GEORGETOWN UNIVERSITY MEDICAL CENTER
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Tutorial in Neural Electrophysiology: Part I

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THE FUNDAMENTALS OF ELECTRICAL FIELDS IN NEURONS

This primer constitutes a brief and cursory introduction to the physical laws that dictate ion movement and their contribution to the electrical properties we can observe in cells such as neurons. The Neuron software package is used for hands-on examples and exercises to consolidate the concepts presented here. You can access the materials and instructions for installation of these exercises on this project's GitHub repository.

DRIFT & DIFFUSION

Here we present 3 fundamental physical laws that describe the movement of ions in an electrical and chemical gradient. These laws can be (and are!) applied to many other fields including developmental biology, MR physics, signal processing and neural network modeling. Learning this now can save you some work later.

For simplicity's sake we will only consider movement in one direction of space (x). Thus, whenever you see this expression $\frac{\partial some\ variable}{\partial x}$, know that we are ignoring all other dimensions (y, z, t, etc.) and only describing the change in $some\ variable$ in one dimension, the dimension of x. If you're interested in 4D dynamics, read up on tensors! These mathematical objects are truly awesome and describe beautiful patterns and phenomena in nature.

FICK'S LAW The first law I will present to you, equation 1.1, is a mathematical definition that describes how any given particle behaves in an aqueous solution. We define a variable, J_{diff}, to describe the *diffusion flux*,a measure of how many molecules are observed moving through a square centimeter at every second (molecules/sec-cm^2).

Equation 1.1: Fick's Law for Diffusion Flux

$$J_{\text{diff}} = -D\frac{\partial[C]}{\partial x} \tag{1.1}$$

Now, let's break equation 1.1 down into its basic parts. J_{diff} is a function of the diffusion coefficient D (cm^2/sec) for a particular ion and the concentration $[C]$ of that ion in $1D$ space along dimension x. Note that the negative sign indicates that J_{diff} flows from high to low concentration.

TMM;DR *Fick's law describes how many ions travel through a unit of area over time as constrained by the propensity of that ion's ability to move in space and the distribution of that ion in space.*[1]

OHM'S (OTHER) LAW To describe how ions behave in a biological system we must also consider an additional force, the electric field, E, that emerges from spatially distributed clusters of charged molecules. The flow of charged particles in an electrical field can be described by:

Equation 1.2: Ohm's Law for Drift

$$J_{\text{drift}} = \partial_{\text{el}} E \tag{1.2}$$
$$J_{\text{drift}} = -\mu z[C]\frac{\partial V}{\partial x}$$

Here J_{drift} is the drift flux (molecules/sec-cm^2), ∂_{el} is electrical conductivity (molecules/V-sec-cm), E is the electrical field (V/cm) defined over space in the direction of x ($E = -\frac{\partial V}{\partial X}$), V is electrical potential (Joules/Coulombs), μ is mobility of the ion ($cm^2/V - sec$), z is the valence of the ion (dimensionless) and $[C]$ is the concentration. Similarly to Fick's law for diffusion, equation 1.2 states the drift of positively charged particles takes place down the electrical gradient scaled by the conductivity of the medium they are travelling through.

TMM;DR *Ohm's Law for electrical drift describes how many ions travel through a unit of area over time as constrained by the electrical field that surrounds them.*

EINSTEIN'S INSIGHT Lastly, we have a mathematical expression that links these two physical laws together. Of course it was the brilliant Einstein (1908) who made the contribution needed to derive the fundamental laws that govern the electrical behavior of cellular processes.

Einstein described diffusion as a random walk process in the language of the laws of thermodynamics. He demonstrated that the frictional resistance exerted by a medium on an ion

[1] Too Much Math; Didn't Read

in motion down an electrical or chemical gradient is the same for drift as it is for diffusion at thermal equilibrium. With this relation, we can link the diffusion coefficient and electrical mobility via equation 1.3.

Equation 1.3: The Einstein Relation between Diffusion and Mobility

$$D = \frac{kT}{q}\mu \tag{1.3}$$

Here, k is the Boltzmann constant (1.38×10^{-23} joules $/°K$), T is the absolute temperature in units Kelvin and q is the charge of the molecule in coulombs.

