

Effect of Whole-body Exposure to High-frequency Electromagnetic Field on the Brain Electrogeny in Neurodefective and Healthy Mice

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Abstract: A direct registration of brain cortical and hippocampal activity during a high-frequency electromagnetic field (HF EMF) exposure was performed. All experimental procedures were done under urethane anaesthesia (20%, 2g/kg i.p.) in Lurcher mutant mice, wild type (healthy littermates) were used as controls. Experimental animals were exposed to the HF EMF with frequency corresponding to cellular phones. Our method is based on the use of gel electrodes (silicon tubes or glass microcapillaries filled with agar) where the connection with classical electrodes is located out of HF EMF space. ECoG evaluation showed a distinct shift to lower frequency components but clear effect has been observed only in wild type (healthy) mice whereas in Lurcher mutant mice only gentle differences between frequency spectra were found. Measurement of hippocampal rhythmicity showed gentle changes with increase of higher frequencies (i.e. opposite effect than in cortex) and changes in theta oscillations registered from a dentate gyrus and CA1 area in both types of animals (healthy and mutant). These findings support the idea about possible influencing the central nervous system by HF EMF exposure and support also some recent results about possible health risks resulting from cellular phones use.

Key words: High-frequency electromagnetic field – EEG recording – Neurodegeneration

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Introduction

New telecommunication technologies have been introduced without full provision of information about their character and without prior discussion about possible consequences for human health. As the costs of mobile phone technology have fallen, their use has increased dramatically and the overall levels of exposure of the population as a whole have therefore increased.

The high-frequency electromagnetic fields (HF EMF) produced by external sources may cause both thermal and non-thermal biological effects. Thermal effects are those caused by the rise in temperature produced by the energy absorbed from oscillating electric fields. Main consequence is the increasing of temperature and it continues to do so until the heat input is balanced by the rate at which it is removed, mostly by blood flowing to and from other parts of the body. It had not yet been proved possible to measure these small changes in temperature directly, except those at the outer skin [1] and, although temperature is a more direct determinant of thermally induced tissue damage, the majority of theoretical studies up to the present time have restricted themselves to the computation of specific absorption rate (SAR) alone. The relationship between the SAR and the resulting temperature rise is complex, and significantly dependent on antenna configuration, location and frequency. The most problematic feature of a temperature calculation is modelling the effect of blood flow on heat transfer. In another published study [2] the heat deposition within the head was computed. The thermal model includes the convective effects of discrete blood vessels, whose anatomy was determined using magnetic resonance angiography of a healthy volunteer. For a 915 MHz dipole antenna with a time-averaged power output of 0.25 W (equivalent to a typical mobile phone), this study results in a SAR of about 1.6 W/kg and predicts a maximum brain temperature rise of 0.11 °C in the steady state.

The principles of non-thermal effects are based on a possible influence of small energy produced during cellular phones activity. The energy quanta of HF EMF at 0.9 and 1.8 GHz equal 4 and 7 meV, respectively. Both these values are extremely small compared with the energy of around 1 eV needed to break the weakest chemical bonds in genetic molecules (DNA). So it seems impossible that HF EMF could damage DNA directly, which might start cells on the tumours pathway, but HF EMF could produce other effects, too. In general, detectable changes can arise only if the effect of the electric field within the biological system exposed to RF fields is not masked by thermal noise. Thermal noise or random motion, also known as Brownian motion, is due to the thermal energy that all objects possess at temperatures above absolute zero. All components of biological tissue – ions, molecules and cells – are therefore in a constant motion. Another mechanism involving cells concerns the attraction between them in the presence of an electric field [3]. The electric field polarises the cell, which may act as an electric dipole and attracts similarly polarised cells. For typical cells and frequencies below about 100 MHz, the energies involved are calculated to become comparable to thermal

