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Frequency Spectrum Investigations on Detection of Radiofrequency Electromagnetic Fields by Biological Cells

Francesca Apollonio, Micaela Liberti, Alessandra Paffi, Guglielmo D'Inzeo, MEMBER IEEE
ICEmB at "La Sapienza" University of Rome, 00184 Rome, Italy,
(ICEmB: Italian Interuniversity Center Electromagnetic fields and Biosystems)

Abstract _ In order to investigate the biological effects of high frequency electromagnetic (EM) fields as those emitted from mobile telecommunication systems, a mechanistic approach is to develop biophysical models. In this paper the effort of elucidating the modalities of interaction of radiofrequency (RF) EM fields has been focused on the frequency spectrum characterisation of the response of the biological system simulated under exposure conditions. Authors improved the representation of the ionic channels as the core of the previously proposed integrated approach to model electromagnetic fields interaction.

I. INTRODUCTION

Since the beginnings of bioelectromagnetic studies cell membrane and its alterations have been the focal issue in scientific investigations moved by the aim of understanding possible induced effects on biological systems. In the recent past [1, 2] authors proposed a theoretical integrated approach focussed on the analysis of a neuronal network behaviour under exposure conditions. In these contributions the neuronal cell has not been considered as basic biological unit of the bio-system: other elementary sub-structures, with defined tasks and functional modalities have been included, namely ionic channels. The definition of a the theoretical approach has led to the identification of a biological scale of complexity where all the structures involved in each level, because of their polar (or multipolar) nature, are intrinsically sensitive to EM fields [1, 2]. In such approach the core of the study of the interaction between this biological system and EM field has been set in the stochastic properties of the ionic channel embedded in the cell membrane. Thank to these properties a "stochastic neuronal network" able to follow fast evolving of RF signals typical of modern telecommunication standards has been achieved [2]. The insertion of Markov Models of three voltage dependent ionic channels: Calcium, Potassium and Sodium, performed by means of stochastic automata, allows direct implementation of primary interaction steps with the EM fields avoiding the limitations implied by the short-circuiting functioning of the membrane capacitance [2].

In this paper, to further characterize the importance of the differences in time pattern of the various type of EM signal (CW, AM modulated, pulsed modulated, pseudo noise, etc.), authors moved to an analysis in the frequency domain of the results, that can more effectively evidence distinct contributions in the signal.

First steps in this direction has faced the limitations in observation times and maximum number of realizations related to the automata implementation. As a consequence authors focalised their attention on the core of the whole model and have realized a new algorithm for the ion proteinic channels model able to overcome the principal limitations and to open the possibility of a frequency domain analysis.

II. MODELS AND METHODS

A. Biophysical model description

Ionic channels are the elementary electrical signalling systems both within and between neurons. They are individual proteins anchored within the bilipid membrane of a cell and can be thought of as macromolecular pores that are permeable to specific ions. Ionic channels are ubiquitous and provide the substratum for all biophysical phenomena underlying information processing: mediating synaptic transmission, determining the membrane voltage, supporting action potential initiation and propagation. In order to study the action of the external environment (temperature, chemical agents, EM field, etc.) on the behaviour of ionic channels it is necessary to set up a suitable model based on experimental observations. In particular traces of a small electrical current (usually some pA) flowing through a single ionic channel as a function of time, obtained by means of patch clamp recording techniques, immediately reveals some of the unique aspects of the channel [3]. In fact the ionic current switches between two states, one defined by zero (the channel is closed) and the other by the maximum current (the channel is open); these fluctuations are probabilistic in character with the channel randomly alternating between the two states.

The way by which it is possible to derive from the experimental current recording a state machine Markov model is well assessed [3]; moreover Markov models have been already used in the past [4, 5] in order to model the most common voltage-gated ionic channels in presence of EM fields and their results have shown good agreement with experimental data [4]. As a starting point in the present paper the attention is focalized on Potassium channel, whose state machine model is reported in Fig.1

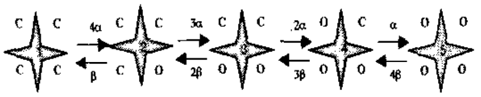

Fig. 1. Potassium channel state machine. Only one is the open state, the closed ones are four.

