A model integrating the cerebellar granule neuron excitability and calcium signaling pathways

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Abstract

The activation of various intracellular biochemical pathways is influenced by the electroresponsiveness of the neuron. There exists a close and constant co-operation between the membrane-bound cellular functions and intracellular signaling. This work aims at integrating the biophysical and biochemical data available for the cultured cerebellar granule neuron. The models of calcium-protein kinase C signaling pathways are combined with the model of the granule neuron excitability. The latter model can be used to provide the realistic stimulus for the signaling pathway models. The integrated model makes it possible to explore the participation of intracellular mechanisms in the neuronal information processing.

Keywords: Calcium ion; Excitability; Intracellular biochemical pathway; Modeling; Neuron.

1 Introduction

The electrical excitability in neurons is known to lead to changes at the molecular and genetic level, introducing a potential mechanism for storing information [2]. As an example, Shibata

et al. [8] have experimentally shown that the expression of ion channels and electrical excitability is crucial for the proper gene expression during cerebellar granule cell development and for formation of synapses during their differentiation. Changes induced in the concentrations of second messengers, such as the free calcium (Ca^{2+}) ions, activate or influence many cascades of biochemical reactions. Therefore, by combining the mathematical models so far created for cell membrane properties and excitability, for intracellular calcium processing and for biochemical pathways, we can study the dynamics of the complex molecular interactions of the cell.

Cerebellar granule cells are small glutamatergic relay neurons in the cerebellar cortex circuitry. The previously developed compartmental model for the cultured cerebellar granule neuron (CGN) excitability is capable of reproducing the major electroresponsive properties, the fast frequent firing up to 300 Hz and linear f-I relation (9 Hz/pA), of its real counterpart under current stimulation conditions [6]. Protein kinase C (PKC) signaling is known to be involved in various calcium-related biochemical pathways inside the cell. We are specifically interested in understanding the role of these pathways in the context of the model for the cultured cerebellar granule neurons [5]. In this work, the compartmental model is used to predict the realistic intracellular calcium concentrations needed for the calcium-protein kinase C (Ca-PKC) signaling pathway activation.

2 Models

2.1 Model for excitability

This paper expands our membrane-level model by incorporating in it some intracellular signaling pathways to study their dynamic interactions with ion channels and excitability. The compartmental model for the cultured cerebellar granule neuron was implemented using the GENESIS neuronal simulator with special emphasis on model parameter and constraint selection, as well as model fine-tuning using semi-automatic optimization procedure [5], [6].

Based on experimental evidence the neuron was assumed to be a sphere containing six different voltage-dependent ion channel/current types. These inluded the fast-inactivating sodium channel (Na_F), the delayed-rectifier type of potassium channel (K_{Dr}), the transient fast-activating A-type potassium channel (K_A), the inward-rectifier type of potassium channel (K_{ir}), the high-voltage activated calcium channel (Ca_{HVA}) and the large-conductance, voltage- and calcium -activated potassium channel (BK_{Ca}), for which we presented Hodgkin-Huxley type reconstructions. The Ca_{HVA} is responsible for loading the calcium ions into the cell. The active participation of calcium ions was assumed to take place in a very narrow volume close to cell membrane.

2.2 Model for calcium signaling pathways

A survey was made to compare the different kinds of simulators for their ease of use, specifically together with the already existing GENESIS scripts, and eventually the Kinetikit graphical user interface was chosen [1], [3]. Kinetikit is an extension to GENESIS simulation software and runs under GENESIS simulator [4]. Kinetikit makes it easy to model large networks of interacting pathways and has its own script language. The signaling pathways are described by reactions, reactions rates, and concentrations of reactants using simple chemical reaction kinetics. GENESIS 2.2.1 ja Kinetikit 9 are here used for all simulations.

Both the realistic calcium stimuli and different types of artificially generated waveforms to activate and test the pathways of interest are used. The earlier developed CGN model is used as a basis to compute the realistic calcium time-series data for activating the pathways. Specifically, we have studied the protein kinase C pathways using basic chemical reaction kinetics and the modified initial parameter values originally presented by Bhalla and Iyengar for a hippocampal neuron [2]. The models for the example pathways were obtained from the database in http://doqcs.ncbs.res.in [9]. The components and connections were first defined in Kinetikit scripts and the reaction kinetics then presented as follows,

$$\mathrm{A} + \mathrm{B} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{C}, \tag{1}$$

where A and B are reactants and C is the product. k_1 is the forward and k_{-1} the backward rate constant. The reactions of the PKC pathway (Eq.1) can be given with the systems of differential equations of the form

$$\frac{\mathrm{d}[\mathrm{C}]}{\mathrm{d}t} = -\frac{\mathrm{d}[\mathrm{A}]}{\mathrm{d}t} = -\frac{\mathrm{d}[\mathrm{B}]}{\mathrm{d}t} = k_1[\mathrm{A}][\mathrm{B}] - k_{-1}[\mathrm{C}]. \tag{2}$$

The differential equations are implemented in Kinetikit and the activities of the pathways are simulated with different kinds of input for the calcium component of the PKC model. Both artificial and realistic stimuli are used; linear and step functions, waveforms resembling a sine wave, and calcium time-series data simulated using the neuronal model by Linne [5]. The concentrations of any other second messengers of the PKC pathway are kept constant.

