry of toxic polar molecules into the brain. It also serves as a regulatory system that stabilises and optimises the fluid environment of the brain's intracellular compartment [19,24].

The intact BBB protects the brain from damage, whereas a dysfunctioning BBB, as induced by epileptic seizures or extreme hypertension, allows influx of normally excluded hydrophilic molecules into the brain tissue. This might lead to cerebral oedema, increased intracranial pressure and in the worst case, irreversible brain damage.

The normal selective permeability of the blood-brain barrier (BBB) can be altered in several neuropathological

conditions and experimental situations. The BBB opens during an acute hypertensive episode [30], in epileptic seizures [15,31], and during therapeutic X-ray irradiation [16].

Several authors have reported that electromagnetic exposure alters BBB permeability [1–4,7,8,10–12,17,20,21,32,33]. Other authors have reported difficulties in confirming these findings [11,14,22,23,35–38]. The lack of agreement among investigators might be due to lack of an assay of BBB permeability that combines sensitivity and specificity.

Shivers et al. [29] observed during exposure to clinical magnetic resonance imaging (MRI) procedures an amplified vesicle-mediated transport of horseradish peroxidase across the endothelium to the extracellular compartment of the brain parenchyma of the rat. They did not, however, report on effects of the exclusive field components. The mechanism involved, however, points strongly to facilitation of *pinocytosis*-like transport of albumin through BBB [18].

In the present investigation we have studied the permeability of BBB to endogenous albumin and fibrinogen during exposure to microwaves. We exposed rats to 915 MHz microwaves as continuous wave (CW) and pulse-modulated at the various repetition rates ($4\text{--}217\text{ s}^{-1}$). Preliminary results are previously reported by Salford et al. [25–28].

Our model does not make use of radioactive tracers and does not supply foreign substances or anaesthetics to the

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rat during exposure. Instead it uses the animals own albumin, that does not penetrate the intact BBB. The passage of even minute amounts of albumin from the capillaries through an opened BBB into the surrounding brain tissue is revealed by our histopathological technique. This also allows for the identification of exactly in which constituents (neurones, glial cells, extracellular space) of the surrounding brain, where the albumin is situated. The perfusion-fixation method washes out all blood and its albumin content from the brain vessels including the capillaries. This facilitates the identification of albumin that has leaked out through the opened BBB into the brain.

2. Material and methods

2.1. Exposure in a TEM-cell

A Transverse Electromagnetic transmission line cell (TEM-cell) used for the RF exposure of rats was designed by dimensional scaling from previously constructed cells at the National Bureau of Standards (Crawford, 1974). TEM-cells are known to generate uniform TEM-fields for standard measurements.

The cell is enclosed in a wooden box that supports the outer conductor and central plate. The outer conductor is made of brass-net and is attached to the inner walls of the box. The centre plate, or septum, is constructed of aluminium and is held up by teflon braces which are screwed at the inner side walls.

To allow access to the inside of the cell both ends can be removed. The inside of the cell is ventilated through 18 holes (diam. 18 mm) in the side walls and top of the box and the brass-net of 50 mesh allows air to circulate. These holes are also used for examination of the interior during exposure. Probes for monitoring temperature inside the cell or of test object are inserted through these holes.

The rats are placed in plastic trays to avoid contact with the central plate and outer conductor. The bottom of the tray is covered with absorbing paper to collect urine and faeces.

The rats were exposed to 915 MHz electromagnetic radiation continuous wave and pulse-modulated with different repetition rates. The modulated RF-radiation consists of square wave shaped pulses with durations of 0.57, 4 or 6 ms and intensities (in Watts) during the presence of the pulse. Transmitted and absorbed power was measured at continuous wave exposure with and without rats in the TEM-cell. From these measurements the average SAR in the whole rat was calculated to be 1.2 ± 0.4 W/kg per watt of input power. This value was in good agreement with the theoretical estimate of 1.6 W/Kg per watt of input power that was used in the evaluation of the experiment [5,9,13,34].