noise in electric fields of $E = 300 \text{ V/m}$. The energies are calculated to become appreciably less for HF EMF but authors suggest that, since these values would depend on the detailed structure of the biological elements involved, the possibility of biological effects for fields of this size cannot be excluded. Other possible biological effects are associated with cell membranes and the movement of currents through the membrane in either direction. Membranes are known to have strongly non-linear electric properties [4]. When a voltage is applied across the membrane, the current that flows is not always proportional to the voltage. Part of this non-linearity may, in fact, be due to the effect of the electric field on the proteins in the membrane or nearby, which assists the flow of the product currents through the membrane. The membrane also acts as a rectifier. If an oscillating voltage (electric field) is applied across a rectifier, the total current that flows when the field is in one direction is not balanced by the current when the field is in the other: An AC field produces a net DC current and hence a net flow of products through the membrane. However, the response time of the ion gates are very much slower than the period of microwave frequencies and, using data obtained from measurements on membranes [4], it has been shown that, for electric fields of 200 V/m , the relative change in the membrane potential is very small [3]. Another hypothesis is that the interaction with biological tissue depends on the coherence of the HF EMF. Experimental evidence supporting this idea has been given by Litovitz et al. [5] but not yet independently replicated. Other recent studies found that HF EMF can affect membrane proteins [6] and can change the movement of ions across membranes [7] and experimental results suggest that HF EMF at levels produced by mobile phones might influence ion channels (especially voltage-gated Ca^{2+}) and other membrane proteins of neurons in the brain under normal conditions. Recent experimental studies described significant neuronal damage in hippocampus, cerebral cortex and basal ganglia after a short-term exposure [8], on the other hand no significant effect of long-term HF EMF on hippocampal structures has been published until now [9]. Studies of the EEG in animals have generally not employed conditions that are directly relevant to mobile phone technology, and the results have been mixed. However, some recent experiments have produced evidence of non-thermal effects from RF fields on brain activity [10, 11].

The main aim of the presented study was an electrophysiological investigation of the influence of high-frequency electromagnetic field (HF EMF) on brain functions in Lurcher mutant mice, which represent an excellent model of neurodegenerative disease, and on healthy control animals.

Materials and Methods

Lurcher mutant mice (Lurchers) represent a natural model of genetically determined olivocerebellar degeneration [12]. Heterozygote individuals (+/Lc) are characterized by the postnatal complete loss of cerebellar Purkinje cells (excitotoxic apoptosis) and by the decreased number of granule cells and inferior

olivary neurons (secondary to the loss of Purkinje cells). Affected homozygots (*Lc/Lc*) are not viable. Unaffected homozygots, wild type mice (+/+) are completely healthy and serve as controls. Our previous results suggest that in Lurchers some cognitive functions are changed – in series of experiments studying the development of spatial learning in *Lc/+* and *+/+* during the first month of life using the standard Morris water maze [13, 14]. Some neurons of Lurchers are more sensitive to neurotoxic substances [15] and other experiments discovered a higher degree of the CNS excitability in *Lc/+* when compared with *+/+* using a method of audiogenic epilepsy [16]. Similar findings were obtained in experiments which measured brain cortical activity after previous electrical and drug stimulation [17, 18]. Also significant changes of hippocampal activity (LTP) were found in anesthetized *Lc/+* in comparison with *+/+* [19].

We expect that some brain structures in Lurchers would be more sensitive (i.e. with lower threshold) to different kind of stimulation and neuronal injury. It is possible to assume a higher effect of other types of stimulation on such neurons, including HF EMF. A direct registration of brain cortical and hippocampal activity during exposure to high-frequency electromagnetic field (HF EMF) and a depiction of possible changes in the hippocampal rhythmicity were performed. All experimental procedures were done under urethane anaesthesia (20%, 2g/kg i.p.) in adult (10-12 weeks) Lurker mutant mice (n=20) and wild-type (healthy littermates) which served as controls (n=20). Experimental animals were exposed to HF EMF with frequency of 880-890 MHz, similar to the range used by mobile phones. As a source of the HF EMF a high-frequency generator with a high-frequency amplifier was used. The power output was 10 W. The radiation was directed by a waveguide to a space with the animals placed (modified stereotaxic frame in the Faraday's chamber). The area of the orifice was 600 cm².

Serious problem in the use of classical EEG technology is the presence of conductive (contact) electrodes in brain tissue resulting to discontinuity of HF EMF and possible electrolytical processes caused by the nonhomogeneity on the boundary line of metal-tissue. Our original method [20] is based on the use of gel electrodes (silicon or glass capillary tubes filled with agar). The corresponding conductivity is achieved by supplementation of saline and final biophysical quality is adequate to the brain tissue. The connection with classical (wire) electrodes is performed out of the HF EMF space. Spontaneous electrocorticogram (ECoG) was measured as 2 min segments from continuously recorded activity either without HF EMF exposure or with it. Final calculation with Fourier analysis and averaging were performed off-line on DISYS-system (Software for data acquisition and analysis). Hippocampal activity was recorded by glass capillary filled with agar (used as a registration electrode), grounding electrode was a silicon tube filled with agar located on the neck. Stereotactic coordinates [21] – ref. point bregma: AP: 2.0; L: 1.5; V: 1.3 (CA1) – 2.0 (hilus DG). DISYS-system for a final averaging according to Fourier analysis with emphasis on a theta-oscillation especially was used.

