Software Experiment #2

WHAT MAKES A NEURON SPIKE?
- Generation Of Action Potential

Content areas: Basic neuroscience covered in biology, physiology, psychology, and engineering courses

Pre-requisite knowledge: Cell biology, human nervous system, first order systems

Learning Objectives: After this lesson, students should be able to:

- Begin to understand the neuron as the basic building block of the nervous system.
- Understand how a neuron works from a biophysical point of view, including the modulation of membrane voltage using depolarizing and hyperpolarizing currents.

Time Required:

Keywords. Active and passive channels, action potential

Summary. This lesson illustrates the mechanisms underlying the generation of an action potential in a neuron. These mechanisms include membrane voltage-dependent (active) and voltage-independent (passive) channel currents. The active currents can be either depolarizing or hyperpolarizing. APs are used by a neuron to communicate with another neuron.

INTRODUCTION / MOTIVATION
(The following introductory material is adapted from
http://www.mindcreators.com/NeuronBasics.htm)

1. Neuron Morphology

The neuron is the cell that animals use to detect the outside environment, the internal environment of their own bodies, to formulate behavioral responses to those signals, and to control their bodies based on the chosen responses. This is of course a very simplistic definition of what neurons do. But it does cut to the basics. All neurons have a body called a Soma. The Soma contains the nucleus and all of the other organelles that are needed to keep the cell alive and functioning. Neurons also have directionality to them. On one side of the neuron are the dendrites. You can think of this side as being the 'input' side. The dendrites are branching structures which connect with the outputs of other neurons. They typically spread over a wide area in the immediate vicinity of the neuron. This allows the neuron to get inputs from a number of different synapses. The other end of is the 'output' end. It contains an axon and ends in a number of synapses which usually connect to the dendrites of other neurons or are connected directly to muscles. The axon is usually quite long compared to the rest of the neuron. In fact, you have some neurons with axons that extend the entire length of your body!

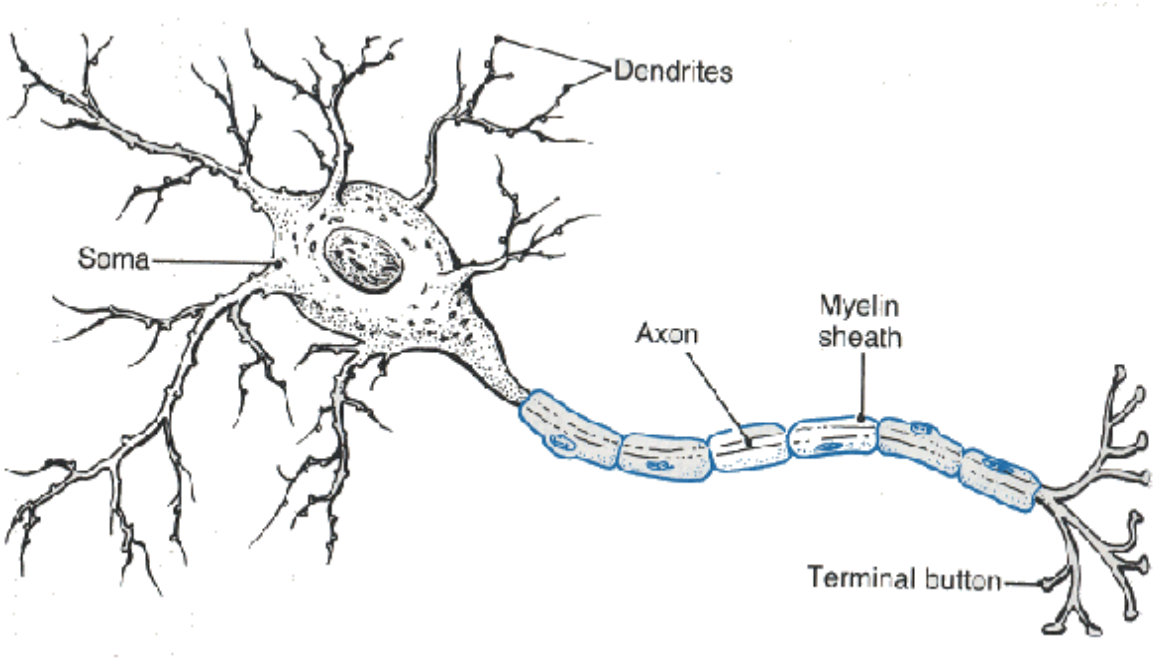


Figure 1. Neuron Diagram.