2.2. Albumin and fibrinogen immunohistochemistry

Fischer 344 rats of both sexes, weighing 119–555 g (median: 202 g; 25% quartiles: 175 g; 75% quartiles: 273 g)

were used in these experiments (own breeding). The rats were not anaesthetised, during the exposure.

Both controls and exposed animals were sacrificed by perfusion-fixation of the brains under chloralhydrate anaesthesia between 20 minutes and 2 hours after the exposure. The brains were perfused with saline for 3–4 minutes, thereafter fixed in 4% formaldehyde for 5–6 minutes and immersion fixed in 4% formaldehyde for more than 24 hours. Whole coronal sections of the brains (3, 7 and 11 mm from the tip of the frontal pole) were dehydrated and embedded in paraffin and sectioned at 5 μ m. The chloralhydrate anaesthesia is necessary to avoid stress and blood pressure rise during perfusion-fixation procedure. Also for ethical reason no animals were sacrificed without chloralhydrate anaesthesia.

Albumin was demonstrated with the IgG fraction of rabbit anti-rat albumin (Cappel Research Products, Organon Teknika, Västra Frölunda, Sweden) diluted 1:16,000. Fibrinogen was demonstrated with rabbit anti-human fibrinogen (Dacopatts AB, Hägersten, Sweden), diluted 1:500. Incubation time for both was over night at 4°C.

Biotinylated swine anti-rabbit IgG was used as a secondary antibody. Then avidin, peroxidase conjugated, was coupled to the biotin and visualised with DAB (diaminobenzidine), counterstained with Meyer-HTX [6]. Standard control procedures were used for both albumin and fibrinogen.

The numbers of immunopositive extravasates were recorded under a microscope. None or occasional minor leakage was rated as normal, whereas one larger or several leakages were regarded as pathological. Immunopositive sites were, however, disregarded when localised in the hypothalamus, basally from the median eminence and laterally including the nucleus lateralis hypothalami, in the immediate vicinity of the third ventricles. These structures are well known for their insufficient blood-brain barrier and within any part of the choroid plexus of the ventricles consistently shows immunopositivities, mostly of a diffuse type, in the strain used in the present experiments.

2.3. Statistics

The frequency of occurrence of albumin extravasation in exposed and control animals were compared with chi-square or Fisher's exact probability test.

3. Results and discussions

Example of pathological leakage around small vessels is demonstrated in figure 1. The number of pathological rat brains among all control rats is 62 out of 372 (ratio: 0.17 ± 0.02). These findings are occasional and rare and are probably due to normal minor disturbances. The frequency of pathological rats among controls in the various groups is not significantly different ($p < 0.4$).

In the present investigation we exposed male and female Fischer 344 rats in a TEM-chamber to microwaves of 915 MHz as continuous wave (CW) and pulse-modulated

