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A new exposure system for the *in vitro* detection of GHz field effects on neuronal networks

Introduction

Different ideas exist about possible targets for biological effects of electromagnetic fields (EMF). One of the favourite systems are the membranes of nerve cells, e.g. in the central nervous system. Our aim is to clarify whether EMF exposure may induce athermal effects. In the experiments, direct electrophysiological *in vitro* measurements are conducted on neuronal networks exposed to EMF, i.e. possible changes in the action potential patterns produced by the networks are detected. The technique was developed as an alternative to the common patch-clamp approach.

Method and Material

Fig. 1 SEM-micrograph of neuronal and glial cells on a micro-sensor electrode
Inner electrode diameter 10 µm (white arrow).

Primary neuronal and glial cells were isolated from the frontal cortex of embryonic (E15 – E17) NRMI mice. Chip [2] cultures were maintained at 37 °C in a 10 % CO_2 atmosphere for 30 days. The action potentials of the neurons were detected in real time by an integrated, electrically passive micro-electrode array (MEA). The neuronal cells grew directly on the MEA (Fig. 1). They reached their full electric activity after about 4 weeks. After this time, the networks were considered mature and suitable for experiments (Fig. 2). For EMF exposure the chips were introduced into a rectangular wave-guide (Fig. 3), that could be operated in the propagating or standing wave modes [1]. The drive signals were either CW (1.9 GHz - 2.2 GHz) or a generic mobile phone signal (UMTS-standard) of up to approx. 8 W (Fig. 4).

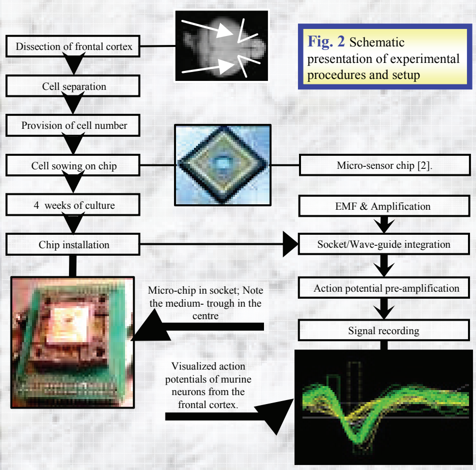

Fig. 2 Schematic presentation of experimental procedures and setup

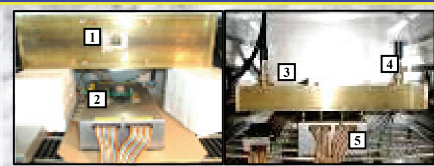

Fig. 3 Left: The wave-guide (1) is countersunk to pick up the micro-sensor chip in the socket (2). Right: View of the wave-guide with incoming signal line (3) and terminating resistor (4). The pre-amplifier is directly located under the chip socket (5). For actual experiments, the terminating resistor was connected via an appropriate extension cord and located outside the incubator to avoid an additional heat source.

In contrast to glass, silicon requires a special surface treatment to enable neuronal adherence. As a trade-off, additional sensors, e. g. for pH and temperature, can be easily integrated [2, 3].

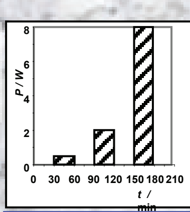

Fig. 4 EMF exposure pattern B

Results

Wave-guide characteristics and EMF distribution

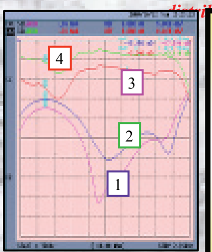

Fig. 5 Reflection and transmission of the wave-guide with and without chip (200 µl DMEM in trough)

Frequency range: 1.75 to 2.25 GHz. Box 1: Reflection without chip below –20 dB. Box 2: Reflection with chip below –18 dB. Box 3: Transmission with chip: loss below 0.2 dB. Box 4: Transmission without chip: loss below 0.1 dB. Conclusion: Reflection and transmission behaviour of the wave-guide are excellent in the exposure frequency range of 1.9 to 2.2 GHz.

