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Abbreviations

BBB	Blood-brain barrier
GSM	Gobal System for Mobile Communications
MRI	Magnetic Resonance Imaging
RF	Radiofrequency electromagnetic fields
TEM-cell	Transverse Electromagnetic transmission line cell

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Abstract

The possible risks of radio-frequent electromagnetic fields for the human body, is a growing concern for the society. We have earlier shown that weak pulsed microwaves give rise to a significant leakage of albumin through the blood-brain barrier (BBB). Now we have investigated whether a pathological leakage over the BBB might be combined with damage to the neurons. Three groups of each 8 rats were exposed for 2 hours to GSM mobile phone electromagnetic fields of different strengths. We found, and present here for the first time, highly significant ($p<0.002$) evidence for neuronal damage in both the cortex, the hippocampus and the basal ganglia in the brains of exposed rats.

Introduction

The largest human biological experiment ever. So has the voluntary exposure of the brain to microwaves from handheld mobile phones by one fourth of the world's population been called (Salford et al.2001).

Within the near future microwaves will be emitted also by an abundance of other appliances in the cordless office and also in the home. The possible risks of radiofrequency electromagnetic fields (RF) for the human body, is a growing concern for the society. For a review see Hyland (Hyland 2000). Most researchers in the field have dwelled on the question whether RF may induce or promote cancer growth. Some have indicated increased risk (Hardell et al.2002; Repacholi et al.1997) while most studies including our own have shown no effects (Salford et al.1997a) or even a decreased risk (Adey et al.1999)

The possible risks of microwaves for the human body has attracted interest since the 1960-ies, e.g. before the advent of mobile phones, when radar and microwave ovens posed a possible health problem. Oscar and Hawkins early performed studies on effects of RF upon the BBB (Oscar and Hawkins1977). They demonstrated that at very low energy levels (< 10 W/m^2), the fields in a restricted exposure window caused a significant leakage of ^{14}C mannitol, innulin and also dextran (same molecular weight as albumin) from the capillaries into the surrounding cerebellar brain tissue. These findings, however, were not repeated in a study using ^{14}C-sucrose (Gruenau 1982). In a recent in-vitro study it has been shown that EMF at 1.8 GHz increases the permeability to sucrose of the BBB (Schirmacher et al. 2000). Shivers (Shivers et al.1987; Prato et al.1990) examined the effect of MRI upon the rat brain. They showed that the combined exposure to RF, pulsed and static magnetic fields gave rise to a significant pinocytotic transport of albumin from the capillaries into the brain.

Inspired by this work, our group has since 1988 studied the effects of different intensities and modulations of 915 MHz RF in a rat model where the exposure takes place in a TEM-cell during various time periods. In series of more than 1600 animals, we have proven that subthermal energies from both pulse-modulated and continuous RF fields – including

those from real GSM mobile phones - have the potency to significantly open the BBB for the animals´ own albumin (but not fibrinogen) to pass out into the brain and to accumulate in the neurons and glial cells surrounding the capillaries (Malmgren1998; Persson et al.1997; Persson and Salford 1996; Salford et al.1992, 1993, 1994, 1997b, 2001) (fig 1). These results are duplicated recently in another laboratory (Töre et al. 2001). Similar results are found by others (Fritze et al.1997).

We and others (Oscar and Hawkins1977; Persson et al.1997) have pointed out that when such a relatively large molecule as albumin may pass the BBB, also many other smaller molecules, including toxic ones, may escape into the brain due to the exposure to RF. We have hitherto not concluded that such leakage is harmful for the brain. It is shown by Hassel, however, that autologous albumin injected into the brain tissue of rats, leads to damage to neurons at the injection site when the concentration of albumin in the injected solution is at least 25% of that in blood (Hassel et al.1994). In the present study, we have investigated whether leakage over the BBB might cause damage to the neurons.

Material and Methods

A Transverse Electromagnetic transmission line cell (TEM-cell) used for the RF exposure of rats was designed by dimensional scaling from previously constructed cells at the National Bureau of Standards (Crawford1974). TEM-cells are known to generate uniform electromagnetic fields for standard measurements. A genuine GSM mobile phone with a programmable power output is connected via a coaxial cable to the TEM-cell. No voice modulation was applied.