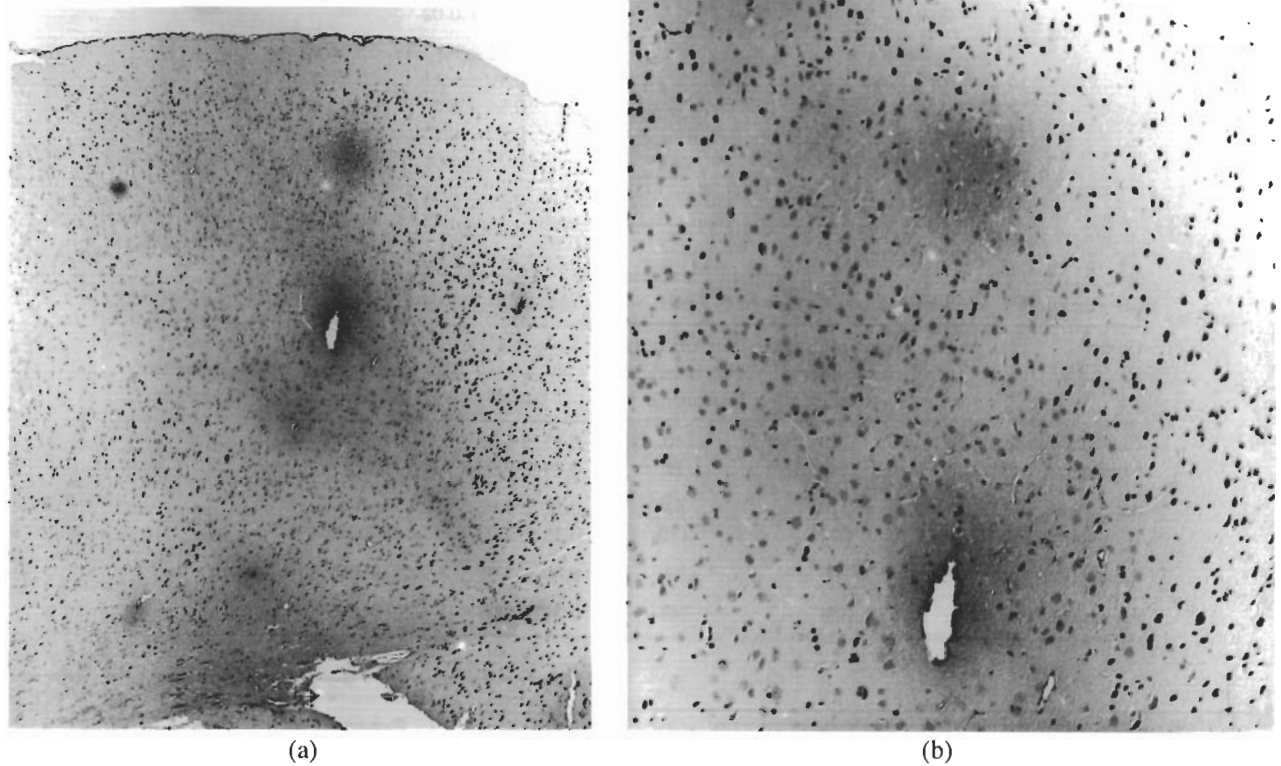


Figure 1. Example of pathological leakage around small vessels demonstrated through immunostaining against albumin. Hematoxylin eosin staining. (a) Section through frontal lobe with cortex and white matter, magnification $\times 5$. (b) Part of (a) with vessel, magnification $\times 10$.

with different pulse power and at various time intervals. The CW-pulse power varied from 0.001 W to 10 W and the exposure time from 2 min to 960 min. In each experiment we exposed 4 rats with 4 controls randomly placed in excited and non-excited TEM-cells, respectively.

We have in total investigated 630 exposed rats at various modulation frequencies and 372 controls. The frequency of pathological rats is significantly increased ($p < 0.0001$) from 62/372 (ratio: 0.17 ± 0.02) for control rats to 244/630 (ratio: 0.39 ± 0.03) in all exposed rats. Grouping the animals according to the level of specific absorbed energy (J/kg) that is normally referred to as "Specific Absorption" (SA), give significant difference ($p < 0.0001$) in all levels of absorbed energy above 1.5 J/kg compared to controls. (see table 1).

The exposure was 915 MHz microwaves either pulse modulated (PW) at 217 Hz with 0.57 ms pulse width, at 50 Hz with 6.6 ms pulse width or continuous wave (CW). The frequency of pathological rats (< 0.2) among controls in the various groups is not significantly different ($p < 0.4$). The frequency of pathological rats was 170/481 (0.35 ± 0.03) among rats exposed to pulse modulated (PW) and 74/149 (0.50 ± 0.07) among rats exposed to continuous wave exposure (CW). These results are both

highly significantly different to their corresponding controls ($p < 0.0001$) and the frequency of pathological rats after exposure to pulsed radiation (PW) is significantly less ($p < 0.002$) than after exposure to continuous radiation (CW) (table 2). The degree of pathological leakage in exposed animals is more severe and more frequent per animal compared to the controls.