2. How is an action potential generated?

The signal output of a neuron can either cause excitation or inhibition in the neuron it is connected to. When a neuron sends an excitatory signal to another neuron, then this signal will be added to all of the other inputs of that neuron. If it exceeds a given threshold then it will cause the target neuron to fire an action potential, if it is below the threshold then no action potential occurs.

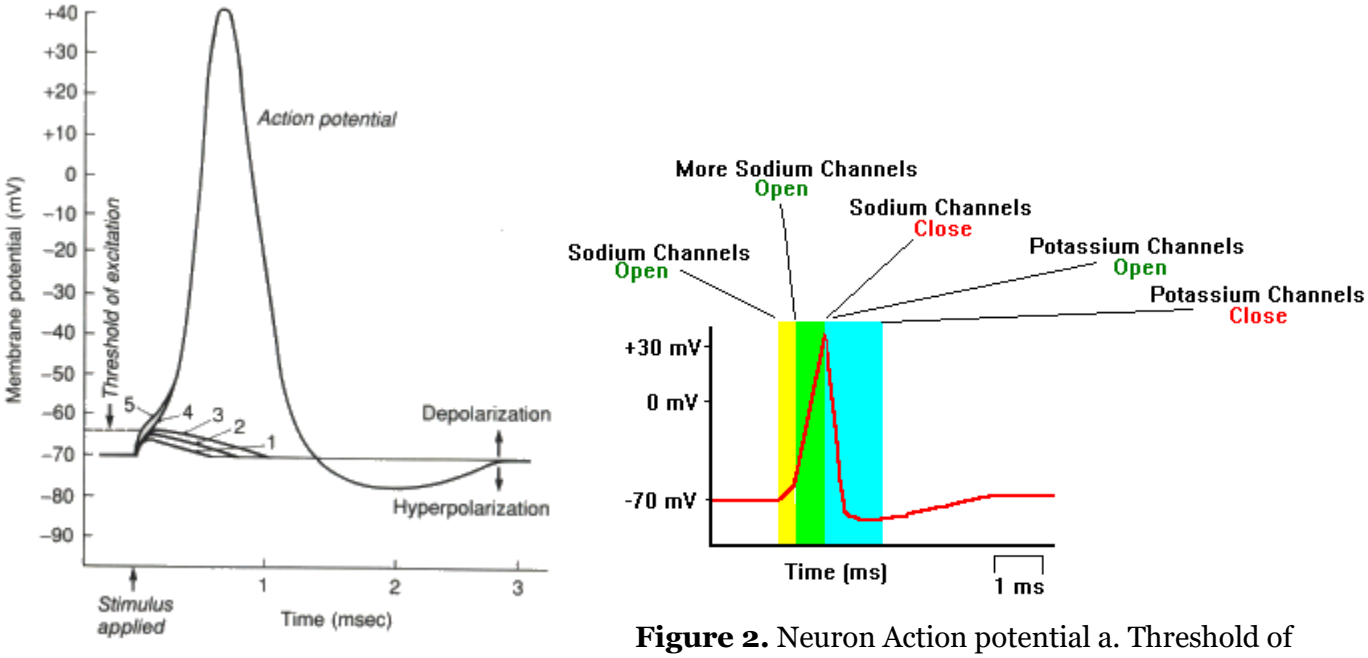


Figure 2. Neuron Action potential a. Threshold of excitation; b. Active and passive channels.

An action potential is an electric pulse that travels down the axon until it reaches the synapses, where it then causes the release of neurotransmitters. The synapses are extremely close to the dendrites of the target neuron. This allows the neurotransmitters to diffuse across the intervening space and fit into the receptors that are located on the target neuron. This causes some action to take place in that neuron that will either decrease or increase the membrane potential of the neuron. If it increases the membrane potential then it is exciting the neuron, and if it decreases the membrane potential it is inhibiting the neuron. If it causes the membrane potential to pass the firing threshold then it will activate an action potential in the target neuron and send it down its axon.

