

## EFFECTS OF NON-IONIZING RADIATION ON NEURAL DEVELOPMENT AND MATURE BRAIN

- An experimental study employing human and rodent, organotypic brain slice cultures and neural stem cells

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### Background and research objectives

The present project focuses on *the brain as target for Radio Frequency Radiation (RFR)*, and more specifically the *hippocampus*, which is located in the temporal lobe of the human brain, deep to the ear and normal position of a hand-held mobile phone. The hippocampus plays key roles in memory and learning, besides being a primary target of Alzheimer's disease, epilepsy and ischemic neurodegeneration following heart failure. Both human and rodent hippocampus moreover harbours a population of neural stem with continuous formation of new neurons and glial cells throughout life.

### Methodology

The primary material employed in the project and subjected to RFR has been slices of rat brain hippocampal tissue, derived from one week old rats and grown as organotypic slice cultures, which allows maturation and survival of developing brain slices for several weeks *in vitro*, with much better mimicking of the *in vivo* organization of the brain than primary and dispersed cell cultures.

Rat hippocampal slice cultures, prepared by standard technique from 1 week old rats, have so far been exposed to a UMTS 2 GHz test-signal (2800 culture, of which 50% exposed to SAR 2 W/Kg; 20 min on, 40 min off) and a GSM 900 MHz test-signal (2880 culture, of which 50% exposed to SAR 0.8 W/Kg; 20 min on, 40 min off), while exposure to a GSM 1800 MHz test-signal current is ongoing.

RF exposure devices were constructed as *radial waveguides* with a centrally placed antenna for excitation of the fundamental transversal electromagnetic wave, and the culture dishes with slice cultures placed in 12 separate wells in the periphery at a constant distance from the antenna. Emission out of the individual waveguides was effectively suppressed by an electromagnetically closed construction with a shortcut at the outer boundary. No absorbing material was used in order to prevent heating effects due to the required high field strength in the order of some hundred V/m. The final design used consisted of two double-decker waveguides with two waveguides stacked on top of each other, with the double-decker waveguides placed on each their shelf in same CO<sub>2</sub> incubator and controlled by one computer. For each experiment set-up the two double-decker waveguides were loaded with a total of (2 x 2 x 12) 48 culture dishes with 1 ml of culture medium. Six hippocampal slices with an area of 2 x 2 mm<sup>2</sup> and an initial thickness of 300 µm were placed on top of semi porous polycarbonate membrane in contact with the from below. With the culture dishes being positioned at the bottom of the waveguide, the highest SARs occur at the surface of the medium at the location of the culture slices, and a mean SAR of the slice

cultures of up to 2W/kg could be achieved. For computation of the field and SAR distribution inside the exposure devices the Finite-Difference Time-Domain (FDTD) method was used. Although the six slice cultures in each culture dish were arranged in a circle the ratio of maximum to minimum SAR was only 1.1. On-line temperature measurements inside the waveguides were performed with electrically shielded PTC resistors which had been calibrated by use of a glass-fibre optical temperature probe.

## Results

### 1. Technical adjustments

Tolerance-limits for temperature inside waveguides were more difficult to keep than predicted, and adjustments, like establishment of pump-driven continuous air flow through the waveguides, were necessary. The measured temperature increase linked to onset of RFR is assumed to result from a microwave effect on the water vapours within the waveguides in particular at high SAR values (2 W/kg *versus* 0.8 W/Kg).

### 2. NonRFR-related biological results (donor age, temperature, antibiotics)

Normal, nonRFR-exposed hippocampal slice cultures, derived from 6 and 8 day old rats, differed in susceptibility to 30 min. oxygen-glucose deprivation (OGD) after 2 weeks in culture, suggesting that an unwanted variability might occur when 7 day old rats are used, as in most hippocampal slice culture experiments today. The donor age effect seems to reflect a persistence in culture of the relative resistance to OGD of newborn and early postnatal rats compared to rats from the second week of life, starting to display adult susceptibility to OGD.

Normal, nonRFR-exposed hippocampal slice cultures, derived from 6 day old rats and grown for 2 weeks at temperature difference of 0.5°C (36.3 versus 36.8 °C) resulted in a difference in susceptibility to OGD, with the cultures grown at 36.3 °C being least susceptible. The observation, prompted by the finding of a 0.5°C difference between UMTS 2000 (SAR 2W/Kg) exposed and control cultures, strengthens the demands for narrow tolerance limits for temperature differences, as well as demands to the equipment used in experimental RFR studies.

Antibiotics belonging to the  $\beta$ -lactam group, like penicillin, were demonstrated to reduce the neuronal susceptibility to OGD, and should accordingly not be added to the culture media used in studies of OGD and glutamate receptor mediated neurodegeneration.

### 3. RFR-related results (preliminary conclusions)

Hippocampal slice cultures, derived from 6-8 day old rats, exposed for 2 weeks from start of culturing to non-ionizing radiation (UMTS 2 GHz; SAR 2 W/Kg; 20 min. on, 40 min. off; - GSM 900 MHz; SAR 0.8 W/Kg; 20 min. on, 40 min. off) display no obvious changes in general survival, histological organization and neuronal content.

Hippocampal slice cultures, derived from 7 day old rats, and grown for 2 weeks during exposure to UMTS 2 GHz (SAR 2 W/Kg; 20 min. on, 40 min. off) did not differ from corresponding non-RFR exposed (control) cultures with regard to basic electrophysiological properties, like resting membrane potential, membrane resistance and size and duration of action potentials

Hippocampal slice cultures, derived from 6-8 day old rats and exposed to non-ionizing RFR (UMTS 2 GHz; SAR 2 W/Kg; 20 min. on, 40 min. off; - GSM 900 MHz; SAR 0.8 W/Kg; 20 min. on, 40 min. off) for 2 weeks from start of culturing, displayed no consistent and significant differences OGD-induced neuronal cell death, compared to corresponding control cultures.

Hippocampal slice cultures, derived from 7-8 day old rats and exposed to non-ionizing RFR (GSM 900 MHz; SAR 0.8 W/Kg; 20 min. on, 40 min. off) for 2 weeks from start of culturing, displayed no difference compared to corresponding control cultures in neuronal susceptibility to acute 24 hr. exposures to graded concentrations of the excitotoxic glutamate receptor agonist NMDA.

**Remaining non-analyzed material**

RFR-exposed and control material, subjected to autoradiographic ligand binding studies for analysis of possible RFR-inducible changes in glutamate receptor expression and distribution, has not been analyzed due to ongoing (prolonged) exposure of the autoradiographic films. Final analysis of the results of the electrophysiological reactions of RFR-exposed and control cultures from the GSM 900 series to OGD awaits completion of the series and breaking of the code. The GSM 1800 series will start end of April/beginning of May and continue for the rest of 2008. – Use of human foetal brain tissue, as originally planned, is not likely to be included, due to unforeseen problems in the practical procurement of the tissue from the hospital partner, despite permission from the regional science-ethical committee.