MOVING FORWARD Now we have everything we need to derive an equation for ion movement across membranes in physiological conditions! Note we must use the contribution of both the concentration and electrical gradient to describe flux across the membrane.

THE NERNST-PLANCK EQUATION (NPE)

We can write the flux of ions across the membrane, J (molecules/sec-cm^2), as a parameter of both the concentration gradient and electric field by combining the equations for diffusion, J_{diff}, and drift flux, J_{drift}.

$$\begin{aligned} J &= J_{\text{drift}} + J_{\text{diff}} \\ &= -\mu z[C]\frac{\partial V}{\partial x} - D\frac{\partial [C]}{\partial x} \end{aligned}$$

Using Einstein's relation, we can express the diffusion coefficient in terms of mobility and simplify the flux equation.

$$J = -\left(uz[C]\frac{\partial V}{\partial x} + \frac{\mu kT}{q}\frac{\partial [C]}{\partial x}\right) \tag{1.4}$$

Equation 1.4 is the Nernst-Planck Equation (NPE) of the ion-flux form (J is in molecules/sec-cm^2). We can obtain a more useful equation by dividing J with Avogadro's number[2] to obtain the NPE in the molar form:

$$\begin{aligned} J &= \frac{J}{N_A} = \frac{-\mu z[C]}{N_A}\frac{\partial V}{\partial x} - \frac{\mu kT}{N_A q}\frac{\partial [C]}{\partial x} \\ &= -\left(uz[C]\frac{\partial V}{\partial x} + \mu\frac{RT}{F}\frac{\partial [C]}{\partial x}\right) \end{aligned}$$

[2] Avogadro's Number is equal to $6.022x10^{23}$ mol^{-1}

Since current is the product of ion flux and the charge it carries, the NPE of the current-density form can be obtained by multiplying the molar flux by the total molar charge, zF.[3]

NPE in the current-density form can now be derived in its full glory:

$$I = J \cdot zF = (J_{\text{drift}} + J_{\text{diff}}) \cdot zF = -\left(\mu z^2 F[C]\frac{\partial V}{\partial x} + \mu zRT\frac{\partial [C]}{\partial x}\right) \tag{1.5}$$

Where the current, I, is expressed in amperes flowing over a unit area, A/cm^2; J is expressed in mol/sec-cm^2; z is the valence of the ion; μ is the molar mobility, $\mu/N_A(cm^2/\text{V-sec-mol})$; R is the gas constant (1.98 cal/° K-mol) and T is temperature in units Kelvin (K).

The NPE in equation 1.5 gives us a somewhat realistic mathematical framework with which to describe ionic current flow driven by the concentration gradient and electrical field.[4]

Note that this equation describes **passive** behavior of ions in an electrical field. Though quite a bit unwieldy, this equation can be used to derive the familiar Nernst equation which we will use to calculate the resting membrane potential given a set of ions and their concentrations.

Nernst Equation

As described above, the equation 1.5 expresses ionic current in terms of the concentration gradient and local electric field. However one may ask the question, under what condition is the membrane at equilibrium? We can answer this question by setting the membrane current to 0, moving V to the left side of the equation and integrating with respect to V.

$$\begin{aligned} I &= -\left(uz^2F[C]\frac{\partial V}{\partial x} + uzRT\frac{\partial [C]}{\partial x}\right) = 0 \\ \frac{\partial V}{\partial x} &= \frac{-RT}{zF}\frac{1}{[C]}\frac{\partial [C]}{\partial x} \rightarrow \int_{x_1}^{x_2}\frac{dV}{dx}dx = -\frac{RT}{zF}\int_{x_1}^{x_2}\frac{d[C]}{[C]dx}dx \end{aligned}$$

We change variables and extract the following:

$$\int_{V_1}^{V_2} dV = \frac{-RT}{zF}\int_{[C]_1}^{[C]_2}\frac{d[C]}{[C]}$$

Then use our handy-dandy rules for integration of fractions:[5]

$$V_2 - V_1 = -\frac{RT}{zF}ln\frac{[C]_2}{[C]_1}$$

[3] F is Faraday's Number, the magnitude of charge of one mole of electrons, and can be defined as Avogadro's Number times the electric charge carried by a single proton, q .

