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A NEW COMPUTER SOFTWARE FOR SIMULATION OF NEURONS

Mahmut ÖZER, Yalçın İŞLER
Zonguldak Karaelmas University, Engineering Faculty, Department of Electrical and Electronics Engineering, 67100-Zonguldak

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ABSTRACT

In this paper, a new computer software package 'Simulron' for simulation of neurons is introduced. Excitable membranes with voltage-gated ionic channels can be modeled by using the software, and current clamp and voltage clamp experiments can be simulated. The program allows user to determine the ionic channel count and set the rate functions of the channels. If the rate functions are not known, the program enables the user to set steady-state and time constant functions. First-order differential equations used to define dynamics of the gate and membrane potential are solved using forward Euler method of integration with variable time steps. Outputs of the simulations are shown on spreadsheet template allowing flexible data manipulation and can be graphically displayed.

Key Words : Simulation, Neuronal modeling, Computer software, Voltage-Gated ionic Channels, Excitable membranes

NÖRONLARIN SİMULASYONU İÇİN YENİ BİR BİLGİSAYAR YAZILIMI

ÖZET

Bu makalede, nöronların simülasyonu için yeni bir bilgisayar yazılımı 'Simulron' tanıtılmaktadır. Yazılım kullanılarak gerilim-kapılı iyon kanallarına sahip uyarılabilir membranlar modellenebilmekte ve akım ve gerilim kenetleme deneyleri simüle edilebilmektedir. Program, kullanıcının iyon kanal sayısını belirlemesine ve kanalların hız fonksiyonlarını tanımlamasına izin vermektedir. Hız fonksiyonları bilinmiyorsa, program kullanıcının sürekli hal ve zaman sabiti fonksiyonlarını ayarlamasına imkan tanımaktadır. İyon kanal kapısı ve membran potansiyeli dinamiğini tanımlamak için kullanılan birinci-dereceden diferansiyel denklemler değişken zaman basamaklarına sahip ileri yönlü Euler integrasyon yöntemi kullanılarak çözülmektedir. Simülasyonun çıktıları esnek data kullanımına izin verecek şekilde tablo şablonu üzerinde gösterilmekte ve grafik olarak görüntülenebilmektedir.

Anahtar Kelimeler : Simülasyon, Nöronal Modelleme, Bilgisayar Yazılımı, Gerilim-Kapılı İyon Kanalları, Uyarılabilir Membranlar

1. INTRODUCTION

Computer simulations of biological neuronal models are powerful tools for neuroscientists on understanding of nervous system physiology and its functioning (Sacchi et al., 1998; De Schutter, 1992). Experimental studies are taken as a basis for constructing neuronal models. Furthermore the simulations based on neuronal models are used to investigate features of neurons that are experimentally inaccessible or not easily controlled (Yamada et al., 1999). In this context, a number of computer software packages such as Nodus (De Schutter, 1989), Neurosim (Revest, 1995), Neuron (Hines and Carnevale, 1997) and Genesis (Bower and Beeman, 1995) are introduced for neuronal modeling. Nodus which runs on Apple Macintosh

(TM) Computers is designed for simulation of the electrical behavior of neurons and small networks. Neurosim is a teaching software and includes a set of programs for use in teaching neurophysiology at basic and intermediate level. The others support the simulation of complex models of single neurons and large networks for experienced modelers. The Neuron and Genesis were developed in the Unix environment, but the Neuron was subsequently ported to MSWindows and MacOS. Their comparative analysis is carried out by De Schutter (De Schutter, 1992). As a cheap alternative, easily understood methodology for solving simple biological models is presented using a Microsoft Excel spreadsheet (Brown, 1999; 2000). His approach involves entering the key parameter values into the spreadsheet and conducting the simulations by solving a set of equations without requiring any programming skills or use of the macro language. Recently a new software tool implemented in Java using Jbuilder4 is reported for modeling biological systems (Burhan and Holcik, 2002).

