Simulation of axonal excitability using a Spreadsheet template created in Microsoft Excel

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Abstract

The objective of this present study was to implement an established simulation protocol (A.M. Brown, A methodology for simulating biological systems using Microsoft Excel, Comp. Methods Prog. Biomed. 58 (1999) 181–90) to model axonal excitability. The simulation protocol involves the use of in-cell formulas directly typed into a spreadsheet and does not require any programming skills or use of the macro language. Once the initial spreadsheet template has been set up the simulations described in this paper can be executed with a few simple keystrokes. The model axon contained voltage-gated ion channels that were modeled using Hodgkin Huxley style kinetics. The basic properties of axonal excitability modeled were: (1) threshold of action potential firing, demonstrating that not only are the stimulus amplitude and duration critical in the generation of an action potential, but also the resting membrane potential; (2) refractoriness, the phenomenon of reduced excitability immediately following an action potential. The difference between the absolute refractory period, when no amount of stimulus will elicit an action potential, and relative refractory period, when an action potential may be generated by applying increased stimulus, was demonstrated with regard to the underlying state of the Na⁺ and K⁺ channels; (3) temporal summation, a process by which two sub-threshold stimuli can unite to elicit an action potential was shown to be due to conductance changes outlasting the first stimulus and summing with the second stimulus-induced conductance changes to drive the membrane potential past threshold; (4) anode break excitation, where membrane hyperpolarization was shown to produce an action potential by removing Na⁺ channel inactivation that is present at resting membrane potential. The simulations described in this paper provide insights into mechanisms of axonal excitation that can be carried out by following an easily understood protocol. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Axon; Hodgkin Huxley; Ion channel; Microsoft Excel; Modeling; Simulation; Spreadsheet

1. Introduction

This article is an extension of a previous study that described in detail how to execute simulations of biological systems using a spreadsheet template created in Microsoft Excel [1]. The focus
of this present study was to implement that simulation protocol to describe some fundamental properties of axonal excitability based on the squid giant axon containing a fast Na\textsuperscript{+} current, which results in the upstroke of the action potential, a delayed rectifier K\textsuperscript{+} current, which results in membrane repolarization, and an ohmic leak current that determines resting membrane potential. The simulation uses the rate constants derived by Hodgkin and Huxley \cite{2} to describe the voltage dependence of ion channel behavior, although they have been updated to reflect modern conventions \cite{1,3}. This simulation serves two purposes. Firstly, it allows the user to conduct simulations to investigate mechanisms of axonal excitation in the squid giant axons. However, other preparations can be modeled simply by changing the appropriate rate constants and conductances. Secondly, the user can study and graphically display the underlying properties of ion channels, such as activation, inactivation and the resulting conductance changes, to see how those properties determine axonal behavior.

Familiarity with the previous paper \cite{1} is essential, as this present study uses it as a stepping stone to demonstrate ‘real life’ scenarios. This paper will be appreciated most by those who are interested in carrying out interactive simulations of ion channel behavior, but who do not wish to expend the time and money necessary to learn programming. The simulation involves using in-cell formulas in which rows and columns of new data are generated from key parameter values typed into the spreadsheet, and solving a set of equations based on those parameters. The features of Excel that make it ideal for this purpose are a user friendly interface, flexible data handling, in-built mathematical functions and instantaneous charting of data. The objective of this study was to use an established, easy to use simulation protocol to demonstrate key features of axonal excitability determined by ion channel properties.

2. Computational method

Full details of the computational method illustrated in this present study have appeared previously \cite{1}. The objective of the simulation was to determine how the membrane potential of a model squid giant axon containing $I_{\text{Na}}$ and $I_{\text{K}}$, responded to a variety of stimuli, i.e. it is a current clamp simulation where changes in membrane potential were modeled in response to constant current injection. Each stimulus paradigm was designed to illustrate an individual property of axonal excitability. Briefly, the simulation is carried out by (1) setting the initial membrane potential, (2) setting the amplitude and duration of current injection, (3) sequentially solving a series of equations describing the rate constants, (in)activation parameters, conductances, currents and finally, change in membrane potential, respectively, based on the initial values input by the user in the first two steps. In current clamp simulations the change in membrane potential ($V$) over time is described by:

$$\frac{dV}{dt} = \frac{I_{\text{total}}}{Cn}$$ \hfill (1)

In the simulation described in this paper

$$I_{\text{total}} = I_{\text{Na}} + I_{\text{K}} + I_{\text{leak}} + I_{\text{inj}}$$ \hfill (2)

where

$$I_{\text{Na}} = 120 m^3 h (-V + 50) \ \mu A \ cm^{-2}$$ \hfill (3)
$$I_{\text{K}} = 36 n^4 (-V - 77) \ \mu A \ cm^{-2}$$ \hfill (4)
$$I_{\text{leak}} = 0.3 (-V - 59.4) \ \mu A \ cm^{-2}$$ \hfill (5)

and $I_{\text{inj}}$ is the injected current input by the user. The (in)activation parameters are described by

$$m(h, n) = \frac{\alpha}{\alpha + \beta}$$ \hfill (6)

rate constants for $\alpha m$, $\beta m$, $\alpha n$, $\beta n$, $\alpha h$ and $\beta h$ can be found elsewhere \cite{1,3}.

