SIMULATIONS OF THE CULTURED GRANULE NEURON EXCITABILITY

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Abstract

We have developed a biophysical model of a cultured rat cerebellar granule neuron and simulated its excitability under different experimental conditions. The basic excitability properties of such a small neuron; the specific action potential waveforms, the overall firing patterns induced by current stimulations, and the linear frequency-current relation, are the main model constraints. Simulations show that for a one-compartmental granule neuron model, the constraints are met using six voltage- and time-dependent ion channel types and calcium dynamics linked to BK_{Ca} ion channel function. This kind of model of a single neuron forms a solid basis for building the increasingly more complex network models of cultured neurons, and specifically for the cerebellar neuronal network.

Keywords

Biophysical model, cell culture, cerebellum, excitability, granule neuron

Introduction

Cerebellum is known to be important in controlling, fine-tuning and predicting movements, and may also be involved in higher cognitive functions. To study the properties of the interesting cerebellar circuitry, we created a biophysically-detailed model of one of the cerebellar neuronal types, the granule neuron (CGN), and simulated its excitability. The CGN model here presented, is capable of reproducing the major electroresponsive properties of its real *in vitro* counterpart when a minimum number of compartments/parameters is used (see also [12,13]). This is the first model developed specifically for cultured CGN, in comparison to the earlier compartmental models for slice preparations [6,8,14]. The CGN model of cultured neurons can further be utilized for simulations of complex interactions between ion channels, intracellular signaling and neuronal excitability (as described by Bhalla [2]), for simulation of the neuronal excitability during differentiation [19], and for models of the behavior of complex networks of cultured neurons. The networks are at present increasingly studied by using multi-electrode arrays as culture platforms (see e.g. Fromherz [7]).

Methods

Model Implementation. We used compartmental modeling techniques, Hodgkin-Huxley type reconstructions of ion channel/current behavior and a simple model for calcium dynamics, to mathematically describe the granule neuron excitability. The CGN model was implemented using the GENESIS script language and neuronal simulator [20]. The starting parameter values of the model equations were selected based on data from *in vivo* and *in vitro* experiments on cerebellar granule neurons. The basic excitability properties of the granule neuron were used as the main constraints of the model. Emphasis was put on data from neurons in culture.

Model Assumptions and Equations. Based on experimental evidence and electrotonic calculations the neuron was assumed as one-compartmental sphere containing six different voltage-dependent ion channel/current types (BK_{Ca}, Ca_{HVA}, K_A, K_{Dr}, K_{ir} and Na_F). In summary, the total current

through the neuronal membrane ($I_{tot}(t)$) was given as the sum of capacitive, passive and active ionic as well as externally applied currents:

$$I_{tot}(t) = d/dt(V_m(t)) \cdot C_M + (V_m(t) - E_M)/R_M + \sum_{i=1}^{n} g_i(V_m(t),t) \cdot (V_m(t) - E_i) + I_{app}(t), \quad (1)$$

where $V_m(t)$ is the transmembrane potential, C_M the cell membrane capacitance, R_M the linear membrane resistance and E_M its equilibrium potential, $g_i(V_m(t),t)$ the voltage- and time-dependent ion conductance and E_i its equilibrium potential, and $I_{app}(t)$ the externally applied current. $g_i(V_m(t),t) = (Y_m^i)^{p_i} \cdot (Y_h^i)^{q_i} \cdot G_i$ and $d/dt(Y_{m/h}^i) = \alpha_{m/h}^i (1 - Y_{m/h}^i) - (\beta_{m/h}^i \cdot Y_{m/h}^i)$, where Y_m^i and Y_h^i are the gating particles for current activation (*m*) and inactivation (*h*), G_i is the maximum conductance, and α_m^i, α_h^i, β_m^i and β_h^i are the forward and backward rate constants for current activation (*m*) and inactivation (*h*). *i* is an index for specific ion channel type. The processing of calcium ions was assumed to take place only in a very narrow volume (shell) close to cell membrane, according to equation:

$$d/dt([Ca]) = (B \cdot I_{CaHVA}/v_{shell}) - (([Ca] - [Ca]_{rest})/\tau_{Ca}), \quad (2)$$

where *B* is a constant for calcium ion transfer into the cell, I_{CaHVA} the calcium current through voltage- and time-dependent calcium ion channels, v_{shell} the volume of the shell, $[Ca]_{rest}$ the intracellular calcium concentration at rest, and τ_{Ca} the time constant for the decay of intracellular free calcium.