In this paper, we present a new software package 'Simulron' for simulation of single-compartment neuronal model as do-it-yourself in which user-defined voltage-gated ionic channels can be set. The program allows user to determine the ionic channel count and set the rate functions of the channels. If rate functions are not known, the program enables the user to set steady-state and time constant functions. Outputs of the simulations are shown on spreadsheet template as in (Brown, 1999; 2000) and can be graphically displayed. The software package is available on request being free of cost.

2. PROGRAM DESCRIPTION

The Simulron was developed in Delphi 6.0 programming language. All parameters used to construct the model are set by the user. When it runs, the main page is screened as shown in Figure 1. Desired menus are accessible through the main page.

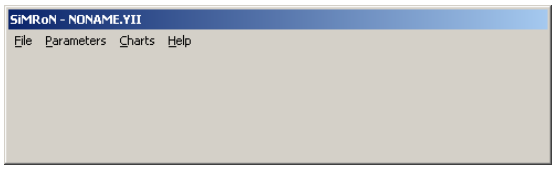


Figure 1. Program main page

2. 1. An Ionic Channel Gate Model

Simulron supports any format using Hodgkin-Huxley mathematical formalism (Hodgkin and Huxley, 1952) for user specified voltage-gated ionic channels. Therefore Hodgkin-Huxley mathematical formalism for voltage-gated ionic channels is discussed briefly in this section.

In the H-H mathematical model, each ionic channel is assumed to have one or more independent gates. An ionic channel gate exists in just two states, closed (C) and open (O). The gate can change from one state to the other at random. The changes are stochastic events-that is to say they occur at random in time domain- and so we can describe their timing only in probabilistic terms. There is some structure or the property of the gate that is concerned with the transition between these two states, and the word *gate* is used to describe this concept (Aidley and Stanfield, 1996). Gating is the process whereby the gate is opened and closed. There may be a number of different closed and open states, so the gating processes may involve a number of different sequential or alternative transitions from one state of the gate to another. In order for the ionic channel to be open, all of its gates must be in the open state. With these assumptions, conductance of an ionic channel through a population of identical ionic channels is defined as:

$$G_X(V,t) = g_{max-x} m^p(V,t) h^q(V,t) \quad (1)$$

Where *m* and *h* show voltage-dependent probability of being open state for activation and inactivation gates respectively, *V* is the membrane potential, g_{max-x} is maximal conductance of ionic channel when all of the gates were in the open state, *p* is the number of activation gates and *q* is the number of inactivation gates.

The activation and inactivation gates open and close over time in response to the membrane potential according to the first-order differential equations as follows:

$$\frac{dm}{dt} = \phi(T)\left[\alpha_m(V)(1-m) - \beta_m(V)m\right] \quad (2)$$

$$\frac{dh}{dt} = \phi(T)\left[\alpha_h(V)(1-h) - \beta_h(V)h\right] \quad (3)$$

Eqs. (2) and (3) state that the closed activation gate (*1-m*) and the inactivation gate (*1-h*) open at a rate $\alpha_m(V)$ and $\alpha_h(V)$ respectively; and the open activation gate m and the inactivation gate h close at a rate $\beta_m(V)$ and $\beta_h(V)$ respectively. The rate functions $\alpha(V)$ and $\beta(V)$ are dependent on the potential across the membrane. The forms of the rate functions are usually determined by using a mix of

theoretical and empirical considerations (Willms et al., 1999). $\phi(T)$ is temperature function involving the effects of temperature (T in ^{0}C) on the rate functions and defined as $\phi(T) = Q_{10}^{\Delta T/10}$ .The gating of many ion channels have Q_{10} near 3 (Hille, 2001). Warming speeds the rate functions (Hodgkin et al., 1952; Frankenhaeuser and Moore, 1963; Beam and Donaldson, 1983; Schwarz, 1986). $\phi(T)$ is taken as $3^{(T-6.3)/10}$ in Simulron. Eqs. (2) and (3) may be also written as :

$$\frac{dm}{dt} = \phi(T)\left[\frac{m_\infty(V) - m}{\tau_m(V)}\right] \tag{4}$$

$$\frac{dh}{dt} = \phi(T)\left[\frac{h_\infty(V) - h}{\tau_h(V)}\right] \tag{5}$$