3 Simulations

The changes induced by action potentials in the intracellular calcium concentrations were first computed using the excitability model and different types of input stimuli to activate the neuron. In Figure 1 we stimulate the neuron's soma using the current injection of 12 pA to generate action potentials. The associated calcium concentration oscillates between 1 and 5.5 μM, the range of which is assumed typical for a small granule neuron during frequent firing. Subsequently, the generated calcium time-series is used as an input signal to the PKC signaling pathway and its second messenger calcium (Fig.2).

Clear changes were found in the simulated activities of interacting molecules when using both artificial and realistic stimuli for the calcium component (Fig.2). The waveform resembling a sine wave, as well as the realistic calcium time-series, induced oscillating PKC activity. There is a delay in the onset and offset of the PKC activity. Furthermore, the longer the

period of the stimuli, the stronger is the activity (i.e. the amplitude of the simulated signal). The pathway modeling also verified that the realistic calcium stimuli needs to be less than 10 μM which is 100 times the normal cytosolic Ca^{2+} concentration of 100 nM. Above 10 μM concentrations the pathway activities will saturate.

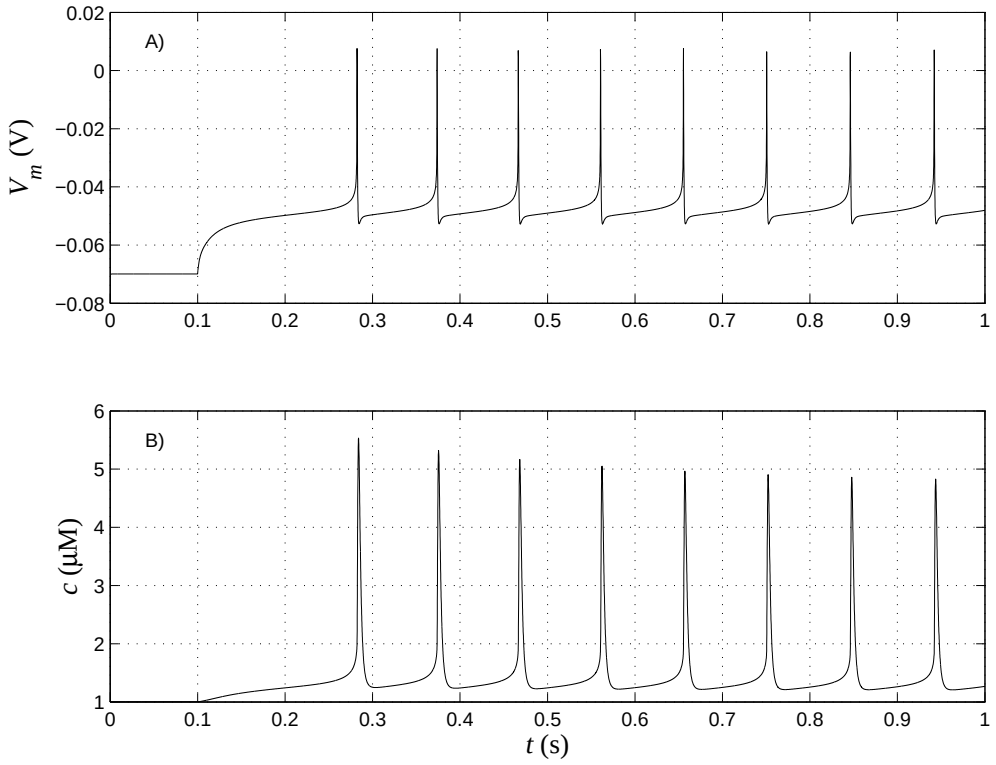

Figure 1: Simulations of the excitability. A) Frequent firing of the CGN model with a 12 pA current injection for 0.9 s, B) Calcium time-series associated with frequent firing.

4 Discussion and conclusions

A model integrating neuronal excitability and intracellular signaling makes it possible to computationally study the complex interactions between ion channels, second messengers and intracellular signaling molecules. The sustained activation of intracellular signaling pathways may serve as a candidate for molecular memory mechanism also in the cerebellar granule

