MetaNeuron Manual

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MetaNeuron was created and written by Eric A. Newman and Mark H. Newman. The computer code was written by Mark H. Newman. It was inspired by Jerome Y. Lettvin's transistor model of the axon, the MetaMembron.

MetaNeuron Manual version 2.02

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Introduction

MetaNeuron is a computer program that models the basic electrical properties of neurons and axons. The program is intended for the beginning neuroscience student and requires no prior experience with computer simulations.

Different aspects of neuronal behavior are highlighted in the six lessons presented in MetaNeuron. The first three lessons, *Membrane Potential*, *Membrane Time Constant and Membrane Length Constant,* illustrate the passive properties of neuronal membranes. The fourth and fifth lessons, *Axon Action Potential* and *Axon Voltage Clamp*, demonstrate how voltage- and time-dependent ionic conductances contribute to the generation of the action potential in the axon. The sixth lesson, *Synaptic Potential and Current*, illustrates how synaptic potentials are generated through the activation of ionotropic neurotransmitter receptors.

The lessons in MetaNeuron do not attempt to model the full complexity of neuronal behavior. The simulations simplify neuronal properties, highlighting the basic principles of neuronal function.

The *Axon Action Potential* and *Axon Voltage Clamp* simulations are based on the Hodgkin-Huxley equations. MetaNeuron represents 1 cm^2 of neuronal membrane and has a capacitance of 1 μF.

Operation of MetaNeuron

MetaNeuron simulations run automatically when the program is opened. A lesson is selected from the "Lesson" pull-down menu or from the function keys. The six lessons in MetaNeuron run independently of each other. If the parameters in one lesson are changed, the changes will not affect the operation of the other lessons.

Changing parameter values

Experiments are run in MetaNeuron by changing the values of the parameters displayed in the top portion of the screen. Parameter values can be changed in two ways:

1. Select the number in a parameter value box by clicking on it, type a new value, and hit "Enter".

2. Click on the gray button to the right of a parameter value box and drag the mouse (Figure 1). Moving the mouse up or to the right increases the value in increments of 1. Moving the mouse down or to the left decreases the value by 1. If the "Shift" or Ctrl" keys are depressed while moving the mouse, the parameter values change in increments of 10 or 0.1, respectively.

Figure 1. Control of parameter values.
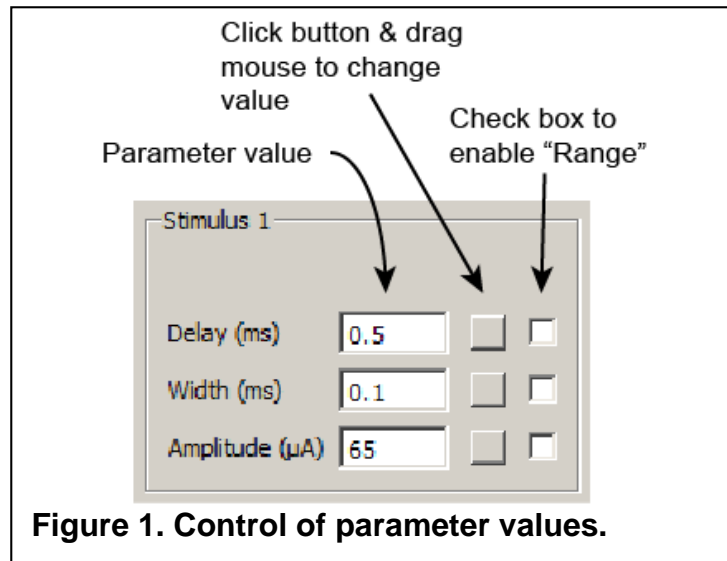
Parameters in boxes that are grayed out cannot be changed. The values of these parameters are determined by other parameters on the screen or are not currently activated.

All parameters can be reset to their default values by selecting "Restore All to Default" (Ctrl+D) in the "File" pull-down menu.

Graphs of parameter values

Some of the parameters in a lesson are plotted in the graph in the lower portion of the screen. The traces are color coded and corresponding trace labels are shown to the right of the graph. Traces in the graph are updated as a parameter value is changed.

The sweep duration, the total time displayed on the X-axis of the graph, is controlled by the "*Sweep duration*" parameter above the graph.

Family of traces

A family of traces can be generated in a graph by selecting the check box to the right of a parameter value box. For instance, to generate a family of traces for a range of stimulus amplitude values, select the "Stimulus 1" "Amplitude" check box. Then enter the desired "Start value", "End value" and "Increment" In the "Range" window above the graph. To return to the single trace mode, click the "Single Value" button in the "Range" window.

3D display

A family of traces can be displayed in three dimensions by clicking the "3D Graph" button above the graph. The display can be rotated by clicking on the window and dragging the mouse.

Measuring traces with the cursor

The X and Y values of any point on a graph can be determined by moving the mouse over the graph and clicking. The X and Y values, in units appropriate for the yellow trace, are displayed in the "Cursor" window to the right of the graph. The Y value where the mouse is clicked is shown in white. The Y value of the yellow trace is shown in yellow. If multiple yellow traces are displayed (range option), the Y value of the yellow trace closest to the cursor is shown.

LESSON 1, RESTING MEMBRANE POTENTIAL

Lesson 1 illustrates how K+ and Na+ channels contribute to the generation of the resting membrane potential. The neuron in this lesson is modeled by passive conductances to K+ and Na+. These conductances are voltage-independent and the neuron does not generate action potentials.

The concentrations of K+ and Na+, both outside and inside the cell, can be varied. The program calculates the electrochemical equilibrium potential for each ion, based on the ion concentration gradient across the membrane, using the Nernst equation,

$$ion\ equilibrium\ potential\ (\mathrm{mV}) = 58 \cdot \log\left(\frac{[ion]_o}{[ion]_i}\right)$$

where $[ion]_o$ and $[ion]_i$ are the concentrations of the ion outside and inside the cell, respectively. The effect of temperature on the equilibrium potential is not included in this version of the equation.

The resting membrane potential of the neuron is determined by the concentrations of K+ and Na+ outside and inside the cell and by the permeability of the membrane to K+ and Na+. The relative membrane permeabilities to K+ and Na+ can be varied. The membrane potential is calculated using the Goldman-Hodgkin-Katz equation,

$$membrane\ potential\ (mV) = 58 \cdot \log\left(\frac{P_K[K^+]_o + P_{Na}[Na^+]_o}{P_K[K^+]_i + P_{Na}[Na^+]_i}\right)$$

where P_K and P_{Na} are the relative membrane permeabilities to K+ and Na+, respectively. The term representing membrane permeability to Cl- is omitted in the MetaNeuron simulation. Active membrane conductances are not modeled in this lesson.

Student exercises

1) Electrochemical Equilibrium Potential. Vary the concentrations of K+ and Na+, both inside and outside the cell. What effect does this have on the electrochemical equilibrium potential of the ion? What is the Na+ equilibrium potential when *Na+ out* equals 100 mM and *Na+ in* equals 10 mM? What is the Na+ equilibrium potential when *Na+ out* equals 100 mM and *Na+ in* equals 100 mM? What is the K+ equilibrium potential when *K+ out* equals 10 mM and *K+ in* equals 100 mM? Why is this?

2) Resting Membrane Potential. Starting with the default parameter values, vary the relative membrane permeability to K^+ and Na^+. What effect does this have on the resting membrane potential? Set *Na+ out* to 100 mM, *Na+ in* to 10 mM, *K+ out* to 10 mM and *K+ in* to 100 mM. What is the membrane potential when the *Na+ permeability* equals 0 and the *K+ permeability* equals 10? What is the membrane potential when the *Na+ permeability* equals 10 and the *K+ permeability* equals 0? What is the membrane potential when the *Na+ permeability* equals 1 and the *K+ permeability* equals 10? Why is this?

3) Membrane Conductance and the Membrane Potential. Starting with the default parameter values, plot the value of the membrane potential as a function of $[K^+]_o$ over a $[K^+]_o$ range of 0.2 to 100 mM. Regraph the data with the membrane potential plotted as a function of $\log([K^+]_o)$. Can you explain why this second plot has the shape that it does?

Reduce P_{Na} to 0 and replot the membrane potential as a function of $\log([K^+]_o)$. What accounts for the difference in the shape of this relation?

LESSON 2, MEMBRANE TIME CONSTANT

Lesson 2 illustrates the effect of the membrane time constant, τ, on the time course of electrical responses in neurons. The neuron in this lesson is modeled by a passive membrane resistance, R_m, a membrane capacitance, C, and a current source. The membrane capacitance equals 1 μF/cm^2, or simply 1 μF, since MetaNeuron represents 1 cm^2 of membrane. The value of the membrane resistance as well as the time course and amplitude of the current source can be varied. The neuron in this lesson does not generate action potentials.

The membrane resistance and capacitance together determine the time constant of the membrane, as described by the equation,

$$\tau = R_m \cdot C$$

The default values of the stimulus current source, a 1 ms depolarizing pulse, are a good approximation of the current generated by a fast excitatory synapse. Using the default current source parameters, MetaNeuron can be used to assess the effect of the membrane time constant on temporal summation of synaptic potentials. The "Threshold" potential shown in the graph (purple line) indicates the approximate voltage at which action potentials will be initiated in a neuron. Note, however, that the neuron model used in this lesson does not include active membrane conductances and will not generate action potentials when the membrane potential exceeds the threshold value.

