

MarkoLAB v 2.0

Dynamic 3D representation of
Markovian States

Workbook for MarkoLAB v 2.0

Robson Rodrigues, PhD

Daniel G. Goroso, PhD

Center of Technology Center

José L. Puglisi, PhD

Donald M. Bers, PhD

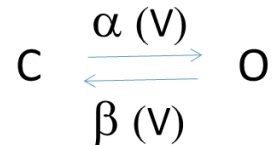
Department of Pharmacology



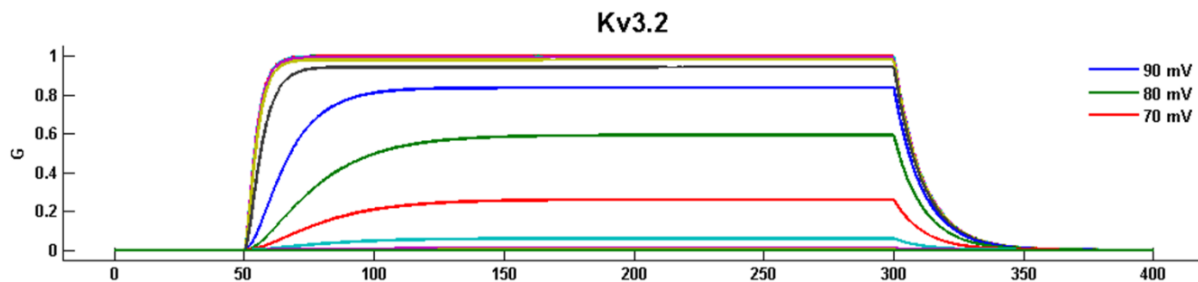
MarkoLAB Exercises on Ionic Channels' Dynamics

Objective: To understand the stochastic nature of the ion channel's dynamics.

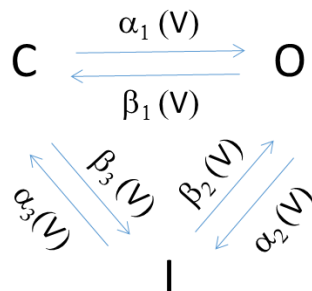
Every ion channel can be represented by a combination of 3 states: C (Closed) O (Open) and I (Inactive).



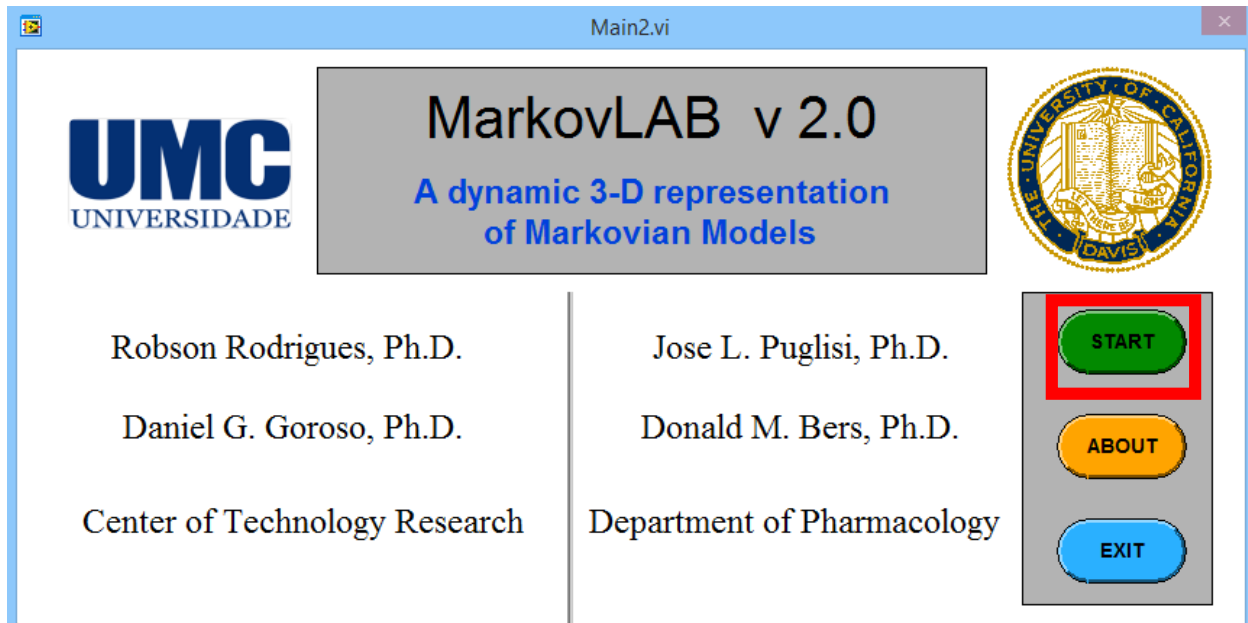
The simplest channel is illustrated below. It consists of a closed and an open state. Alpha and Beta are the rate constants and, in this case, they depend on voltage. These rate constants can also depend on neurotransmitter, ions, mechanical stress. The process of going from close to open is called **activation**. (Going from close to open is **deactivation**). Potassium channel Kv3.2 can be represented with this diagram.



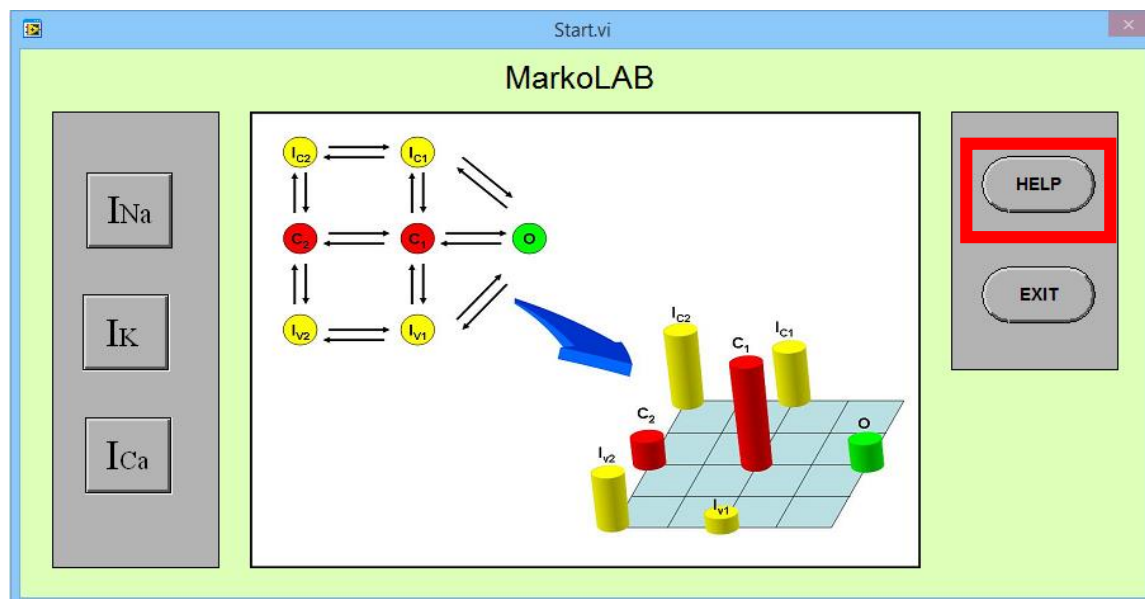
Another channels can be described with a third state Inactive.