Results

Experiments confirmed the possibility of direct EEG registration during exposure to HF EMF. Recording time for each experimental animal was approximately 40 minutes and DISYS system was able to describe spontaneous activity either before influencing by the HF EMF or during it. Examples of five seconds recording in wild type mouse (Fig. 1, 2) and in Lurcher mutant mouse (Fig. 3, 4) are shown below. Final evaluation and averaging of cortical activity showed a distinct shift to lower frequency components. These findings are in accordance with older and recent results but clear effect only in wild type (healthy littermates) was observed (Fig. 5) whereas in Lurcher mutant mice only gentle differences between frequency spectra were found (Fig. 6). On the other hand, changes of hippocampal activity and shift towards higher frequencies in both types of animals (healthy and mutant) were observed (Fig. 7, 8) as well as changes of theta rhythmicity (Fig. 9, 10).

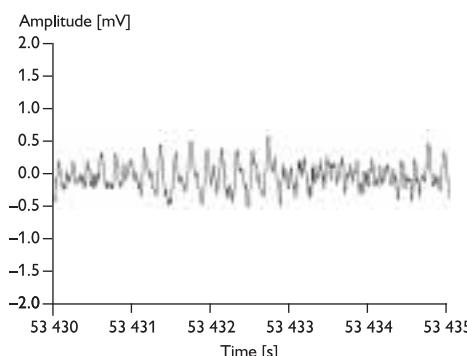


Figure 1 – Spontaneous brain cortical activity (EEG) in wild type mouse without HF EMF exposure; 5 s recording using agar (gel) electrodes.

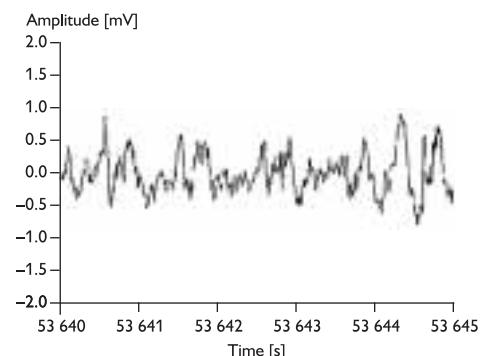


Figure 2 – Spontaneous brain cortical activity (EEG) in wild type mouse affected by the HF EMF; frequency of exposure 885 MHz.

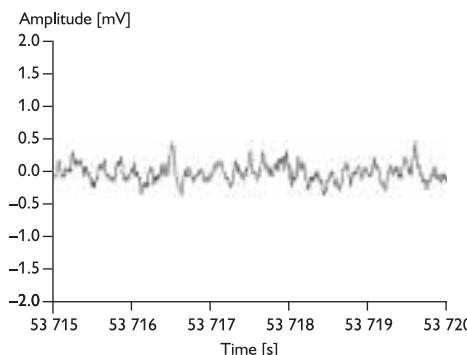


Figure 3 – Spontaneous brain cortical activity (EEG) in Lurcher mutant mouse without HF EMF exposure.

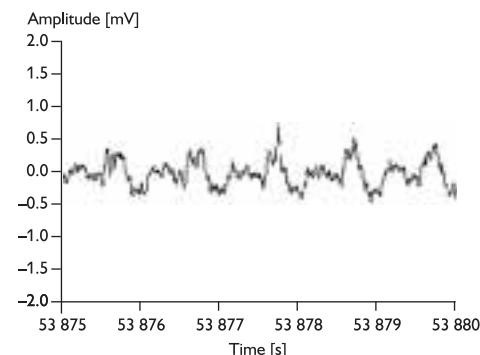


Figure 4 – Spontaneous brain cortical activity (EEG) in Lurcher mutant mouse affected by the HF EMF; frequency of exposure 886 MHz.