The results of BBB-permeability of albumin in rats exposed to 915 MHz microwaves with different modulation frequencies for groups exposed to similar SAR values are displayed in figures 2–5. In figure 2 are displayed the results at SAR values 4×10^{-4} – 8×10^{-3} W/kg. Although the SAR values are very low, around 1 mW/kg, this group indicates the highest fraction of pathological findings recorded in the entire investigation. There seems to be a maximum effect around 8–50 Hz modulation frequency. In figure 3 is given the results at SAR values $(2-8) \times 10^{-2}$ W/kg. In this group the effect of CW and 50 Hz modulation is the same but the effect of 16 Hz and 8.3 & 217 Hz GSM modulation is very low and not significant. The overall effect of pulse modulated exposure is, however, significant. Figure 4 shows the results at SAR values 0.11–0.95 W/kg. The effect of CW exposure is about the same as in previous group but the effect of 217 Hz pulse modulation shows

Table 1
BBB-permeability of rat brain after 915 MHz RF-exposure at various intervals of specific absorption SA (J/kg).

Group of specific absorption (J/kg)	Normal score < 1	Pathological score ≥ 1	Ratio ± SD Pathological/all	Chi-square # Contr. <i>p</i>
Controls SA = 0	310	62	0.17 ± 0.02	
1.5 ≤ SA < 10	32	34	0.52 ± 0.11	< 0.00005
10 ≤ SA < 100	30	20	0.40 ± 0.11	< 0.0002
100 ≤ SA < 1000	116	61	0.34 ± 0.05	< 0.00005
1000 ≤ SA < 10 000	122	78	0.39 ± 0.05	< 0.00005
10 000 ≤ SA < 48 000	90	52	0.37 ± 0.01	< 0.00005
All exposed (630)	386	244	0.39 ± 0.03	< 0.00005

Table 2
BBB-permeability of rat brain after exposure to 915 MHz microwaves either Pulsed (PW) or Continuous Wave (CW).

Group	Number of rats with normal score < 1	Number of rats with pathological score ≥ 1	Ratio ± SD No. score ≥ 1 to all	<i>p</i> Chi-square exposed versus controls	<i>p</i> Chi-square PW versus CW
Controls pulsed (259)	219	40	0.15 ± 0.03	0.68	0.34
Controls CW (113)	91	22	0.19 ± 0.05	0.49	
All controls (372)	310	62	0.17 ± 0.02		
All exposed pulsed (481)	311	170	0.35 ± 0.03	< 0.00005	< 0.002
All exposed CW (149)	75	74	0.50 ± 0.07	< 0.00005	
All exposed (630)	386	244	0.39 ± 0.03	< 0.00005	

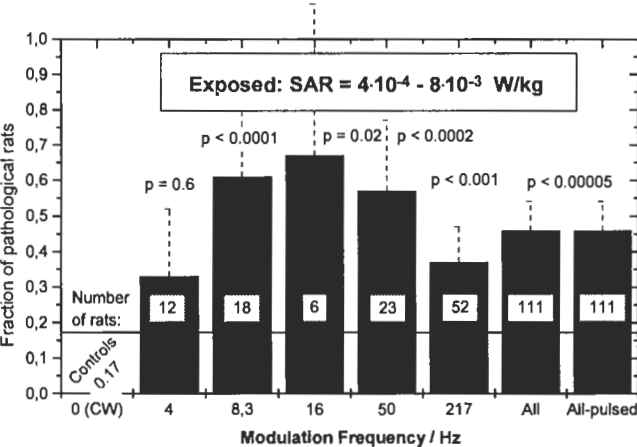


Figure 2. Blood-brain barrier permeability of albumin in rats exposed to 915 MHz microwaves with different modulation frequencies at SAR values 4×10^{-4} – 8×10^{-3} W/kg. The average fraction of pathological leakage in 372 controls was 0.17 ± 0.02 .

a significant effect compared to controls. The effect of 8.3 and 16 Hz modulation is however not significantly different from the controls. Figure 5 displays the results at SAR values 1.7–8.3 W/kg. In this group the SAR levels approach the threshold for thermal effects and the effect of CW exposure is very pronounced with a fraction of pathological rats of 55%. Surprisingly the effect of pulse modulated exposure is not significant at this high SAR level.

