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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.

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

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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^+ current, which results in the upstroke of the action potential, a delayed rectifier K^+ 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 [2] to describe the voltage dependence of ion channel behavior, although they have been updated to reflect modern conventions [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 [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 [1]. The objective of the simulation was to determine how the membrane potential of a model squid giant axon containing I_{Na} and I_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:

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

In the simulation described in this paper

$$I_{total} = I_{Na} + I_K + I_{leak} + I_{inj} \quad (2)$$

where

$$I_{Na} = 120m^3h(-V+50)\ \mu A\ cm^{-2} \quad (3)$$

$$I_K = 36n^4(-V-77)\ \mu A\ cm^{-2} \quad (4)$$

$$I_{leak} = 0.3(-V-59.4)\ \mu A\ cm^{-2} \quad (5)$$

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

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

rate constants for αm, βm, αn, βn, αh and βh can be found elsewhere [1,3].

The spreadsheet template is illustrated in Fig. 1. This template is identical to the one described previously ([1], see Fig. 4 p. 186) and all the expressions used in the calculations are the same ([1], see Table 2 p. 184). Each column contains the solution of a separate voltage dependent parame-

ter (column B contains αm, column C contains βm, etc.). Column A contains the time parameter where the time increment in 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_{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_{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 μA (C) causes an increase in g_{Na} resulting in depolarization of the membrane. This is followed by a delayed increase in g_K, which results in repolarization of the membrane towards rest. The increase in g_K outlasts the duration of the stimulus, a factor that is important in temporal summa-

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1	V	-70																
2	I_{inj}	10																
3	time(ms)	αm	βm	αh	βh	αn	βn	m	h	n	gNa	gK	I_{Na}	I_K	I_{LEAK}	I_{INJECT}	I_{TOTAL}	V
4	0.00	0.16	5.28	0.09	0.03	0.04	0.13	0.03	0.75	0.24	0.00	0.13	0.26	-0.90	3.06	0	2.42	-69.98
5	0.04	0.16	5.27	0.09	0.03	0.04	0.13	0.03	0.75	0.24	0.00	0.13	0.26	-0.90	3.17	0	2.53	-69.87
6	0.08	0.16	5.24	0.09	0.03	0.04	0.13	0.03	0.75	0.24	0.00	0.13	0.26	-0.92	3.14	0	2.49	-69.77
7	0.12	0.16	5.21	0.09	0.03	0.04	0.13	0.03	0.75	0.24	0.00	0.13	0.27	-0.93	3.11	0	2.45	-69.68
8	0.16	0.16	5.18	0.09	0.03	0.04	0.13	0.03	0.75	0.24	0.00	0.13	0.27	-0.94	3.08	0	2.41	-69.58
9	0.20	0.16	5.16	0.09	0.03	0.04	0.13	0.03	0.75	0.24	0.00	0.13	0.27	-0.96	3.05	0	2.37	-69.49
10	0.24	0.16	5.13	0.09	0.03	0.04	0.13	0.03	0.75	0.24	0.00	0.13	0.28	-0.97	3.03	0	2.34	-69.39
11	0.28	0.16	5.10	0.09	0.03	0.04	0.13	0.03	0.75	0.24	0.00	0.13	0.29	-0.98	3.00	0	2.30	-69.30
12	0.32	0.17	5.08	0.09	0.03	0.04	0.13	0.03	0.75	0.24	0.00	0.13	0.30	-0.99	2.97	0	2.27	-69.21
13	0.36	0.17	5.05	0.09	0.03	0.05	0.13	0.03	0.75	0.24	0.00	0.13	0.30	-1.01	2.94	0	2.24	-69.12
504	20.00	0.20	4.34	0.07	0.04	0.05	0.13	0.04	0.65	0.29	0.01	0.27	0.79	-2.86	2.12	10	0.06	-66.08
505	20.04	0.21	4.24	0.07	0.04	0.05	0.13	0.04	0.65	0.29	0.01	0.27	0.79	-2.97	2.00	10	-0.17	-65.68
506	20.08	0.21	4.15	0.07	0.04	0.06	0.13	0.04	0.65	0.29	0.01	0.27	0.81	-3.08	1.89	10	-0.38	-65.30
507	20.12	0.22	4.06	0.07	0.05	0.06	0.13	0.05	0.65	0.29	0.01	0.27	0.84	-3.18	1.77	10	-0.57	-64.92
508	20.16	0.22	3.98	0.07	0.05	0.06	0.12	0.05	0.65	0.29	0.01	0.27	0.90	-3.29	1.66	10	-0.74	-64.55
509	20.20	0.23	3.90	0.07	0.05	0.06	0.12	0.05	0.65	0.30	0.01	0.27	0.96	-3.40	1.55	10	-0.89	-64.19

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 μA reflecting the value of I_{inj} in Cell B2.