Where $m_\infty(V)$ and $h_\infty(V)$ are steady-state activation and inactivation respectively since m and h will get asymptotically close to these values if the voltage is held constant for a sufficient duration; $\tau_m(V)$ and $\tau_h(V)$ show voltage-dependent activation and inactivation time constants so that the time course for approaching these equilibrium values is described by a simple exponential with these time constants, and may be written as:

$$m_\infty(V) = \frac{\alpha_m(V)}{\alpha_m(V) + \beta_m(V)}$$
$$h_\infty(V) = \frac{\alpha_h(V)}{\alpha_h(V) + \beta_h(V)} \tag{6}$$

$$\tau_m(V) = \frac{1}{\alpha_m(V) + \beta_m(V)}$$
$$\tau_h(V) = \frac{1}{\alpha_h(V) + \beta_h(V)} \tag{7}$$

Simulation is carried out based on the single-compartmental model of a neuron which is represented by coupled differential equations according to the H-H model. Current balance equation is

$$C_m \frac{dV_m}{dt} + I_{ion} = I_{inject} \tag{8}$$

where C_m, V_m, I_{ion}, I_{inject} represent membrane capacitance, membrane potential, sum of ionic currents, and injected current respectively. Sum of the ionic currents is given by

$$I_{ion} = \sum_x G_x(V_m - E_x) \tag{9}$$

where E_x is Nernst equilibrium potential which is also called as a reversal potential. The change in membrane potential is expressed as follows:

$$\frac{dV_m}{dt} = \frac{1}{C_m}\left[I_{inject} - I_{ion}\right] = \frac{I_{total}}{C_m} \tag{10}$$

2. 2. Integration Method

First-order differential equations used to define dynamics of the gates and membrane potential are solved in Simulron using the fast forward Euler method of integration with variable time steps. It's necessary to compute *m* and *h* values at each time step before calculating of membrane potential. Eqs. (2) and (3) or Eqs.(4) and (5) have a general form as

$$\frac{dy}{dt} = f(t) = A - By \tag{11}$$

Where $A=\alpha$, $B=\alpha+\beta$. We use forward Euler method to obtain the values of *m* and *h* for each time step. Solution of Eq. (11) for time increment Δt is given as follows (Mascagni and Sherman, 1999):

$$y(t + \Delta t) = y(t) + \Delta t f(t)$$
$$m_{i+1} = m_i + \Delta t \phi(T)\left(\alpha_{m_i}(1 - m_i) - \beta_{m_i} m_i\right) \tag{12a}$$

If the rate functions are not known, then solution of Eq. (11) for time increment Δt is given as follows:

$$m_{i+1} = m_i + \Delta t \phi(T)\left((m_\infty - m_i)/\tau_m\right) \tag{12b}$$

Initial values for *m* and *h* is calculated from steady-state values at initial membrane potential(resting membrane potential). After calculating of *m* and *h* values, it's easy to calculate an ionic current with Eqs. (9) and (1) . Next step at the integration is to calculate the membrane potential according to Eq. (10). When the expression on the right side of Eq. (10) was calculated, it has a constant value. Therefore the integration of membrane potential is done with forward Euler method (Mascagni and Sherman, 1999):

$$V_m(t + \Delta t) = V_m(t) + \Delta t \frac{I_{total}}{C_m} \tag{13}$$

2. 3. File Menu

File menu on the main page contains processes about file manipulation as shown in Figure 2.

