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S-100 protein levels in the blood of Fischer rats, Exposed to 915 MHz CW-Microwaves, and Magnetic fields

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Abstract.

The aim was to study the level of S-100 in blood samples taken from Fischer-344 rats after exposure to 915MHz CW-microwaves and ELF magnetic fields in TEM-cells. Magnetic field exposure took place with the TEM cell in a Helmholtz coil arrangement of either 50 Hz sinusoidal magnetic field of 5μT, or incoherent magnetic field noise IMF at a maximum amplitude of 50 μT.

There seems to be no significant change in the S-100 concentration in blood of rats exposed for 6 hours to high levels (4W) of continuous wave (CW) 915MHz microwaves, 50Hz sinusoidal magnetic fields (5μT), and incoherent magnetic fields (IMF) at 50μT. In contrast, the results of the combined exposure CW + IMF do indicate a decreased concentration of S100 in blood.

This decrease is in agreement with the results of an investigation in 2010 that the extremely low-frequency (ELF) magnetic fields from the fan motor (50Hz AC, 0.3-1.5 μT) reduced the expected BBB-leakage of albumin due to microwave exposure.

The results from other studies also indicate that Bioeffects caused by exposure to microwaves are reduced by overlay with extremely low frequency magnetic fields ELF. It is therefore of the utmost importance when examining Bioeffects from microwaves to also check levels and frequencies of present low-frequency magnetic fields, which may be one of the reasons for the broad spreading in the reported results.

Keywords: Albumin, Blood-Brain Barrier, BBB, S-100, blood, Fischer rats, Electromagnetic fields
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1 Introduction

The enzyme ornithine decarboxylase (ODC) catalyzes the decarboxylation of ornithine to form the diamine putrescine. Litovitz and co-workers showed that the ELF magnetic field exposure parameters should be constant for a minimum time, so-called coherence time to increase ODC activity in L929 fibroblasts (Litovitz et al., 1992). Further studies showed similar coherence time phenomena for maximum increase in ODC activity also requires to exposure with amplitude-modulated 915 MHz microwaves at SAR 2.5 W/kg. With coherence times of

1.0 s or less, the increase of ODC activity is absent while in times of 10 s or longer it increases. Results show that the microwave coherence effects are similar to those observed with ELF fields (Litovitz et al., 1993). Litovitz and co-workers also showed that incoherent electromagnetic noise inhibits biological effects caused by other exposures (Litovitz, 1994, Litovitz et al., 1994).

In 1997, Litovitz and co-workers presented an in vitro study regarding the possibility of using incoherent magnetic field noise IMF, to reduce the microwave bio-response (Litovitz et al., 1997b). All exposures were performed for 8 hours, which in previous experiments was found to give maximum effect of microwave exposure in the device shown in Figure 1. L929 cells were exposed to 60 Hz amplitude modulated microwaves or a 50 Hz pulse-modulated DAMPS (Digital Advanced Mobile Phone System) digital microwave signals at the power levels adjusted to produce an increase in ODC activity. Simultaneously exposure took place with microwaves and band-limited 30–100 Hz IMF magnetic field noise with square root averages (RMS) of up to 10 μT. The simultaneously exposure with DAMPS digital mobile phone signal exposure and, IMF magnetic field noise at RMS levels above 5 μT showed no increase of ODC activity.

These results suggest a possible practical way to inhibit biological effects from microwave exposure through simultaneous exposure to IMF magnetic field noise (Litovitz et al., 1997a). After taking note of these results, we contacted Litovitz, and in 1999 a collaboration was initiated to investigate the effects of microwave exposure in vivo together with the 50 Hz ELF and IMF.

We studied the effects on S-100 which is a calcium binding protein that exists in glial cells in the brain and has an important role in brain maturation. Babies have for example a high concentration of S-100 while it decreases in adulthood.