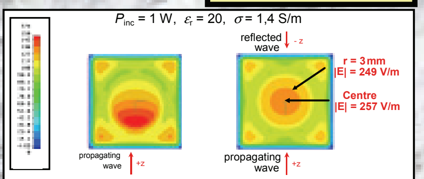

Fig. 6 On-chip EMF distribution for the propagating and standing wave modes
Left: Wave propagating from bottom to top. Right: Standing wave generating a largely homogeneous EMF distribution across the chip trough.

Temperature courses

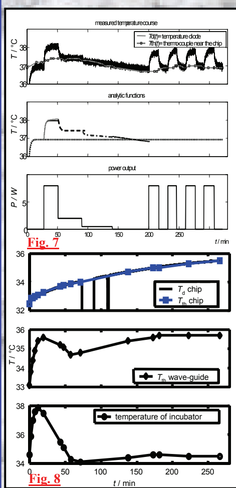

Fig.7 Temperature dependence on the applied EMF power

Top: Temperatures measured by the on-chip sensor (T_d) and in the proximity of the chip (T_{th}).

Centre: Fitted functions (for analytic expressions see Tab. 2).

Bottom: Applied EMF-power.

Fig. 8 Temperature regulation process (no field)

A chip with 200 µl DMEM was installed in the wave-guide.

Top: Temperature at T_d (line). The T_{th}-sensor was submersed in the trough (boxes).

Centre: Temperature at the outer rim of the wave-guide (T_{th} wave-guide).

Bottom: Temperature of Incubator. 0 min corresponds to the first measuring point.

Signal recording & Signal type distinction

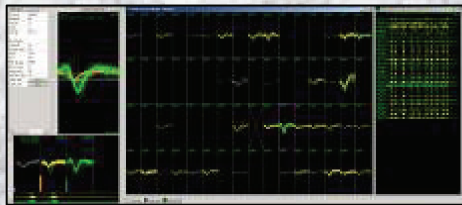

Fig. 9 Screen-shot of action potentials as displayed by the ***MEA Server*** – programme (Plexon Inc., Denton, USA). No EMF exposure. Left: Separation of two action potentials detected by the same electrode. Middle: Sketch of 28 active electrodes. Right: Burst trains obtained for 40 sec-bins.

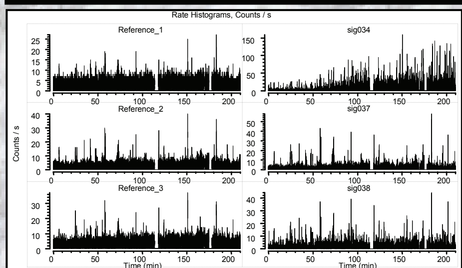

Fig. 10 Signals over time for exposure-pattern B as displayed by the ***NeuroExplorer*** software (Plexon Inc.). Left: Electrodes without neuronal cells exhibit only noisy signals. Right: Neuronal signals with high-noise peaks induced by EMF exposure.

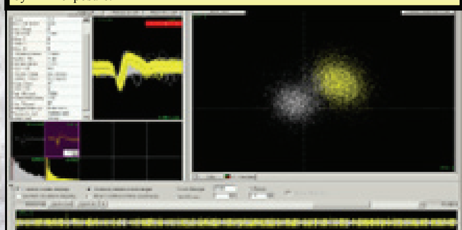

Fig. 11 Separation of neuronal signals from noise. Using the ***Offline Sorter*** (Plexon Inc.). two clusters are obtained. Left: Neuronal signal as waveforms (n=500). Right: Neuronal signals are well separated from noise. Bottom: Signal train from 9000 to 10800 s.

Conclusion

Our preliminary results demonstrate the high potential of the approach.
The wave-guide allows for a CW-EMF exposure in the frequency range from 1.9 GHz to 2.2 GHz in the propagating and standing wave modes and to a generic UMTS field in the propagating wave mode.
Improving the input stage may reduce EMF-induced noise.
Field distributions have been calculated for the the nourishing solution in the standing and propagating wave modes.
Whereas the former generated a homogeneous field, the latter resulted in field gradient over the active chip area.
Specific Absorption Rates can be derived.

Acknowledgments

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