Purpose:

To demonstrate voltage pulse protocols that are used to determine activation and inactivation characteristics of ion channels. The ionic currents in this model are representative of those in a CNS neuron.

General Information:

Program name = "**Vclamp expts.rt**" (Mac)
"**Vclamp expts.exe**" (Win)

Directions:

1) Double click on the icon to load the program.

2) Click on the arrow button or select "**run**" from the "operate" menu to begin execution. This model is NOT intended to run continuously throughout the exercise. Therefore, the parameters for the voltage pulse protocols and graph indicators must be adjusted BEFORE pressing the RUN button..

3) To reset all parameters to their default values, go to the "**operate**" menu and select "**reinitialize all to default**". The default settings are: V_{hold}=V_{cond}=-70; V_{test}=-90, Incr=10;Steps=17, T_{cond}=1; MaxT=20; Show Na; Cursors=auto; Mem trace=OFF; Norm=ON; Sequence=auto.

Problems:

1) Determination of the Current-Voltage Relationship.

a) I-V curve for the Na channel. Reset all variables. Set Normalize = OFF. Set Sequence=STEP. Click the RUN button and then click the "next trace" button (upper left) 17 times to see the individual voltage steps (notice each time how current changes with step potential). The black trace is the most recent trace, the white taces are the previous ones. The START arrow will turn off and the graph at the lower left will update when you finish. As you do each voltage step, notice the traces on the 4 graphs to the right representing the families of gNa, iNa, iTotal and voltage-step traces.With these settings, you generated 17 voltage steps, beginning from -90 mV and increasing to +70 mV in 10 mV increments (see bottom right graph). Since V_{cond} = V_{hold}, **this was a single pulse protocol to test how the channel conducts current over a wide range of voltages.**

Now set Sequence=auto and click RUN again. It will automatically step through the whole sequence of voltage steps and display the same results; this is the default setting.

The graph at the lower left shows the **summary I-V curve for iNa**. What is the magnitude of iNa at the peak ? ______ At what voltage does maximum inward Na current flow? ______. Estimate the reversal potential (E_{Na}) for iNa: _____. Estimate the voltage at which iNa begins to activate: ______.

b) I-V curve for the Ca channel. Use the same settings as in **1a** but set the control on the right of the iNa graph to show Ca current, set steps = 22, and click RUN.

The graph at the lower left now shows the summary I-V curve for iCa. What is the magnitude of iCa at the peak ? ______ At what voltage does maximum inward Ca current flow? ______. Estimate E_{Ca}: _______. Compare the voltage at which iCa begins to activate with that for iNa:_________________ (hint: for easier comparison, turn "trace memory =ON" after running iCa but before running iNa again).

c) I-V curve for the K channel. Use the same settings as in **1b** but set to show iK, then click RUN.

The graph at the lower left now shows the summary I-V curve for iK. What is the magnitude of iK at the peak ? ______ At what voltage does maximum K^+ current flow? ______. Estimate E_K : _______. Compare the voltage at which iK begins to activate with that for iNa: ____________________________ (use the trace memory feature).

d) Activation curve for the Na channel. Reset all variables. Set protocol = MULTIPLE CONDITIONING STEPS, Show iNa, T_{cond} =2 ms, V_{test}=-70 mV, steps = 18, T_{max} = 3 ms, set "Normalize = ON" and click RUN.

This **protocol demonstrates how Na^+ "tail" current is used to measure Na channel activation**. iNa is activated by progressively larger voltage steps but in the middle of each step the voltage is returned quickly back to V_{hold} while the Na^+ channel is still activated. (Therefore we are viewing an expanded time scale). The secondary inward current spike observed at this point in time represent the Na current that is still activated when the voltage pulse ends. The advantage of this technique over the single pulse protocol used in **1a** is that the driving force stays constant for each test.

From the Analysis graph, estimate the threshold voltage at which iNa is just barely activated: _______. Estimate the voltage at which iNa is 50% activated: _______ (this is the value more commonly used to compare channel activation).

e) Activation curve for the Ca channel. Set the graph to show iCa (keep other settings as in 1d), and then RUN.

From the Analysis graph, estimate the threshold voltage at which iCa is just barely activated: ______. Estimate the voltage at which iCa is 50% activated: ____ and explain why this should differ from that for iNa: ______________. Which channel would open first during an action potential?

f) Activation curve for the K channel. Set the graph to display K^+ current, set then RUN.