When a constant current is injected into a neuron, the membrane potential depolarizes with an exponential time course (assuming that there are no active membrane conductances). Similarly, when the stimulus is turned off, the membrane potential returns to the resting membrane potential with an exponential time course.

The rate at which the membrane potential increases and decreases is described by the time constant, τ, of the exponential. τ is defined as the time it takes for the membrane to increase or to decrease to 1-1/e (approximately 63%) of its final value. Remember that sufficient time must be allowed for the membrane potential to reach its plateau level in order to see the entire exponential curve.

Student exercises

1) Membrane Time Constant. Using the default parameter values, determine the time constant of the membrane by measuring the time it takes for the amplitude of the membrane potential to fall 63% of the way back to the baseline value, after the current source is turned off. Use the cursor to measure the values along the membrane potential trace. When you click on the graph, the X and Y values at that point are displayed at the bottom right window in white and the Y value of the trace is shown in yellow.

Does the value of τ that you measured equal the membrane time constant calculated from the equation,

$$\tau = R_m \cdot C$$

Increase the amplitude of the stimulus to 150 μA and repeat your measurement of the membrane time constant. Has it changed?

2) Membrane Resistance and the Time Constant. Using a stimulus amplitude of 150 μA, vary the membrane resistance. What effect does this have on the rising and falling phases of the response? Set the membrane resistance to 2 kΩ·cm². What is the membrane time constant, measured from the falling phase of the response? A family of curves showing the effect of varying the membrane resistance can be displayed by using the range function of MetaNeuron (Figure 2). Check the "Membrane Resistance" range box and choose range values of 0.5, 20, and 2 (begin value, end value, and increment).
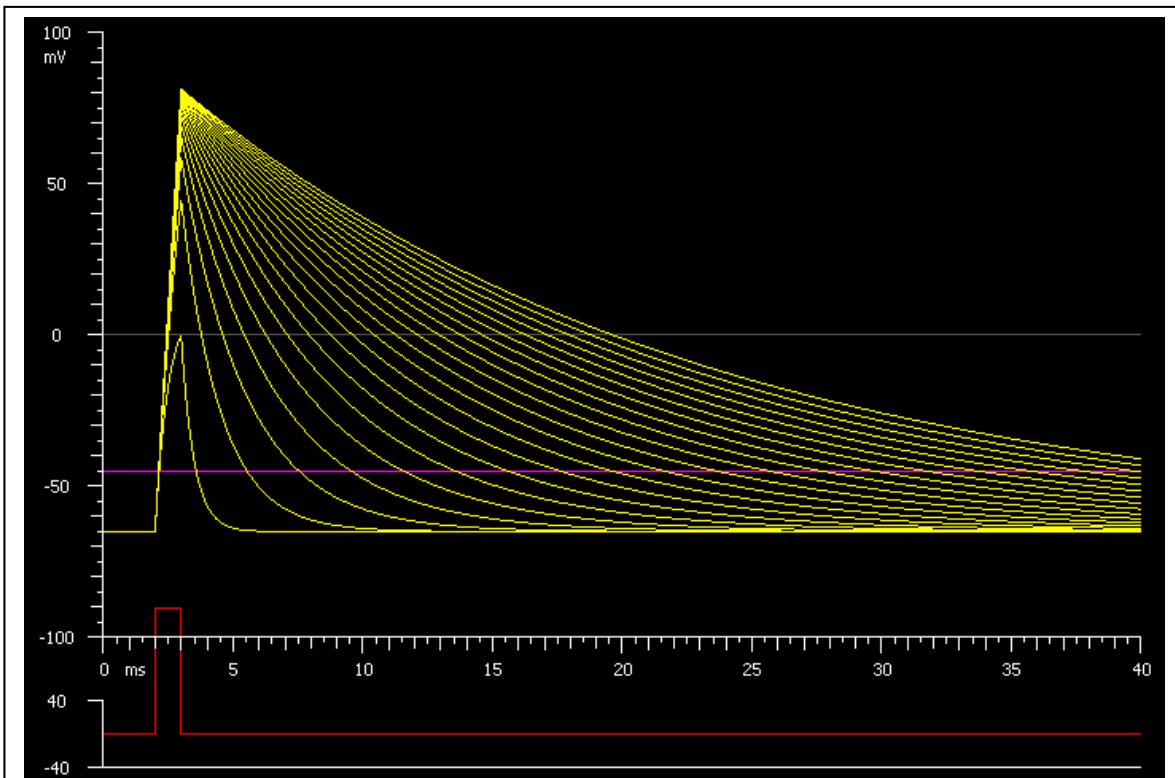

Figure 2. Membrane time constant. A 150 μA, 1 msec current pulse rapidly depolarizes a neuron. At the end of the pulse, the membrane potential decays back towards the resting membrane potential with an exponential time course. The time constant of decay decreases as the membrane resistance is reduced from 20 kΩ to 0.5 kΩ.

3) Temporal Summation. Beginning with the default parameter values, increase the "Number of stimuli" from 1 to 3. This stimulus represents 3 synaptic potentials generated at 2 ms intervals. Note how the responses add together (Figure 3). This superposition of responses is termed *temporal summation*. Now reduce the membrane resistance from 10 to 2 k $\Omega \cdot$cm^2. What effect does this have on temporal summation? Note that changes in temporal summation determine whether the response reaches the threshold level for firing action potentials (the purple line).

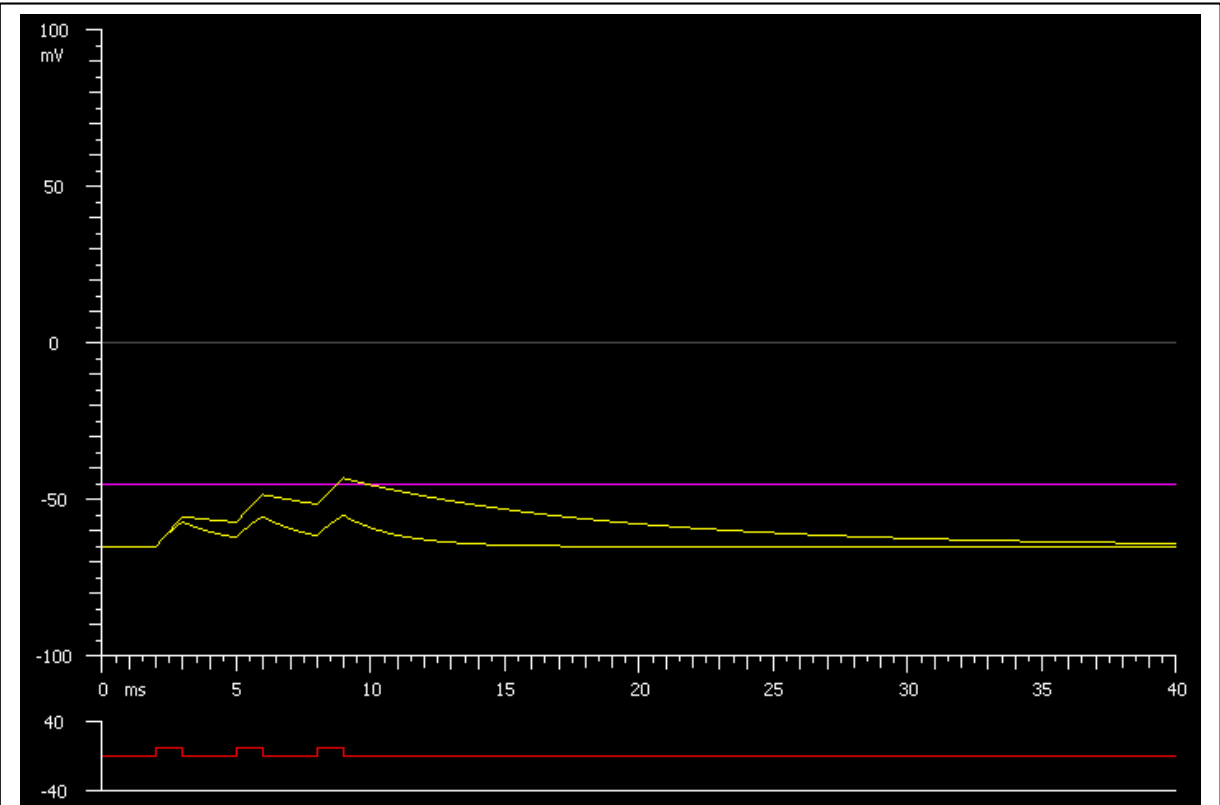**Figure 3. Temporal summation.** The three current pulses (red trace) simulate three synaptic responses. With a long membrane time constant (10 kΩ membrane resistance), the synaptic potentials summate and reach the threshold potential for firing an action potential (purple line). With a short time constant (2 kΩ membrane resistance), the synaptic potentials do not summate and do not reach threshold.

LESSON 3, MEMBRANE LENGTH CONSTANT

Lesson 3 illustrates the effect of the length constant, λ, on the passive spread of voltage down the length of a dendrite or axon. (For simplicity, we will call the process a dendrite hereafter.) The lesson models the dendrite as a cylindrical process of uniform diameter and infinite length having a passive leak conductance. The dendrite has a membrane capacitance of 1 μF/cm^2. Values of the membrane resistance (R_m), the internal (cytoplasmic) resistivity (R_i), and the diameter (d) of the dendrite can be varied. Stimuli are applied to the process at X = 0. Responses are calculated from the cable equations, first developed by Lord Kelvin in the 1850's. The stimulus is scaled to R_m, R_i, and d, such that the steady-state depolarization at X = 0 produced by a stimulus does not change when these parameters are varied.

