PHYSIOLOGY MODELING LAB
Membrane Potential and Stochastic Ion Channels

Organize your code and answers clearly in three .Rmd files: 1) GHK assignment, 2) Ion channel, and 3) Connecting GHK with a single ion channel behavior. Enter all answers to boldface questions as comments in the code.

This is an individual assignment, but you are allowed to work together in groups and discuss coding and answers. That said, you are responsible for all of the material in this laboratory assignment. DO NOT COPY from anyone that you work with. You are NOT allowed to share code. You need to write the code and answer the questions yourself. Try the coding yourself first before seeking help.

Continue working on the two files that you have already generated in the pre-lab coding homework assignment. Rename the files to indicate that this is the lab assignment and be sure to include your name in the file name as follows: lastname_firstname_labday.Rmd. Also, type your full name as the first comment in your .Rmd files. Upload three .Rmd and three knitted files to Canvas.

Due: In one week, before the start of lab during week 3

If you have any questions, please do not hesitate to ask. Best of luck!

Part One: Goldman-Hodgkin-Katz Equation—First .Rmd file

Changes in Permeability

Action Potential Peak

During the peak of an action potential, membrane permeability to Na^+ becomes much greater than it was at rest. We will examine how the membrane potential predicted by GHK changes when Na^+ permeability is increased.

First, change Na^+ permeability from 1 to 100. Run your GHK-equation code.

8. How did the membrane potential change? Is the membrane potential more positive (depolarized) or is it more negative (hyperpolarized) compared to the resting potential? Briefly discuss the biology—what is happening? How is this related to the sodium ion permeability?

After hyperpolarization

Depolarization during the late part of an action potential leads to a decrease in Na^+ permeability and an increase in K^+ permeability. This leads to an afterhyperpolarization, which causes the membrane potential to become temporarily hyperpolarized relative to the resting potential.

Change Na^+ permeability back to its original value and increase K^+ permeability to 100. Run your GHK-equation code.

9. How does this afterhyperpolarization potential compare to the resting membrane potential? Is the membrane potential more positive (depolarized) or is it more negative (hyperpolarized)? Briefly discuss the biology—what is happening? Can you relate this to the behavior of the sodium and potassium ions, and, in particular, to the Nernst potential of potassium?

Range of K+ permeability
Produce a range of values for K^+ permeability to assess what will happen to the membrane potential as K^+ permeability is increased (for example: 10, 20, 30, …, 1000). Hint: the *seq()* function is useful for this. Run your GHK-equation code.

Graph membrane potential vs. K^+ permeability for the various values of K^+ permeability (include units, axes labels, and a title).

10. What do you notice about how the membrane potential changes as K^+ permeability is increased? Does the membrane potential have a constant rate of change, or does it seem to approach a particular value?

11. Given that the Nernst potential for K^+ under these conditions is -98.523 mV, can you explain your observation from #10? Do so in a few sentences.

Neuromuscular Junction
In vertebrates, motor neurons release acetylcholine (ACh) onto muscle fibers, causing the muscle fiber to contract. An initial step in this process is the binding of ACh to an ion channel embedded in the plasma membrane of the muscle fiber -- the acetylcholine receptor (nAChR). ACh binding causes this receptor (ion channel) to open. Once open, Na^+ and K^+ ions pass through the nAChR equally well. However, the nAChR excludes anions such as Cl^-.

You've already used the GHK equation to show that increased Na^+ permeability depolarizes the plasma membrane. And you've shown that increased K^+ permeability hyperpolarizes the plasma membrane. You will now use the GHK equation to see what happens when Na^+ and K^+ permeabilities are both increased at the same time. Assign Na^+ permeability to 100 and K^+ permeability to 81, keeping the Cl^- permeability at the original value. Run your R code and solve for the membrane potential.

12. How did your membrane potential change from previous runs? Is the membrane potential more positive (depolarized) or more negative (hyperpolarized)? If increasing K^+ permeability alone is hyperpolarization, why is it depolarizing when K^+ and Na^+ permeabilities are both increased at the same time? (Hint: Nernst potential K^+=-98.523 mV, Na^+=59.271 mV). Explain your answer in a few sentences.

Changes in Ion Concentration
In this part, you will use the GHK equation to show how the membrane potential depends on the external K^+ ion concentration. You will create a vector for external K^+ ion concentration and see how the membrane potential is affected for each K^+ concentration (each new K^+ concentration will have a membrane potential). Please make sure all the permeabilities are returned to their original values. Create a vector for the external K^+ ion concentration in step of 1 from 1→1000mM. Run your R code and solve for the membrane potentials.

Plot membrane potential vs. external K^+ ion concentration (remember units, axes labels, and a title). Plot membrane potential vs. external K^+ ion concentration <u>on a log x-axis</u> (include units, axes labels and a title; use log="x" in the plot() function).