Neurons in a resting state normally have a membrane potential around -70mV. This means that the voltage difference between the fluid on the inside of the cell relative to the fluid on the outside of the cell is negative. How is this negative difference maintained? It is done with ions like Na^+, K^+, Cl^- , and protein anions. The cell membrane prevents charged particles such as these from freely diffusing into and out of the cell. There are two basics ways that they can get in or out. The first is with passive transport. Basically the cell has a protein in the cell membrane that it can open and close like a water faucet. It is specific for certain kinds of chemicals like these ions. When it opens, then the ions can flow down their gradient from the more concentrated area to the less concentrated area. The other way to get ions in or out of cells is to by active transport. The cell uses some of its own energy to actively pump the chemicals *against*

their gradient. The neuron has a pump that actively pumps three Na^+ ions out and takes in two K^+ ions. This means that a net positive charge flows out of the neuron. This is what gives the cell its negative potential. Ions are also what are responsible for the initiation, and transmission of action potentials. When the neurotransmitters from other firing neurons come in contact with their corresponding receptors on the dendrites of the target neuron it causes those receptors to open or close some of the passive ion transports. This allows the ions to flow into the cell and temporarily change the membrane voltage. If the change is big enough then it will cause an action potential to be fired. Figure 3 shows the basics of how ion flow transmits the action potential down the length of the axon.

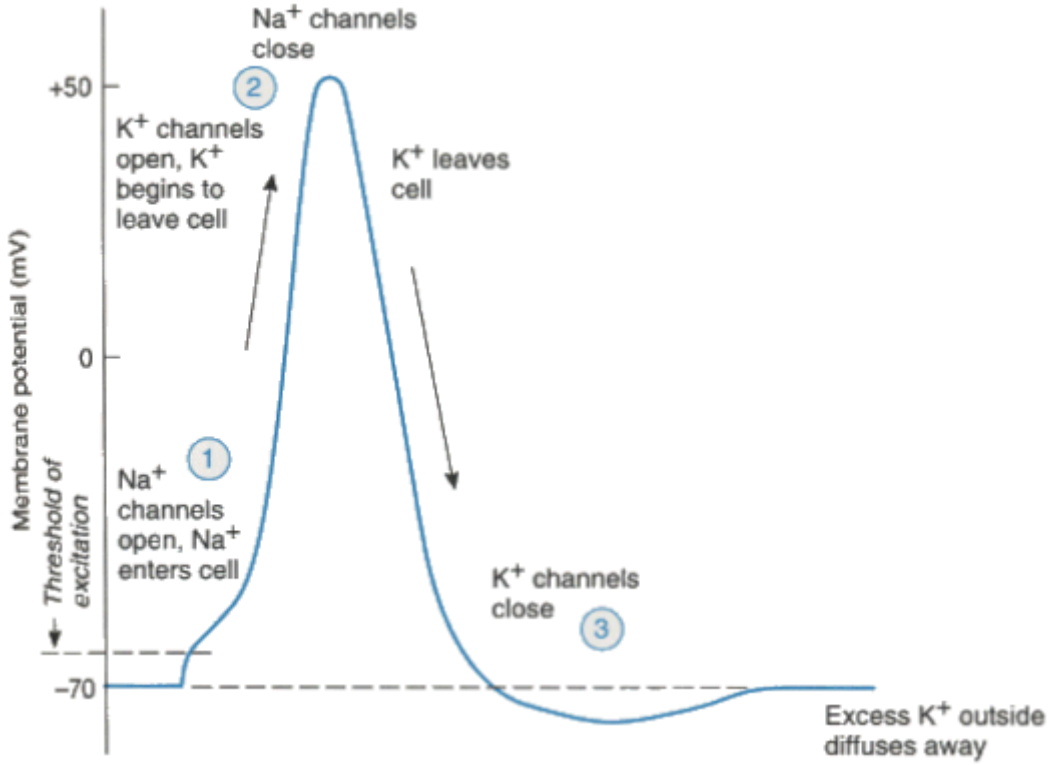


Figure 3.. Ion flow in action potential.

