COMPLETE EVALUATION OF CONFORMATIONAL MW EFFECTS ON ACH CHANNELS WITH PARALLEL COMPUTING

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ABSTRACT

We present here a parallel implementation and optimization of a Markov model simulating the Acetylcholine-receptor (ACh) channel response to 2.45 GHz fields. It overcomes the limitations of a previous implementation, allowing a complete understanding of the simulated phenomenon. The main experimentally observed effects of microwaves on ACh channels are theoretically explained, and an energetical mapping of ACh conformational changes achieved.

INTRODUCTION

Ionic protein channels inside cell membranes are possible sites of interactions between electromagnetic (EM) fields and biological systems. They are complex structures, and their mechanisms of EM interactions need an appropriate theoretical analysis to be understood.

In previous papers [1,2] a modelling technique to simulate the non-thermal effects of EM fields on the gating of ionic channels in cell membranes has been presented. EM effects at low frequencies on voltage-dependent channels [1] and microwave (MW) effects on ligand-dependent Acetylcholine (ACh) channels [2] have been studied. An evaluation of the EM power incident on biological targets has been performed in both cases, verifying that no effective thermal mechanisms could be involved in the observed effects. It has been demonstrated that some conformational effects are induced by MW fields [2], which modify the probabilities for the channel to be in a certain state. Both studies [1,2] are based on a stochastic modelling approach, which is shortly summarized in the following.

The modelling technique presented in [1,2] is based on Markov models. The channel is considered a stochastic automaton, existing in a finite number of possible conformations (states) with some transition rates from one state to another. The transition rates define the frequency of transition from one state to another. Therefore, they are expressed as $(\text{times})^{-1}$.

It can easily be demonstrated [1] that a transition rate from state *i* to state *j* also defines the probability that the transition from *i* to *j* occurs.

Some researchers have proposed possible modellings of ligand-receptor channels. Starting from these studies [2, and references herein], for the ACh-receptor channel a 5 state model has been chosen, with 3 closed and 2 open states (Fig. 1).

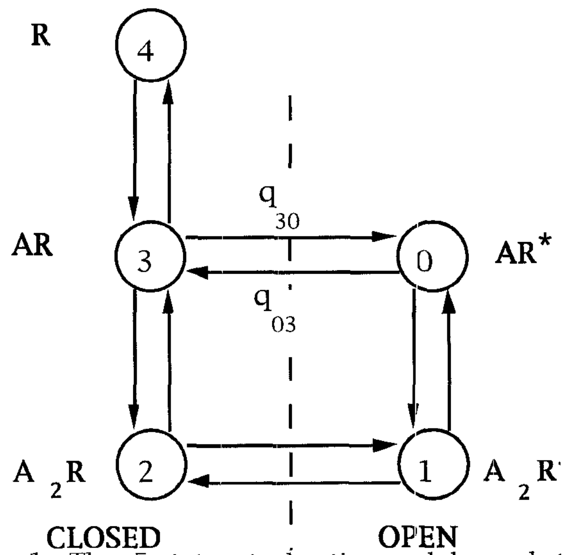

Fig. 1: The 5-state stochastic model used to simulate the ACh-receptor channel.

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R is the receptor-channel in closed conformation, A is the agonist molecule. R* is the open conformation. It can be observed that conformations with no agonist bound to the receptor site, or with one or two ligand molecules are supposed to exist.

A random process is generated, consisting in a succession of transitions among states. The dwell-times T_D in each state are evaluated by using the following formula

$$T_D = a \cdot \ln \frac{r}{b} \quad (1)$$

where a and b are evaluated from transition rates [1,2] and r is a random number, generated at each time-step. In conclusion, a succession of transitions is generated, and each transition is divided by the following by T_D time steps.

As each state corresponds to a certain conductivity of the channel, the succession of states can be easily translated into a signal corresponding to the current flowing through the channel.

