Lab 1 – Introduction to membrane properties

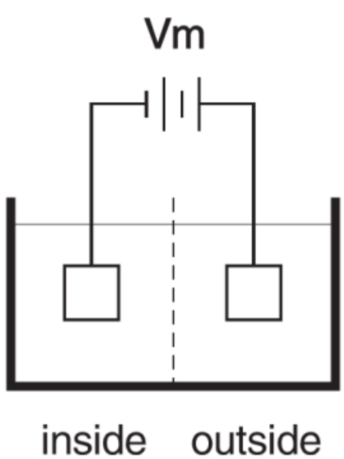
This lab session consists of 3 parts:

1. Equilibrium potential
2. Resting potential
3. Summary questions

****Make sure to paste each plot that you generate in Matlab for full credit****

1. Equilibrium potential

Purpose: to understand the principle of an equilibrium potential.



You have a container with two compartments of equal volume: "inside" (intracellular) and "outside" (extracellular). Each compartment contains an ionic solution in water. They are separated by a semi-permeable membrane that allows one of the ionic species to pass through it. Electrodes from an external voltage source (a "voltage clamp") are inserted into the compartments. The voltage clamp lets you control the transmembrane potential Vm.

In this experiment, you will examine how movement of the permeant ion through the membrane is affected by its ionic charge, z, its concentration, and the externally applied voltage.

Open the Matlab m-file "npe.m"

We begin by studying potassium (K^+), so you should set **z** = +1. Next you must set the initial ion concentrations in both the intracellular and extracellular compartments. The K^+ concentration within mammalian nerve cells is approximately 130 mM, and the extracellular concentration is about 3 mM. Therefore, you would set **Cin** = 130 and **Cout** = 3. You will change the externally applied voltage **Vm** and observe how it affects the ion concentrations in the two compartments.

A. Run the simulation with the applied transmembrane potential **Vm** = 0. Paste a copy of this time course in your lab report. What happens to the concentrations over time? Explain why this happens.

B. Set the transmembrane potential to -40 mV (inside is negative with respect to outside) and repeat the simulation. Paste a copy of this time course in your lab report. What happens to the concentrations now, and why?

C. Use the provided script to find a potential where the concentration gradient does not change. Paste a copy of this time course in your lab report. Calculate the Nernst potential for the initial K^+ concentrations, assuming $\mathbf{z}$ = +1, and $\mathbf{T}$ = 25°C. How does this value compare to the potential you determined using the simulation?

D. Suppose the extracellular K^+ concentration increased from 3 mM to 10 mM. How would this affect the Nernst potential for K^+?

E – G. Repeat A – C for Na^+ (10 mM inside, 124 mM outside).

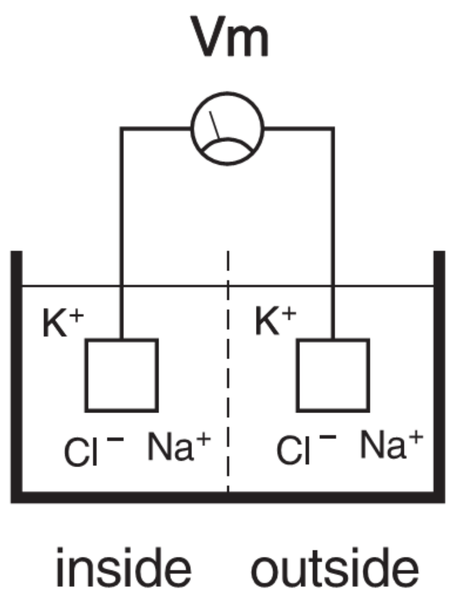
H – J. Repeat A – C for Cl^- (30 mM inside, 130 mM outside).

K – M. In this experiment, the two compartments had equal volumes so their concentration changes were of equal magnitude but opposite sign. Suppose the outside compartment was much larger than the inside compartment. How would this affect the concentration changes? Perform this simulation for Na^+ by changing the value of **vol_o** to 10 in npe.m. Repeat A - C.

2. Resting potential

Purpose: To understand the contribution of different ions to the resting potential.

The resting potential across the cell membrane of a neuron is usually in the range of -40 to -80 mV. Goldman, Hodgkin, and Katz examined how membrane permeability to different ionic species affects membrane potential.



Here, you have a cell with a membrane that is permeable to K^+, Na^+, and Cl^-. The potential Vm that develops across the membrane is monitored by a sensitive electrometer.

You can change the intracellular and extracellular concentrations of these ions. Using pharmacological agents, you can also control the relative permeability of the membrane to each of these ionic species from 0.0001 to 1.

The intracellular and extracellular ionic concentrations are given in this table. At the beginning of the simulation, all the membrane pores have very low permeabilities.

Ion	Intracellular	Extracellular
Na^+	10 mM	124 mM
K^+	130 mM	3 mM
Cl^-	20 mM	130 mM

Generate a Matlab script that enables you to calculate the membrane potential (**Vm**) based on intracellular and extracellular ion concentrations and the membrane's permeability to those ions. Hint – you should be implementing the Goldman-Hodgkin-Katz equation. ****Make sure to submit your code****

Use the following constants:

```
R = 8.3144621; % J/molK = Coulombs * Volts / moles * degrees Kelvin
F = 9.64853399e4; % C/mol = Coulombs / moles
T = 298.15; % in K, 25 degrees Celsius, remember: 0 C = 273.15 K
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A. Set $\mathbf{P_{Cl}}$ to 0.0001.
   1) When $\mathbf{P_K}$ is 0.01 and $\mathbf{P_{Na}}$ is 0.001, what happens to the resting potential and why?
   2) What if $\mathbf{P_{Na}}$ is changed to 10 times larger than $\mathbf{P_K}$?
   3) What is the maximum hyperpolarizing (decrease from the resting membrane potential) or depolarizing (increase from the resting membrane potential) value for each of these two ionic species? What has to occur in terms of permeabilities to hit the max or min?

B. Set $\mathbf{P_{Na}}$ to 0.001 and make $\mathbf{P_K}$ 10 times larger than $\mathbf{P_{Na}}$.
   1) Increase $\mathbf{P_{Cl}}$ from 0.0001. How does this affect **Vm**?
   2) What is the effect of increasing $\mathbf{P_{Cl}}$ if $\mathbf{P_{Na}}$ is much larger than $\mathbf{P_K}$?
   3) Why does $\mathbf{P_{Cl}}$ affect **Vm** in this way?

C. Set $\mathbf{P_K}$ to 0.01, $\mathbf{P_{Na}}$ to 0.001, and $\mathbf{P_{Cl}}$ to 0.0001, and reduce the intracellular chloride concentration to 10 mM
   1) Record the resting potential.
   2) Predict what will happen if $\mathbf{P_{Cl}}$ increases to 0.01, and then check your prediction.
   3) Suppose the intracellular chloride concentration was much larger, say 60 mM – now what do you think would happen if $\mathbf{P_{Cl}}$ increases from 0.0001 to 0.01? Test your prediction.

D. Adjust $\mathbf{P_{Na}}$, $\mathbf{P_K}$, and $\mathbf{P_{Cl}}$ so that $\mathbf{V_m}$ is close to -80 mV. Does this require a unique combination of permeabilities, or is there more than one way to get this resting potential?

3. Summary Questions

1) What determines the equilibrium potential for an ion across a barrier?
2) What is responsible for the resting potential?
3) Why doesn't the resting potential of a real cell equal the equilibrium

potential for potassium?

4) What three mechanisms are responsible for ion concentration gradients?
5) Why is active transport of sodium and potassium (as well as other ions, such as calcium) required to maintain the resting potential?