13. Provide a general description of what you see going on in these 2 graphs. What is the trend (increasing or decreasing) that you observe and why? Overall, what happens to the membrane potential when external K^+ is decreased? How much (a lot or a little) does the membrane potential change when K^+ is decreased 10-fold from 1000mM to 100mM? From 10mM to 1mM?

Compare the Nernst equation with GHK equation

14. Describe two differences between the GHK and the Nernst equation.

Program in R the Nernst potential for K^+ with the equation provided below:

$$E_K = \frac{RT}{F} \ln\left(\frac{K_o}{K_i}\right),$$

where E_K is the Nernst potential for K^+, dependent on external K^+ concentration.

Let's see how changes in the Nernst potential compare with changes in membrane potential when external K^+ ion concentration is changed (in steps of 1 from 1→1000mM).

Plot the following on the same graph: (i) the Nernst potential for potassium and (ii) the GHK membrane potential, with external K^+ ion concentration as the independent variable. Hint:

```
plot(x-axis, y-axis, xlab="External Potassium Concentration mM", ylab="Reversal Potential mV",
    main="Change in Potassium Concentration", type="l", col=c(1))
points(x-axis, y-axis, type="l", col=c(2))
legend("topleft", legend = c("GHK", "Nernst"), fill=c(1,2), lwd = 1, cex = 0.5)
```

Plot the following on the same graph **with a log x-axis**: (i) the Nernst potential for potassium and (ii) the GHK membrane potential, with external K^+ ion concentration as the independent variable.

15. What do you see? Why does the membrane potential deviate from Nernst potential at low K+ concentrations?

Part Two: Probability and Stochastic Behavior – Second .Rmd file

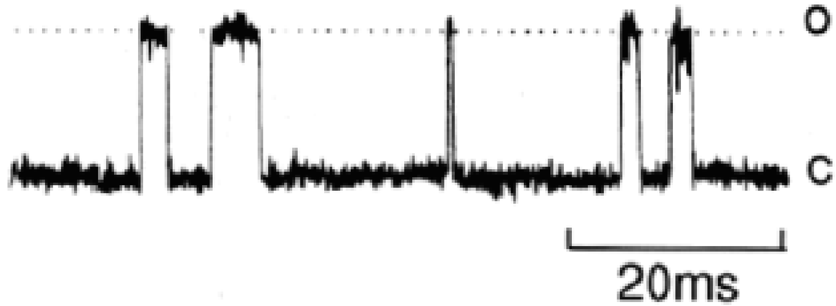
Random Numbers and Ion Channels (this part is similar to the Markov chain lab from the Spring quarter BioMath class, Lab-4)
We are now ready to simulate an ion channel flipping between open and closed states. We'll use a procedure very much like what we did for the fair coin toss.

PROGRAM ion channels in R

- Set the variable endtime to 50, and then use endtime to create a range variable t (t will be used in the for loop as our index number). Then, use t to specify the indices of an array called state, which will store the ones and zeros corresponding to whether our channel is open or closed over time (i.e., the channel's state).

- Initialize state to all zeros (all closed states for time points).
  - endtime <- 50
  - state <- rep(0,endtime)
- Determine the values to enter into state stochastically, i.e., using the runif()function. Assume that roughly 50% of the time the channel is open and 50% of the time that channel is closed (this is exactly like you did for the fair coin toss).
- Find the average value of state. **Is it 0.5? Run again with larger endtime**!
- Plot the state array versus time (t). Add the mean as well using *abline()*. Hint: you can do the second part with the command abline(h=average, col=c(2), lty=2).

16. Compare and contrast the plot you've generated to the plot below. What features are similar? What features are different?



17. Was the hypothesis that *the channel opening, and closing was a simple random event, like a coin toss,* correct? What have we left out?

<u>Ion Channels Simulation</u>

The problem is that the state of a real ion channel is determined not only by one random variable but by two. The first random variable is associated with the probability that a closed channel will become open, and the second random variable is associated with the probability that an open channel will close. These two probabilities could be independent. Think of it like this: at every instant the channel is open, an unfair coin is flipped to decide if the channel is going to close, and at every instant the channel is closed, a different unfair coin is flipped to see if the channel is going to open.

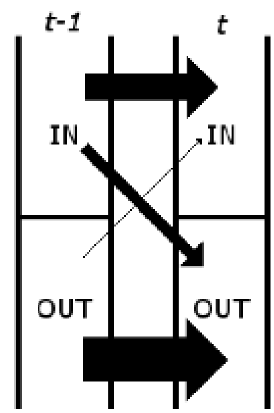
An analogy might help. Imagine that your housemate has a really irritating little dog that barks continuously. He's really annoying, and if he's in the house there is some probability at any given point in time that you'll get fed up with him and put him outside. Of course, the neighbors get annoyed with him as well, so if he's outside there's some probability that they'll call the police, forcing you to let the beast back in the house. The key is that these probabilities are independent of each other, i.e., that your neighbors may be more or less tolerant than you are. If we divide time into discrete steps (i.e., t in 1:endtime), then during any time step we can say that the "state" of the dog is either "inside" or "outside", and the probabilities of the events "you put dog out" and "neighbors call police" govern the transitions made to the dog's state. Let's define dog_state to be equal to 1 if the dog is in the house and 0 if the dog is outside.