1. The first step of the action potential is that the Na^+ channels open allowing a flood of sodium ions into the cell. This causes the membrane potential to become positive.
2. At some positive membrane potential the K^+ channels open allowing the potassium ions to flow out of the cell.
3. Next the Na^+ channels close. This stops inflow of positive charge. But since the K^+ channels are still open it allows the outflow of positive charge so that the membrane potential plunges.
4. When the membrane potential begins reaching its resting state the K^+ channels close.

Now the sodium/potassium pump does it's work and starts transporting sodium out of the cell, and potassium into the cell so that it is ready for the next action potential.

The action potential travels down the length of the axon as a voltage spike. It does this using the steps outlined above. As a section of the axon undergoes the above process it increases the

membrane potential of the neighboring section and causes it to spike. This is like a mini chain reaction that proceeds down the length of the axon until it reaches the synapse. An important thing to keep in mind about the action potential is that it is one way, and all or nothing. The action potential starts at the top of the axon and goes down it. Also, if a neuron fires then the action potential is the same regardless of the amount of excitation received from the inputs. What is important in neurons is the *rate* of fire. Figure 4 demonstrates this principal. A weak stimulus will cause a lower rate of fire than a strong stimulus. So it is not the amplitude of the action potential that is important, but the number of times a neuron fires for a given time period. However, it has been shown in experiments that the rate of fire of a neuron is directly related to the depolarizing current applied to that neuron. This can be seen in figure 5. This fact will be important later on when the neural model is being explained.

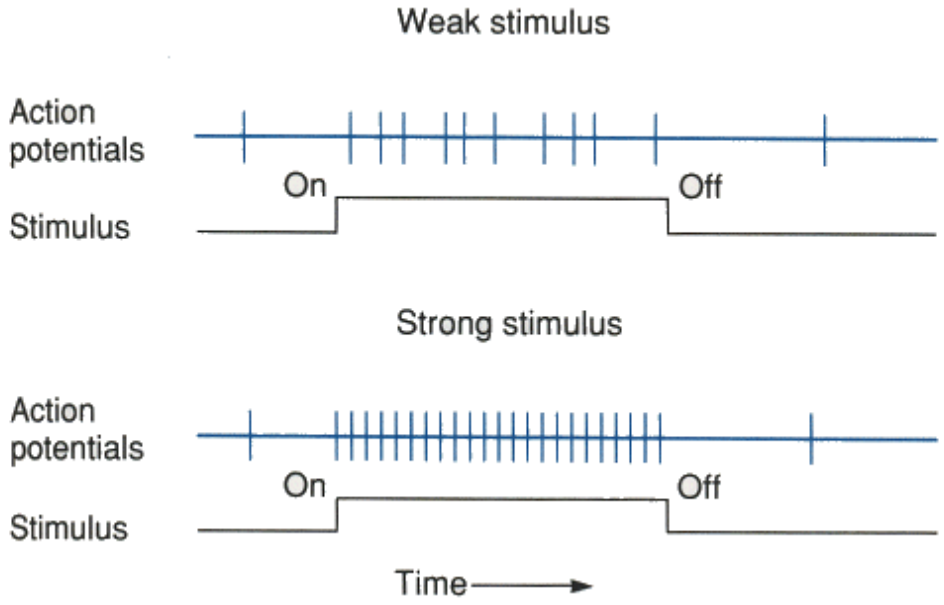


Figure 4. The rate law demonstrates that a stronger stimulus will cause a neuron to fire more often than for a weaker stimulus.