Starting Parameter and Constraint Selection. The selection of starting and fixed parameter values for passive and active membrane properties are presented in detail in the Ph.D. Thesis by Linne [13]. In short, the channel equation parameters are based on the data given in some earlier publications [1,4,15,17,18], but with slight modifications. The basic excitability properties of the granule neuron, used as the main constraints of the model, were gathered from several publications [3,5,10,16,19], as well as from our own recordings [9,11] (see Table 1).

Table 1. Constraints for the CGN Model

Action Potential Waveform	Basic Firing Properties
1. V_{th} (-0.045 V $\le V_{th} \le -0.035$ V	**1.** Frequent, repetitive firing
2. $V_{peak}^{\max}$ (0 V $\le V_{peak}^{\max} \le +0.01$ V)	**2.** Linear conversion of *f-I*
3. $V_{peak}^{\min}$ ($V_{peak}^{\min} < -0.05$ V)	**3.** Delay in firing at threshold stimuli (I_{app}^{th} between 11 and 12 pA)

V_{th} is the threshold membrane potential for firing, $V_{peak}^{\max}$ is the maximum (positive) peak value and $V_{peak}^{\min}$ the minimum (negative) peak value of the membrane potential, I_{app}^{th} is the applied current threshold for initiating firing.

Optimization and Fine-Tuning. The CGN model was optimized and fine-tuned based on a semi-automatic, iterative approach [13]. It utilizes the following sets of parameters (Θ; Table 2): ***(i)*** a set of *known parameters* (Θ_1) which are *fixed* at a physiologically reasonable value (reliable estimates based on large experimental data set are required), ***(ii)*** a first set of *unknown parameters* (Θ_2) which are chosen based on realistic physiological approximations (initial guesses) and *fixed*, and ***(iii)*** a second set of *unknown parameters* (Θ_3 and Θ_4) which are optimized during the *iterative fine-tuning*.

The final parameters used in the model simulations were: $d_{soma} = 6\times10^{-6}$ m, $R_M = 5\times10^{9}$ Ω, $C_M = 3.5\times10^{-12}$ F, $E_M = -0.07$ V (passive model), $E_M = -0.025$ V (active model), $E_{Na} = +0.07$ V, $E_K = -0.075$ V, $E_{Ca} = +0.14$ V, and $E_{BKCa} = -0.085$ V [***(i)***]. Ion conductance parameters were given both fixed (p_i, q_i) parameter values and initial guesses (G_i) according to the protocol in Table 2 (presented as 'channel name (p_i,q_i,G_i)'): BK_{Ca} (1, -, 30 S/m^2), Ca_{HVA} (2, 1, 4.6 S/m^2), K_A (3, 1, 10 S/m^2), K_{Dr} (4, -, 120 S/m^2), K_{ir} (1, -, 28 S/m^2), and Na_F (3, 1, 400 S/m^2) [***(i)***, ***(ii)*** and ***(iii)***]. Final calcium dynamics parameters were fixed in the beginning and given the following values: $B = 5.2\times10^{-6}$ mol/C, $[Ca^{2+}]_{rest} = 100\times10^{-6}$ mol/m^3, $\tau_{Ca} = 1\times10^{-3}$ s, and $d_{shell} = 1\times10^{-7}$ m.

Table 2. Optimization and Fine-Tuning Protocol for the CGN model

1. Define Θ.
2. Select the parameter subspace_1 ($\Omega_i \in \Theta_1$) with known, fixed parameters, and set each Ω_i its corresponding value.
3. Set the initial guesses for the parameter subspace_2, Θ_2, $G_i \in \Theta_2$, i = Na_F, K_{Dr}, K_{ir}. Set G_{Kir} as constant initial guess.
4. Define the lower (V_{th}^1) and upper (V_{th}^2) bounds for V_{th}.
5. If $V_{th}^1 \le V_{th} \le V_{th}^2$ goto 7.
6. Else keep adjusting Θ_2, but keep G_{Kir} constant, until 5 is met.
7. Define initial guesses for parameter subspace_3, Θ_3, $G_i \in \Theta_3$, i = Ca_{HVA}, BK_{Ca}, and adjust $0.5 \cdot G_{KDr}$.
8. Define upper bounds for V_{peak}^{max} ($V_{peak}^{max\,1}$ and $V_{peak}^{max\,2}$).
9. If $V_{peak}^{max\,1} < V_{peak}^{max} < V_{peak}^{max\,2}$ and V_{th} > -0.045 V goto 11.
10. Else keep adjusting Θ_3 until 9 is met.
11. Define initial guess for $G_i \in \Theta_4$, i = K_A.
12. Adjust basic firing properties according to Table 1.