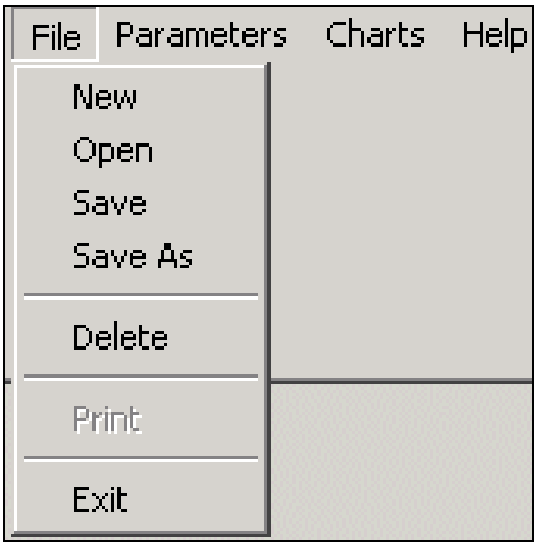


Figure 2. File menu

'New' command opens a new parameters page. 'Open' command loads a parameters file with YII extension saved on disk. It is ensured to save a file on disk with 'Save' command. Current parameters can be saved with a different name by using 'Save As' command. It can be deleted a saved parameters file by 'Delete' command. And finally 'Exit' command gets the user out from the program.

2. 4. Parameters Menu

When 'Parameters' menu on the main page is selected, the Parameters page is screened as shown in Figure 3. All parameters used to construct the model are set by the user through the Parameters page. Firstly, ionic channel count, simulation duration, time step for numerical integration, temperature, resting membrane voltage, specific leakage conductance (G_{leak} in mS/cm^2), reversal potential for leakage current (E_{leak} in mV) and specific membrane capacitance ($C_{membrane}$ in μF/cm^2) are set by the user. The program creates voltage-gated ionic channels as much as 'Channel Count'. 'Next' command on the 'Parameters' page is used to screen 'Parameters for Channel #' page as shown in Figure 4 and set the parameters for the channels. Reversal potential (E_x in mV), maximal conductance of ionic channel (g_{max-x} in mS/cm^2), the number of activation gates (p) and the number of inactivation gates (q) for the channel are set by user at the top of 'Parameters for Channel #' page. There are two options for the gate kinetics. First option (Style 0) allows the user to set the rate functions of the activation and inactivation gates by keyboard as shown in Figure 4. If the rate functions are not known, steady-state and time constant functions must be set by using the second option (Style 1) as shown in Figure 5.

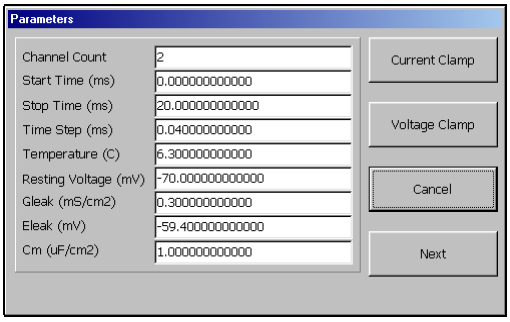


Figure 3. Parameters menu

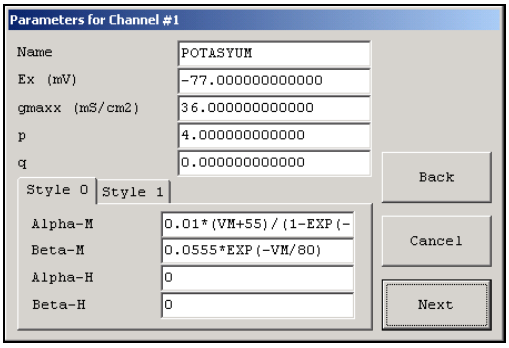


Figure 4. Parameters for Channel Page with Style 0

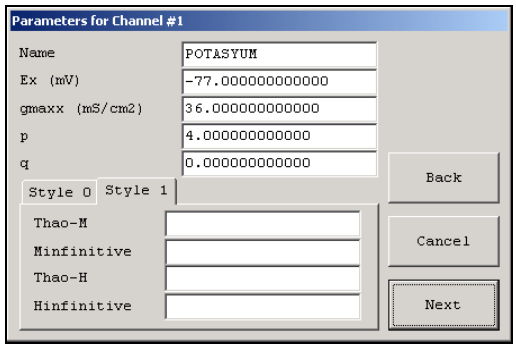


Figure 5. Parameters for Channel Page with Style 1

Parameter setting is canceled with 'Cancel' button. Finally parameter page for the next channel is obtained with 'Next' button. It is returned from 'Parameters for Channel #' page to 'Parameters' page with 'Back' button. After setting the parameters for the channels, voltage clamp or current clamp experiments can be simulated by clicking on 'Voltage Clamp' or 'Current Clamp' button on the 'Parameters' page.