Responses can be displayed in two modes: "Potential vs. Distance" and "Potential vs. Time". The familiar steady-state exponential decay of voltage with distance is seen in the "Potential vs. Distance" mode when both the "Stimulus duration" and the "Graph time" are set to a time much longer than the membrane time constant, as occurs using the default values. The attenuation and slowing of a synaptic potential as it passively spreads down a dendrite towards the soma can be seen in the "Potential vs, Time" mode. When the "Synaptic Potential" box in the "Stimulus" window is checked, a synaptic potential is generated at X = 0. The potential is generated by a conductance having rising and falling time constants of 0.1 and 1.0 times the "Stimulus Width". Voltage vs distance vs time relations can be viewed by selecting the "3D Graph" function.

Student exercises

1) Length constant - steady-state exponential decay of voltage with distance. When a dendrite is depolarized at a point (X = 0), the potential will decay with distance as it is passively conducted down the dendrite. It is useful to examine the characteristics of the decay under steady state conditions, when the dendrite has been depolarized for a long time compared to the membrane time constant.

The default parameter values illustrate the steady-state exponential decay of the membrane potential with distance. The dendrite is depolarized for 50 ms and the membrane potential plotted at t = 50 ms.

Measure the membrane length constant (λ), the distance at which the membrane potential falls to 37% (1/e) of its maximal value. Does this equal the value calculated by the equation,

$$\lambda \, (cm) = 0.5 \sqrt{d \frac{R_m}{R_i}}$$

where λ in the length constant (cm), d is the dendrite diameter (cm), R_m is the membrane resistance (Ω cm^2), and R_i is the internal resistivity (Ω cm)?

2) Length constant - membrane resistance, internal resistivity, and dendrite diameter. Starting with the default parameter values, vary the membrane resistance. How does this affect the length constant? For a range of R_m from 1 to 300 kΩ cm^2, graph the length constant as a function of R_m. What is the relation between the two?

Starting with the default parameter values, vary the internal resistivity. How does this affect the length constant? For a range of R_i from 1 to 500 Ω cm, graph the length constant as a function of R_i. What is the relation between the two?

Starting with the default parameter values, vary the dendrite diameter from 0.05 to 3 μm (Figure 4). How does this affect the length constant? What effect would varying the diameter of an unmyelinated axon have on action potential propagation velocity in the axon?
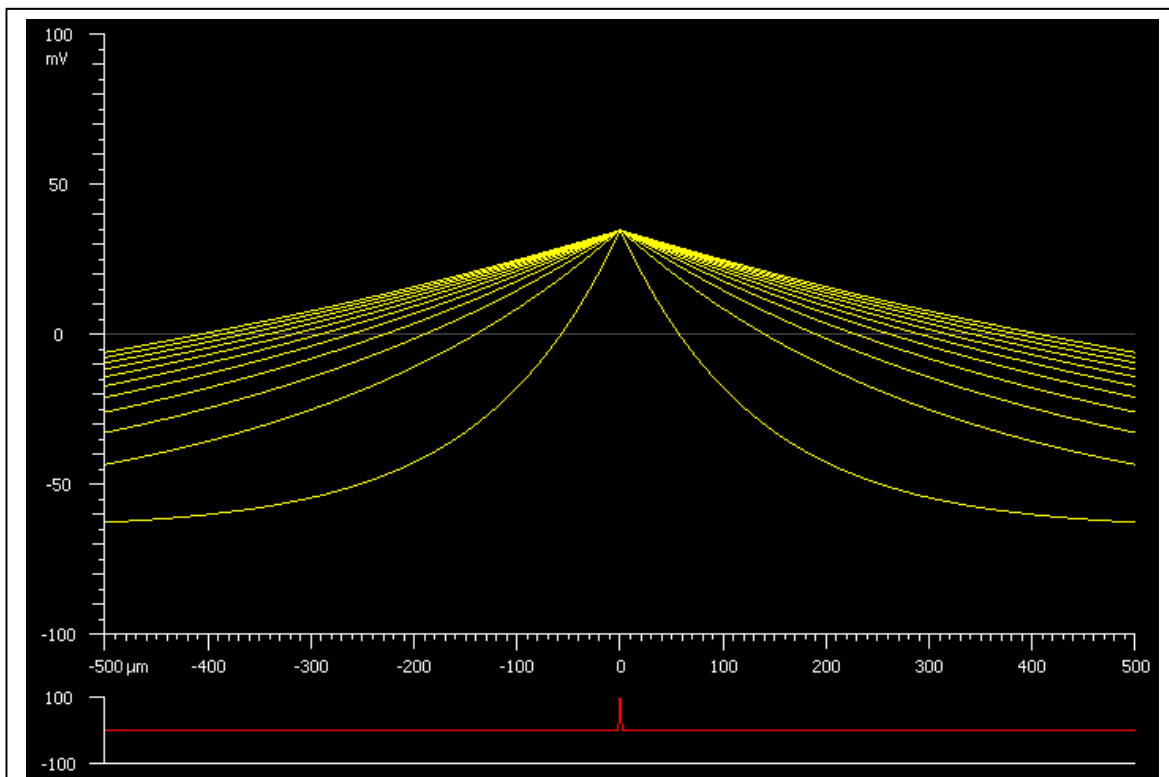

Figure 4. Dendrite length constant. The dendrite is depolarized by a 50 ms current pulse at position X = 0. The membrane potential is plotted at t = 50 ms as a function of distance from the point of current injection. The length constant changes as the dendrite diameter is varied from 0.05 to 5 μm.

3) Passive conduction of a synaptic potential in a dendrite. Starting with the default parameter values, select the "Potential vs. Time" mode and the "Synaptic Potential" stimulus. Set the "Stimulus Width" to 1 ms. The time course of the synaptic potential can be viewed at different distances from the site of generation of the potential by varying the "Position" parameter in the "Potential vs. Time" window. View the time course of the synaptic potential at X = 0 (the site of generation), X = 50 μm, and X = 100 μm (Figure 5). How does the synaptic potential change as it is passively conducted down the dendrite? What accounts for the change in time course of the potential? The Potential vs time vs distance relation can be viewed by selecting the "3D Graph" function. Choose the "Surface" option on the 3D graph (Figure 6).
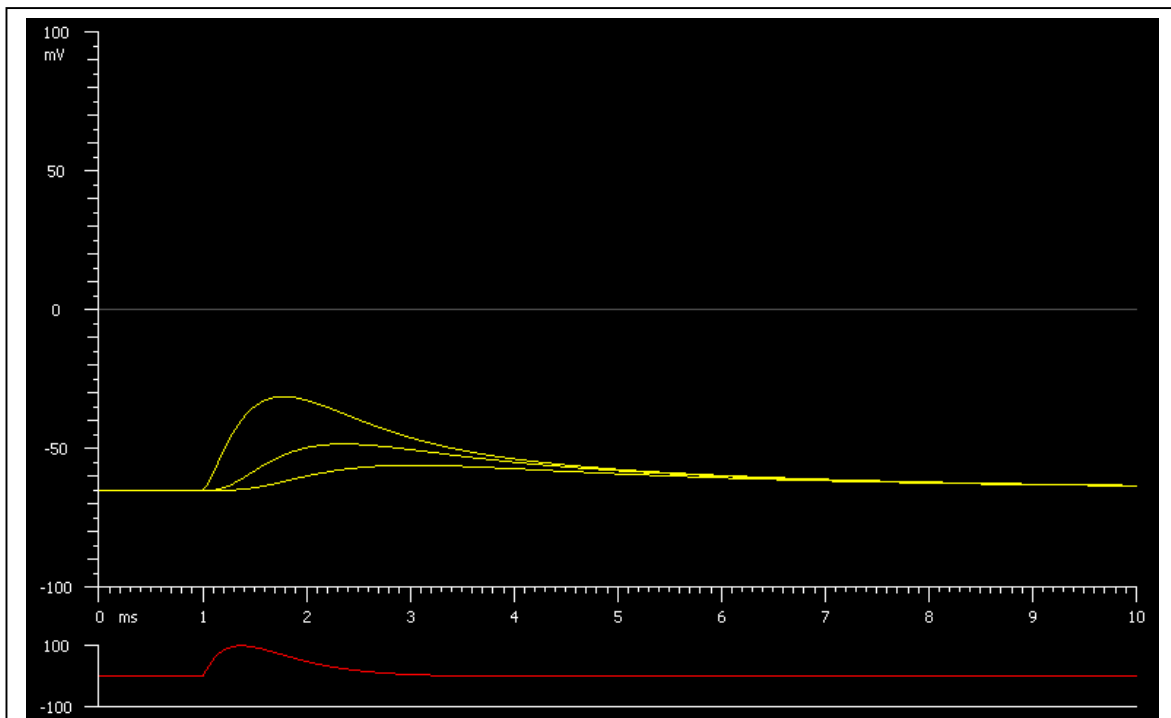

