Project: Simulation of Ion Channel Gating **Lab: Joseph Greenstein**

Introduction: Many of the properties of excitable tissues such as nerves and muscles arise from the properties of individual cells, each of which may be considered a miniature, but still rather complex, electrical, chemical, and (in the case of muscle) mechanical system. The electrical excitation of a single nerve or muscle cell produces an action potential (AP), the characteristic depolarization of the cell membrane that occurs as a result of the passage of electrical currents across the cell membrane. A variety of ion channels and membrane transporters are responsible for controlling membrane currents. Computer simulation techniques can be used to understand how single ion channels behave and how a population of channels gives rise to a whole-cell ionic membrane current.

The L-type Ca^{2+} Channel: The L-type Ca^{2+} current (I_{CaL}) is present in many types of cells including neurons and cardiac myocytes. L-type Ca^{2+} channels (LCCs) are voltage gated, and open upon membrane depolarization. A simplified Markov state model for an LCC is shown in Figure 1.

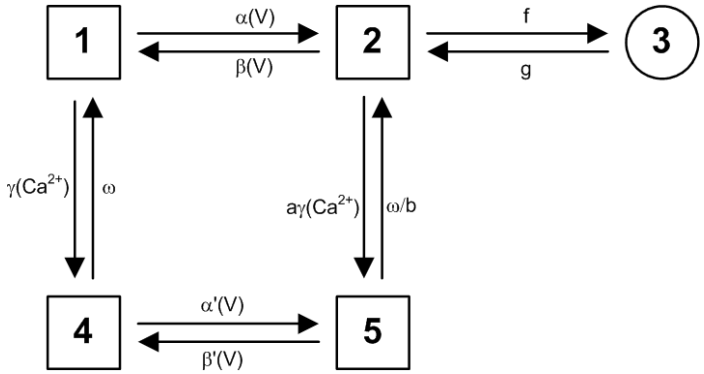


Figure 1: LCC Markov Model

States 1 and 2 are closed states, state 3 is the open state, and states 4 and 5 are inactivated states. State 3 is the only conducting state, all others are non-conducting. Depolarization of the cell membrane promotes transitions of the channel from state 1 to state 2, after which some channels can open (transition to state 3). States 4 and 5 represent Ca^{2+}-dependent inactivation states (i.e. the downward transition rates depend on $[Ca^{2+}]$).

A single LCC can occupy only one of the five possible states at any moment in time. The process of channel gating is stochastic, i.e. the time at which a channel transitions from one state to another is random. The distribution of waiting times between transitions follows an exponential distribution (see below). In addition, since a cell typically contains a large population of LCCs, we can learn a great deal by understanding how the state occupancy probabilities evolve in time. For example, if the occupancy probability of state 3 (i.e. the open probability) is 0.10 at time = 20 ms, then in a large population of LCCs we would expect to find about 10% of the channels open at 20 ms.

Simulation of Single Channel Gating for an LCC: By definition, the dwell time in any state of a Markov process is exponentially distributed with rate λ equal to the sum of all rates exiting that

state. Note that the expected dwell time (or mean dwell time) is 1/λ. To simulate single channel activity it is necessary to generate exponentially distributed random variates. There may be a function which can do this directly in your programming language. An alternative is to calculate these from uniform random variates as described below.

Let *U* be a uniformly distributed random variable on the interval (0,1). Define *X* as:

$$X = -\frac{1}{\lambda}\ln U \tag{1}$$

Then

$$\Pr\{X \le x\} = F(x) = 1 - e^{-\lambda x} \tag{2}$$

Therefore equation (1) can be used to transform a uniformly distributed random variable into an exponentially distributed random variable with parameter λ.

Note that a Markov process can be shown to be memoryless, i.e. the future evolution of the process depends only on its current state, but not the prior history of state occupancies. This property simplifies how a channel's response to voltage clamp is simulated. At the instant that membrane potential changes from one value to a new value (e.g. from holding potential to the test potential), the simulation must be restarted with a new dwell time chosen based on the currently occupied state and the newly calculated transition rates.

Further Exploration: More detail on how to simulate exponentially distributed random variables can be found in Ch 10 of "A First Course in Probability" by Sheldon Ross. A description of the use of Markov models for ion channel simulation can be found in Ch 18 of "Ionic Channels of Excitable Membranes" by Bertil Hille.

Project Statement: Here, we will focus on simulating and understanding the process of activation and deactivation using a simplified model shown in Figure 2. This model consists only of states 1 and 2 of the LCC in Figure 1.

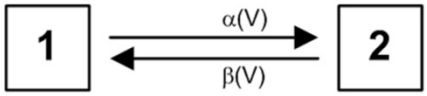


Figure 2: Simple LCC activation/deactivation model.