The spreadsheet template is illustrated in Fig. 1. This template is identical to the one described previously \cite{1}, see Fig. 4 p. 186) and all the expressions used in the calculations are the same \cite{1}, see Table 2 p. 184). Each column contains the solution of a separate voltage dependent parame-
ter (column B contains \(am\), column C contains \(bm\), etc.). Column A contains the time parameter where the time increment is 0.04 ms. The user inputs the amplitude of injected current in cell B2. Column P contains the data referring to injected current. Thus, the duration of the current injection can be altered by increasing or decreasing the number of rows in which it appears. In the first row of calculations (row 4 on the spreadsheet) the parameters are solved based on the initial value of \(V\) input by the user in cell B1. In this instance it is set to \(-70\) mV. The calculations are carried out sequentially from cell B4 to cell Q4. The \(I_{\text{total}}\) (see Eq. (2)) is then used to calculate the new membrane potential \((V)\) in cell R4. The voltage dependent parameters in the next row are solved starting at cell B5 sequentially from B5 to Q5 using the new value of \(V\) in cell R4, and so on, sequentially down the rows. In the following simulations only the value of current injection, initial membrane potential or duration of current injection, were altered once the template has been set up. These parameters can be altered as necessary by changing values in cell B1, B2 or in column P, respectively, to conduct the simulations described below. The data of interest can be studied graphically, by plotting the time parameter (column A) against the column containing the appropriate data (e.g. for \(g_{\text{Na}}\), the data is in column K).

2.1. Action potential threshold

The threshold, or critical depolarization, for action potential firing is considered to be the membrane potential above which regenerative depolarization occurs, resulting in the firing of an action potential. Fig. 2A illustrates the calculated time course of a uniformly propagated action potential and the underlying \(Na^+\) and \(K^+\) conductance changes. Note that the current injection of 10 \(\mu A\) (C) causes an increase in \(g_{\text{Na}}\) resulting in depolarization of the membrane. This is followed by a delayed increase in \(g_{\text{K}}\), which results in repolarization of the membrane towards rest. The increase in \(g_{\text{K}}\) outlasts the duration of the stimulus, a factor that is important in temporal summa-

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Fig. 1. Spreadsheet template used to calculate \(V\). The value for \(V\) in cell B2 is the initial membrane potential and is used in the calculations in row 4. The value of \(V\) in cell R4 is then used as the voltage parameter in the calculations in row 5 to calculate the new value of \(V\). Calculations are carried out sequentially down the rows where the value for \(V\) calculated in column R of the previous row is used in the calculations of the subsequent row to calculate the new value of \(V\), and so on. Rows 14–503 have been omitted to save space. At row 504 it can be seen that the value in column P has changed from 0 (no injected current) to 10 \(\mu A\) reflecting the value of \(I_{\text{inj}}\) in Cell B2.
Fig. 2. Stimulus evoked action potential. (A) The action potential (bold) results from an increase in $g_{Na}$, which depolarizes the membrane, followed by a delayed increase in $g_K$, which results in repolarization of the membrane. An after-hyperpolarization occurs due to $g_K$ outlasting $g_{Na}$. (B) The total conductance is plotted illustrating that when $g_{Na}$ is larger than $g_K$ there is an increase in membrane conductance (arrow) favoring a depolarization of the membrane. The faint line illustrates sub-threshold stimulation for comparative purposes. (C) The current injection of 10 $\mu$A of 5 ms duration used to evoke the action potential.

Fig. 3. Rheobase and chronaxie. (A) The threshold current required to elicit an action potential is 2.5 $\mu$A (the largest illustrated current). Increasing sub-threshold pulses result in increasing membrane potential changes. (B) Chronaxie is the minimum stimulus duration required to elicit an action potential when applying a current of twice the rheobase (5 $\mu$A). In this case the duration was 6.2 ms.
Fig. 4. The initial resting membrane potential determines the profile of the action potential. (A) A hyperpolarized membrane potential (−80 mV, bold trace) results in a larger action potential than a more depolarized membrane potential (−60 mV, faint trace). (B) This is due to removal of Na\(^+\) channel inactivation resulting in larger \(g_{Na}\) due to more available Na\(^+\) channels, and decreased \(g_{K}\) (not shown). (C) Current injection of 20 \(\mu\)A for 5 ms.

2.2. Refractory period

Refractoriness is the period of decreased excitability of an axon immediately after an action potential. There are two types of refractoriness, absolute and relative. The absolute refractory period refers to the period immediately after an action potential, when it is impossible to elicit a further action potential no matter how much current is injected. Fig. 5A illustrates this phenomenon and its underlying mechanism. An initial current injection of 40 \(\mu\)A for 1 ms elicits an action potential. Repeating this stimulus 3 ms later fails to elicit a second action potential, even when current injection is increased by an order of magnitude to 400 \(\mu\)A. The reason for this lack of excitability is shown in the middle panel which displays the K\(^+\) channel activation parameter \(n\), and the Na\(^+\) channel inactivation parameter \(h\).