Discussion

Artificial source of HF EMF used in our experimental paradigm influenced spontaneous cortical activity. A shift towards lower frequencies was observed with gentle differences between Lurcher mutant mice and wild type mice. Our main working hypothesis was that some brain structures in Lurchers should be more sensitive (i.e. they have lower threshold) to different kind of stimulation and neuronal injury. We suppose that thermal gradient arising between lipo- and hydrophylic structures influences ion movement through membranes of neurons and/or glial cells. However, possible delicate changes were identified rather in the brain of healthy animals. These relatively ambiguous findings support our idea about a different vulnerability of the CNS in mice with neurodefective brain to some physical and chemical factors in comparison with controls. The higher CNS excitability in Lurcher mutant mice and its possible suppression or masking effect on

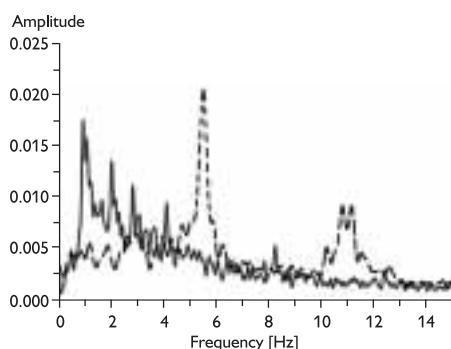


Figure 5 – Fourier analysis of ECoG in wild type mice group (after averaging) before (dashed line) and during (solid line) exposure to HF EMF.

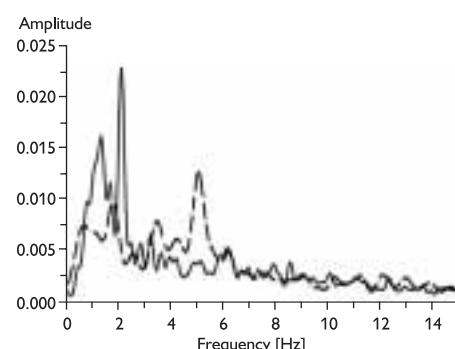


Figure 6 – Fourier analysis of ECoG in Lurcher mutant mice group (after averaging) before (dashed line) and during (solid line) exposure to HF EMF.

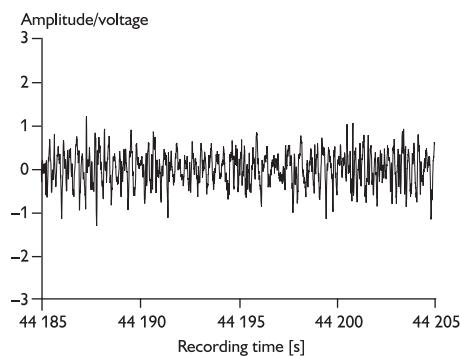


Figure 7 – Example of hippocampal recording in wild type mouse; unaffected animal, area CA1.

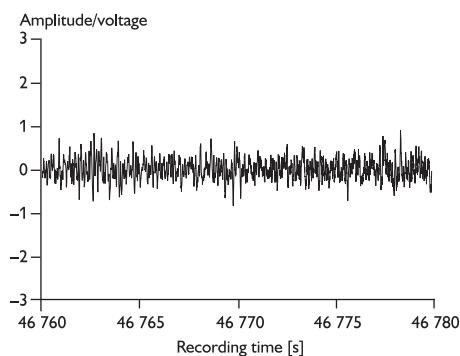


Figure 8 – Example of hippocampal recording in wild type mouse; experimental animal affected by the HF EMF 874 Hz, area CA 1.

the HF EMF influence may be used as an explanation of unsignificant differences between healthy and mice with inborn neurodegeneration. Nevertheless, our general findings are in accordance with previously published investigations of Bawin et al. [22, 23] that exposed cats, which had been previously conditioned to produce selected EEG rhythms in response to a light flash, to low level RF fields. The changes were reported in the performance of the conditioned EEG response task and in various other behavioural parameters. It was argued that the fields acted directly on brain tissue causing a minute release of calcium, resulting in changes in membrane excitability, which could possibly affect EEG rhythms. Takashima et al. [24] reported changes in EEG of rabbits following exposure to a modulated RF field of 1-10 MHz, a frequency range outside the main interest but Shandala et al.[25]; Thuroczy et al.[26] reported about subtle effects on the EEG in rats and rabbits exposed to RF fields within the frequency range of interest. McRee et al. [27] described experiments performed by Rosensteig of the US Environmental Protection Agency, who exposed rats to RF from late fetal life until adult. He saw no changes in either the spontaneous EEG or the electrical responses evoked by flashes of light (visual evoked responses). Mitchell et al. [28]

