COST EMF - MED (Action BM1309):
European network for innovative uses of EMFs in biomedical applications

STSM Report:

Theoretical and in vitro experimental studies on radiofrequency field-induced electrophysiological effects

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Abstract:

The Central Nervous System (CNS) is the most likely target of radiofrequency (RF) exposure in terms of biological effects. In assessing the health and safety risk of RF energy, it is important to determine not only the fields induced in biological tissues, but also the mechanisms underlying its biological interactions with cells. In this context, this work was aimed to explore the consequences on excitable cells due to the RF fields exposure, on one side, directly observing the extracellular electrical activity on neuronal networks exposed to RF field, and, on the other side, developing realistic biophysical models that may help to give an interpretation to the experimental effects observed.

A. Purpose of the STSM

As described in the STSM application, the purpose of the work planned for the STSM was to combine theoretical biophysical knowledge about neuronal models with an experimental exposure of neuronal cultures to pulsed radiofrequency (RF) electromagnetic fields, in order to better understand the mechanisms of neuronal stimulation by RF. In particular, the work was focused, on one side, on the development of a realistic model of a mammalian cortical network and, on the other side, on learning about the use of the microelectrode arrays (MEA) for the recording of the activity of the cortical cells cultured on it in order to perform experimental exposure activity with it.

B. Work Description

The work was structured into two main parts: an experimental part and a modeling one.
The timing of the experimental activity was imposed by the time necessary to obtain an active cell culture from the cortex of embryonic rats. In particular, after the rats sacrifice, it is necessary to wait for 14 days in order to see a significant activity. During the STSM it was possible to work with two different cultures obtained, respectively, the 9th of January and the 6th of February.
The work was thus organized as follows:

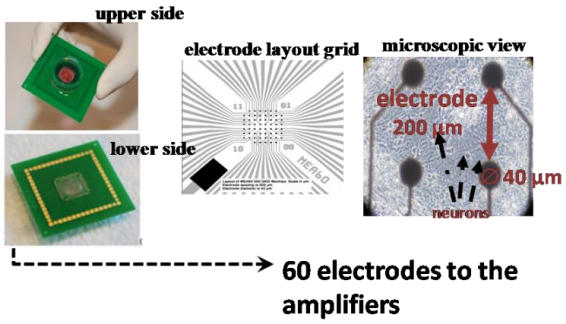

Figure 1: The MEA system

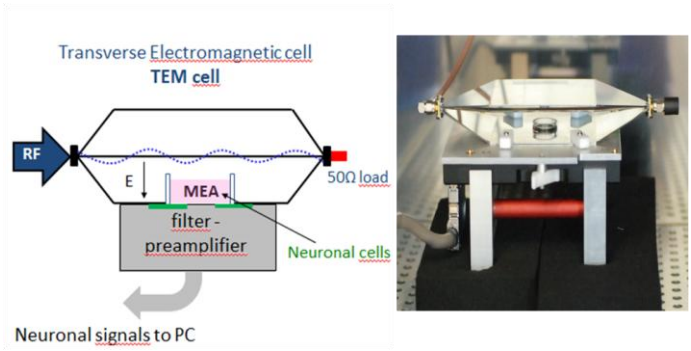

Figure 2: Experimental setup. Left: schematic representation. Right: location inside the incubator at 37 °C and 5% CO_2.

A. **Weeks one and two (19th January-1st February): learning about MEA instrumentation and monitoring of first cells culture activity:** While waiting for the first culture to grow, I learned about the MEA instrumentation, the exposure system and the exposure protocol.
A MEA consists of a glass slide onto which an integrated array of extracellular microelectrodes has been photo-etched. Each electrode extends to the periphery of the chip, and makes contact with an external amplifier, which relays the electrical signals to a computer for analogue-to-digital conversion, filtering, signal detection, storage, and analysis. A glass cylinder sealed with biocompatible silicone is used as cell chamber (Fig.1).
The MEA is placed directly onto the MEA amplifier system. Four mechanical "clips" are used to connect the MEA within a TEM cell to the amplifier; the contact pins of the amplifier are pressed onto the MEA contact pads (Fig. 2).
In the firsts days, I learned how to connect correctly the MEA to the amplifier systems, in order to avoid electrical artefacts due to bad contacts.
The previous studies of the IMS team [1] had shown that the exposure with GSM signal decreases the neuronal activity in terms of burst activity, the focus was to expose with the TEM cell the neuronal cells showing natural bursting activity. After that the cells of the first culture grew in an incubator for 14 days, I checked the electrical activity of all the MEAs with the software McRack; unluckily, some MEAs were too noisy to detect any neuronal activity, while the cells grown on the other MEAs were not active.
As a consequence, no exposure experiments could be done, and it was decided to clean all MEAs to host the cells for another culture.

B. **Week three (2nd-8th February): starting modeling activity and second cell culture:** As a first step for the construction of a realistic network model able to describe the MEA electrical activity, I worked to identify the best single-node representation of the neurons among the extended H-H like models, including additional ionic channels adequately characterized, that can take into account the variability of cortical cells behavior.
Furthermore, on the 6th of February a new neuronal cell culture was obtained from the cortex of embryonic (E18) Sprague-Dawley rats, and I could learn about the protocol for the dissection of the cortices and the treatment of the surfaces of the MEA aimed to improve the "attachment" and growth of the cells culture.