When 'Current Clamp' button is selected, a new page is screened as shown in Figure 6. The user

must set starting time (in ms), stopping time (in ms) and magnitude (in $\mu A/cm^2$) of injected current on this page. When clicking right, two options are screened as 'New' and 'Delete'. 'New' command creates a new row to be set parameters of injected current. Subsequent use of the command will create a subsequent new row. It can be deleted unwanted currents which were set by 'Delete' command.

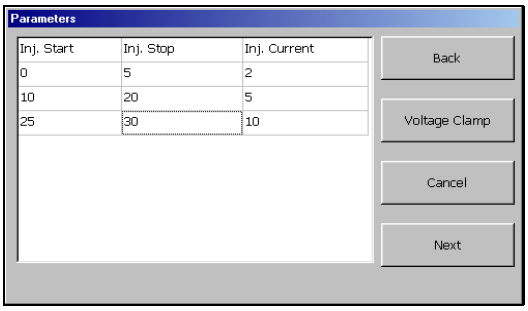
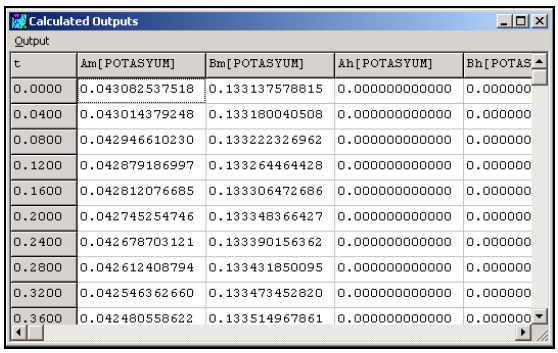


Figure 6. Current clamp page

When 'Voltage Clamp' button is selected, a new page is screened and clamped voltages (in mV) and their intervals (in ms) can be set as in current clamp simulation. 'Next' command on both 'Current Clamp' and 'Voltage Clamp' pages ends the parameter settings and enables the user to return main page.

2. 5. Charts Menu

After defining the model, setting parameter values and selecting the experiment type through 'Parameters' menu, the user must click on 'Charts' button to run simulation. 'Calculated Outputs' page is screened as seen in Figure 7 after ending of simulation. Calculated outputs of the model such as activation and inactivation rate functions, activation and inactivation gate variables, ionic channel currents and membrane potential are shown on spreadsheet template shown in Figure 7.



t	Am[POTASYUM]	Bm[POTASYUM]	Ah[POTASYUM]	Bh[POTAS
0.0000	0.043082537518	0.133137578815	0.000000000000	0.000000
0.0400	0.043014379248	0.133180040508	0.000000000000	0.000000
0.0800	0.042946610230	0.133222326962	0.000000000000	0.000000
0.1200	0.042879186997	0.133264464428	0.000000000000	0.000000
0.1600	0.042812076685	0.133306472686	0.000000000000	0.000000
0.2000	0.042745254746	0.133348366427	0.000000000000	0.000000
0.2400	0.042678703121	0.133390156362	0.000000000000	0.000000
0.2800	0.042612408794	0.133431850095	0.000000000000	0.000000
0.3200	0.042546362660	0.133473452820	0.000000000000	0.000000
0.3600	0.042480558622	0.133514967861	0.000000000000	0.000000

Figure 7. Calculated outputs page

'Output' menu on the 'Calculated Outputs' page allows the user to manipulate calculated values of the parameters used to construct the model as shown in Figure 8.