The frequency of pathological rats was 170/481 (0.35 ± 0.03) among rats exposed to pulse modulated (PW) and 74/149 (0.50 ± 0.07) among rats exposed to continu-

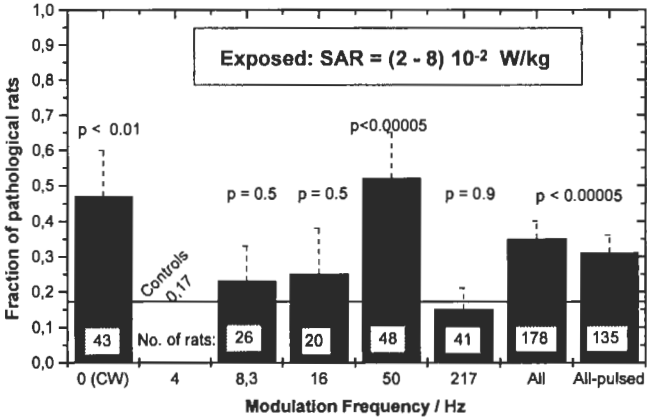


Figure 3. Blood-brain barrier permeability of albumin in rats exposed to 915 MHz microwaves with different modulation frequencies at SAR values $(2-8) \times 10^{-2}$ W/kg. The average fraction of pathological leakage in 372 controls was 0.17 ± 0.02 .

ous wave exposure (CW). These results are both highly significantly different from their corresponding controls ($p < 0.0001$) and the frequency of pathological rats after exposure to pulsed radiation (PW) is significantly less ($p < 0.002$) than after exposure to continuous radiation (CW) (table 2). This is a highly interesting observation as the current opinion is that pulse modulated electromagnetic fields are more potent in causing biological effects.

We have demonstrated that microwave exposure produces an unequivocal effect on the BBB in our rats. The clinical importance of this finding, however, is disputable. Our method for detection of albumin is extremely sensi-

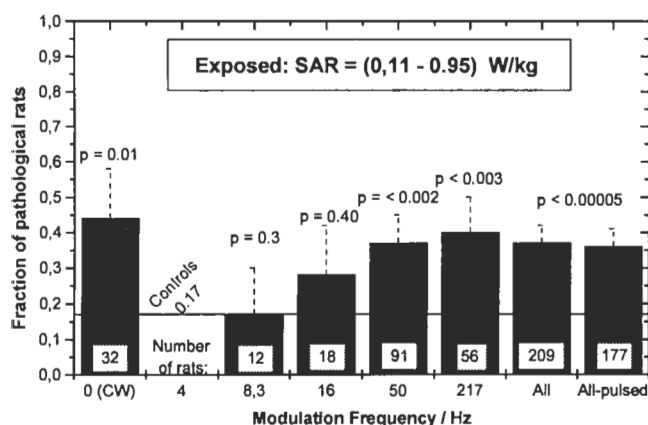


Figure 4. Blood-brain barrier permeability of albumin in rats exposed to 915 MHz microwaves with different modulation frequencies at SAR values 0.11–0.95 W/kg. The average fraction of pathological leakage in 372 controls was 0.17 ± 0.02 .

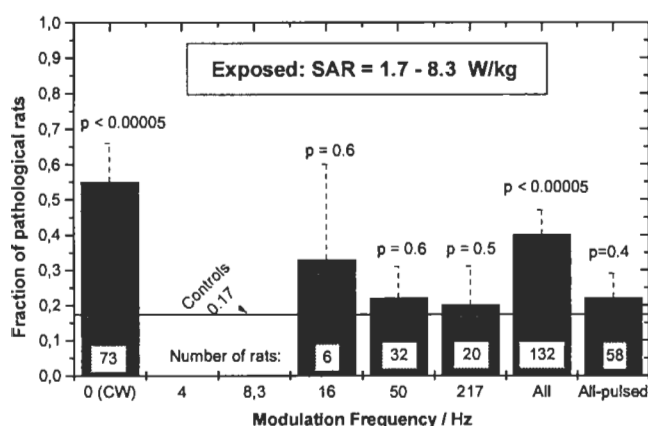


Figure 5. Blood-brain barrier permeability of albumin in rats exposed to 915 MHz microwaves with different modulation frequencies at SAR values 1.7–8.3 W/kg. The average fraction of pathological leakage in 372 controls was 0.17 ± 0.02 .

tive and reveals even minute amounts of albumin leaking through the BBB, so small that they may be harmless to the brain. However, the potential health hazards of the opening the BBB during exposure to wireless communication demands further investigation.