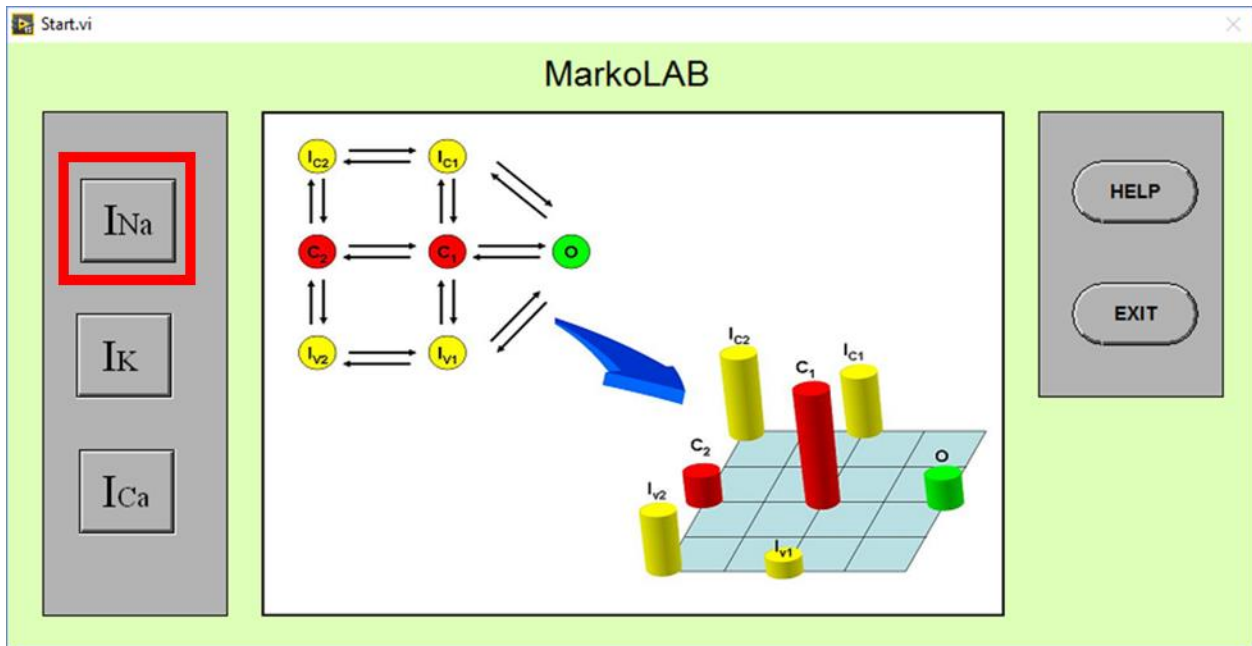
1. MarkoLAB®: Introduction



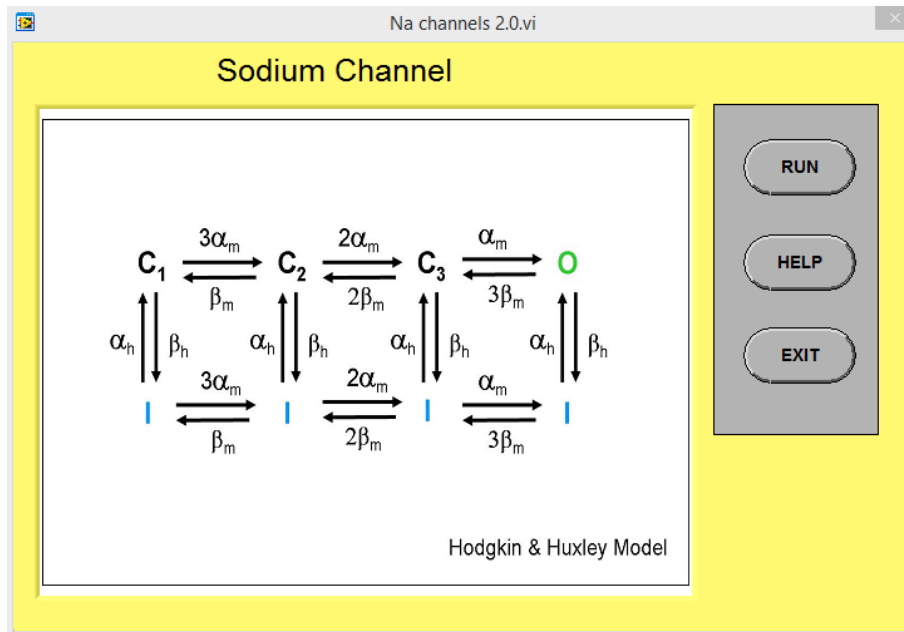
- Begin by opening the program and click on START. The page labeled Start.vi



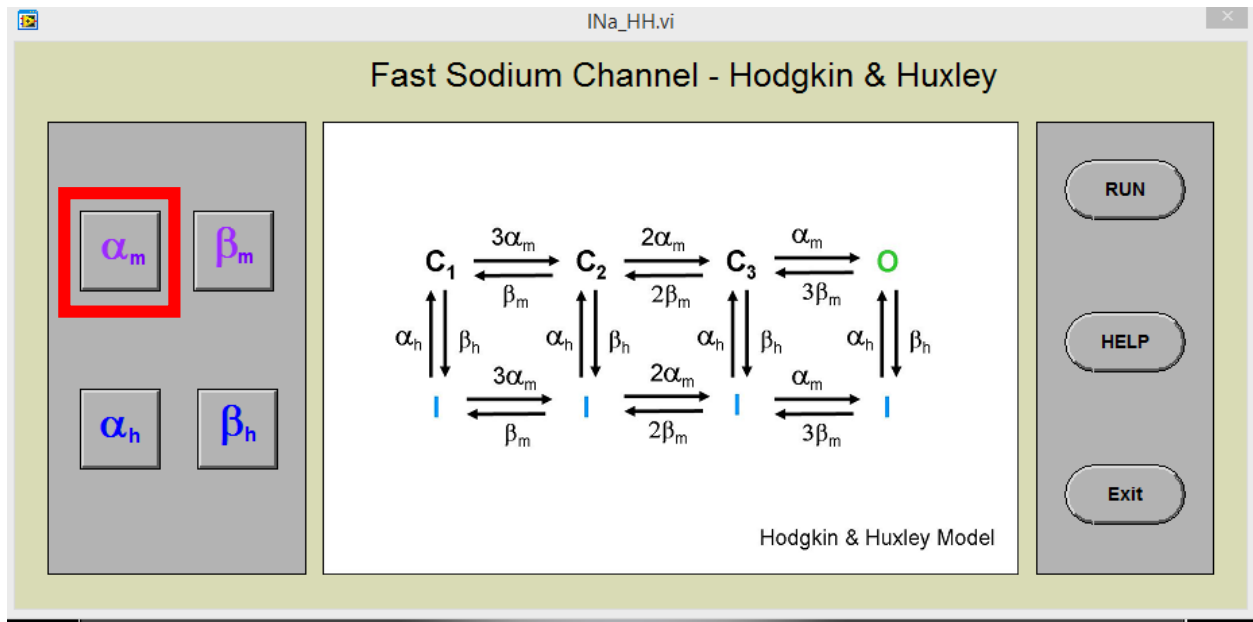
- The Help button will walk you through the menu. Click on the HELP and learn the various functions especially how to measure with the cursors.
- To start MarkoLAB select the type of ion channel that you want to simulate.
- For example, the sodium channel (INa). Press RUN.



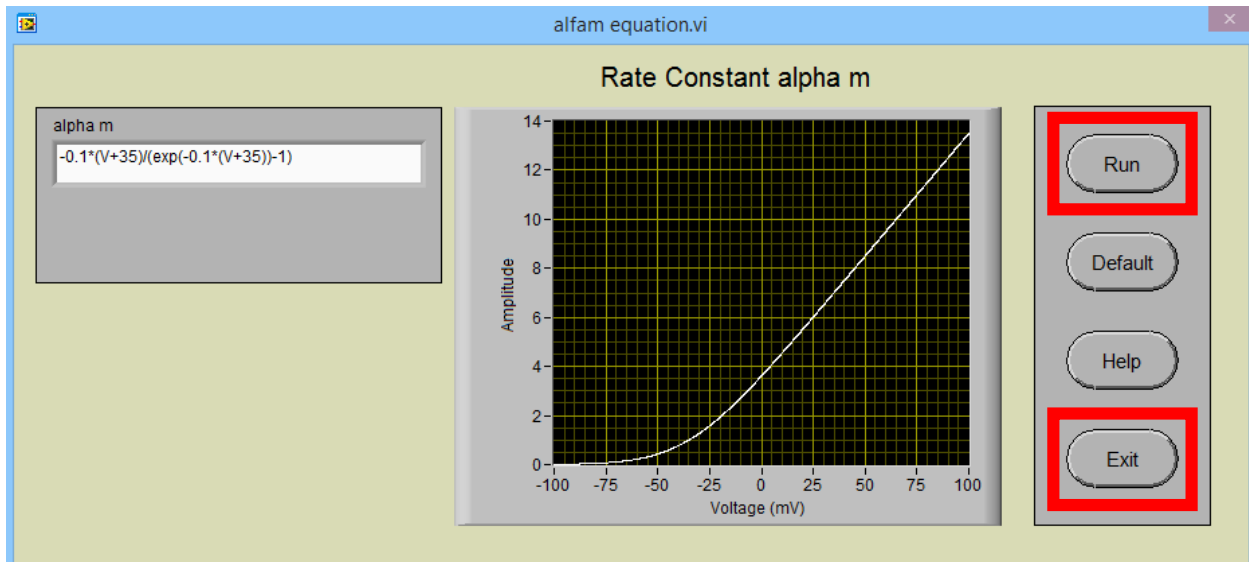
- On the next screen you can view the state diagram that channel.