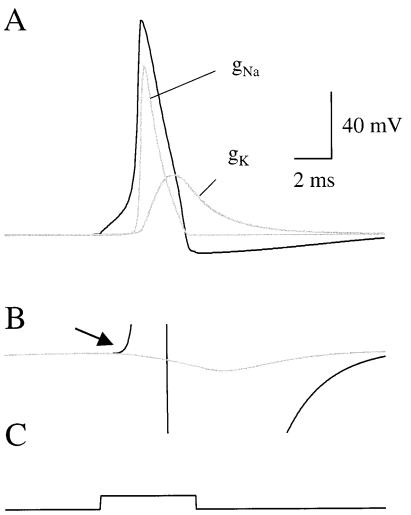

Fig. 2. Stimulus evoked action potential. (A) The action potential (bold) results from an increase in g_{Na}, which depolarizes the membrane, followed by a delayed increase in g_K, which results in repolarization of the membrane. An after-hyperpolarization occurs due to g_K outlasting g_{Na}. (B) The 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 μA of 5 ms duration used to evoke the action potential.

tion (see later). Fig. 2B illustrates the conductance changes critical for action potential firing. In terms of conductance, threshold is defined as the potential at which inward Na^+ conductance is greater than the outward K^+ conductance leading to regenerative membrane depolarization. If the K^+ current is larger than Na^+ current the membrane potential will return to rest (faint trace), however if the Na^+ current is marginally larger that the K^+ current (arrow) membrane potential will become unstable and produce an action potential. This is explained in the Hodgkin cycle of Na^+ excitability where depolarization of the membrane leads to increased Na^+ permeability resulting in net influx of Na^+, which leads to further membrane depolarization and so on [4].

Rheobase is defined as the minimum amount of current required to produce an action potential [3] and is illustrated in Fig. 3A. As the amplitude of current injection increases so membrane depolarization increases. Finally the current injection is sufficiently large (2.5 μA) to cause the all or nothing action potential, due to the larger Na^+ conductance outbalancing the K^+ conductance. Chronaxie is defined as the minimum duration of twice the amplitude of rheobase needed to produce an action potential and is illustrated in Fig. 3B. This illustrates that both duration and amplitude of current injection are important in determining if threshold is reached.

Threshold, however, is not a fixed value but varies depending on axonal membrane potential at the time of current injection (20 μA), a feature illustrated in Fig. 4. It can be seen that for the same amplitude of injected current, a larger g_{Na} occurs at more hyperpolarized potentials (-80 mV) than at depolarized membrane potentials (-60 mV; Fig. 4B). This is because hyperpolarizing the axonal membrane removes Na^+ channel inactivation, resulting in more available Na^+ channels. The current pulse recruits these addi-

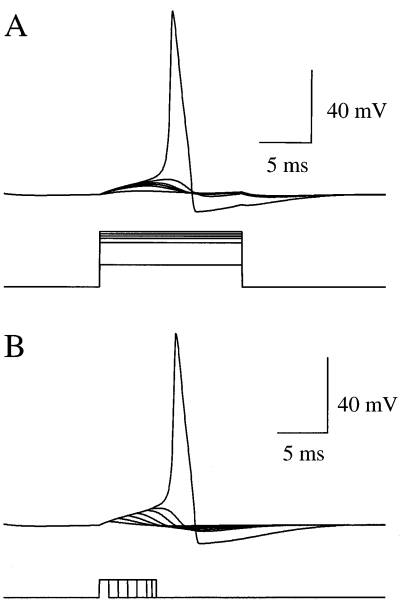

Fig. 3. Rheobase and chronaxie. (A) The threshold current required to elicit an action potential is 2.5 μA (the largest illustrated current). Increasing sub-threshold pulses result in increasing membrane potential changes. (B) Chronaxie is the minimum stimulus duration required to elicit an action potential when applying a current of twice the rheobase (5 μA). In this case the duration was 6.2 ms.

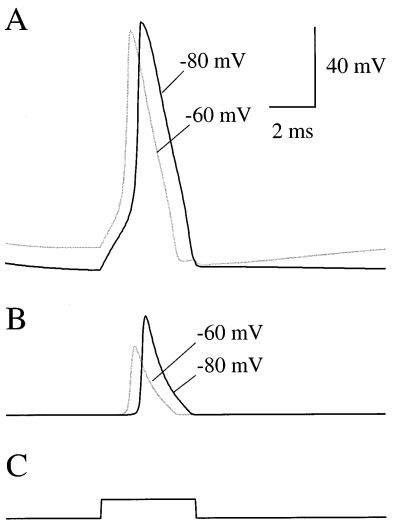

Fig. 4. The initial resting membrane potential determines the profile of the action potential. (A) A hyperpolarized membrane potential (− 80 mV, bold trace) results in a larger action potential than a more depolarized membrane potential (− 60 mV, faint trace). (B) This is due to removal of Na^+ channel inactivation resulting in larger g_{Na} due to more available Na^+ channels, and decreased g_K (not shown). (C) Current injection of 20 μA for 5 ms.

tional Na^+ channels which are available at − 80 mV but are inactivated at − 60 mV, and hence the increased g_{Na}.