1. If the dog is inside (dog_state = 1), then two things can happen
  a. dog stays inside (update dog_state = 1)
  b. dog goes outside (update dog_state = 0)

2. If the dog is outside (dog_state = 0), then two things can happen
  a. dog stays outside (update dog_state = 0)
  b. dog comes inside (update dog_state = 1)

We said that the probabilities of the dog coming inside from outside or going outside from inside are independent, but we didn't say what they are. Let's say that if the dog is inside during a given time step, the event "dog goes outside from inside" (event 1b above) has probability "p"; likewise, if the dog is outside during a given time step, the event "dog comes inside from outside" (event 2b above) has probability "q". Remember that p and q are unrelated to each other; the only constraint on them is that they both must be between 0 and 1 (i.e., they are probabilities). Then, the probability of event 1a is simply 1-p, and the probability of 2a is 1-q. This may seem like a silly degree of formalism to adopt to describe whether a dog is inside or outside, but if you take the time to understand it, the rest of this assignment will make a lot more sense.

Finally, imagine that the neighbors are quite a bit more tolerant than you are such that q < p. In a single timestep, the transition probabilities may look something like the arrows in the following figure, where thicker arrows represent a higher probability of transition, and thinner ones represent a lower probability.



Return to ion channels; see if you can generate a more realistic-looking channel behavior. This will require incorporating two processes that operate independently.

PROGRAM ion channels in R

- Follow the above procedure for defining endtime and state
- Use if/elseif statements to create the two independent processes as outlined in the dog example. For example, **if** the channel is closed, what is the probability it will open, and **if** the channel is open, what is the probability the channel will close. Assign probability values at your discretion.
- Graph your results (remember a title, axis labels, xlim, ylim, and abline(h=average, col=c(2), lty=2))

18. Play with different probability assignments. What do you see in the behavior of the ion channel? Can you get your simulation to look like a realistic ion channel? What probabilities worked the best? Explain.

19. What do you think is missing in this model? What would you include to make the description more realistic? Think about the duration of the channel being open or closed as well as adding a stimulus.

<u>Part Three: Connecting Membrane Potential with Stochastic Behavior of Ion Channels – Third .Rmd file</u>

We individually investigated the 1) effects of changing permeability and concentrations on the overall membrane potential using the GHK equation and 2) the stochastic nature of individual ion channels in their ability to randomly switch from open to closed and closed to open with no external stimulus. Let's ***connect the changes in membrane potential to the individual ion channel state profile***. This will allow us to think about microscopic properties and their overall collective behavior on the macroscopic membrane potential. You may find it helpful to look at your BIOS 20172 Lab #4 if you want a refresher on modeling stochastic processes.

- Run the GHK function with Na+ permeability going from 1 to 50 in steps of 2, setting P_K to 20, P_{Cl} to 5, and the other R, T, and F constants to their proper values.
- Now, graph the membrane potential vector calculated from using GHK function above vs. Na^+ permeability (include units, axes labels and title – please use type="o" in your plot function).
- Next, we will program the ion channel simulation. Make **an outer for-loop that iterates through the sodium permeabilities and set the probabilities of opening and closing (p and q) according to the permeability.** This should follow a scheme that makes sense (depolarization opens the ion channel, etc.…). Use a threshold membrane potential value of ~ -20mV that will open the Na^+ channels. Use runif(1) to stochastically model the evolution of the ion channel state according to the conditionally-defined probabilities p and q.
- Graph the state of the ion channel vs. time for each calculated value of the membrane potential (as the membrane potential changes with Na+ permeability). You will have **25 graphs that correspond to the ion channel profile per calculation of membrane potential**.

Use the pseudocode below if you need any help.

```
P_Na<-seq(1,50,2)
P_K<-20
P_Cl<-5
…
Perm_Na<-GHK()
plot(…)
endtime<-100
state<-rep(0,endtime)
for (iterate through permeabilities){
 if (permeability exceeds a threshold) {
   set p
   set q
 } else if (permeability falls short of a threshold) {
   set p
   set q
 }
 for (iterate through times){
   draw x from U[0,1] distribution
   if (channel is currently open/closed){
     if (x exceeds p/q probability){
       set state accordingly
     }
     else {
       set state accordingly
     }
   }
   else if (channel is currently closed/open){
```

```
    if (x exceeds p/q probability){
      set state accordingly
    }
    else {
      set state accordingly
    }
  }
}
take average of state vector
take standard deviation of state vector
plot(…)
}
```

22. Describe the overall trends in your graphs. Do you observe any notable features on these graphs? If so, what are they?

If you have further questions, please email Dr. Esmael Haddadian haddadian@uchicago.edu