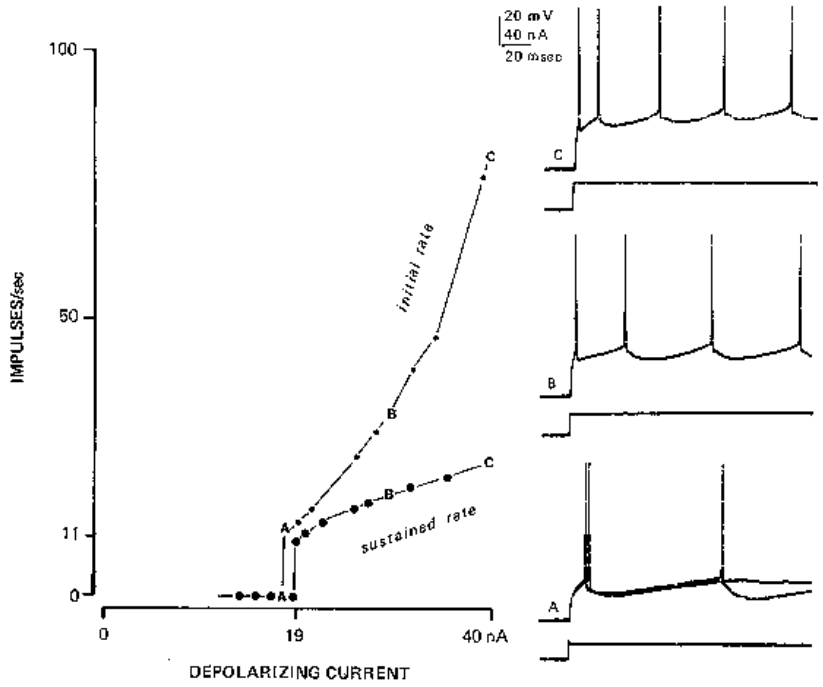


Figure 5. Experimental data showing the relationship between input current and firing rate of a neuron.

3. Taxonomic distribution and evolutionary advantages (adapted from Wikipedia)

Action potentials are found throughout multicellular organisms, including plants, invertebrates such as insects, and vertebrates such as reptiles and mammals. Sponges seem to be the main phylum of multicellular eukaryotes that does not transmit action potentials, although some studies have suggested that these organisms have a form of electrical signaling, too. The resting potential, as well as the size and duration of the action potential, have not varied much with evolution (see table on next page), although the conduction velocity does vary dramatically with axonal diameter and myelination.

Given its conservation throughout evolution, the action potential seems to confer evolutionary advantages. One function of action potentials is rapid, long-range signaling within the organism; the conduction velocity can exceed 110 m/s, which is one-third the speed of sound. No material object could convey a signal that rapidly throughout the body; for comparison, a hormone molecule carried in the bloodstream moves at roughly 8 m/s in large arteries. Part of this function is the tight coordination of mechanical events, such as the contraction of the heart. A second function is the computation associated with its generation. Being an all-or-none signal that does not decay with transmission distance, the action potential has similar advantages to digital electronics. The integration of various dendritic signals at the axon hillock and its thresholding to form a complex train of action potentials is another form of computation, one that has been exploited biologically to form central pattern generators and mimicked in artificial neural networks.

Comparison of action potentials (APs) from a representative cross-section of animals					
Animal	**Cell type**	**Resting potential (mV)**	**AP increase (mV)**	**AP duration (ms)**	**Conduction speed (m/s)**
Squid (*Loligo*)	Giant axon	−60	120	0.75	35
Earthworm (*Lumbricus*)	Median giant fiber	−70	100	1.0	30
Cockroach (*Periplaneta*)	Giant fiber	−70	80–104	0.4	10
Frog (*Rana*)	Sciatic nerve axon	−60 to −80	110–130	1.0	7–30
Cat (*Felis*)	Spinal motor neuron	−55 to −80	80–110	1–1.5	30–120

4. How did Hodgkin and Huxley tease out the components of an action potential?

Brief history:
1902: Bernstein predicted a massive increase in membrane permeability (to all ions) during an action potential. He used Nernst theory and assumed only permeability to K to come up with a resting potential of -75 mV. According to his hypothesis membrane 'breakdown' explained the action potential.

1939: Cole used a Wheatstone bridge to measure membrane conductance during an action potential. The finding was qualitatively consistent with Bernstein's hypothesis but the increase was not as large as one would expect from a membrane breakdown.

1937-38: Hodgkin visited US for his post-doc studies. Met Cole at Columbia Univ. and also at Woods Hole in Summer. He and Curtis almost succeeded in measuring V_m directly by tunneling along the giant axon with a glass micropipette. Eventually they succeeded, albeit with different collaborators – Curtis and Cole, 1940 & Hodgkin and Huxley, 1939.....and found that not only did V_m rise transiently towards zero, but surprisingly there was a substantial overshoot, such that the membrane potential actually reversed in sign at the peak of the action potential.