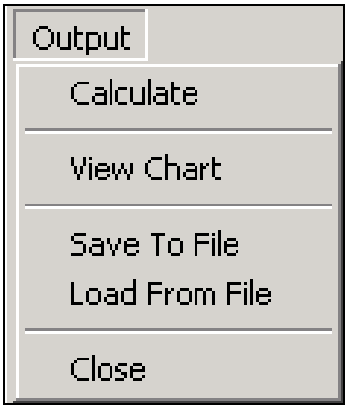


Figure 8. Commands of Output menu

'Calculate' command recalculates all of the values on the spreadsheet template. The variables can be graphically displayed by using 'View Chart' command. When 'View Chart' command is selected, a new page screened with two options as 'Series 1' and 'Series 2' shown in Figure 9. 'Series 1' and 'Series 2' contain the same variables to be graphically displayed.

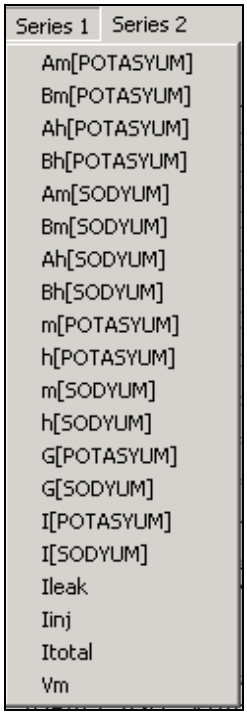


Figure 9. Variables to be graphically displayed in 'Series 1' and 'Series 2' menus

'Series 2' menu is used to display the second variable on the same graph. If the user wants to see any part of the graph in zoom, the user must select

this region by left button of mouse. When the left button of mouse is free, the selected region of the graph is displayed in zoom. It is returned to the original graph by clicking the right button of mouse on the graph.

'Save To File' command on the 'Output' menu is used to save current 'Calculated Outputs' data on a disk. Finally 'Load From File' command is used to load the saved 'Calculated Outputs' data. 'Close' comand is used to close the calculated outputs page.

3. SIMULATION RESULTS

In this section some simulation results are given to illustrate the Simulron based on the squid giant axon. Simulations were run with fixed time increment of 40 μs as in (Brown, 1999; 2000; Ozer, 2001; 2002). Specific membrane capacitance, *CM* is taken as 1 μF/cm^2, leakage conductance as 0.3 mS/cm^2, resting potential as -70 mV, and reversal potentials E_{Na} as 50 mV, E_K as -77 mV and E_L as –59.4 mV. Temperature is taken as 6.3 ^{0}C. Rate functions of the model are given in Appendix A.

In the first step, threshold for action potential firing is examined. A current pulse of 3 μA/cm^2 with 10 ms delay and 5 ms duration is injected into the compartment. Calculated membrane potential and ionic channel conductances are shown in Figure 10 and 11 respectively. As seen in Figure 10, the injected current wasn't enough to fire an action potential. The subthreshold current couldn't cause an increase in *gNa* resulting in depolarizing of membrane. It is seen in Figure 11 that the increase of *gK* is greater than that of *gNa*.

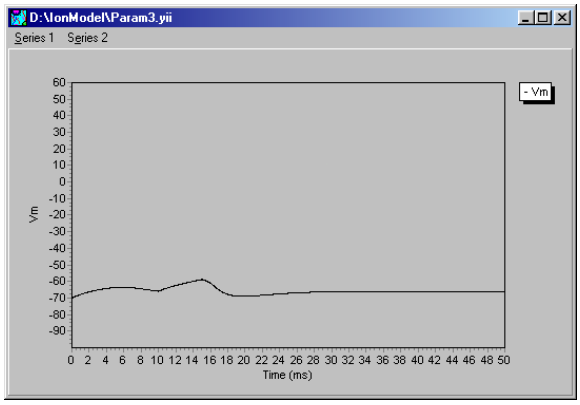


Figure 10. Membrane potential (in mV) under injection of 3 μA/cm^2 current pulse with 10 ms delay and 5 ms duration

