

In order to compare incidence rates, 95% confidence intervals were computed.

The observed number of cancer cases is the number of all the cancer cases in the exposed cohort in the period between 7/97 - 6/98.

In order to estimate relative risk, rate ratios were computed using the rate of 3 different cohorts as the base (the expected values):

The rate in a nearby clinic (which serves a population of 1222 people, all of them living in area B) during the same period of time, i.e. 7/97 - 6/98. In order to compare area A and area B populations we used:

χ^2 test to compare origin and sex division

t- test to compare age means

The national incidence rates of the whole country.

The incidence rates in the whole town of Netanya where the 2 clinics (of area A and B) are located. The data of 2 and 3 were given to us by the Israel cancer registry and are updated to the years 91-94.

We also examined the history of the exposed cohort (of the A area) for malignancies in the 5 years before the exposure began and found only 2 cases in comparison to 8 cases detected one year after the transmitter station came into service.

Results

Of the 622 people of area A, eight cases of different kinds of cancer were diagnosed in a period of only one year (from July 1997 to June 1998). Details on these cases are presented in Table 1. Briefly, we found 3 cases of breast carcinoma, and one case of ovary carcinoma, lung carcinoma, Hodgkin's disease, osteoid osteoma, and hypernephroma.

This rate of cancers in the population of area A was compared both with the rate of 31 cases per 10,000 per year in the general population and the 2/1222 rate recorded in a nearby clinic. To each one of the rates, a 95 percent confidence interval was calculated (Table 2): the rates in area A were significantly higher than both those in area B, and the population as a whole.

A comparison of the relative risk revealed that there were 4.15 times more cases in area A than in the entire population.

The population characteristics of areas A and B were very similar (Table 2-5). The χ^2 test for comparing gender and origin frequencies showed no significant differences in these parameters between the two areas. Age means, as compared by t-test and age distribution stratum also showed no significant difference between the two groups.

Table 2a lists the rates of cancer incidence of areas A and B compared to data of the whole town of Netanya. The comparison clearly indicated that the cancer incidence of women in area A is significantly higher ($p < 0.0001$) compared with that of the whole city.

Discussion

Our study indicates an association between an increased incidence of cancer and living in proximity to a cell-phone transmitter station.

Studies of this type are prone to biases. Possible methodological artefacts to explain our alarming results were considered:

Differences in socioeconomic class and employment status, and demographic heterogeneity due to differences in age, sex and ethnicity were excluded. The two areas that were compared have very closely matched environment, workplace and occupational characteristics.

Confounding variables affecting individuals could not be absolutely adjusted for, however, there was no ionizing radiation that could affect the whole community except the previously mentioned mobile antenna station. There is no traffic density in this area, neither is there any industry or any other air pollution. The population of area A

(on which adequate data could be gathered) did not suffer from uncommon genetic conditions, nor did they receive carcinogenic medications.

Differences in diagnosis and registration of cancer cases. Although we cannot altogether exclude the possibility that higher awareness of the physician responsible for area A led to an artificial increase in cancer cases in this area, this possibility seems to us very unlikely, since both are qualified family physicians.

Several findings are of particular interest:

The measured level of RF radiation (power density) in the area was low; far below the current guidelines based on the thermal effects of RF exposure. We suggest, therefore, that the current guidelines be re-evaluated.

The enormous short latency period; less than 2 years, indicates that if there is a real causal association between RF radiation emitted from the cell-phone base station and the cancer cases (which we strongly believe there is), then the RF radiation should have a very strong promoting effect on cancer at very low radiation!

Although the possibility remains that this clustering of cancer cases in one year was a chance event, the unusual sex pattern of these cases, the 6 different cancer kinds, and the fact that only one patient smoked make this possibility very improbable and remote. It should be noted that 7 out of 8 cancer cases were women, like in the work of Maskarinec (25) who found 6 out of 7 leukemia cases in proximity to radio towers to occur in girls. Such unusual appearances of cancer cases due to one accused factor on two completely different occasions is alarming.

We are aware of at least 2 areas in which a drastic increase in the incidence of cancer cases occurred near a cell-phone antenna, however, the setup was not suitable for a well design study of those cases. In one of them (which also got publication in the daily newspapers) there were 6 out of 7 cancer cases in women working in a store in close proximity to a cell-phone antenna.

In conclusion, the results of this study showed that there was a significantly greater incidence of cancers of all kinds within the vicinity of a cell-phone transmitter station.

It would be certainly too premature to draw any conclusions from our results before they are confirmed and repeated by other studies from other areas, particularly in view of the fact that a great majority of papers on this subject showed that RF fields and mobile telephone frequencies were not genotoxic, did not induce genetic effects in vitro and in vivo, and were not found to be teratogenic or to induce cancers (24). The results of this paper should, however, serve as an alarm and emphasize the need for further investigations.

Addendum

At one year following the close of the study, 8 new cases of cancer were diagnosed in area A and two cases in area B. Among the cases diagnosed in area A was one of osteoid osteoma, the second case from the beginning of the study.

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Acknowledgment

The authors are grateful to Aviva Zeer M.Sc from the Zinman College of Physical Education and Sport Sciences At the Wingate Institute, Israel, for help with the statistical analysis.

The opinions expressed herein are solely those of the writers and do not necessarily reflect the opinions of the institutions with which the writers are associated.

Table 1: Cancer cases in area A

NAME	AGE	SEX	ORIGIN ¹	SMO - KIN G	CANCER TYPE	Measured power density in $\mu\text{w}/\text{cm}^2$
Hemda	52	f	ash	No	Ovary ca stage 1	$0.3\mu\text{w}/\text{cm}^2$
Edna	42	f	sph	No	Breast ca in situ	$0.4\mu\text{w}/\text{cm}^2$
Tania	54	f	ash	No	Breast ca	$0.5\mu\text{w}/\text{cm}^2$
Neli	67	f	ash	Yes	Breast ca	$0.4\mu\text{w}/\text{cm}^2$
Galit	24	f	ash	No	Hodgkins	$0.5\mu\text{w}/\text{cm}^2$
Miriam	61	f	sph	No	Lung ca	$0.3\mu\text{w}/\text{cm}^2$
Masal	37	f	sph	No	Osteoid osteoma	$0.4\mu\text{w}/\text{cm}^2$
Max	78	m	ash	No	Hypernephroma	$0.3\mu\text{w}/\text{cm}^2$

1. Origin: ash - Ashkenazien Jews sph - Spharadic Jews

Table 2: Cancer rates in area A, B and the total population.

	No. of cancer cases	populati on size	Rate per year per 10,000	confide interval lower limit	ce (95%) upper limit	relative risk
Area A	8	622	129	40.1	217.2	4.15
Area B	2	1222	16	-6.3	39.0	0.53
total populat	31	10,000	31	20.1	41.9	1.00

Table 2a: Cancer rates in area A, B and the whole town.

	Male		Female	
	rate	Relative rate	rate	relative rate
Area A	33	1.4	262	10.5
Area B	17	0.7	16	0.6
Whole town	24	1	25	1

Table 3: Comparing area A to area B by gender.

Gender	Area A		Area B	
	N	%	N	%
male	290	49	669	49
female	305	51	685	51

Table 4: Comparing area A to area B by origin.

Origin	Area		Area	
	N	%	N	%
Sfaradic	340	55	551	45
Ashkenaz	239	38	620	51
Russian	41	7	51	4

Table 5: Comparing age means in both areas.

	Area A		Area B	
	mean	Std	mean	std
age	26.5	17.9	25.5	12.4

Table 5: Age distribution by stratum.

	0-1	1-10	10-20	20-30	30-40	40-50	50-60	60-70	>70
IRUS	16	143	157	65	70	88	41	21	21
POLEG	31	285	257	139	180	158	83	55	34



United States Department of the Interior

OFFICE OF THE SECRETARY

WASHINGTON, D.C. 20240

FEB - 7 2014



In Reply Refer To: (ER 14/0001) (ER 14/0004).

Mr. Eli Veenendaal
National Telecommunications and Information
Administration
U.S. Department of Commerce
1401 Constitution Avenue, N.W.
Washington, D.C. 20230

Dear Mr. Veenendaal:

The Department of the Interior (Department) has reviewed the above referenced proposal and submits the following comments and attachment for consideration. Because the First Responder Network Authority (FirstNet) is a newly created entity, we commend the U.S. Department of Commerce for its timely proposals for NEPA implementing procedures.

The Department believes that some of the proposed procedures are not consistent with Executive Order 13186 Responsibilities of Federal Agencies to Protect Migratory Birds, which specifically requires federal agencies to develop and use principles, standards, and practices that will lessen the amount of unintentional take reasonably attributed to agency actions. The Department, through the Fish and Wildlife Service (FWS), finds that the proposals lack provisions necessary to conserve migratory bird resources, including eagles. The proposals also do not reflect current information regarding the effects of communication towers to birds. Our comments are intended to further clarify specific issues and address provisions in the proposals.

The Department recommends revisions to the proposed procedures to better reflect the impacts to resources under our jurisdiction from communication towers. The placement and operation of communication towers, including un-guyed, unlit, monopole or lattice-designed structures, impact protected migratory birds in two significant ways. The first is by injury, crippling loss, and death from collisions with towers and their supporting guy-wire infrastructure, where present. The second significant issue associated with communication towers involves impacts from non-ionizing electromagnetic radiation emitted by them (See Attachment).

In addition to the 147 Birds of Conservation Concern (BCC) species, the FWS has listed an additional 92 species as endangered or threatened under the Endangered Species Act. Together with the bald and golden eagle, this represents 241 species of birds whose populations are in trouble or otherwise merit special protection, according to the varying criteria of these lists. The Department suggests that FirstNet consider preparing a programmatic environmental impact statement (see attachment) to determine and address cumulative impacts from authorizing FirstNet projects on those 241 species for which the incremental impact of tower mortality, when

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added to other past, present, and reasonably foreseeable future actions, is most likely significant, given their overall imperiled status. Notwithstanding the proposed implementing procedures, a programmatic NEPA document might be the most effective and efficient method for establishing best management practices for individual projects, reducing the burden to individual applicants, and addressing cumulative impacts.

Categorical Exclusions

The Department has identified 13 of the proposed categorical exclusions (A-6, A-7, A-8, A-9, A-10, A-11, A-12, A-13, A-14 A-15, A-16, A-17, and A-19) as having the potential to significantly affect wildlife and the biological environment. Given this potential, we want to underscore the importance of our comments on FirstNet's procedural guidance under Environmental Review and Consultation Requirements for NEPA Reviews and its list of extraordinary circumstances in Appendix D.

Environmental Review and Consultation Requirements for NEPA Reviews

To ensure there are no potentially significant impacts on birds from projects that may otherwise be categorically excluded, the Department recommends including the Migratory Bird Treaty Act and the Bald and Golden Eagle Protection Act to the list of requirements in this section.

Extraordinary Circumstances

To avoid potentially significant impacts on birds from projects that may otherwise be categorically excluded, the Department recommends including species covered under the Migratory Bird Treaty Act and the Bald and Golden Eagle Protection Act to the list of environmentally sensitive resources. Additionally, adding important resources to migratory birds such as sites in the Western Hemisphere Shorebird Reserve Network and Audubon Important Bird Areas to the paragraph on areas having special designation or recognition would help ensure their consideration when contemplating use of a categorical exclusion.

Developing the Purpose and Need

The Department recommends inclusion of language that would ensure consideration of all other authorities to which NEPA is supplemental as opposed to simply the FirstNet mission. As currently written, the procedures are limited to ensuring the purpose and need considers the FirstNet mission. If strictly applied, this approach would severely limit the range of reasonable alternatives, and likely preclude consideration of more environmentally benign locations or construction practices.

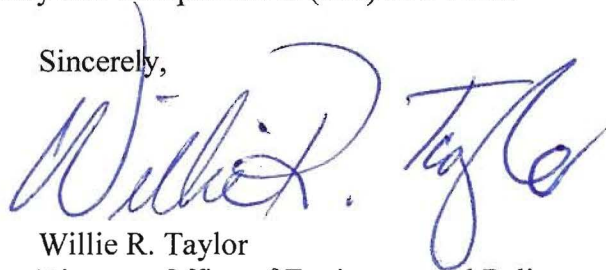
Environmental Review Process, Apply NEPA Early in the Process, Where Action is by Non-Federal Entity

The Department recommends that FirstNet be required to coordinate with federal agencies having jurisdiction by law or special expertise on construction and lighting of its network of towers.

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Thank you for the opportunity to comment on the draft document. If you have any questions concerning the comments, please contact Diana Whittington, NEPA Migratory Bird lead, at (703) 358-2010. If you have any questions regarding Departmental NEPA procedures, contact Lisa Treichel, Office of Environmental Policy and Compliance at (202) 208-7116.

Sincerely,



Willie R. Taylor
Director, Office of Environmental Policy
and Compliance

Enclosure

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Enclosure A

Background

The placement and operation of communication towers, including un-guyed, unlit, monopole or lattice-designed structures, impact protected migratory birds in two significant ways.

The first is by injury, crippling loss, and death from collisions with towers and their supporting guy-wire infrastructure, where present. Mass mortality events tend to occur during periods of peak spring and fall songbird bird migration when inclement weather events coincide with migration, and frequently where lights (either on the towers and/or on adjacent outbuildings) are also present. This situation has been well documented in the U.S. since 1948 in the published literature (Aronoff 1949, see Manville 2007a for a critique). The tallest communication towers tend to be the most problematic (Gehring *et al.* 2011). However, mid-range (~400-ft) towers as proposed by the First Responder Network Authority (FirstNet, a newly created entity under the Department of Commerce) can also significantly impact protected migratory birds, as can un-guyed and unlit lattice and monopole towers (Gehring *et al.* 2009, Manville 2007a, 2009, 2013a). Mass mortalities (more than several hundred birds per night) at unguyed, unlit monopole and lattice towers were documented in fall 2005 and 2011 in the Northeast and North Central U.S. (*e.g.*, Manville 2007a). It has been argued that communication towers including “short” towers do not impact migratory birds, including at the population level (*e.g.*, Arnold and Zink 2011), but recent findings have contradicted that assertion (Manville 2007a, 2013a, Longcore *et al.* 2012, 2013).

The second significant issue associated with communication towers involves impacts from non-ionizing electromagnetic radiation emitted by these structures. Radiation studies at cellular communication towers were begun circa 2000 in Europe and continue today on wild nesting birds. Study results have documented nest and site abandonment, plumage deterioration, locomotion problems, reduced survivorship, and death (*e.g.*, Balmori 2005, Balmori and Hallberg 2007, and Everaert and Bauwens 2007). Nesting migratory birds and their offspring have apparently been affected by the radiation from cellular phone towers in the 900 and 1800 MHz frequency ranges – 915 MHz is the standard cellular phone frequency used in the United States. However, the electromagnetic radiation standards used by the Federal Communications Commission (FCC) continue to be based on thermal heating, a criterion now nearly 30 years out of date and inapplicable today. This is primarily due to the lower levels of radiation output from microwave-powered communication devices such as cellular telephones and other sources of point-to-point communications; levels typically lower than from microwave ovens. The problem, however, appears to focus on very low levels of non-ionizing electromagnetic radiation. For example, in laboratory studies, T. Litovitz (personal communication) and DiCarlo *et al.* (2002) raised concerns about impacts of low-level, non-thermal electromagnetic radiation from the standard 915 MHz cell phone frequency on domestic chicken embryos – with some lethal results (Manville 2009, 2013a). Radiation at extremely low levels (0.0001 the level emitted by the average digital cellular telephone) caused heart attacks and the deaths of some chicken embryos subjected to hypoxic conditions in the laboratory while controls subjected to hypoxia were unaffected (DiCarlo *et al.* 2002). To date, no independent, third-party field studies have been conducted in North America on impacts of tower electromagnetic radiation on migratory birds. With the European field and U.S. laboratory evidence already available,

independent, third-party peer-reviewed studies need to be conducted in the U.S. to begin examining the effects from radiation on migratory birds and other trust species.

Discussion

Collision Deaths and Categorical Exclusions

Attempts to estimate bird-collision mortality at communication towers in the U.S. resulted in figures of 4-5 million bird deaths per year (Manville 2005, 2009). A meta-review of the published literature now suggests, based on statistically determined parameters, that mortality may be 6.8 million birds per year in Canada and the U.S.; the vast majority in the United States (Longcore *et al.* 2012). Up to 350 species of birds have been killed at communication towers (Manville 2007a, 2009). The Service's Division of Migratory Bird Management has updated its voluntary, 2000 communication tower guidelines to reflect some of the more recent research findings (Manville 2013b). However, the level of estimated mortality alone suggests at a minimum that FirstNet prepare an environmental assessment to estimate and assess the cumulative effects of tower mortality to protected migratory birds.

A second meta-review of the published mortality data from scientific studies conducted in the U.S. and Canada (Longcore *et al.* 2013) strongly correlates population effects to at least 13 species of Birds of Conservation Concern (BCC, USFWS 2008). These are mortalities to BCC species based solely on documented collisions with communication towers in the U.S. and Canada, ranging from estimated annual levels of mortality of 1 to 9% of their estimated total population. Among these where mortality at communication towers was estimated at over 2% annually are the Yellow Rail, Swainson's Warbler, Pied-billed Grebe, Bay-breasted Warbler, Golden-winged Warbler, Prairie Warbler, and Ovenbird. Longcore *et al.* (2013) emphasized that avian mortality associated with anthropogenic sources is almost always reported in the aggregate, *i.e.*, "number of birds killed," which cannot detect species-level effects necessary to make effective and meaningful conservation assessments, including determining cumulative effects. These new findings strongly suggest the need for at least an environmental assessment by FirstNet, or more likely, an environmental impact statement.

Radiation Impacts and Categorical Exclusions

There is a growing level of anecdotal evidence linking effects of non-thermal, non-ionizing electromagnetic radiation from communication towers on nesting and roosting wild birds and other wildlife in the U.S. Independent, third-party studies have yet to be conducted in the U.S. or Canada, although a peer-reviewed research protocol developed for the U.S. Forest Service by the Service's Division of Migratory Bird Management is available to study both collision and radiation impacts (Manville 2002).

As previously mentioned, Balmori (2005) found strong negative correlations between levels of tower-emitted microwave radiation and bird breeding, nesting, and roosting in the vicinity of electromagnetic fields in Spain. He documented nest and site abandonment, plumage deterioration, locomotion problems, reduced survivorship, and death in House Sparrows, White Storks, Rock Doves, Magpies, Collared Doves, and other species. Though these species had historically been documented to roost and nest in these areas, Balmori (2005) did not observe these symptoms prior to construction and operation of the cellular phone towers. Balmori and Hallberg (2007) and Everaert and Bauwens (2007) found similar strong negative correlations

among male House Sparrows. Under laboratory conditions, DiCarlo *et al.* (2002) raised troubling concerns about impacts of low-level, non-thermal electromagnetic radiation from the standard 915 MHz cell phone frequency on domestic chicken embryos – with some lethal results (Manville 2009). Given the findings of the studies mentioned above, field studies should be conducted in North America to validate potential impacts of communication tower radiation – both direct and indirect – to migratory birds and other trust wildlife species.

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Anthropogenic radiofrequency electromagnetic fields as an emerging threat to wildlife orientation

Anthropogenic radiofrequency electromagnetic fields as an emerging threat to wildlife orientation

Alfonso Balmori. Anthropogenic radiofrequency electromagnetic fields as an emerging threat to wildlife orientation. *Science of the Total Environment*. Volumes 518-519, 15 June 2015, Pages 58-60.

Abstract

The rate of scientific activity regarding the effects of anthropogenic electromagnetic radiation in the radiofrequency (RF) range on animals and plants has been small despite the fact that this topic is relevant to the fields of experimental biology, ecology and conservation due to its remarkable expansion over the past 20 years. Current evidence indicates that exposure at levels that are found in the environment (in urban areas and near base stations) may particularly alter the receptor organs to orient in the magnetic field of the earth. These results could have important implications for migratory birds and insects, especially in urban areas, but could also apply to birds and insects in natural and protected areas where there are powerful base station emitters of radiofrequencies. Therefore, more research on the effects of electromagnetic radiation in nature is needed to investigate this emerging threat.

Highlights

- The growth of wireless telecommunication technologies causes increased electrosmog.
- Radio frequency fields in the MHz range disrupt insect and bird orientation.
- Radio frequency noise interferes with the primary process of magnetoreception.
- Existing guidelines do not adequately protect wildlife.
- Further research in this area is urgent.

Excerpts

Different animal groups are sensitive to low frequency electromagnetic fields, and many species with receptor organs are provided with important orientation cues from natural electric fields (Kalmijn, 1988). Animals can use the direction of the magnetic field as a compass and the intensity of the magnetic field as a component of the navigational map, with light-dependent reactions in specialised photo-pigments and reactions involving small crystals of magnetite, using one of these systems, or both simultaneously, depending on the animal groups (Kirschvink et al., 2001, Johnsen and Lohmann, 2005, Wiltchko et al., 2007, Hsu et al., 2007, Ritz et al., 2009 and Wajnberg et al., 2010).

Some insects, like bumblebees (*Bombus terrestris*), can interact with floral electric fields and electric field sensing constitutes a potentially important sensory modality. The perception of weak electric fields by bees in nature, which should be considered alongside vision and olfaction, may have an adaptive value (Clarke et al., 2013). An applied static magnetic field affects circadian rhythms, magnetosensitivity and orientation of insects through cryptochromes, and a prolonged weakening of the geomagnetic field affects the immune system of rats (Roman and Tombarkiewicz, 2009 and Yoshii et al., 2009).

In the radiofrequency range, the rapid development and increased use of wireless telecommunication technologies led to a substantial change in the radio-frequency electromagnetic field (RF-EMF) exposure (Levitt and Lai, 2010). This increased exposure was most consistently observed in outdoor areas due to emissions from radio and mobile phone base stations (Urbinello et al., 2014). Current evidence indicates that exposure at levels found in the environment (in urban areas and near base stations), may particularly alter the receptor organs to orient in the magnetic field of the earth, although the species conservation implications are unknown. Radio frequency fields in the MHz range disrupt birds' orientation interfering directly with the primary processes of magnetoreception and therefore disable the avian compass as long as they are present (Wiltschko et al., 2014). Ritz et al. (2004 & 2009) reported the sensitivity for orientation of European robins (*Erithacus rubecula*) to radiofrequency magnetic fields. The orientation of migratory birds is disrupted when very weak high-frequency fields (broad-band field of 0.1-10 MHz of 85 nT or a 1.315 MHz field of 480 nT) are added to the static geomagnetic field of 46.000 nT (Thalau et al., 2006). It was convincingly demonstrated that robins are unable to use their magnetic compass in the presence of urban electromagnetic radiofrequency noise in the frequency range of 2 kHz-5 MHz (Engels et al., 2014). Therefore, electrosmog scrambles birds' magnetic sense and this finding could inform policies written to protect the habitats of endangered species.

As with birds, radio frequency magnetic fields disrupt magnetoreception in insects. The geomagnetic field reception in American cockroach is sensitive to weak radio frequency field causing a disruptive effect (Vacha et al., 2009), so these authors suggest that electromagnetic smog will have to be taken more seriously in animal magnetoreception experiments. In an experimentally-generated electromagnetic field of about 1 V/m with a realistic (and even lower) power intensity similar to those surrounding communication masts, the results and observations suggest that GSM (Global System for Mobile communications) 900 MHz radiation might have a severe impact on the nerve cells of exposed ants, especially affecting the visual and olfactory memory, causing the loss of their ability to use visual cues and suggesting that electromagnetic radiation may have an impact on the orientation behaviour and navigation of animals that use magnetic fields to find their way (Cammaerts et al., 2012 and Cammaerts et al., 2014). Honeybees are sensitive to pulsed electromagnetic fields generated by mobile phones and observable changes in the bee behaviour could be one explanation for the loss of colonies (Favre, 2011). Magnetoreception system in Monarch butterfly orientation (Guerra et al., 2014) may be also suffering interference with anthropogenic radio frequency magnetic fields and this, together with other factors (Brower et al., 2012), may be a cause of their population decline.

Electromagnetic fields act via activation of voltage-gated calcium channels (Pall, 2013). Changes in the size of the magnetic granules upon applying additional magnetic field to the cells of *Apis mellifera* were observed, and this size fluctuation triggered the increase of calcium intracellular (Hsu et al., 2007). Therefore, we may hypothesise that some of the disruptive effects of radio frequency fields on the orientation of animals may be related to the interference with calcium channels.

An aversive effect on bats has been found in habitats exposed to radiofrequency radiation (1-4 GHz) when compared with matched sites where no such radiation can be detected (Nicholls and Racey, 2009). Cattle exposed to radiofrequency emissions (900 MHz) from nearby base stations may suffer changes in the redox proteins and enzyme activities. It was also found that some are sensitive to radiation, while others are not (Hässig et al., 2014).

Exposure to low intensity radiation can have a profound effect on biological processes (Bolen,

1994). Although there is a good degree of evidence on the injurious effects of radiofrequency electromagnetic fields on the immune system, pineal gland, circadian rhythm, oxidative stress and teratogenicity, these topics remain controversial (Lerchl et al., 2008, Takahashi et al., 2009, Jin et al., 2012, Qin et al., 2012, Bilgici et al., 2013, Tsybulin et al., 2013, Yakymenko et al., 2014 and Cao et al., 2015). Conversely, there is a scientific agreement regarding harmful effects of radio frequency radiation on human reproduction (Adams et al., 2014). Low-voltage electricity current-generated electromagnetic field can produce a significantly negative effect on the breeding success of birds (*Ciconia ciconia*) nesting directly on electricity lines (Vaitkuvienė and Dagys, 2014) and these same results have been found in nests exposed to radiofrequency radiation near phone masts (Balmori, 2005).

The health risk of electromagnetic fields to aquatic organisms needs to be addressed (Lee and Yang, 2014). The potential interactions between diadromous fishes of conservation importance and the electromagnetic fields and subsea noise from marine renewable energy developments are being studied (Gill et al., 2012).

In a systematic review of published scientific studies on the potential ecological effects of radiofrequency electromagnetic fields (RF-EMF) in the range of 10 MHz-3.6 GHz, about two thirds of the reviewed studies show ecological effects of RF-EMF at high, as well as at low, dosages (Cucurachi et al., 2013). The low dosages are compatible with real field situations, and could be found under environmental conditions (Cucurachi et al., 2013 and Balmori, 2014). However, studies conducted in real field situations must be made with a sufficient experimental exposure time, since results with a short period of exposure are likely to be ambiguous (e.g. 48 h in Vijver et al., 2013).

A limited number of studies have addressed the effects of radiofrequency radiation on plants indicating that these effects depend on the plant family, growth stage, exposure duration, frequency, and power density, among other factors (Senavirathna and Takashi, 2013 and Halgamuge et al., 2015). There are two papers warning on negative effects of radio frequencies from mobile phone masts on trees (Balmori, 2004 and Waldmann-Selsam and Eger, 2013) and researchers have found very worrying effects in laboratory studies (Pesnya and Romanovsky, 2013). The results of these preliminary findings indicate that further research on this topic is extremely urgent.

These results could have important implications for wildlife, especially in urban and suburban areas, but also in rural, natural and protected areas where there are powerful base station emitters of radiofrequencies (Bürgi et al., 2014). Such effects have not yet been examined, but the consequences continue due to the fact that the existing guidelines of public health protection only consider the effects of short-term thermal exposure (Hyland, 2000) and do not adequately protect wildlife. EMF safety standard should be based on the more sensitive, natural biological response (Blank, 2014). Therefore, more research on the effects of electromagnetic radiation in nature is needed to investigate this emerging threat (Balmori, 2014).

Anthropogenic radiofrequency electromagnetic fields as an emerging threat to wildlife orientation. review

By: Balmori A

Published in: Sci Total Environ 2015; 518 : 58 - 60

Endpoint

- wildlife orientation

(Study character: medical/biological study, survey ⓘ)



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House Sparrows, White Storks, Rock Doves, Magpies, Collared Doves, and other species. Though these species had historically been documented to roost and nest in these areas, Balmori (2005) did not observe these symptoms prior to construction and operation of the cellular phone towers. Balmori and Hallberg (2007) and Everaert and Bauwens (2007) found similar strong negative correlations among male House Sparrows. Under laboratory conditions, DiCarlo et al. (2002) raised troubling concerns about impacts of low-level, non-thermal electromagnetic radiation from the standard 915 MHz cell phone frequency on domestic chicken embryos- with some lethal results (Manville 2009). **Given the findings of the studies mentioned above, field studies should be conducted in North America to validate potential impacts of communication tower radiation both direct and indirect - to migratory birds and other trust wildlife species."**

The full text of the letter, the addendum and citations are available at:

<http://1.usa.gov/1jn3CZg>

Dept. of Interior attacks FCC regarding Adverse Impact of Cell Tower Radiation on Wildlife

The Department of Interior charges that the FCC standards for cell phone radiation are outmoded and no longer applicable as they do not adequately protect wildlife.

FOR IMMEDIATE RELEASE

PRLog (Press Release) - Mar. 24, 2014 - BERKELEY, Calif. -- The Director of the Office of Environmental Policy and Compliance of the United States Department of the Interior sent a letter to the National Telecommunications and Information Administration in the Department of Commerce that addresses the Interior Department's concern that cell tower radiation has had negative impacts on the health of migratory birds and other wildlife.

The Interior Department accused the Federal government of employing outdated radiation standards set by the Federal Communications Commission (FCC), a Federal agency with no expertise in health. The standards are no longer applicable because they control only for overheating and do not protect organisms from the adverse effects of exposure to the low-intensity radiation produced by cell phones and cell towers:

"the electromagnetic radiation standards used by the Federal Communications Commission (FCC) continue to be based on thermal heating, a criterion now nearly 30 years out of date and inapplicable today."

The Department criticized the Federal government's proposed procedures for placement and operation of communication towers, and called for "independent, third-party peer-reviewed studies" in the U.S. to examine the effects of cell tower radiation on "migratory birds and other trust species."

More information is available at:

<http://www.saferemr.com/2014/03/dept-of-interior-attacks-...>

Dependence of non-thermal biological effects of microwaves on physical and biological variables: implications for reproducibility and safety standards

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Abstract

Diverse biological responses, including adverse health effects, to non-thermal (NT) microwaves (MW) have been described by many research groups all over the world. The aim of this paper is to provide an overview of the complex dependence of these effects on various physical and biological parameters, which must be controlled in replication studies.

Besides well-known dependencies on carrier frequency and modulation, emerging data suggest dependencies of NT MW effects on polarization, intermittence and coherence time of exposure, static magnetic field, electromagnetic stray fields, genotype, gender, physiological and individual traits, cell density during exposure. Data also indicate that duration of exposure may be as important as power density (PD) and specific absorption rate (SAR). Further evaluation of these dependencies are needed for understanding the mechanisms by which NT MW affect biological systems, planning *in vivo* and epidemiological studies, developing medical treatments, setting safety standards, and minimizing the adverse effects of MW from mobile communication.

Key words: non-thermal effects of microwaves, mobile (cellular) phones, safety standards.

List of abbreviations:

Anomalous viscosity time dependence (AVTD); blood-brain barrier (BBB); catalase (CAT); Digital Enhanced (former European) Cordless Telecommunications (DECT); circularly polarized (CP); continuous wave (CW); Digital Advanced Mobile Phone System (DAMPS); discontinuous transmission (DTX); electroencephalographic (EEG); electromagnetic field (EMF); embryonic stem (ES) cells; ethidium bromide (EtBr); extremely low frequency (ELF); Gaussian Minimum Shift Keying (GMSK); Ginkgo biloba (Gb); Global System for Mobile Communication (GSM); glutathione peroxidase (GSH-Px); International Commission for Non-Ionizing Radiation Protection (ICNIRP); linearly polarized (LP); malondialdehyde (MDA); micronucleus (MN) assay; microwaves (MWs); N-acetyl-beta-d-glucosaminidase (NAG); nitric oxide (NO); non-thermal (NT); ornithine decarboxylase (ODC); phorbol ester 12-myristate 13-acetate (PMA); phosphorylated H2AX histone (γ -H2AX); power density (PD);

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regional cerebral blood flow (rCBF); Russian National Committee on Non-Ionizing Radiation Protection (RNCNIRP); specific absorption rate (SAR); static magnetic field (SMF); superoxide dismutase (SOD); Time Division Multiple Access (TDMA); tumor suppressor p53 binding protein 1 (53BP1); ultraviolet (UV); Universal Mobile Telecommunications System (UMTS).

Introduction

Exposures to non-ionizing electromagnetic fields vary in many parameters: power (specific absorption rate, incident power density), wavelength/frequency, near field/far field, polarization (linear, circular), continuous wave (CW) and pulsed fields (that include variables such as pulse repetition rate, pulse width or duty cycle, pulse shape, pulse to average power, etc.), modulation (amplitude, frequency, phase, complex), static magnetic field (SMF) and electromagnetic stray fields at the place of exposure, overall duration and intermittence of exposure (continuous, interrupted), acute and chronic exposures. With increased absorption of energy, so-called thermal effects of microwaves (MW) are usually observed that deal with MW-induced heating. Specific absorption rate (SAR) or power density (PD) is a main determinate for thermal MW effects. Several other physical parameters of exposure have been reported to be of importance for so-called non-thermal (NT) biological effects, which are induced by MW at intensities well below any measureable heating¹⁻¹¹. An important question is how these physical parameters could be taken into account in setting safety standards.

Most often, current safety standards are based on thermal MW effects observed in short-term (acute) exposures. On the other hand, NT MW effects, especially those induced during prolonged (chronic) exposures, are accepted and taken into account for setting the national safety standards in some countries such as Russia¹⁰⁻¹². It should be noted that, in contrast to the ICNIRP (International Commission for Non-Ionizing Radiation Protection) safety standards¹³ which are based on the acute thermal effects of MW, the standards adopted by the Russian National Committee on Non-Ionizing Radiation Protection (RNCNIRP) are based on experimental data from chronic (up to 4 month) exposures of animals to MW at various physical parameters including intensity, frequency and modulation, obtained from research performed in the former Soviet Union¹⁰⁻¹².

Since setting the current safety standards, the situation with exposure of the general population to MW has changed significantly. Nowadays, most of the human population is chronically exposed to MW signals from various sources including mobile phones and base stations. These exposures are characterized by low intensities, varieties and complexities of signals, and long-term durations of exposure that are comparable with a lifespan. So far, the “dose” (accumulated absorbed energy that is measured in radiobiology as the dose rate multiplied by exposure time) is not adopted for the MW exposures and SAR or PD is usually used for guidelines. To what degree SAR/PD can be applied to the nowadays NT MW chronic exposures is not known and the current state of research demands reevaluation of the safety standards¹².

There are two main approaches to treat numerous data regarding NT MW effects. The first one is based on the consideration of these effects in dependence on various physical parameters and biological variables as has consistently been described in many experimental studies and will be reviewed in this paper. The second approach is based on neglecting or minimizing the experimentally observed NT MW effects based on the current state of theoretical physical science that is insufficient for comprehensive expla-

nation of the NT MW effects. As a result of such various treatments of the experimental data, the safety standards significantly vary, up to 1000 times, among countries.

The literature on the NT MW effects is very broad. There are four lines of evidence for the NT MW effects: (1) altered cellular responses in laboratory *in vitro* studies and results of chronic exposures *in vivo* studies^{3, 11, 14}; (2) results of medical application of NT MW in the former Soviet Union countries^{4, 7, 15, 16}; (3) hypersensitivity to electromagnetic fields (EMF); (4) epidemiological studies suggesting increased cancer risks for mobile phone users¹⁷⁻¹⁹.

This paper is not intended to be a comprehensive review of this literature. In this review, we will focus on the studies which evaluate dependence of the NT MW effects on physical parameters and biological variables.

Experimental studies

The first data on the NT effects of MW in so-called millimeter range (wavelength 1-10 mm in vacuum) was obtained by Vilenskaya and co-authors²⁰ and Devyatkov²¹. Highly resonant effects of ultra-weak MW (near 70 GHz) on the induction of λ -phage were first established by Webb²², and subsequently corroborated²³. In these and subsequent studies the observed spectra of MW action were found to have the following common properties: (1) the MW effects were strongly dependent on the frequency (frequency windows), (2) there was an associated power (intensity) threshold below which no effect was observed, and above which the effects of exposure depended only weakly on power over several orders of magnitude (so-called S-shaped or sigmoid dependence), (3) the occurrence of MW effects depended on the duration of exposure, a certain minimum duration of exposure was necessary for an effect to manifest itself. These important regularities of the NT MW effects have previously been reviewed^{2, 7-9, 24-27}.

The first investigations of the NT MW effects at lower frequency ranges were performed by Blackman and colleagues²⁸⁻³⁰ and Adey and colleagues^{31, 32}. These groups found dependence of the NT MW effects on modulation.

Since that time, other groups have confirmed and extended the main findings of these pioneering studies as will be reviewed below.

Frequency dependence and frequency windows

The effects of NT MW on DNA repair in *E. coli* K12 AB1157 were studied by the method of anomalous viscosity time dependence (AVTD)^{33, 34}. The AVTD method is a sensitive technique to detect changes in conformation of nucleoids/chromatin induced by either genotoxic or stress factors³⁵⁻⁴⁰. Significant inhibition of DNA repair was found when X-ray-irradiated cells were exposed to MW within the frequency ranges of 51.62-51.84 GHz and 41.25-41.50 GHz. The effects were observed within two “frequency windows”, both displaying a pronounced resonance character with the resonance frequencies of 51.755 GHz and 41.32 GHz, respectively^{33, 34}. Of note, these MW effects were observed at PD well below any thermal effects and could not be accounted for by heating. The frequency windows of resonance type have often been termed “resonances” as also will be used below.

The resonance frequency of 51.755 GHz was stable within the error of measurements, ± 1 MHz with decreasing the PD from $3 \cdot 10^{-3}$ to 10^{-19} W/cm²^{34, 35}. At the same time, the

half-width of the resonance decreased from 100 MHz to 3 MHz revealing an extremely sharp dependence on frequency ($Q \sim 10^4$). This sharp narrowing of the 51.755 GHz resonance with decreasing the PD from $3 \cdot 10^{-3}$ to 10^{-7} W/cm² followed by an emergence of new resonances, 51.675 ± 0.001 , 51.805 ± 0.002 , and 51.835 ± 0.005 GHz^{35, 41}. The half-widths of all these resonances including the main one, 51.755 ± 0.001 GHz, were about 10 MHz at the PD of 10^{-10} W/cm². These data were interpreted in the framework of the model of electron-conformational interactions as a splitting of the main resonance 51.755 GHz by the MW field³⁵.

The MW effects were studied at different PD and several frequencies around the resonance frequency of 51.675 GHz⁴¹. This resonance frequency was found to be stable, ± 1 MHz, within the PD range of 10^{-18} - 10^{-8} W/cm². Along with disappearance of the 51.675 GHz resonance response at the sub-thermal PD of 10^{-6} - 10^{-3} W/cm², a new resonance effect arose at 51.688 ± 0.002 GHz⁴¹. This resonance frequency was also stable within the PD range studied.

Taken together, these data^{34, 35, 41} suggested a sharp rearrangement of the frequency spectra of MW action, which was induced by the sub-thermal MW. The half-widths of all three resonances depended on PD, changing either from 2-3 MHz to 16-17 MHz (51.675 GHz and 51.668 GHz resonances) or from 2-3 MHz to 100 MHz (51.755 GHz resonance)^{35, 41}. The data indicated also that dependencies of half-width on PD might vary for different resonance frequencies.

Significant narrowing in resonance response with decreasing PD has been found when studying the growth rate in yeast cells⁴² and chromatin conformation in thymocytes of rats⁴³. In the Gründler's study, the half-width of the resonance (near 41 GHz) decreased from 16 MHz to 4 MHz as PD decreased from 10^{-2} W/cm² to 5 pW/cm²⁴².

Thus, the results of studies with different cell types indicate that narrowing of the resonance window upon decrease in PD is one of the general regularities in cell response to NT MW. This regularity suggests that many coupled oscillators are involved non-linearly in the response of living cells to NT MW as has previously been predicted by Fröhlich⁴⁴.

Gapeev *et al.* studied effects of MW exposure (frequency range 41.75-42.1 GHz, frequency increment 50 MHz, PD 240 μ W/cm²) on the respiratory burst induced by calcium ionophore A23187 and phorbol ester 12-myristate 13-acetate (PMA) in the peritoneal neutrophils of mice^{45, 46}. MW inhibited the respiratory burst. MW effect displayed resonance-like dependence on frequency, the resonance frequency and half-width of the resonance being 41.95 GHz and 160 MHz, respectively ($Q = 260$)^{45, 46}. In other studies, Gapeev *et al.* analyzed acute zymosan-induced paw edema in mice^{47, 48}. MW exposure of animals at the PD of 0.1 mW/cm² resulted in decrease of the paw edema that was frequency-dependent in the range of 42-43 GHz.

Based on the extrapolation from the data obtained in the extremely high frequency range (30-300 GHz), the values for half-width of resonances at the frequency range of mobile phones (0.9-2 GHz) were estimated to be 1-10 MHz⁴⁰. Effects of GSM (Global System for Mobile Communication) MW on chromatin conformation and 53BP1 (tumor suppressor p53 binding protein 1)/ γ -H2AX (phosphorylated H2AX histone) DNA repair foci in human lymphocytes were studied in this frequency range^{38-40, 49}. Dependence of these MW effects on carrier frequency was observed^{38, 40, 49}. This dependence was replicated in independent experiments with lymphocytes from twenty six healthy and hypersensitive persons^{38, 39, 49}.

Tkalec and colleagues exposed duckweed (*Lemna minor* L.) to MW at the frequencies of 400, 900, and 1900 MHz⁵⁰. The growth of plants exposed for 2 h to a 23 V/m

electric field of 900 MHz significantly decreased in comparison with the control, while an electric field of the same strength but at 400 MHz did not have such effect. A modulated field at 900 MHz strongly inhibited the growth, while at 400 MHz modulation did not influence the growth significantly. At both frequencies, a longer exposure mostly decreased the growth and the highest electric field (390 V/m) strongly inhibited the growth. Exposure of plants to lower field strength (10 V/m) for 14 h caused a significant decrease at 400 and 1900 MHz while 900 MHz did not influence the growth. Peroxidase activity in exposed plants varied, depending on the exposure characteristics. Observed changes were mostly small, except in plants exposed for 2 h to 41 V/m at 900 MHz where a significant increase (41%) was found. The authors concluded that MW might influence plant growth and, to some extent, peroxidase activity. However, the effects of MW strongly depended on the characteristics of the field exposure such as frequency and modulation. These dependences were confirmed in further study of the same group^{51, 52}.

Remondini *et al.* analyzed changes in gene expression in human EA.hy926 endothelial cells using gene microarrays⁵³. Cells were exposed to MW (SAR 1.8-2.5 W/kg, 1 h exposure) either at 900-MHz GSM Basic mode or 1800-MHz GSM Basic mode. Exposure to 900 MHz resulted in up-regulation in 22 genes and down-regulation in 10 genes. No significant change in gene expression was observed after exposure to 1800 MHz.

Sigmoid intensity dependences and power windows

It was found by Devyatkov *et al.* that NT MW effects display sigmoid dependence on intensity above certain intensity thresholds²¹. This type of PD dependence for the MW effects was observed in other studies as previously reviewed^{7-9, 24, 25}.

The data obtained in experiments with *E. coli* cells and rat thymocytes provided new evidence for sigmoid type of PD dependence and suggested that similar to ELF effects, MW effects may be observed within specific “intensity windows”^{35, 41, 43, 54}. The most striking example of the sigmoid PD dependence was found at the resonance frequency of 51.755 GHz³⁵. When exposing *E. coli* cells at the cell density of $4 \cdot 10^8$ cell/ml, the effect reached saturation at the PD of 10^{-18} - 10^{-17} W/cm² and did not change up to PD of 10^{-3} W/cm². In these experiments, the direct measurements of PD below 10^{-7} W/cm² were not available and lower PD was obtained using calibrated attenuators. Therefore, some uncertainty in the evaluation of the lowest PD was possible. The background MW radiation in this frequency range has been estimated to be 10^{-21} - 10^{-19} W/m²/Hz⁵⁵. Based on the experimentally determined half-width of the 51.755 GHz resonance, 1 MHz³⁵, the background PD was estimated as 10^{-19} - 10^{-17} W/cm² within the 51.755 GHz resonance. The resonance MW effects on *E. coli* cells were observed at the PD very close to the estimated background value^{35, 41, 56-58}. These data suggested that the PD dependence of MW effect at the specific resonance frequencies might have a threshold comparable with the background level. Dependence of the MW effect on PD at one of the resonance frequencies, 51.675 GHz, had the shape of “intensity window” in the PD range from 10^{-18} to 10^{-8} W/cm²⁴¹. It is interesting, that no MW effect at this resonance frequency was observed at sub-thermal and thermal PD. This type of PD dependence has supported hypothesis about possible rearrangement of the frequency MW spectra action by the MW field³⁵. The position of the PD window varied between different resonance frequen-

cies and depended on cell density during exposure of cells⁴¹. Despite some uncertainty in the evaluation of PD at the levels below 10^{-7} W/cm² in the referred studies the data indicated that NT MW at the resonance frequencies may result in biological effects at very low intensities comparable with intensities from base stations and other MW sources used in mobile communication.

Gapeev *et al.* have studied dependence of the MW effects at the resonance frequency of 41.95 GHz on the respiratory burst induced by calcium ionophore A23187 and PMA in the peritoneal neutrophils of mice^{45, 46}. Inhibitory effects of MW exposure has been observed at the PD of 0.001 mW/cm² and displayed sigmoid dependence on PD at higher power densities^{45, 46}.

In other study, Gapeev *et al.* analyzed acute zymosan-induced paw edema in mice⁴⁸. MW exposure of animals at the frequency of 42.2GHz and exposure duration of 20 min decreased the paw edema. Sigmoid dependence of this effect on PD has been obtained with a maximum reached at the PD of 0.1 mW/cm².

In their pioneering study on blood-brain barrier (BBB) permeability, Oscar and Hawkins exposed rats to MW at 1.3 GHz and analyzed BBB permeability by measuring uptake of several neutral polar substances in certain areas of the brain⁵⁹. A single, 20 min exposure, to continuous wave (CW) MW increased the uptake of D-mannitol at average power densities of less than 3 mW/cm². Increased permeability was observed both immediately and 4 h after exposure, but not 24 h after exposure. After an initial rise at 0.01 mW/cm², the permeability of cerebral vessels to saccharides decreased with increasing microwave power at 1 mW/cm². Thus, the effects of MW were observed within the power window of 0.01-0.4 mW/cm². Differences in the level of uptake occurred between effects of CW MW and pulsed MW of the same average power. Microwaves of the same average power but different pulse characteristics also produced different uptake levels.

These findings on “power windows” for BBB permeability have been subsequently corroborated by the group of Persson and Salford^{60, 61}. In their recent study, the effects of GSM MW on the permeability of the BBB and signs of neuronal damage in rats were investigated using a real GSM programmable mobile phone in the 900 MHz band⁶². The rats were exposed for 2 h at an SAR of 0.12, 1.2, 12, or 120 mW/kg. Albumin extravasation and also its uptake into neurons increased after 14 d. The occurrence of dark neurons in the rat brains increased later, after 28 d. Both effects were seen already at 0.12 mW/kg with only slight increase, if any, at higher SAR values.

Duration of exposure and time after exposure

Bozhanova with co-authors reported that the effect of cellular synchronization induced by NT MW depended on duration of exposure and PD⁶³. The dependence on duration of exposure fitted to exponential function. The important observation was that in order to achieve the same synchronization of cells, the decrease in PD could be compensated by the increase in the duration of exposure.

Kwee and Raskmark analyzed effects of MW at 960 MHz and various SARs, 0.021, 0.21, and 2.1 mW/kg on proliferation of human epithelial amnion cells⁶⁴. These authors reported linear correlations between exposure time to MW at 0.021 and 2.1 mW/kg and the MW-induced changes in cell proliferation albeit no such clear correlation was seen at 0.21 mW/kg.

MW exposure of *E. coli* cells and rat thymocytes at PDs of 10^{-5} - 10^{-3} W/cm² resulted in significant changes in chromatin conformation if exposure was performed at resonance frequencies during 5-10 min^{33, 43, 65}. Decrease in the MW effects due to lowering the PD by orders of magnitude down to 10^{-14} - 10^{-17} W/cm² was compensated by several-fold increase of exposure time to 20-40 min⁵⁷. At the relatively longer duration of exposure, more than 1 h, the same effect at the lowest PD of 10^{-19} W/cm² was observed⁵⁷.

Gapeyev *et al.* found the frequency and power dependence of anti-inflammatory effect of low-intensity MW exposure (0.1 mW/cm²) using the model of acute zymosan-induced footpad edema in mice⁴⁷. Single whole-body MW exposure of mice at the frequencies of 42.2, 51.8, and 65 GHz after zymosan injection reduced both the footpad edema and local hyperthermia. Some other frequencies from the frequency range of 37.5-70 GHz were less effective or not effective at all. At the frequency of 42.2 GHz the effect had sigmoid dependence on exposure duration with a maximum at 20-80 min. A linear dependence with significantly lower increment was observed at a 10-fold less intensity (0.01 mW/cm²). However, this decrease in the effect was compensated by a slight increase in duration of exposure from 80 min to 120 min.

The MW effects on *E. coli* cells depended on the post-exposure time⁵⁶⁻⁵⁸. This dependence had an initial phase of increase about 100 min post-exposure followed by a phase, which was close to a plateau, around 100 min. A trend to decrease in effect was observed at longer times up to 300 min^{56, 58}.

Significant MW-induced changes in chromatin conformation were observed when rat thymocytes were analyzed in-between 30-60 min after exposure to MW⁴³. This effect nearly disappeared if the cells were incubated more than 80 min between exposure and analysis.

Gapeev *et al.* have studied dependence of the MW effect on the function of the mouse peritoneal neutrophils in dependence on duration of exposure at the frequency of 41.95 GHz and the PD of 240 μ W/cm²^{45, 46}. This dependence had a bell-shaped form with the maximal effects at 20 - 40 min of exposure.

In recent studies, human lymphocytes from peripheral blood of healthy and hypersensitive to EMF persons were exposed to MW from the GSM mobile phones^{38, 39}. MW induced changes in chromatin conformation similar to those induced by heat shock, which remained up to 24 h after exposure. It was found in the same and following studies that GSM MW at the carrier frequency of 915 MHz and UMTS (Universal Mobile Telecommunications System) MW at 1947.4 MHz inhibited formation of 53BP1/ γ -H2AX DNA repair foci and these adverse effects remained at 72 h after an 1-h exposure^{38, 39, 49}.

Of note is that prolonged MW exposures were associated with less prominent effects than shorter exposures in some studies^{51, 66, 67}. This type of dependence on exposure duration was explained by adaptation of the exposed systems to the MW exposure⁶⁷.

The data indicate that there is a time window for observation of the NT MW effects, which may be dependent on endpoint measured, cell type, duration and PD of exposure. The data from different groups suggest also that duration of exposure may have a larger role for some NT MW effects than PD/SAR.

Coherence time

MW exposure of L929 fibroblasts was performed by the group of Litovitz⁶⁸. MW at 915 MHz modulated at 55, 60, or 65 Hz approximately doubled ornithine decarboxylase

(ODC) activity after 8 h. Switching the modulation frequency from 55 to 65 Hz at coherence times of 1.0 s or less abolished enhancement, while times of 10 s or longer provided full enhancement. These results suggested that the microwave coherence effects are remarkably similar to those observed previously with extremely low frequency (ELF) magnetic fields by the same authors.

Intermittence

Diem and colleagues exposed cultured human diploid fibroblasts and cultured rat granulosa cells to intermittent and continuous MW (1800 MHz; SAR 1.2 or 2 W/kg; different modulations; during 4, 16 and 24 h; intermittent 5 min on/10 min off or continuous exposure)⁶⁹. Comet assay was applied to analyze DNA single- and double-strand breaks. MW-induced effects occurred after 16 h exposure in both cell types and after different mobile-phone modulations. The intermittent exposure showed a stronger effect than continuous exposure.

Remondini *et al.* analyzed changes in gene expression in human HL-60 leukemia cells using gene microarrays⁵³. Cells were exposed to MW (SAR 1.0-1.3 W/kg, 1800 MHz DTX mode, 24 h exposure) either continuously or intermittently, 5 min ON/5 min OFF. Gene expression was affected by intermittent exposure but not continuous exposure.

Modulation

There is strong experimental evidence for the role of modulation in the diverse biological effects of NT MW both *in vitro* and *in vivo*^{32, 60, 70-79}. Examples include different types of modulation such as amplitude-, speech and phase modulations: (i) Amplitude modulation at 16 Hz, but not 60 Hz or 100 Hz, of a 450-MHz MW increased activity of ODC⁷⁴. (ii) Speech-modulated 835-MHz MW produced no effect on ODC as compared to the typical signal from a TDMA (Time Division Multiple Access) digital cellular phone⁷¹. (iii) Phase-modulated GSM-1800 MW (Gaussian Minimum Shift Keying, GMSK) at 1.748 GHz induced micronuclei in human lymphocytes while CW MW did not⁷⁵.

Gapeev and co-authors studied production of reactive oxygen species (ROS) in isolated peritoneal neutrophils of mice using a model of synergistic reaction of calcium ionophore A23187 and phorbol ester PMA^{79, 80}. MW exposure at 41.95 GHz, continuous wave mode and 50 $\mu\text{W}/\text{cm}^2$, inhibited ROS production. MW modulated with the frequency of 1 Hz resulted in stimulation of the synergistic reaction. Modulation frequencies of 0.5, 2, 4, and 8 Hz did not cause significant effects, and modulation frequencies of 0.1, 16, and 50 Hz inhibited the synergistic reaction.

In other study, Gapeev *et al.* analyzed acute zymosan-induced paw edema in mice⁴⁸. MW exposure of animals at the PD of 0.1- 0.7 mW/cm^2 and some “effective” frequencies in the range of 42-43 GHz decreased the paw edema. Application of different modulation frequencies from the range of 0.03–100 Hz to MW exposure at the effective carrier frequency of 42.2 GHz did not lead to considerable changes in the effect. In contrast, modulation of MW at the “ineffective” carrier frequencies of 43.0 and 61.22 GHz by frequencies from the ranges of 0.07–0.1 and 20–30 Hz resulted in a maximal anti-inflammatory effects. The results suggested a complex dependence of

the anti-inflammatory action of low-intensity MW on carrier and modulation frequencies.

Huber with co-authors investigated effects of MW similar to those used in mobile communication, a “base-station-like” and a “handset-like” signal (10 g tissue-averaged spatial peak-SAR of 1 W/kg for both conditions), on waking regional cerebral blood flow (rCBF) in 12 healthy young men⁷⁶. The effect depended on the spectral power in the amplitude modulation of the carrier frequency such that only “handset-like” MW exposure with its stronger low-frequency components but not the “base-station-like” MW exposure affected rCBF. This finding supported previous observations of these authors⁷⁷ that pulse modulation of MW is of importance for changes in the waking and sleep EEG, and substantiated the notion that pulse modulation is crucial for MW-induced alterations in brain physiology.

Markkanen *et al.* exposed cdc48-mutated *Saccharomyces cerevisiae* yeast cells to 900 or 872 MHz MW, with or without exposure to ultraviolet (UV) radiation, and analyzed apoptosis⁷⁸. Amplitude modulated (217 pulses per second) MW significantly enhanced UV induced apoptosis in cells, but no effect was observed in cells exposed to unmodulated fields at the identical time-average SAR of 0.4 W/kg that was lower than the ICNIRP safety standards.

Persson and colleagues studied effects of MW of 915 MHz as CW and pulse-modulated with different pulse power and at various time intervals on permeability of the blood-brain barrier (BBB) in Fischer 344 rats⁶⁰. Albumin and fibrinogen were demonstrated immunochemically and classified as normal versus pathological leakage. The CW-pulse power varied from 0.001 W to 10 W and the exposure time from 2 min to 960 min. The frequency of pathological rats significantly increased in all exposed rats. Grouping the exposed animals according to the level or specific absorption energy (J/kg) gave significant difference in all levels above 1.5 J/kg. The exposure was 915 MHz MW either pulse modulated at 217 Hz with 0.57 ms pulse width, at 50 Hz with 6.6 ms pulse width, or CW. The frequency of pathological rats was significantly higher in MW-exposed groups than in controls and the frequency of pathological rats after exposure to pulsed radiation was significantly less than after exposure to CW.

In a study by Lopez-Martin *et al.*⁸¹, GSM-exposed picrotoxin-pretreated rats showed differences in clinical and EEG signs, and in c-Fos expression in the brain, in comparison to picrotoxin-treated rats exposed to an equivalent dose of unmodulated radiation. Neither MW exposure caused tissue heating, so thermal effects could be ruled out. The most marked effects of GSM MW on c-Fos expression in picrotoxin-treated rats were observed in limbic structures, olfactory cortex areas and subcortical areas, the dentate gyrus, and the central lateral nucleus of the thalamic intralaminar nucleus group. Nonpicrotoxin-treated animals exposed to unmodulated radiation showed the highest levels of neuronal c-Fos expression in cortical areas. These results suggested a specific effect of the pulse GSM modulation on brain activity of a picrotoxin-induced seizure-proneness rat model.

Luukkonen *et al.*⁸² investigated effects of MW at 872 MHz and relatively high SAR value (5 W/kg) on intracellular reactive oxygen species (ROS) production and DNA damage in human SH-SY5Y neuroblastoma cells. The experiments also involved combined exposure to MW and menadione, a chemical inducing intracellular ROS production and DNA damage. Both CW and a pulsed signal similar to that used in GSM mobile phones were used. Exposure to the CW radiation increased DNA breakage in comparison to the cells exposed only to menadione. Comparison of the same groups also

showed that ROS level was higher in cells exposed to CW RF radiation at 30 and 60 min after the end of exposure. No effects of the GSM-like modulated signal were seen on either ROS production or DNA damage.

Hinrikus *et al.*⁸³ evaluated the effects of MW (450 MHz) pulse-modulated at the frequencies of 7, 14 and 21 Hz on human electroencephalographic (EEG) rhythms. The field power density at the scalp was 0.16 m W/cm². Modulated microwaves caused an increase in the average EEG alpha (17%) and beta (7%) power but the theta rhythm remained unaffected. Increases in the EEG alpha and beta power were statistically significant during the first half-period of the exposure interval (30 s) at the modulation frequencies of 14 and 21 Hz. The authors concluded that the effect of the 450-MHz MW modulated at 7, 14 and 21 Hz varies depending on the modulation frequency.

Hoyto *et al.*⁸⁴ exposed human SH-SY5Y neuroblastoma and mouse L929 fibroblast cells to MW (SAR of 5 W/kg) at 872 MHz using continuous-waves (CW) or a modulated GSM-like signal under isothermal conditions⁸³. Menadione was used to induce reactive oxygen species, and tert-butylhydroperoxide (t-BOOH) was used to induce lipid peroxidation. Two statistically significant differences related to MW exposure were observed: Lipid peroxidation induced by t-BOOH was increased in SH-SY5Y (but not in L929) cells, and menadione-induced caspase 3 activity was increased in L929 (but not in SH-SY5Y) cells. Both differences were statistically significant only for the GSM-modulated signal.

Franzellitti *et al.*⁸⁵ exposed human trophoblast HTR-8/SVneo cells to MW at 1.8 GHz CW and differently modulated GSM signals (GSM-217Hz and GSM-Talk) during 4 - 24 h⁸⁴. The inducible HSP70C transcript was significantly enhanced after 24 h exposure to GSM-217 Hz signals while being reduced after 4 and 16 h exposure to GSM-Talk signal.