Needed experimental development before further insights into the phenomenon could be obtained – *space clamp* (Marmont 1949 and Cole 1949) and *voltage clamp* (Cole et al.) techniques. Space clamp was achieved by threading the axon with a silver wire to provide a very low axial resistance, thereby eliminating longitudinal voltage gradients. Voltage clamp turned

out to be more complex requiring two pairs of electrodes, one to measure and the other to provide the 'clamping current' using a feedback amplifier.

How to identify individual contributions to current from the different ion species? – Hodgkin and Katz (1949) had demonstrated that both sodium and potassium made important contributions to the current which helped explain the puzzling observations that V_m overshoots zero during the action potential.

Bernstein (1902) – action potential results from an unbounded transient increase in permeability for all ions

Hodgkin and Kats (1949) – hypothesized that changes in permeabilities for different ions could account for the observed changes in V_m. In their view, V_m would tend to the Nernst potential for the ion to which the membrane was dominantly permeable, and this dominance could change with time. At rest, this agreed with the Bernstein theory. But during an action potential upstroke, they postulated that a dramatic shift took place, causing the membrane to become much more permeable to sodium than to potassium. Hence V_m would tend towards E_{Na} (about + 60 mV) and an overshoot of zero potential would be expected. They predicted and showed that the action potential amplitude depended critically on the concentration of external sodium; decreased sodium led to a lower peak for the action potential. In the theoretical section of their paper, they generalized the Nernst equation to predict the steady state potential when the membrane is permeable with different degrees to more than one ionic species – GHK Equation.

The 'sodium hypothesis' was a major conceptual advance. However, the question of how the permeability changes were dynamically linked to V_m was not completely addressed until the papers of 1952.....until they 'disentangled' the contributions of different ionic conductances, assuming they responded independently to V_m (a key assumption). How did they do it – eliminated sodium from the bathing medium, I_{Na} becomes negligible and so I_K can be measured directly. Then I_K can be determined by subtraction of I_{Na} from the normal response. They showed that these currently were strongly influenced by V_m.

Supplementary information:
Link with lots of information on neural science:
http://icwww.epfl.ch/~gerstner/SPNM/node14.html

Another link with lots of information: http://www.mindcreators.com/ModelingTheNeuron.htm

Lecture series based on genesis: http://www.genesis-sim.org/GENESIS/cnslecs/cnslecs.html

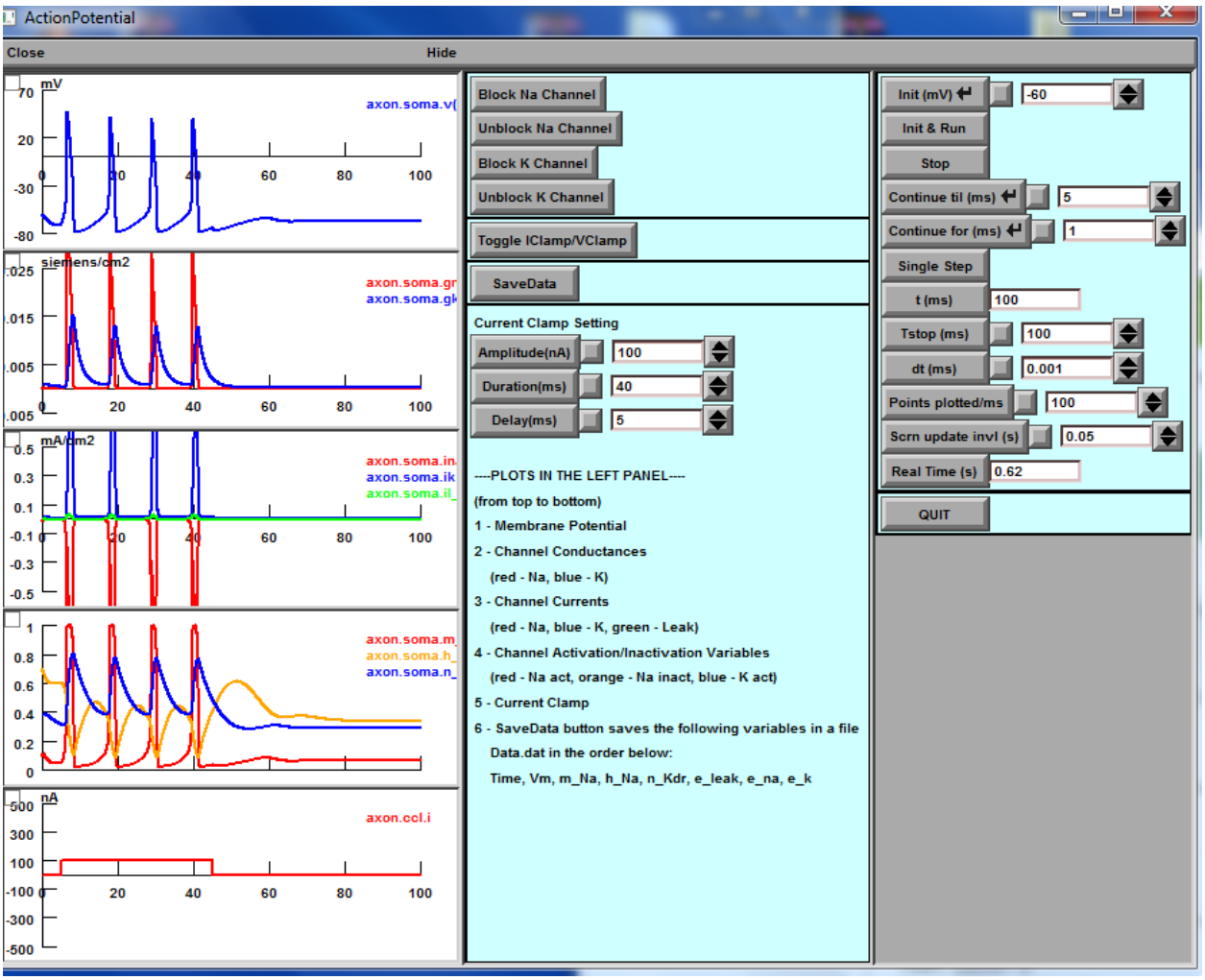
Duke Neuron Page: http://neuron.duke.edu