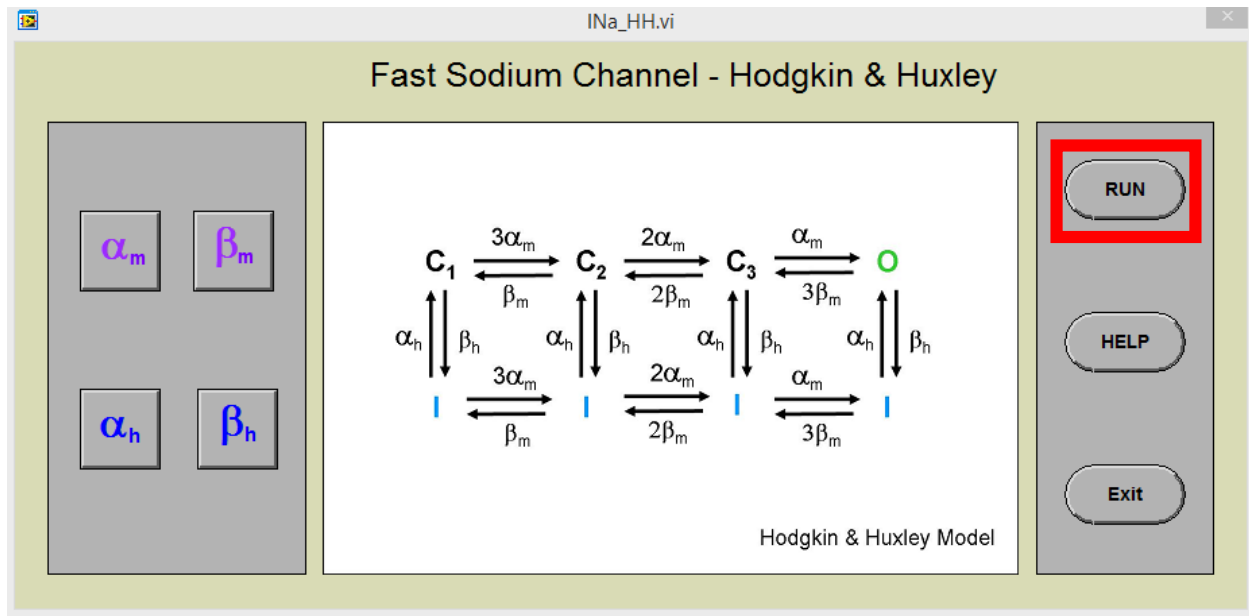
- Press RUN
- Clicking on one of the buttons α_m , β_m , α_h ou β_h , you can access parameter data between these states.



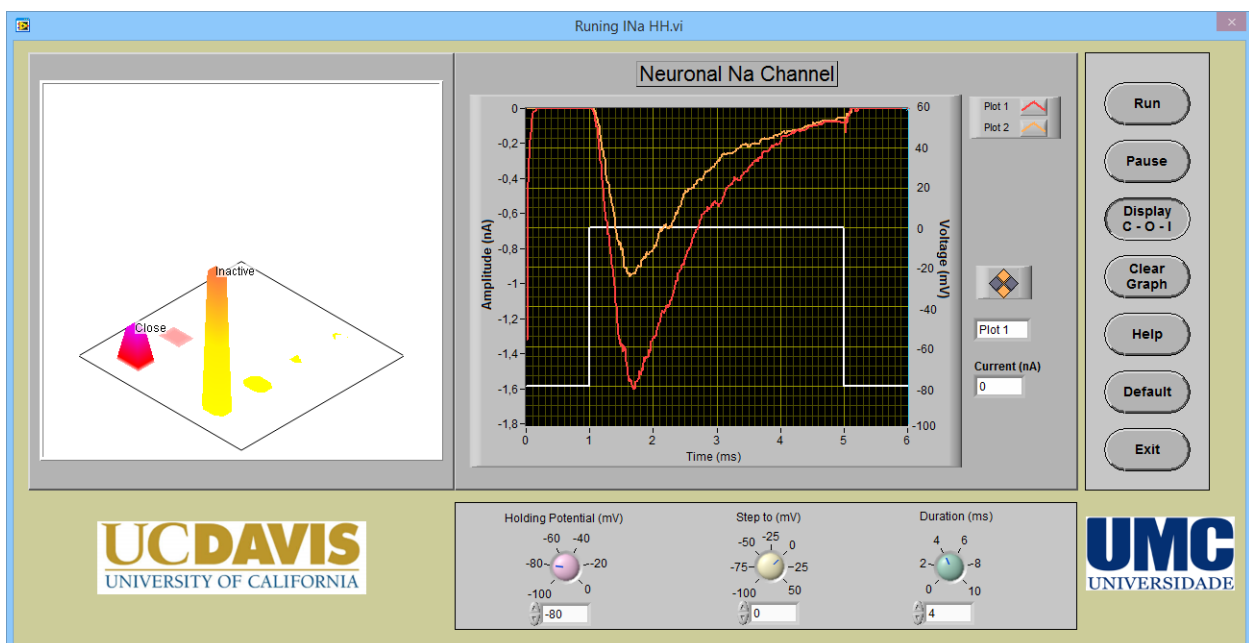
- Screen for parameter



- Press RUN to view the graph or exit to return to the previous screen.



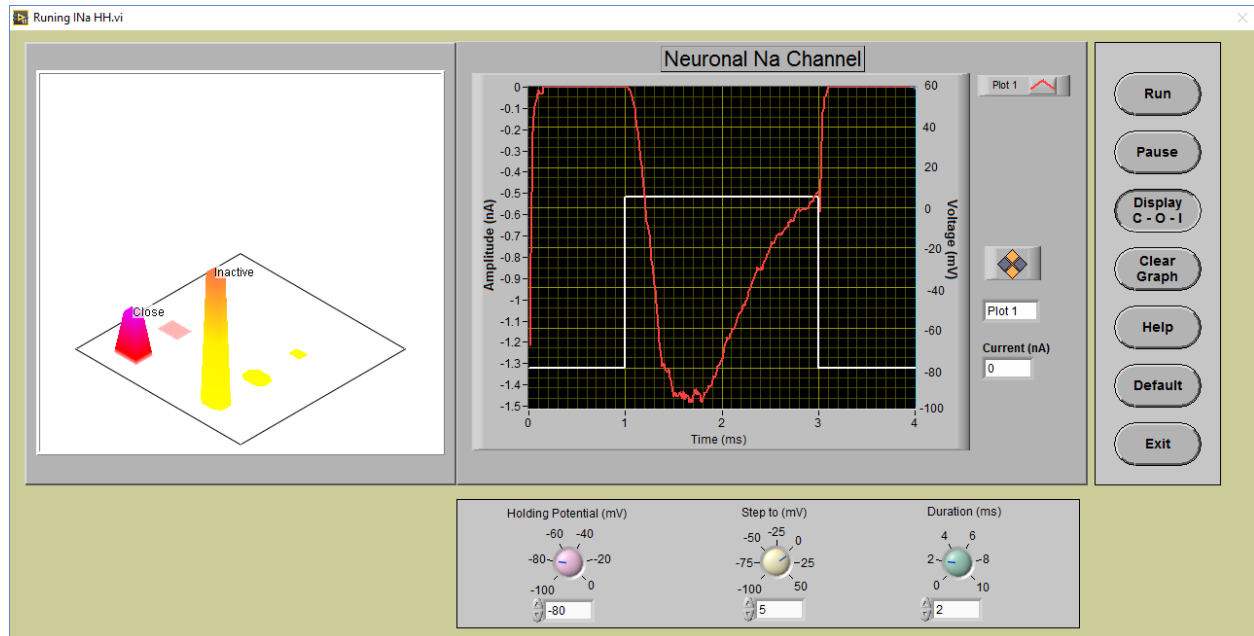
- Click on **RUN** and access the screen for simulations on the current and states of the selected ion channel.