Results

Simulations show, that for a one-compartmental neuron six voltage- and time-dependent ion channel types (BK_{Ca}, Ca_{HVA}, K_A, K_{Dr}, K_{ir}, and Na_F) and simple calcium dynamics reliably reproduce the experimentally recorded basic firing properties of the cultured granule neuron. This suggests that a one-compartmental representation is sufficient for reproducing the constraints (Table 1) for a small cultured neuron. In summary, the CGN model of this study is able to reproduce the following basic electroresponsive properties: **(1)** realistic action potential waveform, specific for a cultured granule neuron (Fig. 1.), **(2)** delay in firing with small applied currents (I_{app}; Fig. 2), **(3)** fast frequent firing, up to 300 Hz (Fig. 2), and **(4)** linear frequency-current (*f-I*) relation, up to 200 Hz (Fig. 3). Furthermore, simulations by eliminating particular ion channel currents from the final fine-tuned model (i.e., resembling pharmacological blockade of ion channels) further validated the model robustness. For example, complete elimination of BK_{Ca} causes reduced hyperpolarization, thereby causing increase in

firing frequency (not shown here). Similarly, complete elimination of K_A causes increase in firing frequency.

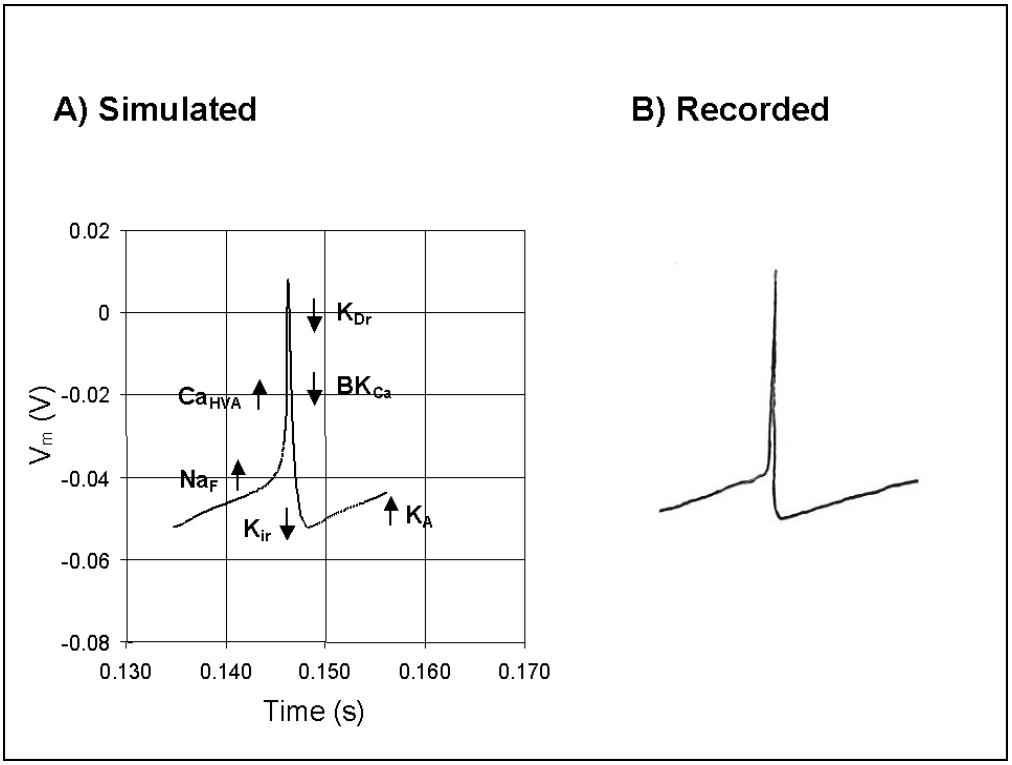

Figure 1. Simulated (A) and recorded (B) action potential waveforms. The waveform of the recorded AP in **B** and its specific phases are satisfactorily reproduced: both the potential preceding the spike and after the spike are reproduced with the simulated model in **A**. The threshold membrane potential for firing is approximately -40 mV. The positive peak of the AP is close to +10 mV and the negative peak to -55 mV. The experimentally recorded AP is taken from Lin and Moran [10].