Significant amount of *in vivo* studies under varying parameters of exposure (intensity, frequency, exposure time, modulation, intermittence) have been performed in Russia/Soviet Union and published in Russian. Retrospective analysis of 52 Russian/Soviet *in vivo* studies with animals (mice, rats, rabbits, guinea pigs) on chronic exposure to MW has recently been published¹¹. In these studies, various endpoints were measured up to 4 month of chronic exposure including analysis of: weight of animal body, histological analysis and weight of tissues, central nervous system, arterial pressure, blood and hormonal status, immune system, metabolism and enzymatic activity, reproductive system, teratogenic and genetic effects. Based on their analysis, the authors concluded that: “exposure to modulated MW resulted in bioeffects, which can be different from the bioeffects induced by CW MW; exposure to modulated MW at low intensities (non-thermal levels) could result in development of unfavorable effects; direction and amplitude of the biological response to non-thermal MW, both *in vitro* and *in vivo*, depended on type of modulation; often, but not always, modulated MW resulted in more pronounced bioeffects than CW MW; the role of modulation was more pronounced at lower intensity levels”.

One review of the Russian/Soviet studies on the role of modulation on MW effects is available in English¹⁵. The authors conclude that “a number of good-quality studies have convincingly demonstrated significant bioeffects of pulsed MW. Modulation often was the factor that determined the biological response to irradiation, and reactions to pulsed and CW emissions at equal time-averaged intensities in many cases were substantially different”.

In conclusion, significant amount of in vitro and in vivo studies from different research groups, although not universally reported, clearly indicated dependence of the MW effects on modulation.

Polarization

It is believed that circular polarization might have been important in inducing chiral asymmetry in interstellar organic molecules that could be subsequently delivered to the early Earth and could explain the origin of the chirality of biological molecules⁸⁶.

The effects of circularly polarized (CP) MW were studied in *E. coli* cells at the frequencies from two frequency windows (resonances) that were identified using linearly polarized (LP) MW, within the frequency ranges of 51.62-51.84 GHz and 41.25-41.50 GHz^{34, 65}. At the resonance frequency of 51.76 GHz, right-handed CP MW inhibited repair of X-ray-induced DNA damages^{34, 65}. In contrast to right-handed polarization, left-handed CP MW had virtually no effect on the DNA repair, while the efficiency of LP MW was in-between of two circular polarizations. Inversion in effectiveness of circular polarizations was observed at another resonance frequency, 41.32 GHz. In contrast to the frequency of 51.76 GHz, left-handed CP MW at 41.32 GHz significantly inhibited DNA repair, while right polarization was almost ineffective. MW of the same CP affected cells at several frequencies tested within each resonance, alternative CP being almost ineffective^{34, 54, 65}. Therefore, specific sign of effective CP, either left- or right-, was the attribute of each resonance. Two different types of installations, based on either spiral waveguides⁶⁵ or quarter-wave mica plates^{34, 41, 54, 87, 88}, were used to produce CP MW. Similar results were observed regardless the way of producing the MW of different polarizations.

Pre-irradiation of *E. coli* cells to X-rays inverted the sign of effective polarization^{34, 54}. This inversion was observed for two different resonances, 41.32 and 51.76 GHz. Neither resonance frequencies, nor half-widths of the resonance changed during the inversions in effective CPs. The effects of left- and right-handed CP MW become the same at 50 cGy³⁴. At this dose, about one single stranded DNA break per haploid genome was induced. X-ray-induced DNA breaks result in relaxation of the supercoiled DNA-domains. It is known that the majority of DNA in living cells has a right-handed helicity (B-form) but a minor part, in order of 1 %, may alternate from the B-form with the form of left-handed helix (Z-form). Supercoiling is connected with transitions between right B-form to left Z-form in these DNA sequences. Therefore, the data suggested that difference in biological effects of polarized MW might be connected with DNA helicity and supercoiling of DNA-domains.

Supercoiling of DNA-domains is changed during cell cycle because of transcription, replication, repair, and recombination. It can also be changed by means of DNA-specific intercalators such as ethidium bromide (EtBr). EtBr changes supercoiling and facilitates the transition of DNA sequences from Z-form to B-form. Preincubation of *E. coli* AB1157 cells with EtBr inverted the effective polarization at the resonance frequency of 51.755 GHz and right-handed MW became more effective than left polarization⁸⁷. EtBr changed the supercoiling of DNA-domains starting at a concentration of 1 µg/ml as measured with the AVTD in different cell types including *E. coli*^{35, 37, 89}. These data provided further evidence that DNA may be a target for the NT MW effects.

The effects of MW on conformation of nucleoids in *E. coli* cells have recently been studied at the power flux density of $100 \mu\text{W}/\text{cm}^2$ ⁹⁰. Linearly polarized MW resulted in significant effects within specific frequency windows of resonance type in the range of 51-52 GHz. The distances between frequency windows were about 55-180 MHz. Only one of the two possible circular polarizations, left-handed or right-handed, was effective at each frequency window. The sign of effective circular polarization alternated between frequency windows.

While most data on polarization have been obtained by the same research group^{34, 41, 43, 54, 56, 65, 87, 88, 90-92}, recent data of others corroborated our findings at least partially⁹³. These authors analyzed the condensation of chromatin in human buccal epithelium cells by the method of vital indigo carmine staining. MW induced chromatin condensation in dependence on polarization⁹³.

Obviously, the difference in effects of right- and left polarizations could not be explained by the heating or by the mechanism dealing with “hot-spots” due to unequal SAR distribution. The data about the difference in effects of differently polarized MW, the inversion of effective circular polarization between resonances and after irradiation of cells with X-rays and incubation with EtBr provided strong evidence for the non-thermal mechanisms of MW effects. These data suggested chiral asymmetry in the target for the NT MW effects, one of which is presumably chromosomal DNA³⁴, and selection rules on helicity if quantum-mechanical approach is applied⁵⁴.

Electromagnetic environment

Hypothetically, background EMF might be of importance for the MW effects. This hypothesis is based on the experimental observations that SMF, ELF magnetic fields, and MW at low intensities induced similar effects in cells under specific conditions of exposure^{1, 39, 94-96}. Despite very little has been achieved for mechanistic explanation of such effects, there are attempts to consider the effects of EMF in a wide frequency range in the frames of the same physical models⁹⁷⁻¹⁰³.

Litovitz and colleagues found that the ELF magnetic noise inhibited the effects of MW on ODC in L929 cells⁷². The ODC enhancement was found to decrease exponentially as a function of the noise root mean square amplitude. With 60 Hz amplitude-modulated MW, complete inhibition was obtained with noise levels at or above $2 \mu\text{T}$. With the DAMPS (Digital Advanced Mobile Phone System) cellular phone MW, complete inhibition occurred with noise levels at or above $5 \mu\text{T}$. Further studies by the same group revealed that the superposition of ELF noise inhibited hypoxia de-protection caused by long term repeated exposures of chick embryos to MW¹⁰⁴.

The effect of a magnetic noise on microwave-induced spatial learning deficit in the rat was investigated by Lai¹⁰⁵. Rats were exposed to MW (2450 MHz CW, PD $2 \text{ mW}/\text{cm}^2$, average whole-body SAR $1.2 \text{ W}/\text{kg}$) alone or in combination with noise exposure (60 mG). Microwave-exposed rats had significant deficit in learning. Exposure to noise alone did not significantly affect the performance of the animals. However, simultaneous exposure to noise significantly attenuated the microwave-induced spatial learning deficit. The author concluded that simultaneous exposure to a temporally incoherent magnetic field blocks MW-induced spatial learning and memory deficits in the rat¹⁰⁵.

Lai and Singh studied combined effects of a temporally incoherent magnetic noise (45 mG) and MW (CW 2450 MHz, PD $1 \text{ mW}/\text{cm}^2$, average whole-body SAR of 0.6

W/kg) in rat brain cells¹⁰⁶. MW exposure induced significant DNA breakages as measured with both neutral and alkaline comet assays. Exposure to noise alone did not significantly affect cells. However, simultaneous noise exposure blocked the MW-induced effects.

Yao and colleagues investigated the influence of the GSM-like MW at 1.8 GHz on DNA damage and intracellular reactive oxygen species (ROS) formation in human lens epithelial cells (hLECs)¹⁰⁷. DNA damage examined by alkaline comet assay was significantly increased after 3 W/kg and 4 W/kg radiation, whereas the double-strand breaks (DSB) evaluated by γ -H2AX foci were significantly increased only after 4 W/kg radiation. Significantly elevated intracellular ROS levels were detected in the 3-W/kg and 4-W/kg groups. After exposure to 4 W/kg for 24 hours, hLECs exhibited significant G₀/G₁ arrest. All the effects were blocked when the MW exposure was superposed with a 2 μ T electromagnetic noise. The authors concluded that superposed electromagnetic noise blocks MW-induced DNA damage, ROS formation, and cell cycle arrest.

We have previously reported that resonance effects of MW on *E. coli* cell depend on the magnitude of static magnetic field at the place of MW exposure⁵⁷. This dependence was explained by the model of electron-conformational interactions that also predicted possible shift of resonance frequencies in dependence on SMF³⁵. More recently, Ushakov with co-authors exposed *E. coli* cells to MW at the PD of 10⁻¹⁰ W/cm² and the frequencies of 51.675, 51.755 and 51.835 GHz⁸⁸. In this study, cells were exposed to MW at various values of SMF: 22, 49, 61, or 90 μ T. The authors observed that the effects of MW exposure on the conformation of nucleoids depended on the SMF during exposure.

Gapeev *et al.* analyzed effects of MW (41.85-42.1 GHz, frequency increment 50 MHz, PD 50 μ Bt/cm², 20 min exposure) on synergistic reaction of calcium ionophore A23187 and phorbol ester PMA in activation of the respiratory burst of the peritoneal neutrophils of mice⁷⁹. The MW exposure was performed at various SMF. At a SMF of 50 μ T, the authors observed frequency-dependent inhibition of the synergetic reaction with maximal effect at the frequency of 41.95 GHz. In the same frequency range, frequency-dependent activation of the synergetic reaction with a maximal effect at the frequency of 42.0 GHz was found at a SMF of 95 μ T. The authors concluded that increasing the SMF from 50 to 95 μ T resulted in the inversion of ten MW effects and the shift of the resonance frequency by 50 MHz^{79, 108}. Moreover, these effects of MW at the 41.95 GHz and 42.0 GHz were not found at the SMF of ± 1 , 28.3, 75.5 or 117.3 μ T suggesting that the NT MMW effects may appear only at specific values of SMF^{79, 108}.

The observations on dependence of the NT MW effects on SMF and ELF stray field may be of significant interest for further development of physical theory for the NT MW effects and development of safe mobile communication.

Cell-to-cell interaction in response to NT MW

The effects of NT MW at the resonance frequency of 51.755 GHz on conformation of nucleoids in *E. coli* cells were analyzed with respect to cell density during exposure⁵⁷. The per-cell-normalized effect of MW increased by a factor of 4.7 $\pm$ 0.5 on average as cell density increased by one order of magnitude, from 4 $\cdot$ 10⁷ to 4 $\cdot$ 10⁸ cell/ml. These data suggested a co-operative nature of cell response to MW, which is based on cell-to-cell

interaction during exposure. This suggestion was in line with the observed partial synchronization of cells after exposure to MW.

The co-operative nature of cell response to MW at the resonance frequency of 51.755 GHz was confirmed in further studies with *E. coli* cells^{35, 41, 58}. In addition, dependence of the per-cell-normalized effect on cell density was found for two other resonances, 51.675 GHz and 51.688 GHz. These data suggested that dependence on cell density during exposure is a general attribute of the resonance response of *E. coli* cells to NT MW. At the cell density of $4 \cdot 10^8$ cells/ml, the average intercellular distance was approximately 13 μm that is 10 times larger than the linear dimensions of *E. coli* cells^{57, 58}. Therefore, no direct physical contact seemed to be involved in the cell-to-cell interaction. Two mechanisms, biochemical and electromagnetic, were considered to account for the co-operative nature in the resonance response to weak EMF in wide frequency range including ELF, MW and ionizing radiation^{57, 109, 110}. The first one, biochemical, is based on release of secondary chemical messengers (ions, radicals, or molecules) by those cells, which were directly targeted. Via diffusion, these messengers can induce response in other cells. The second mechanism, electromagnetic, is based on reemission of secondary photons. According to this mechanism, reemitted photons can induce response in other cells if the intercellular distance is shorter than the length of photon absorption. Our experimental data on MW effects fitted better to the electromagnetic mechanism but a combination of two mechanisms was also possible^{57, 58}. In particular, free radicals with prolonged lifetimes might be involved in the observed cell-to-cell communication during response to EMF¹¹¹.

The absorption length of photons with the frequencies of 10^{12} - 10^{13} Hz corresponds to the intracellular distance at the cell density of $5 \cdot 10^8$ cell/ml, at which saturation in the dependences of EMF effects on cell density was observed^{57, 58, 111, 112}. Such photons may be involved in cell-to-cell communication according to the electromagnetic mechanism and in agreement with the prediction of Fröhlich that biosystems support coherent excitations within frequency range of 10^{11} - 10^{12} Hz⁴⁴. From this point of view, cell suspension may respond to NT MW as a whole. In this case, the number of the exposed cells should be large enough to facilitate cell-to-cell communication during the responses to MW at specific parameters of exposure such as frequency, modulation, and polarization. Interestingly, the cell density for saturation of both MW and ELF effects was about $5 \cdot 10^8$ cell/ml that is close to cell densities in soft tissues of eukaryotes^{58, 111}. Such density of cells in the tissues may be important for regulation of living systems by electromagnetic cell-to-cell communication. Cellular membranes and DNA have been considered as possible sources of coherent excitations and photons, which may be involved in electromagnetic cell-to-cell communication^{35, 44, 111}.

PD dependences of the MW effect at the 51.755 GHz resonance frequency were considerably different between two cell densities, $4 \cdot 10^7$ cells/ml and $4 \cdot 10^8$ cells/ml³⁵. However, the resonance frequency of 51.755 GHz did not shift with the changes in cell density. The half-width of the 51.755 GHz resonance did not depend on cell density either. Contrary to the 51.755 GHz resonance response, the half-width of the 51.675 GHz resonance depended on cell density⁴¹. The data suggested that intracellular interaction during the NT MW exposures at some specific frequencies might affect sub-cellular targets for NT MW. This target is presumably chromosomal DNA that is organized in the DNA-domains^{34, 92, 97}.

In all studies concerning dependence of the MW effects on cell density, the cells occupied a negligible part of the exposed volume and could not change the absorption

of MW even at the highest cell densities^{35, 41, 57, 58}. Striking difference in the cell responses at various cell densities provided further evidence for non-thermal mechanism of the observed MW effects.

Significant MW effect on synchronization of *Saccharomyces carlsbergensis* yeast cells were observed by Golant and co-authors¹¹³. Exposure to MW at 30 $\mu\text{W}/\text{cm}^2$ and 46 GHz induced synchronization as measured by cell density and bud formation. The authors assumed that MW induced cell-to-cell interaction resulting in the observed synchronization.

Genetic background and cell type

We studied effects of MW on *E. coli* cells of three isogenic strains with different length of chromosomal DNA⁹². Bacterial chromosomal DNA in N99 wild type cells was lengthened by inserting DNA from λ and $\lambda\text{imm}^{434}\text{bio}^{10}$ phages. Lysogenic strains N99(λ) and N99($\lambda, \lambda\text{imm}^{434}\text{bio}^{10}$) obtained were used for MW exposure along with the wild type N99 strain. The response of each strain was studied at 10-17 frequencies within the ranges of 41.24-41.37 GHz and 51.69-51.795 GHz. Clear resonance responses to MW at 10^{-10} W/cm² were observed for each strain in both frequency ranges. Significant shifts of both resonance frequencies were found between strains. The shifted resonances had the same amplitude and half-width as for N99 cells⁹². Upon shifting, no changes in effective circular polarization within each shifted resonance were observed. The shifts in resonance frequencies could not be explained by activity of additional genes inserted with the phage DNA. On the other hand, the theoretical consideration based on oscillations of the DNA-domains regarding a whole nucleoid provided a good correlation between the increasing in the DNA length and the shifts in resonances⁹².

A detailed analysis of MW effects on *E. coli* AB1157 cells at 10^{-10} W/cm² and various frequencies revealed the resonance frequency of 51.755 ± 0.001 GHz³⁵. This value was statistically significantly different from the resonance frequency of 51.765 ± 0.002 in response of *E. coli* N99 cells to MW in the same frequency range³⁵. It should be noted that both strains, AB1157 and N99, are considered as wild type strains. Nevertheless, these strains are different in their genotypes by several specific gene markers^{23, 33}. These data suggested that strains of different origin, even being considered as wild type strains, might have different resonance responses to NT MW.

Stagg with colleagues exposed tissue cultures of transformed and normal rat glial cells to packet-modulated MW (TDMA that conforms to the North American digital cellular telephone standard) at 836.55 MHz¹¹⁴. Results from DNA synthesis assays differed for these two cell types. Sham-exposed and MW-exposed cultures of primary rat glial cells showed no significant differences for either log-phase or serum-starved condition. C6 glioma cells exposed to MW at 5.9 $\mu\text{W}/\text{g}$ SAR (0.9 mW/cm²) exhibited small (20-40%) but significant increases in 38 % of [³H]-thymidine incorporation experiments.

Repacholi with co-authors chronically exposed wild-type mice and E mu-Pim1 transgenic mice, which are moderately predisposed to develop lymphoma spontaneously, to plane-wave pulse-modulated MW at 900 MHz with a pulse repetition frequency of 217 Hz and a pulse width of 0.6 ms¹¹⁵. Incident power densities were 2.6-13 W/m² and SARs were 0.008-4.2 W/kg, averaging 0.13-1.4 W/kg. The lymphoma risk was found to be

significantly higher in the exposed transgenic mice. No effects were seen in the wild type mice.

Markkanen with colleagues found that MW affected the UV-induced apoptosis in *Saccharomyces cerevisiae* yeast cells KFY437 (cdc48-mutant) but did not modify apoptosis in KFY417 (wild-type) cells⁷⁸.

Czyz with colleagues exposed pluripotent embryonic stem (ES) cells of wild-type and deficient for the tumor suppressor p53 to pulse modulated GSM MW at 1.71 GHz¹¹⁶. Two dominant GSM modulation schemes (GSM-217 and GSM-Talk), which generate temporal changes between GSM-Basic (active during talking phases) and GSM-DTX (discontinuous transmission, which is active during listening phases thus simulating a typical conversation), were applied to the cells at and below the ICNIRP safety standards. GSM-217 MW induced a significant upregulation of mRNA levels of the heat shock protein hsp70 of p53-deficient ES cells differentiating in vitro, paralleled by a low and transient increase of c-jun, c-myc, and p21 levels in p53-deficient, but not in wild-type cells. These data substantiated the notion that the genetic background determines cellular responses to GSM MW.

Human cultured fibroblasts of three different donors and three different short-term human lymphocyte cultures were exposed to UMTS-like MW at 1950 MHz and the SAR below safety limit of 2 W/kg by Schwarz *et al.*¹¹⁷. The alkaline comet assay and the micronucleus assay were used to analyze genotoxic effects. UMTS exposure increased the comet tail factor (CTF) and induced centromere-negative micronuclei in human cultured fibroblasts in a dose and time-dependent way. No UMTS effect was obtained with lymphocytes, either unstimulated or stimulated with phytohemagglutinin. The authors concluded that UMTS exposure may cause genetic alterations in some but not in all human cells in vitro.

Hoyto *et al.*¹¹⁸, analyzed the effects of MW exposure on cellular ornithine decarboxylase (ODC) activity in fibroblasts, two neural cell lines and primary astrocytes. Several exposure times and exposure levels were used, and the fields were either unmodulated or GSM-like-modulated. Murine L929 fibroblasts, rat C6 glioblastoma cells, human SH-SY5Y neuroblastoma cells, and rat primary astrocytes were exposed to RF radiation at 872 MHz in a waveguide exposure chamber equipped with water cooling. Cells were exposed for 2, 8, or 24 hours to CW MW or to a GSM type signal pulse modulated at 217 Hz. ODC activity in rat primary astrocytes was decreased statistically significantly and consistently in all experiments performed at two exposure levels (1.5 and 6.0 W/kg) and using GSM modulated or CW radiation. In the secondary cell lines, ODC activity was generally not affected. The authors concluded that ODC activity was affected by MW exposure in rat primary neural cells, but the secondary cells used in this study showed essentially no response. In further studies by the same group, the difference in response of human SH-SY5Y neuroblastoma and mouse L929 fibroblast cells to a GSM-modulated MW at 872 MHz was documented⁸⁴.

Nylund and Leszczynski have examined cell response to MW (900 MHz GSM-like signal, average SAR of 2.8 W/kg) using two human endothelial cell lines: EA.hy926 and EA.hy926v1¹¹⁹. Gene expression changes were examined using cDNA Expression Arrays and protein expression changes were examined using 2-DE and PDQuest software. The same genes and proteins were differently affected by exposure in each of the cell lines.

Remondini *et al.* analyzed changes in gene expression in six human cell lines by gene microarrays⁵³. Cells were exposed to MW at 900 MHz GSM Basic mode, SAR 1.8-2.5

W/kg, 1 h exposure. Most cell lines responded to GSM-900 MHz, except for the CHME5 human microglial cells.

Zhao *et al.* studied whether expression of genes related to cell death pathways are dysregulated in primary cultured neurons and astrocytes by exposure to MW from GSM cell phone at the frequency of 1900 MHz for 2 h¹²⁰. Microarray analysis and real-time RT-PCR have shown up-regulation of caspase-2, caspase-6 and Asc (apoptosis associated speck-like protein containing a card) gene expression in neurons and astrocytes. Up-regulation occurred in both “on” and “stand-by” modes in neurons, but only in “on” mode in astrocytes. Additionally, astrocytes showed up-regulation of the Bax gene. The authors concluded that even relatively short-term exposure to the cell phone can up-regulate elements of apoptotic pathways in cells derived from the brain, and that neurons appear to be more sensitive to this effect than astrocytes.

Finally, it follows from the emerging data that MW effects are defined by the genotype and may be cell-type and cell-line dependent. These dependences may explain, at least partly, the discrepancies among replication studies from different laboratories.

Gender- and age-related differences

There are studies indicating that MW may exert a gender-related influence on brain activity¹²¹⁻¹²³. Papageorgiou and co-authors investigated the gender-related influence of MW similar to that emitted by GSM900 mobile phones on brain activity¹²¹. Baseline EEG energy of males was greater than that of females, and exposure to MW decreased EEG energy of males and increased that of females. Memory performance was invariant to MW exposure and gender influences. Smythe and Costall reported the effects of mobile phone exposure on short- and long-term memory in male and female subjects¹²². The results showed that males exposed to an active phone made fewer spatial errors than those exposed to an inactive phone condition, while females were largely unaffected. These results further indicated that mobile phone exposure has functional consequences for human subjects, and these effects appear to be gender-dependent. Nam and colleagues exposed volunteers of both gender to MW emitted by a CDMA cellular phone for half an hour¹²³. Physiological parameters such as systolic and diastolic blood pressures, heart rate, respiration rate, and skin resistance were simultaneously measured. All the parameters for both groups were unaffected during the exposure except for decreased skin resistance of the male subjects¹²³.

Prevalence of women (usually around 70%) among subjects, which report hypersensitivity to electromagnetic fields of wide frequency range including MW, may also be considered as an indirect evidence for the gender-dependent effects of MW.

In his pioneering study concerning age in cancer risk from MW exposure, Hardell and colleagues found that the highest risks were associated with >5-year latency period in the 20-29-year age group for analog phones (OR = 8.17, 95% CI = 0.94-71), and cordless phones (OR = 4.30, 95% CI = 1.22-15)¹²⁴. Of note, no participants of age less 20 years were involved on this study. In further studies from the Hardell's group, highest risk was found in the age group <20 years at time of first use of wireless phones^{125, 126}.

Nam with co-authors reported that skin resistance in teenagers decreased by exposure to CDMA MW from cellular phones whereas no effects were seen in adults¹²³.

Individual differences

We observed significant individual variations in effects of GSM and UMTS MW on chromatin conformation and 53BP1/ γ -H2AX DNA repair foci in studies with lymphocytes from hypersensitive to EMF subjects and healthy persons^{38-40, 49}.

Shckorbatov with colleagues investigated electrokinetic properties of cell nuclei and condensation of heterochromatin in human buccal epithelium cells in response to MW at 42.2 GHz¹²⁷. MW exposure decreased electric charge of cell nuclei and an increased chromatin condensation in dependence on individual traits of donors¹²⁷.

Hinrikus *et al.*⁸³ evaluated the effects of pulse-modulated MW (450 MHz) on human EEG rhythms. Thirteen healthy volunteers were exposed to MW; the field power density at the scalp was 0.16 m W/cm². Differences were found in individual sensitivity to exposure. Increases in the EEG beta power appeared statistically significant in the case of four subjects. In other study, the same authors confirmed and extended their observations on individual sensitivity to exposure with pulse-modulated MW¹²⁸. The experiments were carried out on four different groups of healthy volunteers. A 450-MHz MW modulated at 7 Hz (first group), 14 and 21 Hz (second group), 40 and 70 Hz (third group), 217 and 1000 Hz (fourth group) frequencies was applied. MW exposure, SAR 0.303 W/kg, increased the EEG energy. The proportion of subjects significantly affected was similar in all groups except for the 1000 Hz group: in the first group 16% at 7 Hz modulation; in the second group 31% at 14 Hz modulation and 23% at 21 Hz modulation; in the third group 20% at 40 Hz and 13% at 70 Hz modulation; in the fourth group 16% at 217 Hz and 0% at 1000 Hz modulation frequency.

Zotti-Martelli with colleagues exposed peripheral blood lymphocytes from nine different healthy donors for 60, 120 and 180 min to CW MW with a frequency of 1800 MHz and PD of 5, 10, and 20 mW/cm² and analyzed DNA damage using micronucleus (MN) assay¹²⁹. Both spontaneous and induced MN frequencies varied in a highly significant way among donors, and a statistically significant increase of MN, although rather low, was observed dependent on exposure time and PD. The data analysis highlighted a wide inter-individual and reproducible variability in the response.

Sannino *et al.* evaluated the induction of micronuclei in response to MW (900 MHz, average SAR of 1.25 W/kg) exposure and subsequent treatment with mitomycin C in peripheral blood lymphocytes from five human volunteers¹³⁰. MW exposure reduced the level of mitomycin C –induced micronuclei in cells collected from four donors (i.e., responders). However, the effect of MW was not observed in the remaining donor (i.e., non-responder). The overall data indicated the existence of heterogeneity in the MW response among individuals.

Physiological variables

The importance of physiological variables, which may include all conditions of cell culture growth such as aeration, the composition of the growth and exposure media, on NT MW effects has previously been reviewed⁸.

In our investigations, *E. coli* cells were exposed to CP or LP MW (100 μ W/cm²) at the resonance frequencies of 41.32 GHz and 51.76 GHz^{56, 57}. Both value and direction of the MW effects strongly depended on the phase of culture growth. At logarithmic phase of growth, MW resulted in condensation of nucleoids. In contrast, MW exposure decon-

densed nucleoids in cells if exposure was performed at the stationary phase of growth. It is known, that the state of nucleoid condensation depends on cell activity. In stationary cells nucleoids are more condensed compared to logarithmic cells that divide actively. We concluded that MW are able to either stimulate or inhibit activity of the cells in dependence on stage of growth, stationary or logarithmic, respectively. Higher variability in effects was observed for logarithmic phase and effects were more stable for the stationary phase that is characterized by partial synchronization of cells^{56, 57}. There was no effect at all if cells were exposed at the end of the logarithmic phase where the MW effects changed their direction from inhibition to stimulation⁵⁷. Another peculiarity was observed at the very beginning of the logarithmic stage, where the condensation of chromatin induced by MW was very weak. The AVTD data were confirmed by the electrophoretic analysis of proteins bound to DNA⁵⁶. The main feature of the effect in the stationary phase was a decrease in the quantity of several unidentified DNA-bound proteins with molecular weights of 61, 59, 56, 26, and 15 kDa. In contrast, the main trend was an increase in some proteins, 61, 56, 51 and 43 kDa after exposure at the logarithmic phase. The decrease or increase in the level of proteins bound to DNA correlated with the observed changes in the state of nucleoids, decondensation or condensation, respectively.

The MW effects was studied both at stationary and logarithmic phase of growth during exposure to MW in the PD range of 10^{-18} to $3 \cdot 10^{-3}$ W/cm² at various cell densities⁵⁸. Relatively weak response to MW was observed in exponentially growing cells. Partially synchronized stationary cells were more sensitive, especially at the cell densities above 10^8 cell/ml. The data suggested that the co-operative responses of cells to MW vary in dependence on phase of growth.

Recent data by Ushakov and colleagues indicated that the MW effects on *E. coli* cells depended on concentration of oxygen in the cell suspension during exposure⁸⁸. This dependence might suggest that oxygen concentration should be indicated in order to improve reproducibility in replication studies.

Similar to the effects of ELF⁹⁵, the MW effects were reported to depend on concentration of divalent ions⁷⁹.

Antioxidants and radical scavengers inhibit effects of MW

Lai and Singh described effects of MW on the rat brain cells as measured using a microgel electrophoresis assay¹³¹. These effects were significantly blocked by treatment of rats either with the spin-trap compound N-tert-butyl- α -phenylnitron or with melatonin that is a potent free radical scavenger and antioxidant¹³². These data suggested that free radicals might be involved in the effects of MW.

Oktem and colleagues exposed rats to MW from GSM900 mobile phone with and without melatonin treatment¹³³. Malondialdehyde (MDA), an index of lipid peroxidation, and urine N-acetyl-beta-d-glucosaminidase (NAG), a marker of renal tubular damage, were used as markers of oxidative stress-induced renal impairment. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities were studied to evaluate changes in antioxidant status. In the MW-exposed group, while tissue MDA and urine NAG levels increased, SOD, CAT, and GSH-Px activities were reduced. Melatonin treatment inhibited these effects. The authors concluded that melatonin might exhibit a protective effect on mobile phone-induced renal impairment in rats.

Ozguner and colleagues exposed Wistar-Albino rats to MW from GSM900 mobile phone with and without melatonin and analyzed histopathologic changes in skin¹³⁴. MW induced increase in thickness of stratum corneum, atrophy of epidermis, papillomatosis, basal cell proliferation, granular cell layer (hypergranulosis) in epidermis and capillary proliferation. Impairment in collagen tissue distribution and separation of collagen bundles in dermis were all observed in exposed animals as compared to the control group. Most of these changes, except hypergranulosis, were prevented with melatonin treatment. The authors concluded that exposure to GSM900 MW caused mild skin changes and melatonin treatment could reduce these changes. In other studies of the same group, the ability of melatonin to reduce various MW-induced effects was confirmed and inhibitory potential of the antioxidant caffeic acid phenethyl ester (CAPE) was reported¹³⁵⁻¹³⁸.

Ayata *et al.* analyzed the effects of 900 MHz MW with and without melatonin on fibrosis, lipid peroxidation, and anti-oxidant enzymes in rat skin¹³⁹. The levels of MDA and hydroxypyroline and the activities of SOD, GSH-Px, and CAT were studied. MDA and hydroxypyroline levels and activities of CAT and GSH-Px were increased significantly in the exposed group without melatonin and decreased significantly in the exposed group with melatonin. SOD activity was decreased significantly in the exposed group and this decrease was not prevented by the melatonin treatment. The authors assumed that the rats irradiated with MW suffer from increased fibrosis and lipid peroxidation and that melatonin can reduce the fibrosis and lipid peroxidation caused by MW.

Ilhan with co-authors investigated oxidative damage in brain tissue of rats exposed to GSM900 MW with and without pretreatment with Ginkgo biloba (Gb)¹⁴⁰. MW induced oxidative damage measured as: (i) increase in MDA and nitric oxide (NO) levels in brain tissue, (ii) decrease in brain SOD and GSH-Px activities, and (iii) increase in brain xanthine oxidase and adenosine deaminase activities. These MW effects were prevented by the Gb treatment. Furthermore, Gb prevented the MW-induced cellular injury in brain tissue revealed histopathologically. The authors concluded that reactive oxygen species may play a role in the adverse effects of GSM900 MW and Gb prevents the MW-induced oxidative stress by affecting antioxidant enzymes activity in brain tissue.

Koylu *et al.* studied the effects of MW on the brain lipid peroxidation in rats, and the possible protective effects of melatonin on brain degeneration induced by MW¹⁴¹. The levels of lipid peroxidation in the brain cortex and hippocampus increased in the MW group compared with the control group, although the levels in the hippocampus were decreased by combined administration of MW and melatonin. Brain cortex lipid peroxidation levels were unaffected by melatonin treatment. The authors concluded that melatonin may prevent MW-induced oxidative stress in the hippocampus by strengthening the antioxidant defense system.

Sokolovic *et al.*¹⁴² evaluated the intensity of oxidative stress in the brain of Wistar rats chronically exposed to MW from mobile phones (SAR = 0.043-0.135 W/kg) during 20, 40 and 60 days. A significant increase in brain tissue malondialdehyde (MDA) and carbonyl group concentration was found. Decreased activity of catalase (CAT) and increased activity of xanthine oxidase (XO) remained after 40 and 60 days of MW exposure. Melatonin treatment significantly prevented the increases in MDA content and XO activity in the brain tissue after 40 days of exposure while it was unable to prevent the decrease of CAT activity and increase of carbonyl group contents. The authors

concluded that exposure to the mobile phone MW caused oxidative damage in the brain and that treatment with melatonin significantly prevented this oxidative damage.

To conclude this section, several studies suggest that supplementation with antioxidants and radical scavengers can reduce MW effects.

Summary of experimental studies

Numerous experimental data have provided strong evidence for NT MW effects and have also indicated several regularities in appearance of these effects: dependence on frequency within specific frequency windows of “resonance-type”; narrowing of the frequency windows with decreasing intensity; dependence on modulation and polarization; sigmoid dependence on intensity within specific intensity windows including super-low PD comparable to intensities from base stations; thresholds in intensity and exposure time (coherence time); dependence on duration of exposure and post-exposure time; dependence on cell density that suggests cell-to-cell interaction during response to NT MW; dependence on physiological conditions during exposure, such as stage of cell growth, concentration of oxygen and divalent ions, activity of radicals; dependence on genotype; cell-type and cell-line dependence; gender-, age- and individual differences; and SMF and EMF stray field during exposure may be of importance for the effects of NT MW.

Replication studies

Obviously, not taking into account the dependences of NT MW effects on a number of physical parameters and biological variables may result in misleading conclusions regarding the reproducibility of these effects. Especially important might be the observations that NT MW could inhibit or stimulate the same functions dependent on conditions of exposure². Under different conditions of exposure, MW either increased or decreased the growth rate of yeast cells⁸, the radiation-induced damages in mice¹⁴¹, the respiratory burst in neutrophils of mice⁷⁹, the condensation of nucleoids in *E. coli* cells^{56, 57} and human lymphocytes⁴⁰. Potentially bi-directional effects of MW should be taken into account in replication studies.

Despite of considerable body of studies with NT MW in biology, only a few studies were performed to replicate the original data on the NT MW effects. It should be noted, that these replications are usually not completely comparable with the original studies because of either missing description of important parameters of exposure or significant differences in these parameters between original study and replication.

One well-known attempt to replicate the results of Gründler was the study by Gos and co-authors¹⁴⁴. No MW effects were observed in this replication study. However, the deviations from the Gründler’s protocol might be a simple reason for poor reproducibility. For example, synchronized cells were used in studies of Gründler. Contrary to the Gründler’s original protocol, Gos used exponentially growing cells. If the MW effects in yeast cells are dependent on stage of growth, cell density and intercellular interactions as it has been described for *E. coli* cells^{35, 41, 56, 57}, no response should be expected in the logarithmic phase of growth. Gos and colleagues used *S. cerevisiae* strain with the auxotrophy mutations for leucine and uracil. Gründler used the wild type strain. It might suggest another cause for the deviations between the data of Gründler and Gos. Despite

orientation of SMF in respect to electric and magnetic components of MW was the same, the values of SMF were different. The stray ELF field was 120 nT in the study by Gos, that is higher than usually observed background fields, < 50 nT. The spectral characteristics of the background fields, which were described only in the study by Gos, might be also different. In addition, the conditions of cell cultivation might vary between studies; for example, the data on oxygen concentration in media used in both studies are not available.

Amount of already known physical and biological variables that are important for reproducibility of NT MW effects seem to be far beyond the limits of usually controlled parameters in biological experiments. The knowledge of some of these variables is based on consistent findings following from experimental studies of different research groups. Further evaluation of variables that are important for the NT MW effects would benefit from the developing of the physical and molecular biological models for the MW effects.

Most reviews of the experimental studies do not include analysis of various biological variables and physical parameters when comparing the data on NT MW effects from different studies. As result, misleading conclusion is often made that MW at NT levels produce no “reproducible” effects.

Possible mechanisms

Analyzing theoretically our experimental data on the MW effects at super-low intensities we concluded that these effects should be considered using quantum-mechanical approach⁵⁷. Reanalysis of our data by Binhi resulted to the same conclusion⁹⁷. This is in line with the fundamental quantum-mechanical mechanism that has been suggested by Fröhlich¹⁴⁵. Most probably, the physical mechanisms of the NT MW effects must be based on quantum-mechanical approach and physics of non-equilibrium and nonlinear systems^{44, 98, 146-148}.

Our data indicated also that chromosomal DNA is a target for interaction with MW^{34, 87, 92}. The length of genomic DNA is much longer than the dimension of surrounding compartment. For example, there is about 1.8 m of DNA in a human genome that is compacted in interaction with other compounds such as proteins, RNA and ions to fit into a nucleus with a characteristic diameter of 5-10 μ m. Importantly, concentration of DNA in the nuclei is higher than in crystallization solutions for DNA, 50-100 mM versus 10-30 mM, respectively. Whether DNA is organized in nuclei as a liquid crystal remains to be investigated. However, it is clear that DNA in a living cell cannot be considered as an aqueous solution of DNA molecules in a thermodynamic equilibrium.

The quantum-mechanical physical model for primary interaction of MW with DNA has been proposed¹⁴⁹. We hypothesized that genomic DNA contain two different codes¹⁰⁹. The first one is the well-known genetic triplet code for coding the genes. The second one is a “physical code” that determine the spectrum of natural oscillations in chromosomal DNA including electromagnetic, mechanical and acoustic oscillations, which are hypothetically responsible for regulation of gene expression at different stages of ontogenesis and for genomic rearrangements in evolution¹⁰⁹. The physical model describing these coupled oscillations in chromosomal DNA has been proposed⁹². This model helps to resolve the so-called C-paradox that addresses the issue of a genome size, so-called C-value. Only few percent of DNA encodes genes in almost all eukaryotic genomes. The same amount of DNA is involved in regulation of gene expression by known biochemical mechanisms. The function of the rest of DNA, which does not depend on complexity

of eukaryotic species and is represented by noncoding repetitive DNA sequences, is not understood in molecular biology providing a basement for hypotheses such as “junk DNA”. The function of this major part of genomic DNA became clear given that the whole genomic DNA is responsible for the creation of the natural spectrum of oscillations that is hypothetically a main characteristic of each biological species¹⁰⁹.

The understanding of mechanisms for the NT MW effects is far from comprehensive. Many questions remain to be addressed such as whether resonance effects of MW depend on electromagnetic noise and SMF during exposure.

Urgent needs and further perspectives

At present, new situation arose when a significant part of the general population is exposed chronically (much longer than previously investigated durations of exposures) to NT MW from different types of mobile communication including GSM and UMTS/3G phones and base stations, WLAN (Wireless Local Area Networks), WPAN (Wireless Personal Area Networks such as Bluetooth), DECT (Digital Enhanced (former European) Cordless Telecommunications) wireless phones. It should be anticipated that some part of the human population, such as children, pregnant women and groups of hypersensitive persons could be especially sensitive to the NT MW exposures.

Multiple sources of mobile communication result in chronic exposure of significant part of general population to MW at the non-thermal levels. Therefore, the ICNIRP safety standards, which are based on thermal effects in acute exposures, cannot protect the general population from the chronic exposures to NT MW from mobile communication¹³.

Most of the real signals that are in use in mobile communication have not been tested so far. Very little research has been done with real signals and for durations and intermittences of exposure that are relevant to chronic exposures from mobile communication. In some studies, the so-called “mobile communication-like” signals were investigated that in fact were different from the real exposures in such important aspects as intensity, carrier frequency, modulation, polarization, duration and intermittence. How relevant such studies to evaluation of adverse health effects from MW of mobile communication is not known.

Emerging evidence suggests that the SAR concept, which has been widely adopted for safety standards, may not be useful alone for the evaluation of health risks from MW of mobile communication. How the role of other exposure parameters such as frequency, modulation, polarization, duration, and intermittence of exposure should be taken into account is an urgent question to solve. Solving this question would greatly benefit from the knowledge of the physical mechanisms of the NT MW effects.

So far, most laboratory and epidemiological studies did not control important features of the NT MW effects as described above and therefore, only limited conclusion regarding health effects of MW from mobile communication can be drawn from these studies. It should be noted that one group of epidemiologists with a long-lasting experience in studying relationship between mobile phone usage and cancer risk have consistently been concerned regarding importance of various MW signals and exposure durations^{19, 150-152}. The group of Hardell was the first epidemiologic group in attempting to study separately the MW signals from cordless phones, analogue phones and digital phones. As a rule, analogue phones had the highest association with the cancer risk. Cordless phones were associated with the risk for brain tumors, acoustic neuroma, and

T-cell lymphoma stronger or in the same degree as digital and analogue phones despite significantly lower SAR values were produced by cordless phones^{17, 19, 151, 152}. It should be also noted that epidemiological data are controversial and methodological differences are a subject of debates between various research groups^{17, 153}. However, the approach of Hardell's group is more valid from the mechanistic point of view and this should be taken into account when comparing with results of other groups that ignore or minimize the complex dependencies of the NT MW effects on several parameters/variables.

The data about the effects of MW at super low intensities and significant role of duration of exposure in these effects along with the data showing that adverse effects of NT MW from GSM/UMTS mobile phones depend on carrier frequency and type of the MW signal suggest that MW from base-stations/masts can also produce adverse effects at prolonged durations of exposure and encourage the mechanistic *in vitro* studies using real signals from base stations/masts. Further investigations with human primary cells under well controlled conditions of exposure, including all important parameters as described above, are urgently needed to elucidate possible adverse effects of MW signals that are currently being used in wireless communication, especially in new technologies such as UMTS mobile telephony.

The dependence of adverse effects of NT MW from GSM/UMTS mobile phones on carrier frequency and type of signal should be taken into account in settings of safety standards and in planning of *in vivo* and epidemiological studies. Of note, the data from epidemiological studies should be treated with care. Indeed, it is almost impossible to select control unexposed groups because the whole population in many countries is exposed to wide range of MW signals from various sources such as mobile phones and base stations/masts of various kinds, WLAN, WPAN, DECT wireless phones and given that duration of exposure (must be at least 10 years for cancer latency period) may be more important for the adverse health effects of NT MW than PD/SAR. From this point of view, current epidemiological studies may be either inconclusive, if results are negative, or may underestimate the hazard of MW exposure, if results are positive.

The joined efforts of scientific groups within national or international programs are needed for mechanistic studies of the NT MW effects. In order to take into account all necessary physical parameters and biological variables, these programs should involve scientists with long-lasting experience in studying NT MW effects.

Because NT MW affect not only brain cells, but also blood cells^{38-40, 75}, skin and fibroblasts^{68, 69, 134, 154}, stem cells^{67, 116, 155}, reproductive organs and sperm quality¹⁵⁶⁻¹⁵⁹ the using of hands-free cannot minimize all adverse health effects. Possibilities to minimize the adverse effects of NT MW using various biophysical and biochemical approaches should be studied.

Identification of those signals and frequency channels/bands for mobile communication, which do not affect human cells, is needed as a high priority task for the development of safe mobile communication.

Acknowledgements

Financial supports from the Swedish Council for Working Life and Social Research, the Swedish Radiation Protection Authority, the National Scholarship Program of the Slovak Republic, and the Russian Foundation for Basic Research are gratefully acknowledged.

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Is cognitive function affected by mobile phone radiation exposure?

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Abstract

Behavioral tasks, including the Morris water maze (MWM), radial arm maze and object recognition task, have been extensively used to test cognitive impairment following exposure of rodents to mobile phone (MP) radiation on various frequencies and specific absorption rate (SAR) values. Exposed animals in most of the cases revealed defects in their working memory possibly due to cholinergic pathway distraction. The only experiment on mice at very low SAR did not show statistically significant deficits by 8-arm maze, but our own data in mice exposed to GSM 900 MHz radiation, revealed memory lesions on MWM task; exposed mice had difficulties in memory consolidation and/or retrieval of the stored information. Lastly, a number of studies have been applied to volunteers showing variable results depending on the experimental setup, revealing memory improvement or deficits following MP exposure.

The recorded data from the literature are generally favouring the conclusion that EMF is affecting memory function although a more rigorous and reproducible exposure system has to be adopted in relation to the recently criticized importance of SAR.

Key words: electromagnetic fields, Morris water maze, spatial memory, cognition

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This work has been supported by the Special Account for Research Grants of the National and Kapodistrian University of Athens.

A.F. Fragopoulou is a scholarship recipient of the Hellenic State Scholarship Foundation – “N.D. Xrysovergis” Bequest (PhD fellowship).

Note added in proofs:

A number of studies have appeared after the submission of the manuscript, dealing with EMFs and cognitive memory function. It is worth mentioning that a positive effect was found on transgenic mice for Alzheimer's disease following chronic exposure to MP radiation as reported by Arendash GW, Sanchez-Ramos J, Mori T, et al. *Journal of Alzheimer's Disease* 2010; 19: 191–210.

Introduction

The extended use of mobile phone technology throughout all social levels and all ages, starting from as low as 4 years old, has forced a large number of scientists to get involved in the investigation of the effects. The major issue is that unlike other forms of everyday radiation exposure, the use of the mobile phone and the wireless DECT phone takes place near the user's head and therefore direct or indirect effect on the brain function is highly possible. Thus, the elucidation of the cellular, molecular and behavioural effects has to be explored in depth, especially since the majority of life-time users will be the current teenagers.

The aim of this kind of research is to determine a specific absorption rate (SAR) value threshold below which no obvious effects are detected in any organism, any cell, in order to propose biologically based levels for exposing humans on a daily basis either through cell phones, or base stations or DECT wireless phones or even wi-fi routers and baby monitors.

To approach these questions, extensive research is being performed in various laboratories. Due to the still unknown mode of primary action at the molecular level, many approaches studying the effects of microwaves (MW) have been applied¹.

At the population level, studies deal with the effects by statistically correlating exposure conditions to health symptoms, as severe as brain tumors^{2, 3}, or mild well being discomforts, such as headaches or fatigue⁴. There is also a report on children exposed prenatally to mobile phone radiation showing defects on behavior⁵. In humans, the studies involve mainly volunteers and have investigated possible effects on sleeping conditions and memory function⁶.

Studies on animal models involve every possible aspect of experimental approach (behavioral, molecular, biochemical, biophysical, ultrastructural, physiological). Such models used are mainly rodents and to a less degree insects. Our group has shown DNA fragmentation and induced cell death during oogenesis, along with a decrease in the offspring number in insects and a defect on osteogenesis following prenatal exposure in mice⁷⁻⁹.

Due to the fact that mobile phone use affects mainly the brain tissues, special attention has been given to the study of hippocampus, cerebellum and frontal brain function and structure on rodents (mostly rats). In general there are numerous reports on the effects of electromagnetic fields (EMF) on cognitive functions. Animal learning and memory function have been tracked using mazes, such as the Morris water maze (MWM), the radial arm maze (RAM), as well as the object recognition task (ORT) and the object location task (OLT). It is well documented that these mazes are related to the spatial environment and recognition learning and memory. Extra maze spatial cues are widely applied to facilitate learning and testing any deficits following exposure to MW. Especially RAM is being used to explain hippocampal formation and function¹⁰.

The MWM task is widely used since spatial navigation is a complex cognitive function that depends on several neural and cognitive systems for successful completion^{6, 11}. Unlike the T-maze in which the animals have to make a binary decision (i.e. going left or right), in the MWM successful performance requires continuous monitoring of the animal's position in relation to extra-maze cues: a process that involves "cognitive mapping". Many reports have controversially showed impairment^{12, 13}, or improvement^{14, 15}.

At the cell culture level, a number of studies have been performed in order to clarify under controllable and reproducible conditions, the actual primary damage induced by

EMFs. Thus, in cultured hippocampus neurons a decrease of excitatory synaptic activity and a reduced number of excitatory synapses was detected after exposure to GSM 1800 radiation (15 min/day for 7 days) at a SAR value of 2.4 W/kg¹⁶.

In addition, a recent report has found that EMFs affect the endocytotic activity of murine melanoma cells¹⁷.

Besides MW radiation effects, a limited number of studies has used extremely low frequency (ELF) EMF (50 or 60 Hz depending on the power line) revealing memory deficits on rats¹⁸⁻²⁰, which, interestingly, become less prominent upon exposure of the animals to MW²¹. A similar study but on mice showed reversible effects on cognitive functions as revealed by 8-arm RAM²².

Given the controversial evidence existing on the occurrence or not of any effects following MW exposure, we present herein a comparative analysis of reports on cognitive effects including some of our own recently published experimental data.

Results and discussion

Several pioneer studies concerning the effects of MW on cognitive functions, that examined the short term memory of rats, are published using a 2450-MHz circular waveguide exposure system and a SAR value of 0.6 W/kg²³. These investigators demonstrated significant deficits when exposed rats were performing at the RAM and the MWM and suggested that the reported defects in the working memory of rats are possibly due to cholinergic pathway distraction. On a later report it was shown that rats exposed to the same conditions, pulsed 2450-MHz MW (500 pulses/s, average power density 2 mW/cm², average whole body SAR 1.2 W/kg), for 1 hour just before each training session in a water maze, showed a deficit in their spatial “reference” memory²⁴.

On the other hand, Cobb and collaborators²⁵, replicating the experiments by Lai²³, under the same conditions of exposure, i.e. 2450-MHz, circular polarized waveguide system (CWG), SAR value 0.6 W/kg, but with minor methodological differences, did not find any effects on memory and learning in rats. Additionally, another report that appeared at the same year by exposing rats at similar conditions, did not observe any effects with RAM (Table 1)²⁶. However, it had been reported earlier that MW affect specific cognitive aspects of behavior such as, attention, memory, learning, discrimination, time perception, which may occur even at very low SAR levels²⁷.

Also, using RAM and ORT, no evidence was found at even higher SAR values of 1-3.5 W/kg, by applying head only and not whole body exposure of rats for 45 minutes and at another frequency of 900-MHz²⁸. Cosquer and collaborators on 2005 using a 12-arm maze apparatus, bordered by 30 cm high opaque walls, observed that exposed rats behaved normally. Therefore they concluded that MW exposure under those conditions (2450-MHz, circularly polarized field – Table 1) does not alter spatial working memory, when access to spatial cues was reduced²⁹.

In a recent report, the MWM performance of male Wistar rats was affected following exposure to 50 missed calls/day for 4 weeks by a GSM (900/1800 MHz) mobile phone in vibratory mode³⁰. The phone-exposed animals had significantly (~3 times) higher mean latency to reach the target quadrant in the MWM and spent significantly (~2 times) less time in the target quadrant. Trying to understand the cellular basis of the observed behavioural deficits, Leif Salford and collaborators have reported that a 2-hr exposure of rats at GSM 915-MHz resulted in neuronal damage, 28 and 50 days later³¹. In addition,

Table 1 - Comparative studies of EMF on cognitive performance
(ND=not determined, MWM=Morris Water Maze, RAM=Radial Arm Maze)

Study	Experimental Animal	Exposure source	Frequency	SAR or density	Duration of exposure	Task	Findings
Lai <i>et al.</i> , 1994	Rats	Circular polarized generator	2450 MHz	0.6 W/kg	45' before each trial	12-arm RAM	Deficit in working memory
Wang B, Lai H, 2000	Rats	Circular polarized generator	2450 MHz	1.2 W/kg	1 h before each training	MWM	Deficit in spatial reference memory
Cobb <i>et al.</i> , 2004	Rats	Circular polarized generator	2450 MHz	0.6 W/kg	45' before each trial	12-arm RAM	No effect
Dubreuil <i>et al.</i> , 2003	Rats	RF generator Head only	GSM 900 MHz	1 W/kg 3.5 W/kg	45' before each trial	12-arm RAM ORT	No effect
Cassel <i>et al.</i> , 2004	Rats	Circular polarized generator	2450 MHz	0.6 W/kg	45' before each trial	RAM	No effect
Cosquer <i>et al.</i> , 2005	Rats	Circular polarized generator	2450 MHz	0.6 W/kg	45' before each trial	RAM reduced access to cues	No effect
Nittby <i>et al.</i> , 2008	Rats	TEM cells	GSM 900 MHz	0.6 mW/kg 60 mW/kg	2 hr/week for a year	ORT episodic-like memory test 3 weeks after exposure	Effect
Narayanan <i>et al.</i> , 2009	Rats	Mobile phone	GSM 900/1800 MHz	ND	~ 50'/day (50 missed calls/day for 4 weeks)	MWM	Spatial memory impairment
Lai, 1996 Lai <i>et al.</i> , 1998	Rats	Sinusoidal magnetic fields	60Hz	1 mT	1 hr	12-arm RAM	Effect
Jadidi <i>et al.</i> , 2007	Rats	Sinusoidal magnetic fields	50 Hz	8 mT	20'	MWM	Spatial memory impairment
Sienkiewicz <i>et al.</i> , 2000	Mice	GTEM cells far field	GSM 900 MHz	0.05 W/kg	45'/day for 10 days	8-arm RAM	No effect
Fragopoulou <i>et al.</i> , 2010	Mice	Mobile phone	GSM 900 MHz	0.41-0.98 W/kg	1 hr before each trial and between the trials	MWM	Spatial memory impairment, learning lesions

(continued)

Table 1 - continued

(ND=not determined, MWM=Morris Water Maze, RAM=Radial Arm Maze)

Study	Experimental Animal	Exposure source	Frequency	SAR or density	Duration of exposure	Task	Findings
Sienkiewicz <i>et al.</i> , 1998	Mice	Sinusoidal magnetic fields	50 Hz	7.5 μ T to 7.5 mT	45' before each trial	8-arm RAM	Reversible effects
Preece <i>et al.</i> , 1999	Humans	Local brain exposure analog phone	915 MHz	1 W power	ND	Working memory	Improved performance
Koivisto <i>et al.</i> , 2000	Humans	Local brain exposure by mobile phone	GSM 902 MHz	0.25 W mean power	On and off	Working memory	Improved performance
Edelstyn and Oldershaw, 2002	Humans 20-22 years old	Local brain exposure by mobile phone	GSM 900 MHz	1.19 W/kg	30'	Cognitive neuropsychological tests subtraction and verbal fluency	Improvement
Maier <i>et al.</i> , 2004	Humans	Local brain exposure by mobile phone	GSM 915 MHz	1.0 mW/m ²	50'	Auditory discrimination	Impairment
Besset <i>et al.</i> , 2005	Humans	Local brain exposure by mobile phone	GSM 900	ND	2 hr/day, 5 days/week for 45 days	Cognitive tasks	No effect
Russo <i>et al.</i> , 2006	Humans	Local brain exposure by mobile phone	GSM 888 MHz Modulated CW-unmodulated	1.4 W/kg	40' prior to test	Cognitive tasks	No effect
Krause <i>et al.</i> , 2006	Children	Local brain exposure by mobile phone	GSM 902 MHz	1.4 W/kg	On and off	Auditory memory task	Effects on brain oscillatory responses
Regel <i>et al.</i> , 2007	Humans	Local brain exposure by mobile phone	GSM 900 MHz	1.0 W/kg	30' prior to test	Cognitive tasks	Increased accuracy in a working memory test
Haarala <i>et al.</i> , 2007	Humans	Signal generator and dummy phone	GSM 902 MHz	1.1 W/kg	On and off	Cognitive tasks	No effects
Luria <i>et al.</i> , 2009	Humans	Local brain exposure by mobile phone	GSM Nokia 5110	0.54-1.09 W/kg	On and off	Spatial working memory	Delay on reaction time

(continua)

Table 1 - continued
(ND=not determined, MWM=Morris Water Maze, RAM=Radial Arm Maze)

Study	Experimental Animal	Exposure source	Frequency	SAR or density	Duration of exposure	Task	Findings
Wiholm <i>et al.</i> , 2009	Humans	Headset with a fixed antenna placed on the left side of the head	884 MHz	1.4 W/kg	150' prior to test at 10 p.m.	Spatial memory and learning	Symptomatic group improved their performance

Reports have been ordered according to date published, species exposed and type of radiation

the same group has reported that the blood brain barrier (BBB) has been disrupted in irradiated rats³².

Concerning the long term effects, Salford’s group has shown in rats that whole body SAR values, as low as 0.6 and 60 mW/kg, significantly alter the performance during an episodic-like memory test after 55 weeks of 2-hr exposure once a week³³.

Studies on the effects of MW radiation on mice’ cognitive functions are very limited. In one of them the animals were exposed within GTEM (Gigahertz Transverse Electro-magnetic) cells at GSM 900-MHz frequency but at very low SAR of just 0.05 W/kg. No statistically significant deficits were resolved by 8-arm maze³⁴. Expanding the exploration on the effects of radiation on mice, our group has performed a series of experiments to test spatial memory and learning in mice *Mus musculus* Balb/c using primarily the MWM task. The exposure setup consisted of a commercially available mobile phone, as firstly introduced by our group in insects^{7, 8} and applied recently as well in mice^{9, 35}. In these experiments free moving mice were irradiated within their home plastic cages, as also reported by other studies in rats^{30, 36}. The animals were exposed to a 2-hr daily dose of pulsed GSM 900-MHz voice modulated at a SAR level of 0.41 to 0.98 W/kg, for four consecutive days during the MWM task protocol. Extended analysis of the data revealed that the animals exposed to the near field of a commercially available mobile phone could not transfer the learned information across the training days. Moreover, the data of the memory probe trial showed that the exposed animals had difficulties in memory consolidation and/or retrieval of the stored information of the position of the hidden platform, since they showed no preference for the target quadrant. Before each set of experiments the mean power density of the radiation emitted by the mobile phone handset in the RF range at 900-MHz was measured with the field meter’s probe placed inside the cage with the animals. The measured exposure values were in general within the established exposure limits by ICNIRP on 1998³⁷. We used commercially available digital mobile phone handsets, in order to analyse effects of real mobile telephony exposure conditions. Thus, instead of using simulations of digital mobile telephony signals with constant parameters (frequency, intensity, etc.), or even “test mobile phones” programmed to emit mobile telephony signals with controllable power or frequency, we used real GSM signals which are never constant since there are continuous changes in their intensity³⁵.

The SAR was approximately calculated according to the formula^{37, 38}:

$$SAR = \sigma E^2/\rho$$

where E is the root mean square value of the electrical field, σ is the mean electrical conductivity of the tissues and ρ is the mass density. The SAR is a parameter widely used by many authors to compare the absorbed energy in different biological tissues. Thus, the parameters used for mice and rats were calculated according to Peyman *et al.*³⁹.

Another very promising and significant set of approaches involves experimental studies on volunteers and have focused on human cognitive function following exposure to mobile phone radiation (Table 1). One category of reports has shown memory improvement, i.e. facilitation in attention following exposure to mobile phone¹⁴. In another case, 915-MHz mobile phone exposure improved performance in a working memory task¹³, and in the same direction another study found improvement in cognitive tasks, i.e. verbal memory capacity, sustained attention and visuospatial working memory⁴⁰.