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In 1977 he began to study microwave induced hyperthermia for tumour therapy and since then this project has expanded very rapidly. He developed an equipment for local hyperthermia in patients with breast carcinoma, and recently equipment for heat treatment of benign prostate hyperplasia and menorrhagia. He started already in 1982 with biomedical applications of nuclear magnetic resonance (NMR) and has developed and built a special equipment for performing *in vivo* NMR studies in both animal and man. In close co-operation with several other scientists he is also conducting *in vitro* studies of the NMR relaxation times in tissues of thyroid and brain, also studying the influence of paramagnetic ions and super-paramagnetic particles. He has studied extensively clinical measurements of flow, microcirculation, diffusion and perfusion with NMR. During the past years he has been deeply involved in studying the effects of electromagnetic fields on healthy brain and on brain-tumours. He was co-sponsor for the New York Academy of Science conference on the biological and health effects of clinical NMR examination held in 1991. At present he is deeply involved in studies of the effect of low power pulsed microwave fields on the blood brain barrier and other biological systems. He is also engaged in using electromagnetic fields for tumour therapy and has made a lot of progress in the use of high voltage electric fields for permeabilization of tumour cell membranes *in vivo*. The objective of that investigation is to study the destructive effect of electroporomeabilization on tumours and its ability to introduce toxic compounds into tumour cells. He is studying the therapeutic effect of electroporomeabilization on tumour cells in presence of chemotherapeutic agents (for example, Bleomycin) and radioimmunotherapeutic agents.



Leif G. Salford was born in Malmö, Sweden, in 1941. He received the MD degree from the University of Lund in 1969, and the Ph.D. degree from the same university in 1974 with the thesis "Influence of profound hypoxia on regional metabolism, blood flow and cell morphology in rat brain". During 1972–1973 he was Wrightsman scholar at the Dept. of Neurology, Cornell Medical Center, New York Hospital, NY. In 1977 he became associate professor of neurosurgery, Lund University, and in 1979 Consultant Neurosurgeon. He was Professor and Chairman, Department of Neurosurgery, Kuwait University, 1981–1983, and Professor of Neurosurgery and Director of the Departments of Clinical Neurosciences, Sahlgrenska University Hospital and Göteborg University, Sweden, 1993–1996. His actual positions are: Professor and Chairman, Dept. of Neurosurgery, Lund University Hospital, and Director of the Institute for Clinical Neuroscience, Lund University, Lund, Sweden. Dr. Salford is Chairman of the Neuro-oncology Committee of the World Federation of Neurosurgical Societies, and President of the European Association for Neuro-oncology. He is President of the Swedish Neurosurgical Society, Honorary President of the Scandinavian Neurooncology Society and Expert in the EU Information Society Forum.

Dr. Salford's research is concentrated on the malignant primary brain tumours and on the search for efficient treatment against this hitherto incurable type of cancer. This has also resulted in his studies of opening the



extensive reports and books. His scientific career began in 1963 with studies

blood-brain-barrier by the use of electromagnetic fields in order to reach the tumour cells with cytotoxins, and in his interest in the biophysical effects of EMF upon brain and tumour biology. Dr. Salford is Director of the Laboratory for Experimental Neurooncology, Lund University, where animal models and tissue cultures play an important role in his research in close cooperation with technological institutions such as Medical Radiation Physics. He strives to utilize the latest progress in technology in his search for solutions to medical problems. Another result of this collaboration is the introduction of electroporomeabilisation *in vivo* as a tool for treatment of intraparenchymatous tumours which has proven efficient in connection with cytotoxins in the rodent brain tumour model.



Arne Brun, MD, Ph.D., is a professor of neuropathology, University Hospital, Lund, Sweden. His publications are mainly on central nervous system diseases such as dementias, especially the Alzheimers' disease and frontal dementias, also cerebrovascular incl. BBB-studies.