SOFTWARE EXPERIMENT #2 ASSIGNMENT

Run the NEURON 'Action Potential' tutorial described below and answer the 10 questions at the end.

NEURON tutorial - Action Potential
[adapted from a similar GENESIS tutorial (Bower and Beeman, 2007)]

Assuming that NEURON is already installed on your systems, go to the folder where you saved the Action Potential tutorial and double click on **ActionPotential.hoc.** This will open the NEURON software and start the AP tutorial. A screen similar to the one below will pop up.



CAUTION: *Make sure that you do not close the* ***"nrniv"*** *window. Closing the* ***"nrniv"*** *window will close the NEURON software and you will have to restart the tutorial and redo all your work.*

Resetting/Changing Field Values:

To change the value of a field, simply click on that field and either input your desired value through the keyboard or use the up/down arrows to increase/decrease the present value. Notice that when you click on a field, the fieldname is highlighted yellow. Once you have input the desired value, press enter. This will remove the highlighted fieldname and will cause a check mark to appear right next to the field. This indicates that the value has been changed successfully.

To reset the field value to its default value, simply uncheck the checkboxes. This causes the field values to be reset to their default values.

INPUTS: External concentrations of ions (reversal potentials), + Current Injection [Note: Conductances cannot be changed by user in this tutorial].

OUTPUT: Membrane potential /spiking

Click on the **INIT&RUN** button to run the model with default settings. The upper left window shows the membrane potential for the simulation. You should see 4 action potentials. The lower right window shows the injection current. You should notice that current was injected between 5 and 45 msec at a level of 100 nA. The upper right window shows the conductances for each of the two channels. These are the time varying conductances of each channel. The lower left window shows the current for each channel.

Notice that at the beginning the cell is at rest and you can see the rest values for each of the three activation and inactivation variables. Once the action potential occurs, Na activation variable **m** increases the fastest, followed by an increase in K activation **n** and then the inactivation of Na **h**.