In this work, we complete the analysis of MW (2.45 GHz, a few mW/cm^2 on the target) effects on ACh channels, discussing some limitations of the previous model implementation [2] and overcoming them by an implementation of the Markov model on a parallel distributed memory platform. Finally, results are shown demonstrating the effects of MW on the stability of the different molecular conformations of the ACh channel. These results allow a deep understanding of the mechanisms of interactions between MW and biological systems at molecular level.

EXPERIMENTAL RESULTS AND MODELLING LIMITATIONS

The experimental observations and data on which we optimized our model can be summarized as follows (all of them are non-thermal effects):

1) The receptor seems to desensitize to the ACh action when the field is on [3,4];

2) The opening frequency decreases passing from the unexposed to the exposed condition [3,4];

3) The average closed and open time do not change consistently from unexposed to MW exposed situation [3,4];

4) At least a slow kinetics closed state should exist [5], causing opening bursts after long-lived closed states[5].

The model of Fig. 1 has been optimized on the experimental data of the current flowing through the channel using a combinatorial optimization method called Tabu Search [6], defining a cost function taking into account the differences between the experimental and theoretical curves for the opening and closing times. Two parameter sets, the former for the unexposed data set, and the latter for the exposed one, have been obtained, and are shown in Tab. I.

Transition rate (ms^{-1})	Unexposed channel	Exposed channel
q_{43}	0.01 (unopt.)	0.01 (unopt.)
q_{34}	0.01 (unopt.)	0.01 (unopt.)
q_{32}	1.6	1.6
q_{23}	4.0	1.6
q_{03}	0.2	0.2
q_{30}	1.8	0.6
q_{21}	1.8	2.4
q_{12}	1.8	1.6
q_{01}	0.1	0.4
q_{10}	1.0	0.8

Tab. I: Values obtained from Tabu Search optimization for transition rates. For two transition rates only a rough optimization was possible, due to computational limits.

The resulting models, implemented on a computer, allowed us to verify point 1), 2) and 3). It was also possible to understand some of the conformational effects explaining the observed effects [7].

Anyway, some important limitations were found in the implementation of the model and, above all, in the analysis of the experimental results through the model. The main limitation is computational. In fact, a rigorous implementation of the modelling techinique, and an appropriate optimization of the transition rates, should take into account the observation 4) concerning the existence of a closed state with very slow kinetics. This means that very long simulations should be generated, to optimize the transition rates q_{34} and q_{43} in Fig. 1. Transitions between states 3 and 4 are very slow and not so frequent, and are therefore observed if and only if very long current traces are avalaible (longer than 10 s). Now, as the time step in the simulations should not be longer than 0.001 ms, and being the optimization a computationally hard task, on a serial RISC workstation only an approximate treatment was possible for q_{34} and q_{43}, and consequently for the item 4). Times for the optimization of the simplified model, not optimizing slow kinetic transitions, are shown in Tab. II. This computational limit is here skipped with a parallel implementation of both the model and the optimization module computing the optimum values for the transition rates.

Time Step (ms)	Optimization time (IBM 250 T)
10^{-3}	7' 38"
10^{-4}	3h 38' 34"
10^{-5}	24h 21'

Tab. II: Times for a Tabu Search optimization of transition rates. They refer to an IBM RS6000 250 T, and are given for different time discretizations.

A PARALLEL SOLUTION TO COMPUTATIONAL LIMITS

The optimization of the model and its implementation has been ported to a parallel distributed memory platform, the IBM SP2 at Perugia University. It is an 8 RISC processor architecture, with a high-speed switch. The programming interface is the Parallel Virtual Machine (PVM) [8], which supports a message-passing programming model. The model has been implemented in a master-slave fashion. A master process activates 8 slave processes. Each slave process takes care of generating a fragment of current signal. After a certain number of time steps, the master collects all the signal fragments from slaves, and links them. When a complete signal is ready, the master makes some statistics on it, evaluates the open and closed time distributions, and compute the cost function corresponding to the currently implemented model. These data are sent to slaves, which start a search through each partition of the search space, looking for better values of the transition rates. After a new parameter set is found, the slaves send it to the master, which starts the parallel generation of the new current signal, following the method described earlier. The computing times for this iterative method are shown in Tab. III. Different times are shown, due to the choice of the number of current signals generated at each optimization step. In fact, for an appropriate evaluation of the signal properties, it is necessary to evaluate statistical values (open and closed times, for instance) for more different traces (thousands of traces 30 to 50 s long).