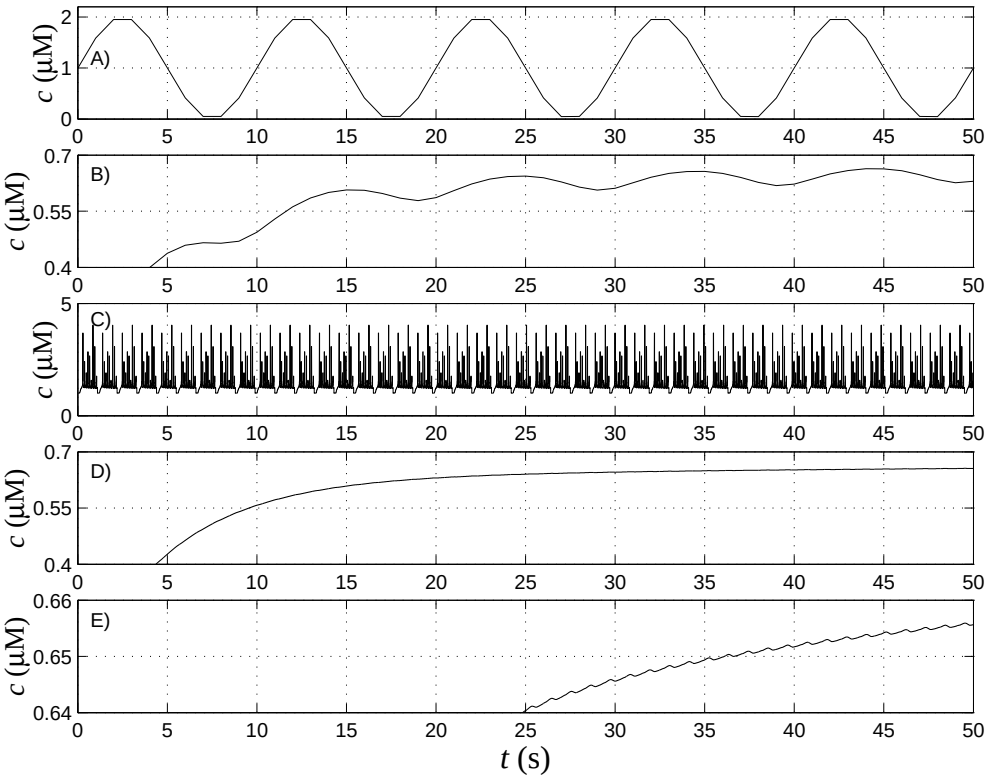

Figure 2: Simulations of the calcium signaling pathways. A) Artificial stimulus (waveform resembling a sine wave) and B) the corresponding PKC activity. C) Calcium time-series generated using the neuronal excitability model. Small, 12 pA current injections are applied to the neuron's soma for 1.1 s. The injections are repeated for the total simulation time of 50 s. D) The corresponding PKC activity for realistic calcium time-series. E) A detailed presentation of the signal trace seen in D. A clear oscillatory behavior is seen in the PKC activity.

neuron as shown by Bhalla and Iyengar [2]. We will use the integrated model presented in this paper to study the modulation of especially Ca_{HVA}, BK_{Ca} and K_A channels by intracellular signaling. Furthermore, we will simulate the effect of intracellular signaling pathways on action potential waveforms and overall neuronal firing patterns. The use of simulation models allows us to predict the effects of various specific intracellular signaling pathways capable of modulating neuronal excitability. Calcium-imaging experiments combined with the current-

clamp data will be used to define the constraints for modulation of intracellular signaling pathways.

In general, it will be necessary for the creation of cellular models to emphasize the importance of taking into account the structural and electrophysiological properties of the cell, as well as the molecular-genetic aspects of the highly complex molecular reactions of the cell. This work presents some aspects of the information processing capabilities of single cells by utilizing the amount of information available from biochemical and molecular biological studies. The integrated model here presented provides a systems biological approach to study and predict the roles of signaling molecules and separate pathways in a neuron. In future studies we will be integrating models also for store-induced release of calcium ions in neurons to explore the compartmentalized actions of intracellular calcium in the functions of the signaling pathways and networks of pathways (preliminary results presented in [7]).

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Tiina Manninen has studied mathematics and physics at the Tampere University of Technology, Finland, and received her M.Sc. degree in mathematics in September 2003. She is specializing in modeling and simulation of the biochemical signaling in neurons with an emphasis on automated model parameter estimation. She is presently pursuing her Ph.D. studies at the Tampere University of Technology, Institute of Signal Processing.

Tuula O. Jalonen got her B.Sc. degree in biochemistry and M.Sc. and Phil.Lic. degrees in animal physiology at the University of Turku, and later her Ph.D. degree in physiology at the Medical School, University of Tampere, Finland. Her main field of research is on cellular level *in vitro* electrophysiology and brain research with studies on receptors, ion channels and calcium signaling, related to various neurological diseases. She has done research and held positions in Finland at the universities of Turku and Tampere, as well as for long periods of time in Sweden and UK. During the years 1991-1997 she held the position of assistant professor at the Department of Neurosurgery, Albany Medical College, Albany, NY, USA. After returning to Finland, she has continued her research at the Institute of Medical Technology in Tampere and since 2002 holds a position of Senior Scientist at the Department of Biological and Environmental Science of the University of Jyväskylä in Central Finland.