The model for the Potassium channel is made up of five states, only one of which is the open state. As it is possible to observe from the figure, each of the states takes into account four different particles: to drive the channel to an open state all the particles must undergo a transition between closed and open. The transition rates α and β are function of the environmental conditions, in particular temperature (T) and membrane voltage (V). From a historical point of view Hodgkin and Huxley, who first studied the dynamics of voltage-dependent channels, postulated a number of fictive "gating particles" (4 for Potassium) in order to satisfactorily describe their macroscopic experimental recording of current [3]. At present, due to novel techniques of molecular biology, it is possible to have an insight on the details of the molecular structure of channels recognizing in the four particles specific regions composed of aminoacid sequences (segments).

By means of classical kinetic theory, starting from the overall state diagram of the system (Fig. 1), it is possible to reconstruct the dynamic behaviour of the potassium channel evaluating its probability to be in the open state as a function of time, ($p_{open}(t)$). This value is necessary to calculate the current flowing through the family of Potassium channels as reported in [2] and here recalled for sake of simplicity in Eq. 1

$$I(t) = \gamma p_{open}(t)(V - V_{rest}) \tag{1}$$

where V_{rest} represents the membrane voltage at equilibrium and γ the conductivity of the channel.

The numerical procedure applied to evaluate the channel current is based on a Monte Carlo technique and has been for the first time described in [4], applied in [1, 2] and here briefly recalled.

Each of the transitions from one state to another are assumed to be characterized by a first-order differential equation, with a single associated time constant. With the aid of a random generation the differential equation is numerically solved. In the two following figures the algorithm, which consist of a two layer modellisation, is reported in a schematic view. Fig. 2 describes the first part of the procedure implemented in order to achieve a kinetics for the single channel. It is assumed that the gating particles flip randomly between closed and open state, hence for each of the particles, given the starting conditions (time and state of the particle), a dwell time in the initial state is calculated. At the end of the dwell time (T_d) the particle switches into the next state. As reported in Fig. 2 the closed and open time are evaluated basing on two contributions, a deterministic one due to the transition coefficients, respectively β and α and a stochastic one associated to the random function generator, characteristic of the Monte Carlo solution of the differential equation. At each transition the particle decides the next state on the base of V and T values. In order to have the channel in the open state all the particles must be in the open state (see Fig. 1), in terms of logical characterization this means an AND of the four particles behaviour. The final result is the behaviour in time of the opening and closing of the channel. As second step, considering that in a small patch of cellular membrane in physiological conditions several channels of the same type exist, an average value of several (N) single ionic channels output has to be evaluated for simulating the global channel open probability behaviour, as reported in Fig. 3.

B. EM Coupling

The modalities of the channel interaction with the EM field assumed as the bases of our investigations has been explained in [1, 2].

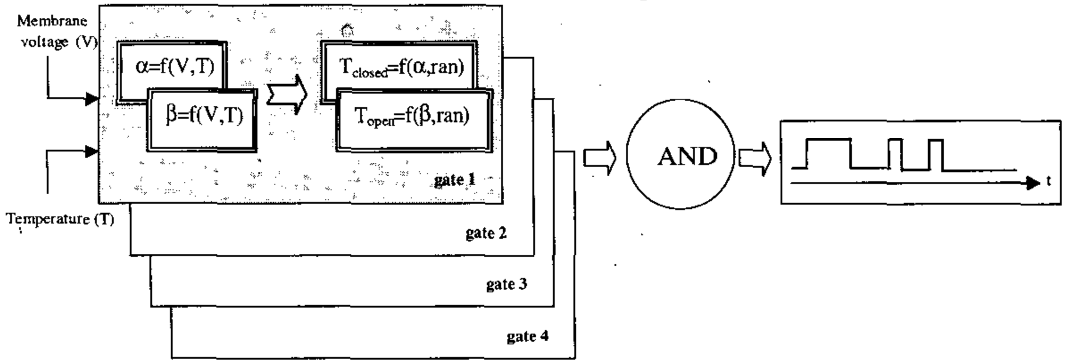

Fig. 2. Time-behaviour of a single ionic channel

The EM field acting on the cell is seen as a perturbing component of the transmembrane voltage [5]. As stated previously ionic channels coefficients α and β depend on transmembrane voltage, which is considered as the sum of two contributions: the first one is related to the physiological membrane, the second one is induced by the EM field. Therefore the EM field can be thought as a perturbation of the equilibrium state that modifies ionic currents [5].