Also, DeSeze' group has studied on 2005 the outcomes from the daily use of mobile phones GSM 900 on cognitive function⁴¹. Fifty-five subjects (27 males and 28 females) were divided into two groups: a group with mobile phone switched on and a group with mobile phone switched off. The two groups were matched according to age, gender, and IQ. This double blind study lasted for 45 days and the neuropsychological test battery composed of 22 tasks, screened four neuropsychological categories: information processing, attention capacity, memory function, and executive function. This neuropsychological battery was performed four times, on day 2, day 15, day 29, and day 43. The results indicated that daily mobile phone use had no effect on cognitive function after a 13-hr rest period.

In a very interesting study Krause and collaborators assessed the effects of EMF emitted by mobile phones on the 1-20 Hz range by event-related brain oscillatory electroencephalogram (EEG) responses in children performing an auditory memory task (encoding and recognition)⁴². What they found was that EMF emitted by mobile phones has effects on brain oscillatory responses during cognitive processing at least in teenagers. Also in an attempt to test MW effects on human attention Russo and collaborators studied on 2006 a large sample of volunteers (168) using a series of cognitive tasks apparently sensitive to RF exposure (a simple reaction task, a vigilance task, and a subtraction task)⁴³. Participants performed those tasks twice, in two different sessions. In one session they were exposed to RF, with half of subjects exposed to GSM signals and the other half exposed to continuous waves (CW) signals, while in the other session they were exposed to sham signals. No significant effects of RF exposure on performance for either GSM or CW were found. On the other hand, it has been shown that in humans, exposure at 1 W/kg, to pulse-modulated radio frequency electromagnetic field 900 MHz, reduced reaction speed and increased accuracy in a working-memory task⁴⁴. The same study showed that exposure prior to sleep alters brain activity. For a summary of the available literature see Table 1.

The possible effects of CW and pulse modulated (PM) EMF on human cognition in 36 healthy male subjects were studied by Haarala and collaborators on 2007. They performed cognitive tasks while the volunteers were exposed to CW, PM, and sham EMF. They found no differences between the different EMF conditions⁴⁵.

In a just recent report, Bengt Arnetz' group investigated the effects of a 2 hr and 30 min RF exposure (884-MHz) on spatial memory and learning, using a double-blind repeated measures design⁶. The exposure was designed to mimic a real-life mobile phone conversation, at a SAR value of 1.4 W/kg. The primary outcome measure was a "virtual" spatial navigation task modelled after the commonly used and validated MWM. The distance travelled on each trial and the amount of improvement across trials

(i.e., learning) were used as dependent variables. The participants were daily mobile phone users, with and without symptoms attributed to regular mobile phone use. The symptomatic group improved their performance during RF exposure while there was no such effect in the non-symptomatic group (Table 1).

Conclusions

In the presented studies the effects of MW radiation deriving either from RF generator providing continuous or modulated mobile phone-like signal, or from conventional mobile phone either computer controlled or under normal communication, were investigated at various carrier frequencies, 900, 1800 and 2450 MHz on the spatial learning and memory of rodents and humans. Several investigators have demonstrated the commonality between the performance of humans on real time spatial navigation tasks as compared to rats, mice and most other mammals studied so far⁴⁶. The role of hippocampus, in particular, in navigation is concordant with neuronal response in rats and we assume in mice as well.

In our experiments using the MWM, Balb/c mice were required to find a submerged platform in the circular pool after 4 days of training by creating a “reference map” (reference memory)⁴⁷. Exposed mice to the near field of a conventional mobile phone showed difficulty in finding the position of the hidden platform during training and could not transfer the learned information across the days. The recorded data from the probe trial indicated that exposed mice had difficulty in memory consolidation and/or retrieval of the stored information³⁵.

A number of studies have used a range of SAR values, from 0.02 mW/kg up to 4 W/kg in order to induce and detect memory deficits in rodents and especially in rats. In the vast majority of the studies the Transversal Electromagnetic Mode (TEM) cells were used, exposing the animals at a given power density from an RF generator. Similar learning and memory deficits revealed with the MWM following exposure to pulsed circularly polarized 2450-MHz MW at 2 mW/cm² power density, have been also reported in rats²⁵. Some studies failed to reveal any effects whereas others have demonstrated that according to the radiation set up used (frequency, power density and duration of exposure) the animals' memory function is somehow affected by EMF (Table 1). In a very recent study Narayanan and collaborators using similar to ours exposure setup protocol irradiated male Wistar rats, 10-12 weeks old, which are developmentally comparable to human teenagers³⁰. The rats were exposed to 50 missed calls/day for 4 weeks from a GSM (900/1800-MHz) mobile phone in vibratory mode (no ring tone). After the experimental period, the animals were tested for spatial memory performance using the MWM test. Both phone exposed and sham exposed animals showed a significant decrease in escape time with training. In the probe trial phone exposed animals had significantly (~3 times) higher mean latency to reach the target quadrant and spent significantly (~2 times) less time in the target quadrant than age- and sex-matched controls. It is crucial to note that this work has used similar to ours experimental protocol having the mobile phone within the cage, but with longer exposure. It seems therefore that mice and rats respond similarly to the radiation stress by exhibiting deficits in their spatial memory operation. Some investigators (including our group) have chosen to perform experiments in animals allowed to move freely in their home cages during exposure to radiation^{9, 30, 35, 36}. Doing so, any possible confounding effects of restraint stress are minimized, since it is well known

that stress affects learning and memory⁴⁸. Exposure conditions were carefully selected in order to simulate as close as possible ordinary mobile phone use (duration and signal strength). EMF with changing parameters are found to be more bioactive than fields with constant parameters^{44, 49, 50}. That is probably because it is more difficult for living organisms to get adapted to them. Experiments with constant GSM or DCS signals can be performed, but they do not simulate actual conditions. International guidelines limit the local SAR to a maximum of 2 W/kg³⁷, or 1.6 W/kg³⁸. Since the maximum SAR value as calculated in our experiments was at most 0.98 W/kg and since this SAR value does not affect the mice's body temperature³⁷, the exposure conditions used in our experiments can be considered nonthermal.

Furthermore, some investigators (including us) selected the age of the experimental animals (50-day-old) to correspond approximately to that of late adolescence in humans, a population in which mobile phone use is particularly prevalent. Similar to our exposure conditions have been used by other investigators⁵¹; they have irradiated rats with conventional mobile phone operating at a maximum power of 0.607 W. They found by mRNA analysis an effect on injury associated proteins leading to cellular damage to the rat brain.

Since it is well known that performance in the MWM is dependent on the hippocampus, it is plausible to assume that MW radiation exposure affected this brain area. Such a notion may be supported by the observation that apoptotic cells have been detected in the hippocampus of rats after a 2 hr for 50 days GSM radiation^{31, 32}. Furthermore, the function of the hippocampus could be affected by the GSM irradiation possibly due to disruption of the blood-brain barrier, which has been reported to occur as a result of GSM irradiation^{52, 53}. However, other investigators using 915-MHz at power levels resulting in whole-body specific absorption rates of 0.0018-20 W/kg failed to reveal such a relationship⁵⁴.

Considering that memory functions are similar in mice and humans with respect to the involvement of the hippocampus⁵⁵, we may assume that upon using the mobile phone in contact with the head, a person may experience cognitive deficits. Interestingly, it has been reported that exposure to GSM 890-MHz radiation results in deficits of human cognitive function⁵⁶. The same research group reported recently using a spatial working memory task that the average reaction time (RT) of the right-hand responses under left-side exposure condition was significantly longer than those of the right-side and sham-exposure groups⁵⁷. These results confirmed the existence of an effect of exposure on RT, as well as the fact that exposure duration (together with the responding hand and the side of exposure) may play an important role in producing detectable radiofrequency radiation (RFR) effects on performance. It is notable that right and left hemispheres did not show similar patterns of activation. Differences in these parameters might be the reason for the failure of certain studies to detect or replicate RFR effects. The question whether the memory impairment is reversible is open for exploration by further experiments which are in progress. Finally the actual molecular impact of the EMF is being studied at the proteomics level in our lab, in an attempt to explain the molecular events underlying the brain cells' malfunction after irradiation.

It has been suggested that behavioral alterations induced by EMF are thermally mediated⁵⁸. That is because in most studies these effects derive from SAR values beyond the reference standard of 2 W/kg. The effects reported at very low SAR values may be explained by free radical formation as suggested⁵⁹. It could also be due to protein conformation changes⁶⁰. It might be possible that these changes cause alterations in cognitive function-related proteins, such as androgen receptors and apolipoprotein A⁶¹.

Finally, as questioned in a recent study by Philips and collaborators⁵⁹: “Are studies unable to replicate the work of others more credible than the original studies? In other words, can negative studies cancel positive studies or may studies showing effects be less valid because no explanation is provided?” The answer is that given the different frequency and modulation and in general the exposure set up conditions used in different studies, the issue remains open as to which of the parameters used in the “exposure cocktail”, is crucial to alter brain cells’ function. Is it the RF itself or the modulation? Or may be the ELF component of the battery switching mode of the cell phone. This issue is more complex than it seems when trying to compare animal studies with human clinical or experimental findings, possibly due to the differences in exposure conditions. Till the final elucidation of the effects, this research task is open for investigation requiring probably more sophisticated approaches and experimentation procedures.

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Provocation study using heart rate variability shows microwave radiation from 2.4 GHz cordless phone affects autonomic nervous system

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Abstract

Aim: The effect of pulsed (100 Hz) microwave (MW) radiation on heart rate variability (HRV) was tested in a double blind study. **Materials and Methods:** Twenty-five subjects in Colorado between the ages of 37 to 79 completed an electrohypersensitivity (EHS) questionnaire. After recording their orthostatic HRV, we did continuous real-time monitoring of HRV in a provocation study, where supine subjects were exposed for 3-minute intervals to radiation generated by a cordless phone at 2.4 GHz or to sham exposure. **Results:** Questionnaire: Based on self-assessments, participants classified themselves as extremely electrically sensitive (24%), moderately (16%), slightly (16%), not sensitive (8%) or with no opinion (36%) about their sensitivity. The top 10 symptoms experienced by those claiming to be sensitive include memory problems, difficulty concentrating, eye problems, sleep disorder, feeling unwell, headache, dizziness, tinnitus, chronic fatigue, and heart palpitations. The five most common objects allegedly causing sensitivity were fluorescent lights, antennas, cell phones, Wi-Fi, and cordless phones. **Provocation Experiment:** Forty percent of the subjects experienced some changes in their HRV attributable to digitally pulsed (100 Hz) MW radiation. For some the response was extreme (tachycardia), for others moderate to mild (changes in sympathetic nervous system and/or parasympathetic nervous system). and for some there was no observable reaction either because of high adaptive capacity or because of systemic neurovegetative exhaustion. **Conclusions:** Orthostatic HRV combined with provocation testing may provide a diagnostic test for some EHS sufferers when they are exposed to electromagnetic emitting devices. This is the first study that documents immediate and dramatic changes in both Heart Rate (HR) and HR variability (HRV) associated with MW exposure at levels

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well below (0.5%) federal guidelines in Canada and the United States (1000 microW/cm²).

Key Words: heart rate variability, microwave radiation, DECT phone, autonomic nervous system, provocation study, sympathetic, parasympathetic, cordless phone, 2.4 GHz, electrohypersensitivity

Introduction

A growing population claims to be sensitive to devices emitting electromagnetic energy. Hallberg and Oberfeld¹ report a prevalence of electrohypersensitivity (EHS) that has increased from less than 2% prior to 1997 to approximately 10% by 2004 and is expected to affect 50% of the population by 2017. Whether this is due to a real increase in EHS or to greater media attention, is not known. However, to label EHS as a psychological disorder or to attribute the symptoms to aging and/or stress does not resolve the issue that a growing population, especially those under the age of 60, are suffering from some combination of fatigue, sleep disturbance, chronic pain, skin, eye, hearing, cardiovascular and balance problems, mood disorders as well as cognitive dysfunction and that these symptoms appear to worsen when people are exposed to electromagnetic emitting devices²⁻⁷.

The World Health Organization (WHO) organized an international seminar and working group meeting in Prague on EMF Hypersensitivity in 2004, and at that meeting they defined EHS as follows⁸:

“... a phenomenon where individuals experience adverse health effects while using or being in the vicinity of devices emanating electric, magnetic, or electromagnetic fields (EMFs) . . . Whatever its cause, EHS is a real and sometimes a debilitating problem for the affected persons . . . Their exposures are generally several orders of magnitude under the limits in internationally accepted standards.”

The WHO goes on to state that:

“EHS is characterized by a variety of non-specific symptoms, which afflicted individuals attribute to exposure to EMF. The symptoms most commonly experienced include dermatological symptoms (redness, tingling, and burning sensations) as well as neurasthenic and vegetative symptoms (fatigue, tiredness, concentration difficulties, dizziness, nausea, heart palpitation and digestive disturbances). The collection of symptoms is not part of any recognized syndrome.”

Both provocation studies (where individuals are exposed to some form of electromagnetic energy and their symptoms are documented) and amelioration studies (where exposure is reduced) can shed light on the offending energy source and the type and rate of reaction.

Several amelioration studies have documented improvements in the behavior of students and the health and wellbeing of teachers⁹, among asthmatics¹⁰, and in both diabetics and those with multiple sclerosis^{11,12} when their exposure to dirty electricity is reduced. Dirty electricity refers to microsurges flowing along electrical wires in the kHz

range that can damage sensitive electronic equipment and, it appears, affect the health of those exposed.

In contrast to amelioration studies, provocation studies, examining the response of people with self-diagnosed EHS, have generated mixed results.

Rea *et al.*¹³ were one of the first to show that sensitive individuals responded repeatedly to several frequencies between 0.1 Hz and 5 MHz but not to blank challenges. Reactions were mostly neurological and included tingling, sleepiness, headache, dizziness, and - in severe cases - unconsciousness, although other symptoms were also observed including pain of various sorts, muscle tightness particularly in the chest, spasm, palpitation, flushing, tachycardia, etc. In addition to the clinical symptoms, instrument recordings of pupil dilation, respiration, and heart activity were also included in the study using a double-blind approach. Results showed a 20% decrease in pulmonary function and a 40% increase in heart rate. These objective instrumental recordings, in combination with the clinical symptoms, demonstrate that EMF sensitive individuals respond physiologically to certain EMF frequencies although responses were robust for only 16 of the 100 potentially sensitive individuals tested.

In a more recent review, Rubin *et al.*¹⁴ concluded that there was no robust evidence to support the existence of a biophysical hypersensitivity to EMF. This was based on 31 double-blind experiments that tested 725 EHS subjects. Twenty-four studies found no difference between exposure and sham conditions and of the seven studies that did find some evidence that exposure affected EHS participants, the research group failed to replicate the results (two studies) or the results appeared to be statistical artifacts (three studies).

Those who live near antennas and those who suffer from EHS often complain of cardiovascular problems such as rapid heart rate, arrhythmia, chest pain, and/or changes in blood pressure^{3,7,15,16}.

Indeed, the doctors who signed the Freiburger Appeal¹⁷ stated the following:

"We have observed, in recent years, a dramatic rise in severe and chronic disease among our patients especially . . . extreme fluctuations in blood pressure, ever harder to influence with medications; heart rhythm disorders; heart attacks and strokes among an increasingly younger population . . ."

Based on these findings we decided to study the affect of microwave (MW) radiation generated by a digital cordless phone on the cardiovascular system by monitoring heart rate variability (HRV). Unlike cell phones that radiate microwaves only when they are either transmitting or receiving information, the cordless phone we used radiates constantly as long as the base of the phone is plugged into an electrical outlet. The phone we used was an AT&T digitalally pulsed (100 Hz) cordless telephone that operates at 2.4 GHz or frequencies commonly used for microwave ovens and Wi-Fi. It resembles its European version know as a Digital Enhanced Cordless Telecommunications (DECT) phone that operates at 1.9 GHz¹⁸.

HRV is increasingly used for screening cardiovascular and neurological disorders¹⁹⁻²⁴. We wanted to determine whether HRV could be used as a tool to diagnose EHS and whether it could be used to predict probability and/or intensity of the reaction to a MW provocation. The HRV analysis, using NervExpress software^{25, 26}, provides information about the functioning of the sympathetic and parasympathetic nervous system with real time monitoring and provides additional information including a pre-exposure fitness score based on the orthostatic test.

Materials and methods

Background electromagnetic environment

Testing was done in two locations, one in Golden and the other in Boulder, Colorado, on three separate weekdays during a 6-day period (Table 1). Background levels of low frequency magnetic fields, intermediate frequency radiation on electrical wires, and radio frequency radiation were monitored at each location and the values are provided in Table 1. All testing of the electromagnetic environment was done in the area where volunteers were tested for their heart rate variability during the provocation study.

The extremely low frequency **magnetic field** was measured with an omni-directional Trifield meter. This meter is calibrated at 60 Hz with a frequency-weighted response from 30 to 500 Hz and a flat response from 500 to 1000 Hz. Accuracy is $\pm 20\%$.

Power quality was measured with a Microsurge Meter that measures high frequency transients and harmonics between 4 and 150 kHz (intermediate frequency range). This meter provides a digital reading from 1 to 1999 of dv/dt expressed as GS units with a $\pm 5\%$ accuracy²⁷. Since we were trying to ensure low background exposure, we installed GS filters to improve power quality. The results recorded are with GS filters installed.

Within at least 100 m of the testing area, all wireless devices (cell phones, cordless phones, wireless routers) were turned off. **Radio frequency radiation** from outside the testing area was measured with an Electrosmog Meter, which has an accuracy of ± 2.4 dB within the frequency range of 50 MHz to 3.5 GHz. Measurements were conducted using the omni-directional mode and were repeated during the testing. This meter was also used to determine the exposure of test subjects during provocation with a digital cordless phone. This **cordless phone** emits radio frequency radiation when the base station is plugged into an electrical outlet. This happens even when the phone is not in use. We used the base station of an AT&T 2.4 GHz phone (digitally pulsed at 100 Hz) to expose subjects to MW radiation¹⁸. The emission of MWs at different distances from the front of the base station is provided in fig. 1.

Testing of subjects

Subjects were **recruited** by word-of-mouth based on their availability during a short period of testing. Of the 27 people who volunteered to be tested, two were excluded, one based on age (less than 16 years old) and another based on a serious heart condition.

Subjects were asked to complete a wellness and EHS **questionnaire**. They were then asked questions about their age, height, weight, blood type, time of last meal, and occupation (in the event of occupational exposure to electromagnetic fields/radiation).

Table 1 - Measurements of the electromagnetic environment at each testing location

Location	Date	Magnetic Field 30 - 1000 Hz mG	Power Quality 4 - 150 kHz GS units	Radio Frequency Radiation 50 MHz - 3.5 GHz microW/cm ²
Colorado				
Golden	10/16/08	3 - 15	140	0.8
Boulder	10/20/08	0.4	37	<0.01
Boulder	10/21/08	0.4	80	<0.01

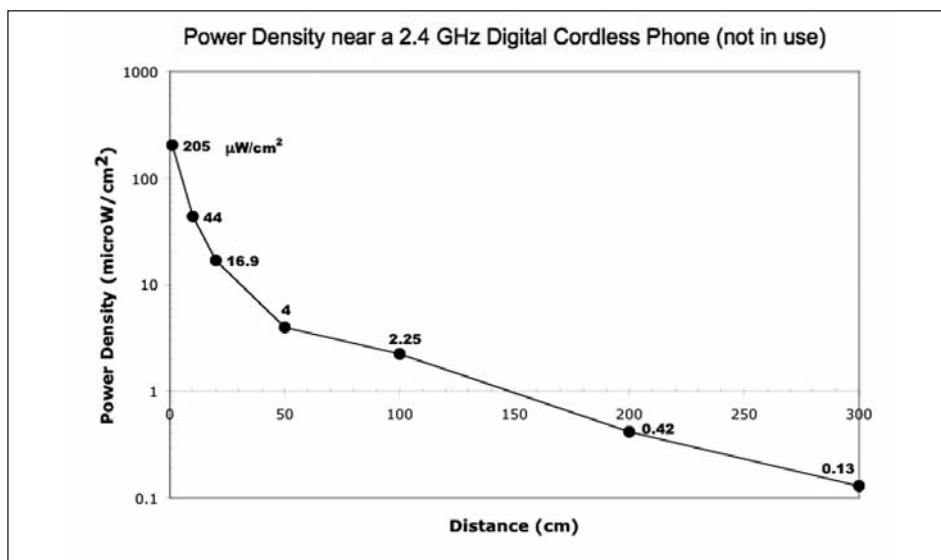


Fig. 1. Radiation near a 2.4 GHz AT&T digital cordless phone when the base station of the phone is plugged into an electrical outlet and the phone is not in use

We measured resting heart rate and blood pressure using a Life Source UA-767 Plus digital blood pressure monitor; saliva pH with pH ion test strips designed for urine and saliva (pH range 4.5-9.0), and blood sugar with ACCU-CHEK Compact Plus.

In an attempt to address the question: “Is there a simple test that relates EHS with the electrical environment of the human body?”, we measured galvanic skin response (GSR), body voltage, and the high and low frequency electric and magnetic field of each subject.

Wrist-to-wrist galvanic skin response was measured as an indicator of stress using a Nexxtech voltmeter (Cat. No. 2200810) set at 20 volts DC and attached to the inner wrist with a Medi Trace 535 ECG Conductive Adhesive Electrodes Foam used for ECG monitoring. Capacitively coupled “body voltage” was measured with a MSI Multimeter connected to a BV-1 body voltage adaptor. The subject’s thumb was placed on one connector and the other connector was plugged into the electrical ground, which served as the reference electrode. High frequency (HF) and low frequency (LF) electric and magnetic fields were measured with a Multidetektor II Profi Meter held at approximately 30 cm from the subject’s body, while the subject was seated.

HRV testing

Two types of HRV testing were conducted. The first was an *orthostatic* test and the second was *continuous monitoring* of heart rate variability with and without provocation (exposure to MW frequencies from a digital cordless phone). NervExpress software was used for HRV testing²⁵. NervExpress has both CE and EU approval and is a Class Two Medical Device in Canada and in the European Union. An electrode belt with transmitter was placed on the person’s chest near the heart, against the skin. A wired HRV cable with receiver was clipped to the clothing near the transmitter and connected to the COM

port of the computer for acoustical-wired transmission (not wireless). This provided continuous monitoring of the interval between heartbeats (R-R interval).

For the *orthostatic* testing subject laid down on his/her back and remained in this position for 192 R-R intervals or heartbeats (approximately 3 minutes), at which time a beep from the computer indicated that the person stand up and remain standing until the end of the testing period, which was 448 intervals (approximately 7 minutes depending on heart rate).

For the *provocation* testing, subject remained in a lying down position for the duration of the testing. A digital cordless phone base station, placed approximately 30 to 50 cm from subject's head, was then connected randomly to either a live (real exposure) or dead (sham exposure) extension cord. It was not possible for the subject to know if the cordless phone was on or off at any one time. Continuous real-time monitoring recorded the interval between each heartbeat. Data were analyzed by timed stages consisting of 192 R-R intervals (heartbeats).

The sham exposures are referred to as either pre-MW exposure or post-MW exposure to differentiate the order of testing. Since type of exposure was done randomly in some instances either the pre-MW or the post-MW is missing. Subjects who reacted immediately to the cordless phone were retested with more real/sham exposures. When subject was exposed multiple times, only the first exposure was used for comparison. Provocation testing took between 9 to 30 minutes per subject.

After the initial testing, treatments (deep breathing, laser acupuncture, Clean Sweep) that might alleviate symptoms were tried on a few subjects but these results will be reported elsewhere.

Interpretation of HRV results

The results for the orthostatic testing and provocation testing were sent to one of the authors (JM) for interpretation. An example of the type of information send is provided in fig. 2 (orthostatic) and fig. 3 (provocation). No information was provided about the subject's self-proclaimed EHS and the information about exposure was blinded. JM did not examine the provocation results until he reviewed the orthostatic results. No attempt was made to relate the two during this initial stage of interpretation.

Predicting response and health based on orthostatic test

For the orthostatic testing JM provided a ranking for cardiovascular tone (CVT), which is based on the blood pressure and heart rate (sum of systolic and diastolic blood pressure times heart rate) and provides information on whether the cardiovascular system is hypotonic (<12,500) or hypertonic (>16,500). We used a 5-point ranking scale as follows: Rank 1: < 12,500, hypotonic; Rank 2: 12,500 to 14,000; Rank 3: 14,000 to 15,500; Rank 4: 15,500 to 16,500; Rank 5: > 16,500, hypertonic.

Non-Adaptive Capacity (NAC)^a was ranked on a 5-point scale with 1 indicating highly adaptive and 5 indicating highly non-adaptive. This was based on a balanced sympathetic (SNS) and parasympathetic (PSNS) nervous system (average orthostatic response within ± 1 standard deviation from center on graph) and on the overall fitness

^a Later Adaptive Capacity (AC) was used, which is the inverse of NAC.

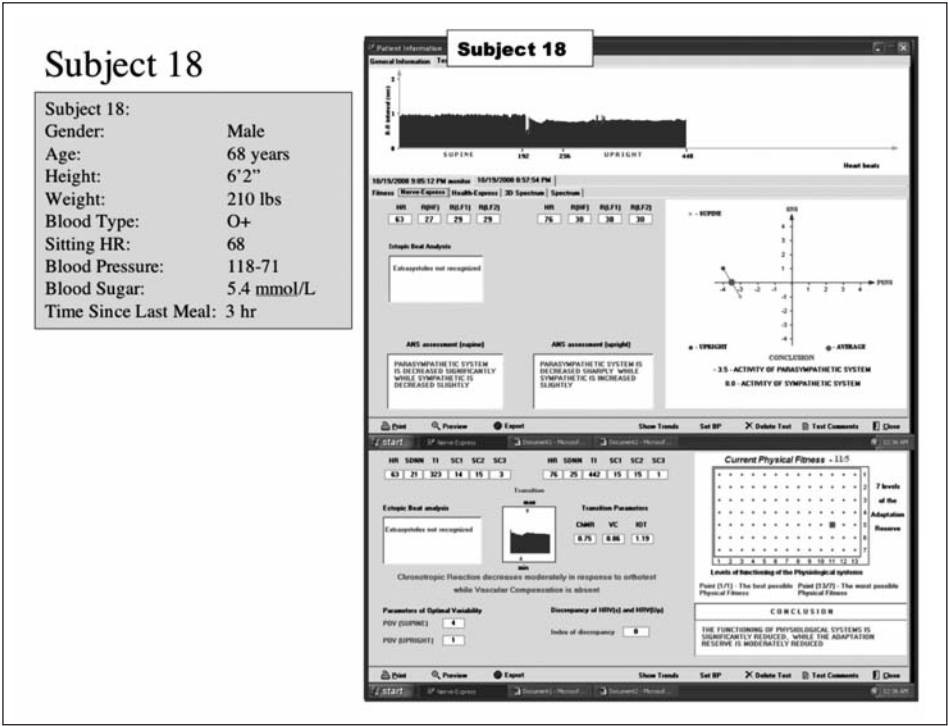


Fig. 2. Orthostatic HRV information provided for blinded analysis of Subject 18

score. The closer to normal value of the autonomic nervous system (ANS) in a given subject, the less likely they are to react, since their adaptive capacity is high. “Normal” refers to the balanced SNS/PSNS and the appropriate direction of movement under stress, in this case when person stood up. Direction of movement is shown in the NervExpress graph (fig. 2). Appropriate direction of movement would be either up 1 standard deviation (small increase in SNS and no change in PSNS); up and to the left 1 standard deviation each (small increase in SNS and small decrease in PSNS); or to left (no change in SNS and slight decrease in PSNS). For those who move further to the left (greater down regulation of PSNS) or further up and to the left (greater up regulation of SNS combined with a greater down regulation of PSNS), the less likely they are to adapt and the more likely they are to react. Likewise, if the fitness score is high or adequate, the individual would be capable of resisting the stressor. An adequate physical fitness score is between 1:1 and 10:6. The first number refers to the functioning of the physiological system and the second is the adaptation reserve. The lower the numbers the greater the level of fitness in each category. Note, if a subject with good or adequate fitness was to be a reactor to MW stress, his/her reaction would be both rapid and strong.

Probability of Reaction (POR) was ranked on a 5-point scale with “1” indicating low probability of a reaction and “5” indicating high probability of a reaction to stress of any kind. Criteria were similar to the NAC. However, greater consideration was given to the Chronotropic Myocardial Reaction Index (ChMR) value and the dysautonomic

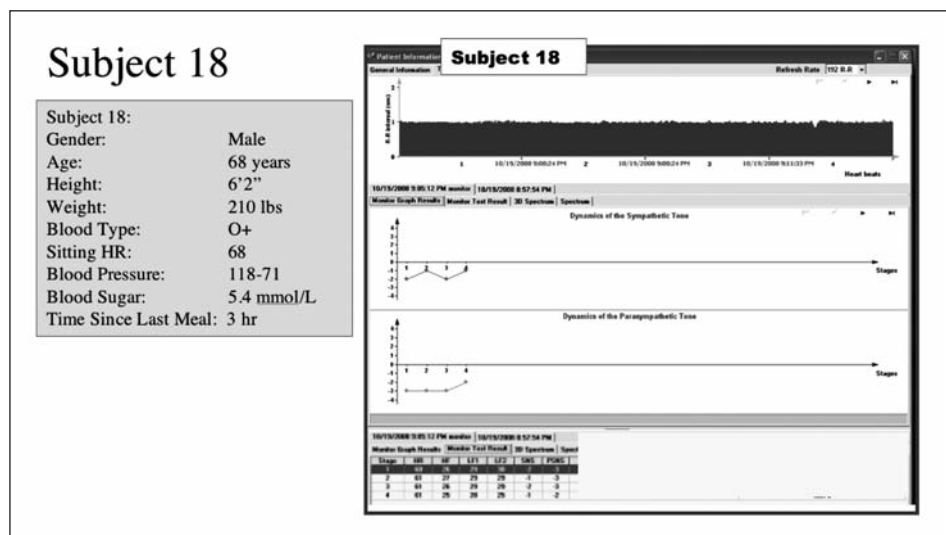


Fig. 3. Continuous monitoring of HRV with real and sham exposure to MW radiation from a digital cordless phone. Information provided for blinded analysis of Subject 18

status (average of orthostatic test is more than two standard deviations from center or up to the right) of the subject, whereby individuals with compromised ANS and a poor ChMR ranking (outside the range of 0.53 to 0.69) would be most likely to react and *vice versa*.

A potential non-responding reactor is someone with low energy, average orthostatic response in lower left quadrate, and a physical fitness score between 10:6 and 13:7. Subject 18 in fig. 2 is a borderline non-responding reactor. Note, this does not necessarily imply that this person is hypersensitive, only that he probably does not have enough energy to mount a reaction even if he was EHS.

JM also provided his comments on the health status of the subject based on the rhythmogram, autonomic nervous system assessment (changes in the SNS and PSNS), Fitness Score, Vascular Compensation Reaction (VC), ChMR, Compensation Response (CR), Ortho Test Ratio (OTR), Parameters of Optimal Variability (POV), Index of Discrepancy (ID); and Tension Index (TI). The interpretation of the HRV parameters is dependant to a certain degree on the integration of all the data provided as a whole with value being given to the total ANS picture presented. Those skilled in the art and science of HRV analysis should reach similar interpretive assessment of the data presented here²⁶.

Blinded analysis of provocation results

The blinded data for the continuous monitoring of heart rate variability with real and sham exposure were sent to JM for analysis (fig. 3). JM attempted to identify the stage during which exposure took place, stage during which the subject reacted, and then ranked symptom probability (5-point scale) and intensity (non-reactive, mild, moderate, intense). The assessment is provided in Appendix A.

Wellness and EHS Questionnaire

Prior to any testing, each subject was asked to complete a wellness and EHS questionnaire. This was designed on surveymonkey (www.surveymonkey.com) and was administered in paper format. This questionnaire was analyzed separately from the HRV data.

Results

Background electromagnetic environment

The two environments, where we conducted the testing, differed in their background levels of EMF and electromagnetic radiation (EMR). The Golden site had high magnetic fields (3-15 mG), high levels of dirty electricity (140 GS units) despite the GS filters being installed, and elevated levels of radio frequency (RF) radiation (0.8 microW/cm²) coming from 27 TV transmitters on Lookout Mountain within 4 km of our testing environment. Despite RF reflecting film on windows the RF levels inside the home were elevated. The Boulder environment was relatively pristine and differed only with respect to power quality on the two days of testing (Table 1).

The cordless phone, used for provocation, produced radiation that was maximal at the subject's head (3 to 5 microW/cm²) and minimal at the subject's feet (0.2 to 0.8 microW/cm²) depending on height of subject and the environment. The cordless phone did not alter magnetic field or power quality.

Participants

A total of 25 subjects were included in this pilot study, ranging in age from 37 to 79 with most (40%) of the subjects in their 50s (Table 2). Eighty percent were females. Approximately half of the participants had normal body mass index and the other half were either overweight (28%) or obese (16%)²⁸. Mean resting heart rate for this group was 70 (beats per minute) and ranged from 53 to 81. Blood pressure fell within a normal range for 40% of participants and fell within stage 1 of high blood pressure for 16% of the subjects²⁹. None of the subjects had pacemakers, a prerequisite for the study. Forty percent had mercury amalgam fillings and 28% had metal (artificial joints, braces, etc.) in their body. This is relevant as metal implants and mercury fillings may relate to EHS³⁰.

Questionnaire

Self-perceived Electrosensitivity

One third of participants did not know if they were or were not electrically sensitive, 40% believed they were moderately to extremely sensitive, 16% stated that they had a little sensitivity, and 8% claimed they were not at all sensitive. Their sensitivity was slightly debilitating for 24% and moderately debilitating for 20% of participants (fig. 4).

Reaction time for symptoms to appear after exposure ranged from immediately (12%) to within 2 hours (4%) and was within 10 minutes for the majority of those who believe they react (28%) (fig. 5). Recovery time ranged from immediately to within 1 day with

Table 2 - Information about participants

		#	%
Gender	Male	5	20%
	Female	20	80%
Age	Mean and Range	60 years	37-79 years
Age Class	20s	1	4%
	30s	1	4%
	40s	2	8%
	50s	10	40%
	60s	5	20%
	70s	7	28%
BMI ^a	obese	4	16%
	overweight	7	28%
	normal	13	52%
	underweight	1	4%
Resting Heart Rate	Mean and Range	70 bpm	53-81 bpm
Blood Pressure ^b	Normal	10	40%
	Pre-hypertension	11	44%
	High Blood Pressure	4	16%
Metal in Body	Pace maker	0	0%
	Mercury fillings	10	40%
	Other metal	7	28%

^a BMI = Body Mass Index based on height and weight²⁸^b Blood Pressure (BP) according to National Heart Lung and Blood Institute (nd)²⁹

only 4% claiming to recover immediately. Several participants noted that the rate of reaction and recovery is a function of the severity of their exposure and their state of health. The more intense the exposure the more rapid their response and the slower their rate of recovery. These results may have a bearing on the provocation study as we are testing an immediate reaction/recovery response (~3 minutes) to a moderate intensity exposure (3 to 5 $\mu\text{W}/\text{cm}^2$) and the percent that claims to respond quickly is low among this group.

Symptoms

The most common symptoms of exposure to electrosmog, as identified by this group of participants, included poor short-term memory, difficulty concentrating, eye problems, sleep disorder, feeling unwell, headache, dizziness, tinnitus, chronic fatigue and heart palpitations (fig. 6, upper graph). Of the symptoms commonly associated with EHS, heart palpitations (10th), rapid heartbeat (18th), arrhythmia (21st), and slower heartbeat (23rd) are the only ones we would be able to identify with HRV testing. For most participants who claim to react, reactions are mild to moderate.

All of the symptoms, except high blood pressure, arrhythmia, and slower heartbeat, were experienced several times per day (daily) or several times per week (weekly) by at least one or more participants. The patterns for symptom severity and frequency are similar (fig. 6, upper vs lower graph). Some of the symptoms (feeling unwell, pain, chronic fatigue, gas/bloat, skin problems) were experienced several times each month (monthly) may relate to menses in pre-menopausal or peri-menopausal women (16 women).

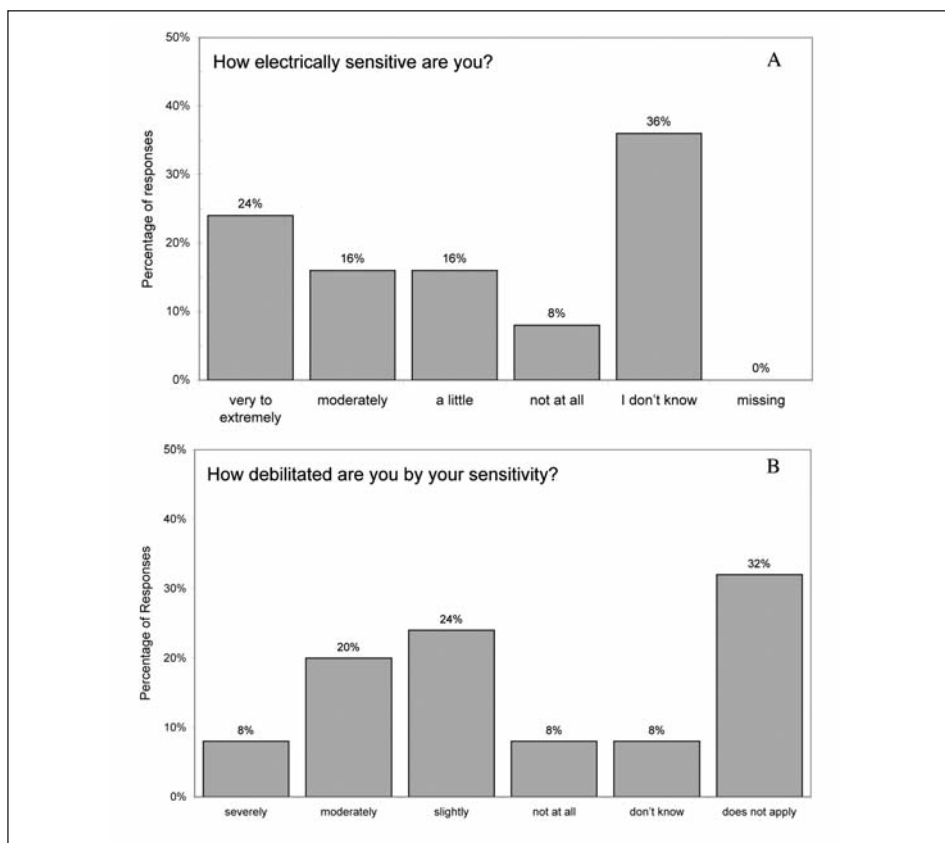


Fig. 4. Self-proclaimed electrosensitivity of participants (n=25)

A large percentage of participants had food allergies (64%), mold/pollen/dust allergies (48%), pet allergies (20%), and were chemically sensitive (36%) (fig. 7).

Some also had pre-existing health/medical conditions (fig. 8). The top five were anxiety (28%); hypo-thyroidism (24%); autoimmune disorder (20%), depression (16%) and high blood pressure (16%). Note these may be self-diagnosed rather than medically diagnosed conditions.

Objects contributing or associated with adverse health symptoms

Among the objects identified as contributing to adverse health symptoms, tube fluorescent lights were at the top of the list with more than 40% of participants reacting *often* or *always* (fig. 9). The next 4 items on the list (antennas, cell phones, Wi-Fi, cordless phones) all emit microwave radiation. According to this figure 16% of subjects respond to cordless phones *often* or *always* and their responses may include headaches, dizziness, depression, which we are unable to monitor with HRV.

Fifty-two percent stated they are debilitated by their sensitivity, 24% slightly, 20% moderately, and 8% severely. Some have difficult shopping, which may relate to

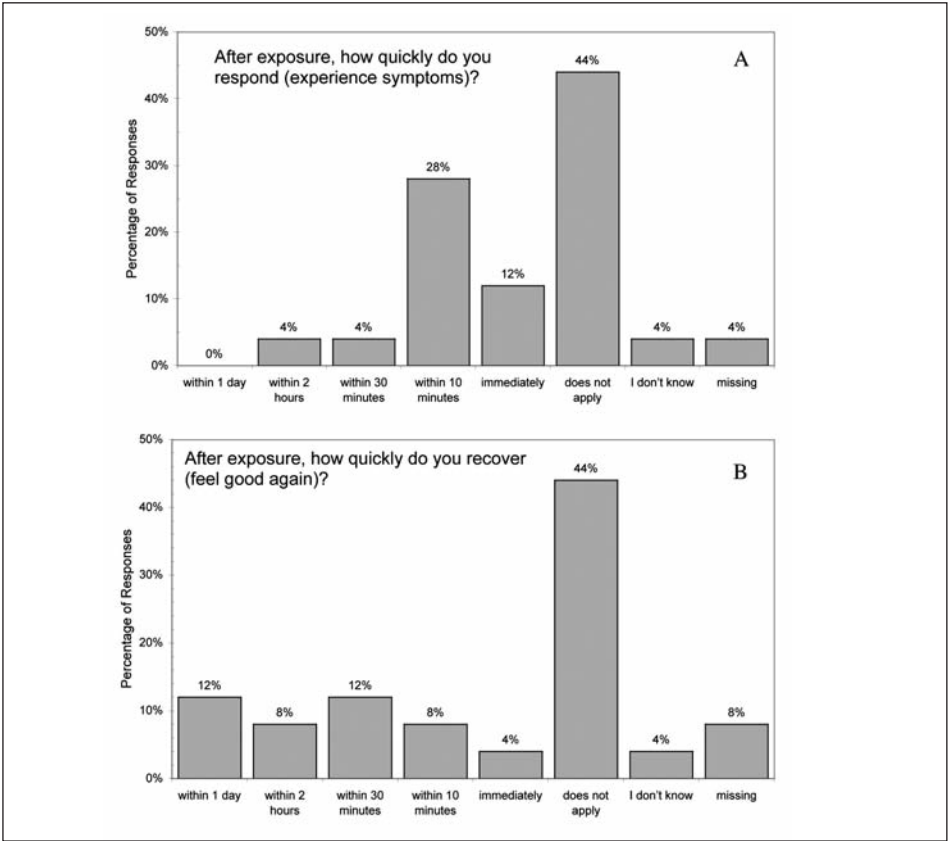


Fig. 5. Self-proclaimed response time of participants to electro-stress and recovery (n=25)

lighting in stores. Others have difficulty flying or traveling by car, perhaps due to microwave exposure on highways and in airplanes. A few subjects are unable to use mobile phones and computers and are unable to watch television. Some are unable to wear jewelry because it irritates the skin and/or watches because they often malfunction (fig. 7).

EHS and person's EMF

The body voltage, as measured by the potential difference between the subject and the electrical ground, differed at the two sites. Subjects at Golden had much higher values than those at Boulder. This was also the case for the high and low frequency electric field and for the HF and LF magnetic field (Table 3). Galvanic skin response was highly variable among subjects prior to testing and did not relate to either sensitivity or the environment. There was no association between any of the EMF measurements (body voltage, GSR, electric field or magnetic field) that we conducted prior to testing and EHS of the subjects tested. In a follow-up study it would be useful to monitor each person's EMF before, during, and after exposure.

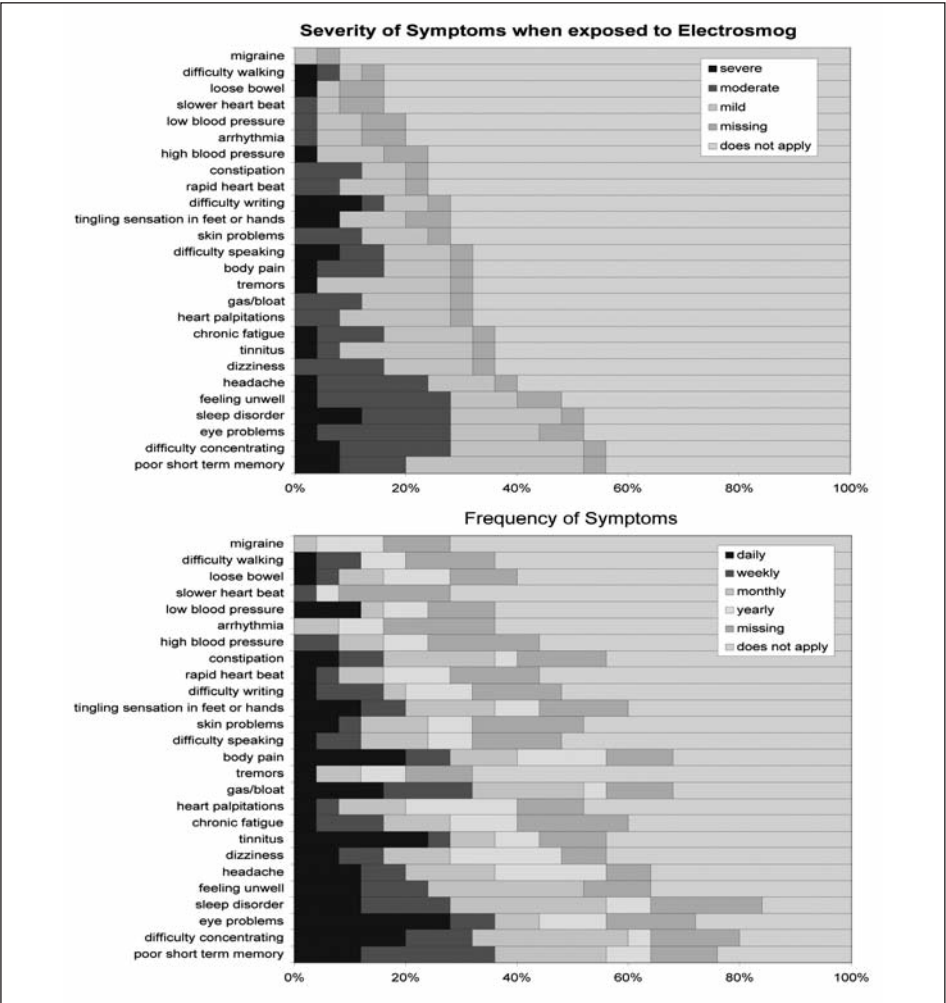


Fig. 6. Severity and frequency of symptoms associated with electrosmog exposure (n=25)

Blind assessment of responses: orthostatic HRV provocation HRV

The Orthostatic HRV provided us with the state of the ANS and the relative fitness score of the individual prior to exposure, which is important for predicting the intensity outcome of exposure.

A summary of the orthostatic HRV (blinded analysis) along with the self-assessment and the provocation HRV (blinded and unblinded) are provide in Appendix A for each subject. For those individuals who had either a moderate or intense response, the blinded predictions show good agreement for stage of exposure and for intensity of exposure.

Based on the orthostatic test, those with high adaptive capacity had a lower probability of reacting to stress, but if they did react, their reaction would be moderate to

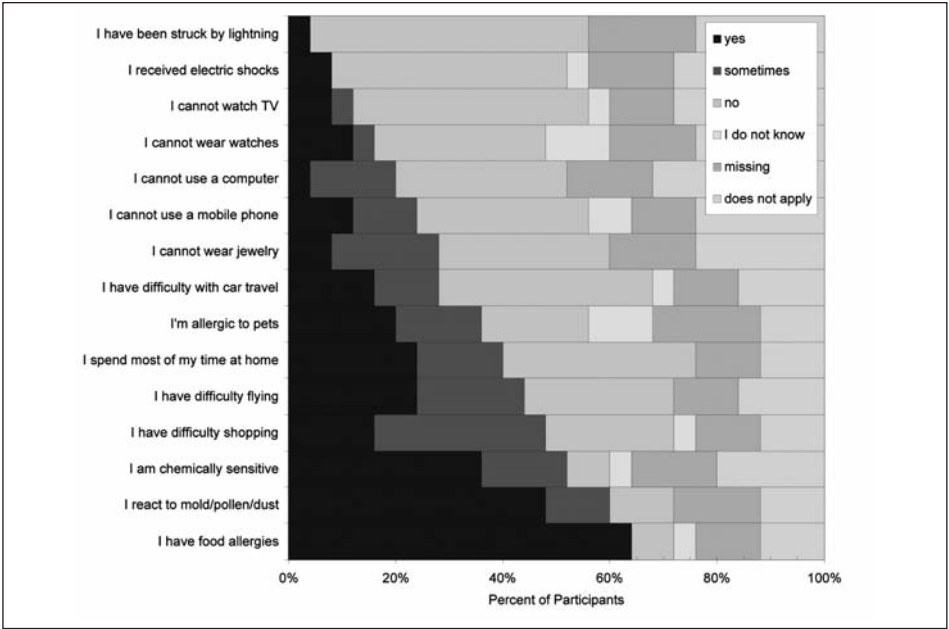


Fig. 7. Response to specific questions that may contribute to or be associated with electrical sensitivity (n=25)

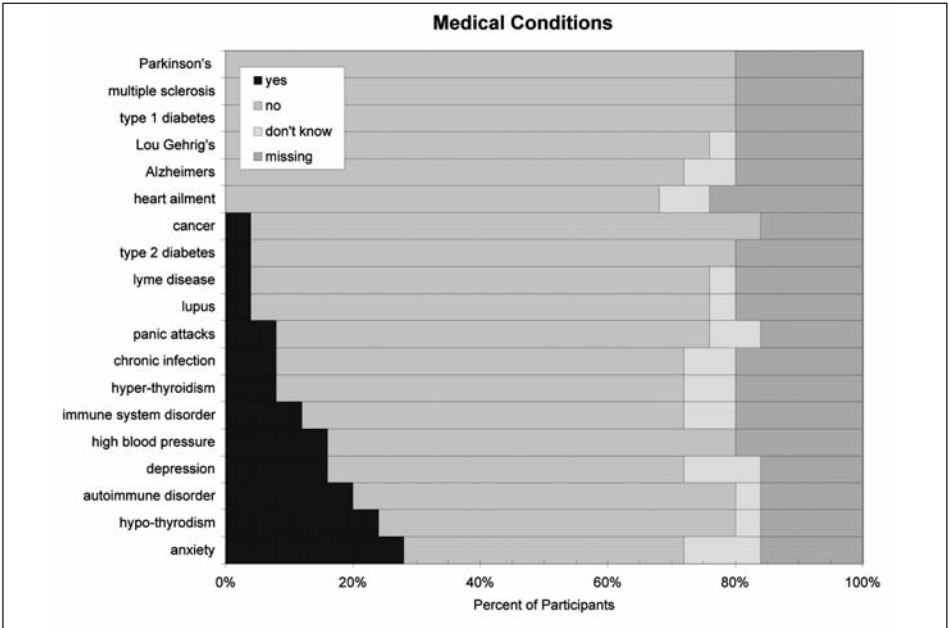


Fig. 8. Existing medical conditions of participants (n=25)

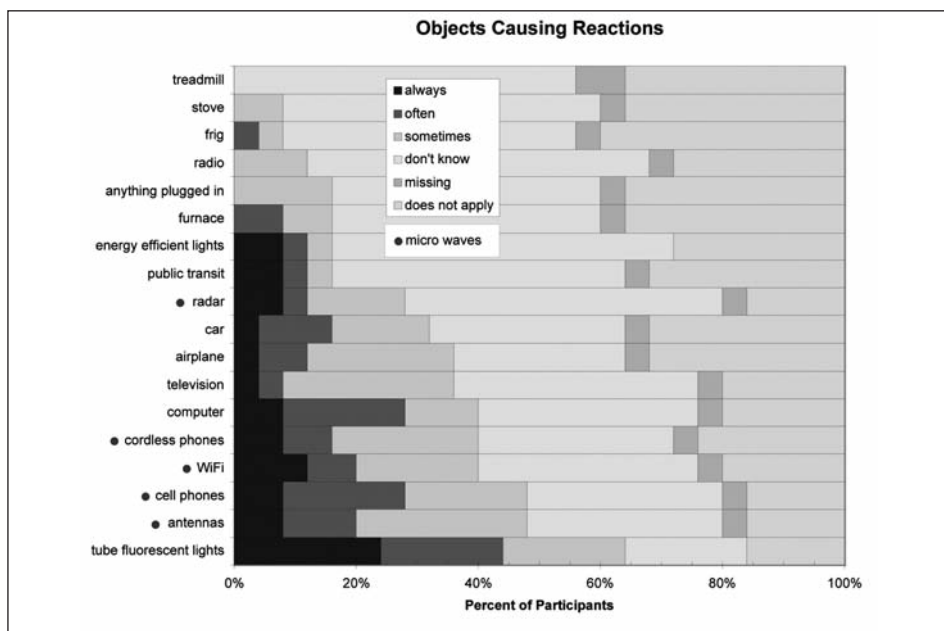


Fig. 9. Objects contributing to adverse health symptoms. Those marked with a dot generate microwave frequencies (n=25)

Table 3 - Personal electromagnetic environment (mean $\pm$ standard deviation) of subjects tested including galvanic skin response (GSR), body voltage, electric (E-field) and magnetic fields (M-field) at both high and low frequency (HF and LF) [$* P \leq 0.05$].

Location	Date	GSR mV	Body Voltage mV	E-field HF mV	E-field LF mV	M-field HF mG	M-field LF mG
Golden	10/16/08	3.5 ± 1.8	$3.4 \pm 0.5^*$	$88 \pm 85^*$	$333 \pm 71^*$	$4.6 \pm 5.7^*$	$17 \pm 14^*$
Boulder	10/20/08	3.2 ± 2.5	0.5 ± 0.5	13 ± 33	63 ± 94	0.2 ± 0.6	$2.7 \pm 0.7^*$
Boulder	10/21/08	4.1 ± 1.3	0.2 ± 0.1	2 ± 0.8	57 ± 50	0.1 ± 0.4	$1.7 \pm 0.6^*$

intense. Conversely, those with low adaptive capacity had a higher probability of reacting but they didn't always have the energy to react and hence their reactions would be mild.

Provocation HRV

Most of the subjects (15/25, 60%) did not respond appreciable to the MW radiation generated by the cordless phone when it was plugged into a live outlet. The rhythmogram was unchanged and the heart rate, parasympathetic and sympathetic tone remained constant (figs. 3, 10, 12).

However, 10 subjects (40%) did respond to the MW challenge. Fig. 13 shows the response for six of those 10. Response and the recovery were immediate. MW provoca-

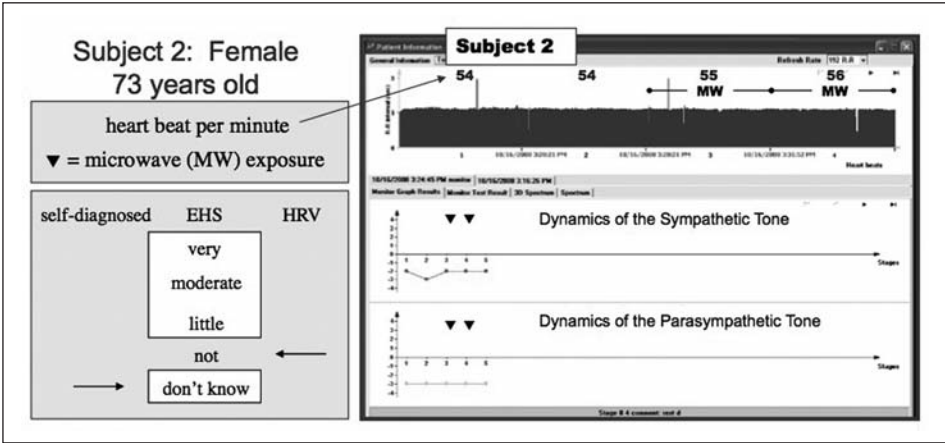


Fig. 10. Continuous monitoring of HRV during provocation part of this study for one subject who was non-reactive

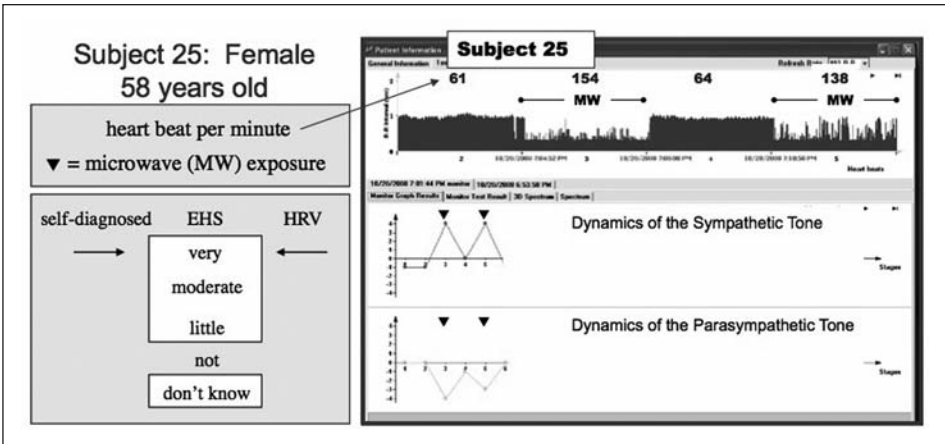


Fig. 11. Continuous monitoring of HRV during provocation part of this study for one subject who reacted to the MW radiation from a digital cordless 2.4 GHz phone

tion differed noticeably compared with sham exposure. Heart rate increased significantly for four of the subjects, resulting in tachycardia for three. The heart rate for subject 25 jumped from 61 bpm to 154 bpm (with real provocation) and returned to 64 bpm (with sham provocation) (fig. 11). The increase in heart rate was accompanied by up regulation of the SNS and down regulation of the PSNS during cordless phone exposure for four subjects in Table 4 (fig. 13). Response of the one subject (Subject 27) was paradoxical in that the heart rate increased from 72 to 82 bpm during which time the parasympathetic tone increased and the sympathetic tone remained constant.

Fig. 14 shows the range of responses of some non- or slightly reactive subjects to provocation.