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 μA 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 μA. 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, indicting 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

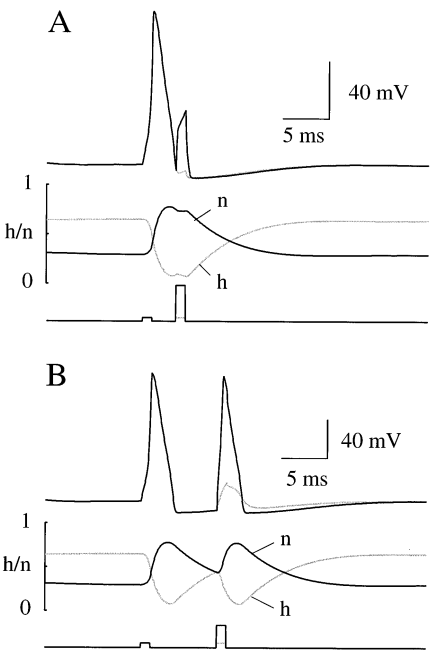

Fig. 5. Refractory period. (A) The absolute refractory period is the period immediately following an action potential when no amount of current injection will elicit a second action potential. Top panel: the first action potential was elicited by a current of 40 μA. Increasing this stimulus amplitude by an order of magnitude failed to elicit a second action potential. Middle panel: this is due to increased g_K reflected in the value of *n* near to 1, and the Na^+ channels still being in the inactivated state, reflected by the value of *h* close to 0. Bottom panel: the current injection profile. (B) The relative refractory period occurs immediately after the absolute refractory period when a second stimulus can elicit a second action potential but the stimulus intensity must be increased. Top panel: a second action potential can be elicited if an increased current is injected (200 μA). The first action potential was elicited by 40 μA. The faint trace shows that a second current pulse of 40 μA failed to elicit an action potential. Middle panel: The values of n and h are returning towards rest when the second pulse is injected. Bottom panel: the current injection profile.

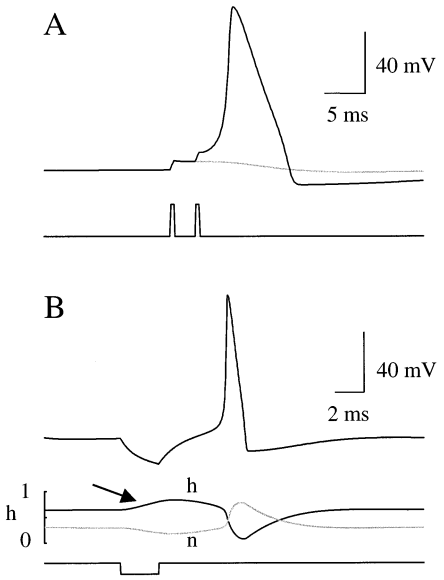

Fig. 6. Temporal summation and anode break excitation. (A) The faint trace shows the effect of only one stimulus, which fails to elicit an action potential. An action potential is elicited when two sub-threshold stimuli are injected (bold trace). (B) Injection of an anodal current pulse (bottom panel) results in membrane hyperpolarization (top panel). 'Breaking' the anodal pulse results in membrane excitation culminating in an action potential. Middle panel: This is due to removal of Na^+ channel inactivation (arrow indicates where *h* increases towards 1 indicating reduction in Na^+ channel inactivation), and decrease in g_K reflected in a decrease in *n* towards 0.

majority of K^+ channels are open resulting in hyperpolarization of the membrane.

The relative refractory period occurs after the absolute refractory period and refers to the fact that a second action potential can be elicited but a larger current must be injected. This is illustrated in Fig. 5B. Delaying the interval between the two stimuli to 8 ms permits current injection, albeit of an increased amplitude (200 μA), to elicit a second action potential (top trace). This is because the increased interval allows h to return towards resting levels resulting in more Na^+ channels available for opening, and n decreases (middle trace) resulting in less K^+ channels being open resulting in a decreased level of membrane hyperpolarization. A larger current pulse must be injected to overcome the fact that neither n nor h has returned to their resting levels.

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 principal 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 signaling 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 μA 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 μA decreased the inter-spike interval to 11.8 ms, increasing the number of action potentials to 11.

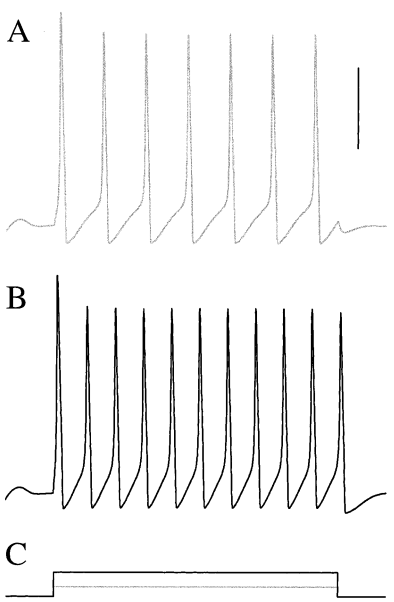

Fig. 7. Repetitive firing. (A) Injection of a 120 ms current pulse of 8 μA (faint trace in C) produces repetitive firing in the model axon. (B) Increasing the current amplitude to 20 μA (bold trace in C) increases the number of action potentials from 7 to 11. The scale bar represents 40 mV in A and B.

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.

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