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Effects of Nanosecond Pulsed Electric Fields on the Activity of a Hodgkin and Huxley Neuron Model

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Abstract— **The cell membrane poration is one of the main assessed biological effects of nanosecond pulsed electric fields (nsPEF). This structural change of the cell membrane appears soon after the pulse delivery and lasts for a time period long enough to modify the electrical activity of excitable membranes in neurons. Inserting such a phenomenon in a Hodgkin and Huxley neuron model by means of an enhanced time varying conductance resulted in the temporary inhibition of the action potential generation. The inhibition time is a function of the level of poration, the pore resealing time and the background stimulation level of the neuron. Such results suggest that the neuronal activity may be efficiently modulated by the delivery of repeated pulses. This opens the way to the use of nsPEFs as a stimulation technique alternative to the conventional direct electric stimulation for medical applications such as chronic pain treatment.**

I. Introduction

Traditional studies on electroporation of plasma membranes were based on the delivery of pulsed electric fields with duration comprised between μs and ms and amplitudes of the order of some kV/m [1]. Only in recent years, biological effects of nanosecond pulsed electric fields (nsPEF), characterized by durations in the nanosecond time scale and amplitudes in the MV/m range, have been widely investigated [2]–[4], evidencing promising applications in medical fields, such as gene expression modulation [2], cancer treatment [3], wound healing and cicatrization [5].

Another important medical application of nsPEFs, which is currently under investigation, is based on the stimulation/inhibition of excitable tissues: neuronal and muscular, both skeletal and cardiac [6]–[10]. In this context the delivered pulses can activate or inactivate action potentials useful for alternative cardiac pacing, defibrillation and relieve of chronic pain. Experimental studies on cardiac cells of rat [8] reported a cell excitation via a mechanism involving the membrane poration. Indeed, a direct ion current activation seems to be unlikely, due to the short pulse duration with respect to the reaction times of the ionic channels. The mechanism of action potential generation by nsPEFs is likely to involve a first permeabilization of the plasma membrane, followed by changes in intracellular Ca^{2+} concentration [8] modulating the electrical activity of the cell. Analogously, experiments on rat neuromuscular system [9] and studies on nerve models [7], [10] reported the block of the conduction of the electric stimulation along a fiber, ascribed to the variation of membrane conductivity related to the pore formation in the plasma membrane [7], [10].

Indeed, although the mechanisms of action of nsPEFs are not yet clear, structural changes in plasma membrane with the opening of stable conductance nanopores has been recently suggested [11]–[13] by molecular dynamics investigations and experimentally confirmed with the use of a nanometric fluorescent dye [11].

The membrane poration determines a change in the membrane permeability to ionic species that can trigger a cascade of events possibly leading to functional changes in any kind of cell. When dealing with excitable cells, such as neurons, where the electrical activity results from the balance of ionic fluxes through the membrane, a change in cell permeability is likely to affect the mechanisms of the action potential generation and transmission. The final outcome could be similar to that induced by other kinds of electric stimulation of the nervous system, such as direct current stimulation (DCS) and deep brain stimulation (DBS), with the advantages of reversibility, suppression of possible self-launched action potentials, and negligible heating or tissue damage [7].

In the aforementioned stimulation techniques, the stimulation signal: a DC current in DCS or a train of "long" pulses (pulse width: 60-90 μs) in DBS, directly affects the dynamics of voltage-gated ion channels. In the case of nsPEFs, pulse duration seems to be too short if compared to the reaction times of voltage sensitive channels and the effect of the stimulation on the electrical activity is indirect and mediated by the induced variations of the passive properties of the cell membrane, i.e. permittivity and conductivity.

In turn, conductivity and permittivity variations depend on the pore density according to analytical formulations making use of empirical parameters [14]. The pore formation and destruction is a stochastic process following, in general, the Smolukowski equation [15]; in the nanosecond time scale, it can be simplified leading to the asymptotic electroporation model [15], [16], where pores with a fixed size (≈ 1 nm) are considered [17]–[19]. Pore density exhibits a highly non-linear dependence on the trans-membrane potential (TMP) induced by the particular nsPEF used, which can be obtained as the outcome of microdosimetric studies [20].