The cell is enclosed in a wooden box (15 x 15 x 15 cm) that supports the outer conductor and central plate. The outer conductor is made of brass-net and is attached to the inner walls of the box. The centre plate, or septum, is constructed of aluminium.

The TEM-cells are placed in a temperature-controlled room and the temperature in the TEM-cells kept constant by circulating room air through holes in the wooden box.

The SAR-distribution in the rat brain has been simulated with the FDTD-method (Martens et a. 1993) and found to vary less than 6 dB in the rat brain.

The rats are placed in plastic trays (12 x 12 x 7 cm) to avoid contact with the central plate and outer conductor. The bottom of the tray is covered with absorbing paper to collect urine and faeces.

Thirty-two male and female Fischer 344 rats aged 12 - 26 weeks and weighing 282 ± 91 g were divided into 4 groups of each 8 rats. The peak output power from the GSM mobile telephone fed into two TEM-cells simultaneously for 2 hours were 10 mW, 100 mW and 1000 mW per cell, respectively. This exposed the rats to peak power densities of 0.24. 2.4 and 24 W/m^2, respectively. This exposure resulted in average whole-body specific absorption rates (SAR) of 2 mW/kg, 20 mW/kg and 200 mW/kg, respectively. For further details about exposure conditions and SAR calculations, see (Martens et al. 1993; Malmgren 1998). The fourth group of rats was simultaneously kept for 2 hours in non-activated TEM-cells. The animals were awake during the exposure and could move and turn within the exposure chamber.

The animals in each exposure group were allowed to survive for about 50 days after exposure. They were carefully observed daily for neurological or behavioural abnormalities during this period at the end of which they were anaesthetized and sacrificed by perfusion-fixation with 4% formaldehyde.

The brains were removed from the skull by non-traumatic technique (resection of bone structures at the skull base, followed by a midline incision from the foramen magnum to the

nose) after an extended in situ post mortem fixation time of 30 minutes. Each brain was sectioned coronally in 1-2 mm thick slices, which all were embedded in paraffin and cut at 5 micrometer, stained for RNA/DNA with cresyl violet to show dark neurons. Applying albumin antibodies (Dakopatts), albumin is revealed as brownish spotty or more diffuse discolorations (Figs 1a and b). The microscopical analysis was performed blind to the test situation.

The occurrence of "dark neurons" was judged semi-quantitatively by the neuropathologist as 0 (no or occasional dark neurons), 1 (moderate occurrence of dark neurons) or 2 (abundant occurrence). The Kruskal Wallis one-way analysis of variance by ranks was used for a simultaneous statistical test of the score distributions for the 4 exposure conditions. When the null hypothesis could be rejected, comparisons between controls and each of the exposure conditions was made with the Mann-Whitney non-parametric test for independent samples.

Results and discussion

Controls and test animals alike showed the normal diffuse positive immuno-staining for albumin in hypothalamus, a kind of built-in method control.

Control animals showed either no or an occasional and often questionable positivity for albumin outside the hypothalamus. In one animal a moderate amount of dark neurons were observed while in all the other animals no such change was present.

Exposed animals usually showed several albumin positive foci around the finer blood vessels in white and gray matter. Here the albumin had spread in the tissue in between the cell bodies, and surrounded neurons, which were either free of albumin or in some foci containing albumin. Also scattered neurons, not associated with albumin leakage between the neurons, were positive.

The cresyl violet staining revealed scattered and grouped dark neurons, which were often shrunken and dark staining, homogenised with loss of discernible internal cell structures. Some of these dark neurons were also albumin positive or showed cytoplasmic microvacuoles indicating an active pathological process. There were no haemorrhages and no discernible glial reaction, astrocytic or microglial, adjacent to changed neurons. Changed neurons were seen in all locations, but especially the cortex, hippocampus and basal ganglia, mixed in among normal neurons (fig 2). The percentage abnormal neurons is roughly appreciated to be maximally around 2 %, but in some restricted areas dominated the picture.

The occurrence of dark neurons under the different exposure conditions is shown in figure 3 which shows a significant positive relation between EMF dosage (SAR) and number of dark neurons.

A combined non-parametric test for the 4 exposure situations simultaneously revealed that the distributions of scores differed significantly between the groups ($p<0.002$).

We present here for the first time evidence for neuronal damage caused by non-thermal microwave exposure. The cortex as well as the hippocampus and the basal ganglia in the brains of exposed rats contain damaged neurons. We realise that our study comprises few animals, but the combined results are highly significant and exhibit a clear dose-response relation.