[4] Note that the negative sign in equation 1.5 indicates I flows in the opposite direction as the voltage over space ($\partial V/\partial x$) and in the opposite (same) direction as $\frac{\partial C}{\partial x}$ if z is positive (negative). **Remember this tid-bit of information** as it will come in handy later on for calculating the resting membrane potential for ions with different valences.

[5] Recall that $\int_{x_1}^{x_2}\frac{dx}{x}$ is evaluated as $ln(x_2) - ln(x_1)$ and can be rewritten as $ln(\frac{x_2}{x_1})$. Yay math! Isn't this fun? ☺

We must provide one last formal definition before we can achieve the familiar form of the Nernst equation. The membrane potential of a cell is defined as such by convention:

$$V_m = V_{in} - V_{out}$$

The equilibrium potential, E_i, of ion i, defined by the cross-membrane potential at which membrane current carried by ion i equals 0 (i.e., where the current $I_i = 0$, or in prose: the resting membrane potential), is therefore written as:

Equation : The Nernst Equation for Resting Membrane Potential

$$E_i = V_m = V_{in} - V_{out} = \frac{RT}{zF} ln \frac{[C]_{out}}{[C]_{in}} \tag{1.6}$$

Voila! You've followed through about 200 years worth of mathematical research and derived the Nernst equation. Go ahead and give yourself a pat on the back, you deserve it (wo)man.

Congratulations aside, note the implications of equation . When the membrane is at equilibrium for a particular ion, the membrane potential and concentration gradient exert equal and opposite forces – *effectively canceling each other out.*

APPLYING WHAT WE'VE LEARNED

Let us take advantage of our new mathematical toy and play with an example.

Given that $[K^+]_{in} > [K^+]_{out}$ in most neurons, the K^+ ions tend to flow out of the cell down their gradient. If left unopposed, the ions would all flow down their gradient and out of the cell. However this doesn't happen because the electrical field is acting in the opposite direction and nullifies the force due to diffusion.

Can we confirm the state of the cell at equilibrium by calculating the direction of the resting membrane potential for potassium?

Given a physiological temperature of $T = 37°C(37° + 273.15 = 310.5K)$, standard concentrations of $[K^+]_{in} = 140mM$, $[K^+]_{out} = 5mM$, constants $RT/F = 62mV$ and $z = 1^+$ for the charge potassium of ions:

$$E_i \quad = \quad 62mV \cdot log_{10} \frac{[C_{out}]}{C_{in}} = -89.7mV$$

This negative value for E_i reflects an inward pointing electrical field driving positively charged potassium ions to flow inward. This is the value at which the driving force out of the cell by the concentration gradient is canceled-out by the electrical field, establishing electro-chemical equilibrium. Feel free to calculate the resting membrane potential for other ionic species given the standard ionic concentrations below.

	Inside (mM)	Outside (mM)
Frog muscle (Conway 1957)		
K^+	124	2.25
Na^+	10.4	109
Cl^-	1.5	77.5
Ca^{2+}	4.9†	2.1
Squid axon (Hodgkin 1964)		
K^+	400	20
Na^+	50	440
Cl^-	40–150	560
Ca^{2+}	0.4†	10
Typical mammalian cell		
K^+	140	5
Na^+	5–15	145
Cl^-	4	110
Ca^{2+}	1–2†	2.5–5

†(10^{-4}) free

1 Exercise: The Equilibrium Potential

Experimental Set-Up To get us started, let us consider a simple two compartment model of equal volume: The intracellular and extracellular compartments. 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 V_m.

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

1.1 Launching `NEURON`

Visit this project's GitHub repository and install Neuron. Follow the instructions for launching the web applet using the terminal or by navigating to the Applications folder and launching the `nrngui` application and a small window titled `NEURON Main Menu` should pop-up near the top-left corner of your screen along with a terminal window.

Next, select `File` from the menu and click on '`load HOC`'. A file-browser will help you find the `NPE.HOC` file we will need for this simulation.