We will begin by exploring the effects of holding voltage on channel inactivation.

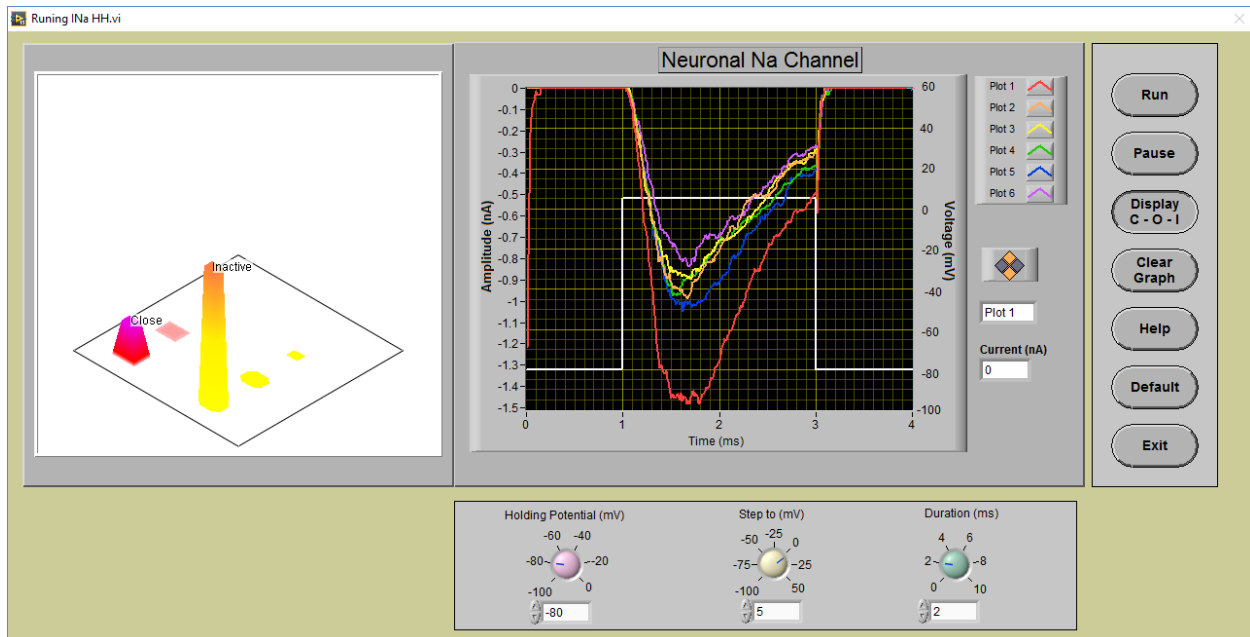
2. Effect of Holding Voltage on Channel Inactivation

In this simulation we keep constant the step voltage while the holding Voltage is changed.

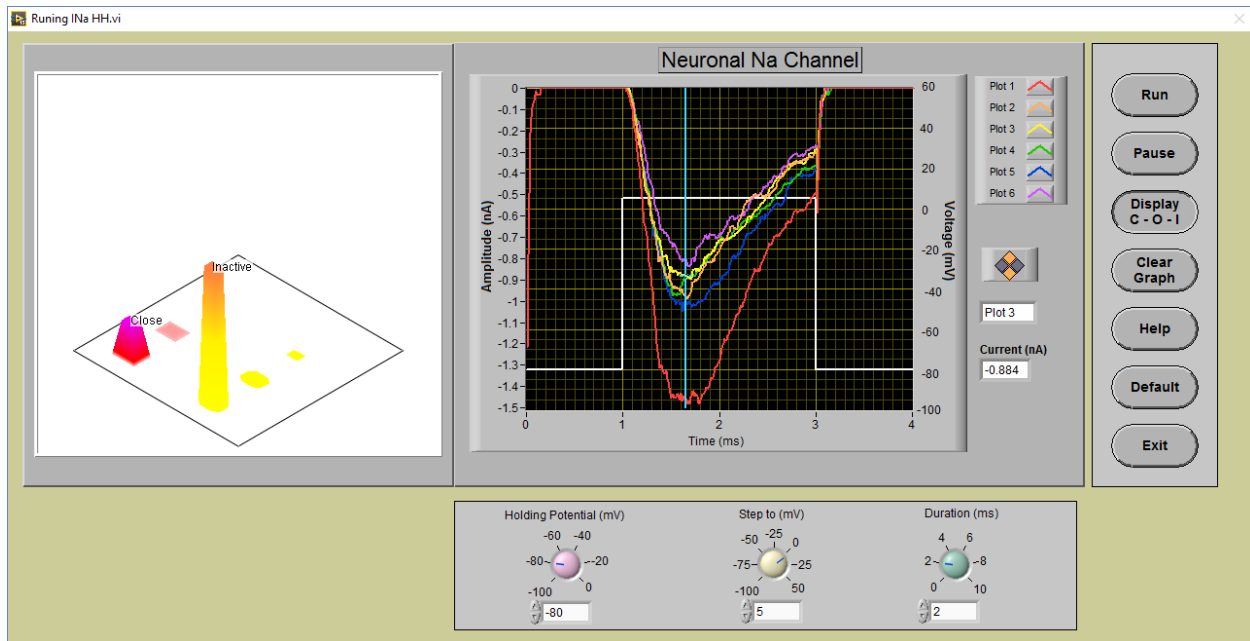


Set the holding Voltage at -80 mV, Step to at 5 mV and Duration at 2 ms.

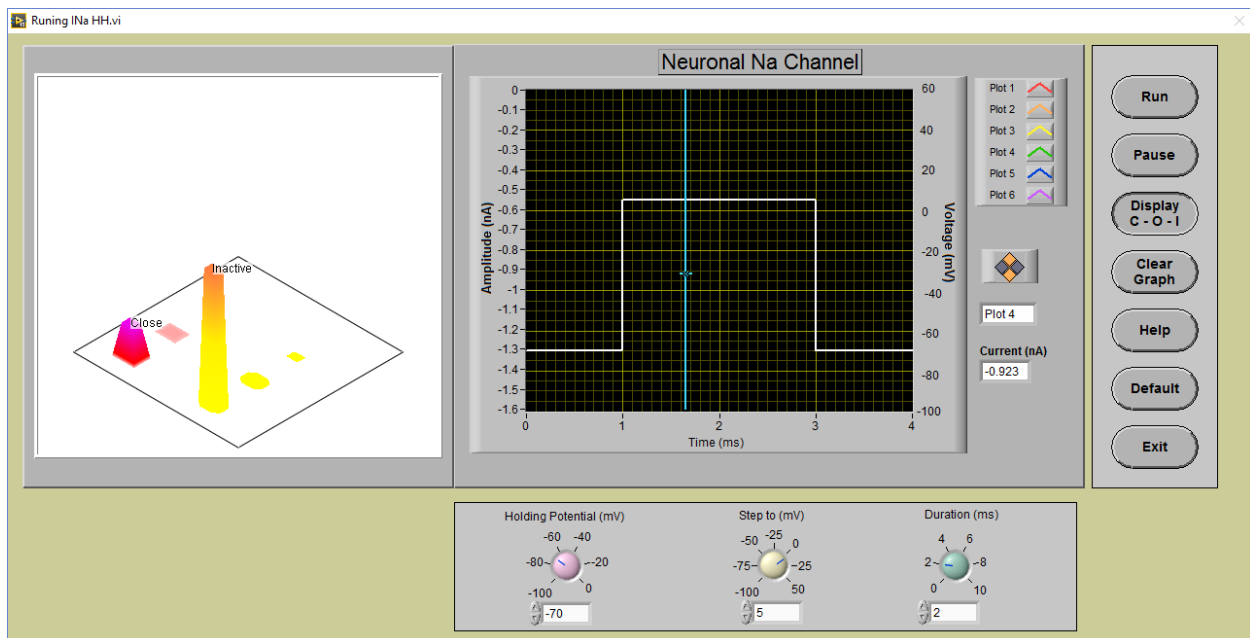
Press RUN.