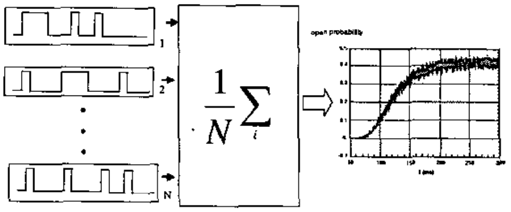

Fig. 3. Behaviour of the averaged channel current.

For what concerns the dynamical interaction in time, referring to section II.A, it is possible to say that once the dwell time T_d has been determined, it is assumed that the channel is insensitive to external stimulation for T_d time steps, when it reaches the next state, and returns to be sensitive and so on, such behaviour is resumed by Fig. 4.

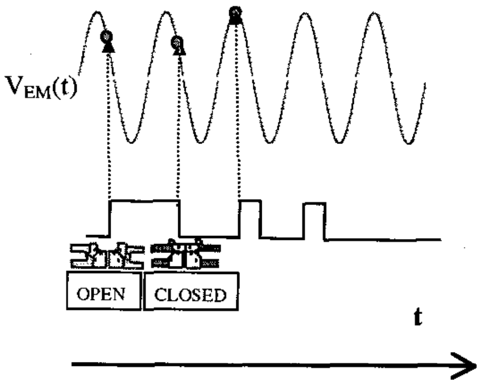

Fig. 4. Time behavioural modalities of EM interaction with the ionic channel.

C. Improved RF algorithm

In [2] the temporal evolution of the dynamic behaviour of the channel was sampled by a fixed temporal step chosen in order to be low enough for a correct sampling of the waveforms involved. This implied that the time step was hundred of thousand of times shorter than the minimum dwell time (around 0.1 ms). As a consequence we had the automata cycling for a large number of time in the same state without any interaction with the external environment driving to very long computational times, redundant considering that the channel is sensitive to the external environment only at state transitions. In this paper an important improvement on the model has been achieved. The overall sampling in time is realized jumping from one transition to the next one, thus implementing a variable temporal step, with a post processing average to be performed. The speed-up obtained in this way can be quantified as ten times, with a memory usage not dependent on the number of realizations (N), which from a physical point of view represent the number of channels of the same family. This overall improvement allows us to increase the number of realizations (N) that is possible to simulate. This is fundamental in order to overcome the necessity, adopted in [2], of evaluating statistical significance of the calculated open probability, here the data are almost directly comparable with the biological ones. Secondly with a number N sufficiently high it is possible to take advantage of ergodicity of the implemented automata and gain more reliable data. In computing the mean value of open probability of the Potassium channel in dependence on the number N of realizations, it has been possible to notice that the value become stable for N=200,000. This value fits well the number of channel actually present in a biological neuron, so it has been chosen as reference value for this work.

III. RESULTS

In order to understand how voltage-dependent channels interact with RF EM fields, an external sinusoidal signal has been considered. Moreover it has been chosen to evaluate the behaviour of the channel under exposure conditions by means of the FFT (Fast Fourier Transform) of the open probability, in the following called $p_{open}(f)$.