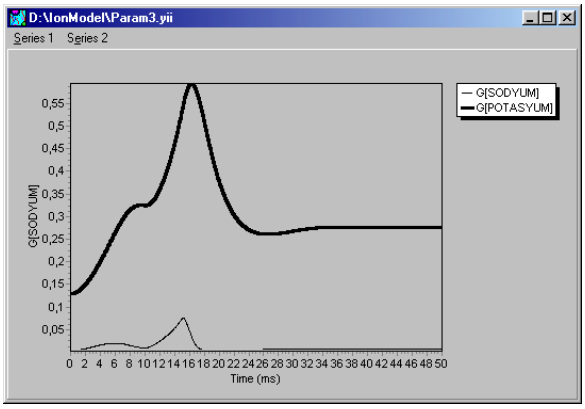


Figure 11. Ionic channel conductances (in mS/cm2) under injection of 3 μA/cm^2 current pulse with 10 ms delay and 5 ms duration

In the second step, A current pulse of 3.5 μA/cm^2 with 10 ms delay and 5 ms duration is injected into the compartment. Calculated membrane potential and ionic channel conductances, are shown in Figure 12 and 13 respectively. As seen in Figure 13, the injected current caused an increase in gNa which results in depolarizing of membrane, and a delayed increase in gK which results in repolarization of membrane. Both of them produced an action potential as shown in Figure 12. So the threshold can be defined in terms of the conductances as a membrane potential point at which inward Na^+ conductance is greater than outward K^+ conductance. Immediately after action potential, membrane potential goes down beyond the resting membrane potential level as seen in Figure 12. This after-hyperpolarization occurs due to *gK* outlasting *gNa* as seen in Figure 13. This fact can be traced from dynamic changes of activation and inactivation gate variables of fast sodium channel in Figure 14 and activation gate variable of delayed rectifier potassium channel in Figure 15.

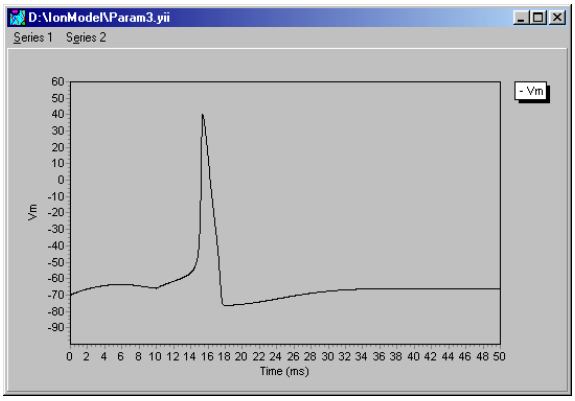


Figure 12. Membrane potential (in mV) under injection of 3.5 μA/cm^2 current pulse with 10 ms delay and 5 ms duration

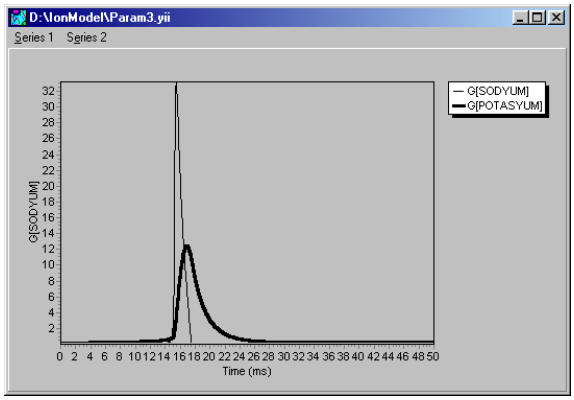


Figure 13. Ionic channel conductances (in mS/cm2) under injection of 3.5 μA/cm^2 current pulse with 10 ms delay and 5 ms duration