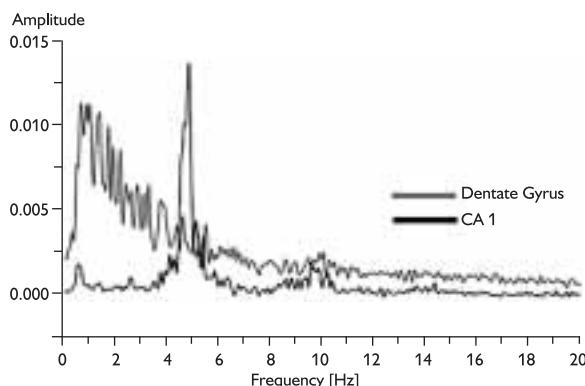


Figure 9 – Fourier analysis of hippocampal activity recorded from area CA1 and dentate gyrus in wild type mouse (without HF EMF exposure); „peak“ of theta oscillation located in CA1.

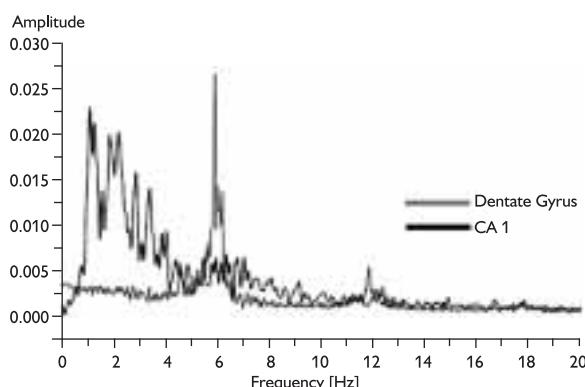


Figure 10 – Fourier analysis of hippocampal activity recorded from area CA1 and dentate gyrus in wild type mouse during HF EMF influence; „peak“ of theta oscillation in dentate gyrus.

reported the findings of a joint project on the same subject carried out in the USA and the former Soviet Union. Both groups exposed rats to fairly intense continuous-wave RF fields for seven hours. Interestingly, both teams found small but statistically significant reduction of power in the EEG, but in different parts of the frequency spectrum.

Laboratory studies investigating the effects of mobile phone signals on the spontaneous EEG in awaked subjects have produced somewhat mixed results. Reiser et al. [29] reported that exposure to GSM signals was associated with increases some 15 minutes later in the power of EEG frequencies of about 10 Hz and higher; Roschke and Mann [30] were unable to detect any differences in EEG spectra related to exposure to GSM signals. A similar inconsistency appears to hold for the study of sleep EEG. Mann and Roschke [31] reported that exposure to GSM-like signals reduced latency to sleep onset, and altered spectral characteristics of REM sleep, although a subsequent study by the same group [32] failed to replicate these findings. In other study [33], exposure to a “pseudo-GSM signal” (15 minute on/off cycles, 900 MHz, duty cycle of 87.5% rather than the 12.5% used in phone signals, and an estimated whole-body SAR of 1 W/kg) was associated with reduced waking after sleep onset and changes in EEG power spectra during the first of the night’s episodes of non-REM sleep. In three studies “event-related potentials” (ERPs) were investigated during exposure to GSM-like signals. In the first [34] visual sensory responses to checkerboard reversal were found to be unaffected during exposure. In two other studies [35, 36] positive effects were reported. In a recent study in Czech Recublic, Jech and colleagues [37] also found changes of visual event related potentials during exposure to HF EMF.

Taken together, main importance of this study was that neurodefective animals and their healthy littermates (as ideal controls) were used. Despite our experimental paradigm (i.e. whole-body HF EMF exposure characterised by lower amount of absorbed energy in comparison with human brain during cellular phone use), presented results suggest that some neuronal populations (cortical and subcortical) respond to this type of radiation. Current WHO recommendation published by Repacholi [38] gives basic principles for individual protection. The limiting of the lenght of calls or using “hands-free” device has probably higher importance in children in which possible thermal effect on the developing brain may be connected with higher healthy risks.

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