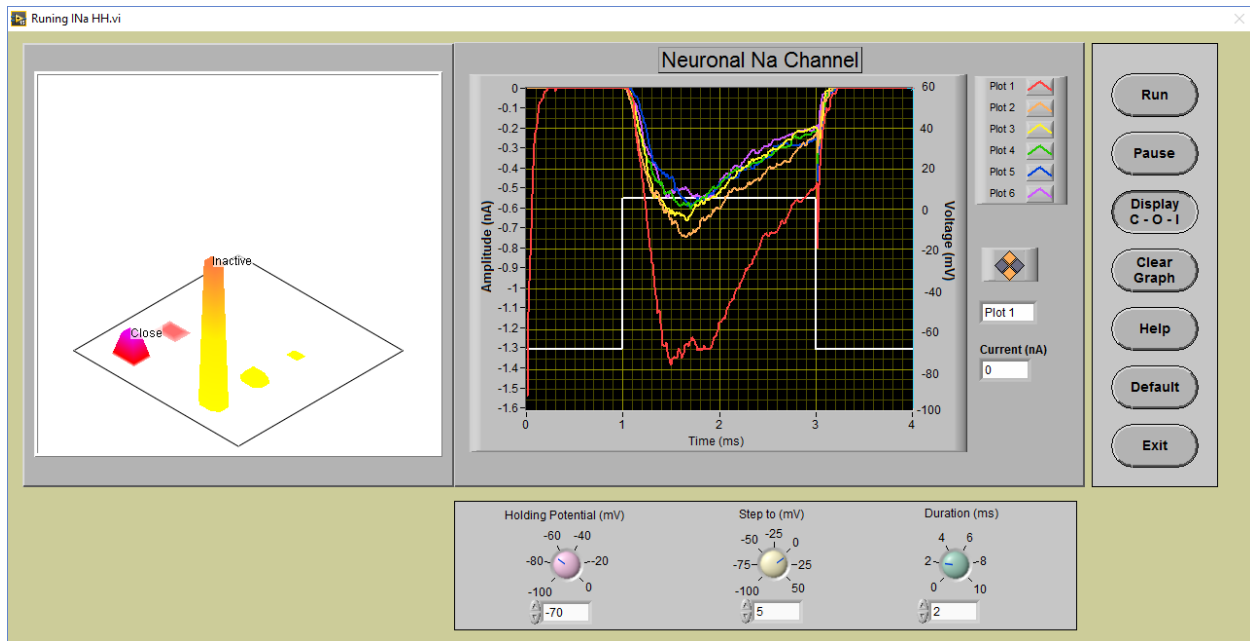
Repeat the procedure 5 more times. You will have 6 plots on the screen



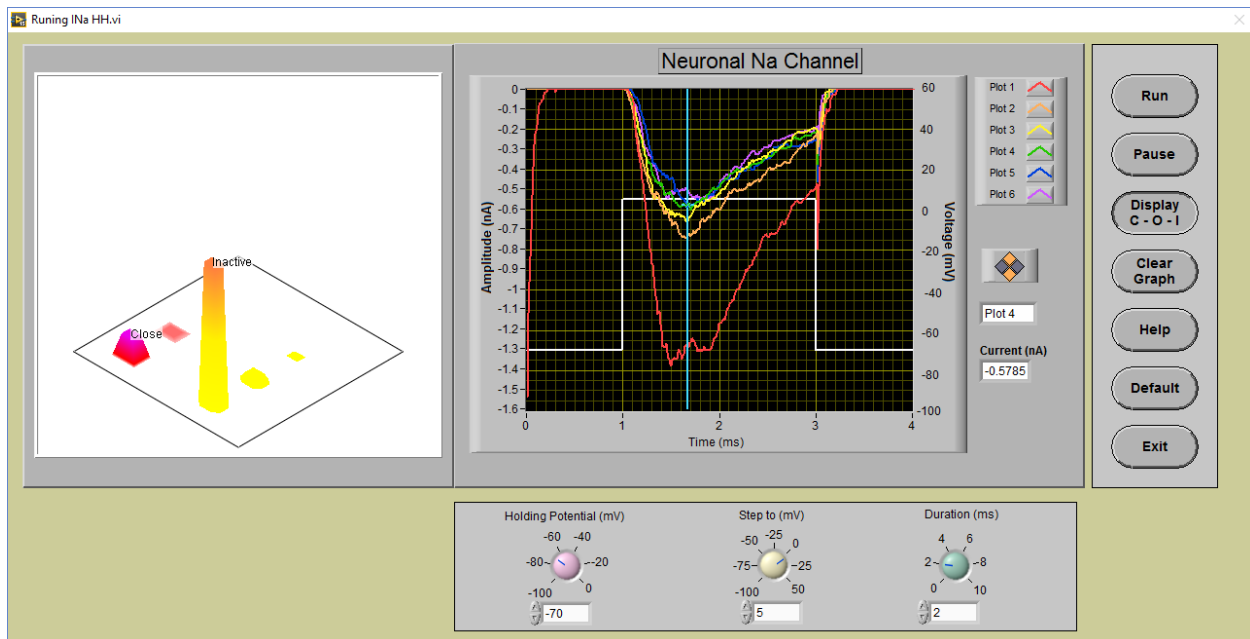
Ignore the first trace (red) and using the cursor measure the average of the 5 other traces. In this particular case is - 0.884 nA. (Note: You can jump from trace to trace by dragging the cursor)



Now set the Holding Potential at -70 mV and keep the other parameters the same (Step to 5 mV, duration 2 ms)



Repeat the same procedure than before. Run a first curve and then repeat 5 more times



Likewise, discard the first trace and measure the average peak of the 5 other traces.

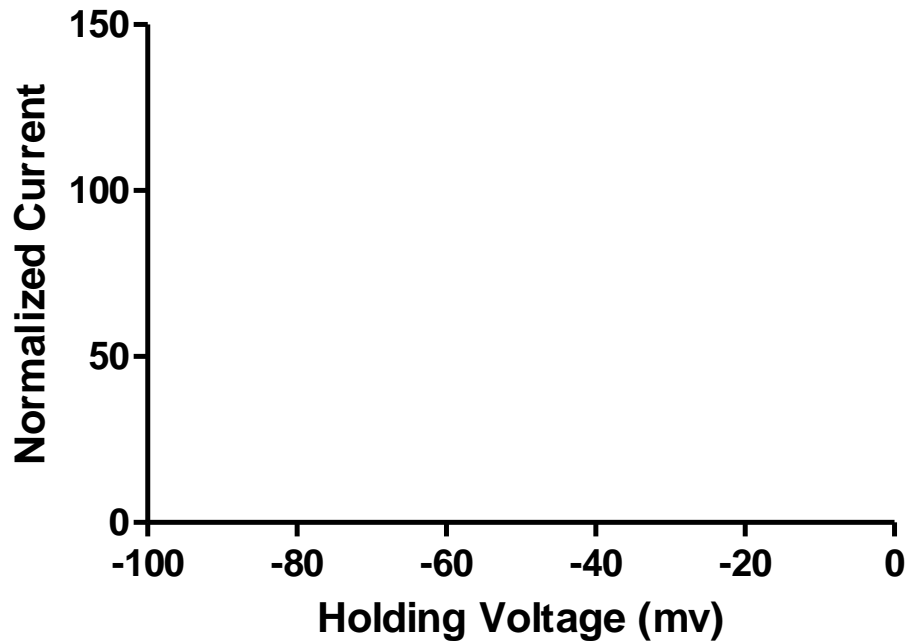
In this example the value is -0.57 nA

Repeat the procedure and complete the following table

Holding Potential (mV)	Current (nA)	Normalized Current (I/I _{max})
-90		
-80		
-70		
-60		
-50		
-40		
-30		
-20		
-10		
0		