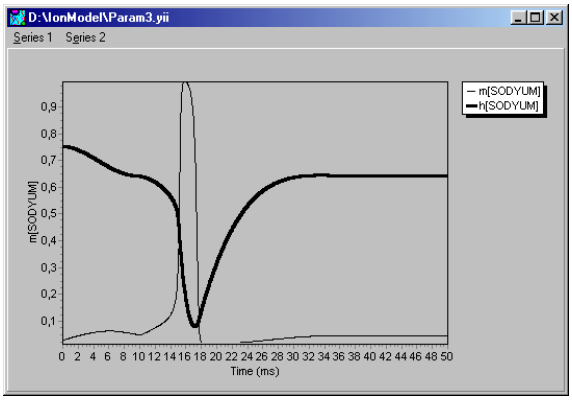


Figure 14. Activation and inactivation gate variables of fast sodium channel under injection of 3.5 μA/cm^2 current pulse with 10 ms delay and 5 ms duration

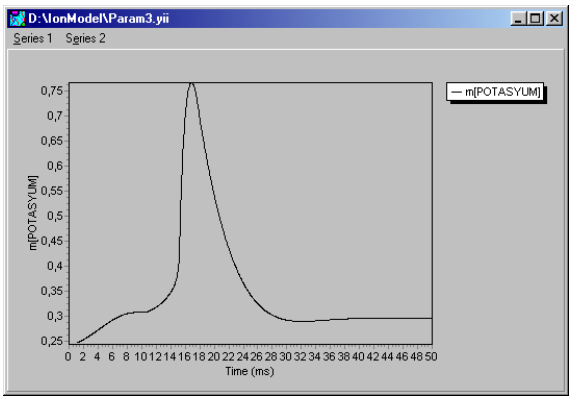


Figure 15. Activation gate variable of delayed rectifier potassium channel under injection of 3.5 μA/cm^2 current pulse with 10 ms delay and 5 ms duration

Fast sodium channel inactivation parameter *h* is close to 0, and delayed rectifier potassium channel activation parameter n is close to 0.75 immediately after the action potential. *h* value indicates that the majority of fast sodium channels are inactivated state, and *n* value indicates that the majority of delayed rectifier potassium channels are open. Therefore membrane hyperpolarizes immediately after the action potential. These two factors(i.e. the majority of fast sodium channels are in inactivated state, and the majority of delayed rectifier potassium channels are open) result in decreased excitability immediately after the action potential, and explain underlying mechanism of refractoriness after the action potential.

4. CONCLUSION

In this paper, we present a new software package 'Simulron' for simulation of single-compartment neuronal model as do-it-yourself in which user-defined voltage-gated ionic channels can be set. The program allows user to determine the ionic channel count and set the rate functions of the channels. If rate functions are not known, the program enables the user to set steady-state and time constant functions. First-order differential equations used to define dynamics of the gate and membrane potential are solved using the fast forward Euler method of integration with variable time steps. Outputs of the simulations are shown on spreadsheet template as in (Brown, 1999; 2000) and can be graphically displayed. The software package is available on request being free of cost. The user can examine the excitability of neurons and dynamics of voltage-gated ionic channels. The software package addresses to ones to run simple simulations of neurons without the need to any programming language or expensive software and also can be used for educational purposes.

5. APPENDIX A

Conductance of fast Na^+ channel is given by

$$G_{Na} = g_{Na} m^3 h \qquad (A.1)$$

where g_{Na} is 120 mS/cm^2. Rate functions of fast Na^+ channel at 6.3 ^{0}C are as follows:

$$\alpha_m(v) = \frac{0.1(40+v)}{1-e^{-(v+40)/10}}$$

$$\beta_m(v) = 0.108e^{-v/18} \qquad (A.2)$$

$$\alpha_h(v) = 0.0027e^{-v/20}$$

$$\beta_h(v) = \frac{1}{1+e^{-(v+35)/10}}$$

Conductance of delayed rectifier K^+ channel is given by

$$G_K = g_K n^4 \quad (A.3)$$

Where g_K is 36 mS/cm^2. Rate functions of delayed rectifier K^+ channel at 6.3 ^{0}C are as follows:

$$\alpha_n(v) = \frac{0.01(v+55)}{1-e^{-(v+55)/10}}$$

$$\beta_n(v) = 0.0555e^{-v/80} \quad (A.4)$$

6. REFERENCES

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