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Aim of this work is to couple analytical expressions for the time behavior of conductance and capacitance of the porated membrane with a functional circuital model of an excitable membrane patch. This will allow us to assess the effects of poration on neuronal electric activity and to quantify the observed modification as a function of significant parameters, such as maximum poration level, pore resealing time, background stimulation level of the neuron model.

II. Models and Methods

The models used to describe the passive properties and the excitable behavior of a porated neuronal membrane patch will be reported in Section II.A and II.B, respectively.

A. Modeling Capacitance and Conductance of the Porated Membrane

The opening of aqueous pores in a membrane patch determines modifications in its capacitance and conductance.

The overall capacitance of the porated membrane can be considered as the sum of two contributions: one due to the aqueous pores and the other to the lipid bilayer, each of them weighted by the respective area [21]. The relative variation of the membrane capacitance due to the pore formation is given by:

$$\frac{\Delta C}{C_m} = \left(\frac{\varepsilon_w}{\varepsilon_m} - 1 \right) \frac{A_p}{A_m}, \quad (1)$$

where C_m is the capacitance of the non-porated membrane, ε_m and ε_w are the permittivity values of lipid bilayer and water, respectively, A_m is the whole area of the membrane patch and A_p the area occupied by the pores. Due to the small dimensions of pores, even for high values of pore density (N above 10^{15} m^{-2}), capacitance variation is always below 2%; thus it can be disregarded.

Different is the case of conductance where the flux of ions through the aqueous pores becomes the dominant contribution due to the higher conductivity of water with respect to the lipid bilayer. Such a pore contribution G_p is given by [22], [23]:

$$G_p = \frac{\pi r_p^2 \sigma_w}{h} NA, \quad (2)$$

with r_p the pore radius, σ_w the water conductivity, h the membrane thickness, N the pore density, and A is a term accounting for the energy barrier experienced by the ions when flowing through the pore [22]. Both N and A depend on the TMP and hence on time. A can be estimated on the bases of the TMP calculated as in [20]; as an example, for a nsPEF of 10 ns and an intensity of 2.2 MV/m, A showed a first significant peak with a fast decay (in the time scale < 1 μs) down to a value approximately equal to 0.1 m^2. Typically N exhibits a very sharp increase (in a time scale of ns) up to a maximum level (N_{MAX}), which, due to the resealing process of the pores, slowly decays (in the time scale up to s) to the initial value N_0. In this paper, N_{MAX} has been calculated as in [20] and is of the order of 10^{15} m^{-2}. Pore resealing has been modeled as a single time constant (τ) process, like in [15]; τ has been chosen from literature data equal to 3, 10, 100 and 1500 ms [15], [24], [25]. A value of σ_w=1 mS/m was used in (2), like the one reported in [26] for bound water.

B. Modeling Neuronal Activity

To account for non-linear and active properties of the neuronal membrane, the well-known Hodgkin and Huxley (HH) circuital model was considered [27]. The circuit is made of the parallel combination of the membrane capacitance C_m and three branches accounting for different ionic currents. Moving from the Kirchhoff law, the balance of currents is given by:

$$C_m \frac{dV_m}{dt} = -g_l(V_m - E_l) - g_K(V_m - E_K) - g_{Na}(V_m - E_{Na}) + I_s, \quad (3)$$

where g_l, g_K, g_{Na}, E_l, E_K, E_{Na} are, respectively, the leakage, Potassium and Sodium specific ion conductances and the reversal potentials of the corresponding currents. In the HH model, Sodium and Potassium conductances present non-linear voltage dependence with time constant of the order of ms, whereas g_l is a constant value equal to 0.3 mS/cm^2. Usually such an equation is solved with the Euler integration method with a time step of 10 μs. I_s is the stimulation current and drives the transition between the resting state and the firing one. The threshold current is equal to 6.3 μA/cm^2.