Immediately after the first action potential \(h\) is close to 0 indicating the majority of Na\(^+\) channels are inactivated, and \(n\) is close to 1, indicating that the majority of K\(^+\) are open, a fact reflected in the hyperpolarized membrane potential immediately after the action potential. Thus there are two factors which result in decreased excitability immediately following an action potential: (1) the majority of Na\(^+\) channels are in the inactivated state and unavailable for opening; and (2) the...
2.3. Temporal summation

This term usually refers to synaptic transmission where sub-threshold postsynaptic potentials overlap and accumulate to drive the membrane potential past threshold resulting in a postsynaptic action potential. Here the basic principle is the same but the inputs are direct current injection into the axon. This effect is demonstrated in Fig. 6A and shows how two sub-threshold stimuli of 40 μA amplitude and 200 μs duration delivered 1 ms apart can elicit an action potential (bold trace), whereas a single stimulus of the same dimensions fails to elicit an action potential (faint trace). The stimulus-induced changes in membrane conductance outlast the duration of the stimulus, and it is possible for the conductance changes brought about by the first stimulus to sum with the conductance changes elicited by the second stimulus if the interval between the two stimuli is sufficiently small. The summed conductances can then drive the membrane potential past threshold.

2.4. Anode break excitation

This phrase refers back to the terminology used in the 1940s where anodal current injection results in a hyperpolarizing of the membrane potential. The anode break refers to removal of a hyperpolarizing stimulus which gives rise to an action potential. Thus, an action potential can fire even after a current injection that results in membrane hyperpolarization. The middle panel illustrates the events underlying this phenomenon. As the anodal current is injected, $h$, the $Na^+$ channel inactivation parameter increases towards 1 signalizing that $Na^+$ channel inactivation is being removed and more $Na^+$ channels are available for opening. Conversely the activation parameter $n$, for $K^+$ channels decreases towards 0 resulting in decreased $K^+$ conductance. During the period of anodal polarization there is a reduced outward $K^+$ current and an increased $Na^+$ current. Thus release of the polarization results in a membrane depolarization that rapidly becomes regenerative and results in threshold being reached and an action potential firing.
2.5. Repetitive firing

In 1928 Adrian outlined a theory of repetitive firing [5], to describe how stimuli of increasing strength resulted in increased frequency of action potential firing. The objective of the Hodgkin Huxley model was to describe membrane permeability changes associated with a single action potential, and predicts repetitive firing over a limited range of frequencies in the squid giant axon. Adding hypothetical K\(^+\) conductances and altering the voltage dependent properties of K\(^+\) currents extends the range of firing frequencies to more accurately describe the repetitive firing patterns seen in squid giant axon [6,7]. However, for demonstration purposes it is viable to use the original Hodgkin and Huxley model to simulate repetitive firing. Fig. 7 illustrates the property of increased stimulus amplitude resulting in increased action potential frequency. (A) Injecting a current stimulus of 8 \(\mu\)A for 120 ms elicited a pattern of seven evenly spaced action potentials with inter-spike intervals of 17.8 ms. (B) Increasing the stimulus amplitude to 20 \(\mu\)A decreased the inter-spike interval to 11.8 ms, increasing the number of action potentials to 11.

3. Discussion

In this paper current clamp simulations of excitability of a model of the squid giant axon, a classic electrophysiological preparation, are described. The large diameter of the giant axon of the squid *Loligo* allowed experimenters to insert microelectrodes inside the axon to record 'intracellular' responses of the axon and thus made it an ideal preparation in which to study excitation [8]. Subsequent investigation using the voltage clamp technique allowed experimenters to control the voltage of a piece of membrane and determine ion movements at fixed voltages [9–12]. These data culminated in the classic description of Na\(^+\) and K\(^+\) permeability changes during excitation and conduction in squid giant axon [2]. Hodgkin and Huxley derived a series of equations that accurately described the changes in permeability in Na\(^+\) and K\(^+\) responsible for an action potential. Their model also predicted some basic properties of an excitable membrane, such as refractoriness and anode break excitation described in this paper. Why go to the trouble of repeating the Hodgkin and Huxley model? The model is still valid almost 50 years after its initial description, and is widely used to describe behavior of voltage-gated ion channels [13]. A great advantage of the model is that it can be successfully applied to other preparations such as the R15 neuron of *Aplysia*, which displays a more complicated pattern of firing that the squid axon [14]. The advantage of the protocol described in this paper is that it does not require any programming knowledge, which in today’s Windows based environment, is costly and very time consuming. It does not require the expense of purchasing specialized software such as Neurosim [15], Neuron [16] or Genesis [17]. It uses a spreadsheet on a standard desktop PC which almost all biologists know how to use, even if at an elementary level. Such is the advance in technology that computation of an action potential which originally took 8
h on a hand held calculator can now be done in under 1 s on a 400 MHz Pentium II computer with 64 MB RAM using the protocol described here.

References