GRAPHICAL USER INTERFACE

- The left panel shows the following physiological variable as a function of time (ms), one below the other: membrane potential V (in mV), channel conductances (Siemens/cm2), channel currents (mA/cm2), activation/inactivation variables (dimensionless), and the injected current.
- The middle panel provides the user the option of changing the cell parameters (dimensions, channel properties, etc). You WILL NOT be using these options and so you can ignore the second panel except the bottom part where you block and unblock channels.
- The third panel is the main CONTROL panel from where you can adjust your inputs. You will only be changing two items in the top half of the panel: (i) initial voltage of the membrane, initV, in mV, and (ii) Tstop (ms).
- Middle of third panel: 'Voltage Clamp' means that you will hold the membrane potential constant (note: this will be step change from Vinit and so will cause transients). Similarly the injection current is held constant (from initial value of 0) in the 'IClamp' mode. Use the button 'Toggle IClamp/VClamp' to switch between the models. As you do this, a panel appears below it so that you can provide the appropriate input parameters. For the 'IClamp' mode, you need to enter the values for the current pulse you will use, 'Amplitude (nA)', 'Duration (ms)' and 'Delay (ms)', and for the 'VClamp' mode you will provide 'Amplitude (mV)'.
- You can change the display features on each plot by using the little box at the top left hand corner of each figure. Clicking on the box will give you several options including 'View' from where you can select 'View=plot' to scale the plot to fit the figure, or use 'New View' to enlarge part of the plot. You can also use the 'crosshair' option to determine (x,y) coordinates of specific points on the plots.

EXERCISES:

1. Reset all fields to their initial values. Click on the **Block Na Channel** button to run a simulation with the Na channel blocked (this simulates the action of tetrodoxin, a neurotoxin derived from the puffer fish. Actually this is how many neurotoxins work). Click **INIT&RUN** again to run the simulation. What do you think will happen? Note below.

2 . Reset all fields to their initial values and unblock the Na channel by clicking on the **Unblock Na Channel button**. Click on **Block K Channel** to block the K channel. Run the simulation. What will happen? Note below.

3. Using the state plots (time history of activation and inactivation functions), explain the generation of an action potential.

4. Increase the magnitude of the injection current and describe what happens to the firing frequency.

5. Perform a voltage clamp experiment to estimate the sodium reversal potential. Explain the logic of how you obtained it (including equation) and attach the relevant plots.

6. Determine approximate values for mNa, hNa and mKd for the following cases:
   - at the rest state (inject = 0)
   - at the peak of the action potential (with I_inject = 100 nA)
   - at the lowest point of the action potential (of the down swing with I_inject = 100 nA)

7. Determine the values of all currents at equilibrium (by hand) and make sure they sum up to zero when inject = 0. The parameters used in the model are as follows:
Maximal Conductances for the ion channels in S/cm^2: gbarNa= 0.12; gbarKd= 0.036;gbarleak= 0.0003;
Other parameters: Asoma = 0.00785 cm^2; Csoma = 1μF/cm^2. Reversal potentials (mV): get from NEURON. Run the model, and then use the OC window to get the values of the parameters. For this, first type "access axon.soma", and then get the values using ena, ek, el-leak, m_na, h_na, n_kdr, v).

8. By counting the number of spikes generated in a 100 *msec* window, construct a plot of firing frequency vs. injected current, starting at the rheobase current (minimum injected current that elicits spiking) and working up to a value of about 10 times rheobase. (Suggested settings: base current = 0 *nA*; onset delay 1 = 0 *msec*; pulse width 1 = 100 *msec*; simulation time, tstop = 100 *msec*.) How much does a 10-fold increase in injected current increase the firing rate? What happens if you increase the injected current to 100 times rheobase?

9. In problem 3 we saw that single action potentials can be elicited by small *sustained* levels of current injection. Single action potentials can also be elicited by *transient* pulses of current injection, even when the duration of the pulse is shorter than the duration of the action potential. As the length of the pulse decreases, however, the amplitude necessary to elicit an action potential increases. Generate a plot of single spike threshold current vs. pulse duration for pulse widths between 0.1 and 2.0 *msec*. Is there a simple relationship between pulse width and threshold current? (Use an integration time step of 0.01 *msec* for this study.)

10. All of the injection pulses in the previous current clamp problems have been depolarizing. In this problem you will look at the effect of hyperpolarizing current pulses.
Set the pulse amplitude to --100 *nA* and set the pulse duration to 5 *msec*. What happens? What is the threshold, in terms of current magnitude and pulse duration, for eliciting this so-called *anode break* excitation? What mechanisms in the model are responsible for this behavior? (Hint: look at the time course of the state variables *m*, *n* and *h*.)