At a stroke, or a violent blow to the head the glial cells get swollen and proteins begins to leak out into the blood. The blood concentration of S-100, is normally vanishingly small but can be measured by a special blood test. An elevated concentration in blood thus denotes a brain injury. The higher the level, the more severe damage to the brain. This information applies in several different ways within neurosurgery. By following the S-100 concentration in blood of injured patients it is possible to get a prediction of possible recovery. The concentration of S-100 increase in CSF and/or serum after a vast number of cerebral diseases, e.g. traumatic brain injury (TBI), cerebral infarction, and subarachnoid haemorrhage. Its median concentration in blood in healthy adults is about 5 μg/l (Astrand, et al. 2012).

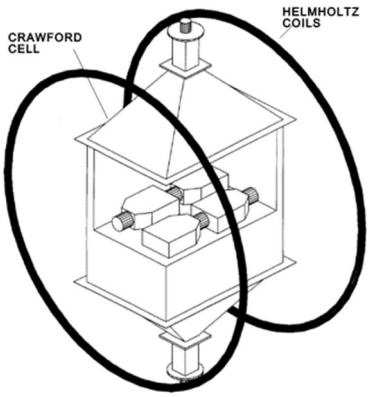
Our study aims to find out if the level of S-100 in blood samples taken from rats, changes after exposure to various types of electromagnetic fields, and became reduced by incoherent white magnetic field noise IMF.

2 Material and methods

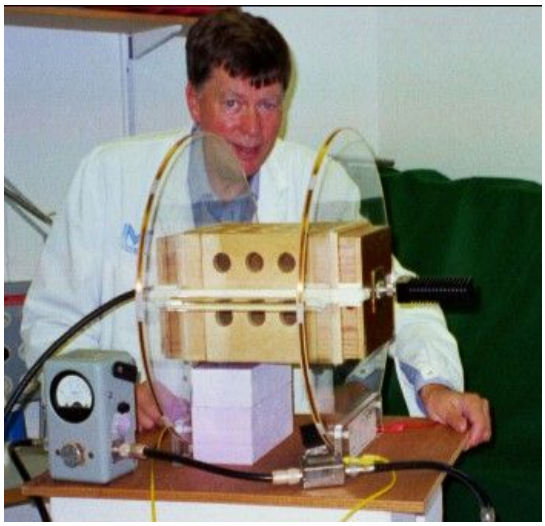
2.1 Exposure system

Lars Malmgren, and his companions Kjell-Åke Carlsson and Bernt Böhmer designed and built an exposure system consisting of a TEM cell for 900 MHz microwaves. Figure 2 shows the TEM-cell placed in a Helmholtz coil with ten turns of copper wire wound on a Plexiglas support frame. The ELF magnetic field is generated with a recorded signal on a cassette in a

tape recorder connected to a sound power amplifier whose speaker output was connected to the Helmholtz coils. The signal cassettes for incoherent white magnetic field noise IMF. were obtained from Litovitz.



Figur 1
Litovitz exposure system for L929-fibroblast cell culture flasks.



Figur 2
Our exposure system for Fischer rats.

The exposure system was designed to enable simultaneous exposure to ELF and microwave fields. Since the TEM cell has an inner shell constructed of brass mesh, the level of the ELF magnetic field inside the TEM cell was checked. In this experimental set-up no fan were applied for ventilation to avoid ELF exposure from the fan motor (50Hz AC, 0.3-1.5 μT) used in most other in vivo TEM cell experiments (Nittby et al., 2011).

Figure 3
Cassette recorder with output signal connected to a sound power amplifier. The Helmholtz coils are connected to the amplifier's speaker output. The signal cassettes were obtained from Litovitz.

In the present study, we exposed Fischer-344 rats in a TEM-cell which outer conductor is made of brass-net attached to the inner walls of the supporting wooden box. The aluminium center plate held up by Teflon braces attached at the inner sidewall.

To allow access to the inside of the cell both ends can be removed. The inside of the cell is ventilated through 18 holes (diam. 18 mm) in the sidewalls and top of the box and the brass-net allows air to circulate without using a fan. These holes are also used for examination of the interior during exposure, and probes for monitoring temperature inside the cell or test object are inserted through these holes.