Regarding the selection of frequency values to be investigated, the following consideration has to be considered: the channel, as explained in Section II.B, acts as a sort of sampler of the induced waveform. Hence if the period of the sinusoidal signal is higher than the average open and closed times, resulting in several opening or closing of the channel during the same period, the signal is clearly reconstructed. On the contrary, when the period is shorter than the mean gating time, namely in the range of RF and Microwaves (MW), is assumed that the channel behaves as a sort of random down-sampler, which looks for the EM induced values at times, randomly allocated around average values of the order of some ms (mean value of dwell time). All the simulations have been done using the same value of 1 mV of EM induced voltage. This choice even though not realistic permits us to be sure that an effect of the interaction, if present, can be readily identified. In fact if we think to the channel as an electronic device, it is possible to consider its amplitude dynamics as about 60 dB, starting from a minimum voltage value of almost 20 μV, as reported in [2]. The value of 1 mV, located on the higher zone of the dynamics, leads to a frequency spectrum in which single components are clearly detectable over the noise.

First result is relative to the $p_{open}(f)$ for a CW (continuous wave) of 217 Hz (Fig. 5). This frequency has been chosen since it is one of the components in the low frequency range of the GSM signal. It is easy to observe a strong component located around 200 Hz. We expected this kind of result on the base of the observations previously reported.

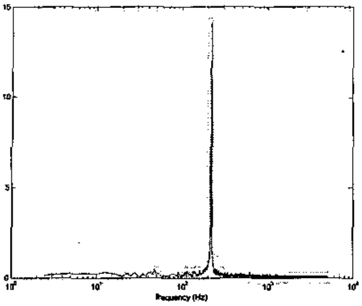

Fig. 5. Frequency spectrum of the open probability exposed to a CW of 217 Hz.

Considering the behaviour of the channel exposed to RF and MW EM fields we have chosen two different frequencies: 10 MHz and 915 MHz (carrier of mobile telecommunication systems). A comparison of the $p_{open}(f)$ spectrum obtained for the two signals is reported in Fig. 6.

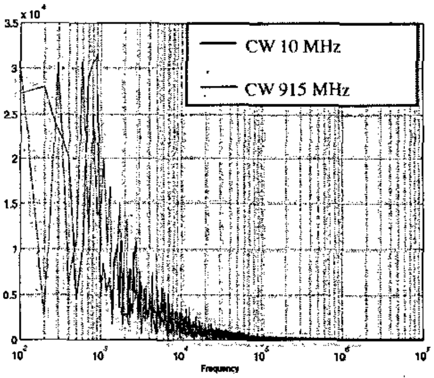

Fig. 6. Comparison between CW 10 MHz and 915 MHz

Unlike the case of a CW of 217 Hz no component at respectively 10 and 915 MHz has been evidenced, thus it has been decided to represent only the low frequency range, most significant in terms of frequency contributions.

Regarding the low frequency range reported in Fig. 6, it is possible to observe a significant spectral content for frequencies up to 10 kHz which could be correlated to the typical channel kinetics. In fact the channel, looking at the external environment (i.e. the EM field) when it switches from open to closed and vice versa, down-samples the external field thus introducing aliasing components. A further analysis has been carried out considering an AM (Amplitude Modulated) signal. We have taken into account the 915 MHz carrier, amplitude modulated by a 217 Hz. The result is reported in Fig. 7.

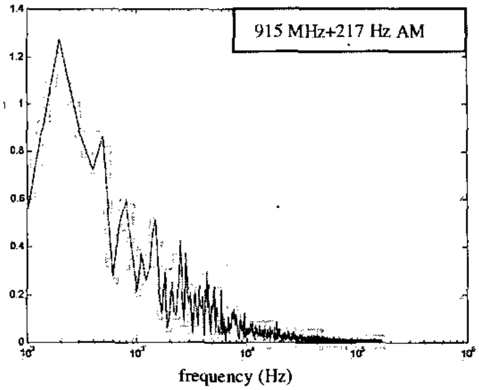

Fig. 7. Frequency spectrum of a 915 MHz, with a 217 Hz amplitude modulation

It is possible to observe a clear component at around 200 Hz, indicating a sort of demodulation carried out by the channel. Other components, as in the case of CW could be related to possible aliasing.

IV CONCLUSIONS

It is possible to conclude that the ionic channel model set-up can clearly reconstruct frequency components when the signals are located in the low frequency spectrum. For what regards RF signals it operates a frequency shift, probably related to its proper dwell (sampling) time values. It is interesting to note that modulation properties seem to emerge when the channel deals with AM signals.

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