The observed dark neurons are deemed not to be artefacts for the following reasons. The brains were perfusion fixed in situ and removed atraumatically. The dark neurons were intermingled with normal appearing neurons (see fig 2a,b). Further, the presence of vacuoles in several of the dark neurons is a clear sign that damage occurred in the living animal. We cannot exclude that the neuronal change described may represent apoptotic cell death.

The neuronal albumin uptake and other changes described would seem to indicate a serious neuronal damage, which may be mediated through organelle damage with release of

not only hydrolytic lysosomal enzymes but also e.g. sequestered harmful material, such as heavy metals, stored away in cytoplasmatic organelles (lysosomes).

The time between last exposure and sacrifice is of great importance for the detection of foci of leakage since extra-vasated albumin rapidly diffuses down to, and beyond, concentrations possible to demonstrate accurately immunohistologically. However, the initial albumin leakage into the brain tissue (seen within hours in about 40% of exposed animals in our previous studies) may start a secondary BBB opening, leading to a vicious circle – as we demonstrate albumin leakage even 8 weeks after the exposure.

The reason for our choice of 12 to 26 weeks old rats is that they are comparable to human mobile phone addicted teen-agers with respect to age. The situation of the growing brain might deserve special concern from the society since biological and maturational processes are particularly vulnerable. The intense use of mobile phones by youngsters is a serious memento. A neuronal damage of the kind, here described, 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 maybe already in their middle age.

Figure Legends

Fig. 1 . (a) Slightly enlarged cross section of central parts of the brain of an unexposed control rat, stained for albumin which appears brownish in the central inferior parts of the brain, the hypothalamus, a normal feature. In the left lower corner (arrow) a brown spot representing an occasional focal leakage.

(b) As (a) for an RF exposed rat , stained for albumin , which appears brownish in multiple small foci representing leakage from many vessels.

Fig. 2. (a) Row of nerve cells in a section of the pyramidal cell band of the hippocampus in a RF exposed rat. Among the normal big and pale blue nerve cells there are interspersed black and shrunken nerve cells, so called dark neurons . Microscopical picture stained with Cresyl violet, high magnification

(b) The cortex of an RF exposed rat, showing normal nerve cells pale blue, intermingled with abnormal, black and shrunken " dark neurons " at all depths of the cortex but least in the superficial upper layers. Microscopical picture stained with Cresyl violet, high magnification.

Fig 3. Distribution of scores for the occurrence of "dark neurons" as function of exposure condition. The dotted line connects mean values for each condition. A simultaneous non-parametric comparison of all 4 conditions revealed significant differences ($p<0,002$). The p-values in the figure depict comparisons between each experimental condition and controls.