Figure 5. Passive conduction of a synaptic potential. A synaptic potential (yellow traces) is attenuated and slowed as it is conducted down a dendrite. The time course of the synaptic potential is seen at the site of generation and at 50 and 100 μm from the site of generation.
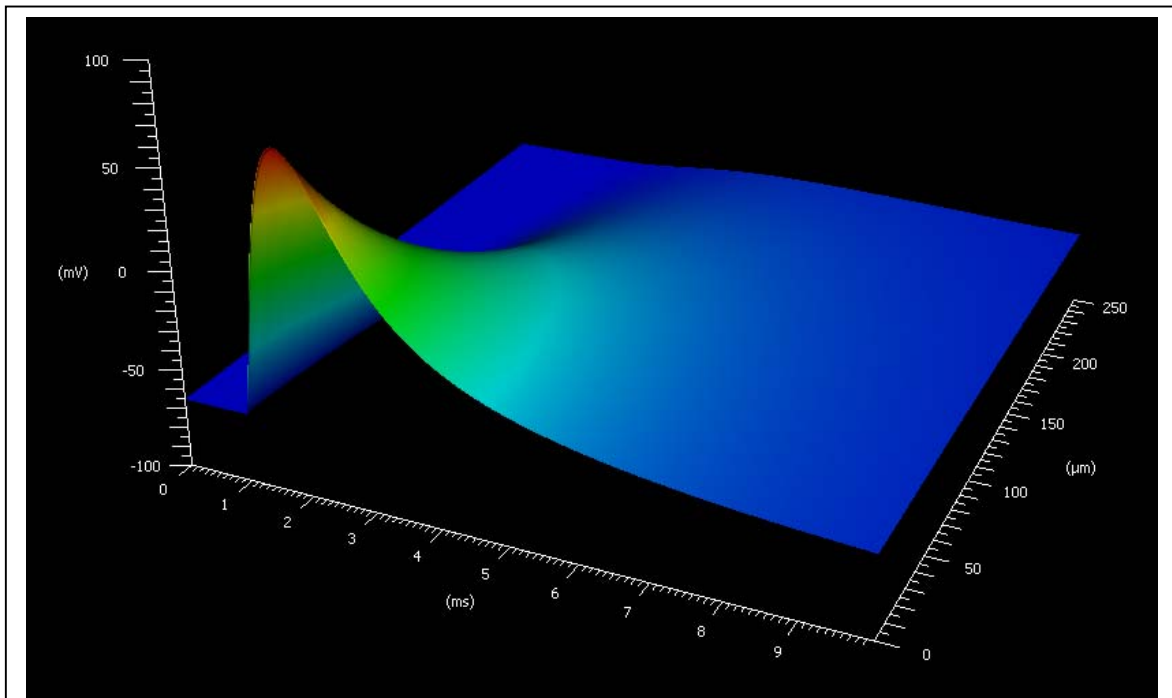
Figure 6. 3D plot of passive conduction of a synaptic potential. The amplitude of a synaptic potential (mV axis) is plotted as a function of time (ms axis) and distance (μm axis) from the site of generation. Note that the synaptic potential is attenuated and slowed as it is conducted down the dendrite. The amplitude of the synaptic potential has been increased for illustrative purposes.

LESSON 4, AXON ACTION POTENTIAL

Lesson 4 illustrates how voltage- and time-dependent Na$^+$ and K$^+$ conductances generate the action potential. The MetaNeuron model in this lesson is based on equations published by Alan Hodgkin and Andrew Huxley in 1952 to describe the voltage-dependent Na$^+$ and K$^+$ conductances of the squid giant axon. The Na$^+$ conductance in the MetaNeuron model represents fast, tetrodotoxin-sensitive Na$^+$ channels. The K$^+$ conductance represents delayed rectifier K$^+$ channels. A leak conductance is also included. The values of some of the parameters in the equations have been adjusted to simulate action potential generation in a vertebrate axon.

Several parameters controlling Na$^+$, K$^+$, and leak conductances in the model can be adjusted:

- The Na$^+$, K$^+$, and leak equilibrium potentials. Modifying these values will change the driving force for ion flux through the Na$^+$, K$^+$, and leak channels.

- gNa$^+$ max, gK$^+$ max, gLeak, the maximum membrane conductances. These parameters represent the number of Na$^+$, K$^+$, and leak channels in the membrane.

- TTX (tetrodotoxin) and TEA (tetraethylammonium). Selecting TTX blocks the Na$^+$ conductance. Selecting TEA blocks the K$^+$ conductance, but not the Leak conductance.

- Temperature. Raising the temperature reduces the time constants controlling Na$^+$ conductance activation and inactivation and K$^+$ conductance activation, resulting in a faster action potential. The temperature factor has a Q10 of 3.0.

Activation of the neuron is controlled by several stimuli:

- Stimulus 1. The delay, width and amplitude of this current pulse can be varied.

- Stimulus 2. A second current pulse is added when the "Stimulus 2 On" box is selected.

- Holding Current. This continuous current source can be used alone or together with the current pulses.

Na$^+$ and K$^+$ conductances or currents are displayed along with the membrane potential when the appropriate boxes are selected in the "Conductances and Currents" window.

Student exercises

1) Action Potential Threshold. Vary the amplitude of "Stimulus 1". What effect does this have on the cell response (Figure 7)? What is the threshold stimulus amplitude (in μA) for initiating an action potential? The threshold stimulus is best measured by adjusting the stimulus amplitude in 0.1 μA increments until it is just large enough to initiate an action potential. Click on the "Stimulus 1 Amplitude" gray button and move the mouse while holding down the ctrl key.
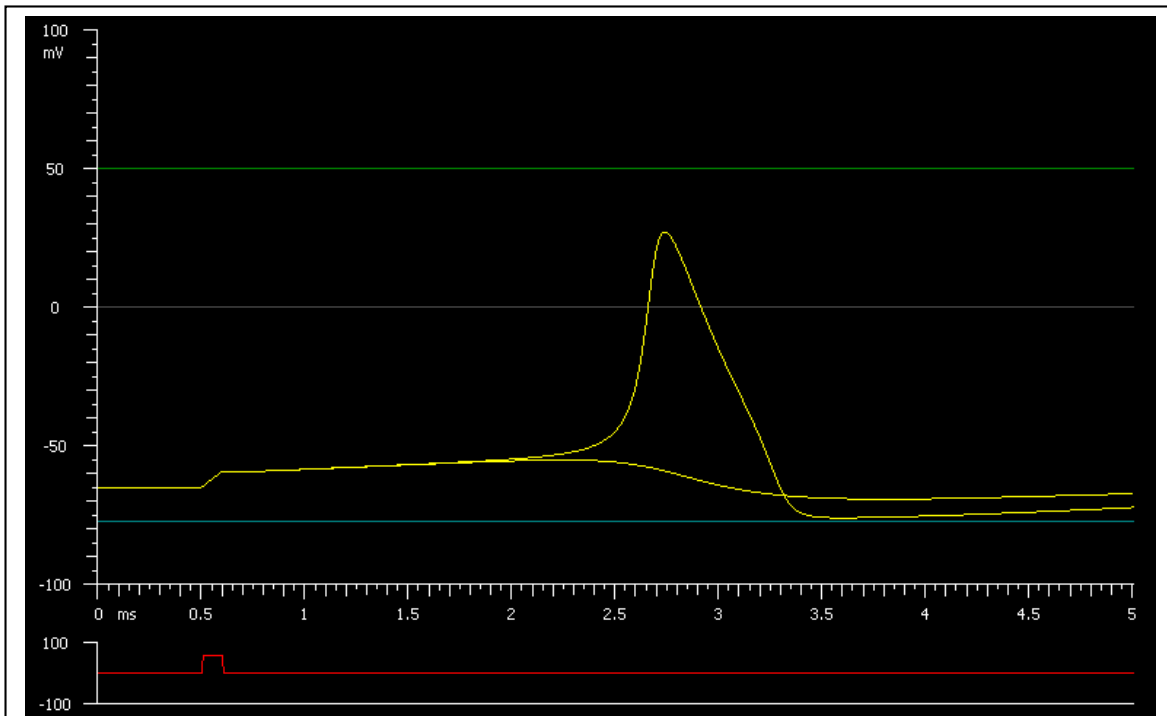

Figure 7. Action potential generation. Response of MetaNeuron to two stimuli of slightly different amplitudes (red line). One evokes an action potential while the other does not. The peak of the action potential approaches the Na+ equilibrium potential (green line). The after hyperpolarization reaches the K+ equilibrium potential (blue line).

2) Action Potentials and the Na+ equilibrium potential. Set "Stimulus 1 Amplitude" to 150 μA. The stimulus will evoke an action potential. Vary the "Na+ equilibrium potential". What effect does this have on the action potential? Why?

3) Action potentials and *gK max.* Using a "Stimulus 1 Amplitude" of 150 μA and a "Na+ equilibrium potential" of 50 mV, vary "gK max". What effect does this have on the time to action potential initiation and the width of the action potential? Why?

4) Na⁺ channels and Action Potential Initiation. Action potentials are often initiated at the axon hillock, where the axon leaves the cell body. The axon hillock has a high density of Na+ channels, which lowers the threshold for initiating an action potential. MetaNeuron can be used to measure the effect of Na+ channel density on the threshold of action potential generation. Vary "gNa max" from 200 to 380 mS/cm^2 in 20 mS/cm^2 steps. (Na+ channel density is proportional to gNa max.) For each "gNa max" value, determine the threshold stimulus amplitude (in μA) for initiating an action potential. Plot the threshold current as a function of gNa max. Why does the action potential threshold vary as Na+ channel density is changed?