Such a well-assessed model has been modified to account for membrane poration as follows. TMP, calculated as in [20] for a pulse of 10 ns duration, has completely recovered to its initial value after a time lag lower than 1 μs. Therefore, given the typical response times of the neuron, the time course of the TMP due to the pulse has not been directly considered in the equation (3). Moreover, it has been postulated that the currents through the ionic channels are not affected by poration [7]; finally, as noted in Section II.A, C_m is minimally affected by poration, so we have maintained its constant value equal to 1 μF/cm^2. As a consequence, the only parameter depending on poration that we inserted in the model is the leakage conductance which has been modeled as a decreasing exponential function starting from the value assumed by the equation (2) after 1 μs (≈ 3 mS/cm^2) and with the time constant equal to τ. Even such a modified model has been implemented in the C++ environment using the direct Euler integration method with time step of 10 μs. Simulations have been carried out for stimulation currents I_s ranging from 6.3 up to 20 μA/cm^2.

III. Results

The first effect induced by poration on the neuron activity is the introduction of a new electrical regime consisting in an initial inhibition period followed by the onset of a regular firing. In Fig. 1 such a behavior is shown, together with the exponential decay of $g_l(t)$, for τ=1500 ms and I_s= 20 μA/cm^2. To note that the firing activity restores before that $g_l(t)$ recovers to its initial value of 0.3 mS/cm^2. The possibility of temporarily silencing the neuron and the duration of such a reversible effect revealed to be dependent on the choice of I_s and τ, as reported in Fig. 2.

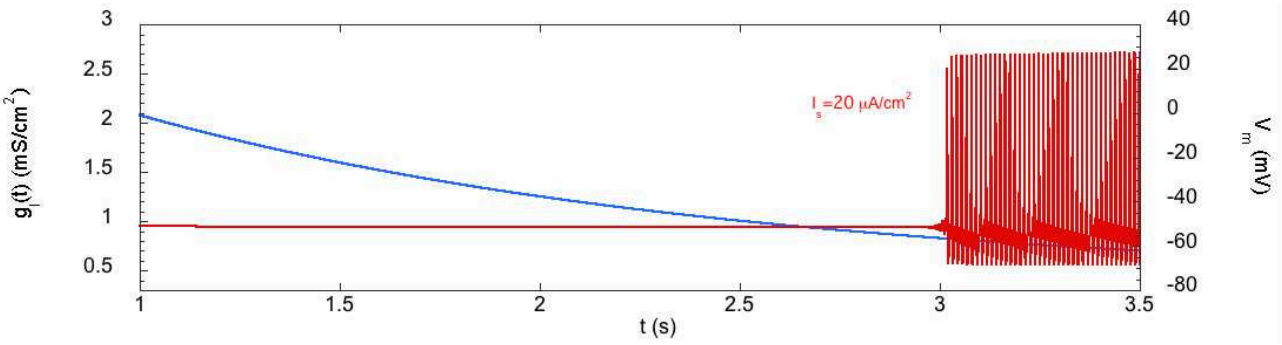

Figure 1. Time courses of the leakage conductance and of the transmembrane voltage for I_s= 20 μA/cm^2, τ=1500 ms, N_{MAX}=10^{15} m^{-2}, T=6.3°C.

The figure shows, for each time constant τ, the observed electrical regime in correspondence of each I_s value, highlighting those values representing the thresholds between two regimes. The first interesting effect is an enlargement of the resting regime with a translation of the threshold for the upper regime in all cases. Moreover, one can observe that the temporary silencing is possible only for τ>10 ms and I_s> 10 μA/cm^2.

If one wants to investigate the characteristics of the new "temporary silencing" regime, it is possible to observe that the duration of such an inhibition is in turn strongly dependent on I_s and τ as evident from Fig. 3. Clearly, the longer the decay times of the membrane conductance, the longer the inhibition periods obtained; this can be explained considering that the longer permanence of pores in their open state leads to a sort of membrane short circuit, which determines the TMP decrease under the threshold for the action potential generation. However, it seems that higher stimulation currents I_s drive more quickly the neuron back to its regular firing state. This can be justified by a higher I_s value counterbalancing the negative contribution due to g_l in eq. (3). In addition, variations in the inhibition times seem to depend even on the choice of N_{MAX}, as shown in Fig. 4.