From the Analysis graph, estimate the threshold voltage at which iK is just barely activated: ______. Estimate the voltage at which iK is 50% activated: ______. Compare this to iNa ______. (use the trace memory). Which is steeper?_________Why? ____

2) Inactivation of voltage-gated channels.

a) The Na channel. Reset all variables. Set protocol = multiple conditioning steps, set Vhold=Vcond=-100,T_{cond} = 20 ms, V_{test} = 0 mV, Incr=10, steps = 10, MaxT=40, Normalize = ON, Sequence=STEP and then RUN.

This is a **double pulse protocol to test inactivation of the channels as a function of voltage**. The first pulse activates the channel, then allows enough time to pass so that inactivation is (nearly) complete. Then a constant second voltage step is delivered to test the degree of inactivation (i.e. how much current is available to be activated). The peaks of the second pulses are measured and normalized and plotted on the analysis graph. STEP through this procedure and observe carefully what is happening.

The settings above produce an Analysis graph showing the inactivation curve for the Na^+ channel. Notice that the slope of this curve is opposite to that of the activation curve in **1d**. The 50% level indicates the voltage at which the Na channel is 50% inactivated. Estimate this value: ______.

b) The Ca channel. Use the same settings as for **2a**, except: set the graph to Show Ca currents, set T_{cond} = 100 ms, T_{max} = 140 ms, Vhold=Vcond=-90, Sequence=Auto, then RUN.

The Analysis graph shows the inactivation curve for the Ca^{2+} channel. Estimate the voltage at which the Ca^{2+} channel is 50% inactivated: ______.

c) The K channel. Set to show K^+ current but leave the settings the same as for Ca. What happens? _______________________________ Why? ________________

3) Time course for removal of inactivation.

a) The Na channel. Reset all variables. Set protocol = multiple TIME steps, set T_{cond} = 20 ms, V_{cond} =V_{test} = 0 mV, Vhold=-90, steps = 12, T_{max} = 120 ms, Incr = 5, Normalize = ON and then RUN.

This protocol uses **conditioning and test pulses of the same magnitude, with the test pulses progressively delayed in time**. This allows the current to fully activate, then the degree of inactivation remaining (the test pulse) is measured by a second depolarizing pulse delayed in time. Note that iNa is inactivated quite soon (<32 ms) after the conditioning pulse, but completely reactivated only after ≈55 ms.

Estimate the time constant (tau ≈ the time required for recovery to 63% of max) for recovery of iNa inactivation: _____.

b) The Ca channel. Keep the same settings as in **3a**, with these changes: Show Ca, set V_{hold} = -50 mV, T_{cond} = 150 ms, incr = 20 ms, steps = 11, T_{max} = 400 ms, then RUN. Estimate tau: _____; speculate as to why this might be different than for iNa: ______________________________________.

c) The K channel. Hopefully, you've realized this K^+ channel shows no time dependent inactivation.

4) Miscellaneous experiments.

Experiment with any of the protocols above, using different values for Vtest or Vcond, a different number of pulses or increments and different display times (Tmax). For example:

How does changing Vcond affect the Na inactivation curve?

How does changing the number of steps affect the shape of the I-V curve?

Try to determine the "**window current**" for Na: run protocol 1D, set trace memory ON, but do NOT reset all variables, then run protocol 2A. To repeat this procedure for Ca: run 1E followed by 2B.

If the traces or the analysis graph look strange, you may not have selected the appropriate protocol --- start with the settings listed in one of the experiments above and then alter the parameters. You may also try to manually position the cursors[1] after setting cursors = MAN (note the cursor changes will not alter the measurements until the program is re-RUN.

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1. There are two sets of cursors in this graph (black and purple) which may be dragged horizontally using the mouse. With "cursors" set to "AUTO", both sets of cursors are positioned automatically. The black cursors are positioned in a time window to measure the peaks in the iNa trace; the purple cursors are used to read the voltages of the conditioning steps (in some protocols). Sometimes it will appear as if only one cursor is displayed - in actuality, the two cursors are overlaying each other. The cursor windows determine the measurements reflected in the analyzed data graph displayed at the lower left. For example, after exercise **1a**, set "cursors" to "MAN", move the left black cursor to 8 ms and re-run the program while watching the I-V plot (lower left). The shape of the curve will not change but the magnitude of the currents at each voltage step will, due to the fact that the program measured the largest iNa values after the iNa trace had peaked.