5) Action Potential Membrane Conductances. Starting with the default parameter values. Select "Show ionic conductances" in the "Conductances and Currents" window. The green and blue traces show the time course of the Na⁺ and K⁺ conductances that are activated during the action potential. Why does the K⁺ conductance turn on slower and last longer than the Na⁺ conductance?

6) Action Potential Membrane Currents. Select "Show ionic currents" in the "Conductances and Currents" window. The green and blue traces show the time course of the inward (negative) Na⁺ current and the outward (positive) K⁺ current that generate the action potential. Why does the initial phase of the Na⁺ current have an inflection point?

7) Anodal Break excitation. Starting with the default parameter values, set "Sweep Duration" to 10 msec. Now *decrease* the "Stimulus 1 Amplitude" until it reaches –225 μA. An action potential is evoked by the *hyperpolarizing* stimulus! This phenomenon is called *anode break excitation*. Can you explain why a hyperpolarizing stimulus evokes an action potential?

8) Spreading Depression. Starting with the default parameter values, set "Stimulus 1 Amplitude" to 0 and "Sweep Duration" to 20 ms. Now slowly raise the "K+ equilibrium potential" to more and more positive values. As the K+ equilibrium potential is raised, you will observe the following sequence of events.

- The membrane potential depolarizes.

- Spontaneous action potentials are generated.

- The frequency of the action potentials increases.

- The amplitude of the action potentials diminishes.

- The action potentials die out as the membrane potential continues to depolarize.

The same sequence of events occurs during spreading depression episodes in the cortex. During spreading depression, extracellular K^+ concentration increases from a normal level of 3 mM to 50 mM or more. Spreading depression is thought to underlie the symptoms of migraine headache in the visual cortex, with the spontaneously generated action potentials resulting in visual auras that sometimes accompany migraines, and the loss of action potential generation resulting in a visual scotoma.

Can you explain why this sequence of events occurs when the K^+ equilibrium potential is raised?

9) Multiple Sclerosis. The symptoms of multiple sclerosis are caused by conduction failure due to the demyelination of CNS axons. The symptoms of multiple sclerosis are alleviated by lowering a patient's core body temperature while symptoms are exacerbated by an exercise-induced rise in body temperature. MetaNeuron can be used to investigate the effect of temperature on action potential generation. Starting with the default parameter values, adjust the stimulus amplitude so that it is just below threshold for initiating an action potential. Now vary the temperature. How much does the temperature have to be lowered to evoke an action potential? Starting again from the default parameter values, adjust the stimulus amplitude so that it is just above threshold for initiating an action potential. How much does the temperature have to be raised to block initiation of the action potential? In a patient with conduction block, lowering their core body temperature will restore conduction by lowering the threshold for action potential generation.

Why does lowering the temperature reduce the threshold for action potential generation?

10) Refractory Period. MetaNeuron can be used to measure the time course of the refractory period which follows the action potential. Two stimulus pulses are used to determine the change in threshold that occurs during the refractory period (Figure 8). The amplitude of the first stimulus is set to well above threshold and reliably evokes an action potential. The amplitude of this stimulus remains fixed. The second stimulus, which is initially set to a delay of 6 ms after the first stimulus, is used to determine the change in threshold of the axon. The amplitude of the second stimulus is adjusted until it is just strong enough to evoke a second action potential. This stimulus amplitude is a measure of the threshold of the axon. The higher the threshold of the axon, the stronger is the stimulus needed to trigger the second action potential.

Starting with the default parameter values, set "Sweep Duration" to 10 ms and "Stimulus 1 Amplitude" to 150 μA. Turn "Stimulus 2" ON and set "Stimulus 2 delay" to 6 ms.

With "Stimulus 2 Delay" at 6 ms, adjust "Stimulus 2 Amplitude" until it is just large enough to evoke an action potential. (Use the click and drag method of

varying "Stimulus 2 Amplitude".*)* This is the threshold amplitude.

Reduce "Stimulus 2 delay" to 5 ms and once again adjust "Stimulus 2 Amplitude" to the threshold level. Repeat this measurement for Stimulus 2 Delays of 4 to 1 ms. As you approach a Stimulus 2 Delay of 1 ms, reduce the delay in smaller increments so that the threshold amplitude vs. delay relation can be determined more accurately. Note that at small delays, the second action potential becomes smaller and is not strictly an "all or none" event. Use your judgment in determining a criterion for when the action potential is evoked.

Plot the Stimulus 2 threshold amplitude as a function of Stimulus 2 delay. The plot shows the time course of the refractory period following an action potential.

What is the duration of the absolute refractory period and the relative refractory period, based on this experiment?
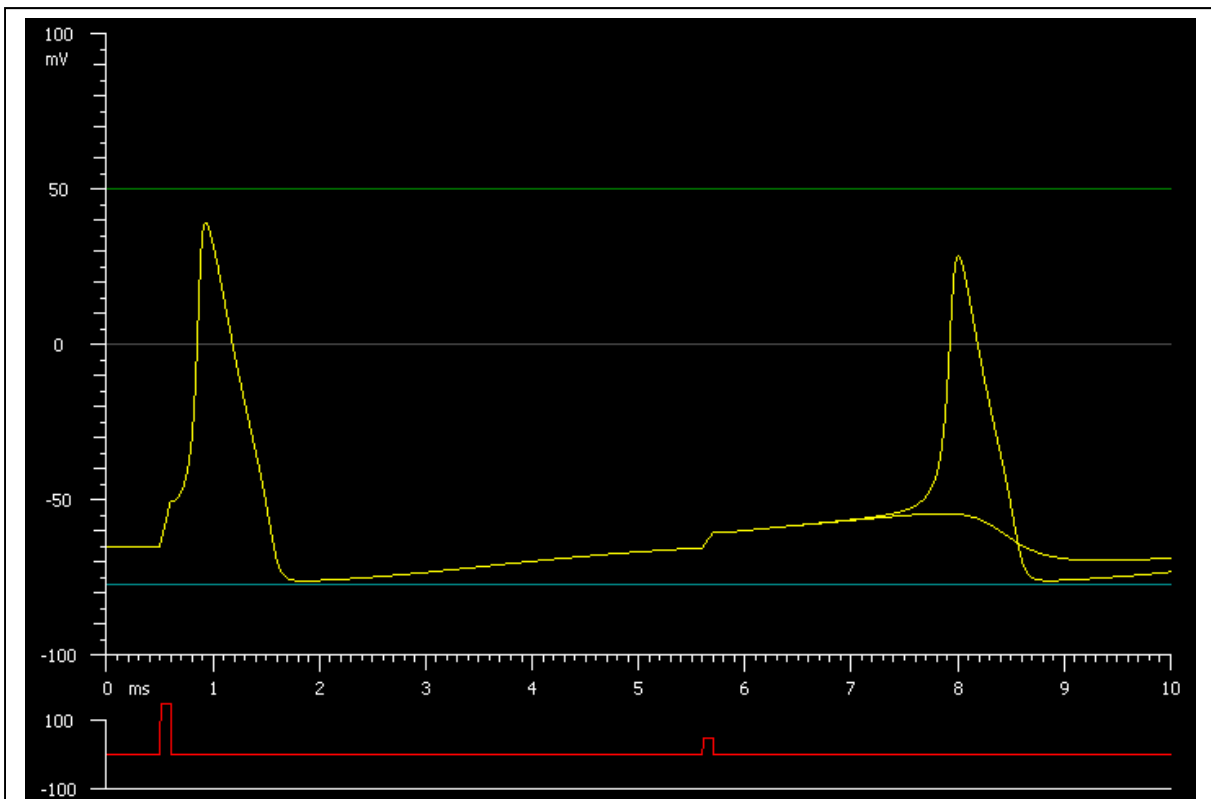

Figure 8. The refractory period. The time course of the refractory period following an action potential can be determined using a two stimulus protocol. The first stimulus is well above threshold and reliably evokes an action potential. The second stimulus, at a delay following the first action potential, is adjusted so that it just triggers an action potential. The amplitude of the second stimulus is a measure of the membrane threshold at that delay.

LESSON 5, AXON VOLTAGE CLAMP

Lesson 5 uses the voltage clamp technique to illustrate the voltage- and time-dependent properties of the Na$^+$ channels and delayed rectifier K$^+$ channels that generate the action potential. The voltage clamp technique uses a negative feedback electronic circuit to hold the membrane potential to a value (the command voltage) specified by the experimenter. The circuit then measures the time-dependent currents flowing through Na$^+$ and K$^+$ channels at that voltage. Typically, the membrane potential is first held at a negative voltage near the resting membrane potential, the "Holding Potential", and is then stepped to a more positive voltage, determined by the "Stimulus 1" and "Stimulus 2" Amplitudes in MetaNeuron.

It is important to remember that action potentials cannot be generated when an axon is voltage clamped. The currents that are measured under voltage clamp contribute to the generation of the action potential, but these currents are not, in themselves, action potentials.