Table 4 - Real-time monitoring of heart rate, sympathetic and parasympathetic tone before, during, and after exposure to a 2.4 GHz digital cordless phone radiating 3-5 microW/cm²

EHS	Subject Code	EHS Ranked	Heart Rate (bpm)				Sympathetic Response				Parasympathetic Response			
			bgrnd	pre	MW	post	bgrnd	pre	MW	post	bgrnd	pre	MW	post
Intense	25	1	61	61	154	64	-1	-1	4	0	0	0	-4	-1
	17	2	66	68	122	66	0	0	4	0	0	-2	-3	0
	26	3	59	61	106	61	-1	-1	3	0	1	2	-3	1
	27	4	72	nd	82	69	0	nd	0	0	-3	nd	2	-2
Moderate	5	5	66	66	66	65	1	1	3	0	-1	-1	-3	-1
	9	6	77	75	75	73	1	1	0	1	-2	0	-3	-1
	3	7	48	50	53	nd	2	-2	0	nd	2	0	0	nd
	16	8	61	nd	62	63	0	nd	-2	0	-2	nd	-2	-2
	8	9	81	nd	81	80	1	nd	1	1	0	nd	-2	-1
	10	10	69	68	70	70	0	0	0	0	-2	-2	-3	-1
Mild	2	11	54	54	55	56	-2	-3	-2	-2	-3	-3	-3	-3
	23	12	59	nd	58	60	-1	nd	0	-2	-2	nd	-2	-3
	12	13	71	nd	69	74	0	nd	1	0	-1	nd	-1	-1
	18	14	60	61	61	61	-2	-1	-2	-1	-3	-3	-3	-2
	19	15	63	62	62	61	-1	0	-1	-1	-3	-3	-3	-2
	6	16	65	66	66	65	0	0	0	0	-3	-3	-4	-3
	4	17	61	62	61	61	-2	-1	-1	-2	-3	-2	-3	-2
	24	18	71	72	71	69	0	0	0	0	-3	-2	-1	-2
None	1	19	71	70	71	71	0	0	0	1	-3	-1	-1	-1
	11	20	57	nd	57	58	0	nd	0	0	3	nd	3	2
	21	21	78	78	78	nd	1	1	1	nd	-2	-3	-3	nd
	7	22	70	71	70	69	0	0	0	0	-3	-3	-3	-3
	14	23	69	68	67	66	0	0	0	0	-1	-2	-2	-1
	20	24	67	nd	66	66	0	nd	0	0	-1	nd	-1	-1
	13	25	80	78	76	nd	1	1	1	nd	-3	-2	-2	nd
Response			Mean Heart Rate (bpm)				Mean Sympathetic Response				Mean Parasympathetic Response			
Intense			65	63	116	65	-0.5	-0.7	2.8	0.0	-0.5	0.0	-2.0	-0.5
Moderate			67	65	68	70	0.8	0.0	0.3	0.4	-0.8	-0.8	-2.2	-1.2
Mild			63	63	63	63	-1.0	-0.8	-0.6	-1.0	-2.6	-2.7	-2.5	-2.3
None			70	73	69	66	0.3	0.4	0.3	0.2	-1.4	-2.2	-1.3	-0.8
All			66	66	74	66	-0.1	-0.3	0.4	-0.2	-1.5	-1.7	-2.0	-1.4

Note:

EHS categories described in text: bgrnd = background; pre=sham exposure before real exposure; MW=microwave exposure; post=sham exposure after real exposure; nd=no data

The pre- and post-MW cordless phone response (SNS & PSNS) differed significantly for this group (fig. 15) with up regulation of the SNS and down regulation of the PSNS with MW exposure and the reverse for post-MW exposure suggesting a recovery phase.

The severe and moderate responders had a much higher LF/HF ratio than those who either did not respond or had a mild reaction to the MW exposure from the cordless phone (fig. 16B). This indicates, yet again, a stimulation of the SNS (LF) and a down-

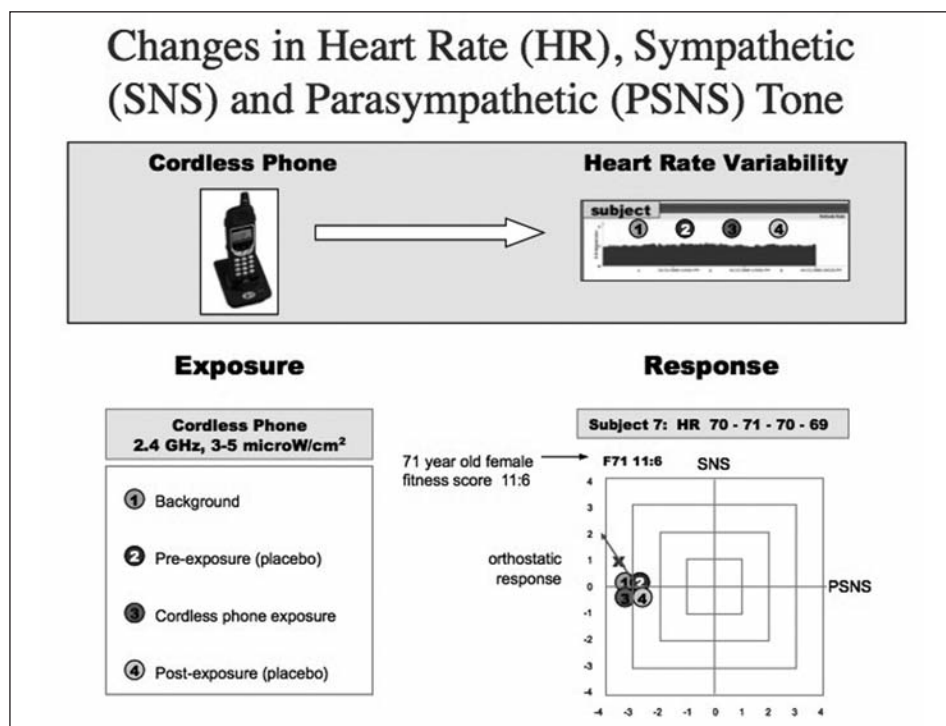


Fig. 12. Subject 7: no changes in heart rate, sympathetic, and parasympathetic tone before, during, and after blind provocation with a 2.4 GHz cordless phone generating exposure of 3 to 5 microW/cm²

regulation of the PSNS (HF). The up regulation was greater for LF2 than for LF1 (fig. 16A).

Based on self-assessment and the results from the provocation study, 2 subjects (8%) underestimated their sensitivity and 5 subjects (20%) overestimated their sensitivity to the cordless phone provocation. However, only two of the 5 claim to experience mild heart palpitations and only one of those responds “sometimes” to cordless phones.

Discussion

The most intriguing result in this study is that a small group of subjects responded immediately and dramatically to MW exposure generated by a digital cordless DECT phone with blinded exposure. Heart rate (HR) increased significantly for 4 subjects (16%) (10 to 93 beats per minute) and the sympathetic/parasympathetic balance changed for an additional 6 subjects (24%) while they remained in a supine position. This is the first study documenting such a dramatic change brought about immediately and lasting as long as the subject was exposed and is in sharp contrast to the provocation studies reviewed by Levallois³, Rubin *et al.*¹⁴, and Bergqvist *et al.*³¹. Authors of these reviews generally conclude that they were unable to establish a relationship between low or high frequency fields and electromagnetic hypersensitivity (EHS) or with symptoms typically occurring

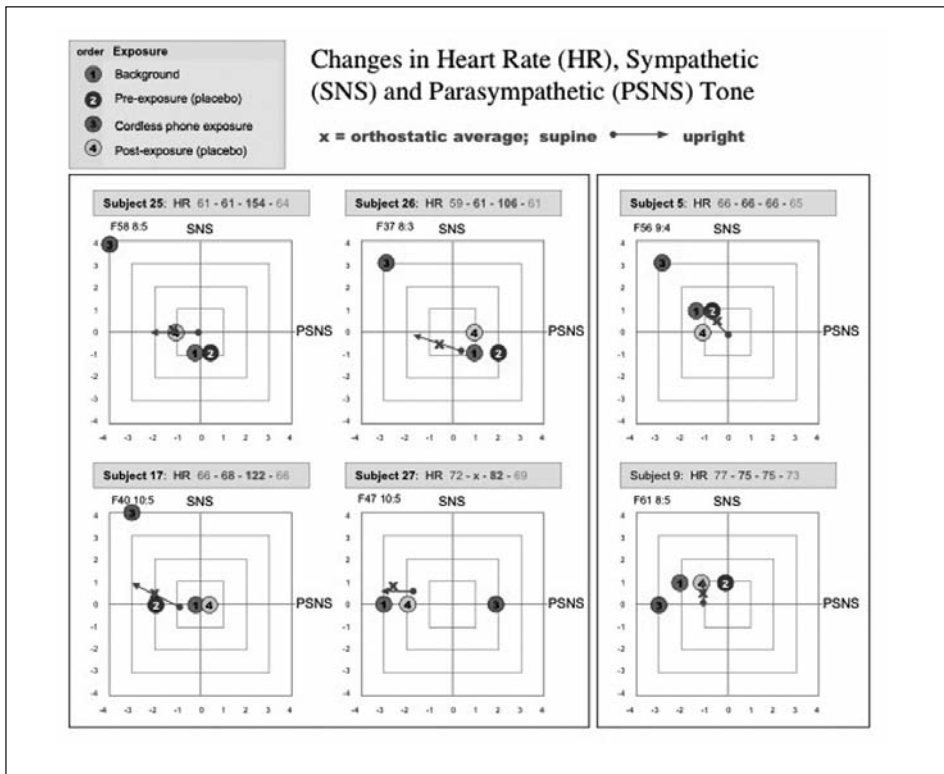


Fig. 13. Reactive Subjects: changes in heart rate, sympathetic, and parasympathetic tone before, during, and after blind provocation with a 2.4 GHz cordless phone that generates exposure of 3 to 5 microW/cm²

among such afflicted individuals. Furthermore, several studies report no effect of mobile phones (various exposure conditions) on human HRV-parameters³²⁻³⁹.

Our results clearly show a causal relationship between pulsed 100 Hz MW exposure and changes in the ANS that is physiological rather than psychological and that may explain at least some of the symptoms experienced by those sensitive to electromagnetic frequencies. Dysfunction of the ANS can lead to heart irregularities (arrhythmia, palpitations, flutter), altered blood pressure, dizziness, nausea, fatigue, sleep disturbances, profuse sweating and fainting spells, which are some of the symptoms of EHS.

When the SNS (fight or flight response) is stimulated and the PSNS (rest and digest) is suppressed the body is in a state of arousal and uses more energy. If this is a constant state of affairs, the subject may become tired and may have difficulty sleeping (unable to relax because of a down regulated PSNS and/or up regulated SNS). Interestingly, Sandstrom⁴⁰ found a disturbed pattern of circadian rhythms of HRV and the absence of the expected HF (parasympathetic) power-spectrum component during sleep in persons who perceived themselves as being electrically hypersensitive.

If the dysfunction of the ANS is intermittent it may be experienced as anxiety and/or panic attacks, and if the vagus nerve is affected it may lead to dizziness and/or nausea.

Our results show that the SNS is up regulated (increase in LF) and the PSNS is down regulated (decrease in HF) for some of the subjects during provocation. The greatest

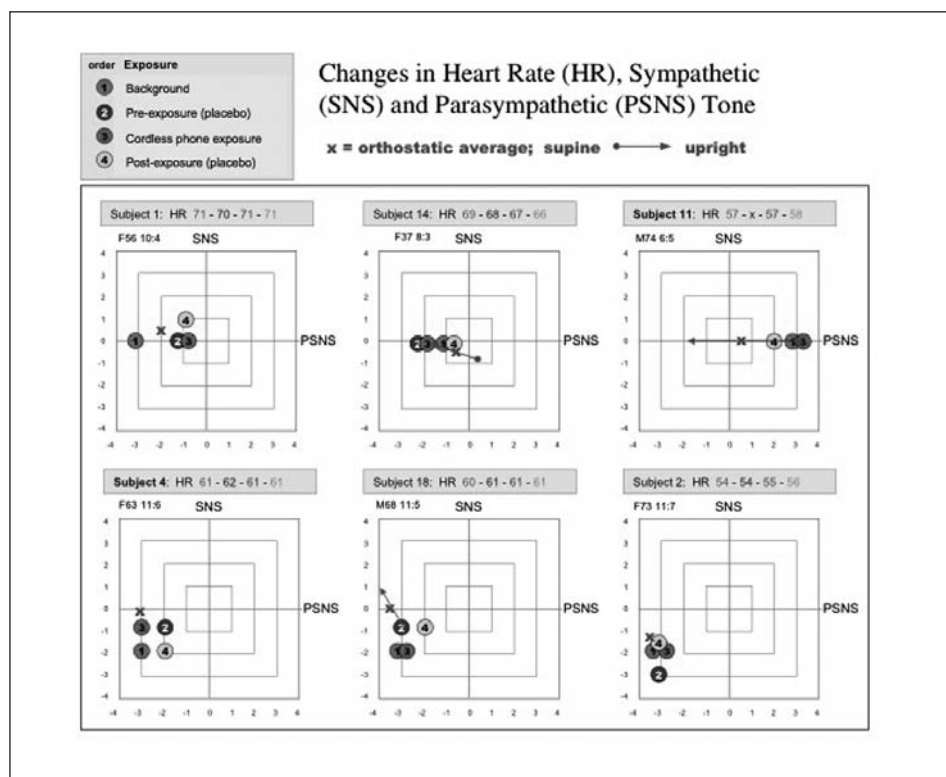


Fig. 14. Non or slightly reactive subjects: patterns of response for before, during, and after blind provocation with a 2.4 GHz cordless phone that generates exposure of 3 to 5 microW/cm²

increase is in LF2, which is the adrenal stress response, although LF1 also increases. We not know the degree to which this is due to the 100 Hz pulse, the MW carrier, or their combination.

Several studies lend support to our results.

Lyskov *et al.*⁴¹ monitored baseline neurophysiological characteristics of 20 patients with EHS and compared them to a group of controls. They found that the observed group of patients had a trend to hypersympathotone, hyper-responsiveness to sensor stimulation and heightened arousal. The EHS group at rest had on average lower HR and HRV and higher LF/HF ratio than controls. We found that subjects with intense and moderate reactions to the MW provocation also had higher LF/HF ratios than those who did not respond.

Kolesnyk *et al.*⁴² describes an “adverse influence of mobile phone on HRV” and Rezk *et al.*⁴³ reports an increase of fetal and neonatal HR and a decrease in cardiac output after exposure of pregnant women to mobile phones.

Andrzejak *et al.*⁴⁴ reports an increased parasympathetic tone and a decreased sympathetic tone after a 20-minute telephone-call. While these results are contrary to our findings, the effect of speaking cannot be ruled out in Andrzejak’s study. In our study the subject remained in a supine position, silent and still during the testing.

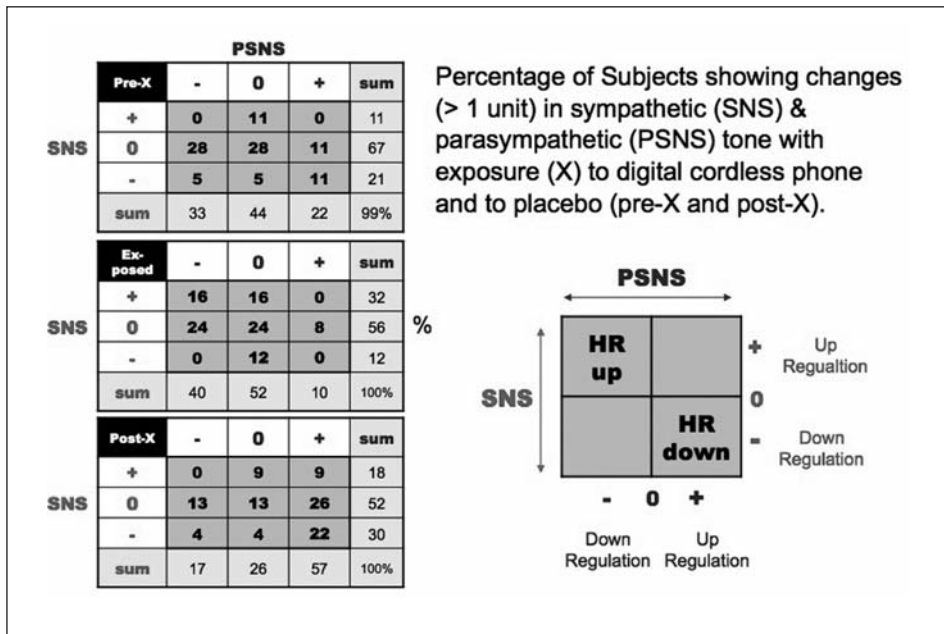


Fig. 15. Response of 25 subjects to blind provocation by a 2.4 GHz digital cordless phone that generates exposure of 3 to 5 microW/cm²

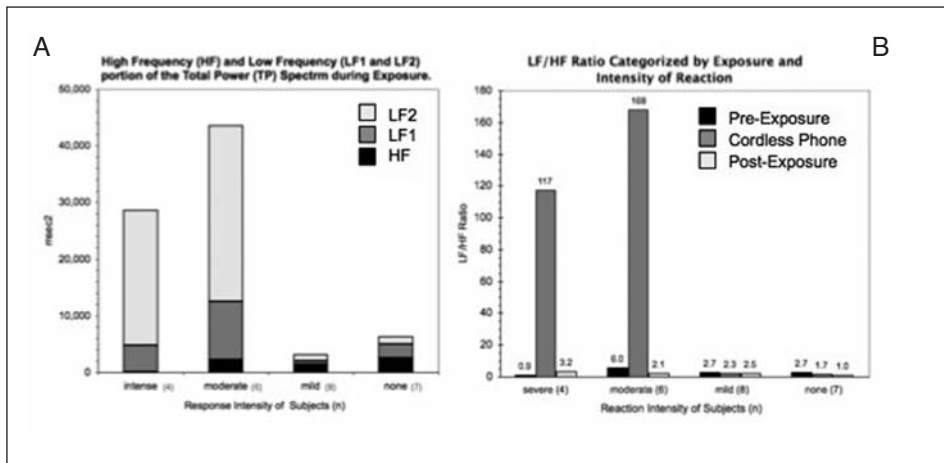


Fig. 16. A. Mean high frequency (parasympathetic) and low frequency (sympathetic) spectral distribution as a function of response intensity of 25 subjects exposed to a 2.4 GHz cordless phone. B. Low frequency (LF1 + LF2) to high frequency (HF) ratio for different exposures

Workers of radio broadcasting stations have an increased risk of disturbances in blood pressure and heart rhythm. They have a lower daily heart rate, a decreased HR variability, higher incidences of increased blood pressure and disturbances in parameters of

diurnal rhythms of blood pressure and HR-all of no clinical significance, but showing a certain dysregulation of autonomic cardiac control⁴⁵⁻⁴⁸.

Bortkiewicz *et al.*⁴⁹ reported that exposure to AM radio frequency EMF within hygienic standards affects the functions of the ANS of workers. Workers had higher frequency of abnormalities in resting and 24-h ECG than controls and an increased number of heart rhythm disturbances (ventricular premature beats). As in our study, RF exposure was associated with a reduced HF power spectrum suggesting that the EMF field reduce the influence of the PSNS on circulatory function.

Several studies report changes in blood pressure with electromagnetic exposure^{50, 51}. Others show an increase of oxidative stress and a decrease of antioxidative defense-systems in heart-tissue irradiated with 2.45 GHz and 900 MHz respectively^{52, 53}. Still others show a stress-response reaction following exposure to radio frequency radiation either in the form of heat shock proteins (hsp) or changes in enzymatic activity. Irradiation of rats with a low-intensity-field (0.2-20 MHz) resulted in an increase of myocardial hsp70⁵⁴. Similarly 1.71 GHz MW exposure increased hsp70 in p53-deficient embryonic stem cells⁵⁵. Abramov and Merkulova⁵⁶ report pulsed EMFs increase the enzymatic activity of acetylcholinesterase in the animal heart, which suppresses the parasympathetic and allows the sympathetic to dominate.

Most of the studies on humans, that did not show any effects of MW radiation in some of the studies mentioned above, were conducted with young, healthy subjects, giving rise to the question whether the experiments would have yielded different results with subjects with a "higher level of pathologic pre-load" and thus fewer possibilities to acutely compensate the possible stressor of radiation.

The studies on work-exposure to MW radiation were able to show different levels of effects on the cardiovascular system, and this could be interpreted as the necessity to remain regularly, repeatedly, and for a longer time under the influence of a certain EMF exposure, hence pointing out the great importance of the electromagnetic exposures in the work and home environment. Perhaps only chronic exposure to MW-EMF can influence various rhythms (e.g. cardiovascular biorhythms) sufficiently to cause detectable effects. Perhaps it is these individuals who become EHS and then respond to stressors if they have sufficient energy to mount a reaction.

In our study, half of those tested claimed to be moderately to extremely sensitive to electromagnetic energy and they ranged in age from 37 to 79 years old. The symptoms they identified are similar to those reported elsewhere and include poor short-term memory, difficulty concentrating, eye problems, sleep disorder, feeling unwell, headache, dizziness, tinnitus, chronic fatigue, and heart palpitations^{2, 7, 57}.

The common devices attributed to stress generation included fluorescent lights, antennas, cell phones, Wi-Fi, and cordless phones. The last 4 items all emit MW radiation.

Many of those claiming to have EHS also had food allergies, mold/pollen/dust allergies and were chemically sensitive. With so many other sensitivities it is difficult to determine whether the sensitivity to electromagnetic energy is a primary disorder attributable to high and/or prolonged EM exposures or a secondary disorder brought about by an impaired immune system attributable to other stressors.

Interestingly, the younger participants (37 to 58) displayed the most intense responses presumably because they were healthy enough to mount a response to a stressor. Those who did not respond to the MW exposure were either not sensitive, or they had a low adaptive capacity coupled with a poor fitness score and did not have enough energy to

mount a reaction. Orthostatic HRV combined with provocation monitoring may help distinguish these three types of responses (sensitive, not sensitive, non-responsive reactors).

The term EHS was deemed to imply that a causal relationship has been established between the reported symptoms and EMF exposure and for that reason the WHO⁸ has labeled EHS as *Idiopathic Environmental Intolerance* (IEI) to indicate that it is an acquired disorder with multiple recurrent symptoms, associated with diverse environmental factors tolerated by the majority of people, and not explained by any known medical, psychiatric or psychological disorder. We think this labeling needs to be changed especially in light of this study.

Conclusions

The orthostatic HRV provides information about the adaptive capacity of an individual based on fitness score and on the state of the SNS and PSNS. A person with high adaptive capacity is unlikely to respond to a stressor (because they are highly adaptive) but if they do respond the response is likely to be intense. Orthostatic HRV was able to predict the intensity of the response much better than the probability of a response to a stressor, which in this case was a 2.4 GHz digital cordless phone that generated a power density of 3 to 5 microW/cm².

Forty percent of those tested responded to the HRV provocation. Some experienced tachycardia, which corresponded to an up regulation of their SNS and a down regulation of their PSNS (increase in LF/HF ratio). This was deemed a severe response when the HR in supine subjects increased by 10 to 93 beats per minute during blinded exposure. HR returned to normal during sham exposure for all subjects tested. In total, 16% had a severe response, 24% had a moderate response (changes in SNS and/or PSNS but no change in HR); 32% had a slight response; and 28% were non-responders. Some of the non-responders were either highly adaptive (not sensitive) or non-responding reactors (not enough energy to mount a reaction). A few reactors had a potentiated reaction, such that their reaction increased with repeated exposure, while others showed re-regulation with repeated exposure.

These data show that HRV can be used to demonstrate a physiological response to a pulsed 100 Hz MW stressor. For some the response is extreme (tachycardia), for others moderate to mild (changes in SNS and/or PSNS), and for some there is no observable reaction because of high adaptive capacity or because of systemic neurovegetative exhaustion. Our results show that MW radiation affects the ANS and may put some individuals with pre-existing heart conditions at risk when exposed to electromagnetic radiation to which they are sensitive.

This study provides scientific evidence that some individuals may experience arrhythmia, heart palpitations, heart flutter, or rapid heartbeat and/or vasovagal symptoms such as dizziness, nausea, profuse sweating and syncope when exposed to electromagnetic devices. It is the first study to demonstrate such a dramatic response to pulsed MW radiation at 0.5% of existing federal guidelines (1000 microW/cm²) in both Canada and the US.

Acknowledgements

We thank those who offered their homes for testing and those who volunteered to be tested. Special thanks goes to Evelyn Savarin for helping with this research.

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M. Havas, et al: Microwave radiation affects autonomic nervous system

APPENDIX A: Summary of data based on blind assessment.

1	2	3	4	5			6		7			Notes				
EHS	Subject Code	EHS Ranked	EHS Self Assessment	CV Tone	IOR	AC	POR	Actual Stages Exposed	HR	SNS	PSNS	Stages Exposed	Blind Assessment Stages showing reaction	POR	IOR	
intense	25	1	very	3	3.5	4.5	1.5	3, 5 (6, 7, 9)	93	5	-4	3, 5	3, 5	5	high to extreme	8
	17	2	very	3	3.5	3.5	3.0	3, 5 (6)	54	4	-1	3, 5, 6	3, 5, 6	4	moderate	9
	26	3	moderate	1	5.0	5.0	1.5	3, 5, 6 (7, 8)	45	4	-5	3, 5, 6, 7	3, 5, 6, 7	5	moderate to intense	10
	27	4	little	3	3.0	3.0	3.5	2, 4 (5, 6)	10	0	5	2, 4, 5	2, 4, 5	5	mild	11
moderate	5	5	moderate	2	4.0	3.5	3.5	3, 5	0	2	-2	3, 5	3, 5	5	moderate to high	12
	9	6	don't know	2	3.5	4.5	2.0	3, 5, 6, 8	0	-1	-3	3, 5, 6	3, 5, 6	4	high	13
	3	7	don't know	-1	5.0	4.0	1.0	3, 4	3	2	0	2, 3	3, 4	4	moderate	14
	16	8	very	1	4.0	4.0	3.5	2, 4, 6	1	-2	0	2	2	1	mild	15
	8	9	not	5	2.5	1.5	3.5	2, 3	0	0	-2	2, 4	2, 4	1	mild	16
mild	10	10	don't know	2	3.5	3.0	3.0	3 (7, 8, 9)	2	0	-1	3, 7, 8, 9	3 mild, 7, 8, 9 intense	5	intense	17
	2	11	don't know	2	2.0	1.0	3.5	3, 4	1	1	0	unknown	none	1.75	mild	18
	23	12	little	-1	5.0	1.5	4.5	2, 4 (5, 6)	-1	1	0	2, 5	2, 5	2	mild to moderate	19
	12	13	little	3	4.0	4.0	2.5	2, 3 (5)	-2	1	0	3	3, 4, 5	3	mild to moderate	20
	18	14	don't know	2	2.5	2.5	4.5	3	0	-1	0	3	3	1.5	mild	21
	19	15	don't know	4	2.0	2.5	3.0	3	0	-1	0	2, 4	2, 4	3	mild to moderate	22
	6	16	very	2	3.5	2.0	4.5	3, 4	0	0	-1	2	2	2	mild	23
	4	17	little	1	2.0	1.0	4.0	3, 4	-1	0	-1	3, 4	4, 5	2.25	moderate	24
none	24	18	little	2	3.0	4.0	3.0	3, 5	-1	0	1	2, 4, 5	2, 4, 5	2.5	mild	25
	1	19	don't know	5	3.5	3.5	3.5	3, 4	1	0	0	3, 4	4, 5	1	mild	26
	11	20	not	1	1.0	5.0	1.5	2, 4, 5	0	0	0	3	3	1	mild or non-symptomatic	27
	21	21	little	3	2.5	2.0	3.5	3 (4, 5)	0	0	0	2, 5	2, 5	1.5	none to mild	28
	7	22	very	2	2.5	1.5	4.0	3, 4	-1	0	0	unknown	unknown	1.5	mild	29
	14	23	don't know	5	2.5	3.5	3.0	3, 4	-1	0	0	2	2, 3, 4	1.75	mild	30
	20	24	little	3	3.5	4.0	4.5	2	-1	0	0	possibly 5?	6	1	mild	31
13	25	very	4	3.0	2.5	3.5	3, 4	-2	0	0	unknown	unknown	1	mild	32	
		code	code	code	code	code	code									
		5	hypo	intense	high	high										
		4		strong												
		3	normal	moderate	moderate	moderate										
		2		mild												
		≤1	hyper	don't know	low	low										

Notes:

- 1 Electrohypersensitivity (EHS) response categories are based on HR = heart rate; SNS = sympathetic nervous system; PSNS = parasympathetic nervous system.
- 2 EHS was ranked based on changes in HR and changes in the SNS and PSNS during exposure to microwave (MW) radiation.
- 3 Self-assessment of sensitivity based on questionnaire response.
- 4 Cardiovascular (CV) Tone is based on the HR times the sum of the systolic and diastolic blood pressure; values at 1 or lower are hypotonic and values at 5 are hypertonic.
- 5 Intensity of reaction (IOR); adaptive capacity (AC), which is 6 - non adaptive capacity (NAC); and probability of reaction (POR) are based on the orthostatic heart rate variability (HRV) results and are described in the text.
- 6 Subjects were exposed to MW radiation at different stages. Stages in parentheses were not used in the study as they reflect multiple exposures with interference from other agents.
- 7 Blind assessment was based on the HRV during continuous monitoring with real and sham exposure to MW radiation from a 2.4 GHz digital cordless phone radiating and at a power density between 3 and 5 microW/cm².
- 8 Excellent subject.
- 9 Symptomatic at stage 3, parasympathetic rally begins to recovery but feels anxiety, stage 3 faint or dizziness predicted. Decent Chronotropic Myocardial Reaction Index (ChMR) and vascular compensation reaction (VC). Middle of bell curve.
- 10 The healthier a subject the more likely the reaction. This person has the energy to become symptomatic.
- 11 Mildly inflamed. Mildly fatigued but highly adaptive. ChMR and VC good. Has ability to react.
- 12 Adaptive person. Could use Mg and/or K based on high standing HR.
- 13 Has plenty of energy. Moderate response due to weakening. Stage 7 body re-regulating from exposure.
- 14 Shows a weakening reaction (down regulation of SNS). Positive reactor. Very healthy for age. Highly adaptive geriatric.
- 15 Lot of adaptive capacity. If she is exposed her reaction would be a fairly strong reaction.

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- 16 Has diminished energy capacity (11:6). This person doesn't have enough energy to have a robust response.
- 17 Potentiated reactor, time sensitive, couldn't tolerate re-exposure. If she reacts it will be moderately strong because of ChMR. Needs minerals for VC factor slowed her down.
- 18 May be on heart medication. Cardiac rate and rhythm non-adaptive. CV tone hypertonic.
- 19 Any neurological insult will be met with a hard reaction since she has inverted response when she stands up.
- 20 If reactor, it will be strong because of ChMR strong. Highly adaptive capability and reserve. Slow VC could be mineral or vitamin D deficiency.
- 21 Don't have a strong PSNS resistance. Reactivity is based on inability to go parasympathetic, and then they will go more sympathetic if they have the energy to do so. No energy. Either a delayed reaction or a weak reaction.
- 22 A fibrillation, palpitations of heart probable. Strong girl. 11:6 fitness is OK for a person this age.
- 23 May have dental problems based on S/P response. Neurologically compromised.
- 24 Neurologically compromised. May be overmedicated on CV drug.
- 25 Strong gal. Decent reserve capacity but temporary fatigue. Doesn't feel bad but poor health for her age.
- 26 Normal reaction to stress, mild non-toxic reaction. Potential for reaction: moderately high because of the 10.4 but may tolerate an amount of exposure before they react because of the reserve capabilities.
- 27 Ridiculously healthy. Poster boy for his age. He can take a lot based on fitness of 6:5.
- 28 Lower end of bell curve. Doesn't have energy to react although may be symptomatic.
- 29 Either highly adaptive or non-reactive. Orthostatic response indicates that person doesn't have enough energy to have a robust response.
- 30 Normal CV tone for age, Decent Tension Index (TI). Good geriatric pattern. If she reacts it would be moderate to mild.
- 31 Strong girl. Has strong adrenal capacity. If she reacts it will be strong. May have chronic fatigue.
- 32 Moderate inflammation. Tired and has low adaptive reserve. If stressor comes along it will produce more stress. If reacting it would be medium.

Comparative assessment of models of electromagnetic absorption of the head for children and adults indicates the need for policy changes

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Abstract

Globally more than four billion phones are in use, with more than half of all users believed to be children and young adults. Over the past two decades, models of the human head have been devised based on imaging studies and used to estimate the extent and rate of radiation energy absorption to the brain, the Specific Absorption Rate (SAR). IEEE and ICNIRP SAR recommendations rest solely on avoiding thermal effects on the adult male head under conditions of a six minute long call and do not take into account the long-term cell phone use, the length of calls, non-thermal biological effects, the smaller size and greater physiological vulnerability and increased absorption to the heads of children and females. Currently recommended approaches by the IEEE calculate peak spatial average SAR for safety compliance testing of cell phones based on a physical model of an adult male head with an added 10 mm plastic spacer to model the ear (pinna). By incorporating such a spacer, the IEEE model assumes that the RF energy absorption in the ear (or pinna) may be treated like extremities of the body such as the legs and the arms that are not proximate to the brain. The 10 mm spacer artificially results in 2 to 4 times lower exposures to the head. Recent epidemiologic studies of adults from those few nations where cell phone use has been extensive for a decade or longer indicate significantly increased risk of a variety of brain tumors. These findings, together with the limitations of currently used head models and the growing use of phones by the young and females, indicate a clear and compelling need for improved, biologically-based

models of the head in order to better estimate population-wide exposures of children and women to cell phones and provide the grounds for improved policies to reduce those exposures.

Key Words: health effects, mobile phones, Specific Absorption Rate (SAR), children and adults, radio frequency radiation, brain and cell phone.

Introduction

Cell phone use has grown exponentially throughout the world in less than a decade. More than half of the world's population uses cell phones today as telephones as well as clocks, radio, video, and tools for exchanging information. Current technology of 2G and 3G phones operates in the microwave range, from 800 to 2450 megahertz (MHz). Standards for these phones rest on guidance developed by two non-governmental engineering-based groups, the Institute of Electrical and Electronics Engineers (IEEE) and International Commission on Non-Ionizing Radiation Protection (ICNIRP)^{1, 2}. For compliance with IEEE and ICNIRP exposure limits, the quantification of exposure to the head, the 1 or 10 gram (g) Specific Absorption Rates (SAR), is based on a physical model of an adult male head with a 10 mm spacer at the ear, or pinna, to estimate radiofrequency (RF) thermal energy absorption that can take place in the course of a call with no accounting for the duration of the call assuming that it will not result in change in temperature of the brain. In the U.S., Canada, and most industrial nations, there is no independent review of these standards, monitoring of the cell phone manufacturers for compliance with these standards, or monitoring of cell phone use in real life.

A growing number of *in vitro* and *in vivo* studies have confirmed that both 2G and 3G signals at non-thermal levels are genotoxic^{3, 4}. Potential mechanisms of such impact include changes in free-radical formation, alterations in electron conformation, and inhibition of proteins and other factors involved in DNA repair and synthesis. While molecular mechanisms for possible adverse effects have not been completely elucidated, energy absorption of higher frequency signals emitted by recently developed 3G, or even the new generation 4G cell phones, may result in greater biological effects. Based on these considerations, a growing number of national governmental agencies have issued precautionary advisories, urging that children avoid regular use cell phones next to their heads, restricting the marketing and development of cell phones for children, and recommending general methods for reducing direct exposure to the head of adults⁵.

To complement such general precautions, this paper briefly reviews the underlying engineering and biology of RF signals associated with different generations of phones, synthesizes evolving evidence on the health effects of RF, clarifies and considers the strengths and limits of currently used models of the head used for testing phones, and summarizes efforts to promote precaution regarding the use of phones.

The changing nature of RF cell signals

Over the past four decades, cell phone types and uses have radically changed. The first generation, known as 1G, was a bulky cell phone introduced in the 1980s based on analog modulation with output power typically around 2 to 3 Watts (W). Examples of these systems are the Advanced Mobile Phone System (AMPS) in North America, Asia

Pacific, Russia, Africa and Israel in the frequency band between 800 and 900 MHz, and the Nordic Mobile Telephone (NMT) 900 system since 1986 in Scandinavia, Netherlands, Switzerland and Asia. The RF from 1G phone was presumed to produce mainly thermal effects, with any potential risks resulting from heating of the tissues.

The advanced generations of cell phones, namely 2G and 3G, employ higher data rates and a broader range of multimedia services and were launched in 1991 and 2001. Unlike 1G cell phones, the maximum radiated power was now controlled by the base station (cell tower or mast). The base station reduced the power emitted by 2G and 3G cellphones to a level that produces a good signal to noise ratio (SNR). These phones rely on digital modulation with mean (rms) output power typically around 250 or 125 mW (maximum 1-2W). Typical examples of these systems are: the North American Digital Cellular (NADC) system (824-894 MHz) since 1991 in USA; the Personal Communication Services (PCS) system (1850-1990 MHz) since 1996 in USA; the Global System for Mobile Communications (GSM) system (880-960 MHz) since 1991 in Europe and Asia Pacific; and the Digital Cellular System (DCS) 1800 (1710-1880 MHz) employed since 1993 in Europe. The modulation signals used in these digital systems are complex with the lowest rate of 217 Hz (e.g., GSM is encoded at 217 pulses/sec). This lower rate was reported to result in greater interaction with the biological tissues, inducing non-thermal effects and increased risks to living cells, even at low absorbed average powers⁶. Current 3G and 4G phones involve modulation with even lower minimum pulse rates and much higher data rates. As a result, 3G phones can result in greater cumulative average exposures, a result of the higher data rates.

Most contemporary cell phones use monopole or helix type antennas, which produce similar radiation patterns. The radiation pattern determines how the energy is distributed in the space. This can be represented by two planes that are orthogonal to each other, one is the electric field, the other is the magnetic field. When a monopole or helix antenna rests in a vertical direction and is unimpeded by any RF absorbing obstacle like the human head or body, it produces a nearly symmetrical pattern of RF around this antenna. In actual use about one half of the RF energy radiated by a cell phone is absorbed by the human head. The closer the cell phone is to the head the greater is the absorbed energy in the head tissues.

Biologic effects of non-ionizing radiation

Ionizing radiation (IR) is well known to have potent biological effects that break chemical bonds creating ions. This breakage of bonds results in diseases ranging from cancer to developmental and reproductive impairment, to death.⁷ These biological impacts arises because 15% of the IR directly breaks ionic bonds at the backbone of DNA causing mutations that can lead to cancer; 85% of IR damage is caused by the creation of free radicals in the cell's cytoplasm near the DNA molecule, also resulting in DNA mutations, or through other mechanisms that are still being elucidated.

Non-ionizing radiation (NIR), found at all frequencies with energy levels too low to break chemical bonds from low-frequency electric power systems to microwave (MW) frequencies used by cell phones also produces biological effects when studied in cell cultures and in experimental animals. At low levels, equivalent to exposure from radiation from mobile phones, RF has been shown to result in damage to biological tissues, including both single and double DNA strand breaks, alterations in the permeability of

the blood-brain barrier (BBB), oxidative stress, and damage to neural cells of the brain^{8,9}.

Two mechanisms have been identified thus far to explain the variety of non-ionizing electromagnetic fields (EMFs) interactions with biological systems: thermal effects and non-thermal effects. Thermal effects arise directly from the increased movement of molecules results in tissue heating as a result of the absorption of EMFs in a dissipative medium. Absorption of energy at MW/RF frequencies is largely due to the motion of water dipoles and dissolved ions. At high frequencies (such as for the MW/RF band), tissues with high water content, such as occurs in the brains of young children, show electrical conductivity increasing with frequency. Thus, the net thermal response of the body will vary depending on SAR, ambient temperature, clothing, thermoregulatory system and physiological condition.

Non-thermal effects can result from direct interaction of the MW/RF fields on molecules or tissue components, changing electron conformation, altering stress proteins (previously known as heat shock proteins), immune-system function and having other impacts that remain to be clarified. Non-thermal effects are still not very well understood and their exact consequences on human health are still being investigated. Some reported non-thermal effects on tissue are biochemical and electrophysiological effects and can result in changes in the nervous, immune and cardiovascular systems, as well as in metabolism and hereditary factors^{4,10,11}.

In a pioneering research effort that created the widely used Comet Assay, Lai and Singh demonstrated that two hours of microwave radiation, comparable to that emitted by a cell phone, damaged DNA of the rat brain¹². A European study team of a dozen collaborators under the aegis of REFLEX [Risk Evaluation of Potential Environmental Hazards from Low Energy Electromagnetic Field (EMF) Exposure Using Sensitive in vitro Methods], found evidence that low (non-thermal) energy levels of RF exposure induced double strand breaks in DNA of cells exposed to between 0.3 and 2 W/kg¹³. Although the mechanism(s) underlying such non-thermal effects of NIR remains unclear, it seems quite plausible, as with the cancer-promoting effects of inflammatory lesions, that mutagenic damage to DNA could be induced by generated free radicals. In contrast, many other studies of non-thermal or thermal effects of RF issue have yielded no evidence of DNA damage. But, the great preponderance of these negative studies have not reflected independent research but resulted from studies directly funded by the cell phone industry¹⁴.

Current SAR calculations rest solely on avoiding thermal impacts. In principle, as the newer generation of digital phones radiate lower mean power in comparison to the analogue phones, the risk associated with the heating of tissues should be correspondingly reduced. However, most mobile communication systems are pulse-like in nature and modulated at low frequencies with high data rates. As a result, these newer systems can induce low-levels of currents in the brain tissues that have been linked with a variety of non- or thermal effects, e.g., BBB alterations, single and double strand DNA breaks, chromosomal aberrations, etc., at RF energy levels substantially below the thermal threshold.

Despite the growing industry-independent evidence that NIR has a range of biological impacts, intense controversy surrounds the interpretation of the limited available public health investigations regarding risk for cancer or other chronic diseases. Human studies on both cancer and non-cancer impacts of NIR are inconsistent for reasons that have been thoroughly discussed by a number of authors¹⁵.

Epidemiologic studies

The biology and epidemiology of the often lethal cancer of the brain is complex. It is unreasonable to expect to be able to detect an increased risk of brain tumors in less than a decade, because brain tumors are known to have latencies that can be between a decade to four decades long¹⁶. Recently several authors have produced meta-analyses that show that only when studies have followed people for a decade is there evidence of increased risk (Table 1).

For more than a decade, Hardell and his colleagues conducted a series of studies in Sweden, a country where proportionally more of the population has heavily used cell phones for a longer period of time than in many other industrialized nations. Regarding acoustic neuroma (AN), the Swedish group reported an 2.7 to 5.1 fold increased risk of AN for those regularly using an analog cell phone for five years or more compared to those who never or rarely used a cell phone^{17, 29}. Hardell's team also found long-term analogue cell phone use significantly increased the risks of meningioma and astrocytoma^{22, 29}. Recently, Hardell and Carlberg found that persons who had used cell phones for 10 years or more also had the highest risk for astrocytoma. This study also included persons who had begun to use cell phones before age 20. Cases with first mobile phone use younger than 20 years age had five times more brain cancer for 1 or more years of use (OR=5.2, 95% CI=2.2-12). For AN, the highest risk was found for greater than 10 years of ipsilateral mobile phone use (OR=3.0, 95% CI=1.4-6.2)³⁰.

The International Agency for Research on Cancer (IARC) began an international collaborative case-control study on cell phone use and the incidence of brain tumors in 13 countries in 1997 (the INTERPHONE study). Among six INTERPHONE reports from different countries, which included persons who had used phones episodically for less than a decade, none reported a relationship between cell phone use and AN^{18-20, 31-33}. They did not report any significant relationship between long term cell phone use and glioma, meningioma or other brain tumors^{21, 24, 25, 27, 28}. However, the recently published Interphone study found that the heaviest cell phone users, cumulative call time ≥ 1640 hours have increased risk of glioma (OR=1.40, 95% CI=1.03-1.89) and meningioma (OR=1.15, 95% CI=0.81-1.62)³⁴. Brain tumor risk was not found to be higher among those who use cell phone less frequently.

The lack of an observed association between published studies of cell phone use and risk for malignant or benign tumors in other published studies could reflect a number of methodological limits of study design. Most of these negative studies involved relatively short time periods of cell phone use, infrequent use of cell phones, or a small number of cases. In an effort to refine evaluation of the issue, studies have been carried out that separate out extent and type of cell phone use, including side of the head on which phones are typically used. The Hardell group found a consistent pattern of an association between ipsilateral AN and cell phone use providing that there was a 10-year latency period or longer (OR=2.4, 95% CI = 1.1-5.3)²³. Two additional studies from other investigators in the Nordic region^{19, 20} produced similar results. A study used interphone protocol that pooled data from 5 North European countries similarly found an increased glioma risk after a decade of use for ipsilateral cell phone exposure (OR=1.4, 95% CI=1.0-1.9)³⁵. A significant excess risk for reported ipsilateral phone use to the tumor was also found for glioma regardless of the duration of cell phone use²⁶.

A recent meta-analysis of studies produced by a team from California and Korea has corroborated this analysis, noting that the Hardell's work consistently reflects high

Table 1 - Summary of published articles on brain tumors and long term (≥ 10 years) cell phone use

Study	Population	Period	Study type	No. cases	No. controls	OR (95% CI)	Cell phone exposure
Acoustic Neuroma							
Hardell <i>et al.</i> , 2002 ¹⁷	Sweden	2000-2002	Case-control	46	26	1.8 (1.1-2.9)	regular analogue phone use
Christensen <i>et al.</i> , 2004 ¹⁸	Denmark	2000-2002	Case-control	2	15	0.2 (0.04-1.1)	regular use
Lönn <i>et al.</i> , 2004 ¹⁹	Sweden	1999-2002	Case-control	14	29	1.8 (0.8-4.3)	regular use
				12	15	3.9 (1.6-9.5)	ipsilateral exposure
Schoemaker <i>et al.</i> , 2005 ²⁰	4 Nordic countries and UK	1999-2004	Case-control	47	212	1.1 (0.7-1.5)	regular use
				23	72	1.8 (1.1-3.1)	ipsilateral exposure
Schüz <i>et al.</i> , 2006 ²¹	Denmark	1982-2002	Cohort	28	42.5	0.7 (0.4-1.0)*	regular use
Hardell <i>et al.</i> , 2006 ²²	Sweden	1997-2003	Pooled case-control	19	84	2.2 (1.4-3.8)	regular analogue phone use
				1	18	0.6 (0.1-5.0)	regular digital phone use
Hardell <i>et al.</i> , 2008 ²³	Sweden		Meta-analysis	83	355	1.3 (0.6-2.8)**	regular use
Glioma							
Christensen <i>et al.</i> , 2005 ²⁴	Denmark	2000-2002	Case-control	6***	9	2.4 (1.1-5.3)****	ipsilateral exposure
Lonn <i>et al.</i> , 2005 ²⁵	Sweden	2000-2002	Case-control	22	33	1.6 (1.4-6.1)	regular use
				14	15	0.9 (0.5-1.6)	regular use
						1.8 (0.8-3.9)	ipsilateral exposure
Hepworth <i>et al.</i> , 2006 ²⁶	UK	2000-2003	Case-control	48	67	1.1 (0.7-1.7)	regular use
Schüz <i>et al.</i> , 2006 ²⁷	Germany	2000-2003	Case-control	12	11	2.2 (0.9-5.1)	regular use
Lahkola <i>et al.</i> , 2008 ²⁸	5 European countries		Case-control	143	220	0.9 (0.7-1.3)	regular use
				77	117	1.4 (1.0-1.9)	ipsilateral exposure

(continued)

Table 1 - Summary of published articles on brain tumors and long term (≥ 10 years) cell phone use

Study	Population	Period	Study type	No. cases	No. controls	OR (95% CI)	Cell phone exposure
Meninglioma							
Lönn <i>et al.</i> , 2005 ²⁵	Sweden	2000-2002	Case-control	8 4	32 15	0.7 (0.3-1.6) 1.4 (0.4-4.4)	regular use ipsilateral exposure
Christensen <i>et al.</i> , 2005 ²⁴	Denmark	2000-2002	Case-control	6	8	1.0 (0.3-3.2)	regular use
Hardell <i>et al.</i> , 2006 ²²	Sweden	1997-2003	Pooled case-control	34 8	84 18	1.6 (1.0-2.5) 1.3 (0.5-3.2)	regular analogue phone use regular digital phone use
Schüz <i>et al.</i> , 2006 ²⁷	Germany	2000-2003	Case-control	5	9	1.1 (0.4-3.4)	regular use
Lahkola <i>et al.</i> , 2008 ²⁸	5 European countries		Case-control	42 21	130 73	0.9 (0.6-1.3) 1.0 (0.6-1.7)	regular use ipsilateral exposure
Astrocytoma							
Hardell <i>et al.</i> , 2006 ²⁹	Sweden	2000-2003	Case-control	40 16	40 18	3.7 (2.0-7.0) 2.2 (0.8-6.5)	regular analogue phone use regular digital phone use
All Malignant Brain Tumor							
Hardell <i>et al.</i> , 2006 ²⁹	Sweden	2000-2003	Case-control	48 19	40 18	3.5 (2.0-6.4) 3.6 (1.7-7.5)	regular analogue phone use regular digital phone use

* Standardized incidence ratio was calculated based on observed and expected numbers

** Based on 4 case-control study (Lönn et al 2004, Christensen et al. 2004, Schoemaker et al. 2004, and Hardell et al., 2006)

*** Results from a Meta-analysis, based on three case-control studies (Lönn et al., 2004, Schoemaker et al., 2005 and Hardell et al., 2006)

**** low-grade glioma

quality methods and design. The researchers examined 465 articles published in major journals and focused on 23 studies involving 37,916 participants. In eight of the studies – those that were conducted with the most scientific rigor – cell phone users were shown to have a 10% to 30% increased risk of all types of tumors studied compared with people who rarely or never used cell phones (OR=1.2, 95% CI=1.0-1.3). The risk was highest among those who had used cell phones for 10 years or more³⁶.

The results of the entire literature on epidemiology and cell phone use remain controversial, because most studies suffer from a number of methodological shortcomings including: insufficient statistical power to detect an excess risk of brain tumors; reliance on small populations; short-term exposure periods; problems in recollection of past practices and difficulty in characterizing changing exposures throughout a lifetime in large populations. As a number of researchers have suggested, retrieving billing records from cell phone network providers to obtain cumulative duration and frequency of cell phone use and corroborating personal interview would provide the capability to validate self-reported cell phone exposure in future studies³⁷. Assuring independent funding for future research will also be critical, given the widely reported biases associated with the design and interpretation of industry-funded studies to date.

Regarding short-term health impacts from RF exposure such as insomnia, impairment of short-term memory, headache, alteration of EEG and other behavioral problems, evidence has been fairly consistent that such effects are worsened in longer term cell phone users^{38, 39}. Whether these relatively benign perturbations signal the likelihood that more serious health impacts will occur after longer-term RF exposure is a matter of critical importance for future studies.

Models of the head used to evaluate compliance with safety standards

Given the concerns that have been raised from the biological and epidemiological studies, it is important to establish standards for RF exposures from cell phones that incorporate the best scientific information regarding differences in the heads of people of various sizes, genders and ages. Children's skulls are thinner and their brains are less dense and more fluid, making them more vulnerable than adults to RF signals. Size alone affects absorption. In addition, other physiological properties such as permittivity, electrical conductivity and density also affect transmission and absorption of RF signals, as does myelination of the nerves of the brain, which is not complete until the early to mid-twenties⁴⁰.

The relative permittivity of a material under given conditions is measuring the extent to which it concentrates lines of flux. The relative permittivity of any material is expressed as the ratio of the amount of stored electrical energy when a potential is applied, relative to the permittivity of the vacuum. The relative permittivity or dielectric constant of the air is 1, while that of an adult brain is around 40 and that of a young child's brain is higher closer to 60 to 80⁴¹. This means that peak SAR in a child's head may be 50% to 100% higher than that for an adult⁴².

Conductivity and absorption of RF signals are a function of the dimensions and dielectric properties of the tissues that are directly exposed, as well as their neural density, with nerve cells being much more active than bone, hair, or skin. Conductivity is a parameter relating the electric field to the current density. For the same intensity of electric field, the increase in the conductivity will increase the current density and the

SAR. The absorption of RF energy will then increase, resulting in greater electromagnetic dissipation. Based on the measurements described by Peyman *et al*, the permittivity and the conductivity in the children's head tissues are estimated to be around 20% greater than in adults^{41, 43, 44}.

The combination of both effects, the increase in the concentration of the electric field due to the increase in the electrical permittivity together with the increase of dissipation of RF/MW energy due to the increase in the conductivity, can result in a substantial SAR increase in the children's head in comparison to the adults.^{42, 43}

The weight and size of the tissue being used for estimating the SAR will also affect assessments, with exposures averaged over 1 gram of the head being more stringent than those averaged over 10 grams of the whole body, as the latter involves bone and tissue of more varying electrical conductivities and mass densities than the former. The process of myelination of the brain protects nerves from damage by surrounding them with myelin sheaths, with myelination incomplete until the MID-205 could be yet another factor of concern for children and young adults using cellphones.

Recently, the use of cell phones by young and children has been modeled through a variety of simulations; some based on magnetic resonance imaging (MRI) others based on computerized tomography (CT) scans. Some studies have produced SAR simulations for the heads of adults^{45, 46}, while others took children into consideration⁴²⁻⁴⁴. A range of results was obtained (Table 2). In the Utah Model⁴⁷, the children's head was based on a scaled adult model and a SAR increase (compared with adult) of up to 153% was obtained.

In Schonborn's study, the head model was based on MRI using similar electromagnetic parameters as those for adults, and no significant differences between adult and children SAR results were observed⁵⁴. In another study, the head model was approximated by spheres considering some variation of the electromagnetic parameters, and an increase of around 20% in the calculated SAR was shown⁵⁵.

Using a scaled model for the children's head with adult electromagnetic parameters, no significant variation for the average SAR in the whole head was observed, and when considering the brain, an increase of around 35% in the SAR was calculated⁵¹. In De Salles's study, a 10 year old child head was developed based on CTI from a healthy boy⁴³. The physical and the electromagnetic parameters, such as the permittivity, the equivalent conductivity and the density were fitted to this age. SAR results around 60% higher than those simulated for the adults were observed for the children with fitted parameters.

Wart and his colleagues developed child head models based on MRI. The combined results of these studies indicate that the maximum SAR in 1 g of peripheral brain tissues of the child models aged between 5 and 8 years is about two times higher than in adult models⁵². More recently in an internal IT'IS Foundation Report, Kuster *et al*.⁵³ report that spatial peak SAR of the CNS tissues of children is "significantly larger (~2x) because the RF source is closer and skin and bone layers are thinner".

In all models used, it is readily apparent that smaller heads will absorb proportionally more RF than larger heads, but size is not the only property of interest in estimating differential SAR absorption of younger and older brains. Neuro-development of the brain is an exquisitely complex process that occurs at a more rapid pace in young children than in adults. As a result, even if exposures were equal in persons of all ages, the brains of children are more vulnerable than those of adults. In 1996, Gandhi published a report modeling the greater absorption of RF into the brain of a child compared to that of an adult⁴⁷. Subsequent work refined this analysis, taking into account a range of

Table 2 - Some tissue-classified models of the head and the whole body for estimating radiofrequency absorption of humans

Author, Year	Model	Height, Weight, Sex	Derived From	Voxel Size	# of Tissues, Organs	Percentage SAR Underestimation	Cumulative Percentage SAR Underestimation for Child	Comments
Gandhi <i>et al.</i> , 1996 ⁴⁷	Utah Model	1.75 m ht, 71 kg wt; also scaled models of 5- and 10-year old children	MRI scans	1.974x1.974x2.9 mm for the model of the adult; smaller cell sizes for models of children	32	<153%	<383%	Child's heads scaled from adult's head
Dimbylow, 1998 ⁴⁵	NORMAN*	1.7 m ht, 70 kg wt to correspond to "reference man" ICRP23 ⁴⁵	MRI scan single subject	2x2x2 mm, 2.04x2.04x1.95 mm	37			
Peyman <i>et al.</i> , 2001 ⁴¹						40%	40%	Permittivity & conductivity in children is 60-80 compared to adult's 40
Gandhi and Kang, 2002 ⁴²	Utah Model	MRI-derived model of the adult and scaled models ⁴⁸ of 5- and 10-year old children	MRI scans	Different scaling factors for the head and the rest of the body		50% + >100% from 10 mm spacer + 80% for electrical parameters	~200% @ 1900 MHz; 144% @ 835 MHz	10% smaller head results in 50% underestimation of SAR
Kang and Gandhi, 2002 ⁴⁸		model of the adult	MRI scans				15%/mm of spacer	
Wang and Fujiwara, 2003 ⁴⁹	Japanese Adult Model	Scaled Models of 7- and 3-year old children of the adult	MRI scans of the					Multiple studies find children absorb more radiation than adults. See also references 42, 47, 50-52, and 54.

(continued)

Table 2 - Some tissue-classified models of the head and the whole body for estimating radiofrequency absorption of humans

Author, Year	Model	Height, Weight, Sex	Derived From	Voxel Size	# of Tissues, Organs	Percentage SAR Underestimation	Cumulative Percentage SAR Underestimation for Child	Comments
Gandhi and Kang, 2004 ³⁰	Specific-anthropomorphic phantom (SAM)	Plastic head-shaped phantom with a plastic spacer to represent the pinna	90 th percentile head size of military personnel		Filled with homogenous fluid	Underestimates SAR by a factor larger than 2	Not tested for the size of a child's head	Use of a 6-10mm thick plastic spacer makes it impossible to measure the highest SAR for the pinna
Martinez-Burdalo <i>et al.</i> , 2004 ³¹	Child	Child	Scaled model from adult electrical parameters				35%	As head size decreases, the percentage of energy absorbed in the brain increases
Fernandez <i>et al.</i> , 2005 ⁴⁴	10 years old Brazilian Model	10 year old child (1.2 m height, 35 kg, male)	102 CT scans	0.946 mm x 2.044 mm x 1.892 mm (3.10 mm ³)	10			Permittivity & conductivity of 10 year old
De Salles <i>et al.</i> , 2006 ⁴³	10 years old Brazilian Model	10 year old child (1.2 m height, 35 kg, male)	102 CT scans	0.946 mm x 2.269 mm x 1.601 mm (3.43 mm ³)	10	60%		permittivity & conductivity of 10 year old
Wiat <i>et al.</i> , 2008 ³²		Child's Head, 5 to 8 years old	MRI scans			100% (2x)		Antenna closer to skin and bone layers are thinner; penetration of radiation is twice as deep in child
Kuster <i>et al.</i> , 2009 ³³		Child				>100% CNS tissues		SAR of CNS of children ~twice that for adults

* NORMAN=NORmalized Man

** Scaled models of 5- and 10-year old children derived from the Utah Model using external dimensions typical of children from Geigy Scientific Tables (C. Lentner-Geigy Scientific Tables, Vol. 3, CIBA-Geigy, Basel, Switzerland, 1984).

anatomic differences between adults and children, including conductivity, density and dielectric constants. Gandhi and Kang reported that models with a head that was only about 10% smaller in size could have more than 50% greater SAR with two different antenna lengths, with proportionally deeper penetration of SAR⁴². This work also showed that incorporating a plastic ear model or pinna with a 10 mm spacer gave artificially lowered SAR-values, which are up to two or more times smaller than for realistic anatomic models, as a result of the larger distance to the absorptive tissues. The higher dielectric constant and conductivities likely for younger subjects will result in still higher SAR (up to 80% more) for children.

The peak 1-g body tissue SAR for the smaller head sizes calculated using the widely accepted Finite-Difference Time-Domain (FDTD) computational EMFs method can be up to 56% higher at 1900 MHz and up to 20% higher at 835 MHz compared to the larger models. For brain tissue, the proportionality was even higher where the peak 1-g SAR for the smaller model was up to 220% higher at 1900 MHz and up to 144% higher at 835 MHz of the SARs of the larger models. Similar to the results reported in the earlier 1996 paper for head models of adult and children, these latter results confirmed that there is a deeper penetration of absorbed energy for the smaller head models e.g. the children compared to that for the larger head models representative of adults.

In 2004, a IEEE Standards Coordinating Committee introduced a standard anthropomorphic mannequin (SAM) Model, with a 6-10 mm thick plastic spacer instead of “pinna” for determination of SAR of mobile phones for compliance testing against IEEE and ICNIRP Safety Guidelines (IEEE, 2003). That same year, Gandhi and Kang demonstrated that the “SAM model” with plastic spacer used for compliance testing (preferred by industry) gives SARs that grossly underestimate exposures⁵⁰. In two different published studies, the use of plastic spacers results in an underestimation of the SAR by up to 15% for every additional millimeter of thickness of such spacers^{48, 50}. Thus, the SAR obtained for SAM is up to two or more times smaller than for the anatomic models of the adult head. When other developmental variables are taken into account, this underestimation is even higher for exposure to the smaller heads of the children.