The forward and reverse transition rates (in ms^{-1}) are defined by equations (3) and (4), respectively, as follows:

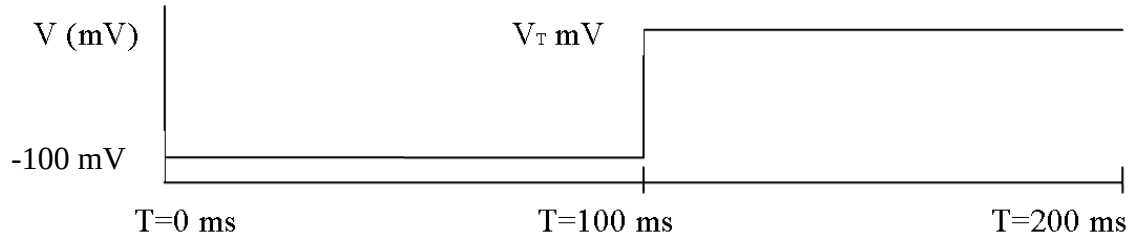
$$\alpha = 0.835e^{0.027(V-35)} \tag{3}$$

$$\beta = 0.033e^{-0.093(V-35)} \tag{4}$$

where *V* is membrane voltage (in mV).

1. Simulate a single L-type Ca^{2+} channel
   a. Write a program to perform a long duration simulation (20 seconds) of a single channel using the two-state model with membrane potential held at -20 mV. Assume the channel is initially in state 1. Observe when the channel transitions between state 1 and 2. Calculate the mean occupancy time for state 2 (i.e. average duration of a visit to state 2) and compare to the theoretical mean dwell time. Repeat for membrane potentials of 5 mV and 40 mV. Show a graph of your simulation output for each potential.
   NOTE: The output of your code should take on the form of two columns of data, the first will consist of transition times, and the second will indicate which state the channel entered at that time (in this case it can only be either 1 or 2).

   b. Write a program to simulate random state transitions of the 2-state LCC activation model in response to the following protocol:



   Assume the channel is initially in state 1. Hold the membrane potential at -100 mV for 100 ms and then step to the test potential V_T = 5 mV for an additional 100 ms. Show an example of how a single channel behaves in response to this protocol. Repeat for V_T = 40 mV. Describe how you formulate your model and simulation and be specific about how you handle the change in membrane potential at 100 ms. Explain what you observe in your simulation results.

2. Simulate a population of channels
   a. Write a program (building from that of Problem 1) to simulate a population of 50 channels using the protocol from Problem 1b with V_T = 5 mV (see NOTE below). Plot an estimate of the state 2 occupancy probability (as fraction of channels in state 2). Do you get identical results when you repeat this simulation?
   NOTE: In order to add single channel data together to obtain state occupancy probabilities for a population of channels, you will need output that is synchronized in time. You will need to write a function that converts the output (in the form generated in problem 1) into a time series. You will want to convert your data into a format with two columns. The first column will be uniformly spaced time points (use an increment of 0.5 ms), and the second column will indicate the state of the channel at the corresponding time points. In this way, you will be able to combine data from multiple simulations to determine what fraction of the population is in state 1 and state 2 at each point in time.
   b. Repeat problem 2a with a population of 500 channels. How does the output differ from that of problem 2a?

Challenge: The occupancy probability of the states in the model of Figure 2 can be described by the following equations:

$$\frac{dp_2}{dt} = \alpha p_1 - \beta p_2 \quad (5)$$

$$p_1 + p_2 = 1 \quad (6)$$

where p_i is the probability of occupancy of state i. Write a program to solve equation 5, which is an ordinary differential equation, for the same protocol described in problem 1b with V_T = 5 mV. Compare the state 2 occupancy probability obtained with those simulated in problem 2 by plotting both on the same graph. Do your answers agree (they should if they are correct)?

Notes:

- For Problems 1 and 2, it makes more sense to plot your results as stair-step plots:
  -
  - In R, use the type="s" option
  - In MATLAB, use the stairs(...) function
- The challenge problem is required. It might even be helpful to do the challenge problem before you do Problem 2, so that you can compare your results and check whether your implementation of Problem 2 is correct.
- For the challenge problem, please plot your results for the occupancy probability of state 2 on the same graphs as in Problem 2 (indicating which line is which).
- For Problem 2, when you add single channel data together to get the occupancy probabilities, you have two options:
-
  1. Convert the single-channel data into a time series by rounding the time points to the nearest 0.5 ms, as stated in part 2a.
  2. Combine and sort the original single-channel time points to get a master list of when any channel in the population changes state.
- Use whichever method is easiest for you, but be careful when counting channels--this part can be tricky.
- Your pdf write-up should include complete but concise answers to all of the questions asked in the problems, as well as all of the output/graphs.
- Attach all of the scripts used to generate data, graphs, etc. in original text format (.py, .R, .m files)