Lesson 5 uses the same Hodgkin-Huxley model of the axon that is used to generate action potentials in Lesson 4. The parameters in Lesson 5 are similar to those in Lesson 4 as well. The only changes in Lesson 5 are that the axon is now voltage-clamped and the stimuli used are command voltages rather than currents. The "Holding Potential" parameter determines the voltage of the axon at the beginning of the experiment. The "Stimulus 1" and "Stimulus 2" Amplitudes determine the voltage to which the membrane is stepped during the two pulses. Note that these stimulus amplitudes represent absolute voltages, not relative voltages added onto the Holding Potential.

A family of voltage clamp traces can be displayed by using the "Range" feature of MetaNeuron. Use the 3D Graph feature to view the family of traces in 3D.

Student exercises

1) Na⁺ and K⁺ voltage clamp currents. Increase "Stimulus 1 Amplitude" and note the development of a transient inward (negative) current and a sustained outward (positive) current. What is the origin of these two components of the current?

A family of voltage clamp traces can be generated in a graph by using the "Range" function of MetaNeuron (Figure 9). Select the Range function. The range of traces can be changed by specifying the begin, end, and increment range values. A 3D plot of the family of traces can be displayed by clicking the 3D Graph button (Figure 10). Note that the 3D plot can be displayed either as individual traces or as a surface. The 3D plot can be rotated by clicking on the plot and dragging the mouse.
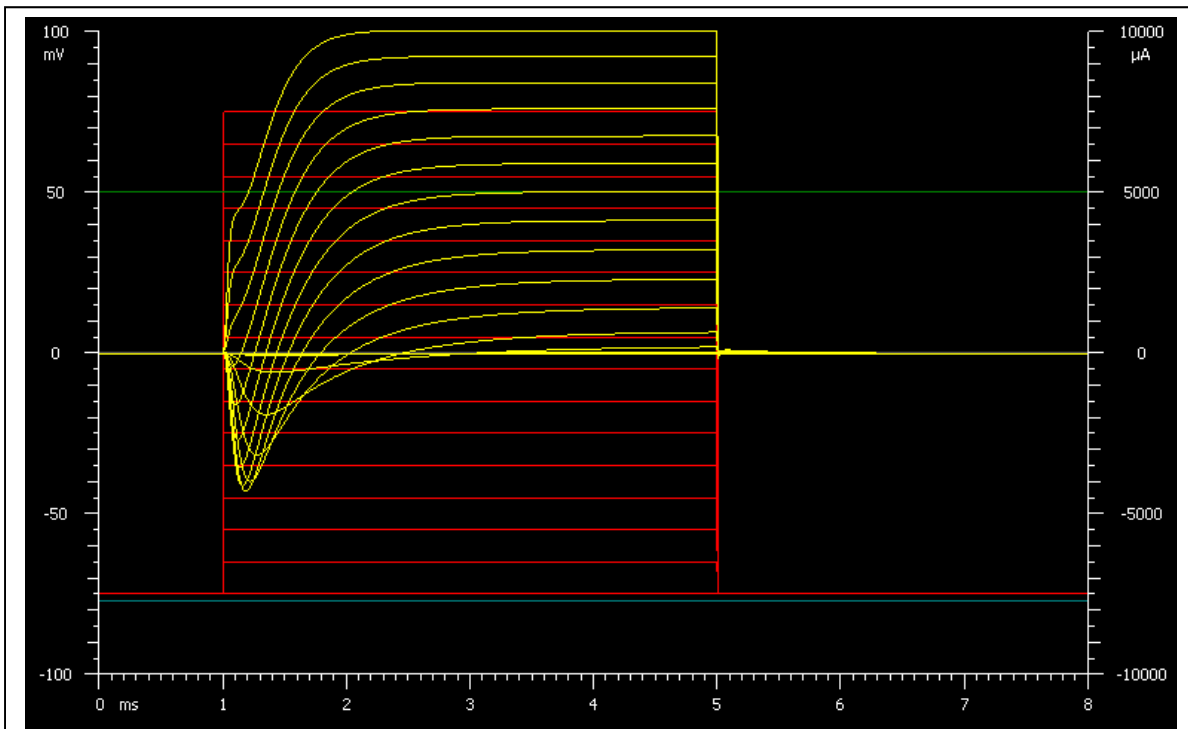

Figure 9. Family of voltage clamp traces showing inward (negative) Na⁺ currents and outward (positive) K⁺ currents. The membrane potential was raised from a holding potential of -75 mV to more positive potentials (-65 mV to +75 mV in 10 mV increments). Membrane currents are shown in the yellow traces and the command voltages in the red traces.
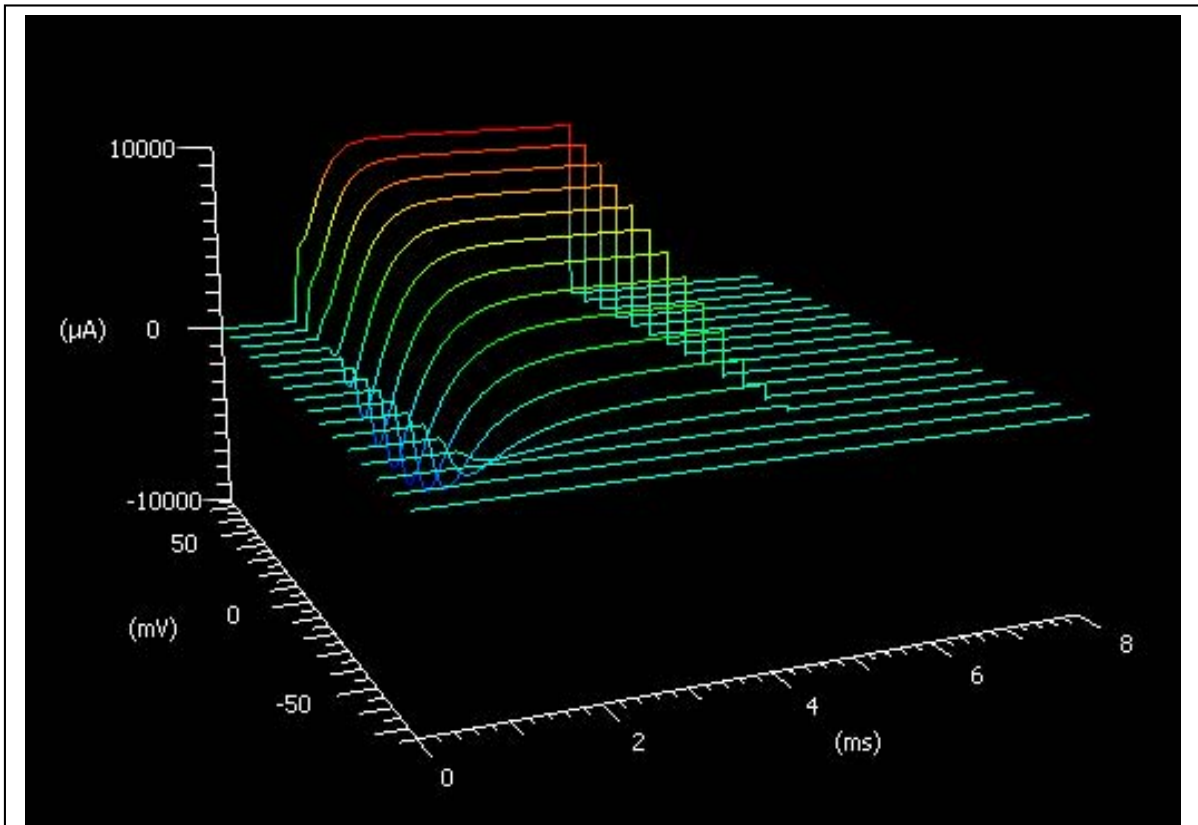
Figure 10. 3D plot of voltage clamp traces. The current traces shown in Figure 9 are replotted in 3D. The 3D plots can be rotated by clicking on the plot and dragging the mouse.

2) TTX and TEA. Starting with the default parameters, set "Stimulus 1 Amplitude" to 0 mV. What happens when the TTX or TEA boxes are selected? Why is this?

3) K$^+$ current-voltage and conductance-voltage relations. The K$^+$ current can be viewed in isolation by blocking the Na$^+$ current with TTX. With the TTX box selected, measure the amplitude of the K$^+$ current as a function of the voltage of the test pulse (Stimulus 1 Amplitude). The K$^+$ current should be measured from the pre-pulse baseline (which you can assume equals 0) to its plateau level, at the end of the voltage pulse. Measure the current over a stimulus amplitude range of −70 to +70 mV in increments of 10 mV. Plot the amplitude of the K$^+$ current as a function of the test pulse voltage.

The K$^+$ conductance over this range of voltages can be calculated using the chord conductance equation,

$$\text{conductance} = \frac{current}{V_m - V_{equilibrium}}$$

where V_m is the test pulse voltage and $V_{equilibrium}$ is the equilibrium potential for the ion, in the case K$^+$. Use this equation to calculate the K$^+$ conductance at each

test pulse voltage and plot the conductance as a function of voltage. What does the conductance-voltage relation tell you about the voltage dependence of K$^+$ channels?