C. **Weeks four and five: modeling activity (9th-22nd February):** Since the cell culture had to grow for almost 14 days, I spent these two weeks working on the model.
Based on the literature overview, I chose to describe the cortical electrical activity with the augmented H-H-type model described in the work of Pospischil et al. [2], beside this I studied the Neuron software to perform the numerical simulations with it.
The single-compartment model chosen [2] is described by the following equation:

$$C_m \frac{dV}{dt} = -I_{leak} - I_{Na} - I_{Kd} - I_M - I_L - I_T$$

where V is the membrane potential, C_m = 1 μF/cm^2 is the specific capacitance of the membrane, I_{leak} is the leakage current, I_{Na} and I_{Kd} are the sodium and potassium currents responsible for action potentials, I_M is a slow voltage-dependent potassium current responsible for spike-frequency adaptation, I_L is a high-threshold calcium current and I_T is a low-threshold calcium current.
Since this equation describes the behavior of one single neuron, while the experimental network is of 10^5 cells, a synaptic current was added in the equation to take into account the stochastic fluctuations due to the activity of the neighbor cells.
The parameters of this stochastic term were tuned in order to obtain the same typical trend of previously recorded unexposed traces, that I had the occasion to analyze.

D. **Week six (23rd February-1st March): exposure activity on the second cells culture:** In the last week, I tested the MEA to check the second cell culture. This time, bursting activity appeared on two MEA, so I was able to proceed with the exposure experiments.
The exposure protocol was applied: MEA were sham exposed twice for 15 min ("pre" phases), exposed to GSM or CW signals with a SAR of 0.1 W/kg for 15 min ("expo" phase) and finally sham exposed twice for 15 min ("post" phases). The duration of the single phase was of 15 min in order to assess the effect of longer exposures.
After that, the traces recorded were post-processed with the software SpyCode in order to obtain the main features of the neuronal activity.
The Mean Burst Rate (number of bursts of all the electrodes/min, MBR) was used to quantify the effect of RF exposure.

C. Results

To express the changes observed before and during exposure, two mean values of the MBR were compared: the one obtained averaging the bursting rate on the two "pre" sham phases, and the one obtained averaging the "expo" phase and the first "post" sham phase.
The MEA exposed to the GSM signal showed a decrease in the MBR of almost the 50%, while the exposure with the CW signal with the same SAR did not elicit such effect, confirming the results of [1] (Fig. 3).
Moreover, for what concerns the modelling activity, the bases of a model for the unexposed cells have been set up.

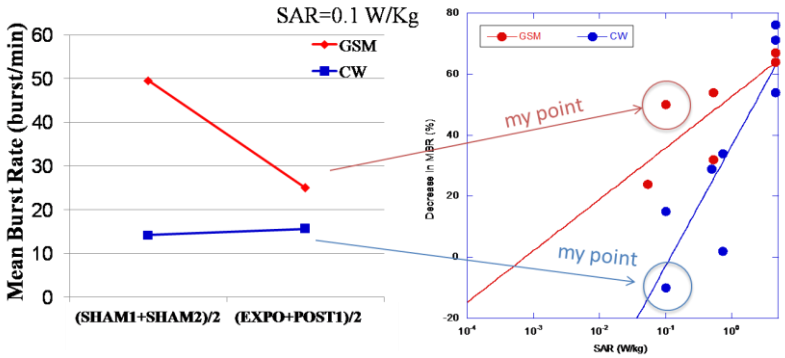

Figure 3: Left: Comparison of the Mean Burst Rate before (sham1+sham2/2) and during (expo+post/2) the exposure conditions Right: Effect in terms of decreasing in MBR as a function of SAR.

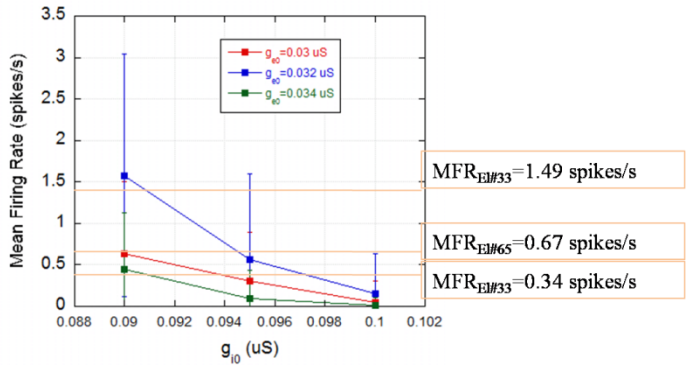

Figure 4: Mean Firing Rate (MFR) obtained on the model varying the synaptic conductances compared with the ones obtained experimentally on the cultures unexposed

Tuning the synaptic conductances it was possible to obtain the same trend of the electrodes traces of the cultures unexposed in terms of Mean Firing Rate (Fig.4).
Future works has to be done in order to improve the model of the unexposed traces to obtain other features such as the bursting rate, number of spikes per bursts, etc. The second step will be focused on the model of synapses or of the channels in order to mimic the action of RF obtaining the same effect attested experimentally.

[1] Moretti, Daniela, et al. "In-vitro exposure of neuronal networks to the GSM-1800 signal." *Bioelectromagnetics* 34.8 (2013): 571-578.
[2] Pospischil, Martin, et al. "Minimal Hodgkin–Huxley type models for different classes of cortical and thalamic neurons." *Biological cybernetics* 99.4-5 (2008): 427-441.

Foreseen publications/articles resulting or to result from STSM (if applicable):

These preliminary results need further work both modellistic and experimental. In particular, the neuronal model need to be improved for a more realistically description of the behavior of the entire network in order to move toward a scientific pubblication.

Confirmation by the host institution of the successful execution of the STSM:

We confirm that Francesca Camera has performed the research work as described above.

Bernard Veyret	Micaela Liberti	Francesca Camera