The current application threshold (I_{app}^{th}) for firing was set between 11 and 12 pA during the final step of the optimization process (Table 2). The CGN model of this study was found to fire at 9 Hz frequency with 12 pA current stimulus. The so-called "K_A effect", a delay in the firing due to the early, strong activation of the K_A type potassium channels with current stimulus pulses close to the threshold (I_{app}^{th}), is reproduced (Fig. 2A), similarly to the effect shown for granule neurons in slice preparations [1]. The constrained CGN model is frequently firing when current stimulus pulses of $I_{app} \geq 13$ pA are used. As can be seen from Fig. 2, the model neuron reproduced reasonably realistic, frequent firing,

showing no adaptation. When the applied current increases, the model neuron is capable of reproducing frequent, repetitive firing up to 200 Hz without saturation ($I_{app} \leq 35$ pA). With relatively high current steps ($I_{app} \geq 40$ pA), the CGN model is still frequently firing, but shows mild saturation of the *f-I* curve. The highest firing rate the model neuron can attain is 300 Hz. The turtle granule neuron model was found to achieve 100 Hz without damped oscillations, when implemented as one-compartmental representation based on the original model by Gabbiani et al. [8] within the framework of this study using GENESIS (Fig. 3).

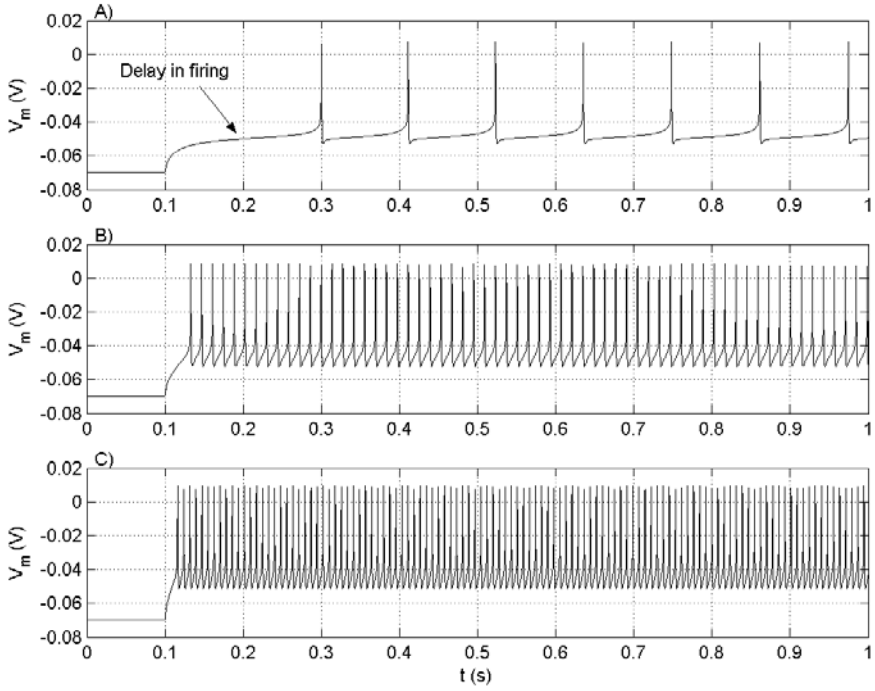

Figure 2. Frequent firing of the CGN model showing no adaptation. I_{app} is 11.9 pA **(A)**, 18 pA **(B)** and 24 pA **(C)**. The delay in firing is successfully reproduced **(A)**.

Fig. 3 shows the *f-I* relations for our model and other earlier CGN models. The present model reproduces the linear *f-I* relationship and fairly reliably follows the experimentally obtained *f-I* relations [3,6]. Therefore, CGN model of this study with linear input-output conversion of applied currents predicts reliable translation of inputs into action potential discharges, even with relatively high firing frequencies. The slopes of the *f-I* curves are as follows: 9 Hz/pA for the CGN model of this study, 8

Hz/pA for slice and a model granule neuron by D'Angelo et al. [5,6], 6 Hz/pA for slice granule neuron by Brickley et al. [3], and 4 Hz/pA for the turtle models when the model is reduced to one compartment and simulated with GENESIS neuronal simulator ([8,14]; linear part of the curve).