A modified SAM model with a lossy pinna similar to living tissue for which 1- and 10-g SARs are relatively close to those for anatomic models, could remedy this systematic underestimation of exposure of the children by using a fluid of higher conductivity than that currently used for compliance testing⁴². Without this correction, current IEEE limits⁵⁶ effectively allow RF that may be 8-16 times higher⁵⁰ than those permitted by previous IEEE guidelines^{56, 57}. This is also due to increasing the SAR limit in the pinna from 1.6 W/kg for any 1-g of tissue to 4.0 W/kg for a larger 10-g of tissue that was originally suggested to apply only to the extremity tissues for the arms and the legs^{57, 58}.

In fact, multiple studies have reported that the brains of young children absorb more radiation compared to those of adults^{43, 47-49, 51-53}. As the brains of children lack neural integration and are not fully myelinated until the twenties, the impact of such greater absorption may be considerable. In addition, this differential absorption of the brain may well render children more vulnerable to the development of both benign and malignant brain tumors, a point indicated in the review of this subject by the National Research Council⁵⁹. Studies by Wiart for French Telecom published last year⁵² and other work by Kuster⁶⁰ confirmed that a given signal is absorbed about twice as deeply into the bone marrow of the head and cortex of a child in contrast with that of an adult, even though systemic absorption may not differ substantially. A series of papers by De Salles also offers important modeling information regarding the increased vulnerability of a child's

Table 3 - Summary of the results confirming that children absorb more radiated electromagnetic energy of the cell phones resulting in higher specific absorption rate (SAR) as compared to adults

Author, Year	Highlights of results
Gandhi <i>et al.</i> , 1996 ⁴⁷	Deeper penetration of absorbed energy for models of 10- and 5-year old children; peak 1-g SAR for children up to 53% higher than adults.
Gandhi and Kang, 2002 ⁴²	Deeper penetration of absorbed energy for smaller heads typical of women and children; peak 1-g SAR for smaller heads up to 56% higher than for larger heads.
Wang and Fujiwara, 2003 ⁴⁹	Compared to peak local SAR in the adult head, we found “a considerable increase in the children’s heads” when we fixed the output power of radiation.
Martinez-Burdalo <i>et al.</i> , 2004 ⁵¹	As head size decreases, the percentage of energy absorbed in the brain increases; so higher SAR in children’s brains can be expected.
DeSalles <i>et al.</i> , 2006 ⁴³	The 1-g SAR for children is about 60% higher than for the adults.
Wiat <i>et al.</i> , 2008 ⁵²	1-g SAR of brain tissues of children is about two times higher than adults.
Kuster <i>et al.</i> , 2009 ⁵³	Spatial peak SAR of the CNS of children is “significantly larger (~2x) because the RF source is closer and skin and bone layers are thinner”; “bone marrow exposure strongly varies with age and is significantly larger for children(~10x)”

head⁴³. Based on CT images of a 10 year old boy, these models confirm the greater absorption of the child and add further support regarding the need to eliminate the plastic spacer at the ear or pinna in estimating exposures to children. A summary of the results confirming that children (and smaller heads typical of women) absorb more radiated energy of cell phones resulting in higher SAR is given in Table 3.

Implications of modeling limitations for current standards

Both the IEEE and ICNIRP guidelines are based only on short-term EMFs exposure and long-term EMFs exposures are not considered. Please refer to page 496²:

“Induction of cancer from long-term EMFs exposure was not considered to be established, and so these guidelines are based on short-term, immediate health effects such as stimulation of peripheral nerves and muscles, shocks and burns caused by touching conducting objects, and elevated tissue temperatures resulting from absorption of energy during exposure to EMFs. In the case of potential long-term effects of exposure, such as an increased risk of cancer, ICNIRP concluded that available data are insufficient to provide a basis for setting exposure restrictions, although epidemiological research has provided suggestive, but unconvincing, evidence of an association between possible carcinogenic effects and exposure at levels of 50/60 Hz magnetic flux densities substantially lower than those recommended in these guidelines”.

The increase in the SAR in the whole head, between the adult and the child, is expected due to the reduced dimensions in the child head, as well as the higher values of the permittivity and of the electrical conductivity of the child brain tissues. Also, children's skulls are thinner than those of adults, and therefore less resistant to radiation.

Another concern is that only thermal effects of RF are considered when estimating the SAR. However, since most mobile communication systems now are pulse-like in nature, modulated at low frequencies, such as in 2G and 3G (e.g., the GSM, UMTS, CDMA, TDMA systems), they are able to induce pulses of currents in the brain tissues and this can result in some low level non-thermal effects, e.g., BBB alterations, single and double strand DNA breaks, chromosomal aberrations, etc., at RF energy levels substantially below the thermal threshold. Several papers and reports have already shown adverse health effects at exposure levels well below the thermal limits^{4, 6, 12, 13, 61}. Further epidemiological studies have shown a many-fold increase in risk for malignant brain tumors, with a larger than 10 years latency period for long-term mobile phone and cordless phone users²³. As a substantial percentage of the population now uses mobile phones for a long time during each day and for several years, operating the antenna very close to their head, then this exposure can not be classified as short term and effectively may represent a serious risk for their health.

Future research needs

There is a need for exposure assessment of juveniles, children, pregnant women and fetuses from personal wireless devices (the wireless devices considered here are the cell phones, wireless PCs and text messaging devices), waist and pocket-mounted devices since mostly adult male models have been considered to date. These studies will focus on development and exposure quantification of anatomic models of several heights and weights of men, women and children of various ages as well as pregnant women and fetuses.

There is an urgent need for characterization of microwave radiated fields from the currently used multi-frequency, multi-element base station antennas; identification of exposed individuals and their locations e.g. school children, building maintenance personnel, etc. There is a paucity of data in regard to radiated electromagnetic fields and the daily variation in time for the newer 4-6 element or more collocated base station antennas and the exposures these antennas entail for the school children and the civilian population living close to such antennas.

An updated survey is needed of the civilian exposure to microwave electromagnetic fields strengths in the U.S. due to the rapidly expanding wireless infrastructure in the last 10-15 years. The last survey involving selected 15 metropolitan areas and mostly focused on VHF and UHF TV stations was reported back in 1980.⁶² This data is totally out of date at the present time.

An expert (non-industry dominated) evaluation of the current IEEE and ICNIRP RF/microwave safety standards in the light of more recent biological experiments is also critical. All of the current safety standards are based on extrapolation from acute short-term exposures and do not account for the modulated signals used in cell phones and other personal wireless devices.

Discussion

The summary of modeling research presented here indicates three major shortcomings of the current IEEE and ICNIRP approaches: 1) the assumption that only thermal effects can occur is not valid. There is growing evidence from in vitro and in vivo studies indicating that RF exposures at levels not known to induce thermal effects commonly encountered today have a range of biological effects, affecting production of free radicals, permeability of the BBB, expression of in heat shock proteins, and direct damage to DNA, as indicated by the comet assay and a variety of in vitro measures of genotoxicity; 2) properties of the head models currently used fail to take into account differences in dielectric constant and conductivity and improper modeling of the pediatric brain, as well as developmental differences such as myelination between the young and older brains; 3) the assumptions as to typical use patterns used in setting these standards, with a six minute average call time, do not reflect current patterns, according to reports from the cell phone industry, where monthly use can easily top 2000 minutes with many calls well in excess of 6 minutes.

Excepting the occasional advertisement, there is no publicly accessible, independently confirmable, information on the details of rapidly expanding markets and uses of cell phones, which makes the development of standards especially challenging. Cell phones are used by many people for much of their waking hours, having replaced traditional phones, alarm clocks, newspapers, radios, global positioning devices, video-cameras and televisions.

Regarding young children, we do not know the typical practice of the young at this point, because those behaviors are changing rapidly. However, we do know school districts are being urged to adopt cell phones for all middle school students as learning tools. This may well be an excellent idea for the purposes of learning, providing that phones are not used and held directly to the developing brain. Whether the use of cell phones as phones proves a potential hazard to the long-term health of the pediatric brain is an issue that merits serious attention. Radiation compliance standards for operation of cell phones are based exclusively on adult male models of the head. Emerging research indicates that long-term heavy users of cell phones face a doubled risk of several forms of brain tumors and risks may well be greater for those who begin regularly using phones before age 20. In light of these facts, the European Environment Agency and several other national advisory groups have adopted a precautionary approach to keep cell phone exposure to a minimum through use of ear-pieces and speaker phones, wired headsets, and to urge that children generally not use cell phones.

To enhance the ability to protect public health and foster better design of this widely used technology, we advise a three-pronged approach: major studies should be undertaken to construct and validate gender and age-appropriate head models further. More research is needed to identify and evaluate the mechanisms through which non- or thermal effects of RF arise and to determine more definitively the extent of health risks from long term use of cell phones, particularly by children. While that work is proceeding, precautionary policies should be advanced to limit potential harm to the developing brain. This should include consideration of directional antennas designed to send signals away from the head since the tissues absorb almost all of the energy radiated in the direction of the head anyway. Responsible public health authorities around the world should disseminate warnings for cell phone users such as those advocated recently in France, Finland and Israel. This involves advising children and their parents

along with the young to make only short and essential calls, to use text messaging when possible, to use always hands free kits and wired headsets, and maintain the antenna far away from their body during the calls. Given the prevalence of this revolutionary technology, some evidence of its chronic toxicity, and the lack of solid information regarding its potential hazards to humans, it is important that major independent, multi-disciplinary research programs be carried out to study and monitor the long-term impact of RF exposures.

Acknowledgement

Support for this work was provided in part by grants from the National Institute of Environmental Health Science, the Heinz Endowments, the Jennie Zoline Foundation, the Environmental Health Trust, and center grants from the National Cancer Institute to the University of Pittsburgh Cancer Institute. The authors declare that they have no competing interests. Constructive comments have been provided by Lloyd Morgan and Allan Frey.

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Investigation on blood-brain barrier permeability and collagen synthesis under radiofrequency radiation exposure and SAR simulations of adult and child head

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Abstract

The effects of Radiofrequency Radiation (RFR) in the frequencies of mobile phones (835, 900, 1800 MHz) on the permeability of blood-brain barrier and hydroxyproline formation along with the modeling studies performed at the Gazi Biophysics Laboratory are reviewed in this paper. The close proximity of a mobile phone to a user's head leads to absorption of part of the mobile phone emitted energy by the head and the brain of the phone user. Permeability of the blood-brain barrier (BBB) of female and male rat brain tissues was examined under 900 MHz and 1800 MHz continuous-wave radiofrequency radiation (CW-RFR) exposure. Increase in BBB permeability was found to be statistically significant in all male rats exposed, whereas no significant difference was observed in female rats. Investigations of the mobile phone radiation effects on biomolecules were also carried out with guinea pigs. Alterations in protein synthesis were quantified by measuring hydroxyproline level in exposed and non-exposed liver tissues by using three different biochemical methods. There was no significant difference on hepatic hydroxyproline levels of RFR exposed guinea pig. In a simulation study, the effects of 835 MHz and 900 MHz RFR exposures on human head while using cellular phone (CP) were investigated. The effects of CP usage on specific absorption rate (SAR) were calculated by SEMCAD X software which uses FDTD method in details. Some parameters as the different head dimensions and dielectric properties of the head (adult and child), positions of the mobile phone (cheek and tilt), and rectangular metal frame spectacles as a widely used metallic accessory were considered. With this aim, dose values in the tissue for 10 g peak spatial-average SAR value were calculated. At both of the frequencies of 835 MHz and 900 MHz, higher SAR values were obtained in the cheek positions than the tilt positions for conditions of with or without metal frame spectacles.

Key words: Radio Frequency Radiation (RFR), Blood-Brain Barrier (BBB), Collagen Synthesis (CS), Specific Absorption Rate (SAR), FDTD

Introduction

During recent years, mobile communication systems have experienced wide and rapidly growing use all over the world. Many studies have investigated whether mobile phone use and radiofrequency (RF) fields in general could have biological effects. The close proximity of the antenna of a mobile phone to the human body and especially the head has raised concerns about the biological interactions of electromagnetic radiation (EMR). Conflicting results were reported on whether low levels of radiofrequency fields increase the permeability of the barrier that keeps harmful substances from entering the brain (blood-brain barrier). In 2008, there was a review on the blood-brain barrier (BBB) which includes a complex picture indicating that some studies showed effects on the blood-brain barrier, whereas others did not. Possible mechanisms for the interactions between electromagnetic fields and living organisms were also discussed in that paper¹. One of the important aims of the present study was to investigate the effects of 900 MHz and 1800 MHz continuous wave (CW) RFR on the permeability of BBB of young adult male and female rats.

Effects of static and ELF electric and magnetic fields on collagen have been studied at the Gazi Biophysics Department and hydroxyproline levels of skin, liver, kidney and lung tissues were found to change after exposure to these fields²⁻⁸. There is very limited number of studies on the effect of RFR at mobile phone range on the tissue level of collagen⁹⁻¹⁰. In this paper, we report our investigation on the effects of mobile phone radiation on collagen synthesis. Collagen was examined by using three different hydroxyproline detection methods such that we could repeat and cross-check our biochemical work and results by these three methods¹¹.

Dosimetry is an important issue on monitoring the biological effects of RFR exposure¹². In a Specific Absorption Rate (SAR) simulation study, the aim was to investigate how SAR changes with various anatomical human head models^{13, 14}. Generic Mobile Phone model which is accepted by the Mobile Manufacturers Forum (MMF) were used in this study¹⁵. Frequencies were selected as 835 MHz and 900 MHz to compare the dose rates of cellular phones (CP) which have been used in the United States and Europe, respectively. Dielectric properties and sizes of phantoms studied were according to the standards of IEEE 1528-2003 and IEC 62209-1 for adult SAM phantom. Children are more affected by RFR with respect to adults¹⁶⁻¹⁸ because of the dimensions and the dielectric properties of their head. Furthermore, SAR simulations of children head models were done for the same frequencies by applying the data from the studies of Peyman and Gabriel's according to the standards of IEEE 1528-2003 and IEC 62209-1 2005^{19,20}.

Materials and methods

Blood brain barrier study

Twenty five male (268.13 ± 41.92 g) and twenty seven female (216.85 ± 24.72 g) young adult Wistar albino rats were used in the study. Four exposure and two control groups were used in the experiment: Group I (n=8)- control males, Group II (n=9)- control females, Group III (n=8)- 900 MHz exposed males, Group IV (n=9)- 900 MHz exposed females, Group V (n=9)- 1800 MHz exposed males, Group VI (n=9)-1800 MHz exposed females. Animals in the control groups were sham-exposed. The animals were

anesthetized with ketamine (45 mg/kg) and xylazine (5 mg/kg) by intramuscular injection prior to the experiments²¹.

Exposed groups were kept at 10 cm away from a horn antenna to satisfy the near field condition. Control (sham) groups were kept in the same setting without any RFR exposure. Synthesized signal generator was used for propagating the RF signal. Field strengths were monitored with a Narda EMR 300 and its appropriate probe (8.3) during the exposures. Background E-field level to which controls were exposed, was measured to be 0.265 ± 0.02 V/m. E-field levels at 900 MHz and 1800 MHz were 13.51 ± 0.41 V/m and 12.62 ± 0.22 V/m, respectively^{22, 23}. RFR or sham exposure duration was 20 minutes for all animals. The experiments were performed with the anesthetized rats in a quiet laboratory with little noise to limit stress. ICNIRP general public E-field limits for these frequencies are 41.25 V/m and 58.34 V/m²⁴. Since the E-field levels in this study are well below currently accepted limits, the exposure level used in this study can be considered non-thermal.

We investigated permeability of BBB using Evans Blue (EB) dye as a tracer which is known to bind to serum albumin after intravenous injection. Quantative method was used for measuring the amount of dye in the brain^{25, 26}. EB dye (2% in saline, 4 ml/kg) was injected into the tail vein of a rat and was allowed to circulate for 20 min. An animal was then exposed to RFR or sham fields for 20 min period. At the end of each exposure, its chest was opened under anesthesia. Brains were perfused with saline through the left ventricle for approximately 15 min until fluid exiting from the right atrium became colorless. Brain was then removed and dissected into four regions: left and right cerebrum, left and right cerebellum. Each brain region was weighted for quantative estimation of EB dye - albumin extravasations. The samples were then homogenized in 2.5 ml phosphate buffered saline-PBS and mixed with a vortex after the addition of 2.5 ml of 60% trichloroacetic acid to precipitate the protein molecules, then centrifuged for 30 min in 3000 rpm (at 1000xg). The supernatant was measured at 620 nm for absorbance of EB dye using a spectrophotometer. The concentration of EB per gram of brain was determined from the absorption measurements using a standard curve. E-field levels and EB contents are presented as the mean $\pm$ SD for each group. Mann-Whitney U-Test was used to assess significance and $p < 0.01$ was considered statistically significant.

Radio frequency radiation effect on collagen

In this investigation, 30 three-month-old male Guinea pigs (250-300 g) were used. They were divided into three groups: sham exposed, 10 minutes mobile phone-exposed, and 20 minutes mobile phone-exposed. Animals that had their own private cage were placed inside the cage just at the beginning of the experiment in order to reduce stress. Cages, made of transparent plastic with the dimensions of 8 cm x 10 cm x 18 cm, have efficient holes for ventilation. RF source was a Nokia 3210 mobile phone with 0.81 W/kg digital SAR value was positioned on the cage where the antenna of the mobile phone is maximum 5 cm above the head of the guinea pig. While mobile phone is at off mode for the sham exposure condition, it was in talking position during the exposure conditions. Measurements were taken instantaneously during the experiment by NARDA EMR 300 and a type 8.3 probe and the data saved to the computer connected to device via fiber optic cable. Guinea pigs were exposed to RFR averaged as 11.2 ± 0.5 V/m for 10 minutes²⁷ and 20 minutes a day during 7 days and analyzed for the effects on liver tissue hydroxyproline level.

After the last day of mobile phone exposure, liver tissues were removed from animals after decapitation. They were immediately frozen in liquid nitrogen and stored at -80°C until analysis. Changes of hydroxyproline level were analyzed biochemically by three different hydroxyproline determination methods: “H. Stegemann-K. Stalder”²⁸⁻³¹, “I.S. Jamall-V.N. Finell”³² and “ISO 3496”³³.

Principle of the first method, named “H. Stegemann-K. Stalder”, is to get the hydroxyproline of the hydrolysis of the tissue sample after homogenization and measuring the optical density of the color formed by adding p-dimethylaminobenzaldehyde, perchloric acid and propan-2-ol at pH 8 and at λ (wavelength) = 560 nm.

The “I.S. Jamall-V.N. Finell” method is based on oxidation of hydroxyproline after the hydrolysis of the tissue sample by kloramin-T and formation of chomofor composites via the reaction with Ehrlich reactive including p-dimethylaminobenzaldehyde and perchloric acid. Optical density of the solution at pH 6 was measured with respect to water at $\lambda = 560$ nm.

The third one known as “ISO 3496” is to get the hydroxyproline of the hydrolysis of the sample after homogenization and measuring the optical density of the color formed by adding sulphuric acid at pH 6.6 at $\lambda = 558$ nm.

For each method, hydroxyproline contents of the tissue samples were determined using standard curves for samples containing known concentrations of hydroxyproline (Sigma H-1637). Two samples were taken from each homogenized tissue, and the concentrations measured by spectrometry were averaged. For each group, hydroxyproline contents of tissues from groups exposed to RF radiation and their controls were compared with ANOVA, Welch ANOVA tests.

SAR simulations of adult and child head

SAR levels resulted from CP exposures were determined by the SEMCAD-X software. SAM phantom and generic CP model were used to assess peak SAR values averaged over 10 g of tissue. The effects of some parameters such as metallic accessories like spectacles, different positioning of CP, different head dimensions and different dielectric properties on SAR were determined at 835 MHz and 900 MHz frequencies^{13, 14}. Selected general cell phone model which is approved by the Mobile Manufacturers Forum has three parts: a monopole antenna, a plastic chassis and a printed circuit board made by perfect electric conducting material inside this plastic chassis. SAR values were obtained by normalizing antenna input power to 1 Watt. It was assumed that phone model sizes are 102 mm x 42 mm x 21 mm (height x width x thickness) and it consists of a hard plastic chassis. Antenna was mounted on the top part of the chassis at the center. Antenna height was modeled as 20% shorter than quarter wave ($\lambda/4$) height to obtain reasonable input impedance near different head models^{15,34}.

Adult head phantom's circumference was scaled with 0.9 factors in order to obtain a child phantom for a 7-year-old child³⁵. Dielectric properties of SAM phantom for adult and child are given in Table 1^{19, 20, 36, 37}.

In the study, a spectacles frame was modeled presuming that it was 37 mm width and 63 mm height and made of Perfect Electric Conducting metal. The length of spectacles' arm is 140 mm and Perspex lens was selected.

Table 1 - Dielectric properties of adult and child SAM phantoms

Frequency	Adult*		Child**	
	ϵ_r	σ (S/m)	ϵ_r 109,85 %	σ (S/m) 116 %
835 MHz	41,5	0,90	45,59	1,0440
900 MHz	41,5	0,97	45,59	1,1252

* Dielectric properties of adult SAM phantom taken from IEEE 1528 and IEC 62209-1

** Dielectric properties of child SAM phantom which was extrapolated from IEEE 1528 and IEC 62209-1 by using Gabriel and Peyman studies ¹⁹⁻²⁰

Results

Blood brain barrier study

In the study, we investigated the effects of exposure to continuous-wave (CW) RFR at 900 MHz and 1800 MHz for 20 min on the permeability of BBB of rats. Male and female rats (Groups III and IV, respectively) were exposed to 900 MHz at an electric (E) field of 13.51 ± 0.41 V/m and rats in 1800 MHz groups (Groups V and VI) were exposed to an E field of 12.62 ± 0.22 V/m. In all exposed and sham-exposed groups, albumin extravasations occurred largely from leptomeningeal blood vessels which, together with those in the choroid plexus and circumventricular organs, have no recognizable blood-brain barrier.

In the male groups Evans Blue dye content in the whole brain was found to be 0.072 ± 0.01 mg % in the controls, 0.1325 ± 0.02 mg % in 900-MHz exposed group and 0.1123 ± 0.02 mg % in the 1800-MHz exposed group (fig. 1). Difference between the exposed groups and controls was found to be significant ($p < 0.01$). No statistically significant difference was found between the two RFR-exposed groups.

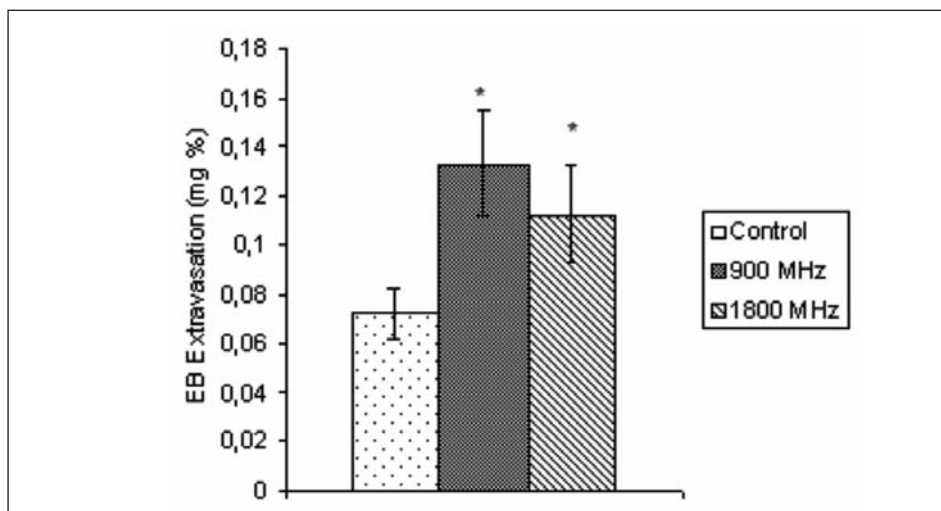


Fig. 1. Brain EB content of male rats. Data is shown as mean $\pm$ standard deviation of the mean (SD)

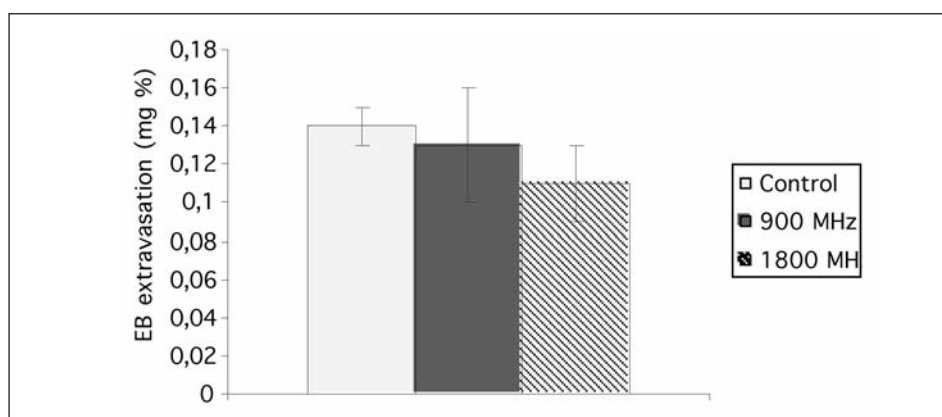


Fig. 2. Brain EB content of female rats

In the female groups, dye content in the whole brain was found to be 0.14 ± 0.01 mg % in controls, 0.13 ± 0.03 mg % in the 900-MHz exposed and 0.11 ± 0.02 mg % in the 1800-MHz exposed groups (fig. 2). No statistically significant difference was found between two RFR-exposed groups ($p > 0.01$). There was also no statistically significant difference between the exposed females and the controls ($p > 0.01$).

Our results showed that a 20-min exposure to 900-MHz and 1800-MHz RFR induced an increase in permeability of BBB of young adult male rats. However, similar exposure to RFR did not induce an effect on the permeability of BBB in young adult female rats.

Radiofrequency radiation effect on collagen

Results are shown in Table 2 and fig. 3. The outcome of the biochemical analysis indicated that hydroxyproline level increased with respect to control but this increase was not statistically significant for all three methods of analysis ($p > 0.05$). The results showed no significant effect of RFR exposure on liver hydroxyproline in the guinea pig. However, difference in hydroxyproline determination accuracy of ISO 3496 method with respect to the other two methods was found to be statistically significant ($p < 0.05$) (Table 2 - fig. 4).

Table 2 - Comparison of liver tissue hydroxyproline levels ($\mu\text{g/g}$ tissue) in groups exposed to RFR for 10 and 20 minutes with controls measured by three different methods. The values in the table represent the least squares means $\pm$ standard deviation (mean $\pm$ Sd)

	H. Stegemann-K. Stalder	I.S. Jamall-V.N. Finell	ISO 3496
Sham exposed group	0.2716 ± 0.0289	0.2897 ± 0.0622	0.3054 ± 0.0125
10 min. Exposure group	0.2773 ± 0.0251	0.2907 ± 0.0185	0.3058 ± 0.0186
20 min. Exposure group	0.2794 ± 0.0282	0.2907 ± 0.0240	0.3075 ± 0.0124

SAR simulations of adult and child head

Variations of 10-g averaged SAR values for 835- and 900-MHz exposure in SAM phantom for adult and child with or without metal frame spectacles, for cheek and tilt positions of CP are given in fig. 5^{13, 14}.

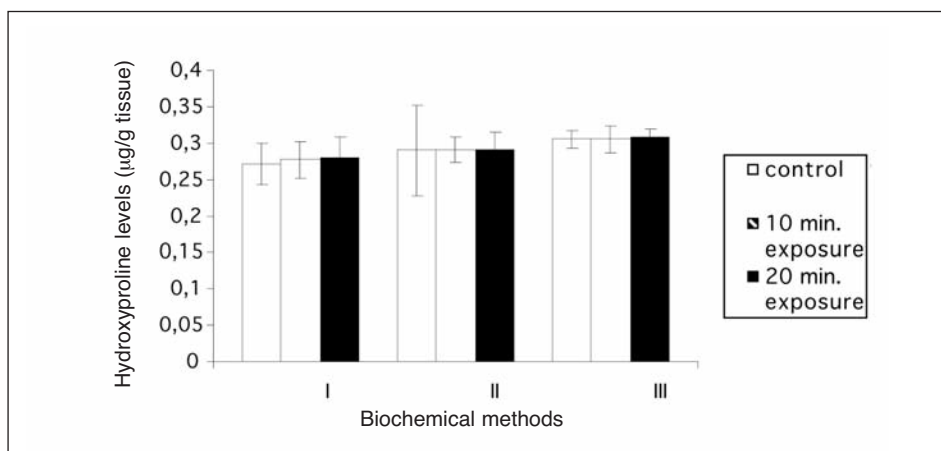


Fig. 3. Liver tissue hydroxyproline level determined by using three different biochemical methods. I: H. Stegemann-K. Stalder's method, II: I.S. Jamall-V.N. Finell's method and III: Method of ISO 3496

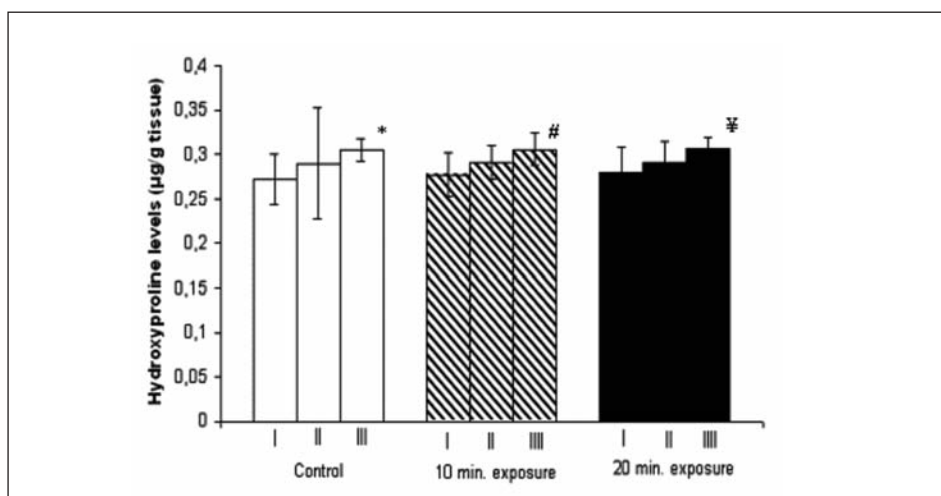


Fig. 4. Liver tissue hydroxyproline level determined by using three different biochemical methods for controls and exposure groups. I: H. Stegemann-K. Stalder's method, II: I.S. Jamall-V.N. Finell's method and III: Method of ISO 3496. *: $p < 0.05$ as compared to the hydroxyproline levels of controls determining by methods of I and II; #: $p < 0.05$ as compared to the hydroxyproline levels of 10 min. exposure determining by methods of I and II; ¥: $p < 0.05$ as compared to the hydroxyproline levels of 20 min. exposure determining by methods of I and II

It was found that usage positions of CP were the most significant parameter affecting SAR values. The obtained 10-g SAR values from the cheek positions were significantly more those that of tilt positions. Higher SAR values were determined on cheek position at both frequencies.

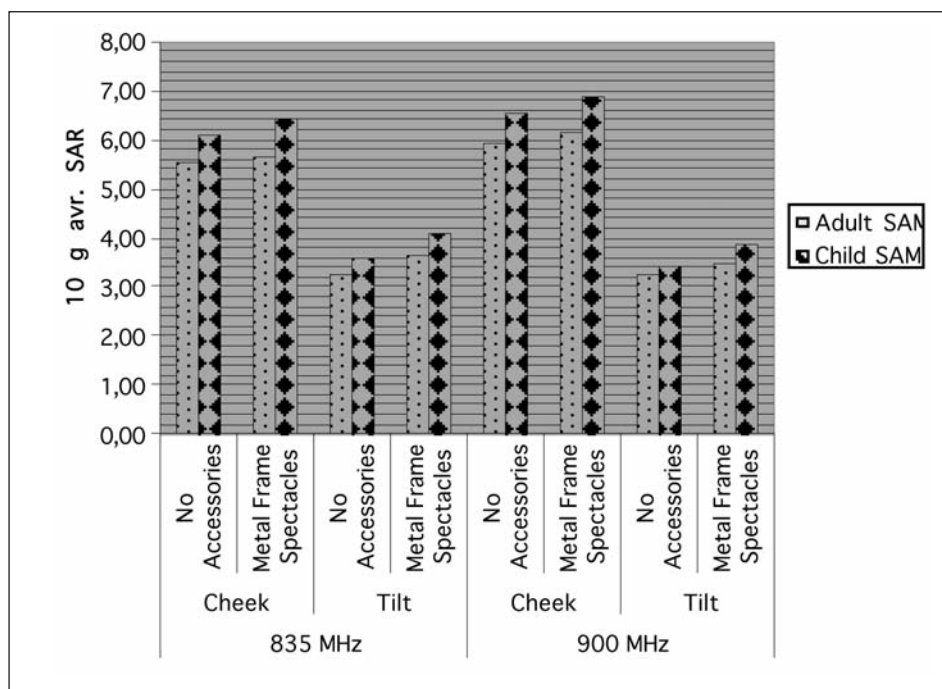


Fig. 5. Variations of peak SAR values for 835-MHz and 900-MHz RFR absorption in SAM phantoms for adult and child with or without metal frame spectacles, for cheek and tilt positions of CP

With the SAM phantom modeled for the child's dielectric characteristics and head size, increased SAR values were determined compared to adults. The reason why this increase occurred may be the change in head sizes, but the main reason is the difference in dielectric characteristics between the child and the adult. In the condition of usage of metal frame spectacles, higher SAR values were determined both at the cheek and tilt positions at both 835 MHz and 900 MHz compared to having no spectacles. It was also observed that local SAR values were higher at the head model near to the spectacles. It might be resulted from the currents induced at the metal frame of the spectacles.

Discussion

Blood brain barrier study

Our results indicate that RFR at non-thermal levels can induce disruption of the BBB. Disturbances to the integrity of BBB and external influences on its functions are critical to central nervous function and could influence and accelerate neurodegenerative processes. One of the possible mechanisms for tumor development is increase in the permeability of BBB, which may result in the entry of carcinogenic substances into the brain.

Our results suggest that 20 minutes of acute exposure of young adult male rats to CW RFR cause disruption to BBB integrity, whereas no significant change was found for the

female rats. Gender differences have been reported for many structures and functions of central nervous system³⁸. Lin *et al.*³⁹ argued that EB dye in the rat brains is closely related to intense RFR hyperthermia. Wijsman and Shivers⁴⁰ demonstrated that BBB permeability to Horse Radish Peroxidase (HRP) was increased in response to heat stress. We present here evidence for BBB disruption caused by non-thermal RFR exposure. Our observation finds support in the work of Salford *et al.*⁴¹ which showed the short-term exposure effects of CW RFR on the BBB at non-thermal levels. It is unlikely that this increase of permeability in male exposed groups could be due to immobilization stress⁴², since animals were exposed to 900-MHz and 1800-MHz RFR under anesthesia. Prato *et al.*⁴³ shown a temporarily increase in BBB permeability to HRP under MRI procedure. Fritze *et al.*⁴⁴ investigated the effects of 900-MHz RFR exposure on the permeability of BBB for duration of 4 h at SAR ranging from 0.3, 1.5 and 7.5 W/kg. The increase in serum albumin extravasations after RFR exposure reached significance only in the group exposed to the highest SAR of 7.5 W/kg. Gruenau *et al.*⁴⁵ evaluated the effects of CW or pulsed RFR at a frequency of 2.8 GHz on the permeability of BBB of unanesthetized rats and the findings indicated that RFR radiation under the given experimental conditions did not damage BBB.

Possible mechanisms of disruption of BBB by RFR are still under discussion. Some authors suggest pinocytotic transport across the endothelial cells⁴⁶. Neubauer *et al.*⁴⁷ described that permeability increase of BBB to rhodamine-ferritin at whole body averaged SAR of 2 W/kg was almost blocked when rats were pretreated with colchicine. These results also suggest that pinocytotic mechanisms may be involved. Some authors argued that an increase of heat shock proteins (HSP) results in oxidative stress and this stress gives rise to brain tumors or the increase in the permeability of BBB^{48, 49}. RFR exposure might produce an increase in HSP level. Researchers are also discussing the link between RFR exposure and the changes of BBB permeability and headaches and the dopamine opiate systems of brain⁵⁰. An alternative explanation could be an opening of tight junctions or an increase of ornithine decarboxylase (ODC) activity which correlates with BBB disturbances⁵¹.

We conclude that our data support the hypothesis that 900-MHz and 1800-MHz CW RFR at non-thermal RFR levels is related to an increase in the permeability of BBB in young adult male rats.

Radio frequency radiation effect on collagen

Since 1960, collagen draws the scientists' interests because it has piezoelectric characteristics and could be affected by external and/or internal natural electromagnetic fields because of its electrical charge. There are researches that focused on effects of electromagnetic radiation on collagen in several tissues but most are related with electric current, static, and ELF electromagnetic fields^{2-8, 52-61}. In addition to these studies, some studies also investigated RFR effect on collagen. For instance, Masuda *et al.*⁹ studied on hairless female rats exposed or sham-exposed for 2 h to GSM 900 or GSM 1800 signals, using a loop-antenna located on the right part of the rats' back. The local Specific Absorption Rate (SAR) at skin level was approximately 5 W/kg. Results on filaggrin, collagen and elastin levels showed an insignificant influence of RFR. Ozguner *et al.*¹⁰ investigated the effects of 900-MHz RFR on the induction of histopathologic changes in skin and they found increased thickness of stratum corneum, atrophy of epidermis, papillomatosis, basal cell proliferation, increased granular cell layer (hyper-

granulosis) in epidermis and capillary proliferation, impairment in collagen tissue distribution and separation of collagen bundles in dermis.

In the present study, effects of RFR generated by GSM 1800 mobile phones on liver tissue collagen were examined by using three different hydroxyproline detection methods. The outcome of the biochemical analysis pointed out that RFR did not significantly affect hydroxyproline level.

Since this is a pioneer study on the effect of mobile phone radiation on hydroxyproline level, using three different methods was needed to ensure validity. In addition to this, collagen composed of the amino acids: glycine (33.5 %), proline (12%) and hydroxyproline (10%), so especially liver hydroxyproline level determination is a difficult procedure because of low level of tissue collagen (4%). In the light of our evidences, hydroxyproline levels obtained by using ISO 3496 method is statistically more significant than the other two methods ($p < 0.05$). In this study, “H. Stegemann-K. Stalder”, “I.S. Jamall-V.N. Finell” and “ISO 3496” were chosen as biochemical methods of liver tissue hydroxyproline level determination after literature search. In each of these three methods, tissue hydrolysis of hydroxyproline was measured by spectrometry after adding Chloramin-T reactive which stains the solution. “ISO 3496” is a method which is nowadays used for determining the absolute value of hydroxyproline in the meat and meat product industry which should be very little collagen content in order to be fine product.

Even though our findings of hydroxyproline levels in liver tissue of RFR-exposed guinea pigs were statistically insignificant with respect to controls. A question to be asked is what would be the consequence of longer duration or prolonged exposure. It would be interesting to study prolonged exposure in further research.

SAR simulations of adult and child head

There is a rapid increase in the usage of wireless communication. While the working frequency of the cellular phone increases, the value of the SAR increases¹⁵. In this study, SAR values resulted by CP operating in 835-MHz and 900-MHz frequency bands were calculated in human head models for both adult and child. Moreover, the feature of this study gives a chance to compare the SAR levels resulted by the frequencies of 835 MHz and 900 MHz which are the CP operating frequencies of Europe and USA.

CPs were positioned near the head models in two positions according to IEEE 1528-2003, IEC 62209-1 2005 standards. In the first condition, CP was located near the cheek, and at the second one, CP was in tilt position. Consequently, SAR level was found to be less in the tilt position than the condition that CP was near the cheek. Our results are consistent with the results of other studies in the literature¹⁵. SAR level in the tilt position of CP was 40% less than the cheek position of CP for 835 MHz. Furthermore, this decrease was 55% for 10 g SAR value for 900 MHz frequency. This may be caused by the location of the current density in phone chassis being closer to the head phantom in the cheek position of CP.

Children of the growing age are more vulnerable to influence of environmental factors. Because of the size of children's head and their dielectric properties, their RF radiation dose rates caused by CP usage are higher than adults. For this reason, scaled head models are usually used for children head simulation. Gandhi et al¹⁸ studied with scaled head models for the 5-year-old and the 10-year-old children for simulating the effect of CP with $\lambda/4$ monopole antenna operating both at 835-MHz and 1900-MHz

frequency bands. They reported that 1 g peak spatial average SAR at 835 MHz frequency was 50% increased in the scaled model of the 5-year-old child head¹⁸.

De Salles *et al.* found that 1 g peak spatial average SAR increased by 60% in the scaled model of 10-year-old child head exposed to CP with patch antenna and $\lambda/4$ mono-pole antenna at the operation frequencies of 835 MHz and 1850 MHz⁶².

In this study, a significant increase was found in the child SAM phantom, modeled according to the dielectric properties of the children with respect to the adult model. 10 g peak spatial average SAR increases for 835 MHz and 900 MHz were calculated as 10% in the cheek position. It was determined that increasing ratios were 10% for 835 MHz and 6% for 900 MHz in the tilt position of CP.

It should be considered that children will be affected from CP more than adults and they should have precaution in using this technology.

According to the SAR calculated in this study, it is observed that the positioning of CP is the most effective parameter affecting SAR level. The spectacles, one of the most widely used accessories in daily life may be one of the important parameters that affect SAR values. Furthermore, sensitive organs like the eye can be exposed to high SAR because of the induced current at the spectacles. The rectangular metal frame spectacles used in this modeling study have a perfect electrical conductivity. Simulation revealed that metal frame spectacles increased the spatial peak SAR for 835-MHz and 900-MHz frequencies as 2-3% in cheek position, but this increase was 7-11.5% in CP's tilt position. In addition to this, it was observed that local SAR levels in the head model near spectacles were high.

SAR calculations for the studies of BBB and collagen synthesis is planned to be evaluated in our further study.

Acknowledgements

RFR measurements were performed with devices purchased by a grant from the Gazi University Research Foundation, Project No: 01/2003-62.

BBB study was supported by a grant from the Gazi University Research Foundation, Project No: 01/2005-78.

Study of "Analysis of Radio Frequency Radiation Effect on Collagen" was funded by a grant from the Gazi University Research Foundation, TF.01/2004-04.

SAR study was supported by the Gazi University Scientific Research Grant SBE-01/2006-22.

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Effects of microwave radiation upon the mammalian blood-brain barrier

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Abstract

Our research group has studied the effects of electromagnetic fields (EMF) upon the mammalian brain (rats) since 1988. Our major field of interest during the period has been the effects upon the blood-brain barrier (BBB) of the rat. The mammalian brain is protected by the BBB from potentially harmful compounds circulating in the blood. In the normal brain, the passage of compounds over the BBB is highly restricted. Our studies have revealed that the EMF radiation of the kind emitted by mobile phones leads to increased permeability of the BBB both immediately after 2 hours of exposure, but also after 7 days, 14 days and 50 days, all at non-thermal exposure levels. Also, damaging effects from radiofrequency EMF upon neurons has been shown after 28 days and 50 days. Of what is known today, the human BBB is very similar to the rodent BBB. With our research into the field, and comparison to other studies of BBB permeability in connection to EMF exposure, it is our sincere belief, that it is more probable than unlikely, that non-thermal EMF from mobile phones and base stations do have effects upon the human brain.

Key words: blood-brain barrier, dark neurons, electromagnetic fields, mobile phone, rats

Introduction

The environment for life on Earth has changed dramatically during the last decades. During the billions of years when life was formed, it was shaped to function in harmony with the naturally occurring physical forces such as gravitation, cosmic irradiation, heat and cold, mechanical forces and the terrestrial magnetism.

The power density of the microwave (MW) background in space is about $0.4 \mu\text{W}/\text{m}^2$, as obtained by integration of recorded spectral data. This results in a power density of

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an extremely low natural MW background on earth, estimated to be in the order of 10^{-15} to $10^{-8} \mu\text{W}/\text{m}^2$.

Artificial MWs were not produced by humans until 1886. At that point, the German physicist Heinrich Hertz was the first to broadcast and receive radio waves. From then on, MWs have been the carriers of telegraphic data between stations on Earth and also between ground-based stations and satellites. In the 1950's, the high frequency Radio Frequency (RFs) became increasingly used as FM and television. Then the use of MWs in the mobile phone communications society has expanded drastically. Today about half of the world's population is owners of mobile phones, and an even larger number are exposed to the MW fields through the passive mobile phoning and MW-emitting base stations placed everywhere around us. All this results in an artificially produced general MW background in our environment in the order of 10^{11} to 10^{18} times the levels generated by the MW background radiation from space. The important question is, whether the exposure to these omnipresent MWs is only of good. The generation of today is the first to be exposed during a whole lifetime. Possibly, this may result in harmful effects. If so, these must be studied, revealed and reduced or avoided.

Our research group has studied the effects of EMF upon the mammalian brain (rats) since 1988. In later years we have included studies on cognition and gene expression where we have demonstrated significant effects of exposure to RF-EMF from mobile phones. However, our major field of interest during the period has been the effects upon the blood-brain barrier (BBB) of the rat. These studies have also revealed damaging effects from RF-EMF upon neurons. We report here our results on BBB effects and to a lesser extent on neuronal damage.

Review of the literature

The blood-brain barrier

The existence of the BBB was discovered in the late 19th century by the German bacteriologist Paul Ehrlich and his student, Edwin Goldman. Paul Ehrlich found, that when he injected dyes into the systemic blood circulation, the brain tissue did not take up any of the staining. However, Goldman described in 1909 that the brain tissue was stained after direct injection of trypan blue into the brain ventricular systems. A barrier surrounding the brain tissue at the site of the brain micro vessels seemed to be a logic explanation to these findings.

Today, it is well known that the mammalian brain is protected from potentially harmful compounds circulating in the blood by the BBB. In the normal brain, the passage of compounds over the BBB is highly restricted. Other barriers in the mammalian body include the eye (a protrusion of the brain), the blood-testis-barrier, the ovarian blood-follicle barrier and the less restrictive placental barrier.

A BBB exists not only in vertebrates, but also in insects¹, crustaceans and cephalopod molluscs (such as the cuttlefish)² and in elasmobranchs (cartilaginous fishes such as sharks)³ and helices (landsnails)⁴, maintaining ionic integrity of the neuronal bathing fluid. Several studies describe well developed blood-barrier functions in these invertebrates where the similarities with the vertebrate BBB are striking.

Anatomy of the mammalian blood-brain barrier

The BBB is formed by the vascular endothelial cells in the capillaries of the brain with glial cells wrapped around. The endothelial cells are sealed together with tight junctions, composed of the tight junction proteins occludin, claudin and zonula occludens⁵. No fenestrations are left between the endothelial cells (fig. 1).

The abluminal membrane of the capillary surface is covered to 25% by pericytes⁶. The pericytes are a type of macrophages, with macrophage markers and capacity for phagocytosis and antigen presentation and seemingly, they are in a position to significantly contribute to central nervous system (CNS) immune mechanisms. They help maintain the stability of blood vessels by regulating the endothelial cells and the vascular permeability⁷.

Surrounding the endothelial cells and the pericytes, there is a bilayer basal membrane. This basement membrane (basal lamina) supports the abluminal surface of the endothelium and may act as a barrier to the passage of macromolecules.

The outer surface of the basal membrane is surrounded by protoplasmic astrocytes. These are implicated in the maintenance, functional regulation and repair of the BBB. Their protrusions, called end feet, cover the basal membrane and form a second barrier to hydrophilic molecules, but also connect the endothelium to the neurons.

The BBB is not only a physical barrier, but is also an enzymatic barrier with the capability of metabolizing certain solutes, such as drugs and nutrients⁸. Many of these enzymes reside selectively in the cerebral endothelial cells. For instance, enzymes like monoamine oxidase A and B, catechol O-methyltransferase, or pseudocholinesterase are involved in the degradation of neurotransmitters present in the CNS⁹.

Differences between the human and the rodent BBB

The mammalian brain at large seems to have a uniform anatomy of its BBB constituents preserved through the evolution, and very little information about differences between mammalian species has been available. However, recently very inter-

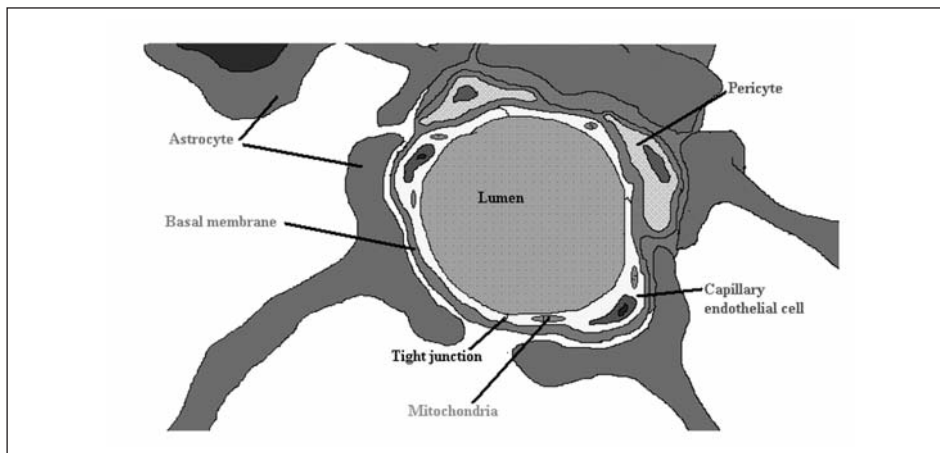


Fig. 1. The mammalian BBB

esting observations have been published. Humans have evolved protoplasmic astrocytes that are both larger (27-fold greater volume) and far more elaborate than their rodent counterparts. These astrocytes reside near blood vessels, and their processes contribute to the BBB¹⁰. When the end feet of human and rodent protoplasmic astrocytes are compared, it is shown that nearly all astrocytes in both species contact the vasculature, but in the human brain, the end feet completely encompass the vessels while the rodent astrocytes form rosettes of end feet around the vasculature. The number of mitochondria is however equally abundant in human and rodent end feet¹¹. Even if the endothelial cells are considered as the major component of the BBB, it cannot be excluded that the observed astrocytic differences may be of importance for how the EMFs affect the BBB in rodents vs humans.

Comparisons between mammalian species concerning enzymatic functions in the BBB are few in number. Similarities are described: mouse vs human¹² and rat vs human¹³, while differences are demonstrated between rodent and dog BBB leading to the conclusion that the canine BBB may be preferable to that of the rat as a model for studies of glucose transport relevant to human brain¹⁴.

Transport across the blood-brain barrier

The microvasculature of the CNS differs physiologically from that of peripheral organs. The endothelial cells are characterised by the low number of pinocytotic vesicles for nutrient transport through the cytoplasm and they have a five-fold higher number of mitochondria as compared to the muscular endothelium¹⁵.

The size and hydrophobic or hydrophilic characteristics of substances affect whether or not they can pass the BBB:

- water, most lipid-soluble molecules, oxygen and carbon dioxide can diffuse from blood to the nerve cells;
- the BBB is slightly permeable to ions such as Na^+ , K^+ , Cl^- ;
- proteins and most water-soluble chemicals pass poorly.

The flux of solutes into the brain parenchyma can be controlled by at least four mechanisms. First, the tight junctions and low number of pinocytotic vesicles guarantee that proteins cannot pass freely into the brain parenchyma. Second, solutes which are not highly lipid soluble, or which do not bind to selective transporters with high affinity, are excluded from free exchange. Thus, the passage of sugars and many aminoacids depends on other, active mechanisms. Third, the BBB has a capacity to metabolize certain solutes, such as drugs and nutrients⁸. Fourth, active transporters maintain the levels of certain solutes at specific values within the brain interstitial fluid. This is made possible by active transport against the concentration gradients. These enzyme systems are differently distributed between the luminal and the abluminal membranes of the endothelial cells, thus gaining the BBB polarity properties.

For the substances, which cannot diffuse over the BBB, certain mechanisms could be used to pass the BBB. These include:

- paracellular routes;
- transcellular routes, with pinocytosis or transcytosis, transendothelial channels, or disruption of endothelial cell membrane.

During certain pathological conditions, the selective permeability of the BBB is disturbed, resulting in a temporary increased BBB permeability. Such conditions include tumours, infarcts, infections or traumas. The BBB itself might play an active role in the

mediation of the neuroimmune response seen in different conditions, by production of inflammatory mediators or by the expression of adhesion molecules⁹. The selective permeability of the BBB is altered also in cases of epileptic seizures^{16, 17} and severe hypertension¹⁸. The result of this can be cerebral oedema, increased intracranial pressure and irreversible brain damage. Also, toxic substances from the blood circulation now reach the neurons.

In the study by Sokrab *et al.*¹⁸, hypertensive opening of the BBB was induced by clamping the upper abdominal aorta in rats for 8-10 minutes. BBB leakage was demonstrated in all 3 rats surviving 2 hours after the clamping and in 5/12 rats sacrificed 7 days after the clamping, although the intensity in the BBB leakage had been reduced in the animals with a 7-day recovery time. The BBB leakage could be visualised in cortex, basal ganglia, hippocampus, cerebellum and the brain stem. Also, importantly, it was concluded that even transient openings of the BBB can lead to permanent tissue damage¹⁸.

There is a time-dependence regarding insults leading to BBB opening. Hardebo evaluated the scale for opening and closure of the BBB after a reversible opening, achieved by a hypertensive or hyperosmolar insult¹⁹. The degree of Evans blue-albumin complex was estimated by gross inspection of the brain surface, and extravasation of inulin and noradrenaline was expressed as tissue radioactivity quotient. The absolute values for extravasation of inuline and noradrenaline were very similar, and all three test substances had an identical time profile. Thirty minutes after the hypertensive insult and 60 minutes after the hypertonic insult, the barrier was reclosed. With electron micrographs of the microvessels of the cortex, micro-pinocytotic vesicles within endothelial cells were seen. Also, vesicles were being formed and disintegrated in the luminal membranes of the endothelial cells. This increased transendothelial pinocytosis was observed as long as the barrier was open.

Hardebo and Nilsson also found that intracarotid infusions of hyperosmolar solutions induced cerebral vasodilatation and flow increase²⁰. It was proposed that BBB opening caused by the acute hypertension could be related to a pressure-forced over-distension of the vessels along the vascular tree, and that increased transendothelial pinocytosis under these experimental conditions might be due to the dilatation and/or distension of the brain vessels.

The importance of the BBB is also revealed by its presence not only in vertebrates, but also in invertebrates. For instance, a glial vertebrate-like BBB has been found in scorpions²¹. Using radio-labelled polyethylene glycol and EDTA it was shown that the cuttlefish *Sepia* has a BBB as tight as the endothelial barrier of mammals². Furthermore, it was concluded that the *Sepia* BBB is formed by perivascular glial processes in the microvessels and venous vessels, but by pericytes in the arterial vessels. Possibly, the glial BBB could be the primitive condition and a barrier associated with vascular elements such as endothelium or pericytes could be a later development²².

Importantly, the BBB seems to be present very early in the foetal development. Also, at an early stage, there seems to be a cerebrospinal fluid barrier, which excludes cerebrospinal fluid (CSF) protein from the brain extracellular space²³. By measuring the protein composition and concentrations in the CSF and plasma of *Mondelphis domestica*, a small rodent-like marsupial, from birth until adulthood, it was found that protein content increased during day 5 and 10 after birth, and later on decreased and reached very low levels. Notably, these marsupials are born at a very early stage of their development, when almost all organ systems are at an embryonic level of development. This

is different from many other animals, in which the development has reached a much more mature stage at the time of birth; for example, in rats the peak concentrations of proteins within the CSF are reached at birth or just before/after this, the protein content is kept low.

Electromagnetic fields

EMFs are produced by the mutual interaction of electric and magnetic fields; by the movement of a charge generating a magnetic field or a changing magnetic field generating an electric field. An Electromagnetic (EM) wave is characterised by its intensity (the amplitude), the frequency of the time variations of the electric and magnetic fields, the pulse width and the number of pulses per second. The different frequencies of EMFs result in a spectrum ranging from 1,000 MHz (10^9 cycles per second) to 300,000 MHz (3×10^{11} cycles per second) and with wavelengths between 1 mm and 1 m.

An EMF spreads indefinitely in the empty space. Any charged object in the vicinity of this field is affected by the electromagnetic interactions. The result of this interaction depends on the amplitude of the field, but also seemingly weak amounts of electromagnetism can mediate significant effects through resonance interactions with sensitive systems.

The rate of EM energy absorbed in tissue per unit mass is called specific absorption rate (SAR). The maximally allowed SAR-value for occupational exposure is 10 W/kg, and 2 W/kg is the maximally allowed SAR-value for public exposure (localized SAR, head and trunk) according to limit values from the International Commission of Non-Ionizing Radiation Protection²⁴. These values are set in order to avoid thermal effects of the EMF radiation, such as whole-body heat stress and excessive localized tissue heating.

In our laboratory, in order to generate uniform EMFs for standard measurements, we have used transverse electromagnetic transmission line chambers (TEM-cells) in the majority of our experiments on rats²⁵⁻³². In each TEM-cell, two animals can be placed, one in an upper compartment and one in a lower compartment (fig. 2). It is important to point out that the position of the animals in upper or lower compartments does not effect the magnitude of observed albumin leakage. Also, we have concluded, with our total series of more than 2000 exposed animals, that there is no difference in the sensitivity to EMF exposure between male and female animals as far as albumin leakage is concerned.

The TEM-cells have mainly been used for exposure in the 900 MHz range. For generation of 1800 MHz-fields, an anechoic chamber has been used³³. The EMFs are generated by means of a directional antenna placed in the top part of the anechoic chamber.

The experimental models used in our studies allow the animals, which are un-anaesthetized during the whole exposure, to move and turn around in the exposure chambers, thus minimising the effects of stress induced immobilization³⁴.

Early studies of electromagnetic field induced blood-brain barrier permeability

Already in 1968, Frey, a pioneer in the field, noted that “in recent years it has been recognized that low-power-density modulated RF energy can affect the functioning of higher living organisms”. In the 1970's, he discussed possible mechanisms by which RF

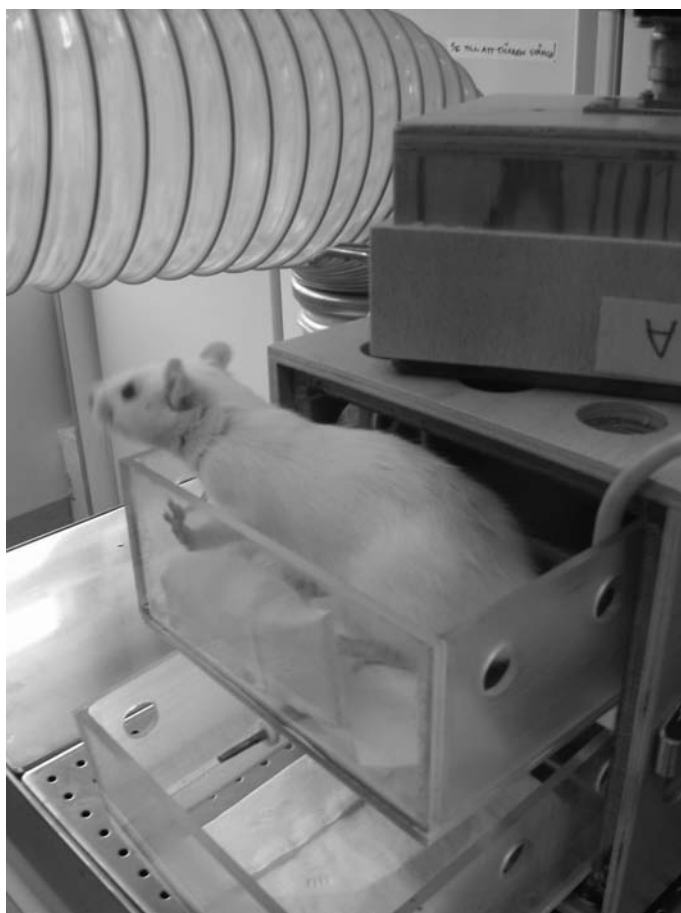


Fig. 2. Rat in the upper compartment of a TEM-cell

energy could affect biological systems, and it was concluded that: “The question is not whether there is a possible mechanism, but rather which of numerous possible mechanisms”³⁵. In order to try to find an answer to that question, the relationship between neural function and behaviour was investigated by Frey *et al.* in 1975. They demonstrated an increased leakage of fluorescein after 30 minutes of pulsed and Continuous Waves (CW) exposure at 1200 MHz³⁵. In general, the fluorescence was seen at the diencephalon level of the brain. Fluorescence was particularly conspicuous in the vicinity of the lateral ventricles and often near the third ventricle. There was a significant difference between the pulsed and continuous waves, and both of these conditions were significantly different from the control condition.