. We exposed rats for 6 hours to high levels (4W) of continuous wave (CW) 915MHz micro-waves. The output from the cell is terminated in a 50 Ohms dummy load. Both forward and reflected average powers are measured, with a Bird model-43 power meter, at the inputs and outputs of the cells.

Magnetic field exposure took place with the TEM cell located in a Helmholtz coil as shown in Figure 2. Sinusoidal magnetic fields 50Hz at a field strength of 5 μT and IMF random white magnetic noise at a maximum amplitude of 50 μT generated by the tape recorder with signal tapes obtained from Litovitz.

2.2 S-100b analysis

Directly after the exposure of the rats, blood was sampled in 600 μl SST capillary tubes (BD microtainer®), containing a separating gel without additives. The samples were kept for at least 30 min for clotting. Then they were centrifuged for 10 min at 2200 g. Thereafter they were frozen and stored at −80 °C.

After a few years, the samples were analysed concerning for S-100B using immune luminometric assays (LIAISON Sangtec 100, Sangtec Medical, Bromma, Sweden). That assay measure A1B isoforms of the protein S-100 (present mainly in glial cells) and BB isoforms (occurring mostly in glial cells and Schwann cells) by assessing its B-subunit as defined by 3 monoclonal antibodies (SMST 12, SMSK 25, and SMSK 28). The serum samples were diluted with phosphate buffer and incubated with a plastic bead which were coated with monoclonal S100b antibodies. After 1 hour of incubation, S100b is bound to the antibody-coated bead. Then the beads were washed and incubated for 2-hours with iodine-125-labelled anti-S100b antibodies. After washing away unbound anti-S100b antibodies, the amount of radioactive labelled S100b-antibodies bound to immobilized S100b on the plastic beads was measured by gamma counting. The precision was 7.0 CV% (coefficient of variation) and sensitivity was 0.02 μg/l (Jonsson et al., 1999, Stalnacke et al., 2005).

3 Results and discussion

3.1 Magnetic field and CW microwave exposure

We have studied the level of S-100 in blood samples taken from rats after exposure to various types of electromagnetic fields. **Figure 4 s**hows the average results of S-100 in blood from unexposed controls and from rats exposed during 6 hours for: CW 915 MHz microwaves, magnetic field 50 Hz ELF 5 μT, IMF 50 μT and a combination of CW + IMF 50μT. The

upper row in Figure 4 "N=" displays the number of rats in each group.

There seems to be no significant change in the S-100 concentration in blood of rats exposed for 6 hours either to high levels (4W) of continuous wave (CW) 915 MHz microwaves, sinusoidal 50Hz magnetic fields (ELF 5 μT) or white noise magnetic fields (IMF) at 50 μT.

However, the results displayed in the Figure 4 do indicate a decreased S100 average value of the combined exposure CW + IMF.

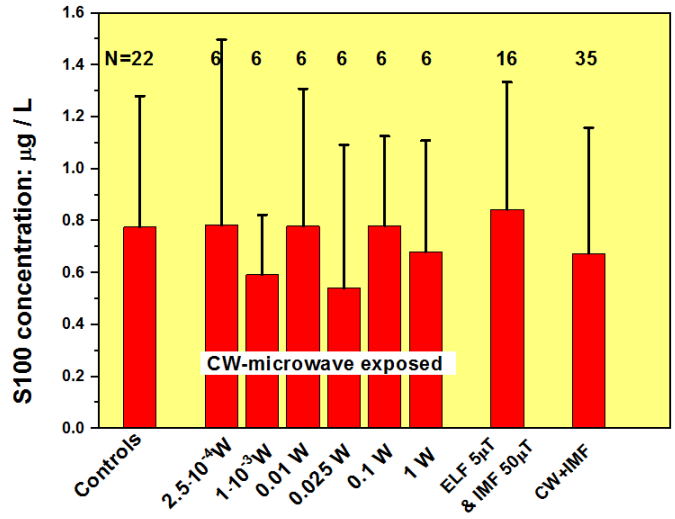


Figure 4

Results of S-100 in blood from unexposed controls and from rats exposed during 6 hours for:

- CW 915 MHz microwaves ,
- Magnetic field 50 Hz ELF 5 μT & IMF 50 Hz
- Combination of CW + IMF 50Hz

The upper row N=, displays the number of rats in each group

4. Discussion

There seems to be no significant change in the S-100 concentration in blood of rats exposed for 6 hours to either high levels (4W) of continuous wave (CW) 915MHz micro-waves, ELF sinusoidal 50Hz magnetic fields (5μT) or white noise magnetic fields (IMF) at 50 μT. However, the results do indicate a decreased S-100 concentration value after the combined exposure CW + IMF.

In most experiments studying the albumin blood-brain barrier (BBB) leakage, the animals are exposed in TEM cells ventilated with external fans powered by electric fan motors. Nittby and co-workers investigated in 2010 whether the extremely low-frequency (ELF) magnetic fields from the fan motor (50Hz AC, 0.3-1.5 μT) affect the expected BBB-leakage of albumin due to microwave exposure (Nittby et al., 2011). Following four groups of rats, with 16 in each, were examined.

1. MW exposure only
2. ELF exposure only
3. Combined MW + ELF exposure
4. Control group. sham-exposed

The MW exposure was performed with GSM-900 MHz at the SAR level 0.4 mW/kg. The normal extravasation of albumin in the basal hypothalamus was used as a positive control for the immunostaining of albumin.

As shown in Figure 5 twenty-five per cent of the MW-exposed animals showed a significant ($p = 0.05$) pathological albumin leakage, in contrast to the 19% for the ELF and 13% for the MW + ELF groups which did not differ significantly from the control group. The

proportion of animals with any albumin leakage at microwave exposure was 50% but decreased to 25% at combined exposure MW + ELF, which agrees with the sham-exposed group (Nittby et al., 2011).

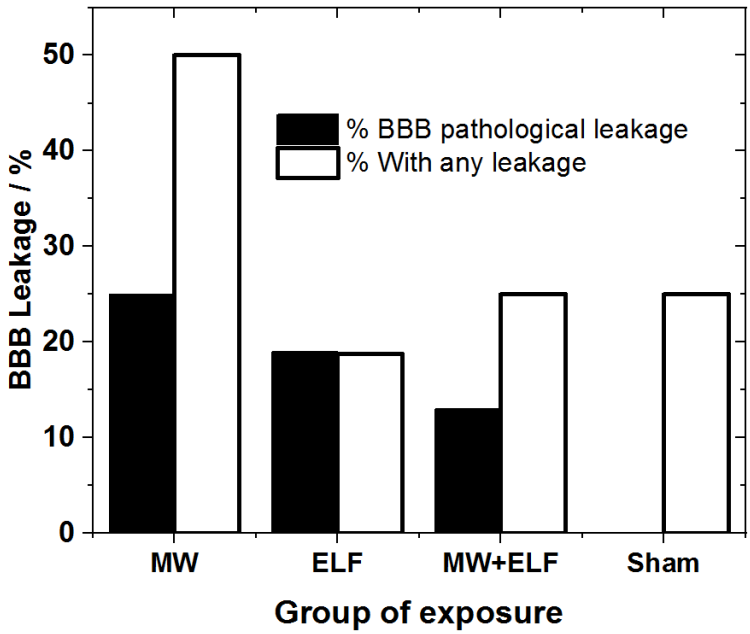


Figure 5
Percentage of animals with pathological albumin leakage foci and with any albumin leakage in the various exposure groups according to Table 2 in the publication (Nittby et al., 2011).