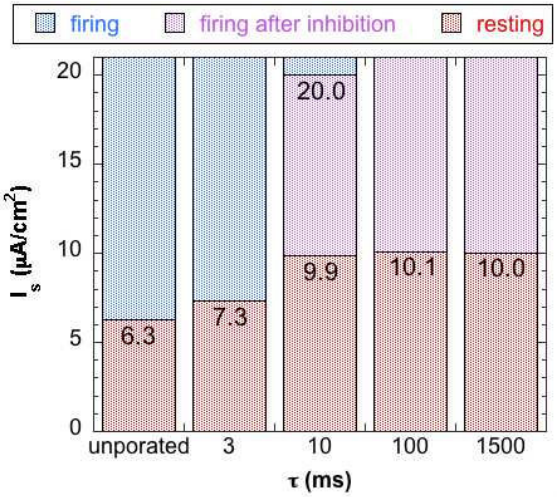

Figure 2. Electrical regimes exhibited by the HH model for the non-porated membrane and for the porated one with different resealing time constants τ and different stimulation currents I_s. Current thresholds between regimes are highlighted.

In this case one can observe that, for the same stimulation current I_s, the increase of the number of the membrane pores leads to a corresponding increase of the inhibition time. Such an increase is almost constant and equal to 1 s for all the I_s values, except for I_s= 10 μA/cm^2 where a delay of 675 ms is obtained. This behavior is justifiable only through the non-linear characteristics of the two processes involved: electroporation and action potential generation.

IV. Conclusions

We demonstrated how a temporary increase of the membrane conductance, due to the electroporation induced by nsPEFs, could disrupt the regular activity of a neuronal model. Such an effect has resulted in both a translation in the threshold between resting and firing regimes and in a temporary silencing of the neuron. The investigation on such a temporary silencing regime has evidenced a strong dependence of the inhibition time on the stimulation current I_s and the pore resealing time constant τ. Further data would be necessary in order to fully elucidate the mechanism of the observed phenomenon. However, the observed effects confirm the firing blocking shown in [7] and suggest the possibility of modulating the electric activity of neurons by inhibiting the action potential initiation through a suitable pulse train.

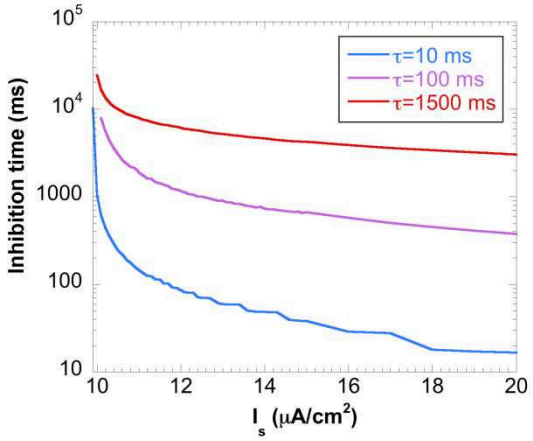

Figure 3. Inhibition time versus the stimulation current I_s for different values of the resealing time constant τ.

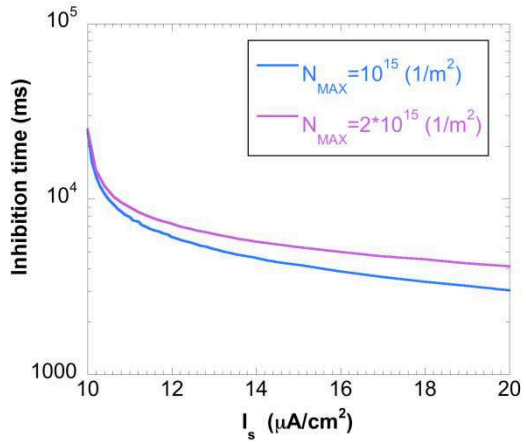

Figure 4. Inhibition time versus the stimulation current I_s for τ=1500 s and two maximum levels of poration (N_{MAX}).

Thus, the eletroporation induced by nsPEFs could be used as an alternative approach to the direct electrical stimulation of neuronal system for medical application such as the chronic pain treatment. However, in order to obtain reliable and predictive results, the use of a realistic poration model is unavoidable, especially for what concerns the estimate of empirical parameters. Thus, an in depth knowledge of the membrane response to nsPEFs, in terms of variations in the ion flux through the pores, like the ones achievable with molecular dynamics, is required.

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