Similar findings were made by Oscar and Hawkins, with 10 minutes of RF exposure at 1300 MHz leading to an increased uptake of D-mannitol in the brains of exposed rats³⁶. The increased permeability was seen both immediately and 4 hours after the exposure, however, not after 24 hours. Notably, MWs of the same average power but with different pulse characteristics produced different uptake levels. Regarding CWs, the uptake of

mannitol increased with increasing power up to 1.0 mW/cm² (corresponding to SAR of 0.4 W/kg), but at higher power densities it started to decrease. For pulsed MWs, a similar phenomenon was seen, but at different power densities. A power window was suggested to explain the fact that increase in the power above certain levels did not result in a corresponding increase of the BBB permeability. Comparing the CWs and pulsed MWs, there were differences in the permeability changes at the same average power. Also, different pulse characteristics of pulsed MWs resulted in different mannitol uptake, although the power density was the same. However, in later studies, Oscar *et al.*³⁷ emphasised that changes of BBB permeability after MW exposure partly could be explained by an increase of local cerebral blood flow. In accordance with this, they concluded that their initial findings³⁶ might be of less magnitude than originally thought³⁷.

Merritt *et al.*³⁸ tried to replicate the findings both by Frey *et al.*³⁵ from 1975 and Oscar and Hawkins³⁶ from 1977. Regarding the findings by Frey *et al.*³⁵, Merritt *et al.*³⁸ could not replicate them in rats exposed to a similar dose of RF radiation at 1,200 MHz, both CW and pulsed. However, Frey commented upon this in an article in 1998, where he pointed out that, in fact, statistical analysis by the editor and reviewer of the data from the study by Merritt *et al.*³⁸ provided a confirmation of the findings of Frey *et al.*³⁵ from 1975³⁹. Regarding the findings by Oscar and Hawkins³⁶, the same lack of replication was reported, as Merritt *et al.*³⁸ found no significant change in the permeability of neither mannitol nor inulin after RF exposure similar to that of Oscar and Hawkins³⁶ from 1977. Similar attempts to replicate the Oscar and Hawkins³⁶ study from 1977 were made by Preston *et al.*, but no increase in the uptake of C-mannitol was found after 30 minutes of exposure to CW MWs at 2450 MHz⁴⁰.

Further lack of EMF induced BBB permeability was reported by Ward *et al.*⁴¹ and by Ward and Ali⁴² for C-sucrose and inulin (CWs exposure during 30 minutes at power densities of 0, 10, 20 and 30 mW/cm²), or by Gruenau *et al.*⁴³ for sucrose (CW and pulsed exposure at 2.8 GHz at power densities between 0 and 40 mW/cm²).

Ward *et al.*⁴¹ found no increased permeation if inulin or sucrose after 2450 MHz irradiation (0-30 mW/cm² for 30 minutes), and with exposure concentrated to the head of the rat⁴² (at 1700 MHz and the same power densities), similar lack of effects were reported. Absence of EMF induced BBB permeability was also reported by Gruenau *et al.*⁴³ (C-sucrose, 30 minutes pulsed or CW radiation at 2.8 GHz between 0-40 mW/cm²).

With horseradish peroxidase (HRP) as an indicator of the BBB permeability, Albert and Kerns⁴⁴ found increases of the tracer in the brains of Chinese hamsters after RF exposure (2 hours CWs at 2450 MHz at 10 mW/cm²). An increased number of pinocytotic vesicles were seen in the endothelial cells of the irradiated animals, but in animals recovering 1 or 2 hours after the RF exposure, almost no horseradish peroxidase permeation could be detected.

Effects of thermal irradiation

With more research into the area of EMF-induced BBB permeability, it became evident that with high-intensity EMF exposure resulting in tissue heating, the BBB permeability is temperature dependent⁴⁵. Thus, the importance of differentiating between thermal and non thermal effects on the integrity of the BBB was realized.

In a series of studies, Williams *et al.*⁴⁵⁻⁴⁸ investigated parameters affecting the BBB passage. Fluorescein was significantly elevated in the brains when rats had been subjected to thermal heating (> 41° C.), corresponding to CW exposure at SAR-levels of

approximately 13.0 W/kg for 30 or 90 minutes. However, the authors believed that these findings were rather due to technical artefacts and not a breakdown of the BBB. Regarding HRP, no HRP leakage could be attributed to MW or thermally-induced breakdown of the BBB (2450 MHz CWs at 0, 20 or 65 mW/cm² for 30, 90 or 180 min)⁴⁷. Regarding sucrose, MW exposure at 2450 MHz for 30 minutes at SAR approximately at 13 W/kg resulted in a decrease of the sucrose uptake, but this decrease was not apparent after 90 minutes⁴⁸.

It was speculated that thermal MW effects could be used to facilitate drug delivery over the BBB. Quock *et al.*⁴⁹ noted that 10 minutes of exposure to 2.45 GHz at 23.7 W/kg facilitated the transport methylatropine, a derivate of atropine. Under non-thermal conditions, the methylatropine does not normally cross the BBB, but after the single thermal MW exposure, anticholinergic effects of methylatropine could be identified (as a shift in the dose-response curves for both pilocarpine and oxotremorine).

Magnetic Resonance Imaging

With the introduction of the magnetic resonance imaging (MRI) technique, combined exposure to RF, pulsed and static magnetic fields was increasingly investigated.

Shivers *et al.* observed that the EMF exposure of the type emitted during a MRI procedure resulted in a temporarily increased permeability in the brains of rats⁵⁰. HRP was used as an exogenous tracer. After 30 minutes of MRI exposure of rats, an amplified vesicle mediated transport could be detected. The vesicles were often attached to the luminal or abluminal cell membrane. These vesicular structures appeared to extend from the luminal to the abluminal cell membrane in some cases, thereby creating transendothelial passageways. Fifteen-thirty minutes after the exposure, the exclusion of protein tracer from subendothelial basal lamina and neuropil was completed. The distribution of the vesicles of the MRI exposed animals was compared to that of sham exposed rats, in which the tracer could be found only in the vascular lumen and luminal sides of the vessels. In neither the MRI or sham exposed rats, the tight junctions of the BBB were permeated with the tracer. This lead to the question, whether the RF radiation might modify the physiochemical membrane properties, thereby leading to the increase of vesicle mediated transport. This study was replicated by Garber *et al.*⁵¹, whereas Adzamli *et al.*⁵² and Preston *et al.*⁵³ could not repeat the findings. The Shivers group later produced quantitative support of their initial findings^{54, 55}. In rats exposed to MRI, the BBB permeability to diethylenetriaminedipentaacetic acid (DTPA) increased. A suggested mechanism explaining the increased permeability was a stimulation of endocytosis, made possible through the time-varying magnetic fields.

Research from our laboratory

Stimulated by the work of the London Ontario group, two from our group visited professor Shivers and his colleagues in 1988. LGS in the hope to find an elegant way to open the BBB by the use of controlled EMFs in order to facilitate passage of cytotoxins into the brain, surrounding the tumours of patients with malignant gliomas, BP with the goal to learn more about possible risks of the MRI technology. Thus, our group started work on effects of MRI on rat brain in 1988 and found, by the use of Evans Blue, the same increased permeability over BBB for albumin²⁷.

Our work was continued by separating the constituents of the MRI field: RF, time varying magnetic field and static magnetic field. Since RF turned out to be the most efficient component of the MRI in this aspect, the following studies focused mainly on the RF effects. In order to simulate the actual real-life situation, endogenous substances, which naturally circulate in the vessels of the animals, were used. Albumin and leakage over the BBB was identified with IgG fraction of rabbit anti-rat. All brains were examined histopathologically by our neuropathologist. 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 semiquantitatively.

We started our RF experiments with the frequency modulation 16 Hz and its harmonics 4, 8, 16 and also 50 Hz, which was felt relevant as it is the standard line frequency of the European power system, with a carrier wave of 915 MHz. At an early stage also 217 Hz modulation was added as this was the frequency of the then planned GSM system. This work was published in 1994²⁹ and 1997²⁶ and comprised sham or 915 MHz exposure for in most cases 2 h but in a minority of the experiments lasting between 2 and 960 min (both continuous and pulsed modulated waves). These results based on 246 rats (1994) and more than 1,000 rats (1997) (the majority EMF exposed and about 1/3 sham-exposed) concluded that there was a significant difference between the albumin extravasation from brain capillaries into the brain tissue between the differently exposed groups and the controls.

It is important to point out that even though all animals in the 1997 series (and basically all of our experiments) are performed in inbred Fischer 344 rats, only at the most 50% of the identically exposed animals display albumin extravasation in CW animals and somewhat less in the other exposed animals. Also the sham-exposed animals have some albumin leakage though only in 17% as a mean of all controls (fig. 3). The leakage observed in unexposed animals presumably is due to our very sensitive immunohistochemical methods. The peculiar fact that at the most only every second exposed inbred animal displays leakage, is difficult to explain.

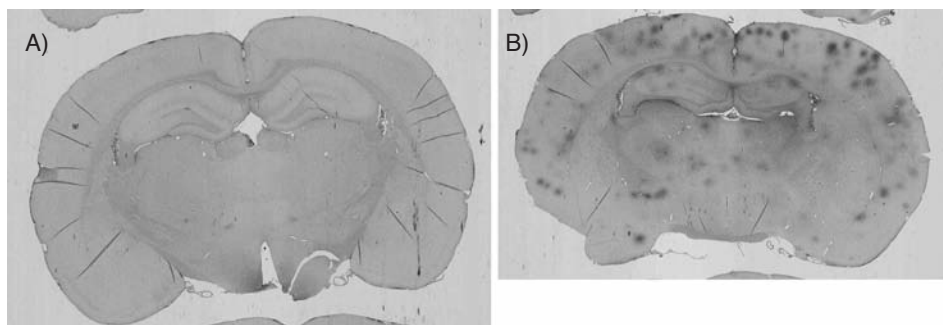


Fig. 3. A) Sham-exposed animal no albumin leakage. Note normal albumin extravasation in Hypothalamus (inbuilt control). B) RF-exposed animal, albumin leakage, Albumin score 3 (on a semi-quantitative score with 3 defined as pronounced albumin leakage and 0 as no albumin leakage)

In a statistical re-evaluation of our material published in 1997²⁶ where only exposed rats with a matched unexposed control rat are included, we found for the most interesting modulation frequency 217 Hz, i.e. that of GSM, that at SAR-values of 0.2 to 4 mW/kg 48 exposed rats had a significantly increased albumin leakage ($p < 0.001$) as compared their 48 matched controls. On the other hand, SAR-values of 25-50 mW/kg, gave no significant difference between 22 exposed rats vs their matched controls (Wilcoxon's Rank Test, 2-sided p-value).

Thus, the most remarkable observation was that exposure with whole-body average power densities below 10 mW/kg gave rise to a more pronounced albumin leakage than higher power densities, all at non-thermal levels. If the reversed situation were at hand, we feel that the risk of cellular telephones, base-stations and other RF emitting sources could be managed by reduction of their emitted energy. The SAR value of around 1 mW/kg exists at a distance of more than 1 m away from the mobile phone antenna and at a distance of about 150–200 m from a base station (figs. 4 and 5).

In all our earlier studies we showed albumin extravasation immediately after exposure as described above. In later years we have performed a series of experiments where the animals were allowed to survive for 7 days⁵⁶, 14 days, 28 days⁵⁷ or 50 days³¹ after one single 2-hour exposure to the radiation from a GSM mobile phone. All were exposed in TEM-cells to a 915 MHz carrier wave as described above. The peak power output from the GSM mobile phone fed into the TEM-cells was 1, 10, 100 and 1000 mW per cell respectively for the 7-14-28-days survival animals, resulting in average whole-body SAR of 0.12, 1.2, 12 and 120 mW/kg for four different exposure groups. The 50-days survival animals were exposed to SAR-values of 1.2, 12 and 120 mW/kg, corresponding to 10, 100 and 1000 mW fed into the TEM-cells.

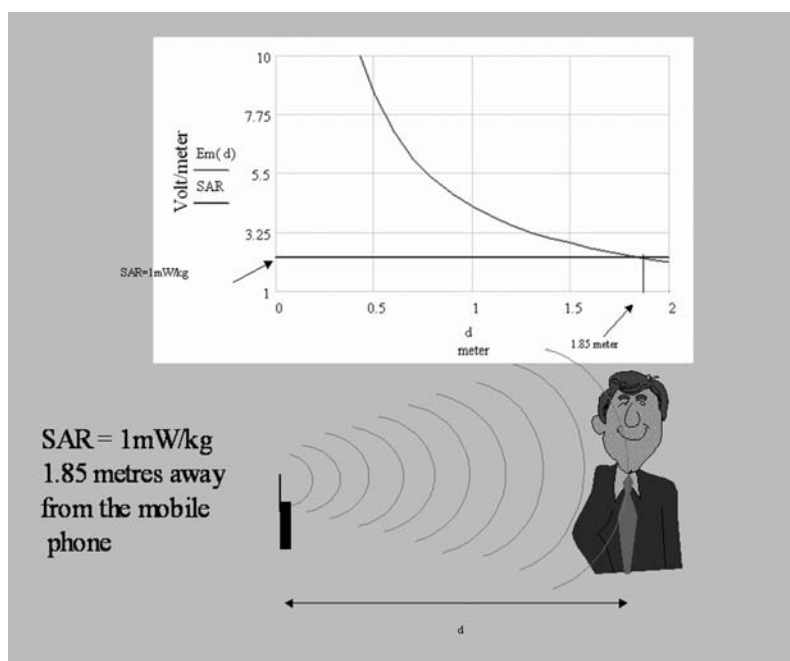


Fig. 4. The SAR-value of around 1 mW/kg exists at a distance of 1.85 meter away from the mobile phone

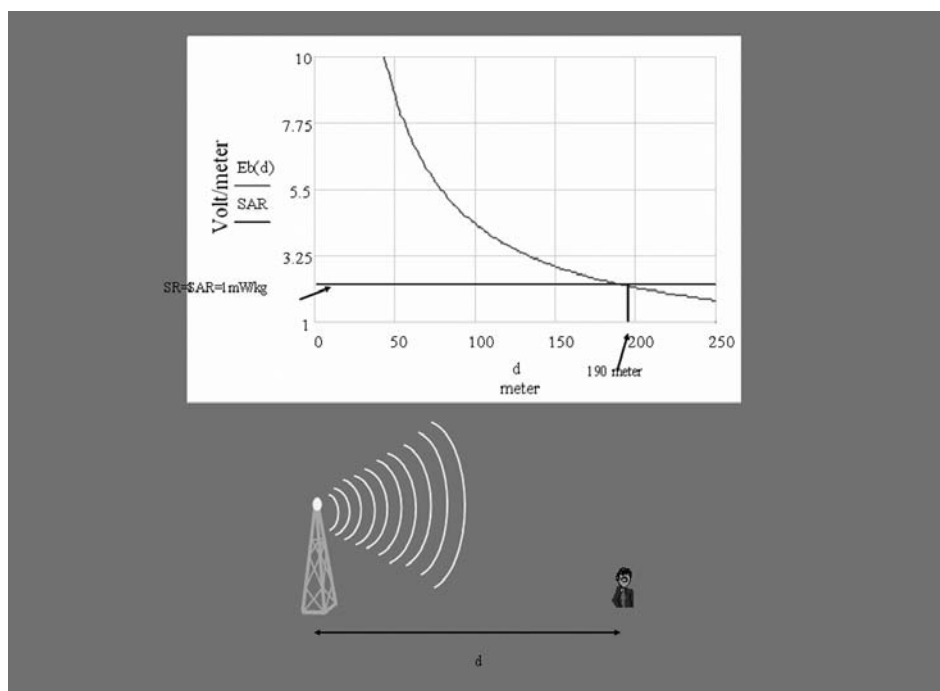


Fig. 5. The SAR-value of 1 mW/kg exists at a distance of 150-200 metres from a base station

Albumin extravasation over the BBB after GSM exposure seemed to be time-dependent, with significantly increased albumin in the brain parenchyma of the rats, which had survived for 7 and 14 days, but not for those surviving 28 days. After 50 days, albumin extravasation was significantly increased again, with albumin-positive foci around the finer blood vessels in white and gray matter of the exposed animals (fig. 6).

In connection to the albumin passage over the BBB, albumin also spread in the surrounding brain tissue. A significantly increased uptake of albumin in the cytoplasm of neurons could be seen in the GSM exposed animals surviving 7 and 14 days after exposure, but not in those surviving 28 or 50 days.

Neuronal uptake

Extravasated albumin rapidly diffused down to, and beyond, concentrations possible to demonstrate accurately immunohistologically. However, the initial albumin leakage into the brain tissue (seen within hours in ~40% of exposed animals in our previous studies) most likely started a vicious circle of further BBB opening.

It has been postulated that albumin is the most likely neurotoxin in serum⁵⁸. Hassel *et al.*⁵⁹ have demonstrated that injection of albumin into the brain parenchyma of rats gives rise to neuronal damage. When 25 µl of rat albumin is infused into rat neostriatum, 10 and 30, but not 3 mg/ml albumin causes neuronal cell death and axonal severe damage. It also causes leakage of endogenous albumin in and around the area of neuronal

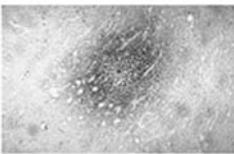
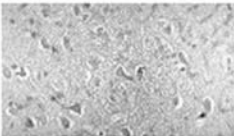
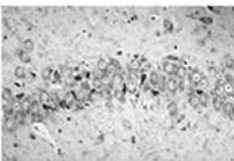
Exposed vs sham		7d	14 d	28 d	50 d
	Albumin foci	0.01	0.02	ns	0.04
	Neuronal albumin	0.03	0.005	ns	ns
	Dark neurons	ns	ns	0.01	0.001
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Fig. 6. Albumin extravasation, neuronal albumin and dark neurons in rats 7, 14, 28 and 50 days after 2 hours of GSM exposure

damage. Albumin in the dose 10 mg/ml is approximately equivalent to 25% of the serum concentration. However, it is less likely that the albumin leakage demonstrated in our experiments locally reaches such concentrations. However, we have seen that in the animals surviving 28 and 50 days after 2 hours of GSM exposure, there was a significantly increased incidence of neuronal damage as compared to the sham controls. In the 7-days and 14-days survival animals, on the other hand, no such increase of neuronal damage was seen.

The damaged neurons took the shape of so-called dark neurons. Three main characteristics of the damaged dark neurons have been proposed⁶⁰: 1) irregular cellular outlines, 2) increased chromatin density in the nucleus and cytoplasm and 3) intensely and homogenously stained nucleus. The damaged dark neurons found in the 50 days-survival animals were investigated regarding signs of apoptotic markers, but we found no positive staining for Caspase-3, a marker for apoptosis⁶¹. However, the albumin leakage out in the neuropil in connection to EMF exposure might start other deleterious processes, leading to the formation of the dark neurons.

In a recent long-term study from our laboratory, rats were exposed to GSM radiation 2 hours weekly during 55 weeks (two different exposure groups with 0.6 mW/kg and 60 mW/kg at the initiation of the exposure period). After this protracted exposure, behaviour and memory of the exposed animals were tested. Whereas the behaviour of the animals was not affected, the GSM exposed rats had significantly impaired episodic memory as compared to the sham controls⁶². After the finalization of these tests, that is 5-7 weeks after the last exposure, the animals were sacrificed by perfusion fixation. Albumin extravasation, an indicator of BBB leakage, was increased in about 1 animal in each group of low GSM exposed, high GSM exposed, sham exposed and cage control

rats. About 40% of the animals had neuronal damage. GFAP staining, as an indicator of glial reaction, revealed positive results in 31-69% of the animals for different groups and the aggregation product lipofuscin was increased in 44-71% of the animals for different groups. With the Gallyas staining (aiming at cytoskeletal structures), no changes were seen. When comparing the results between the different groups, it turned out that there was no statistically significant difference for any of these parameters due to GSM exposure⁶³. When comparing these findings to those from animals which had been exposed only once for 2 hours, it seems likely that during the 55 weeks of repeated exposure, albumin leakage at an initial stage of the experimental period could have been absorbed after some time. At a certain but unknown time point during this protracted, more than 1 year long-exposure period, some adaptation process might have been activated. However, this could not compensate for cognitive alterations.

Other studies of blood-brain barrier permeability including the effects of GSM mobile phones

Since the 1990's, mobile phones have been increasingly used. The RF radiation emitted from these devices was initially of the CW type in NMT mobile phones, but were later almost replaced by the GSM mobile phones with pulsed fields, at frequency levels of 900 MHz (GSM-900) or 1,800 MHz (GSM-1800), with pulse modulations of 217 Hz.

As mentioned above, in the Lund studies, it has been found that the pulsed fields of the GSM mobile phones increase the permeability of the BBB in exposed rats as compared to sham controls. In order to repeat these findings, studies have been performed by Fritze *et al.*⁶⁴ and Töre *et al.*^{65, 66}. Töre *et al.* (Bordeaux) found that 2 hours of GSM-exposure at SAR-values at 0.5 and 2 W/kg increased the BBB permeability, with more pronounced effects seen for exposure at 2 W/kg as compared to 0.5 W/kg. An interesting aspect of this study is the measurement of the blood pressure of the exposed animals, since it is known that the BBB is prone to hypertensive opening. Töre *et al.* found that during the EMF exposure, there was no increase of the blood pressure; it remained within the 100-130 mmHg range. In order to open the BBB through hypertensive mechanisms, it would have been necessary to increase the blood pressure up to 170 mmHg. Another finding in the studies by Töre *et al.* was sympathectomised rats were more sensitive to GSM radiation with a more pronounced increase of the BBB permeability as compared to the non-sympathectomised rats.

In the study by Fritze *et al.*⁶⁴, rats were exposed during 4 hours to GSM-900 MHz radiation with SAR of 0.3, 1.3 and 7.5 W/kg. In the paper published in 1997, Fritze *et al.* reported that there was a significant difference between exposed and sham controls only for the power level of 7.5 W/kg. However, when the Fisher exact probability test was used on the original data, there was a significant difference between the GSM and sham exposed rats also when the 10 animals in each of the power level groups of 0.3 and 1.3 W/kg were pooled ($p=0.01$ Fisher exact probability test)³⁰.

A major concentration of the involved research groups took place at Schloss Reisensburg in Germany in 2003, where the technical approaches in the studies of BBB effects especially were discussed. Two world-renowned researchers in the BBB field, Dr. David Begley of Kings College, London, and Prof. Olaf Poulsen of Copenhagen, Denmark, chaired the FGF/COST 281 Reisensburg, November 2-6 meeting. They made the final statement as a summary of the meeting: "It seems clear that RF fields can have some

effects on tissues''. The statement was made to a large extent on the basis of the concordant findings of the Bordeaux group, represented by Prof. Aubineau, and the Lund group, represented by Prof. Salford and Prof. Persson.

The permeability of the BBB was investigated after exposure to pulsed RF radiation at 2450 MHz for 15, 30, 60 or 120 minutes⁶⁷. Immediately after the exposure, capillary endothelial cells from the cerebral cortex were isolated and with a fluorescein technique, the amount of rhodamine-ferritin complex within these cells was determined. The uptake of rhodamine-ferritin was increased after exposure at an average power density of 10 mW/cm² (corresponding to a SAR-value of 2 W/kg), but not at the power density of 0.5 mW/cm². Also, the duration of exposure influenced the uptake of the substance; with increased uptake after 30, 60 and 120 minutes, but not after 15 minutes. A pinocytotic-like mechanism was proposed to explain the increased uptake after RF exposure⁵⁰. A very interesting finding in this study was that the RF induced rhodamine-ferritin uptake could be blocked by pre-treatment with colchicine. Colchicine inhibits the microtubule function. Thus, it could be seen that RF induced uptake of the systemically administered rhodamine-ferritin by capillary endothelial cells of the cerebral cortex depended both on the power and the duration of the RF exposure, as well as well-functioning microtubules.

In other studies, no EMF induced BBB permeability has been reported⁶⁸⁻⁷¹. Finnie *et al.*⁶⁸ exposed mice to GSM-900 radiation at the SAR-level of 4 W/kg. Albumin immunohistochemistry was used for evaluation. In a second study of BBB permeability, Finnie *et al.*⁶⁹ reported the same lack of GSM EMF induced BBB permeability, in this case after long-term exposure of mice for 104 weeks at SAR-levels of 0.25, 1.0, 2.0 and 4.0 W/kg. Tsurita *et al.*⁷¹ exposed rats to RFs at 1, 439 MHz at SAR-values of 0.25 W/kg. Immunostaining was used to detect albumin extravasation, which however was not increased in this small group of totally 12 EMF exposed animals. Kuribayashi *et al.*⁷⁰ investigated EMF induced BBB permeability in immature and young rats after exposure to 1439 MHz at SAR-levels of 0.2 and 6 W/kg. A dextran tracer was used to evaluate BBB permeability, which was not changed after the exposure. The same group also reported that the immature BBB was insensitive to mobile phone exposure, seen after GSM-900 irradiation of pregnant mice from day 1 to day 19 of gestation (SAR of 4 W/kg, exposure for 60 minutes daily). No increased albumin extravasation was seen in the new-born mice immediately after parturition⁷². Further lack of BBB disruption in young rats, as seen using the Evans blue tracer, was reported by Kumlin *et al.*⁷³ (GSM-900 EMF exposure of young male Wistar rats for 2 hours daily, 5 days weekly for totally 5 weeks at average whole-body SAR of 0.3 and 3 W/kg). However, of the 48 exposed rats, only 12 were examined histopathologically. The remaining animals were included in behavioural tests, where an improvement of learning and memory was seen in a water maze test when comparing the EMF exposed animals to the sham controls. Notably, in all these above mentioned studies with lack of observable EMF induced BBB effects, the SAR-values for exposure are relatively high; never including the low SAR-values in the range of < 10 mW/kg.

Recently, *in vitro* models of the BBB have been used in order to evaluate the EMF induced permeability alterations. Schirmacher *et al.*⁷⁴ used a co-culture consisting of rat astrocytes and porcine brain capillary endothelial cells as a BBB model, including zona occludens proteins, the markers for tight junctions, and with no intercellular clefts. Exposure to GSM-1800 EMFs was found to increase the permeability for sucrose. In a second model, with an improved BBB tightness, the BBB was less sensitive to the EMF exposure, with no increased sucrose passage after GSM-1800 exposure⁷⁵. In a third study

by the same group, the BBB permeability in connection to EMF exposure of the kind emitted by a UMTS mobile phone (3G) was investigated, however, with no findings of increased permeability in connection to the exposure⁷⁶.

Opinions and implications

Mechanisms

Taken together, a large number of studies have been performed within the field of EMF effects upon the mammalian brain. What can be concluded is that the picture of response is highly complex. Whereas some studies show clear effects of increased brain tumour incidence, genetic alterations, EEG changes, altered memory functions and changed neurotransmitter levels; other studies show no significant changes at all. A problem within the field is that the underlying mechanisms are not yet understood. If these had been clearly defined, the possibilities of replicating previous positive findings would have increased significantly. Therefore, the need to define these mechanisms should be obvious. Ways of doing this include both genetic investigations and studies of cell signalling pathways, but also physical and mathematical models are needed in order to clearly define the relationships between EMF radiation and biology.

As described above, in our studies of BBB permeability, we have seen significant biological response at very low SAR levels. This could possibly represent the “inverse U-curve response”, which has also been reported in connection with other kinds of MW exposure previously^{36, 77, 78}. Along these lines, we have specifically studied a Quantum-mechanical model for interaction with protein-bound ions involving Ca^{2+} -transport with resonances at certain frequencies⁷⁹. Appropriate combinations of frequency and amplitude affected the Ca^{2+} -ion transport systems at various degrees and directions. At fixed values of the static and time varying magnetic fields, resonances were found at certain frequencies (7, 21, 24 and 31 Hz). The interaction of ELF magnetic fields with calcium bound proteins fitted extremely well with the quantum mechanical interaction model described by Blanchard and Blackman⁸⁰ and it was concluded that the resonance could be attributed to 45 Ca^{2+} .

In this connection it might be of interest to mention the recent statement that “astrocytic complexity may be the basis for the superior functional competence of the human brain”¹¹. Human protoplasmic astrocytes propagate Ca^{2+} waves with a speed of 35 $\mu\text{m/s}$, which is fourfold faster than rodent astrocytes. Human astrocytes are larger and structurally more complex than those of rodents¹¹. If EMFs exert their effects, at least to some extent, upon the astrocytes, our experimental findings in spinach vesicles are clearly interesting. It may also give rise to different effects upon the human and the rodent brain.

Other approaches for explaining these effects have been suggested.

The EMF interaction with free ions, where external oscillating fields induce forced vibrations of the ions, leading to increase of intra cellular ion concentration and an osmotically driven entrance of water. This in turn would lead to disruption of plasma membranes⁸¹.

Auto-oxidative processes induced by externally applied MWs. For example, GSM exposure increased the levels of malondialdehyde (MDA), an index for lipid peroxidation, nitric oxide (NO), xanthine oxidase (XO) and adenosine deaminase (ADA) in rats.

These increased were prevented by treatment with anti-oxidant (Ginko Biloba)⁸². Reactive oxygen species also mediated a rapid activation of ERK/MAPKs (mitogenactivated protein kinase) after EMF exposure⁸³. The resulting signalling cascade could ultimately affect transcription, by the central key role of ERKs in signalling pathways. Another signalling pathway activated by MW exposure includes the hsp27/p38MAPK stress signalling pathway, which might lead to stabilisation of endothelial stress fibres⁸⁴.

Alterations of protein conformation of serum albumin, where it has been shown that EMFs can affect the conformation of proteins and thus their biological function. For example, the aggregation of bovine serum albumin is enhanced *in vitro* after exposure to MW radiation at 1.0 GHz and 0.5 W⁸⁵. Both exposure duration and the surrounding temperature influenced the aggregation process. At 60°C amyloid fibril formation of bovine insulin was promoted. Importantly, the alterations of protein conformation were not accompanied by measurable temperature changes. The possibility of protein conformation changes in connection to EMF exposure raises the questions of links to human diseases such as the amyloidopathies (including Creutzfeldt-Jakob disease, Alzheimer's and Parkinson's diseases).

Recently, we described a soliton model, which could be the link between mathematical explanations of EMF interactions and the biological response⁸⁶. A soliton is a non-linear wave. It has been shown that solitons are generated and propagated along the microtubule protofilaments in neurons of the brain⁸⁷. The propagation of solitons in the lipids of biological membranes could play a vital role in the action potential propagation along nerve membranes⁸⁸. Interestingly, the transcription bubble could correspond to a soliton travelling along the DNA chain⁸⁹. The diverse actions of the solitons could be the explanation for the vast number of biological responses, which have been seen throughout the years of studies of EMF effects.

Translation to the human situation

Very few studies on the effects of EMF upon biology include the very low whole-body average power densities that our group works with, e.g. below 10 mW/kg. Our observation that it is SAR values at this level that give rise to the most pronounced albumin leakage, whilst higher power densities, still at non-thermal levels, give less leakage. This is complicated! If the reverse situation were at hand, we feel that the risk of radiation from cellular telephones, base-stations and other RF emitting sources could be managed by reduction of their emitted energy. The SAR value of around 1 mW/kg exists at a distance of more than 1 m away from the mobile phone antenna and at a distance of about 150-200 m from a base station. This also means that when the mobile phone is held next to the ear, the SAR value of about 1 mW/kg exists in the most central portion of the brain (fig. 7), and when a hands-free is used and the phone is e.g. in the pocket, there will still be microwaves reaching the brain, though the value of around 1 mW/kg will exist in more superficial portions of the brain.

A new tool to directly study the human BBB has recently been presented⁹⁰. It provides a non-radioactive methodology for *in vivo* non-invasive, real-time imaging of BBB permeability for conventional drugs, using nitroxyl radicals as spin-labels and MRI. This technology should have a chance to substantially advance our direct knowledge of the human BBB permeability.



Fig. 7. Mobile phone antenna 1.4 cm from the human head, operating at 915 MHz. The very low SAR-levels of 10 mW/kg exist in deep-lying parts of the human brain such as the basal ganglia, and the power density of 1 mW/kg is absorbed in thalamus

Non-thermal vs thermal effects

These non-thermal effects are very important to clarify, considering that the exposure limits set up today mainly focus on preventing thermal effects. In many safety standard documents, a SAR-limit of 4 W/kg is referred to localized SAR of limbs and 2 W/kg for localized SAR of head and trunk²⁴. The reason for choosing this SAR-value is a series of studies performed by deLorge and co-workers in the 1970's and early 1980's. In these studies, the trained behavioural performance of rats, squirrel monkeys and rhesus monkeys was tested after MW exposure. It was found that body temperature increases of 1°C or more above the baseline body temperature resulted in changes of this kind of behaviour in the animals. Notably, a SAR of near 4 W/kg was needed to produce this 1°C increase of body temperature^{91, 92}.

These safety limits for thermal exposure are inadequate for all the described non-thermal effects! New standards are required for the non-thermal effects.

Positive vs negative effects

In a situation where series of studies show significant effects of radiation and other studies have failed to show effects, it is important to remember, that the demonstrated effects cannot be disregarded because other studies have shown no effects. According to the Rio declaration, the precautionary principle has to be followed. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing effective measures to prevent damage. Thus precautionary measures are needed, including, not least, extensive future research within this field.

Conclusion

Having personally demonstrated a long series of significant effects of RF-EMF in our animal models, it is our sincere belief, that it is more probable than unlikely, that non-thermal electromagnetic fields from mobile phones and base stations do have effects upon the human brain.

In this context it should, however, be remembered that recently, observations on differences between astrocytic endfeet in the human and the rodent BBB have been published¹¹. More research in this field is important for the translation of results from animal studies to the human situation.

If mobile communication, even at extremely low SAR values, causes the users' own albumin to pass the BBB, which is meant to protect the brain, also other unwanted and toxic molecules in the blood, may pass into the brain tissue and concentrate in and damage the neurons and glial cells of the brain.

The intense use of mobile phones, not least by youngsters, is a serious memento. A neuronal damage may not have immediately demonstrable consequences, even if repeated. It may, however, in the long run, result in reduced brain reserve capacity that might be unveiled by other later neuronal disease or even the wear and tear of ageing. We can not exclude that after some decades of (often), daily use, a whole generation of users, may suffer negative effects such as autoimmune and neuro-degenerative diseases maybe already in their middle age.

We conclude that the suppliers of mobile communication - and our politicians - have an extensive responsibility to support the exploration of these possible risks for the users and the society.

Acknowledgements

This work has been supported by the Hans and Märta Rausing Charitable Foundation, the Swedish Council for Working-life and Social Research and the Lund University Hospital Research Funds.

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In: Mobile Telephones...

ISBN: 978-1-60456-436-5

Editors: A.C. Harper and R.V. Buress, pp. 107-149

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Chapter 3

MOBILE TELEPHONY RADIATION EFFECTS ON LIVING ORGANISMS

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Abstract

A number of serious non thermal biological effects, ranging from changes in cellular function like proliferation rate changes or gene expression changes to cell death induction, decrease in the rate of melatonin production and changes in electroencephalogram patterns in humans, population declinations of birds and insects, and small but statistically significant increases of certain types of cancer, are attributed in our days to the radiations emitted by mobile telephony antennas of both handsets and base stations. This chapter reviews briefly the most important experimental, clinical and statistical findings and presents more extensively a series of experiments, concerning cell death induction on a model biological system. Mobile telephony radiation is found to decrease significantly and non thermally insect reproduction by up to 60%, after a few minutes daily exposure for only few days. Both sexes were found to be affected. The effect is due to DNA fragmentation in the gonads caused by both types of digital mobile telephony radiation used in Europe, GSM 900MHz, (Global System for Mobile telecommunications), and DCS 1800MHz, (Digital Cellular System). GSM was found to be even more bioactive than DCS, due to its higher intensity under equal conditions. The decrease in reproductive capacity seems to be non-linearly depended on radiation intensity, exhibiting a peak for intensities higher than $200 \mu\text{W}/\text{cm}^2$ and an intensity "window" around $10 \mu\text{W}/\text{cm}^2$ where it becomes maximum. In terms of the distance from a mobile phone antenna, the intensity of this "window" corresponds under usual conditions to a distance of 20-30 cm. The importance of different parameters of the radiation like intensity, carrier frequency and pulse repetition frequency, in relation to the recorded effects are discussed. Finally, this chapter describes a plausible biophysical and biochemical mechanism which can explain the recorded effects of mobile telephony radiations on living organisms.

Keywords: mobile telephony radiation, GSM, DCS, RF, ELF, electromagnetic fields, non-ionizing electromagnetic radiation, biological effects, health effects, *Drosophila*, reproductive capacity, cell death, intensity windows.

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Introduction

As mobile telephony becomes more and more a necessary tool in our daily life enabling modern man to communicate easily with everyone at any place and any moment, serious threats arise from the exposure of all living organisms and the environment to a type of radiation unknown until now. Man made electromagnetic fields and radiations differ substantially from natural electromagnetic radiations like natural light, mainly because artificial ones are polarised, able to induce coherent forced vibrations to any electric charge in their space. All living organisms are made of cells and all cellular functions are of electrical nature, involving movements of electrical charges like clouds of free ions or charged macromolecules. Certain movements of certain type of charges within the cells induce or interrupt corresponding cellular functions. Any wrong, synchronized net movement of charge within the cell, would induce a wrong cellular function. The cell as a highly organized unit of life, has protective mechanisms against wrong cellular function, for example by activating certain genes and consequently producing certain proteins like the “heat shock” ones, made to protect the cell from excessive heat. But if the cell fails to protect itself from an external disturbance, a malfunction may start which can be transferred to a whole tissue or the whole organism. Electromagnetic fields (EMFs) are perceived by the cells as external disturbances or external stress but the cells don’t seem to have special genes to be activated for protection against electromagnetic stress. This might be the reason why in response to electromagnetic stress, cells activate heat shock genes and produce heat shock proteins very rapidly (within minutes) and at a much higher rate than for heat itself, ([Weisbrot et al, 2003](#)). It seems to be for the same reason why electromagnetic stress from mobile telephony radiation induces cell death to the reproductive cells much more than other types of external stress examined before like food deprivation or chemicals, ([Panagopoulos et al 2007a](#)). Thus it seems that cells are much more sensitive to man-made electromagnetic fields (EMFs) than to other types of stress previously known. This is probably due to the fact that man-made EMFs constitute a new and perhaps more intense type of external stress, against which, cells have not developed defensive mechanisms. If cells activate heat shock genes to protect themselves from electromagnetic stress and this happens at a much higher rate than for heat itself, this might be dangerous, since repetitive stress leading to continuous expression of heat shock genes may result to cancer induction, ([French et al, 2001](#)).

A number of biological effects induced by man-made (EMFs) and radiations of different frequencies including digital mobile telephony and microwave radiations, have already been reported and documented by many research groups. These include changes in intracellular ionic concentrations, changes in the synthesis rate of different biomolecules, changes in cell proliferation rates, changes in the reproductive capacity of animals, changes in gene expression and even DNA damage and cell death,, ([Aitken et al 2005](#); [Bawin and Adey 1976](#); [Bawin et al. 1975; 1978](#); [Barteri et al 2005](#); [Belyaev et al 2005](#); [Blackman et al 1980; 1989](#); [Caraglia et al 2005](#); [Diem et al 2005](#); [Dutta et al 1984](#); [Kwee and Raskmark 1998](#); [Velizarov et al 1999](#); [Magras and Xenos 2001](#); [Xenos and Magras 2003](#); [Panagopoulos et al 2004; 2007a; 2007b](#); [Lai and Singh 1995; 1996; 1997; 2004](#); [Remondini et al 2006](#); [Nylund and Leszczynski 2006](#); [Diem et al 2005](#); [Salford et al 2003](#)). At the same time, some epidemiological studies are starting more and more to indicate a connection between the use

of cellular mobile phones and certain types of cancer, (Hardell et al 2007a; Hardell et al 2006; Hardell and [Hansson-Mild, 2006](#); [Kundi 2004](#)).

In several cases, melatonin, a hormone which controls the daily biological cycle and has an oncostatic action, produced by the epiphysis (pineal gland) in mammals, mainly during the night, is found to reduce the action of EMR exposure, but the synthesis of melatonin itself seems to be reduced by EMR, ([Burch et al, 2002](#); [Ozguner et al, 2006](#); [Oktem et al, 2005](#)).

Technical Characteristics of Digital Mobile Telephony Radiation

Both systems of Digital Mobile Telephony Radiation used in Europe, GSM 900 MHz and DCS 1800 MHz and also the system used in USA, GSM 1900 MHz, use different carrier frequencies, (900, 1800, and 1900 MHz respectively), but the same pulse repetition frequency of 217 Hz, (Hillebrand 2002; Clark 2001; Hyland 2000; Hamnerius and Uddmar 2000; Tisal 1998). As is obvious, the signals of Digital Mobile Telephony Radiation, combine “radio frequencies” (RF) and “extremely low frequencies” (ELF). All three systems use the “Time Division Multiple Access” (TDMA) code to increase the number of people that can simultaneously communicate with a base station. The radiation is emitted in frames of 4.615 msec duration, at a repetition rate of 217 Hz. Each frame consists of eight “time slots” and each user occupies one of them. Within each time slot the microwave radiation uses a type of phase modulation called “Gaussian Minimum Shift Keying” modulation (GMSK) to carry the information, (Tisal 1998; Hamnerius and Uddmar 2000). The transmitted frames by both handsets and base stations are grouped into multi-frames of 25 by the absence of every 26th frame. This results to an additional multi-frame repetition frequency of 8.34 Hz. Finally, handsets emit an even lower frequency at 2 Hz whenever the user is not speaking, for energy saving reasons, (“non-modulated” or “non-speaking” emission or “discontinuous transmission mode”- DTX), (Hyland 2000). Of course, when the handsets operate at DTX mode, the average emitted power is much less (about one tenth of the emitted power when they operate at “speaking” mode, ([Panagopoulos et al, 2000a; 2004](#)).

Except of the carrier frequency, another important difference between the three systems of digital mobile telephony radiation is that GSM 900MHz antennas of both mobile phones and base stations operate with double the output power than the corresponding DCS 1800MHz ones or the GSM 1900 MHz ones. GSM 900 MHz handsets operate with 2 W peak power output, while DCS 1800 MHz and GSM 1900 MHz ones operate with 1 W peak power output.

Radiation from base station antennas is almost identical to that from mobile phones of the same system (GSM or DCS), except that it is about 100 times more powerful, or to be more accurate, from several tens up to several hundred times more powerful. Thereby, effects produced by mobile phones at certain distances, can be extrapolated to represent effects from base station antennas at about 100 times longer distances. Another difference is that handset signals include one pulse per frame occupying one time slot, whereas base station signals include again one pulse per frame but this pulse may occupy 1-8 time slots depending on the number of subscribers each moment. In other words the ratio between pulse peak power and time-averaged power is usually higher for the handset signals compared to the base station signals, (Hillebrand 2002; Clark 2001; Hyland 2000; Hamnerius and Uddmar 2000; Tisal 1998).

Established Exposure Criteria for Mobile Telephony Radiations

The most stringent international exposure limits in the western world for RF radiation used by digital mobile telephony were set by the International Radiation Protection Association (IRPA) and the International Commission on Non-Ionizing Radiation Protection (ICNIRP). These criteria were established to protect biological tissue from temperature increases, (thermal effects).

The ICNIRP exposure limits are given either in terms of Radiation Intensity (Power Density) usually in mW/cm^2 , either in terms of Specific Absorption Rate (SAR) which is defined as the radiation power, absorbed by the unit mass of tissue, in W/kg . Only the radiation intensity in air outside the body can be readily and objectively measured in exposed individuals. The SAR is difficult to be determined for every single tissue as is different for different tissues and radiations. The best way for determining SAR is by computational approximate methods like the Finite Difference Time Domain (FDTP) method, the Finite Element Method (FEM), or the Method of Moments (MoM), (Meyer and Jacobus, 2003).

According to the ICNIRP exposure criteria, the maximum permitted radiation intensity (in mW/cm^2) for the general population exposure, is given according to radiation frequency and it is $f/2$ (f in GHz). Therefore, at 900MHz, the intensity limit according to these criteria is $0.45\text{mW}/\text{cm}^2$. At 1800 MHz the corresponding limit is $0.9 \text{ mW}/\text{cm}^2$, e.t.c). In terms of SAR the ICNIRP limits for the general population are $0.08 \text{ W}/\text{Kg}$ (for whole-body average absorbed power) and $2 \text{ W}/\text{Kg}$ (for the head and trunk). All the above values are to be averaged over any 6min period during the 24-h day. (IRPA 1988; ICNIRP 1998).

For the frequency 25-800 Hz, the IRPA-ICNIRP limits for the general population are for electric field intensity E , the value $250/f$ and for magnetic induction B , the value $50/f$, (E in kV/m , B in G, f in Hz). Therefore, at 217 Hz, (the pulse repetition frequency of digital mobile telephony radiations), the ICNIRP limits are $1.15\text{kV}/\text{m}$ and 0.23 G for up to 24h exposure during the day, (IRPA 1990; ICNIRP 1998).

As we shall see, during the years after the establishment of the IRPA-ICNIRP exposure criteria, it has been shown that the vast majority of health effects of digital mobile telephony radiations are non-thermal and a lot of biological effects were recorded at radiation intensities much lower than the values of these criteria. This is the reason why several countries in Europe have established much more stringent national exposure criteria, like Italy, Poland, Russia ($10 \mu\text{W}/\text{cm}^2$), or Salzburg (Austria), ($0.1 \mu\text{W}/\text{cm}^2$), ("EMF World Wide Standards").

A Review of Biological, Clinical and Epidemiological Data

There is already a very large number of published studies regarding research on possible health risks from cellular mobile telephony radiations. While a large and increasing number of studies (biological, clinical and epidemiological) have recorded a variety of non-physiological changes with increased probabilities for health hazards including several types of cancer, a lot of other studies find no connection between exposure to mobile telephony radiations and health risks. Inconsistencies observed between studies are partly expected since no identical conditions can ever be attained between different studies and different labs, but also they are explained by some authors to be due to biased samples. According to a recent article in which possible secret ties between industries and University researchers are

discussed, (Hardell et al, 2007b). Since a large number of studies are funded by companies, a matter arises on how much independent these studies can be.

In the present review we shall emphasize on the studies that indicate different possible effects on living organisms, since we consider that we must take most seriously and focus the most on the possibility that is worse for living organisms and the natural environment. Additionally because of the large number of studies relating RF-microwave radiations in general, we shall concentrate on those that regard to radiations with frequencies and intensities close to those utilized by digital mobile telephony radiations (800-2450 MHz).

A. Biological Effects

Microwaves are found to produce thermally and non-thermally a large number of biological effects, in many cellular and animal studies, (Banik et al, 2003). In the case of radiations emitted by mobile telephony antennas at intensities that people are normally exposed, the effects are non-thermal as verified by different experimenters, (Diem et al, 2005; Panagopoulos et al, 2004; 2007a; 2007b; Leszczynski et al, 2002; Schirmacher et al, 2000; Velizarov et al, 1999)

Regarding non-thermal effects of RF radiations, it is a must to refer to the pioneer works of Bawin et. al. and Blackman et. al. back in the seventies and eighties although these works were relating lower frequency RF radiations. In those pioneer experiments, RF radiation with carrier frequencies 147 and 450 MHz, modulated by sinusoidal ELF signals 0-40 Hz, was found to decrease Ca^{2+} concentration in chicken brain cells. The effect was found to become maximum at modulation frequencies 6-20 Hz and at intensities $0.6\text{-}1\text{ mW/cm}^2$, (Bawin et al 1975; 1978). Non-modulated RF signals were not found to be as bioactive as modulated ones by ELFs and additionally, these effects were found to be non-linearly depended on radiation intensity and frequency, exhibiting “windows” within which the phenomena appeared and then disappeared for values outside, (Blackman et al, 1980; 1989).

Repairable DNA damage and increased expression of heat shock protein Hsp 70 without changes in cell proliferation rates was detected in human lens epithelial cells after 2h exposure to 1.8GHz RF field, amplitude modulated at 217 Hz with 3 W/kg SAR. The DNA damage was determined by use of the comet assay, (Lixia et al, 2006).

Increased expression of genes encoding ribosomal proteins and consequently up-regulating the cellular metabolism in human cell types, was found after in vitro exposure to 900 and 1800MHz mobile phone radiation, (Remondini et al, 2006). In an other study, gene and protein expression were altered in human endothelial cell lines, after 900 MHz GSM mobile phone radiation exposure at an average SAR of 2.8 W/kg. Genes and proteins were differently affected by the exposure in each of the cell lines, suggesting that cell response to this type of radiation might be genome and proteome- dependent which in turn might explain to some extend the discrepancies in replication studies between different laboratories, (Nylund and Leszczynski, 2006).

Exposure of human endothelial cells in vitro, to GSM 900 MHz mobile phone radiation for 1h at non-thermal levels, average SAR 2 W/kg, caused transient increase in heat shock protein hsp27 phosphorylation and transient changes in protein expression levels, (Leszczynski et al, 2002).

Rapid (within minutes) induction of heat shock protein hsp70 synthesis, was found in the insect *Drosophila melanogaster*, after in vivo exposure to GSM 1900 MHz mobile phone radiation, (Weisbrot et al, 2003).

According to a theoretical report, repetitive stress caused by mobile phone radiation, leading to continuous expression of heat shock genes in exposed cells and tissues may result to cancer induction, (French et al, 2001).

Two hours of exposure by a cellular mobile phone, changed the structural and biochemical characteristics of acetylcholinesterase, an important central nervous system enzyme, resulting to a significant alteration of its activity. The enzyme was exposed within an aqueous solution at 5 cm distance from the mobile phone, (Barteri et al, 2004).

Exposure of myoglobin solution to 1.95 MHz microwave radiation for 3h at non-thermal levels was found to affect the folding of the protein and thereby changing its biochemical properties, (Mancinelli et al, 2004).

In vitro exposure for 1h of human skin fibroblasts to GSM radiation, induced alterations in cell morphology and increased the expression of mitogenic signal transduction genes, cell growth inhibitors and genes controlling apoptosis, (Pacini et al, 2002).

In an earlier study, 960 MHz GSM-like signal at SAR 0.021, 0.21 and 2.1 mW/cm² with exposure times 20, 30 and 40 min respectively, was found to decrease the proliferation rate of transformed human epithelial amnion cells. The maximum effect was reached at lower power level with a longer exposure time than at higher power level, (Kwee and Raskmark, 1998).

In another study, in vitro exposure of human peripheral blood lymphocytes to continuous 830 MHz radiation, with average SAR 1.6-8.8 W/kg, was found to produce losses and gains of chromosomes (aneuploidy), a somatic mutation leading to cancer. The effect was found to be activated via a non-thermal pathway, (Mashevich et al, 2003).

Long term exposure of rats to 900 MHz mobile phone radiation produced oxidative stress (increased oxidant products of free radicals) in retinal tissue. Melatonin and caffeic acid phenethyl ester (CAPE)- component of honeybee propolis administered daily to the animals prior to their EMR exposure, caused a significant reduction in the levels of the oxidant products, (Ozguner et al, 2006). In a previous study of the same group, melatonin was found to reverse oxidative tissue injury in rat kidneys, after 10 days exposure-30 min per day, to 900 MHz GSM radiation emitted by mobile phone, (Oktem et al, 2005).

Male mice were exposed to 1800 MHz GSM-like microwaves, 0.1 mW/cm² for two weeks on workdays, 2h per day. Then mice were anesthetized and blood samples were taken for hematology, serum chemistry and serum testosterone determinations. Additionally, testicles, epididymes, adrenals, prostates and pituitary glands were removed for histology. Red blood cell count and serum testosterone level were found to be significantly higher in the exposed groups but no significant alterations were found in the other investigated variables, (Forgacs et al, 2005).

Mice prone to the development of lymphomas, exposed for two 30 min periods per day for up to 18 months, to 900 MHz pulsed microwave radiation with a 217 Hz pulse repetition frequency at SAR ranging from 0.007 to 4.3 W/kg, developed twice the number of tumors than the unexposed ones, (Repacholi et al, 1997).

Male Wistar 35-day-old rats were exposed to 2.45 GHz radiation for 2 h/day for a period of 35 days at a power density of 0.344 mW/cm², (SAR 0.11 W/kg). After 35 days the rats were sacrificed and whole brain tissue was isolated for protein kinase C (PKC) assay. The study revealed a decrease in PKC activity. Electron microscopy study showed an increase in

the glial cell population in the exposed group. The results indicated that chronic exposures may affect brain growth and development, (Paulraj and Behari, 2006a). In another study of the same group, single strand DNA breaks were measured as tail length of comet. Fifty cells from each slide and two slides per animal were observed. The study showed that chronic exposure to microwave radiation at non-thermal levels (SAR 1 and 2 W/kg) causes statistically significant increase in DNA single strand breaks in rat brain cells, (Paulraj and Behari, 2006b).

In another study mice placed within an RF antenna park were repeatedly mated for five times while they were continuously exposed at very low levels of RF radiation (0.168-1.053 $\mu\text{W}/\text{cm}^2$). A progressive decrease in the number of newborns per maternal mouse was observed after each mating, which ended to irreversible infertility, (Magras and Xenos, 1997). In a more recent study of the same group, it was found that exposure of pregnant rats to GSM-like 940 MHz radiation at 5 $\mu\text{W}/\text{cm}^2$, resulted in aberrant expression of bone morphogenetic proteins (BMP)-(major endocrine and autocrine morphogens known to be involved in renal development), in the kidneys of newborn rats, (Pyrpasopoulou et al, 2004).

Increase in the number of micronuclei in rat bone marrow erythrocytes, a sign of genotoxicity, was observed after 30 days exposure for 2h daily, to 910 MHz microwave radiation, (Demsia et al, 2004).

In several other mammal studies, no effects were found, in regards to genotoxicity of second generation mobile telephony (GSM, DCS) and third generation, "universal mobile telecommunication system" (UTMS) radiations, (Sommer et al 2007; Oberto et al 2007; Juutilainen et al 2007; Tillmann et al 2007; Gatta et al 2003).

The mortality of chicken embryos was found to increase to 75% from 16% in the control group, after exposure to radiation from a GSM mobile phone, (Grigor'ev, 2003). This result is in agreement with the increased mortality of fertilised chicken eggs that was recorded after irradiation by low power 9.152 GHz pulsed and continuous-wave microwaves, (Xenos and Magras, 2003).

Several studies have reported that microwave exposures increase the permeability of the blood-brain barrier (BBB), an hydrophobic barrier made by endothelial cells to protect the mammalian brain from harmful compounds in the blood. A Swedish group has reported that 915 MHz microwaves at non-thermal intensities causes leakage of albumin into the brain through the BBB in rats, accumulating in the neurons and glial cells which surround the capillaries in the brain, (Salford et al, 1994). The same group reported that GSM mobile phone radiation from a test mobile phone with a programmable constant power output, opens the BBB for albumin, resulting to damage of brain cells in rats. The power density and SAR were within the ICNIRP limits, (Salford et al 2003). These were the first experiments that indicated cell damage caused by mobile phone radiation although this radiation was not a real mobile phone signal. However in an earlier study of the same group, continuous-wave and pulsed 915 MHz radiation at relatively high intensities, 1 W and 2 W respectively, was not found to damage brain or promote brain tumour development in rats, (Salford et al. 1993).

Exposure of an in vitro BBB model, consisted by rat brain cells growing in a culture with pig blood cells, exposed to 1800 MHz microwave radiation pulsed at 217 Hz repetition rate (DCS-like), at SAR 0.3-0.46 W/kg, increased the permeability to sucrose of the BBB twice compared to the control culture. No significant temperature rise was detected during the exposures, (Schirmacher et al, 2000). In a latter study of the same group, in vitro exposure of

three other BBB models with distinctly higher barrier tightness than the previously used one, did not cause any effect on the permeability of the BBB of the models, (Franke et al, 2005).

In regards to DNA damage or cell death induction due to microwave exposure, in a series of early experiments, rats were exposed to pulsed and continuous-wave 2450 MHz radiation for two hours at an average power density of 2 mW/cm² and their brain cells were subsequently examined for DNA breaks by “comet” assay. The authors found a dose-dependent (0.6 and 1.2 W/kg whole body SAR) increase in DNA single-strand and double-strand breaks, four hours after the exposure to either the pulsed or the continuous-wave radiation, (Lai and Singh 1995; 1996). The same authors found that melatonin and PBN (N-tert-butyl-alpha-phenylnitrone) both known free radical scavengers, block the above effect of DNA damage by the microwave radiation, (Lai and Singh 1997). Although these experiments were the first to report DNA damage by microwaves, the radiation intensity (2mW/cm²) was relatively high, exceeding the international exposure limits (ICNIRP 1998) and additionally the radiation frequency was the same as in microwave ovens. This is why the authors of this review cannot be sure on whether the reported effects were thermal or non-thermal.

In vitro exposure of mouse fibroblasts and human glioblastoma cells to 2450 MHz, (Malyapa et al, 1997a), 835.62 MHz and 847.74 MHz (Malyapa et al, 1997b), radiations at SAR 0.6 W/kg, was not reported to damage DNA as measured by comet assay.

A number of recent studies have reported DNA damage, or cell damage, or cell death, induced by mobile telephony or similar RF radiations at non-thermal intensity levels, (Aitken et al, 2005; Diem et al 2005; Panagopoulos et al 2007; Salford et al, 2003; Markova et al, 2005; Caraglia et al, 2005; Nikolova et al, 2005), while some other studies did not find any such connection, (Hook et al, 2004; Capri et al, 2004a; 2004b; Meltz 2003; Cranfield et al, 2003). Aitken et al 2005, reported damage to mitochondrial genome and the nuclear beta-globin locus in the spermatozoa of mice exposed to 900 MHz, 0.09 W/kg SAR, for 7 days, 12h per day. Diem et al 2005, reported single and double-strand DNA breakage in cultured human and rat cells exposed to 1800 MHz mobile phone-like radiation. Panagopoulos et al 2007a, found DNA fragmentation at a very high degree, caused in the reproductive cells of female *Drosophila* insects only by few min daily exposure to a real mobile phone signal for only few days. These were the first experiments that showed extensive DNA damage and cell death by real digital mobile phone GSM and DCS signals. Previous experiments of the same group had shown a large decrease in the reproductive capacity of the same insect, caused by real mobile phone similar exposures, (Panagopoulos et al, 2004).

