Computer Lab Worksheet - CNS Neuron Voltage-Clamp

DAVIS
MSCI 602
1997

Purpose:

To demonstrate voltage clamp experiment on a CNS neuron containing Na and Ca channels.

General Information:

Program name = "**CNS vclamp.rt**"
Location = Any of the Power Macintosh computers in the MSL

Directions:

1) Double click on the "**CNS vclamp.rt**" icon to load the program.

2) Click on the ARROW button or select "**run**" from the "operate" menu to begin execution. You may **let the program continue to run** throughout the exercise.

3) Any of the parameters (e.g. the stimulator or ionic concentrations) can be altered "on the fly" to test their effects on the currents.

4) To reset all parameters to their default values, go to the "**operate**" menu and select "**reinitialize all to default**".

Problems:

1) Characteristics of voltage-gated sodium, calcium and potassium currents.

a) Start the program. The default values show the currents elicited following a rapid voltage step from -70 to -20 mV. Compare and contrast the direction and **time course** of the Na^+, K^+, Ca^{2+} currents: ____.
Explain why iNa returns to zero within 10 ms while iK remains elevated: ________________________
____.
Why is the time course faster than for iCa? ____.
Now turn "Trace Memory" ON, then set Vstep to +10 mV. This allows a direct comparison of the traces obtained at -20 mV (white lines) with those at +10 mV (colored lines). How are the iNa, iCa and iK traces at these two voltages similar ?____. How are they different?____.

b) Reset all variables so that V_{step} = -20 mV. Turn "Trace Memory" ON and set V_{step} = +50 mV. Explain why:
gNa is now larger but iNa is smaller: ____
____.
iCa is now larger: ____
____.
gK is now larger: ____.
iK is now larger:____.

c) Now increase V_{step} in 1 mV increments from +50 to +80 mV while observing iNa. Describe what happens to Na^+ current: ____.
At what value of V_{step} does iNa change direction? _____ (= reversal potential). You should have been able to calculate this number in advance; how? _________________________. Set Vstep = +20 mV and alter the reversal potential for Na+ using the Na_{out} control. What is the $[Na]_{out}$ which makes E_{rev} for Na^+= +20 mV? ____.
Repeat the above procedure for iCa (if you can). E_{rev} for iCa = _____ .What is the $[Ca]_{out}$ which makes E_{rev} for Ca^{++}= +20 mV? ____.

d) Reset all variables. To determine the **threshold** for activation of iNa, set V_{step} to -70 mV and increase it in 1 mV increments until Na^+ current just begins to activate. What is the potential at which this happens? _______. Repeat this procedure while observing iK (or gK) and determine the activation threshold for K^+ current: ______. Repeat this procedure while observing iCa (or gCa) and determine the activation threshold for Ca^{++} current: ______. Why would this be different from Na^+? _____________
_____________________.

2) Effects of changing ion concentrations

a) Reset all variables. Set Na_{in} to 140 mM. What will be the new value of the Na^+ equilibrium potential?____. How could this be verified experimentally? (try it) ______________________________. Do gNa, gK or iK change with changes in Nai? ___ Why or why not? ___________________________
____
Do iNa or iTot change with changes in Nai? ___ Why or why not? _________________________
____.
How do changes in internal or external Ca^{++} affect iNa? ____.
(In a real axon preparation, increases in external Ca^{++} would shift the activation threshold for Na^+ channels).
How do changes in internal or Ca^{++} affect iCa? _______________________________________ (the changes occur in the same direction in a real cell but result from a different process).

3) Effects of selective blockade.

a) Reset all variables. Now look only at the iTot curve. Note the complex shape of iTot as the membrane is progressively depolarized. Sketch below the family of iTot curves one would obtain if V_{step} were progressively increased from -30 mV to +90 mV in 30 mV steps.

This should look similar to diagrams shown in most textbooks. Why does iTot have a slight "hump" at 1 ms when V_{step} = +100mV? ____________________
____.

b) Set V_{step} = 0 mV and turn Trace Memory ON. Lower Na_{out} from 140 to 14 mM and note the effect on iTot: ____.
This will simulate an experiment in which external Na^+ is substituted with an impermeable ion .

A second way to eliminate inward current is to block Na^+ channels with tetrodotoxin (TTX). Set Na_{out} back to 140 mM and increase [TTX] to "hi". Record the effect : _________________________________.

A third way to eliminate Na^+ current is to clamp the membrane at E_{Na} so that no Na^+ current flows (you already did this in #**1c**).

c) Reset all variables. Set Vstep to 0 mV and turn Trace Memory ON. Now lower K_{in} from 140 to 5 mM; this simulates an experiment in which K^+ inside the axon is substituted with a less permeable ion such as Cs^+. Note the effect on iTot: ____.

A second way to eliminate outward current is to block K^+ channels with tetraethylammonium (TEA). Set K_{in} back to 140 mM and increase [TEA] to "hi". Record the effect : ______________________________.

A third way to eliminate K^+ current is to clamp the membrane at E_K so that no K^+ current flows . Why is this a useless experiment ? ____.

d) Reset all variables. Set Vstep to 0 mV and turn Trace Memory ON. Eliminate inward Ca current using the dihydropyridine channel blocker nifedipine. Record the effect on iTot: __________________.
____.
Keep [nifedipine] hi and increase [TTX]; explain the change in iTot: ____________________________.