4) Na$^+$ current-voltage and conductance-voltage relations. Repeat this procedure to determine the current-voltage and conductance-voltage relations for Na$^+$. Select the TEA box and de-select the TTX box in order to view the Na$^+$ current in isolation. Measure the amplitude of the Na$^+$ current as a function of voltage over a stimulus amplitude range of –70 to +80 mV. Measure the Na$^+$ current from the pre-pulse level to the transient peak of the current. Note that the peak current will be negative at some voltages and positive at other voltages. Plot the current-voltage relation. Calculate the conductances using the chord conductance equation and plot the conductance-voltage relation. What does the conductance-voltage relation tell you about the voltage dependence of Na$^+$ channels? In the current-voltage relation, why is Na$^+$ current negative at some voltages and positive at other voltages?

5) Recovery from Na$^+$ channel inactivation. Na$^+$ channels first activate and then inactivate when the membrane potential is stepped from the resting membrane potential to a depolarized potential. The channels do not begin to recover from inactivation until the membrane is repolarized to a negative level. MetaNeuron can be used to measure the time course of recovery of the Na$^+$ channels from inactivation by voltage clamping the axon and using two voltage command pulses.

Starting with the default parameter values, select the TEA box to view the Na$^+$ current in isolation, turn on "Stimulus 2", set "Sweep Duration" to 14 msec, "Stimulus 1" and "Stimulus 2 Amplitude" to +5 mV, "Stimulus 1" and "Stimulus 2 Width" to 2 msec and "Stimulus 2 Delay" to 2 msec.

Note that the amplitude of the peak Na$^+$ current evoked by the second stimulus is smaller than that evoked by the first stimulus (Figure11). This occurs because many of the Na$^+$ channels have not yet recovered from inactivation following the first depolarizing pulse.

The time course of recovery of Na$^+$ channels from inactivation can be displayed in MetaNeuron by using the Range function to plot a range of Stimulus 2 delays. Select the "Stimulus 2 Delay" range box and specify begin, end, and increment range values of 0, 11, and 0.1 ms. The envelop of Na$^+$ current traces displays the time course of recovery of Na$^+$ channels from inactivation (Figure 12).

How does the time course of recovery from Na$^+$ channel inactivation compare to the time course of the refractory period following an action potential? Are these two processes related?
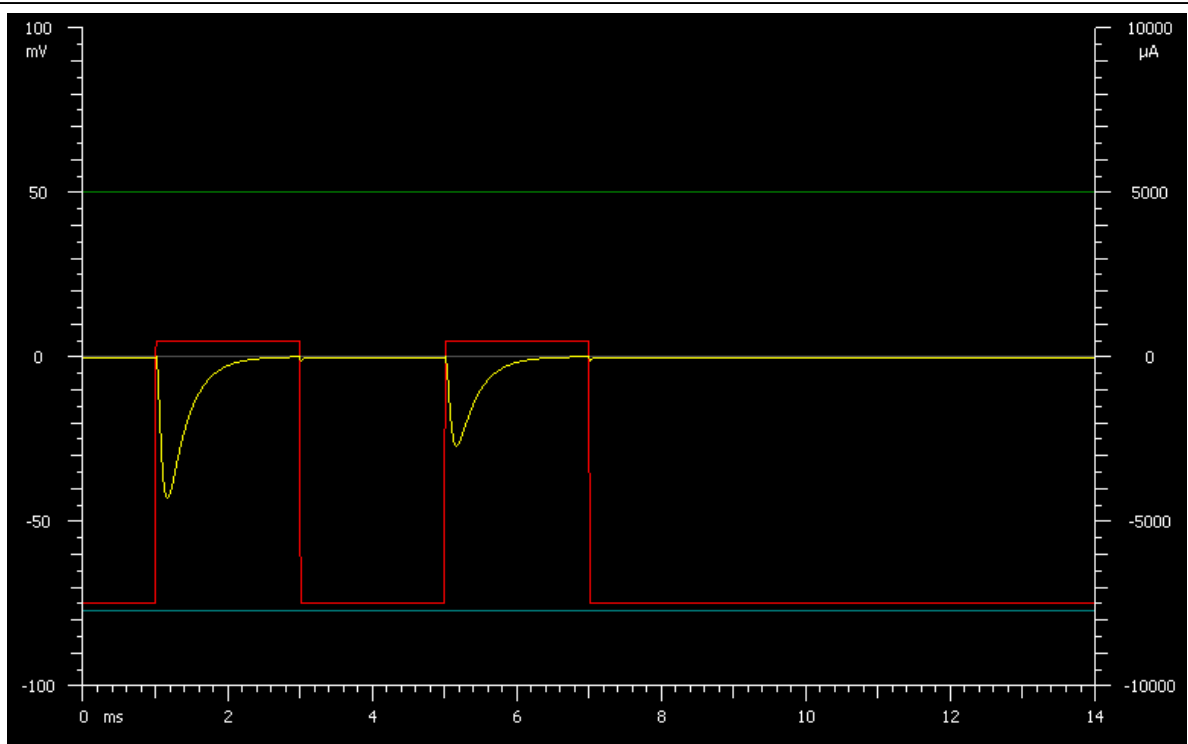
Figure 11. Na+ channel inactivation. The first depolarizing voltage step (red trace) evokes a large inward Na$^+$ current (yellow trace). Following a delay of 2 ms, a second depolarization evokes a smaller current, demonstrating that many Na$^+$ channels have not yet recovered from inactivation. TEA has been selected to block K$^+$ currents.
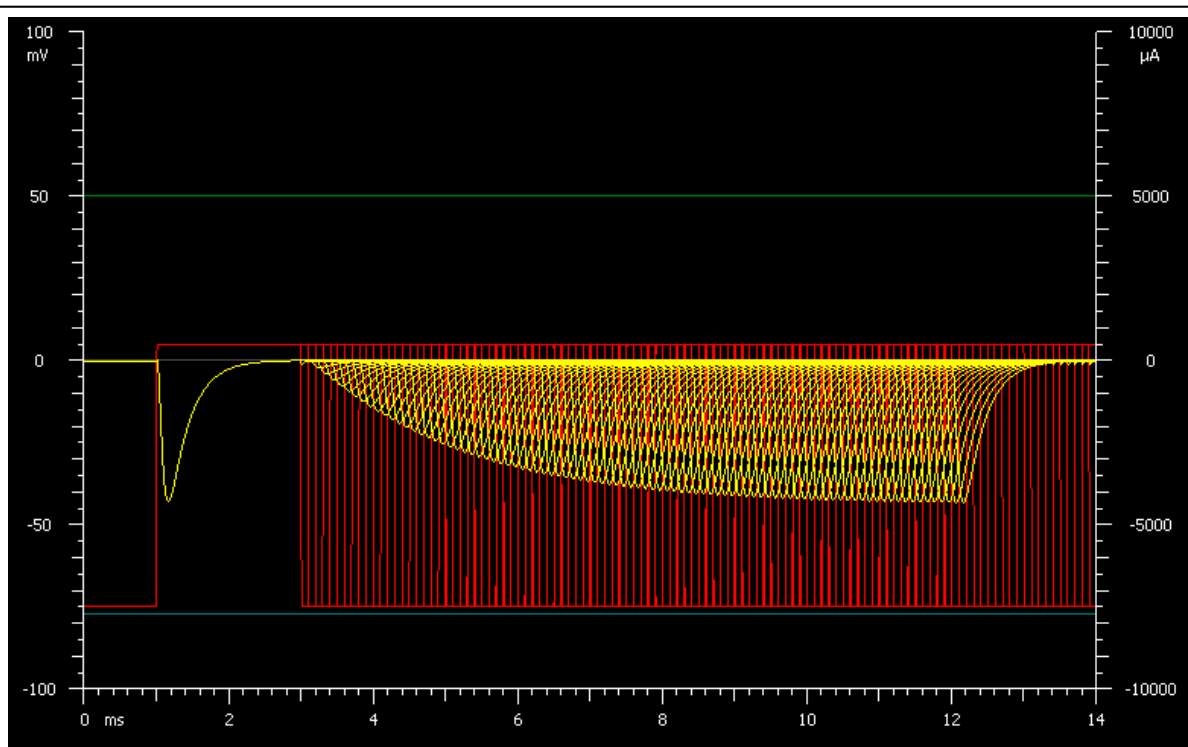

Figure 12. The time course of recovery of Na+ channels from inactivation is revealed in this family of voltage clamp traces. The two pulse experiment illustrated in Figure 11 is repeated for delays ranging from 0 to 9 ms. The peaks of the inward Na$^+$ current traces define the time course of recovery from inactivation.

6) Multiple sclerosis and the effect of temperature on Na$^+$ current. As discussed in Lesson 4, lowering the temperature reduces the threshold for action potential generation and alleviates the symptoms of multiple sclerosis in MS patients. The effect of temperature on Na$^+$ channel currents can be investigated with the voltage clamp.