B. Clinical Studies on Humans. Effects on EEG, EDA, Melatonin, etc

Mobile telephony radiation is found in several studies to affect electroencephalograms (EEG), electrodermal activity (EDA) and the synthesis rate of hormones like melatonin, in humans.

In a series of early experiments performed by a Finish group, GSM mobile phone exposure was found to alter the EEG oscillatory activity of healthy adult subjects, in the 6-8 and 8-10 Hz frequency bands during cognitive (visual memory) tasks, (Krause et al, 2000). In more recent experiments of the same group, exposure of 10-14 year old children to mobile phone GSM field while performing an auditory memory task, induced changes in their brain oscillatory EEG responses in the frequencies 4-8 Hz and 15 Hz, (Krause et al, 2006).

Exposure for 30 min to pulse modulated 900 MHz mobile phones-like EMF, increased waking regional cerebral blood flow (rCBF) and enhanced EEG power in the alpha frequency range (8-12 Hz) prior to sleep onset and during sleep. Exposure to the same field without pulse modulation did not enhance power in waking or sleep EEG, (Huber et al, 2002). In another set of experiments of the same group, 30 min exposure to the same 900 MHz GSM-like field during waking period preceding sleep, increased the spectral power of the EEG in non-rapid eye movement sleep. The maximum increase occurred in the 9.75-11.25 Hz and 12.5-13.25 Hz frequency ranges during the initial part of the sleep. Since exposure during waking, modified the EEG during subsequent sleep, the changes in the brain function induced by mobile telephony radiation are considered to outlast the exposure period, (Huber et al, 2000).

Mobile phone exposure prior to sleep was found to decrease rapid eye movement sleep latency and to increase EEG spectral power in the 11.5-12.5 Hz frequency, during the initial part of sleep following exposure, (Loughran et al, 2005).

Some other studies have failed to find any effects of mobile phone-microwave exposures on EEG during cognitive testing, or to replicate earlier findings, (Röschke and Mann, 1997; Wagner et al., 1998).

Mobile phone radiation was found to affect the evoked neuronal activity of the central nervous system (CNN) as represented by EDA, an index of the sympathetic nervous system. Mobile phone exposure was found to lengthen the latency of EDA (Skin Resistance Response), irrespectively of the head side next to mobile phone, (Esen and Esen, 2006). Therefore, mobile phone exposure may increase the response time of users with different negative consequences, like for example the increase in the risk of phone-related driving hazards, e.t.c.

A statistically significant increase of chromosomal damage was found in blood lymphocytes of people who used GSM 900 MHz mobile phones, compared to a control group of non-users, matched according to age, sex, health status, drinking and smoking habits, working habits, and professional careers. The increase was even greater for users who were smoker-alcoholic, (Gadhia et al, 2003)

In another type of clinical study, exposures of humans to GSM 900 MHz and DCS 1800 MHz mobile phones fields for 35 min, were not found to change significantly arterial blood pressure or heart rate during or after the exposure, (Tahvanainen et al, 2004).

Prolonged use of mobile phone, (more than 25 min per day), was found to induce a reduction in melatonin production among male users. The effect was enhanced by additional exposure to 60 Hz ELF magnetic field, (Burch et al, 2002).

Two studies about possible immediate- short term effects of GSM and UTMS (third generation of mobile networks)-like exposure on well being and cognitive performance in humans based on questionnaires, found contradictive results. The first (Zwamborn et al, 2003) reported no effects of GSM-like exposure, while the UTMS-like exposure was found to reduce well-being and cognitive performance. The second, (Regel et al, 2006) reported no effects at all from either type of radiation. The opinion of the authors of this review is that studies based on questionnaires cannot be as much objective as studies based on measurable indexes like EEG or EDA. Besides, it would be unlikely that subjects would report themselves immediate effects on their well-being.

C. Epidemiological Studies

According to the Swedish Prof. L. Hardell and his research group, the concluding results of up to date epidemiological studies among users for more than ten years use of mobile phones indicate consistently an increased risk for acoustic neuroma and glioma, especially for ipsilateral exposure, (Hardell et al, 2007a). Earlier work of the same research group had found a connection between digital (2nd generation) and analogue (1st generation) mobile phones use and malignant brain tumors, highest for more than ten years latency period, (Hardell et al, 2006).

Another review study of the Austrian Prof. M.Kundi conducted few years ago, states as the resume from several epidemiological and experimental studies, that long term exposure to mobile phone emissions (analogue and digital) constitutes a small to moderate increased risk for developing certain types of cancer, (Kundi, 2004).

Several other studies had not found any association between mobile phone use and cancer, (Inskip et al, 2001; Johansen et al, 2001; Muscat et al, 2002).

A major difficulty in epidemiological studies among mobile phone users is the variation of parameters governing the exposure from hand held mobile phones, i.e. the distance from the nearest base station which can considerably change the intensity of the radiation emitted by the phone, the actual duration of daily use, e.t.c. Nevertheless, the studies done on habitants living close to base stations are more consistent since the station emits a more constant radiation level on a daily basis and therefore a person residing nearby, receives a measurable radiation at least for several hours per day.

A recent Egyptian study (Abdel-Rassoul et al, 2007) found that inhabitants living nearby mobile telephony base stations may develop a number of neuropsychiatric problems like headaches, memory changes, dizziness, tremors, depression, sleep disturbances, reported also in previous studies as “microwave syndrome” (Navarro et al 2003), plus changes in the performance of neurobehavioral functions. Similar results were found by other studies in different countries like in France, (Santini et al 2003), Poland (Bortkiewicz et al 2004), Spain (Navarro et al 2003), Austria (Hutter et al 2006).

Other epidemiological studies have reported diminishes in the populations of birds around mobile telephony base stations at distances 100-600m from the masts in Belgium, (Everaert and Bauwens 2007) and within 200m from the masts in Spain (Balmori 2005). These studies are in agreement with earlier biological studies which had reported increased mortality of avian embryos, exposed to low levels (5-120 $\mu\text{W}/\text{cm}^2$) of RF antennae radiation, (Xenos and Magras, 2003).

The Design of Bioelectromagnetic Experiments and a Reason for Inconsistencies

As described in the previous paragraphs, there are frequently contradictory results in the bioelectromagnetic experiments performed by different labs. One factor that we have found to be very important and able to completely change the results of a biological experiment is the influence of the stray electromagnetic fields that exist inside any lab.

Within a usual room inside a house or laboratory there are 50-60 Hz fields due to the electric wirings and electrical appliances. Close to the walls, near to sockets or close to electrical appliances one can measure electric fields up to 50 V/m and magnetic fields up to 10 mG. Such fields are found to affect biomolecules, cells and whole organisms in different ways and therefore to affect the outcome of any biological experiment, (Goodman E. et al. 1995; Panagopoulos et al. 2002; Weaver and Astumian 1990). Prior to the design of any biological experiment, a careful scanning of stray fields inside the lab is necessary. The experiments should be performed at the place with the minimum stray fields and special care should be taken in having the control under identical conditions with the exposed groups except only for the factor studied. Temperature, light and humidity are additional important factors that should be identical between exposed and control groups.

Before the relatively recent evolution of knowledge in the field of Bioelectromagnetism, ambient electromagnetic fields within the labs were not taken into account in biological experiments. But living organisms are very sensitive to external electromagnetic fields, natural or artificial ones. Rooms or devices used as incubators, are constructed to keep a constant temperature, humidity, e.t.c. in their internal space, but usually are sources of EMFs from their own electrical circuits. A specialized physicist should always be member of any experimental team for taking good care of such factors.

Effects of Mobile Telephony Radiation on a Model Organism

Introduction

In order to study the ability of the electromagnetic signals emitted by cellular mobile telephony antennas to affect the biological function of living organisms, we used a biological model, the reproductive capacity of the insect *Drosophila melanogaster*, a well studied experimental animal with many advantages, including its short life cycle and the good timing of its metamorphic stages and developmental processes, (King 1970). Especially the good timing of this insect's early developmental stages (oogenesis, spermatogenesis, embryogenesis, larval and pupal stages), under certain environmental conditions (i.e. temperature, humidity, food e.t.c.), is a very important feature, on which our experimental protocols were based.

In order to study the effects of mobile telephony radiation on the reproductive capacity, we exposed the insects to real mobile phone signals, emitted by commercially available handsets.

The basic cellular processes are identical in insect and mammalian cells. In addition, insects (particularly *Drosophila*) are much more resistant, at least to ionizing electromagnetic radiation, than mammals, (Koval and Kazmar 1988, Koval et al 1979, 1977, Abrahamson et al 1973). Therefore, a proper experimental protocol relating *Drosophila* can be very useful in assessing the bioactivity of electromagnetic radiation in general, (including non-ionizing radiation and electromagnetic fields).

Our experiments, regarding few minutes daily exposure of this model organism for only few days, to cellular mobile phone signals, have shown a large decrease in the reproductive capacity, affecting both sexes (Panagopoulos et al 2004). Both systems of digital mobile telephony radiation used in Europe, GSM 900 MHz and DCS 1800 MHz were found to

decrease the insect's reproductive capacity, but GSM 900 MHz was found to be even more bioactive than DCS 1800 MHz, mainly due to the higher intensity of GSM 900 MHz antennas compared to DCS 1800 MHz ones, (Panagopoulos et al 2007b; 2007a). The decrease in the reproductive capacity was found to be due to induced cell death (DNA fragmentation) in the gonads, caused by both types of mobile telephony signals, (Panagopoulos et al 2007a). Unpublished experiments of ours presented here for the first time, show that the bioactivity is strongly and non-linearly dependent on the intensity of the radiation, becoming maximum for intensities higher than $200 \mu\text{W}/\text{cm}^2$ and within an "intensity window" around $10 \mu\text{W}/\text{cm}^2$.

Materials and Methods

Experimental Animal

We used *Drosophila melanogaster* flies, wild-type strain, Oregon R, held in glass bottles with standard food, kept in incubator at 25°C , with 12-h periods of light and darkness and 70% relative humidity, cultured according to standard methods, (Panagopoulos et al 2004).

The food consisted of 450ml water, 4g agar, 13g yeast, 32g rice flour, 16g sugar, 25g tomato pulp. The mixture was boiled for over 10min to ensure sterility, which was preserved by the addition of 2ml propionic acid and 2ml ethanol. This food quantity was enough for 25-30 glass vials which were sterilized before the food was added.

In each experiment, we collected newly emerged adult flies from the stock early in the afternoon, anesthetized them lightly with diethyl ether and separated males from females. We divided the collected flies in groups of ten in standard laboratory cylindrical glass vials, with 2.5cm diameter and 10cm height, with standard food, which formed a smooth plane surface, 1cm thick at the bottom of the vials. The vials were closed with cotton plugs.

Exposure System

Before each set of experiments we measured the mean power density of the radiation emitted by the mobile phone handset in the RF range at 900MHz and/or 1800MHz, with the field-meter, "RF Radiation Survey Meter, NARDA 8718", with its probe inside a glass vial similar to the ones we used for the insects in our experiments. In addition, we measured in the same way the mean electric and magnetic field intensities at the Extremely Low Frequency (ELF) range, with the field-meter, "Holaday HI-3604, ELF Survey Meter".

The experimenter's position in relation to the mobile phone during the measurements was the same as during the exposures. The mobile phone was held close to the experimenter's head with its antenna facing downward. The exposures and the field measurements, took place in a quiet but not sound-isolated room to simulate the actual conditions to which a user is subjected during a normal conversation on the mobile phone. The room conditions and the positions of all items around the experimental bench were always the same. Exposures and measurements of mobile phone emissions were always conducted at the same place where the mobile phone had full perception of both GSM and DCS signals. The handset was fully charged before each set of exposures or measurements.

In the most new digital cell phone handsets, the antenna is in the back and upper side of the device. This can be easily verified by measuring the emitted radiation holding the probe of the field meter in contact with different parts of the handset's surface.

The measured exposure values were in general within the established exposure limits, (ICNIRP 1998).

We used commercially available digital mobile phone handsets in all the sets of our experiments, in order to analyze effects of real mobile telephony exposure conditions. As far as we know, we were the first to use a commercially available mobile phone handset itself in biological experiments, (Panagopoulos et al 2000a). The obvious reason was that these devices are the most powerful RF transmitters in our immediate daily environment. Thus, instead of using simulations of digital mobile telephony signals with constant parameters (frequency, intensity etc), or even “test mobile phones” programmed to emit mobile telephony signals with controllable power or frequency, we used real GSM, DCS signals which are never constant, since there are continuous changes in their intensity and frequency. Electromagnetic fields with changing parameters are found to be more bioactive than fields with constant parameters, (Goodman E.M. et al 1995; Diem et al 2005), probably because it is more difficult for living organisms to get adapted to them. Experiments with constant GSM or DCS signals can be performed, but they do not simulate actual conditions. Later other experimenters also started to use mobile phone handsets as exposure devices apparently for the same reasons, (Weisbrot et al 2003; Barteri et al 2005).

We exposed the flies within the glass vials by placing the antenna of the mobile phone outside of the vials, in contact with or at different distances from the glass wall and parallel to the vial's axis. The total duration of exposure was 6min per day in one dose and we started the exposures on the first day of each experiment (day of eclosion). The exposures took place for a total of 2 to 6 days in each experiment depending on the kind of the experiment, as described below. The daily exposure duration of 6min, was chosen in order to have exposure conditions that can be compared with the established exposure criteria, (ICNIRP 1998). Besides, early experiments had shown that only few minutes of daily exposure were enough to produce a significant effect on the insect's reproductive capacity (Panagopoulos et al, 2000a).

The experimenter could speak on the mobile phone during connection (this we called, “modulated” or “speaking” emission), or could just stay silent, (“non-modulated” or “non-speaking” emission, or DTX mode). The intensity of the emitted radiation increases about ten times when the user speaks during connection, than when there is no speaking, (Panagopoulos et al, 2000a).

Exposure Procedures

We carried out six sets of experiments: In the first set, we exposed the insects to the mobile phone's GSM 900 MHz field while the mobile phone was operating in non-speaking mode, (non-modulated emission or DTX). In the second set of experiments, the mobile phone was operating in speaking mode, (modulated emission) during the exposures. In the third set of experiments we investigated the effect of the mobile phone signal on the reproductive capacity of each sex separately. In the fourth set of experiments we compared the bioactivity between GSM 900 MHz and DCS 1800 MHz types of mobile telephony signals. In the fifth set of experiments we exposed the insects to different distances (intensities), from the mobile phone antenna from 0 to 100 cm, for both types of radiation. Finally, in the sixth set of experiments we tested the ability of GSM and DCS fields to induce DNA fragmentation (cell death) in the ovarian cells of the female insects during oogenesis.

In every single experiment we separated the newly emerged collected adult flies to exposed (E) and sham-exposed (SE)/control (C) groups. Each one of the groups consisted

always of ten female and ten male, newly emerged flies. The sham exposed groups had identical treatment as the exposed ones, except that the mobile phone during the 6-min “exposures”, was turned off.

Every time before each exposure, the cotton plugs were pushed down in the glass vials in order to confine the flies to a small area of about 1cm height between the cotton and the food so as to provide roughly even exposure to all flies. After the exposure, the cotton plugs were pulled back to the top of the vials, and the vials were put back in the culture room.

In every group of insects in all the sets of experiments, we kept the ten males and the ten females for the first 48h of the experiment in separate glass tubes. At eclosion, adult female flies have already in their ovaries eggs at the first preovulatory stages and oogenesis has already started. The eggs develop through 14 distinct stages, until they are ready to be fertilized and laid, and the whole process of oogenesis lasts about 48h. By the end of the second day of their adult life, the female flies have in their ovipositors the first fully developed egg chambers of stage 14th, ready to be fertilized and laid, (King 1970; Panagopoulos et al 2004). At the same time, the first mature spermatozoa, (about 6h after eclosion) and the necessary paragonial substances (about 12h after eclosion) in male flies have already been developed (King 1970; Stromnaes and Kvelland 1962; Connolly and Tully 1998). Keeping males separately from females for the first 48h of the experiment ensures that the flies are in complete sexual maturity and ready for immediate mating and laying of fertilized eggs.

After the first 48h of each experiment, the flies were anesthetized very lightly again and males and females of each group were put together (ten pairs) in another glass tube with fresh food, allowed to mate and lay eggs for 72h. During these three days, the daily egg production of *Drosophila* is at its maximum (from the 3rd to 5th day of its adult life), then stays at a plateau or declines slightly for the next 5 days and diminishes considerably after the 10th day of adult life (Bos and Boerema 1981; Shorrocks 1972; Ramirez et al 1983).

On the sixth day of each experiment in all six sets of experiments, the flies were removed from the glass vials and the vials were maintained in the culture room for six additional days, without further exposure.

After the last six days, most F₁ embryos (deriving from the laid eggs) are in the stage of pupation, where they can be clearly seen with bare eyes and easily counted on the walls of the glass tubes, as at the last stages before pupation, the larvae leave the food, crawling up the walls of the glass vials. There may be a few embryos still in the last stages as larvae, which are big enough and ready for pupation (on the surface or already away from the food), so that they can be easily counted. [If the remaining larvae are still many and the counting is imprecise, the experimenter can wait an additional day and recount the pupae]. There may be also already a few newly emerged F₁ adult flies, which can also be counted easily.

During the last six days, we inspected the surface of the food within the glass vials under the stereo-microscope for any non-developed laid eggs or dead larvae, something that we did not see in our experiments (empty egg-shells can be seen after hatching). The number of observed exceptions (non-developed eggs or dead larvae), both in exposed and control groups (less than 5%) was within the Standard Deviation of progeny number. [The insignificant percentage of F₁ egg and larvae mortality is due to the fact that the paternal-maternal flies were newly emerged during the first 2-5 days of their adult lives]. Therefore the number of pupae in our experiments corresponded to the number of laid eggs (oviposition). Furthermore, the counting of pupae can be done without any error at all, whereas the counting of laid eggs under a stereo-microscope is subject to considerable error.

The oviposition of *Drosophila* is influenced by many factors, like temperature, humidity, prior anesthesia, crowding, food, (King 1970). Special care must be taken to keep all these factors constant. Experience in handling the flies is necessary to prevent accidental deaths.

This number of F₁ pupae under the above described conditions, during the insect's three days of highest oviposition, is that we have defined as the Insect's Reproductive Capacity and this is the biological index we have used to examine the bioactivity of electromagnetic radiation-field.

The temperature during the exposures was monitored within the vials with a mercury thermometer with an accuracy of 0.05°C.

In the sixth set of experiments, after the additional last exposure in the morning of the sixth day from the beginning of each experiment, the flies were removed from the glass vials, and the ovaries of females were dissected into individual ovarioles and fixed for TUNEL assay. The vials were then maintained in the culture room for six additional days, without further exposure, in order to count the F₁ pupae as in all the sets of experiments.

TUNEL Assay

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

Each *Drosophila* ovary consists of 16 to 20 ovarioles. Each ovariole is an individual egg assembly line, with new egg chambers in the anterior moving toward the posterior as they develop, through the 14 successive stages as described, until the mature egg reaches the oviduct.

To determine the ability of GSM and DCS radiation to act as possible stress factors able to induce cell death during early and mid oogenesis, we used TUNEL assay, as follows: Ovaries were dissected in Ringer's solution and separated into individual ovarioles from which we took away egg chambers of stages 11-14. In egg chambers of stages 11-14 programmed cell death takes place normally in the nurse cells and follicle cells. Thereby we kept and treated ovarioles and individual egg chambers from germarium up to stage 10. Samples were fixed in PBS solution containing 4% formaldehyde plus 0.1% Triton X-100 (Sigma Chemical Co., Germany) for 30min and then rinsed three times and washed twice in PBS for 5 min each. Then samples were incubated with PBS containing 20 µg/ml proteinase K for 10 minutes and washed three times in PBS for 5 min each. In situ detection of fragmented genomic DNA was performed with Boehringer Mannheim kit containing fluorescein dUTP for 3h at 37°C in the dark. Samples were then washed six times in PBS for 1h and 30 min in the dark and finally mounted in antifading mounting medium (90% glycerol containing 1,4-diazabicyclo (2.2.2) octane (Sigma Chemical Co., Germany) to prevent from fading and viewed under a Nikon Eclipse TE 2000-S fluorescence microscope.

Results and Discussion

In the first two sets of experiments, we separated the insects into two groups: a) the Exposed group (E) and b) the Sham Exposed group (SE). The 6-min daily exposures took place for the first five days of each experiment.

In the first three sets of experiments, the exposures were performed by GSM 900 MHz mobile phone radiation-field. Before the exposures, we measured radiation and field intensities, as described above. In the RF range, the measured mean power density for 6min of modulated emission (M), with the antenna of the mobile phone outside of the glass vial in contact with the glass wall and parallel to the vial's axis was 0.436 ± 0.060 mW/cm². The non-modulated (NM) corresponding measured mean value, was 0.041 ± 0.006 mW/cm². In the ELF range, the measured values for modulated field, excluding the ambient electric and magnetic fields of 50Hz, were 6.05 ± 1.62 V/m electric field intensity and 0.10 ± 0.06 mG magnetic field intensity. The corresponding non-modulated values were 3.18 ± 1.10 V/m and 0.030 ± 0.003 mG. All given values are average from eight separate measurements of each kind $\pm$ Standard Deviation (SD). These values are typical for all commonly used GSM 900 MHz mobile phone handsets.

1. Effect of Non-Modulated GSM radiation-field on the Reproductive Capacity

We carried out four experiments (1.1-1.4) with non-modulated field, (non-speaking emission). The exposure parameters in this case simulate the situation when a user listens through the mobile phone during connection.

Results are listed in Table 1.

Table 1 shows the mean number of F₁ pupae (corresponding to the number of laid eggs) per maternal fly in the groups E(NM) exposed to Non-Modulated (NM), GSM 900 MHz mobile phone field and in the corresponding sham exposed (control) groups SE(NM) during the first three days of the insect's maximum oviposition.

The Non-Modulated GSM 900 MHz signals, decreased the insect's reproductive capacity by up to 20% in relation to the unexposed groups with six min daily exposure for five days. No temperature increases were detected within the vials during the exposures.

Table 1. Effect of Non-Modulated GSM field on the Reproductive Capacity of *Drosophila melanogaster*

Experiment No	Groups	Mean Number of F ₁ Pupae per Maternal Fly	Deviation from Control
1.1	E(NM)	9.7	-16.38%
	SE(NM)	11.6	
1.2	E(NM)	10	-15.96%
	SE(NM)	11.9	
1.3	E(NM)	9.8	-20.16%
	SE(NM)	12.4	
1.4	E(NM)	10.4	-19.38%
	SE(NM)	12.9	
Average $\pm$ SD	E(NM)	9.975 ± 0.31	-18.24%
	SE(NM)	12.2 ± 0.57	

Statistical analysis, (single factor ANOVA test) shows that the probability that mean oviposition differs between the exposed and the sham exposed groups, owing to random

variations, is $P < 5 \times 10^{-4}$. Therefore, the decrease in the reproductive capacity is due to the effect of the GSM field.

2. Effect of Modulated GSM Radiation-field on the Reproductive Capacity

We carried out four experiments (2.1-2.4), with modulated emission (the experimenter was speaking close to the mobile phone's microphone, during the exposures). The exposure parameters in this case simulate the situation when a user speaks on the mobile phone during connection. Results are listed in Table 2.

Table 2 shows the mean number of F_1 pupae (corresponding to the number of laid eggs) per maternal fly in the groups E, exposed to "Modulated" GSM field and in the corresponding sham exposed groups, SE, during the first three days of the insect's maximum oviposition.

The Modulated GSM 900 MHz signals induced a large decrease in the insect's reproductive capacity up to 60% as compared to the unexposed groups. No temperature increases were detected during the exposures and thus these effects are considered as non-thermal.

Table 2. Effect of Modulated GSM field on the Reproductive Capacity of *Drosophila melanogaster*

Experiment No	Groups	Mean Number of F_1 Pupae per Maternal Fly	Deviation from Control
2.1	E(M)	6.7	-48.85%
	SE (M) (Control)	13.1	
2.2	E	5.1	-56.78%
	SE (M) (Control)	11.8	
2.3	E	5.6	-53.72%
	SE (M) (Control)	12.1	
2.4	E	6	-53.125%
	SE (M) (Control)	12.8	
Average $\pm$ SD	E (M)	5.85 ± 0.67	-53.01%
	SE (M) (Control)	12.45 ± 0.6	

The reproductive capacity was much more decreased by modulated emission, (50-60%), than by non-modulated emission, (15-20%). Thus the effect is strongly dependent on radiation-field intensity. At the same time, the intensity of the modulated signal, is about ten times more powerful than the non-modulated signal. Thereby, the effect is not linearly dependent on radiation intensity.

The results from the first two sets of experiments are represented, in Figure 1.

The statistical analysis shows that the probability that mean oviposition differs between the exposed and the sham exposed groups, owing to random variations, is very small, $P < 10^{-5}$. Thus the recorded effect is due to the GSM signal.

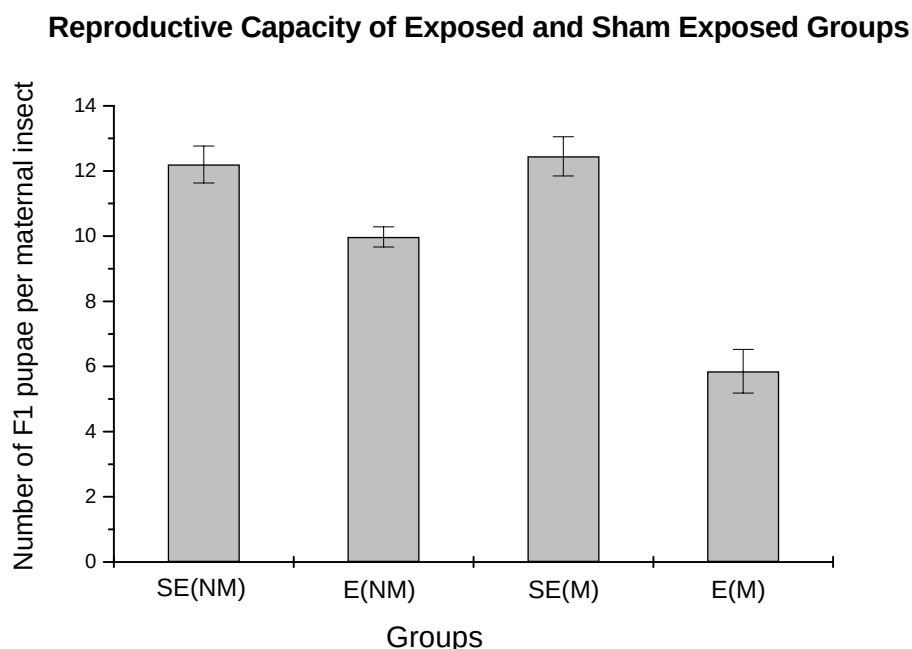


Figure 1. Reproductive Capacity of the groups exposed to non-modulated and modulated GSM 900 MHz field [E(NM), E(M)] and the corresponding sham exposed, [SE(NM), SE(M)], groups. [The error bars correspond to Standard Deviation].

3. Effects on the Reproductive Capacity of Each Sex

A third set of experiments (C) was carried out in order to record the effect of the GSM 900 MHz field on the reproductive capacity of each sex separately. The mobile phone was operating in speaking mode during the 6 min exposures, and the insects were separated into four groups (each one consisting again 10 male and 10 female insects): In the first group (E1), both male and female insects were exposed. In the second group (E2), only the females were exposed. In the third group (E3), we exposed only the males and the fourth group (SE) was sham exposed (control). Therefore in this third set of experiments, the 6-min daily exposures took place only during the first two days of each experiment while the males and females of each group were separated and the total number of exposures in each experiment was 2 instead of 5.

The results from this set of experiments are listed in Table 3 and represented graphically in Figure 2.

The results of this set of experiments show that the GSM field affects the reproductive capacity of both female and male insects. The female insects (E2) were more affected than males (E3) in these experiments. This is expected to be due to the fact that, by the time we started the exposures, spermatogenesis was already almost completed in male flies, while oogenesis had just started, (King 1970; Panagopoulos et al 2004).

Statistical analysis (single factor ANOVA test) shows that the probability that mean oviposition differs between the four groups because of random variations is $P < 10^{-7}$.

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Table 3. Effect of “Modulated” GSM field on the Reproductive Capacity of each sex

Experiment No	Groups	Mean Number of F ₁ Pupae Per Maternal Fly	Deviation from Control
3.1	SE(Control)	13.2	
	E1	8.5	-35.61%
	E2	9.4	-28.79%
	E3	11.7	-11.36%
3.2	SE (Control)	13.8	
	E1	7.6	-44.93%
	E2	8.9	-35.51%
	E3	12.1	-12.32%
3.3	SE (Control)	12.9	
	E1	7.8	-39.53%
	E2	9.3	-27.91%
	E3	11	-14.73%
3.4	SE (Control)	13.5	
	E1	6.9	-48.89%
	E2	7.8	-42.22%
	E3	12.2	-9.63%
Average $\pm$ SD	SE (Control)	13.35 $\pm$ 0.39	
	E1	7.7 $\pm$ 0.66	-42.32%
	E2	8.85 $\pm$ 0.73	-33.71%
	E3	11.75 $\pm$ 0.54	-11.985%

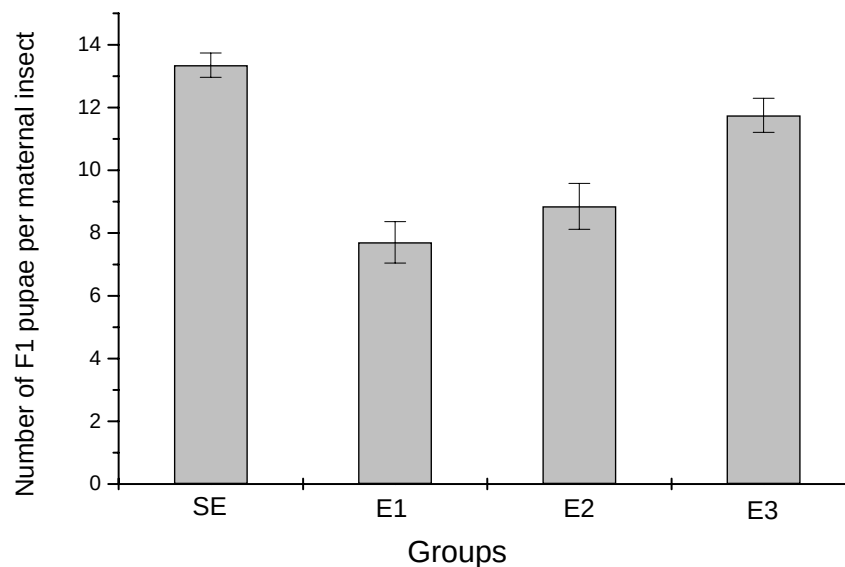
Effect of GSM field on the Reproductive Capacity of each sex

Figure 2. Effect of Modulated GSM field on the reproductive capacity of each sex of *Drosophila melanogaster*. Average mean number of F₁ pupae $\pm$ SD per maternal insect. SE: sham exposed groups, E1: groups that both sexes were exposed, E2: groups in which only the females were exposed, E3: groups in which only the males were exposed.

In the following fourth, fifth and sixth set of experiments, we used a dual band cellular mobile phone that could be connected to either GSM 900 or DCS 1800 networks simply by changing SIM (“Subscriber Identity Module”) cards on the same handset. The highest Specific Absorption Rate (SAR), given by the manufacturer for human head, was 0.89 W/Kg. The exposure procedure was the same. The experimenter spoke on the mobile phone’s microphone during the exposures. The GSM and DCS fields were thus “modulated” by the human voice, (“speaking emissions” or “GSM basic”).

4. Comparison of Bioactivity between GSM 900 MHz and DCS 1800 MHz

In this set of experiments we separated the insects into four groups: a) the group Exposed to GSM 900MHz field with the mobile phone antenna in contact with the glass vial containing the flies (named as “900”), b) the group exposed to GSM 900MHz field with the antenna of the mobile phone at 1cm distance from the vial (named as “900A”), c) the group exposed to DCS 1800MHz field with the mobile phone antenna in contact with the glass vial (named as “1800”), and d) the Sham Exposed (Control) group (named as “SE”). The comparison between first and third group represents comparison with the usual exposure conditions between GSM 900 and DCS 1800 users, while comparison between second and third group represents comparison between possible effects of the RF frequencies of the two systems under equal radiation intensities. Therefore the second group (900A) was introduced for better comparison of effects between the two types of radiation.

Measured mean power densities in contact with the mobile phone antenna for six min of modulated emission, were 0.407 ± 0.061 mW/cm² for GSM 900 MHz and 0.283 ± 0.043 mW/cm² for DCS 1800 MHz. As was expected GSM 900 MHz intensity at the same distance from the antenna and with the same handset was higher than the corresponding DCS 1800 MHz. For the better comparison between the two systems of radiation we measured the GSM power density at different distances from the antenna and found that at 1cm distance, the GSM 900 MHz intensity was 0.286 ± 0.050 mW/cm², almost equal to DCS 1800 MHz at zero distance. Measured electric and magnetic field intensities in the ELF range for modulated field, excluding the ambient electric and magnetic fields of 50Hz, were 22.3 ± 2.2 V/m electric field intensity and 0.50 ± 0.08 mG magnetic field intensity for GSM at zero distance, 13.9 ± 1.6 V/m, 0.40 ± 0.07 mG correspondingly for GSM at 1 cm distance and 14.2 ± 1.7 V/m, 0.38 ± 0.07 mG correspondingly for DCS at zero distance. All these values are averaged over ten separate measurements of each kind $\pm$ standard deviation (SD).

Except for the power density - field measurements of the mobile phone emissions, we obtained the spectra of both types of radiation, plus the background spectrum in our lab, (Fig. 3). Each one of the two types of radiation gave a unique frequency spectrum. While GSM 900MHz gives a single peak around 900MHz, (Fig. 3b), DCS 1800MHz gives a main peak around 1800MHz and a smaller one around 900MHz, (Fig. 3c). The spectra were obtained by a Hewlett Packard 8595 E, (9 kHz-6.5 GHz), spectrum analyzer (USA).

We carried out ten replicate experiments. Results are listed in Table 4 and represented graphically, in Figure 4.

The results from this set of experiments show that the reproductive capacity in all the exposed groups is significantly decreased compared to the sham exposed groups. The decrease is maximum in the 900 groups, (48.25% compared to SE) and smaller in the 900A and the 1800 groups, (32.75% and 31.08% respectively), (Table 4). Although the decrease was even smaller in the 1800 groups than in 900A, differences between the 900A and 1800 groups were found to be within the standard deviation, (Table 4, Figure 4).

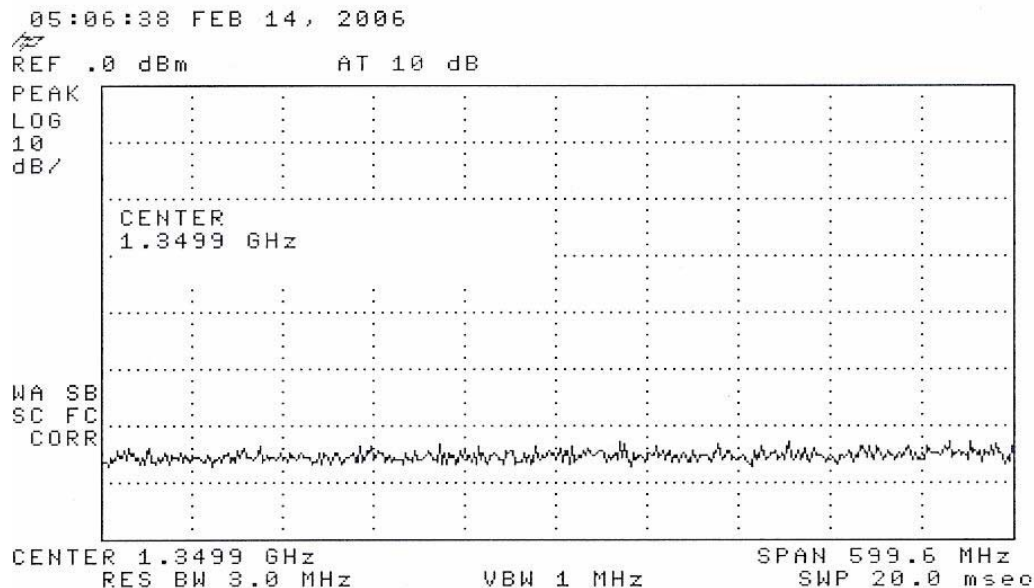
The statistical analysis shows that the probability that the reproductive capacity differs between groups, owing to random variations, is negligible, $P < 10^{-18}$.

Again, we did not detect any temperature increases, within the glass vials during the exposures.

The differences in the reproductive capacity between the groups were greater between 900 and 900A (owing to intensity differences between the two types of radiation) and much smaller between 900A and 1800, (owing to frequency differences between GSM and DCS), (Table 4).

This set of experiments shows that there is a difference in the bioactivity between GSM 900 MHz and DCS 1800 MHz and this difference is mainly due to the higher intensity of GSM 900 under the same exposure conditions, (differences between groups 900 and 900A) and not due to the different RF carrier frequencies, (differences between 900A and 1800 groups).

Intensity differences between the two types of cellular mobile telephony radiation depend also on the ability of communication between the antennas of the mobile phone and the corresponding base station. Even if GSM 900 usually has a higher intensity than DCS 1800, this situation can be reversed in certain places if GSM 900 has a much better signal perception between its antennas than DCS 1800, (Tisal 1998). Our results count for equal signal perception conditions between the two types of radiation.

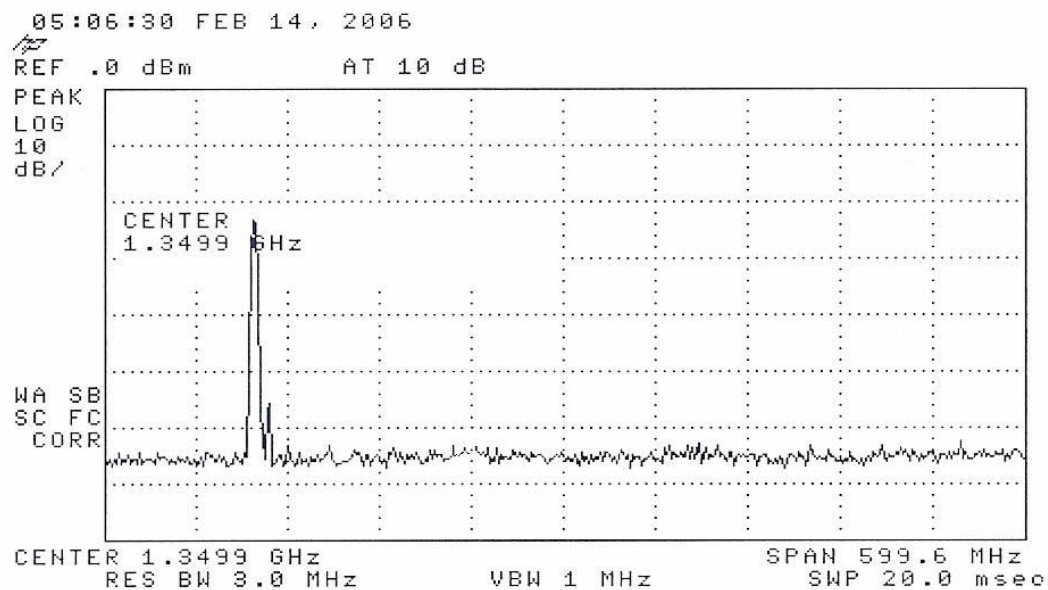


a. Background spectrum.

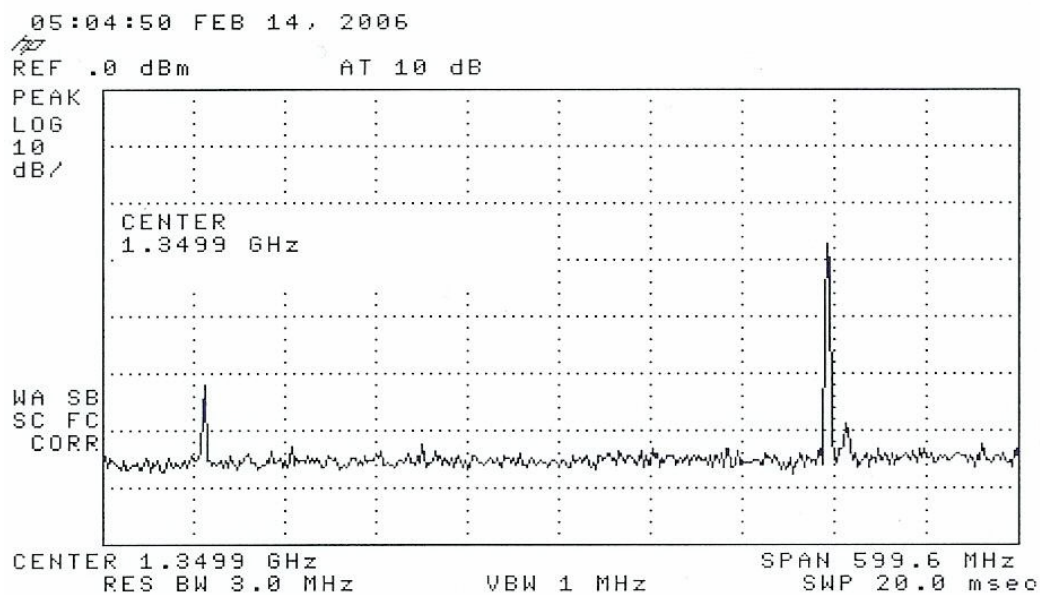
Figure 3. Continued on next page.

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b. Spectrum of GSM 900 MHz.



c. Spectrum of DCS 1800 MHz.

Figure 3. Background, GSM 900 MHz and DCS 1800 MHz spectra.

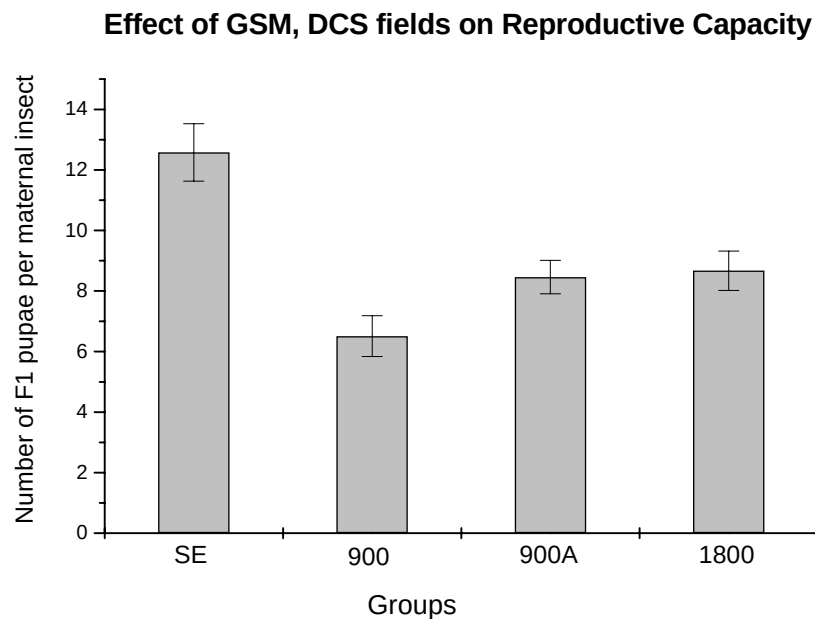


Figure 4. Reproductive Capacity (mean number of F1 pupae per maternal fly) of exposed (900, 900A, 1800) and sham exposed (SE) groups.

Table 4. Effect of Modulated GSM and DCS fields on the Reproductive Capacity of *Drosophila melanogaster*

Experiment No	Groups	Mean Number of F ₁ Pupae per Maternal Fly	Deviation from Control
1	900	7.7	-42.54%
	900A	8.9	-33.58%
	1800	9.2	-31.34%
	SE (Control)	13.4	
2	900	5.8	-51.26%
	900A	8.1	-31.93%
	1800	7.9	-33.61%
	SE (Control)	11.9	
3	900	6.8	-46.03%
	900A	7.9	-37.30%
	1800	8.7	-30.95%
	SE (Control)	12.6	
4	900	7.4	-47.52%
	900A	9.7	-31.21%
	1800	9.9	-29.79%
	SE (Control)	14.1	
5	900	6.2	-52.31%
	900A	8.5	-34.62%
	1800	8.2	-36.92%
	SE (Control)	13	

Table 4. Continued

Experiment No	Groups	Mean Number of F ₁ Pupae per Maternal Fly	Deviation from Control
6	900	6.1	-43.52%
	900A	8.2	-24.07%
	1800	7.8	-27.78%
	SE (Control)	10.8	
7	900	6.7	-47.66%
	900A	8.3	-35.16%
	1800	9	-29.69%
	SE (Control)	12.8	
8	900	6	-48.72%
	900A	7.9	-32.48%
	1800	8.4	-28.21%
	SE (Control)	11.7	
9	900	6.7	-49.24%
	900A	8.8	-33.33%
	1800	9.1	-31.06%
	SE (Control)	13.2	
10	900	5.7	-53.66%
	900A	8.3	-32.52%
	1800	8.5	-30.89%
	SE (Control)	12.3	
Average $\pm$ SD	900	6.51 $\pm$ 0.67	-48.25%
	900A	8.46 $\pm$ 0.55	-32.75%
	1800	8.67 $\pm$ 0.65	-31.08%
	SE (Control)	12.58 $\pm$ 0.95	

5. Radiation Bioactivity According to its Intensity (or According to the Distance from the Antenna)

The aim of this set of experiments was to investigate the dependence of GSM 900 MHz and DCS 1800 MHz bioactivity on their intensity, at different intensity levels that people are exposed to, from mobile phones and base station antennas. The radiation from base station antennas is almost identical to that of corresponding mobile phones but it is about 100 times stronger. Thus distances from mobile phones antennas correspond to about 100 times longer distances from base station antennas of the same type of radiation.

It is difficult to set up experiments regarding exposures from base station antennas since there is no way to have a sham exposed group of experimental animals under identical environmental conditions but without being exposed to the radiation at the same time. Thus we thought that the only way to simulate the reality of the exposure by a base station antenna is to expose the animals at different distances from a mobile phone within the lab.

Biological effects of mobile telephony signals at different intensities- distances from the antenna of a mobile phone handset, resembling effects from base station signals within residential areas, were not performed until now.

In each single experiment of this set, we separated the collected insects into thirteen groups: The first group (named "0") was exposed to GSM 900 MHz or to DCS 1800 MHz

field with the mobile phone antenna in contact with the glass vial containing the flies. The second (named “1”), was exposed to GSM 900 MHz or to DCS 1800 MHz field, at 1cm distance from the mobile phone antenna. The third group (named “10”) was exposed to GSM 900 MHz or to DCS 1800 MHz field at 10 cm distance from the mobile phone antenna. The fourth group (named “20”) was exposed to GSM 900 MHz or to DCS 1800 MHz field at 20 cm distance from the mobile phone antenna, etc, the twelveth group (named “100”) was exposed to GSM 900 MHz or to DCS 1800 MHz field at 100 cm distance from the mobile phone antenna. Finally, the thirteenth group (named “SE”) was the sham exposed. Each group consisted of ten male and ten female insects as previously.

Radiation and field measurements in contact and at different distances from the mobile phone antenna, for six min of modulated emission, for GSM 900 MHz and DCS 1800 MHz in the RF and ELF ranges excluding the background electric and magnetic fields of 50 Hz, are given in Table 5. All the values shown in Table 5, are averaged over ten separate measurements of each kind $\pm$ standard deviation (S.D.).

The measurements reveal that although ELF electric and magnetic fields fall at almost zero levels for distances longer than 50 cm from both GSM 900 and DCS 1800 mobile phone antennas, the RF components of the signals are still evident for distances up to 100 cm, (Table 5).

The Average mean values of reproductive capacity (number of F₁ pupae) from six identical experiments with each kind of radiation are shown in Table 6 and represented in Figures 5, 6. The statistical analysis (single factor Anova test) shows that the probability that the reproductive capacity differs between groups, owing to random variations, is negligible, $P < 10^{-8}$. Once again there was no temperature increases within the vials during the exposures.

The results show that the effect of mobile telephony radiation is maximum at zero distance (intensities higher than $200 \mu\text{W}/\text{cm}^2$) and then becomes maximum at a distance of 20-30 cm from the antenna, depending on the intensity of radiation (GSM or DCS). This distance corresponds to an intensity around $10 \mu\text{W}/\text{cm}^2$ for both types of radiation in regards to the RF components.

Table 5. Radiation and Field Intensities in the Microwave and ELF regions

Distance from Antenna (cm)	GSM Radiation Intensity at 900 MHz, (mW/cm^2)	GSM Electric Field Intensity at 217 Hz, (V/m)	GSM Magnetic Field Intensity at 217 Hz, (mG)	DCS Radiation Intensity at 1800 MHz, (mW/cm^2)	DCS Electric Field Intensity at 217 Hz, (V/m)	GSM Magnetic Field Intensity at 217 Hz, (mG)
0	0.380 ± 0.058	19 ± 2.5	0.9 ± 0.15	0.250 ± 0.048	13 ± 2.1	0.6 ± 0.08
1	0.260 ± 0.047	12 ± 1.7	0.7 ± 0.13	0.068 ± 0.015	6 ± 0.8	0.4 ± 0.07
10	0.062 ± 0.020	7 ± 0.8	0.3 ± 0.05	0.029 ± 0.005	2.9 ± 0.48	0.2 ± 0.05
20	0.032 ± 0.008	2.8 ± 0.4	0.2 ± 0.04	0.012 ± 0.002	0.7 ± 0.12	0.1 ± 0.02
30	0.010 ± 0.002	0.6 ± 0.09	0.1 ± 0.02	0.007 ± 0.001	0.3 ± 0.06	0.06 ± 0.01
40	0.006 ± 0.001	0.2 ± 0.03	0.05 ± 0.01	0.004 ± 0.0007	0.1 ± 0.04	0
50	0.003 ± 0.0006	0.1 ± 0.02	0	0.002 ± 0.0003	0	0
60	0.002 ± 0.0003	0	0	0.0016 ± 0.0002	0	0
70	0.0017 ± 0.0002	0	0	0.0014 ± 0.0002	0	0
80	0.0012 ± 0.0002	0	0	0.0008 ± 0.0002	0	0
90	0.0010 ± 0.0001	0	0	0.0005 ± 0.0001	0	0
100	0.0004 ± 0.0001	0	0	0.0002 ± 0.0001	0	0

Table 6. Effect of Modulated GSM and DCS radiation-fields on the Reproductive Capacity at different Distances-Intensities from the antenna

Groups -Distance from mobile phone antenna, (cm)	Average Mean Number of F ₁ Pupae per Maternal Fly, for GSM 900 MHz	Deviation from Sham Exposed Group	Average Mean Number of F ₁ Pupae per Maternal Fly, for DCS 1800 MHz	Deviation from Sham Exposed Group
0	7.45 ± 0.72	-46.01 %	9.26 ± 0.68	-34.00 %
1	9.38 ± 0.61	-32.03 %	11.36 ± 0.54	-19.03 %
10	11.29 ± 0.80	-18.19 %	11.93 ± 0.71	-14.97 %
20	11.52 ± 0.79	-16.52 %	9.19 ± 0.62	-34.50 %
30	7.33 ± 0.58	-46.88 %	13.03 ± 0.83	-7.13 %
40	12.88 ± 0.98	-6.67 %	13.76 ± 0.85	-1.92 %
50	13.48 ± 0.81	-2.32 %	13.85 ± 0.74	-1.28 %
60	13.61 ± 0.84	-1.38 %	14.00 ± 0.91	-0.21 %
70	13.70 ± 0.91	-0.72 %	14.21 ± 0.89	+1.28 %
80	13.97 ± 0.77	+1.23 %	14.07 ± 0.79	+0.29 %
90	13.74 ± 0.96	-0.43 %	14.02 ± 1.03	-0.07 %
100	14.02 ± 1.01	+1.59 %	14.31 ± 1.08	+2.00 %

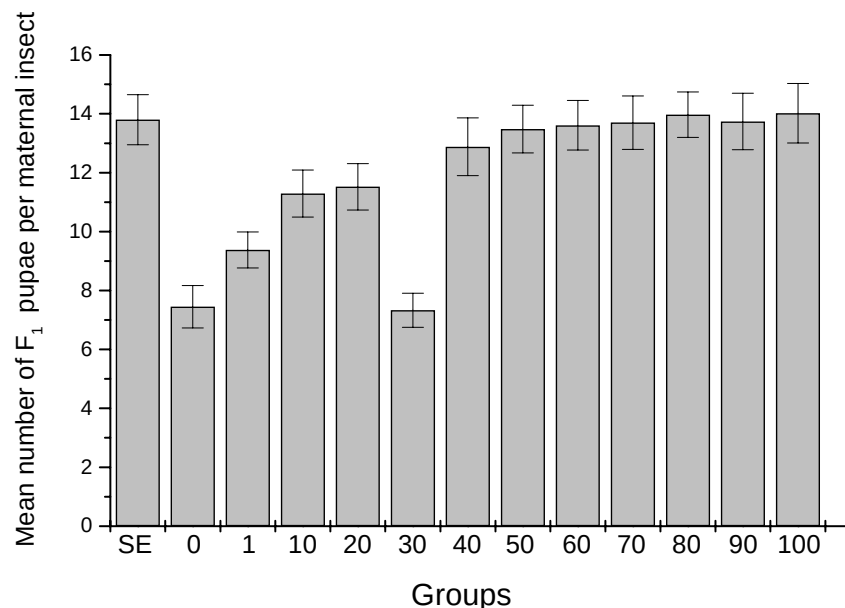
Intensity Effect of GSM 900 MHz Radiation

Figure 5. Reproductive Capacity in relation to the Distance from a GSM 900 MHz mobile phone antenna. The decrease in reproductive capacity is maximum at zero distance and at 30 cm distance from the antenna, corresponding to RF intensities 380 μ W/cm² and 10 μ W/cm² (Table 5).

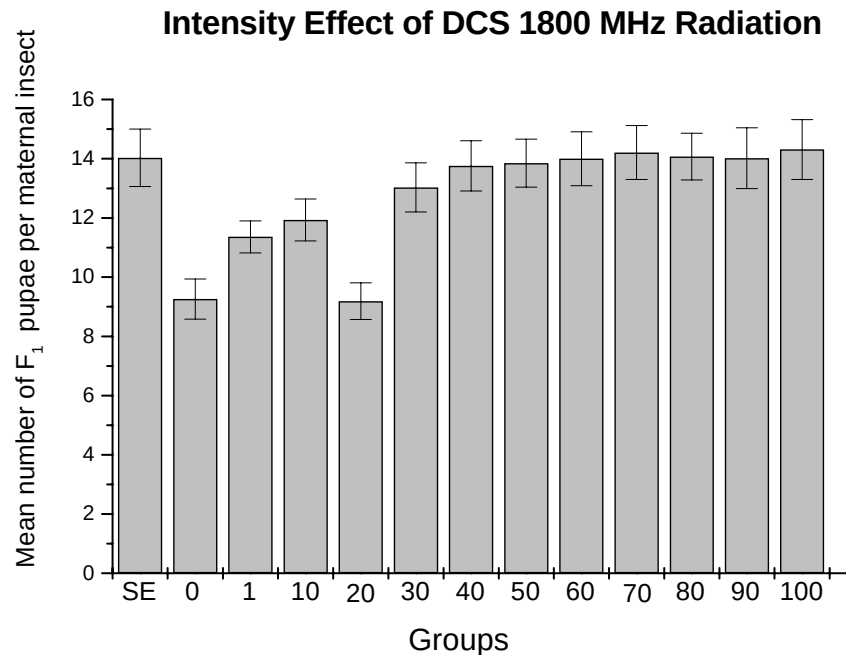


Figure 6. Reproductive Capacity in relation to the Distance from a DCS 1800 MHz mobile phone antenna. The decrease in reproductive capacity is maximum at zero distance and at 20 cm distance from the antenna, corresponding to RF intensities $250 \mu\text{W}/\text{cm}^2$ and $12 \mu\text{W}/\text{cm}^2$ (Table 5).

The effect on the reproductive capacity diminishes considerably for distances longer than 50 cm from the mobile phone antenna and disappears for distances longer than 80-90 cm, corresponding to radiation intensities smaller than $1 \mu\text{W}/\text{cm}^2$. For distances longer than 50 cm where the ELF components fall within the background, the decrease in reproductive capacity is within the standard deviation. This might suggest that the ELF components of digital mobile telephony signals, play a key role in their bio-activity, alone or in conjunction with the RF carrier wave.

We have recorded the existence of an “intensity window” around $10 \mu\text{W}/\text{cm}^2$ (in regards to the RF intensity) where the bio-effect becomes even more intense than at intensities higher than $200 \mu\text{W}/\text{cm}^2$. This intensity window appears at a distance of 20-30 cm from a mobile phone antenna, which corresponds to a distance of about 20-30 meters from a base station antenna. Since mobile telephony base station antennas are usually located within residential areas, at distances 20-30 m from such antennas there are often houses and work places where people are exposed up to 24 hours per day.

Although intensity windows on the bio-effects of RF radiations have been recorded since many years, (Bawin et al 1975; 1978; Blackman et al, 1980), there is still no widely accepted explanation for their existence.

6. The Decrease in Reproductive Capacity is due to Cell Death in the Gonads

In each experiment of this final sixth set, we separated the collected insects into five groups. The first four groups were the same just as in the No 4 experiments: The first group (named

“900”) was exposed to GSM 900 MHz field with the mobile phone antenna in contact with the glass vial containing the flies. The second (named “900A”), was exposed to GSM 900 MHz at 1cm distance from the mobile phone antenna. The third group (named “1800”) was exposed to DCS 1800 MHz field with the mobile phone antenna in contact with the glass vial. The fourth group (named “SE”) was sham-exposed. Finally there was an additional fifth group (named “C”) which was the control. While sham-exposed animals were treated exactly as the exposed ones except that the mobile phone was turned off during the “exposures”, control animals were never exposed in any way or even taken out of the culture room. Each group consisted as always of ten male and ten female insects.

In this set of experiments, there was an additional 6 min exposure in the morning of the sixth day, and one hour later female insects from each group were dissected and prepared for TUNEL assay. This additional exposure time was the only difference in the exposure procedure from the previous sets of experiments. Since we were studying the effect on early and mid oogenesis during which the egg chambers develop from one stage to the next within few hours, (King, 1970), an additional exposure, one hour before dissection and fixation of the ovarioles, was proven to be important in recording immediate effects on DNA fragmentation.

The most anterior region of the ovariole is called the germarium. The most sensitive developmental stages during oogenesis for stress-induced apoptosis, are region 2 within the germarium referred to as “germarium checkpoint” and stages 7-8 just before the onset of vitellogenesis, referred to as “mid-oogenesis checkpoint”, (Drummond-Barbosa and Spradling, 2001; McCall 2004). The nurse cells (NC) and follicle cells (FC) of both checkpoints, were found to be very sensitive to stress factors like poor nutrition, (Drummond-Barbosa and Spradling, 2001; Smith et al., 2002), or exposure to cytotoxic chemicals like etoposide or staurosporine, (Nezis et al., 2000). Apart from these two check points, egg chambers were not observed before to degenerate during other provitellogenic or vitellogenic stages, (germarium to stage 10), (Drummond-Barbosa and Spradling, 2001; McCall 2004).

To determine the ability of GSM and DCS radiation to act as possible stress factors able to induce cell death during early and mid oogenesis, we used TUNEL assay, as described above. The samples from different experimental groups were blindly observed under the fluorescence microscope (i.e. the observer did not know the origin of the sample) and the percentage of egg chambers with TUNEL positive signal was scored in each sample. Statistical analysis was made by single factor Analysis of Variance test.