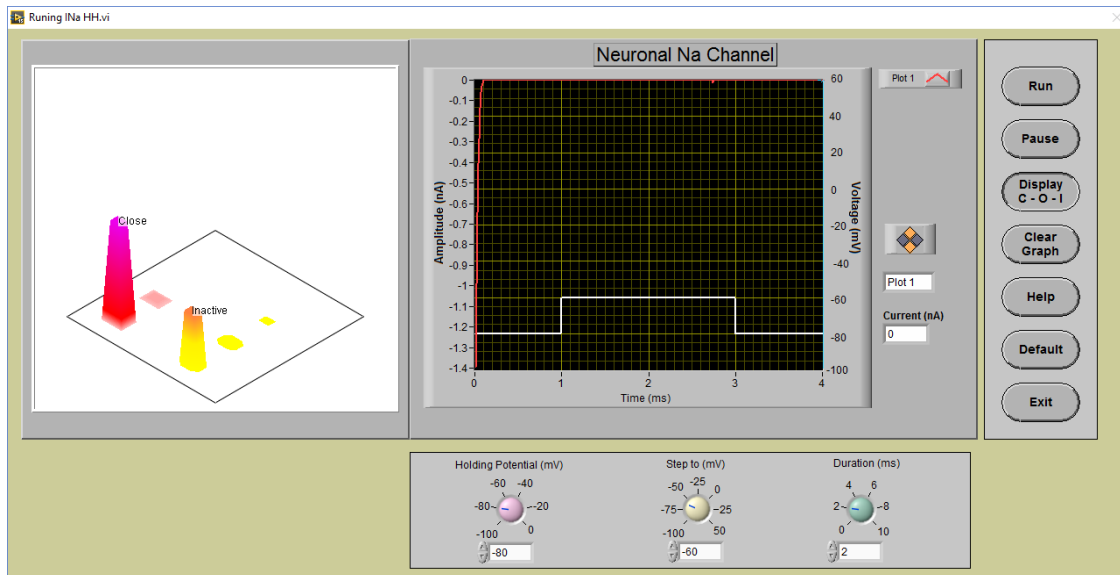
Construct the graph below

Effect of Holding Voltage

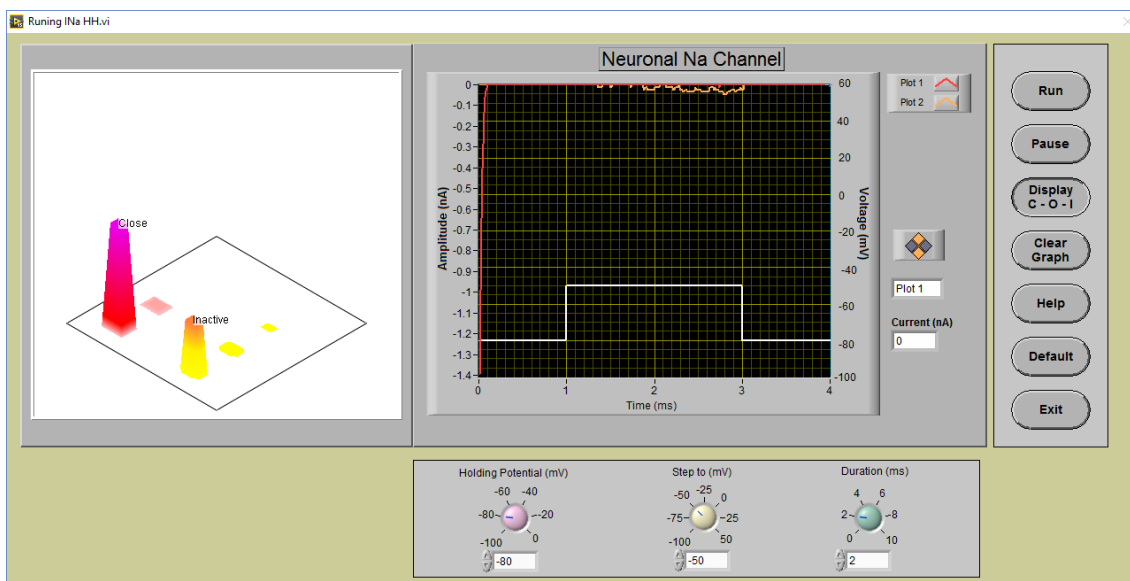


3. Activation Voltage and Current amplitude

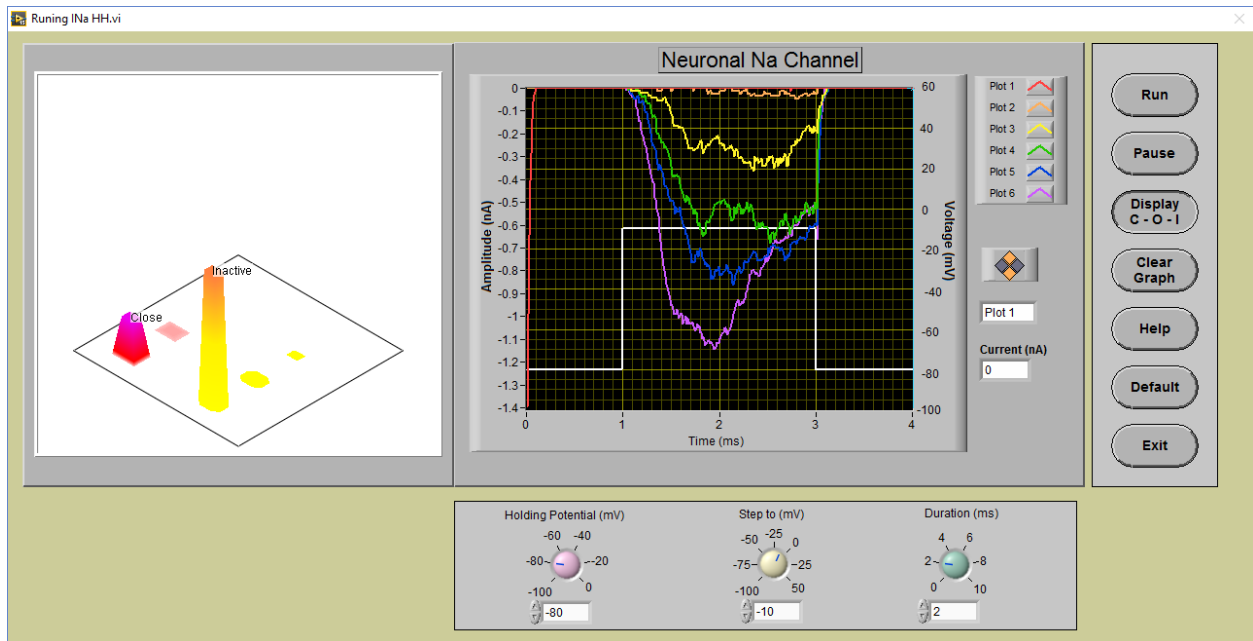
In this simulation we keep the holding voltage constant and change the step voltage (or depolarizing voltage).



Set the holding voltage at -80 mV, the step voltage to -60 mV and the duration at 2 ms. Press **RUN**, almost no current will pass through the channel.



Repeat the experiment with step to voltage at -50 mV. There is no need to equilibrate the system as in the previous exercise.