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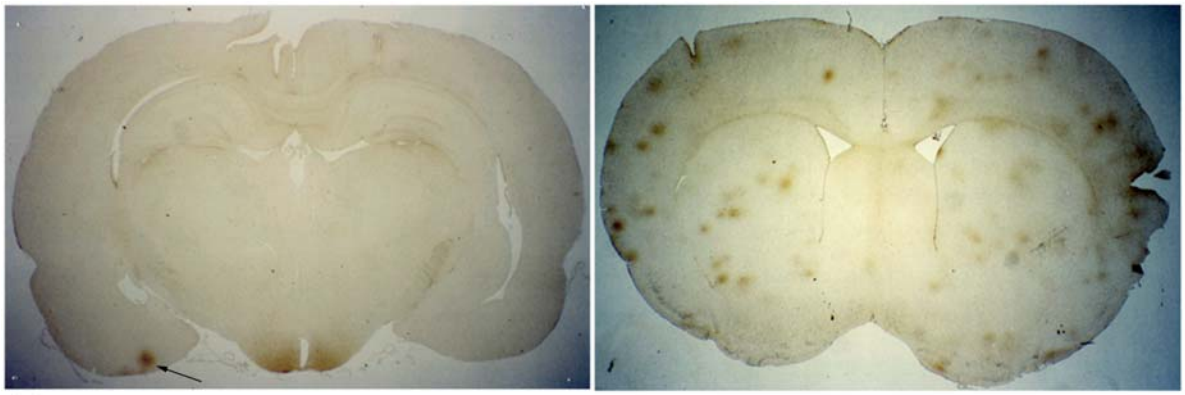
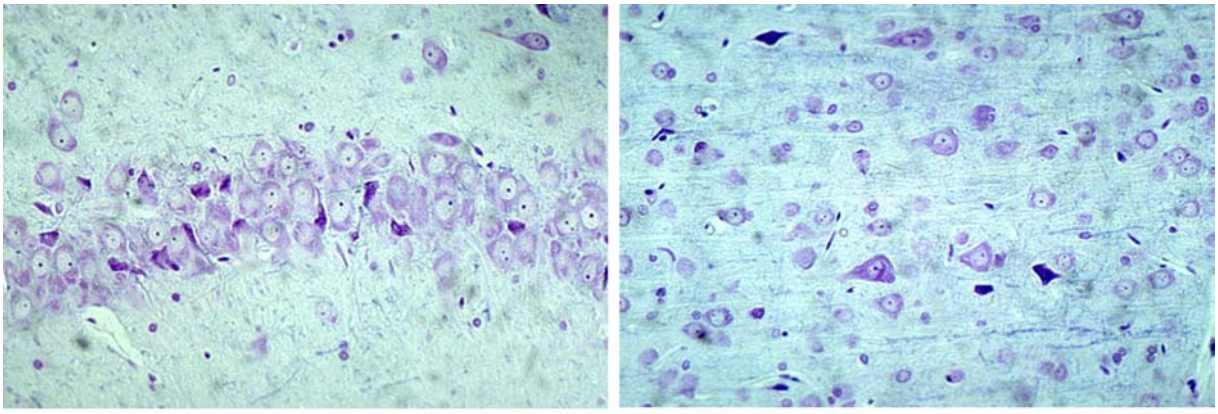


(a) (b)

Figure 1



a) b)

Figure 2

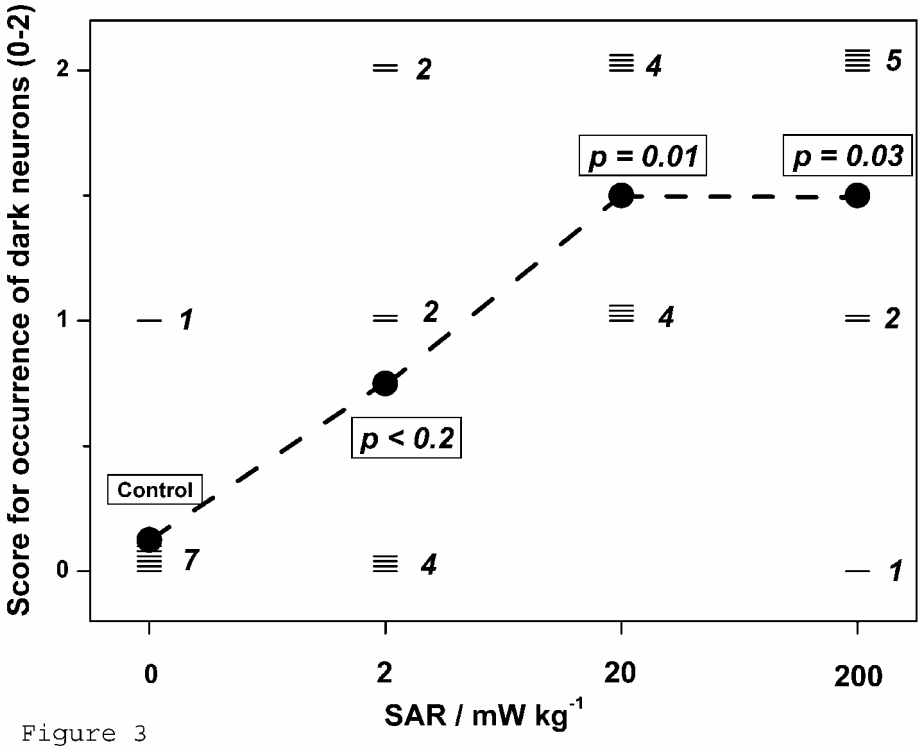

Score for occurrence of dark neurons (0-2)
2
1
0
2
4
5
p = 0.01
p = 0.03
1
2
4
2
p < 0.2
Control
7
4
1
0
2
20
200
SAR / mW kg-1

Figure 3