In Table 7 the summarised data from 8 separate experiments are listed. The data reveal that both GSM 900 and DCS 1800 mobile telephony radiations strongly induce cell death, (DNA fragmentation) in ovarian egg chambers of the exposed groups, (63.01% in 900, 45.08% in 900A and 39.43% in 1800), while in the SE and C groups the corresponding percentage of cell death was only 7.78% and 7.75% respectively.

Ovarian cell death between the control group and the sham exposed group did not differ significantly, (differences were within standard deviation) and this is why the data from the C group are omitted in Table 7.

Electromagnetic stress from mobile telephony radiations was found in our experiments to be much more bioactive than previously known stress factors like poor nutrition or cytotoxic chemicals, inducing cell death to a higher degree not only to the above check points but to all developmental stages of early and mid oogenesis and moreover to all types of egg chamber cells, i.e. nurse cells, follicle cells and the oocyte (OC), (Panagopoulos et al, 2007a).

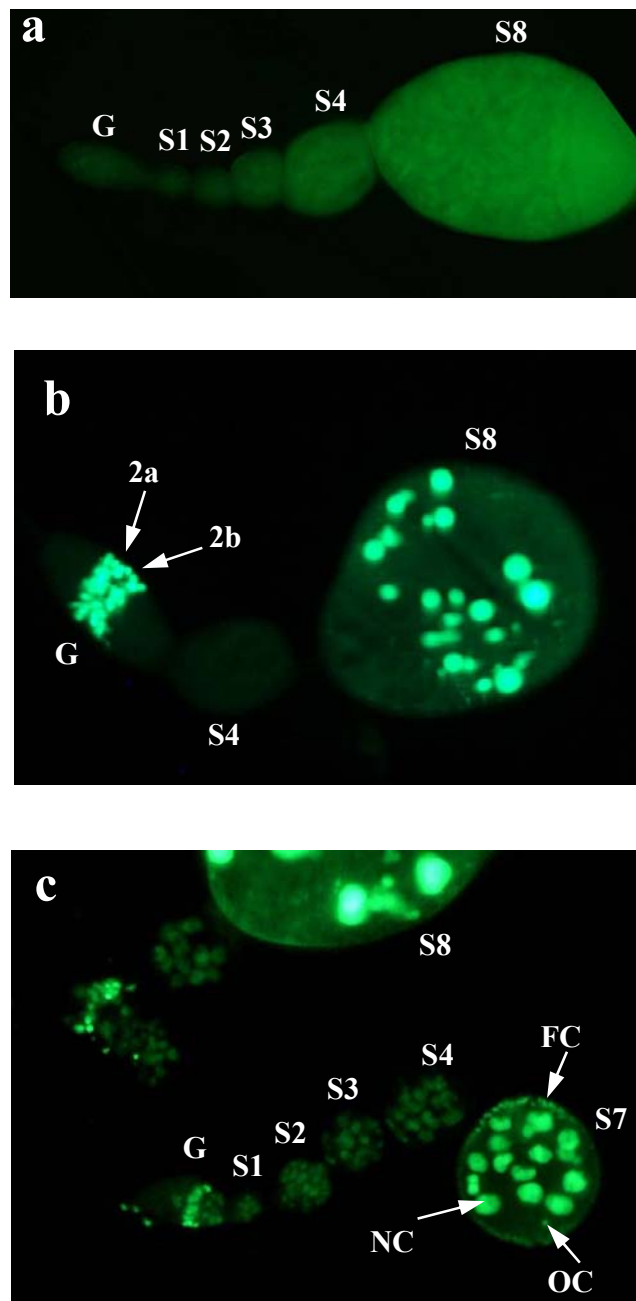


Figure 7. a) Ovariole of a sham exposed female insect with TUNEL negative egg chambers at all the developmental stages from germarium (G) to stage 8. b) Ovariole of exposed female insect with TUNEL positive signal at both check-points, germarium and stage 8 and TUNEL negative signal at the intermediate stages. c) Ovarioles of exposed female insects with TUNEL positive signals at all the developmental stages and in all types of egg chamber cells, nurse cells (NC), follicle cells (FC) and the oocyte (OC).

Table 7. Effect of GSM, DCS fields on Ovarian Cell Death

Groups	Dev. Stages	Ratio of TUNEL Positive to Total Number of Egg-chambers of each dev. stage	Sum Ratio of TUNEL Positive to Total Number of Egg-chambers of all stages	Percentage of TUNEL Positive Egg chambers	Deviation from Sham Exposed Groups
SE	Germarium	37/186	154/1980	7.78%	0%
	1-6	32/1148			
	7-8	78/364			
	9-10	7/282			
900	Germarium	165/189	1315/2087	63.01%	+55.23%
	1-6	675/1252			
	7-8	310/384			
	9-10	165/262			
900A	Germarium	116/184	930/2063	45.08%	+37.30%
	1-6	484/1248			
	7-8	213/374			
	9-10	117/257			
1800	Germarium	101/169	776/1968	39.43%	+31.65%
	1-6	388/1202			
	7-8	196/358			
	9-10	91/239			

Figure 7a, shows an ovariole from a sham exposed female insect, containing egg chambers from germarium to stage 8, all TUNEL negative. This was the typical picture in the vast majority of ovarioles and separate egg chambers from female insects of the sham exposed and control groups. In the SE groups, only 154 egg chambers (including germaria) out of a total of 1980 in 8 replicate experiments (7.78%), were TUNEL positive (Table 7), a result that is in full agreement with the rate of spontaneously degenerated egg chambers normally observed during *Drosophila* oogenesis, (Nezis et al., 2000; Baum et al., 2005).

Figure 7b shows an ovariole of exposed female insect (group 900A), with a TUNEL positive signal in the nurse cells at both checkpoints, germarium and stage 8, while egg chambers of intermediate stages are TUNEL negative. Corresponding pictures from 900 and 1800 (data not shown) had identical characteristics. The two checkpoints in all groups (exposed and SE/C) had the highest percentages of cell death compared to the other developmental stages 1-6 and 9-10, (Table 7). While in the SE groups the sum ratio of TUNEL positive to total number of egg chambers was slightly higher in stages 7-8 (78/364) than in the germarium (37/186), in all three exposed groups this ratio was higher in the germarium than in stages 7-8, (Table 7).

Figure 7c, shows ovarioles of exposed female insects (group 900A), with a TUNEL positive signal at all developmental stages from germarium to 7-8 and in all the cell types of the egg chamber, (nurse cells, follicle cells and the oocyte).

Although in most pictures the TUNEL positive signal was most evident in the nurse cells, in the majority of the egg chambers in all the exposed groups, a TUNEL positive signal was detected in all three kinds of egg chamber cells, (figures 1c).

Ovarian Cell Death induced by GSM and DCS Radiations

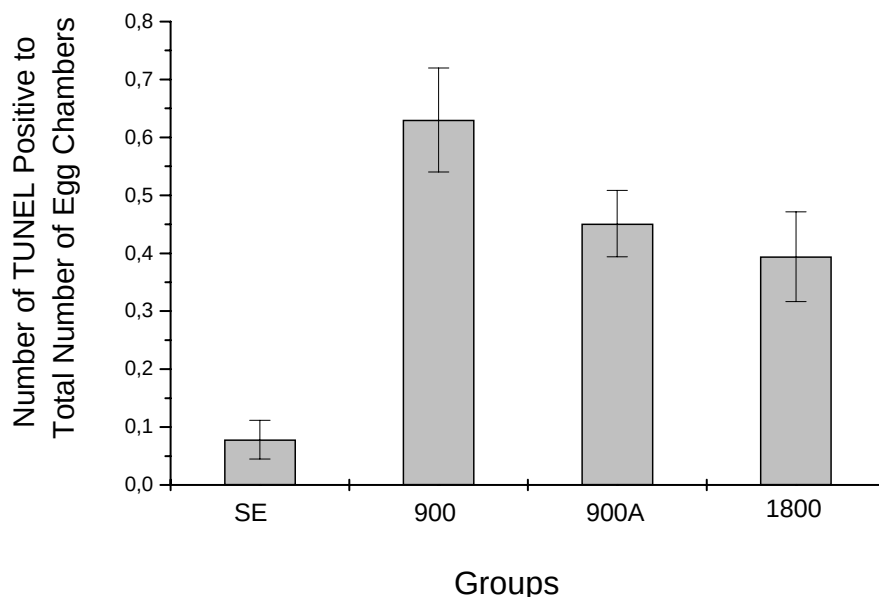


Figure 8. Mean ratio of Ovarian Cell Death (Number of TUNEL Positive to Total Number of Egg Chambers), in each experimental group $\pm$ SD, (0.078 ± 0.0335 in SE, 0.630 ± 0.0898 in 900, 0.451 ± 0.0574 in 900A and 0.394 ± 0.0777 in 1800).

In the SE groups the ratio of TUNEL positive egg chambers of stages 9-10 was very small (7/282). In contrast, the corresponding ratio in all three exposed groups was significantly higher, (165/262 in 900, 117/257 in 900A and 91/239 in 1800).

The summarised data of Table 7 are represented in Fig.8.

The statistical analysis, (single factor Analysis of Variance test), showed that the probability that groups differ between them because of random variations, is negligible, $P < 10^{-13}$.

Our experiments and the statistical analysis show that genomic DNA fragmentation of the egg chambers cells is induced by the mobile telephony radiation. Both types of radiation, GSM 900MHz and DCS 1800MHz induce cell death in a large number (up to 55% in relation to control), of ovarian egg chambers in the exposed insects with only 6 min exposure per day for a limited period of 6 days.

DNA fragmentation is induced in all cases predominantly at the two developmental stages named checkpoints, germarium and stages 7-8. Since the above check points were already known to be the most sensitive stages in response to other stress factors, (Chao and Nagoshi 1999; De Lorenzo et al., 1999; Nezis et al., 2000; Drummond-Barbosa and Spradling 2001; McCall 2004), such an observation could be expected. Our results show that these two checkpoints are the most sensitive stages also in response to electromagnetic stress. However the germarium checkpoint was found to be even more sensitive than stages 7-8 in response to this particular stress. Thereby the two check points are not equally responsive to distinct types of stress and may therefore also respond differentially to other types of stress stimuli. A possible explanation for the more sensitive germarium stage is that it may be more effective

in evolutionary terms for the animal to block development of any defective egg chamber at the beginning rather than at later stages, in order to prevent the waste of precious nutrients.

In the sham exposed/control groups, induced DNA fragmentation was observed almost exclusively at the two developmental stages named check-points (37/186 in the germarium and 78/364 in stage 7-8) and only in few cases at the other previtellogenic and vitellogenic stages, 1-6 (32/1148) and 9-10 (7/282), correspondingly. In contrast, ovarian egg chambers of animals from all three exposed groups, were found to be TUNEL positive to a high degree at all developmental stages from germarium to stage 10, (Table 7).

In all cases (both in the sham exposed/control and also in the exposed groups), the TUNEL positive signal was more intense at the two check points, germarium and stages 7-8, than at the other developmental stages.

There was no detectable temperature increase within the vials during the exposures, therefore the effects are considered as non-thermal.

In this set of experiments, cell death was detected for the first time during all the developmental stages of early and mid oogenesis in *Drosophila*, from germarium to stage 10 and in all types of egg chamber cells, (nurse cells, follicle cells, oocyte). A possible explanation for these effects is that the electromagnetic stress induced in the ovarian cells by the GSM and DCS fields, is a new and probably more intense type of external stress, against which ovarian cells do not have adequate defence mechanisms like they do in the case of poor nutrition or chemical stress.

It is important to emphasize that the recorded effect in the oocyte which undergoes meiosis during the last stages of oogenesis, may result in heritable mutations upon DNA damage induction and repair, if not in cell death.

The results of this set of experiments reveal that the large decrease of reproductive capacity found in the previous sets of experiments is due to elimination of large numbers of egg chambers during early and mid oogenesis, either via stress induced apoptosis or necrosis of their constituent cells, caused by the mobile telephony radiation.

Our present results are in agreement with results of other experimenters reporting DNA damage in other cell types, assessed by different methods than ours, after *in vivo* or *in vitro* exposure to GSM radiation, (Diem et al., 2005; Markova et al., 2005; Salford et al., 2003; Lai and Singh 1995; 1996).

We do not know if the ovarian cell death found in our experiments to be induced by mobile telephony radiation is due to apoptosis, i.e. caused by the organism in response to the electromagnetic stress, or the result of necrosis caused directly by the electromagnetic radiation. This important issue remains to be uncovered.

A Plausible Mechanism for Mobile Telephony Radiation Bioeffects

As we have previously reported, (Panagopoulos et al. 2000b; 2002; Panagopoulos and Margaritis 2003b), any external oscillating electromagnetic field can induce a forced-vibration on the free ions that exist in large concentrations inside and outside all living cells in biological tissue playing a key role in all cellular functions initiating or accompanying all cellular biochemical processes.

The forced-vibrational movement of the free ions is described by the equation,

$$m_i \frac{d^2 x}{dt^2} + \lambda \frac{dx}{dt} + m_i \omega_o^2 x = E_o z q_e \sin \omega t \quad [1]$$

in the case of an external harmonically oscillating electric field: $E = E_o \sin \omega t$ with circular frequency: $\omega = 2\pi\nu$, (ν , the frequency), where: z is the ion's valence, $q_e = 1.6 \times 10^{-19}$ Cb, the electron's charge, $F_2 = -m_i \omega_o^2 x$, a restoration force proportional to the displacement distance x of the free ion, m_i the ion's mass and $\omega_o = 2\pi\nu_o$, with ν_o the ion's oscillation self-frequency if the ion were left free after its displacement x . In our case, this restoration force is found to be very small compared to the other forces and thus does not play any important role. $F_3 = -\lambda u$ is the damping force, where $u = \frac{dx}{dt}$, is the ion's velocity and λ , is the attenuation coefficient for the ion's movement, which for the cytoplasm or the extracellular medium is calculated to be $\lambda \cong 10^{-12}$ Kg/sec, while for ions moving inside channel proteins, is calculated to have a value: $\lambda \cong 6.4 \times 10^{-12}$ Kg/sec, (in the case of Na^+ ions, moving through open Na^+ channels), (Panagopoulos et al 2000b).

We have shown that the general solution of equation [1], is:

$$x = \frac{E_o z q_e}{\lambda \omega} \cos \omega t - \frac{E_o z q_e}{\lambda \omega} \quad [2]$$

Since the second term of [2] is constant, the vibrational movement is described by the equation:

$$x = \frac{E_o z q_e}{\lambda \omega} \cos \omega t \quad [3]$$

Eq. [3] shows that the forced - vibration is in phase with the external force. The amplitude of the free ions forced vibration is,

$$A = \frac{E_o z q_e}{\lambda \omega} \quad [4]$$

Thus, the amplitude is proportional to the intensity and inversely proportional to the frequency of the external oscillating field.

Once this amplitude exceeds some critical value the coherent forces that the ions exert on the voltage sensors of voltage-gated membrane channels can trigger the irregular opening or closing of these channels, thus disrupting cell's electrochemical balance and function.

We have shown that in the most bioactive case of pulsed fields and for double valence cations (i.e. Ca^{+2}) interacting with the channel sensor, the condition for irregular gating of the channel becomes:

$$E_o \geq \nu \times 0.625 \times 10^{-4} \quad [5]$$

(ν in Hz, E_o in V/m). Whenever [5] is satisfied, the external field E can irregularly gate the ion channel.

Relation [5] declares that external ELF electric fields with intensities less than tenths of a mV/m should theoretically be able to disrupt cell function by irregular gating of ion channels (!)

According to this mechanism, lower frequency fields are the most bioactive ones and additionally pulsed fields are shown to be more bioactive than continuous, (uninterrupted), ones, (Panagopoulos et al., 2002).

Thereby, the ELF components of the mobile telephony signals are certainly within the criteria of this theory and thus able to produce the reported effects on living organisms.

Somebody may wonder, how could be possible that irregular gating of ionic channels on a cell membrane could lead to cell death.

Let us consider the irregular gating of ion channels on a cell's plasma membrane. If the electrochemical balance is destroyed by irregular increase of intracellular ion concentration, then water molecules may enter the cell driven by osmotic forces, proportional to the concentration increase. Such an effect could be able to cause the cell to swell out and the plasma membrane to get ruptured, resulting to cell necrosis.

It is known that perturbations of intracellular Ca^{+2} concentrations are responsible for apoptotic triggering, (Zhou et al., 1998; Sheikh and Huang, 2004; Santini et al. 2005). Therefore, another scenario of cell death, caused by irregular gating of ion channels, could be that due to altered intracellular Ca^{+2} concentrations, a false signal may be given to initiate apoptosis.

A common event leading to both apoptosis and necrosis is mitochondrial membrane permeabilization, (Armstrong 2006). This can also be done by direct action of an external EMF on mitochondrial membrane Ca^{+2} channels. Apoptosis is connected with increased mitochondrial concentration of Ca^{+2} ions, released from the endoplasmic reticulum, (Santini et al., 2005). A false uptake of Ca^{+2} ions by mitochondria can be due to irregular opening of mitochondrial Ca^{+2} channels, or due to increased cytosolic Ca^{+2} concentration, caused by irregular release either through the membrane of endoplasmic reticulum or through the plasma membrane. In all cases this could be done by irregular gating of electrosensitive Ca^{+2} channels which exist in all cell membranes.

We have just described few of the many hypothetical but very possible biochemical scenarios which could very explain by means of the above described biophysical theory, the effects of DNA damage recorded in our experiments as well as in other labs experiments, (Diem et al., 2005; Markova et al., 2005; Salford et al., 2003; Lai and Singh 1995; 1996).

Conclusions

As shown by increasing number of biological, clinical and epidemiological studies, the radiations emitted by mobile telephony, at levels that people are daily exposed, are highly bioactive producing a variety of effects on living organisms.

Our studies regarding the effects of mobile telephony radiations on a biological model, the reproductive capacity of the insect *Drosophila melanogaster*, have investigated different

physical parameters of these radiations, like intensity, carrier frequency, pulse repetition frequency, distance from the antenna, e.t.c.

Our experiments have shown a large decrease in reproductive capacity caused by the GSM and DCS fields-radiation. The recorded effect is due to extensive DNA fragmentation on reproductive cells of the experimental animal, induced by these fields-radiation.

Thus, digital mobile telephony radiations nowadays exert an intense biological action able to kill cells, damage DNA, or decrease dramatically the reproductive capacity of living organisms. Diminishes of bird and insect populations can be explained according to reproduction decreases. Phenomena like headaches, fatigue, sleep disturbances, memory loss e.t.c. reported as “microwave syndrome” can possibly be explained by cell death on a number of brain cells during daily exposures from mobile telephony antennas.

Our experiments show that radiation intensities higher than $1 \mu\text{W}/\text{cm}^2$ are able to decrease reproduction of living organisms by killing reproductive cells. Our opinion is that the international exposure limits for these radiations should be set not higher than $1 \mu\text{W}/\text{cm}^2$. Since short term exposures for few minutes per day are able to produce so intense effects on living organisms, the criteria should not be set according to average values but according to maximum values during the exposure periods.

Our experiments reveal that exposure at a distance of 20-30 cm from a mobile phone can be even more bioactive than exposure in contact with the antenna, due to the existence of an “intensity window” around $10 \mu\text{W}/\text{cm}^2$. This intensity, in the case of a usual base station antenna corresponds to a distance of about 20-30 m from the antenna.

Although both types of radiation examined are found to be highly bioactive, GSM 900 MHz seems to be even more bioactive than DCS 1800 MHz, mainly due to higher intensity, but also even when it is emitted at almost the same intensity. Since differences in bioactivity between the two types of radiation under the same intensity are within standard deviation, it seems that RF carrier frequency plays a minimal role in the bioactivity of this radiation, in contrast to the ELF pulse repetition frequencies and the radiation and field intensities that seem to be of great importance in regards to bioactivity.

The ELF components of the mobile telephony signals, seem to play a key role on their bio-effects, since the recorded effects are considerably diminished at distances that these components fall within the background of stray 50 Hz electric and magnetic fields. This supports that lower frequency fields are more bioactive than higher frequency ones with the same rest characteristics, as it is predicted by our theory, (Panagopoulos et al 2000b; 2002), and supported by other experimental evidence, (Lin Liu and Adey 1982; Penafiel et al 1997).

A plausible explanation of the effects of mobile telephony radiations on living organisms is given by the biophysical mechanism that we have proposed, (Panagopoulos et al. 2000b; 2002; Panagopoulos and Margaritis 2003b). According to this mechanism, altered intracellular ionic concentrations due to irregular gating of ion channels on the cell membranes by an external electromagnetic field can initiate cell death through apoptosis or necrosis.

Similar effects on humans with those recorded in our experiments on insects, are considered to be possible because first, insects are found to be more resistant to radiations than mammals, (Koval and Kazmar 1988, Koval et al 1979, 1977, Abrahamson et al 1973) and second, our results are in agreement with reported effects on mammals, (Lai and Singh 1995; 1996; Aitken et al., 2005; Salford et al., 2003).

Scientific evidence implies the need of reconsideration of the current exposure criteria to account for non-thermal effects which constitute the large majority of the recorded biological and health effects. Since Mobile Telephony has become part of our daily life, a better design of base station antenna networks towards the least exposure of residential areas and a very cautious use of mobile phones, is necessary.

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Volume 5

NON-THERMAL EFFECTS AND MECHANISMS OF INTERACTION BETWEEN ELECTROMAGNETIC FIELDS AND LIVING MATTER

An ICEMS Monograph



Edited by
Livio Giuliani and Morando Soffritti

National Institute for the Study and Control of Cancer and
Environmental Diseases "Bernardino Ramazzini"
Bologna, Italy
2010



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isbn
978-88-6261-166-4

pubblicazione
FIDENZA 2010



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JA 02357

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Preface

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Electromagnetic fields are waves that transport energy through space. They are characterized by wavelength and frequency, the two of which are inversely correlated. The shorter the wavelength, the greater the frequency.

Electromagnetic fields include the following (in order of decreasing wavelength and increasing frequency): electromagnetic fields of extremely low frequency (from electric sources), electromagnetic fields of low frequency, electromagnetic fields of radiofrequency and microwaves (from mobile telephones, television antennas etc), ultrasounds, infrared rays, ultraviolet rays, X rays and gamma rays. Gamma rays, given their energy charge, are also defined as ionizing radiation, and are capable of altering genetic cellular material. Indeed, the carcinogenic effects of ionizing radiation have been known for decades.

Scientific data regarding the long-term effects, in particular carcinogenic risk, of the exposure to non-ionizing electromagnetic fields were not reported in the literature until the 1970s. In 1979 two American researchers, Wertheimer e Leeper, published for the first time the results of an epidemiological study that demonstrated an increased carcinogenic risk, specifically leukemic, in children residing in close proximity to electric installations and therefore exposed to non-ionizing electromagnetic fields from electrical current at extremely low frequency.

As was to be expected, concern about the possible carcinogenic risks of non-ionizing radiation has now expanded beyond electricity to include other types of non-ionizing radiation, such as electromagnetic fields of radiofrequency and microwaves from cellular telephones and other wireless technologies such as cordless telephones, computers etc.

The expansion of mobile telephone technologies in the last 10 years is without precedent. In 1996 the number of cellular telephones in Italy was circa 4 million, today this figure is estimated to be 40 million. In the US, cellular telephones in the 1990s numbered 9 million, today more than 150 million Americans use cell phones, including children. It is estimated that more than 2 billion people use cell phones worldwide. In addition, many citizens are exposed to electromagnetic fields originating from the antennas of radio base stations that transmit cellular signals. Indeed, exposure to electromagnetic fields of radiofrequency and microwave, in both the work and general environment, has never before experienced this type of growth. For this reason it is fundamentally important to address the issue of safety, using all available tools to evaluate the potential risks of exposure. These tools include both epidemiological and experimental laboratory studies, as well as basic research.

This book provides updated information concerning mechanism of interaction between non ionising radiation fields and living matter, with particular reference to potential non-thermal toxic effects.

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The scientific knowledge available today regarding electromagnetic fields remains limited. Nevertheless, on the basis of recent epidemiological studies, and while awaiting new experimental data, it is advisable to limit exposure to electromagnetic fields as much as possible. This is especially true for children and adolescents, the most vulnerable segments of the population, and has been recommended by both the Swedish and UK health authorities.

Why investigate the non thermal mechanisms and effects of electromagnetic fields on living systems?

An introduction

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A Fairy Tale

Protection against Non Ionizing Radiation is based on a paradigmatic assumption:

“We know very well the interaction between electromagnetic fields and living organisms: it is a thermal interaction; thus the standards internationally accepted are adequate to protect people and workers”¹.

This is a fairy tale.

Since the 1970s the *non thermal* effects of electromagnetic fields on living organisms have been well known and also the *non thermal* mechanisms have been investigated^{2,3}. Nevertheless, until today, we have been condemned to listen to representatives from international institutions repeating the old refrain above. Furthermore when scientists participating in the ICEMS agreed to edit a monograph – the present one - with the aim of illustrating the non thermal mechanisms and effects due to the electromagnetic interaction with living organisms - mechanisms that are well known today - some of us withdrew their contribution because they did not share the locution “*non thermal*” in the title. The following discussion, which many ICEMS scientists and the coauthors of this monograph took part in, focused on some basic points, maybe obvious but not infrequently forgotten.

To be able to speak about a thermal effect on a *system*, we must first observe a variation in the *temperature* of the *system*.

Temperature

In order to define the temperature of a system it is necessary to include the philosophical concept of ensemble: in extension a collection of independent and indistinguishable particles each having a well defined velocity. In such a picture the temperature will emerge as an average property of the system as the average kinetic energy defined on the ensemble. In the case of a biochemical system made up of many *non*-independent particles the very basic concept of temperature has to be defined through an oversimplification of the system description (useful in most applications): we assume that each molecule can be labelled with a mean velocity energy which, in turn, defines an average energy associated with each degree of freedom of the molecule itself. In such

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a picture a perturbation is termed “thermal” if it is able to change the average kinetic energy associated to each degree of freedom, in such a way that the average of the energies on the ensemble is changed.

The rotating motion of water molecules induced by microwaves is the most evident achievement of such a thermal effect, but we need not think it is unique. In our monograph we focus on an effect – the coupling of RF/MW with cancerous tissues – discovered by E.H. Frick and S. Morse (1924) and re-discovered by C. Vedruccio, as reported in this monograph.

The Energy transfer mechanism described by the classical or semi-classical model of biological matter is based on “hopping” along discrete energy levels. However, as is widely known in the literature, such a model cannot account for the energy transfer process in biological systems such as photo-synthesis, where the light-absorbing molecules can funnel energy with a near-unit quantum efficiency across mesoscopic distances. Such a conundrum implies a deeper re-thinking of the molecular biology model based upon independent and indistinguishable particles. The solution implies a high degree of correlation among a great number of molecules and the entry in play of quantum phenomena. Quantum mechanics teaches us that energy transfer can happen in a quantum-correlated system without entailing kinetic knocks.

Non Thermal effects

In such a picture it is paramount to distinguish between “thermal” and “non-thermal” effects. In fact, the existence of the latter implies a model of biological matter well beyond the classical or semi-classical representation. Hence the deep meaning of the thermal-non thermal *querelle* : to minimize this distinction could lead us to underestimate what is probably the watershed of modern biology.

However, because we are concerned with biology or biophysics - not atomic physics - we may be focused on much more complex systems than atoms and we may fail to monitor the variation of energy of single electrons or single atoms. Even an aqueous solution of aminoacids, in a quantity such as in the electrolytic cell of Zhadin described in this monograph, has millions of billions of billions of molecules, as Avogadro taught us. Thus we should not be deceived by the fact that a certain molecule receives energy during a reaction into concluding that this reaction is based on a thermal mechanism of interaction. We must look at the temperature of the system. We must observe the system and the average of the energies of all components involved.

For instance, in the aqueous solution of the Zhadin experiment, we witness an ion current peak - that can be detected in the order of 10-100 nA - when we apply a suitable combination of DC-AC magnetic fields. But the AC field is very weak: in the order of 10nT! And the DC field is like the geomagnetic one: there is no transfer of energy able to induce an alteration in the system temperature. It is not only a non thermal effect; it is an *athermal* effect!

Thermal/Non thermal in EMF risk assessment

Lastly, let us consider the current meaning of ‘thermal effects’ in RF/MW risk assessment. According to ANSI (1981), interactions inducing a temperature increase lower

than 0.5 °C in the human body are commonly accepted, even by the WHO. The corresponding value in terms of of WHOLE BODY AVERAGE SPECIFIC ABSORPTION RATE (WBASAR) is 4 W/kg. Furthermore, the absorption of 0.4 W/kg – corresponding to a temperature increase equal to a 0.05°C in the body – is considered negligible for workers and the absorption of 0.08 W/kg – corresponding to a 0.01 °C increase – seems to be negligible. WHO, IEEE and ICNIRP assure us that under such a threshold we can be protected against all health effects due to RF/MWs. On this view, biological non thermal effects are only to be considered as reversible effects. But non-reversible effects are detected under the same threshold by epidemiologists –see the assay by Lennart Hardell in this monograph -: such effects can be considered ‘*non thermal*’ effects in this context. What about mechanisms inducing temperature increases lower than 0.001 °C (corresponding to 0.008 W/kg SAR)? They can be considered ‘*non thermal*’ in the same context, in accordance with the usual convention that perturbation of a system, when the parameters are lower by three orders of magnitude than the corresponding parameters of the system, can be considered not related to such parameters.

Perhaps we should specify the meaning of the terms thermal/non thermal in the present monograph. With reference to the usual meaning adopted in the context of *protection against radiation*, we can consider as *non thermal* all mechanisms that are not able to induce an increase in temperature higher than 0.01°C, when we are considering a system like a living organism, or lower than 0.001 °C when a system like a cell is considered, or again lower than 0.0005 °C when a sub-cellular system is studied.

Several mechanisms and effects are considered in this monograph with the collaboration of many scientists who have joined this ICEMS initiative.

Our book does also include thermal mechanisms and effects as well as macroscopic phenomena (see the various sections of the book).

The point is, *protection against non ionizing radiation*, based on parameters adopted by international standards organizations, seems not to be adequate, despite the statement of Ms Van Deventer, nor able to protect people and workers. This is convincingly shown in the paper by Devra Davis, Om Ghandi and colleagues in this monograph.

Acknowledgment

The author is very grateful to Dr. Antonella De Ninno for her precious suggestions about the vexata quaestio thermal/non thermal

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On mechanism of combined extremely weak magnetic field action on aqueous solution of amino acid

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Abstract

The fundamental Physical mechanisms of the resonant action of an extremely weak (40 *nanoT*) alternating magnetic field at the cyclotron frequency combined with a weak (40 *mcT*) static magnetic field, on living systems are analyzed in the present article. The experimental effects of such sort of magnetic fields were described in different articles: the very narrow resonant peaks in electrical conductivity of the aqueous solutions in the *in vitro* experiments and the Biomedical *in vivo* effects on living animals of magnetic fields with frequencies tuned to some amino acids. The existing experimental *in vitro* data had a good repeatability in different laboratories and countries. Unfortunately, for free ions such sort of effects are absolutely impossible because the dimensions of an ion rotation radius should be measured by meters at room temperature and at very low static magnetic fields used in all the above experiments. Even for bound ions these effects should be also absolutely impossible from the positions of Classic Physics because of rather high viscosity of biological liquid media. Only modern Quantum ElectroDynamics of condensed media opens the new ways for solving these problems. The proposed article is devoted to detailed analysis of Quantum mechanisms of these effects.

Key words: extremely weak magnetic fields, aqueous solution, amino acids, cyclotron resonance, coherence domain

Introduction

About 25 years ago Profs Abraham Liboff¹ and Carl Blackman² in the USA discovered that weak (several tens of *mcT*) combined alternating and static magnetic fields resonantly affect different biological objects when the alternating magnetic field frequency was equal to the cyclotron frequency of some biologically active metal (calcium, potassium, magnesium) ions. The cyclotron frequency is determined by the following way:

$$\nu_{CF} = \frac{qB_s}{2\pi m}, \quad (1)$$

where q is an ion charge, m is its mass, and B_s is the static magnetic field. After some

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discussions and theoretical analysis^{1,2} it was accepted that such sort of effect is impossible for free ions, because the dimensions of an ion rotation radius should be measured by meters at room temperature and at very low static magnetic fields used in all the above experiments. But they could arise for ions bound in molecules³⁻⁶.

However, in 90s after discovering^{7,8} the resonant effects in aqueous solutions of alpha amino acids the situation became much more complicated. The static magnetic field was of 40 *mcT*, which is close to the natural geomagnetic field as earlier, but the alternating magnetic field of about 40 *nanoT* was thousand times less than in Liboff's^{1,9} and Blackman's² experiments. The two difficulties befogged the understanding of Physical mechanisms of these effects. The first one was the fact that in this case the ions were free, and the second one was connected with that the alternating field was thousand times less than in Liboff's effect¹, not counting even the fact that amino acids are not metals at all. The editorial staff of Bioelectromagnetics journal firstly delayed the publication of our submitted manuscript and asked to give some kind of Physical explanation of such unusual effect. This theoretical analysis was given by us four years later, when we pointed that similar effect could arise in solutions containing microcrystals of dissolved matter. But situation with the extremely weak alternating magnetic field nevertheless stayed unclear. Fortunately, both our articles^{8,10}, experimental and theoretical ones, were published^{5,8} in this journal. Later, our experiments were successfully replicated in Italy¹¹⁻¹³ and in Germany¹⁴, and now the different articles appeared in international scientific press¹⁵⁻¹⁷ [and others], which were experimentally developing the investigations of Biological effects of the extremely weak alternating magnetic fields *in vivo* on animals. However, till 2002 an obstacle in understanding such sort of the ionic cyclotron resonance effect remained insuperable. It was the impossibility of essential acceleration of an ion at the real viscosity of an aqueous solution under the influence of extremely weak combined magnetic fields. The Classical Physics was giving the well defined negative answer to the possibility of such effect. This problem was solved by Quantum ElectroDynamics of condensed matter.

Physical mechanisms of extremely weak combined magnetic fields action

At the end of 20th century in the famous Institute of Nuclear Physics (Italy) Prof. Giuliano Preparata and his colleagues elaborated a new branch of Quantum ElectroDynamics – the theory of condensed matter¹⁸⁻²¹. Among different liquid media the specific attention was drawn to water with its multitude of unsolved problems which now are successfully solved by this new branch of Quantum Physics. Quantum ElectroDynamics of water convincingly evidenced that the liquid water consists of two components: coherent and incoherent ones. The coherent component is contained within spherical structures, the so called “coherence domains”, where all molecules have the wave functions, oscillating synchronously with the same mutual phase. Coherence domains are surrounded by the incoherent component where the molecular wave functions are oscillating with casual phases relative to each other. As a matter of fact, the incoherent component is the water from the point of view of Classical Physics. Diameters of coherence domains are measured by tenths of a micron, and at room temperature the total volume of the domains is about 40% of the whole water volume. Within a domain, the features of coherent water sharply differ from ones of incoherent water and from the water as a whole. Within domains the water viscosity and oscillation

damping are about ten times less than viscosity and damping in the whole water. The fluidity in the domain is essentially increased, and the diffusion rate of foreign inclusions is much higher than within the incoherent water. The theoretical estimates of all electrical constants of the whole water, being earlier inexplicable by Classical Physics, for the first time turned out to be close to the experimental values, being analyzed by Quantum ElectroDynamics of water. And the unusual dependence of water density on temperature was explained too. The stability of coherence domains is rather high, because the bond energy of water molecules within coherence domains is much more than the thermal noise energy.

In our recent work²² we considered the amino acid ionic exchange between incoherent medium and coherence domains (using a glutamic acid ion as an example) under the influence of weak combined magnetic fields. (In this work we name the aqueous coherence domain containing one or more foreign molecules or ions as a “mixed domain” for brevity. We’ll use this term further for the same purpose). In the above article we studied the formation of mixed coherence domains in aqueous solutions of some amino acids and revealed the mechanisms of capture of some amino acid ions in zwitterion forms. Far from all soluble matters are able to form the mixed domains, but only ones which have the main spectral lines, common with the lines in water spectrum. In the present article we’ll analyze in detail the mechanism of escape of amino acid ion from the mixed coherence domains under the action of resonant combined magnetic fields.

In our analysis of magnetic field effect on ion motion within coherence domains we shall consider the domain wall without traditional easy-to-use approximation of a vertical potential well because the resonant increase in kinetic energy of an ion is impossible at this very rough approximation. Here we shall consider the domain wall as a layer with the finite thickness, in the range of which the density and viscosity have the same values as within the whole domain. Earlier we⁵ derived, analyzed and solved the equation of the ion motion in the centrally symmetric potential field under the influence of parallel combined static and alternating magnetic fields. Here we’ll use some important achievements of the above work. The equation will have the following form:

$$\frac{d^2 \mathbf{r}_o}{dt^2} = -\gamma \frac{d\mathbf{r}_o}{dt} - \omega_o^2 \mathbf{r}_o + \frac{q}{m} \left[\frac{d\mathbf{r}_o}{dt} \times \mathbf{B} \right] + \frac{q}{2m} \left[\mathbf{r}_o \times \frac{d\mathbf{B}}{dt} \right] + \mathbf{F} \quad (2)$$

The equation (2) is given in the vector form. Here is the radius-vector of the ion position originating at the equilibrium point of the ion; t is the time; q and m are the charge and mass of the ion; $\mathbf{B}$ is the total static and alternating magnetic fields; γ is the damping coefficient inhibiting ion circulation around the center ω_o ; is the natural frequency of the ion oscillation in a coherence domain; $\mathbf{F}$ is the total force of an action of surrounding particles on the ion that causes the thermal motion of the ion near its equilibrium point; the bold letters denote vectors; the square brackets symbolize vector products. On the left the general form of a potential well inside a coherence domain is shown. On the right the first term takes into account the passive friction, and the second one is determined by the force of the intradomain potential field restoring the ion to its equilibrium point; the third term is the Lorentz force of the magnetic field action on the moving ion which manifests itself in the rotation of the trajectory of the ion thermal motion around the magnetic field line; the fourth one results from the force made by the curl

field generated by the time-varying magnetic field. In the following, we considered the parallel magnetic fields algebraically summed: the static field, $\mathbf{B}_0$, and the alternating field, $\mathbf{B}_{AC}$, harmonically varying.

On fig. 1A the drawing of the approximate form of the potential well inside a coherent domain is shown. In the center of a domain the potential slowly nonlinearly increases, step by step enlarging the rate of its rise. Within the peripheral region (between two vertical dotted lines R_i and R_e) its rising becomes especially sharp – it is the before mentioned domain border of finite thickness. In the area with $R_e > R_i$ the incoherent medium is located. On the right-hand the drawing of the coherence domain is shown, where the incoherent component outside the coherence domain border is shown too.

When the combined magnetic fields with a cyclotron frequency, corresponding to dissolved amino acid, become switched on, the dissolved amino acid ions can be located in arbitrary points, others than the domain center. All these ions started their comparatively slow rotation around different centers, other than the domain center. But these centers will begin to slide automatically step-by-step toward the domain center, because the minimal potential energy is located there. In some time, all the amino acid ions will gather on concentric orbits around domain center, forming the stable configuration with minimal potential energy. After that they become their rotation along the concentric orbits inside the domain, increasing their kinetic energy. It is rather effective because it will be not only due to the increase in the radius elongation, but especially because the kinetic energy will be especially grow within the high potential gradient in the layer $R_e > R_i$ of high nonlinearity in its potential growth. The group of ions leaves the coherence domain at the border R_i and enters into the incoherent medium, creating its contribution into formation of the prominent peak of the current through the solution. The viscosity of the coherent water inside the domains is about an order lower than in the incoherent media. It permits to increase the ion energy (which is proportional to squared ion velocity) to one or even two orders that is quite enough for leaving the ion from the domain. These processes would not practically influence on the total temperature of the domain and the total solution because of low mass of the total amino acid ions compared to the total mass of the surrounding water. Of course, the effectiveness of such sort of accelerator is extremely low, but it is quite enough for ion leaving a domain.

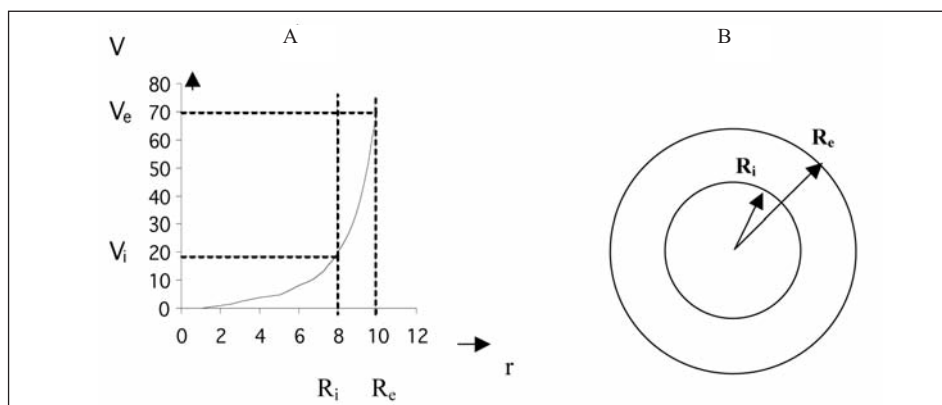


Fig. 1. A) The general form of a potential well inside a coherence domain. B) The coherence domain with the part of incoherent component $R_e > R_i$ area are shown. (Details are explained in the text of this article)

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Immunotropic effects of low-level microwave exposures *in vitro*

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Abstract

The reasons are presented for which the interest of many investigators is directed to the possible immunotropic influences of low energy microwave (MW) electromagnetic fields (EMFs), in terms of their potential harmful effects and also in the perspective of possible therapeutic applications. The available literature data on the influence of MWs on the immune system are up to now fragmentary, describing the changes of a few immune functions, mainly phagocytosis, lymphocyte proliferation, or antibody production, and are frequently controversial or not confirmed by the results of repeated experiments. On the grounds of results of the two series of own experiments the authors indicate which methodological elements, including precise dosimetric circumstances and the timing of exposure in relation to the cell cycle and the initial functional state of exposed cells may be decisive for the final effect of exposure *in vitro*.

Key words: microwave immunotropic effects, sensitivity of immune cells to MWs, cell cycle, functional state of exposed cells.

Introduction

Rapid development of radiocommunication and radiolocation, and widespread use of different electronic devices (mobile phones, radar and microwave broadcast stations) increase the environmental level of electromagnetic radiation. This, in turn, increased the interest of many investigators on possible pathogenic influences of electromagnetic emitters and, on the other hand, on the potential of their therapeutical applications.

After 30 years of research into this area, there is still insufficient information on the specific biological influence of nonthermal intensity of electromagnetic fields (EMFs)¹. According to WHO Environmental Health Criteria WHO², nonthermal inten-

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sities of microwaves (MW) are presently recognized as a “weak factor of biological influence”. This imprecise description has initiated searches for biological detectors sensitive enough to measure “weak biological influence” of MWs, and one of main candidates is the immune system, which is able to react in a measurable way to discrete environmental stimuli. As an important part of homeostatic neuroendocrine-immune network of the organism, the immune system is responsible for efficient defense against infections, regenerative support for injured tissues, and maintenance of immune tolerance toward self or foreign but neutral elements³⁻⁵. These different reactions of the immune system can be investigated using *in vitro* or *in vivo* tests to evaluate possible influences of external stimuli (e.g., drugs or physicochemical factors). Available data on the influence of MWs on the immune system are fragmentary, report on changes of few immune functions, mainly phagocytosis, lymphocyte proliferation, or antibody production, and are frequently controversial or not confirmed by the results of repeated experiments⁶⁻⁹.

Some authors¹⁰ conclude that studies of MW-exposed immune cells have shown no damage or change until the cells were heated, while others¹¹⁻¹³ report immunosuppressive or immunostimulatory phenomena in animals with long-term exposure to low-level MW fields.

Depending on conditions of exposure, frequency and modulation of the radiation, as well as on animal species used in the experiments, various symptoms of either stimulation or inhibition of certain immune reactions have been reported. Guy *et al.*¹⁴ in the lifetime exposure of rats to MWs (pulsed 2450 MHz, SAR 0.15 - 0.4 W/kg) found lowered response of blood lymphocytes to mitogen phytohaemagglutinin (PHA), while Śmiałowicz¹⁵ after exposure at the same wave frequency, although at higher power intensities (SAR 1 – 5 W/kg) reported increased mitogenic response of lymphocytes. Investigating the humoral immune response in mice exposed to 9.4 GHz at SAR 0.015 W/kg, depending on the carrier wave modulation, Vayert *et al.*⁹ found enhancement or lowering the response.

Even the epidemiological investigations of workers exposed to MW radiation did not confirm the existence of measurable shift in the immune status of the investigated populations, despite some observations on abnormalities in single immune parameters in several individuals (e.g. changed number of blood lymphocytes, lowered level of serum immunoglobulins or weaker response of lymphocytes to mitogens). In the available literature no reports exist on the complex assessment of immune phenomena under EMF influence, all investigations were aimed to evaluate only selected, fragmentary reactions of the system or selected types of immune cells. At the present state of knowledge it is, therefore, not possible to conclude about the specific immunotropic potencies of MW radiation, as the assessment of the immunotropic potency requires a general insight into the whole complex immune network, taking in advance the determination of immune status of the host or the investigated cellular population prior to the MW exposure.

The final effect of exposition of biological material to MW radiation depends on the physical properties of applied electromagnetic field on the one side, and on the functional state of exposed living target on the other. The EMFs used in different experiments may differ in countless dosimetric elements, including wave length and frequency, pulse modulation, intensity of EMF influencing the degree of specific absorption rate (SAR) and duration of the exposure. The functional characteristics of biological material, e.g. blood mononuclear cells mainly used for *in vitro* studies, is

even more complex. The EMF exposure may affect the cell at different levels of its structure: the surface receptors changing their distribution and conformation, the cellular membrane changing its rigidity and permeability, mitochondrial metabolic activity, transcription and translation processes or several of these elements at different intensities.

The mononuclear cells isolated from peripheral blood remain in their most stable and inert metabolic state, the G0 phase of cell cycle, in which the cell represents low sensitivity to external influence^{16,17}. When the cell cultured *in vitro* enter more active phases of cell cycle (G1, S, G2, M), its sensitivity to EMF influence may change significantly. In these circumstances the cells exposed to EMF after isolation from the blood, like in the most published studies *in vitro*, and after that cultured, specifically stimulated and tested for their different activities, may not display any significant changes. The exposition to EMF during the culture, of already activated cells, although methodically much more difficult, may deliver better insight into the potential immunotropic effects of the exposition.

One of the best methods of evaluation of immunotropic influences of EMF administered *in vitro* is the system of microcultures of mononuclear cells isolated from the blood (PBMC), representing *in vitro* the abilities of the immune system *in vivo*. The advantages of the method are accessibility of human cells, donor safety, and wide repertoire of immune tests which can be performed.

Recently, using these methods, we investigated the behavior of PBMC in a microculture system after exposure to pulsed (5 μ sec pulses) 1300MHz microwaves (10W/m², SAR 0.18W/kg)¹⁸. The exposure resulted in the increased immunoregulatory activity of T cells, increased production of IL-10, increased IL-1 production by monocytes, and decreased concentration of IL-1ra in culture medium. We concluded that MW may support the inductive phase of immune response, increasing the activity of monocytes and T cells. The special feature of this experiment was that cells were exposed to EMF before the culture, indicating that at the time of exposure they remained metabolically neutral (G⁰ phase of cell cycle), which is normal for lymphocytes freshly isolated from blood.

In the *in vivo* situation, the accidental or deliberate exposure of the individual to MW may influence neutral or active immune cells, both normally present in the body. We have questioned how the active cells, e.g., stimulated *in vitro* with mitogens and entering G1 and S phases, will react to the subsequent exposure to MW. To evaluate the problem, a special anechoic chamber was constructed and technically tested in the Department of Microwave Safety, Military Institute of Hygiene and Epidemiology in Warsaw, Poland¹⁹. The chamber, containing the microplate with cultured cells and MW-emitting antenna, was installed inside the ASSAB CO2 incubator so the PBMC could be exposed to MW at different periods of culturing without removing them from the incubator.

The miniature anechoic chamber (MAC) was a cube of 40 × 40 × 40cm of external dimensions. The internal walls of the chamber were covered by pyramid absorbers to guarantee the absorption of incident field only by the samples. MW reflected from absorbers could be neglected. The absorbers also protected the test samples from the radiation reflected from metal walls of incubator and maintained the homogeneity of MW field around the samples inside the chamber. The plate with cultured cells was located in the middle part of chamber, while the mobile handset used as a MW-emitting antenna was placed on the floor of chamber. The internal dimensions of the chamber were 23 × 23 × 23 cm.

Experiment I. Immunotropic influence of 900 MHz microwave GSM signal on human blood immune cells activated in vitro

Blood samples were collected by vein puncture from healthy donors. PBMC were isolated on Ficol-Paque gradient, and after determination of cell viability (usually no less than 80% viable cells), the microcultures were set up in triplicates (10^5 cells/0.2 ml RPMI + 15% autologous inactivated serum) in Nunclon microplates. Respective triplicates were left without stimulation or stimulated with phytohemagglutinin (PHA, HA16, Murex Biotech Ltd Dartford U.K., 0.4 μ g/cult.) or with concanavalin A (Con A, Sigma, 8 μ g/cult.). The plates were placed inside the anechoic chamber in the ASSAB incubator at 37°C and 5% CO₂. An identical plate of control cultures was also set up and placed in the ASSAB incubator beyond the chamber. At 24h of incubation, rearrangements of the cultures were performed as described elsewhere^{18,20,21}.

As a result of rearrangements of cultures performed at 24 h, the following parameters of T cell and monocyte activities were measured at the end of cultures: T lymphocyte response to PHA and to Con A, saturation of IL-2 receptors, T cell suppressive activity (SAT index), and the index of monocyte immunogenic activity (LM) related to the ratio of produced monokines (IL-1 β versus IL-1ra)²⁰.

For the last 18h of incubation, ³H-labelled-thymidine (³HTdR), Amersham, U.K., spec act. 5 Ci/mM) was added into the cultures in a dose of 0.4 μ Ci/cult.

At the beginning of each of the three consecutive days of incubation, the cultures placed in the anechoic chamber were exposed to MW (900MHz, 20V/m, SAR 0.024W/kg) for 15 min. Control cultures were not exposed to MW.

At 72h the cultures were harvested and incorporation of ³HTdR was measured in Packard Tri carb 2100 TR scintillation counter. The results were calculated as a mean value of dpm (desintegrations per minute) per triplicate of cultures $\pm$ SD. The experiments were repeated 10 times, and the results observed in the exposed cultures were compared with those obtained in the control cultures. The data were analyzed with STATGRAPHICS PLUS 4.0 version (Nr. 471000349). The differences between the mean values were assumed statistically significant if the p values, calculated with the use of U Mann-Whitney's test, were lower than 0.05.

Results and discussion

The relatively short time of exposure of cultured cells to MW (15 min, administered repeatedly at the beginning of each of the three consecutive days of culturing) was chosen deliberately. First, our intention was to check the effects of exposure similar in duration to the average use of a mobile phone. Second, the cells, stimulated with mitogens, were exposed immediately after entering the G1 phase of cell cycle (first day exposure), again when the majority of cells responding to mitogen entered the S phase (second day exposure), and finally when the responding cells, after replication of DNA, reached stage G2 and mitosis (third day exposure). In this way the repeated exposures to MW covered the main periods of metabolic activity during the cell cycle of cultured cells^{16,19}.

The results of 10 experiments are presented in Table 1. The data obtained indicate that activity of lymphocytes and monocytes tested in vitro increased significantly under the influence of MW administered during the culture. The proliferative response of T lymphocytes exposed to MW increased from the value of 60.7 to 82.8 dpm in response to PHA (p < 0.001) and from the value of 55.9 to 73.8 dpm in response to Con A

Table 1 - Influence of MW (900 MHz) on the activity of T lymphocytes and monocytes in microcultures

Cultures (N = 10)	Tested parameter				
	Response to PHA dpm x 10 ³ /cult	Response to Con A dpm x 10 ³ /cult	Saturation of IL-2 receptors (%)	T cell suppressive T activity SAT (%)	LM index
Control	60.7 ± 18.7	55.9 ± 18.3	85 ± 3	36 ± 2	8 ± 2
Exposed to MW	↑ 82.8 ± 26.2	↑ 73.8 ± 25.7	84 ± 2	34 ± 2	↑ 18 ± 3
Statistical significance	p < 0.001	p < 0.001	p = 0.3920	p = 0.0964	p < 0.001

($p < 0.001$). The exposure to MW also increased the immunogenic activity of monocytes. The value of LM index, which depends on the ratio of IL-1 β to IL-1ra²⁰, (both monokines produced by monocytes), increased from the value 8.0 to the value 18.0 ($p < 0.001$). In contrast to the suppressive activity index (SAT), which represents regulatory function of T cells, the saturation of T lymphocyte receptors with interleukin 2 did not change under the influence of exposure to 900 MHz MWs.

The experiments presented here show for the first time that human lymphocytes and monocytes, induced in culture into active phases of their cell cycle (G1 in terms of monocytes and G1 and S in terms of T cells), further accelerate their metabolic activity under additional stimulus created by the exposure to 900 MHz GMS signal.

In contrast to the *in vitro* conditions, where freshly isolated PBMC remain in G0 phase, the immune cells of living organism represent all possible stages of cell cycle. To mimic *in vitro* the *in vivo* situation, we have used for our experiments the anechoic chamber installed in the ASSAB incubator. This technique opens the way to evaluate the possible influence of EMF on different phases of the cell cycle of immune cells. Our observations suggest that a 900 MHz GSM signal is immunostimulatory and may increase the immune reaction of lymphocytes and monocytes already participating in the immune response.

Testing possible immunotropic influences of 900 MHz GSM signal on human blood lymphocytes Scarfi *et al.*²² did not found any changes in proliferative rate of cells exposed for 24 hour before setting up the cultures. Similar timing of exposure (irradiation before the culturing) was applied for human lymphocytes by Tuschi *et al.*²³. They found no changes in several cytokine production and cytotoxic potential of lymphocytes exposed to 1950 MHz, SAR 1 mW/g. The both groups of authors conclude that tested radiofrequencies did not evoke any adverse influences on human immune cells. Nevertheless, in the light of cited above our experiments, the improper timing of irradiation could be responsible for observed negative results.

Experiment II. Immunotropic influence of 1300 MHz MW on cultures of blood mononuclear cells derived from normal donors or patients suffering from chronic virus B hepatitis.

The effect of irradiation may also be dependent on the initial immune state of the donor of blood lymphocytes. Two groups of blood donors, one of healthy individuals

(HD) and the other of patients suffering from chronic virus B hepatitis (HV) were enrolled into our experiments in which blood lymphocytes were exposed to 1300 MHz pulse modulated microwaves at 330 pps with 5 μ s pulse width, or left without irradiation²⁴. The specific absorption rate (SAR) was measured and the value of SAR = 0.18 W/kg was recorded. The microcultures of PBMC were subsequently set up to determine several parameters characterizing the T cell immunocompetence and monocyte immunogenic activity, including: proliferative response to mitogens (PHA, Con A), saturation of IL-2 receptors, T cell suppressive activity (SAT index), monocyte immunogenic activity (LM index) and production of chosen cytokines.

Results

The same power density of 1 mW/cm² reduced response to PHA in HD cultures and significantly increased this response in HV cultures, increased values of SAT and saturation of IL-2 receptors in the both HD and HV cultures (Table 2) and significantly increased production of interferon gamma (IFN γ) and production of tumor necrosis factor alpha (TNF γ) in the HV cultures but not in the HD cultures (Table 3). The results suggest that microwave irradiation (1300 MHz, pulse modulated) may exert distinct immunotropic influence and may enhance the effector immune response in patients with chronic virus B hepatitis, including considerable stimulation of the production IFN γ by immune cells.

Conclusion

The presented data suggest, that exposition in vitro of human blood mononuclear cells to different radiofrequencies of low energy MW (e.g. 900 and 1300 MHz) is potent to modulate the immune activity of lymphocytes and monocytes. The range of affected

Table 2 - Immunomodulatory effects in PBMC cultures exposed to EMF

Test	HD cultures		HV cultures		Statistical significance
	control	EMF exposed	control	EMF exposed	
Spontaneous ³ HTdR incorp.(dpm x 10 ³)	1.9 $\pm$ 0.6	1.6 $\pm$ 0.2	2.9 $\pm$ 0.7	$\downarrow$ 1.8 $\pm$ 0.3	HDc/HVc p< 0.01 HVc/e p< 0.01
Response to PHA (dpm x 10 ³)	67.1 $\pm$ 8.7	$\downarrow$ 45.8 $\pm$ 13.7	75.8 $\pm$ 9.8	$\uparrow$ 98.2 $\pm$ 13.7	HDc/e p< 0.01 HVc/e p<0.05
Response to Con A (dpm x 10 ³)	37.2 $\pm$ 11.7	46.9 $\pm$ 2.8	40.2 $\pm$ 16.8	47.7 $\pm$ 2.4	HDc/e NS HVc/e NS
SAT index	11.7 $\pm$ 9.4	$\uparrow$ 29.7 $\pm$ 7.3	19.8 $\pm$ 11.4	$\uparrow$ 28.9 $\pm$ 11.8	HDc/e p< 0.01 HVc/e p< 0.05
Saturation of IL-2 receptors	72.3 $\pm$ 4.6	$\uparrow$ 91.1 $\pm$ 11.1	72.1 $\pm$ 7.6	$\uparrow$ 87.1 $\pm$ 10.4	HDc/e p< 0.01 HVc/e p< 0.01
LM index	5.7 $\pm$ 3.1	7.6 $\pm$ 4.2	9.7 $\pm$ 4.2	$\uparrow$ 19.7 $\pm$ 8.2	HDc/e NS HVc/e p< 0.01

HD: cultures of PBMC from healthy donors, HV: cultures of PBMC from patients with chronic virus B hepatitis.

W. Stankiewicz et al: Immunotropic effects of low-level microwave exposure *in vitro***Table 3** - Cytokine production in control and EMF exposed PBMC cultures (pg/ml)

Cytokines	HD cultures		HV cultures		Statistical significance
	control	EMF exposed	control	EMF exposed	
IL-1 β	287 $\pm$ 120	298 $\pm$ 189	510 $\pm$ 212	741 $\pm$ 259	HDc/e NS HVc/e p< 0.05
IL-1ra	1312 $\pm$ 692	↓ 670 $\pm$ 256	2312 $\pm$ 672	2670 $\pm$ 1456	HDc/e p< 0.01 HVc/e NS
IFN γ	630 $\pm$ 92	510 $\pm$ 118	673 $\pm$ 92	↑ 1367 $\pm$ 847	HDc/e NS HVc/e p< 0.01
TNF α	1987 $\pm$ 986	2421 $\pm$ 475	1983 $\pm$ 936	↑ 3425 $\pm$ 875	HDc/e NS HVc/e p< 0.01
IL-10	311 $\pm$ 123	↑ 623 $\pm$ 193	471 $\pm$ 149	↓ 166 $\pm$ 59	HDc/e p< 0.01 HVc/e p< 0.01

HD: cultures of PBMC from healthy donors, HV: cultures of PBMC from patients with chronic virus B hepatitis

immune parameters depend not only on the wave length, frequency and intensity of EMF but also on the timing of exposures (before or during the culture) and on the initial immune status of the donor of immune cell.

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