Starting with the default parameter values, raise "Stimulus 1 Amplitude" to +5 mV. Block the K$^+$ channel current by selecting the TEA box. Lower the temperature from 18^0 to 10^0 C (Figure 13). What effect does this have on the time course of the Na$^+$ current? What effect would this have on the total influx of Na$^+$ into the axon? Would this have an effect on action potential generation and propagation?
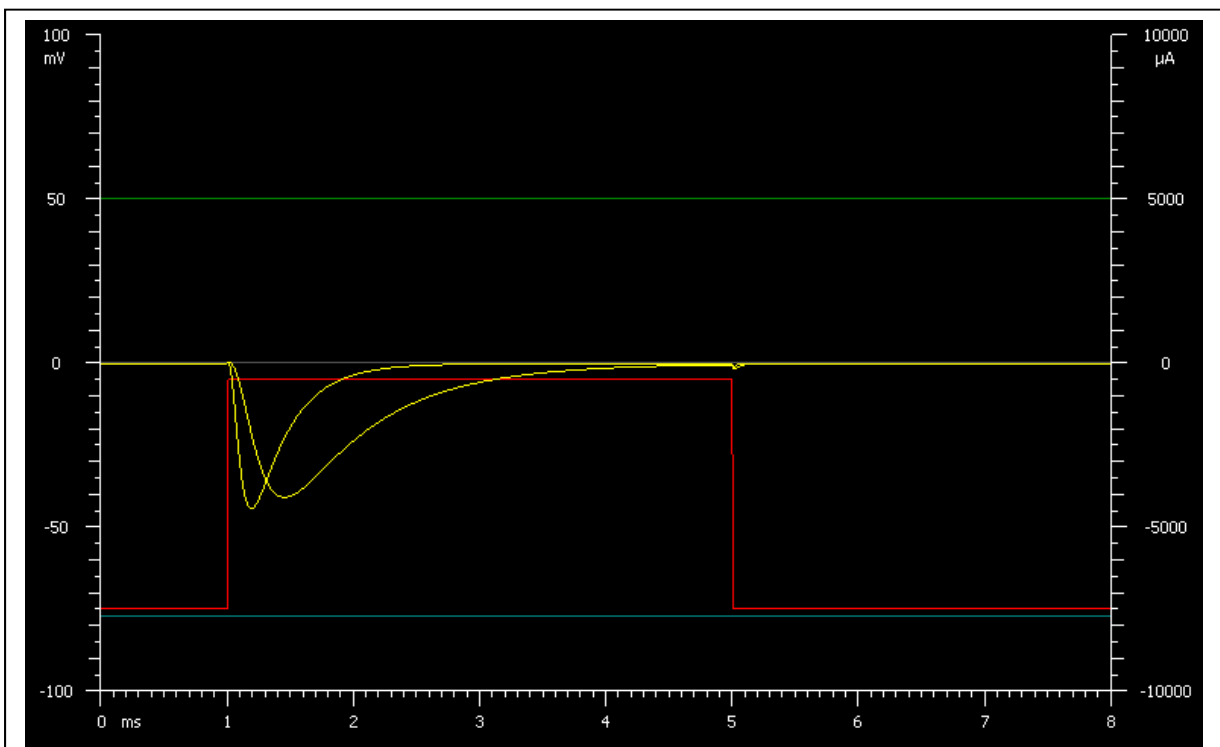

Figure 13. Effect of temperature on Na$^+$ channel current. Lowering the temperature from 18^0 to 10^0 C slows both activation and inactivation of Na$^+$ current. When Na$^+$ channel kinetics are slowed, there is a greater total Na$^+$ influx into the axon, making it easier to initiate and propagate action potentials.

LESSON 6, SYNAPTIC POTENTIAL AND CURRENT

Lesson 6 illustrates the synaptic voltages and currents generated at fast ionotropic synapses. The neuron in Lesson 6 is modeled by a leak conductance and by the opening of an ionotropic receptor conductance, simulating a synaptic response. A fast excitatory synapse is modeled by a receptor that is permeable to both Na^+ and K^+. The relative permeabilities of the two ions determine the reversal potential of the synapse. A fast inhibitory synapse is modeled by a Cl^- permeable receptor. The neuron in lesson 6 does not generate action potentials.

The equilibrium potentials for Na^+, K^+ and Cl^- in this model are +50, -77 and -75 mV, respectively. The resting membrane potential is determined by a leak conductance having a reversal potential of -65 mV. The synaptic conductance is modeled with rising and falling time constants of 0.1 and 1.0 ms.

The reversal potential of the synapse can be determined by varying the membrane potential of the neuron (yellow trace) and noting the potential at which the synaptic response reverses polarity. The membrane potential is varied by adjusting the "Holding Current". The currents flowing through the ionotropic receptor are shown to the right: Na^+ (green) and K^+ (blue), for the excitatory receptor and Cl^- (orange) for the inhibitory receptor.

Student exercises

1) Reversal potential of a fast excitatory synapse. Starting with the default parameter values, note that a depolarizing response (yellow trace) is generated. This represents an excitatory postsynaptic potential (EPSP) generated by the opening of an excitatory ionotropic receptor. Using the default values, the receptor has an Na^+ : K^+ permeability ratio of 1.2 :1. This is a good approximation of an AMPA glutamatergic receptor. The depolarization is generated by a large Na^+ influx (green trace) which is partially offset by a smaller K^+ efflux (blue trace).

Depolarize the cell by injecting a positive holding current into the cell. At what voltage does the EPSP reverse polarity? This potential is known as the "reversal potential". Note the magnitudes of the Na^+ and K^+ currents. The reversal potential can be seen clearly when a range of holding currents are plotted (select the "Holding Current" range option), producing a family of membrane potential traces (Figure 14). Plot the amplitude of the EPSPs as a function of membrane voltage. Measure the amplitude from the baseline to the peak of the synaptic response. What is the shape of the relation? Why does it have this shape?

Change the K^+ permeability from 1.0 to 0.5. Once again, plot the amplitude of the EPSPs as a function of membrane voltage. How does the EPSP reversal potential change? How does the Amplitude-Voltage plot change? Why do these changes occur?

Note that when the membrane potential is at the equilibrium potential for an ion, the current of that ion deviates from zero because the membrane potential changes during generation of the synaptic potential. The ion current does not remain at zero because the cell is not voltage-clamped.
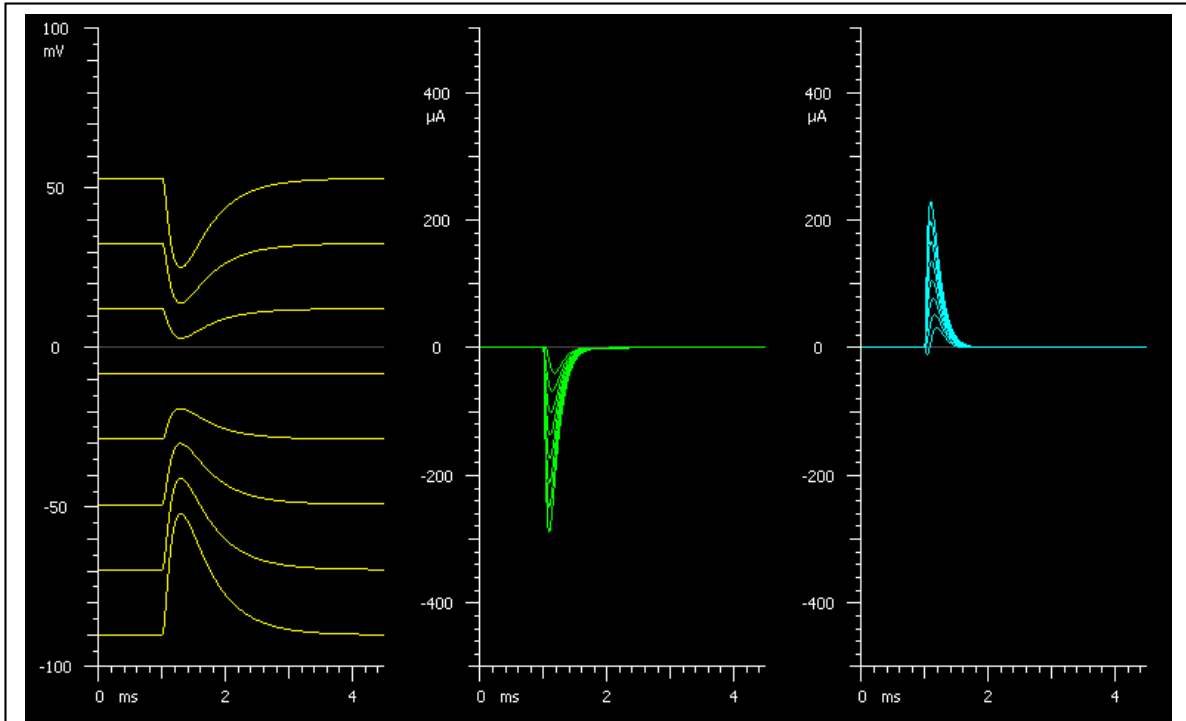

Figure 14. Reversal potential of an excitatory synaptic response. A family of membrane potential traces (yellow) is shown as the membrane potential is varied by injecting current into the cell. The synaptic potential changes from a depolarizing to a hyperpolarizing response as the membrane potential is raised. The membrane potential at which the synaptic response reverses polarity is known as the reversal potential and is determined by the relative permeability of the synaptic receptor to Na^+ and K^+. The Na^+ and K^+ currents flowing through the receptor are shown in the green and blue traces, respectively.

2) Reversal potential of a fast inhibitory receptor. Starting with the default parameter values, select the "Fast inhibitory synapse" from the "Synapse Type" window. Note that a hyperpolarizing response, the inhibitory postsynaptic potential (IPSP) is generated. This represents an inhibitory receptor, such as the $GABA_A$ receptor, which is permeable to Cl^-. What is the reversal potential of the IPSP? Why does it have this value?