These results are in agreement with the findings in another study of rat exposure with concurrent MW+EMF. In 2004, Henry Lai presented the results of a study on the learning ability of rats to locate a submerged platform in a circular maze, when exposed to either MW or IMF and concurrent exposure to MW + IMF. Four treatment groups of rats were studied:

1. MW: 2450MHz continuous microwaves SAR 1.2 W / kg
2. IMF: incoherent magnetic field noise RMS 6μT (60 mg)
3. MW + IMF: simultaneous exposure
4. Controls: Light exposed

The animals were exposed to these conditions for one hour immediately before each workout. One hour after the last training session, the animals were tested in the labyrinth. The results show that microwave-exposed rats had, compared with the controls, much more difficulty in learning to locate the submerged platform. However, no difference was recorded between IMF exposed rats and controls. However, when co-exposed with MW and IMF, the rats learned significantly better than the microwave-exposed rats alone to locate the submerged platform. Thus, simultaneous exposure with MW and IMF inhibits the expected microwave effect of impaired ability of the rats to learn to locate the submerged platform (Lai, 2004).

In 2005, Lai presented the results of a study on the effect on microwave-induced strand breaks in DNA in brain cells upon exposure with either MW or IMF and simultaneous exposure with MW + IMF.

Four groups of rats were exposed as follows:

1. MW: continuous 2450MHz microwaves with a SAR of 0.6W / kg
2. IMF: Incoherent magnetic field noise with RMS 4,5μT (45mG),
3. MW + IMF: simultaneous exposure
4. Sham exposed as Controls

Four hours after exposure, single- and double-strand breaks of DNA were analysed in brain cells, using micro-gel electrophoresis. The results show that brain cells in only microwave-exposed rats had a significantly higher proportion of single- and double-strand breaks in DNA compared with the control group. The IMF-exposed group's share of single- and double-strand breaks in DNA does not differ from the control group. I contrast no expected microwave-induced increase in DNA strand breaks was noted in the co-exposure group of MW and IMF. Thus, these results indicate that co-exposure with MW and IMF counteracts the expected microwave-induced DNA strand breaks in rat brain cells (Lai and Singh, 2005).

Sun and co-workers reported in 2013 that phosphorylation of EGF receptor clusters in human amniotic epithelial cells (FL-cells) induced by exposure to only 1.8 GHz pulse-modulated microwaves was inhibited by concomitant exposure to IMF noise. Epidermal growth factor receptor clusters on the cell membrane surface were analysed using confocal microscopy. After indirect immunofluorescence staining, the degree of phosphorylation of the EGF receptors was measured by Western blot technique.

Three groups of FL-cells were exposed for 15 minutes to either MW (1.8 GHz pulse modulated microwaves at 217 Hz), or IMF (same incoherent magnetic field noise 30-90Hz as used in Litovitz´s experiment at RMS 2μT), and simultaneously with both MW and IMF.

The results show:

1. 15 min exposure of 1.8 GHz MW FL-cells at SAR values of 0.5, 1.0, 2.0 or 4.0 W / kg induced EGF receptor clustering and increased phosphorylation on tyrosine-1173 residual, but with MW at SAR of 0.1 W/kg no significant effects were caused.
2. IMF noise exposure at RMS 2μT did not significantly affect EGF receptor clusters and phosphorylation of the EGF receptor in FL-cells. When superimposed on MW exposure with IMF noise, EGF receptor clusters and phosphorylation completely inhibited at SAR of 0.5; 1.0 and 2.0 W/kg, but did not inhibit at SAR of 4.0 W/kg.

These *in vitro* results indicate that the membrane-bound EGF receptor interacts with pulse-modulated microwaves and that the interaction is inhibited by simultaneous exposure to incoherent magnetic field noise IMF (Sun et al., 2013).

5. Conclusion

The results from several studies indicate that Bioeffects caused by exposure to microwaves became reduced by overlaying with extremely low-frequency magnetic fields ELF. It is therefore of the utmost importance when examining Bioeffects from microwaves to also consider taking into account the levels and frequencies of environmental magnetic fields, which may be one of the reasons for the large spreading in the reported results.

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