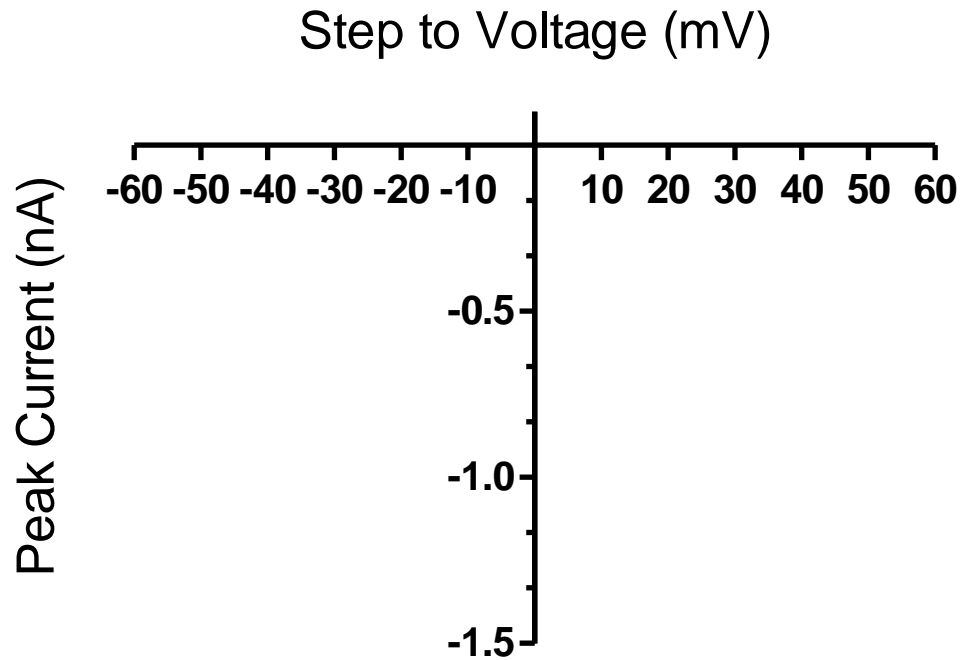


Run the simulations for -60, -50, -40, -20, -10 mV and measure the peak current and time of occurrence. Press "Clear Graph" and repeat for 0, 10, 20, 30, 40, 50, 60 mV.

Fill up the following table

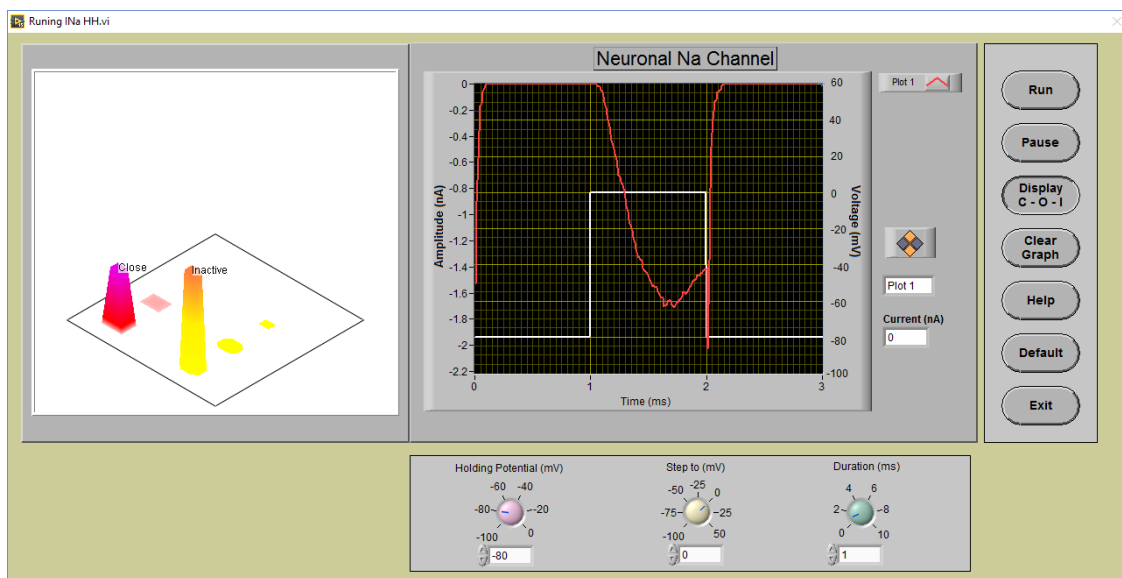
Step to Voltage (mV)	Peak Current (nA)	The peak time of occurrence
-60		
-50		
-40		
-30		
-20		
-10		
0		
10		
20		
30		
40		
50		
60		

Construct the graph below

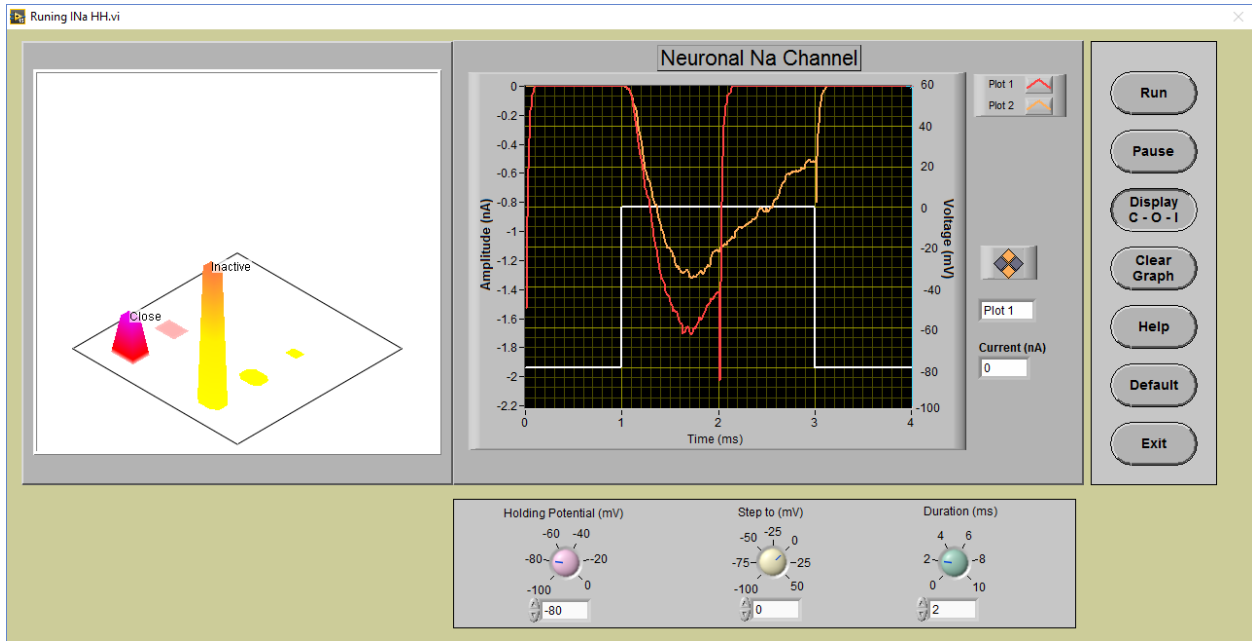


4. Time dependent Inactivation

We will explore the time dependent inactivation

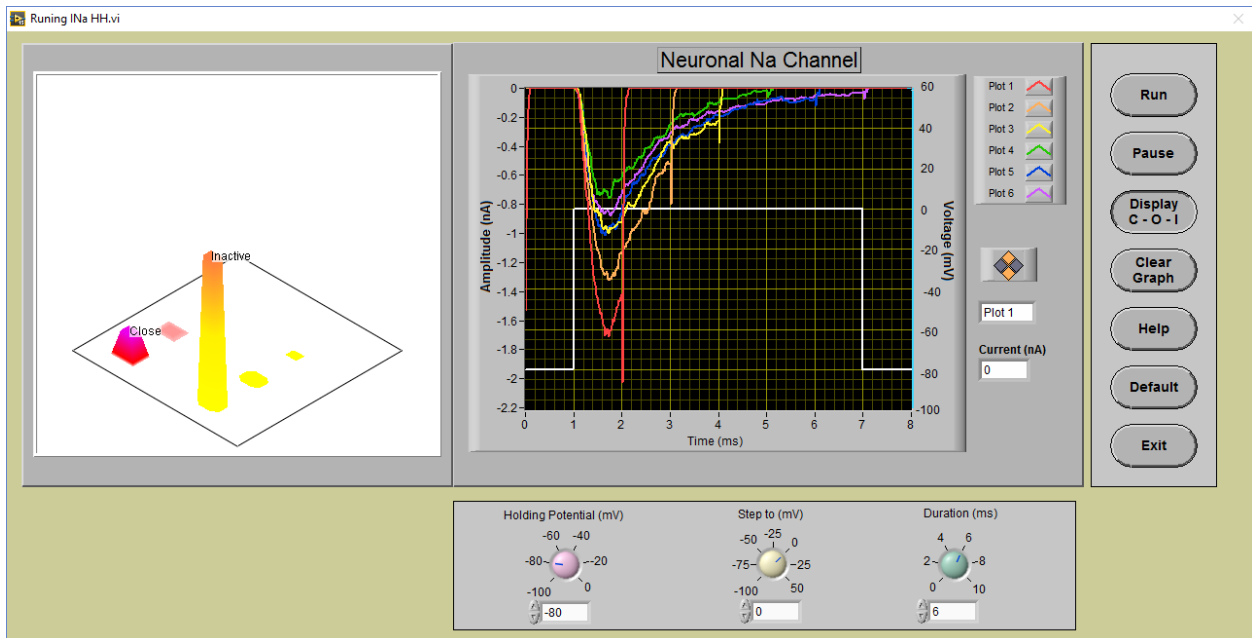


Clear the graph, set the holding potential to -80 mV and Step to 0 mV. We will vary the step duration. Start with 1 ms.