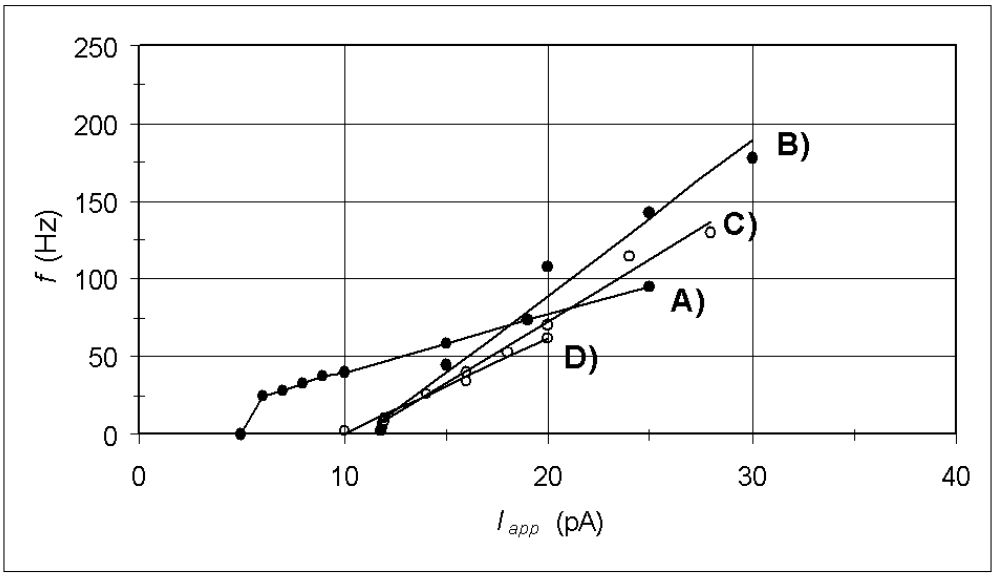

Figure 3. Comparing *f-I* relations for existing model and *in vitro* granule neurons. A) Turtle granule neuron model [8,14], **B)** CGN model of this study, **C)** *in vitro* granule neuron of slice preparation [5] and model neuron [6], and **D)** *in vitro* granule neuron of slice preparation [3].

Discussion and Conclusions

In conclusion, we have developed a biophysical model of a cultured rat cerebellar granule neuron, which is capable of reproducing the major electroresponsive properties of its real counterpart (see also [12,13]). The presented model has the following strengths: **(1)** it consists of a realistic set of ion channels, characterized for a rat cerebellar granule neuron, **(2)** it is able to reproduce the basic electroresponsive properties, specifically the linear *f-I* relation, under current stimulation conditions, **(3)** it is reliable and robust to changes in key parameters, being able to mimic the behavior of real neurons under similar experimental conditions, and **(4)** it is implemented and optimized on a GENESIS

platform, which makes it more flexible to be used for further implementations at network and subcellular levels.

This model is a further addition to the earlier compartmental models for cerebellar granule neurons, which include models for a turtle [8] and for a rat [6,14]. Those three models, however, are mainly aimed at mimicking neuronal behavior in slice preparations. Furthermore, the turtle granule neurons differ from the rat granule neurons in that they have larger soma and dendritic volumes, thereby making the reptile turtle granule neurons electrotonically less compact compared to mammalian rat ones. In addition, turtle granule neurons express different types of ion channels, compared to rat CGNs. Single neuron models, such as the model here presented for a neuron in culture conditions, can be used for various kind of simulations of the complex interactions between ion channels, intracellular signaling and excitability. For the future subcellular modeling of e.g. cellular signaling pathways a lot of biochemical kinetic and molecular data, specifically obtained from the cultured granule neuron, is already available. Therefore, the present model would serve as an ideal template for simulating the excitability during neuronal differentiation in various culture preparations, such as the explant cultures used by Shibata et al. [19]. Single-cell culture models can be further utilized as basis for complex network models of cultured neurons, which have been recently increasingly studied by multi-electrode array systems.

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