Number of current signals	Computing Times (8 processors, IBM SP2)
1000	3h 20'
5000	14h 12'
10000	25h 25'

Tab. III: Times for the generation of a random process with different numbers of realizations of the current signal through the ACh-channel. Times refer to an IBM SP2 with 8 processors.

RESULTS

The implementation of the model and optimization module on an IBM SP2 has allowed a complete and severe evaluation of the model's parameters. Two parameter sets, for the unexposed and exposed channel's model, have been found. The optimum transition rates q_{34} and q_{43} are shown in Tab. IV.

Transition Rates (ms^{-1})	Unexposed channel	Exposed channel
q_{43}	0.0009	0.0009
q_{34}	0.0007	0.0014

Tab. IV: The optimization of transition rates has been completed by a parallel implementation of the Markov models and optimization strategies.

This complete optimization has allowed the simulation of the experimentally observed phenomena of very long lived closed states and of bursting of openings after long lived closures.
Moreover, values obtained for q_{34} and q_{43} explain the experimentally observed phenomena 2) and 3). In fact, as q_{43} is increased because of MW effects, the probability of trapping the channel into a long lived closed state increases because of MW effects.

This causes a complementary decrease in the opening frequency.
Finally, from basic equations of thermodynamics, it has been demonstrated that an evaluation of the energetical gaps among states in the model can be achieved [7].
Now, a complete energetical mapping of the ACh channel and of its conformations under EM exposure is possible, as shown in Fig. 2. Energetical gaps among different conformations are shown (values in J/mole) both for the unexposed and for the exposed channel.

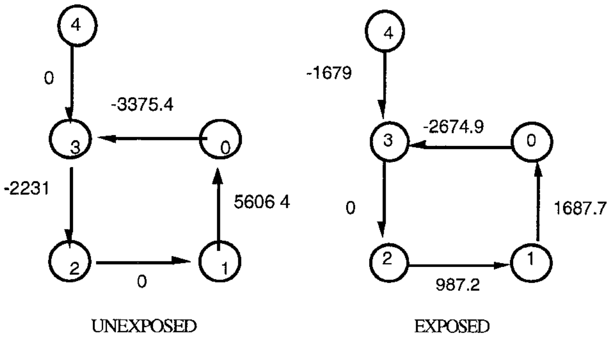

Fig. 2: Energetical gaps among the possible channel conformations in sham and exposed condition.

Several considerations on the effects of MW fields on the stability of this macromolecular structure can be done on these bases. Referring to appropriate biochemical parameters (ligand affinity and efficacy), we demonstrate that the global conformational effects of MW are the increase of the probability that the ligand bind the receptor and a decrease of the probability that the channel open due to the ligand binding. These results inform about the biochemical processes modified by MW effects, and about the structural changes induced by MW on the molecular chains of protein ACh receptor channels. Moreover, from the energetical mapping of the ACh-channel shown in Fig. 2, we understand how the stability of the channel conformations is modified by MW. State 0 is very stable, in both conditions. State 1, 2 and 4 are more stable under MW exposure. State 3 stability decreases substantially, and this is the most evident effect.
These results explain why the same channel can have different behaviours for different exposure conditions. Moreover, the knowledge of the energetical gaps is a first step towards a severe evaluation of all physical and chemical processes involved by MW exposure inside ACh-receptor channels.

CONCLUSIONS

A parallel implementation of a modelling technique based on Markov models has been proposed. The use of a multiprocessor architecture has allowed the solution of computation problems limiting the investigation on MW effects on ACh receptor channels with the above mentioned modelling approach. A parallelization of the model and of the optimization module has allowed the appropriate evaluation of all the model parameters. The complete modelling of the ACh channel gating under MW exposure has given a deeper information on the energetical coupling of the stimulating field with the ACh channel. The biochemical processes modified by MW effects have been individuated and their energetical modifications quantified.

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