Then increase to 2, 3, 4, 5 and 6 ms.

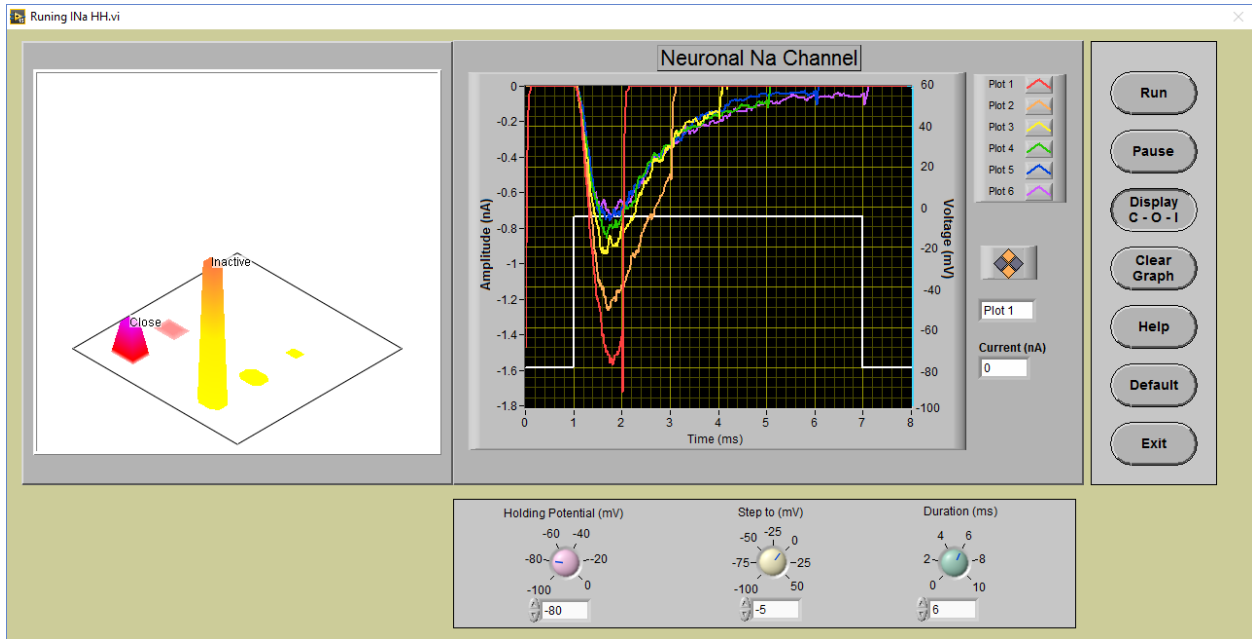
The Peak current when the step to pulse ends is called the ***“Tail Current”***.



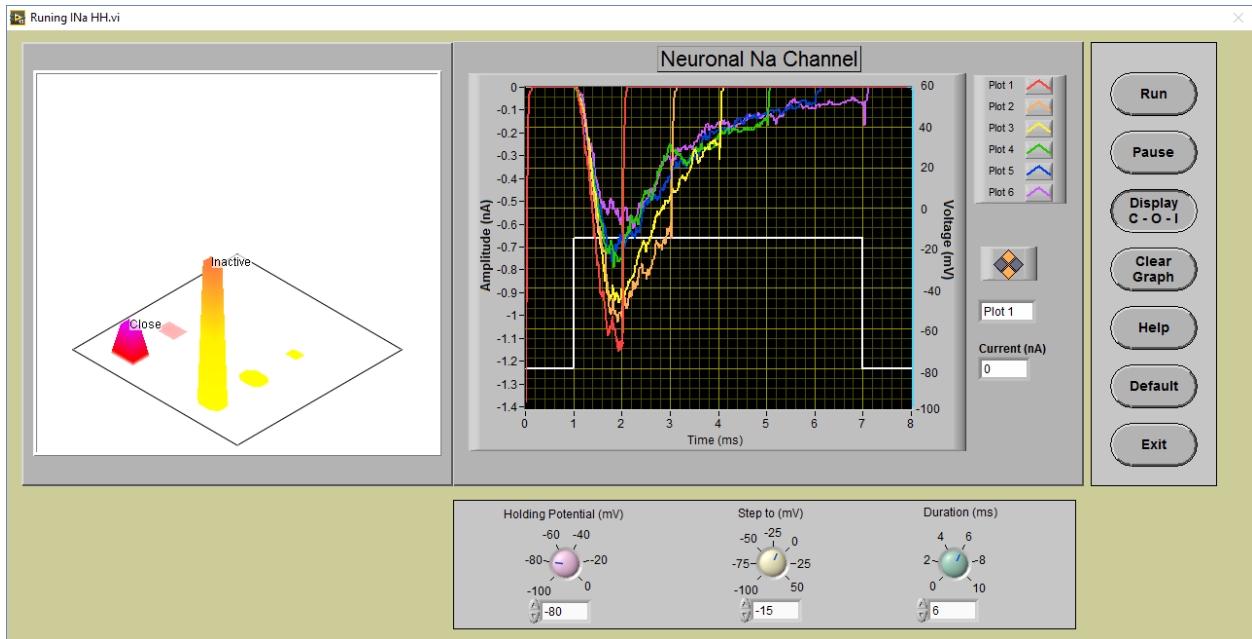
You will obtain a screen similar to the one above.

Measure the peak values of the tail current (white circles).

Why the peak tail current decreases?

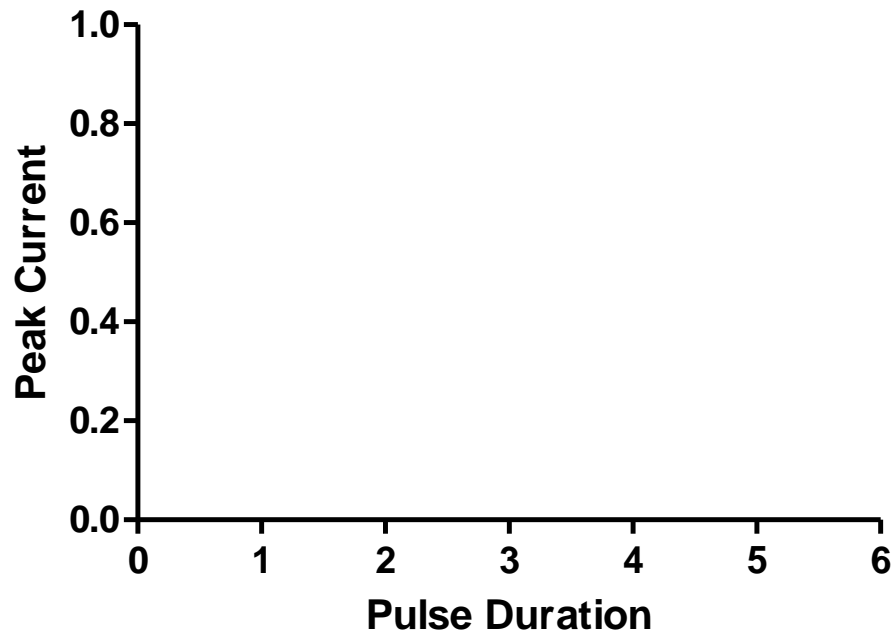


Repeat the procedure using an step voltage of -5 mV.



Similarly repeat the experiment with Step to voltage of -15 mV.

Construct the graph below



Try to explain your results by looking at the formula values α_m , α_h , β_m and β_h .