Use the mouse to navigate through folders. If you accidentally click a folder, click the '`/..`' string to go back a level. If the file-browser does not find the `NPE.HOC` file you may have to manually enter the full path to the file (e.g. '`~/Downloads/NPE.HOC`', where '~' indicates the path to the home user's directory). If you are successful in opening this file you should see the following window:

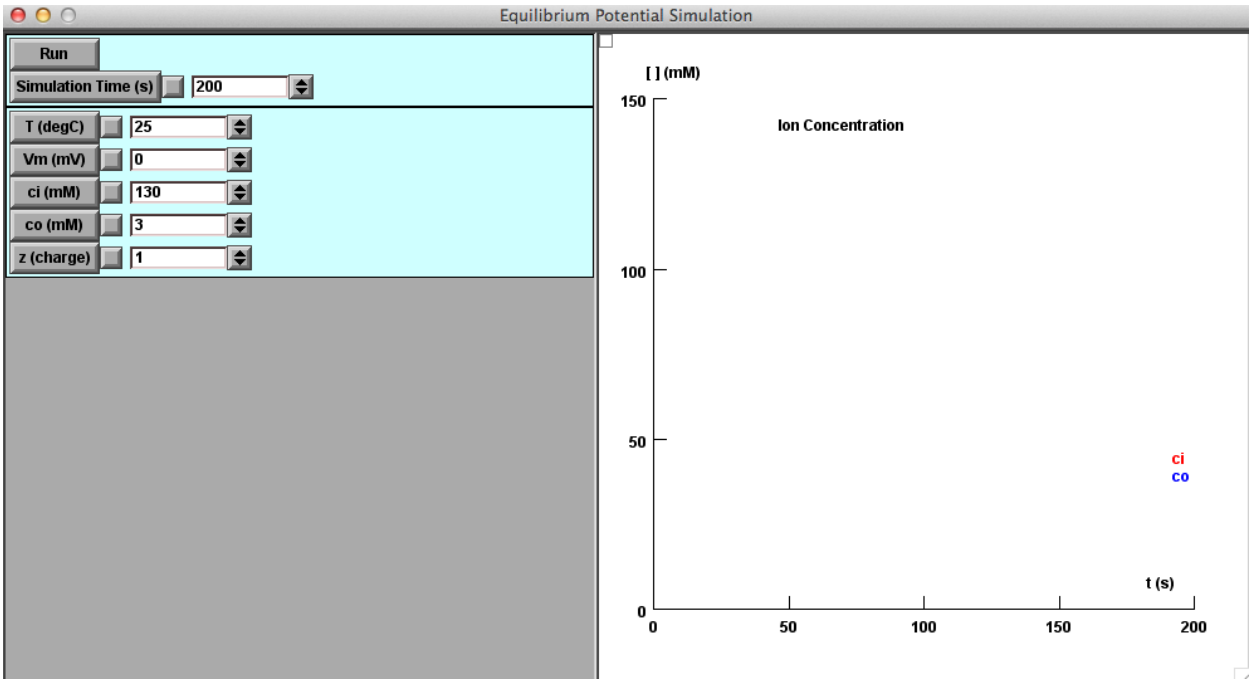

Figure 1: Oh yeah - time to simulate some ionic equilibrium!

1.2 Nernst Potential Simulation

We will begin by studying potassium (K^+), so you should set $z = +1$ (the valence charge of this ion). Next, you must set the initial ion concentrations in the intracellular and extracellular compartments.

The $K+$ concentration inside mammalian nerve cells is approximately $130mM$ and the extracellular concentration is about $3mM$. Therefore you should set $ci = 130$ and $co = 3$. You will change the externally applied voltage, V_m and see how it affects the ion concentrations in the two compartments.

As you go through this lab, it is helpful to take a screenshot of your output at each step and annotate it as you review your results when answering the summary questions on the following page.

A Run the simulation with transmembrane potential $V_m = 0$. What happens to the concentrations over time? Take a screenshot or sketch this in your notebook. Why does this happen?

B Set the transmembrane potential to $-40mV$ (inside is negative with respect to outside) and repeat the simulation. What happens to the concentrations now and why? Take a screenshot and annotate the curves!

C Can you use the simulation to find a potential where the concentration gradient does not change? Calculate the Nernst potential for the initial K^+ concentration you used, assuming $z = +1$ and $T = 25°C$. How does this value compare to the potential you determined using the simulation?

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

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

F Repeat A-C for Cl^- ($30mM$ inside, $130mM$ outside).

G In this experiment, the two compartments had equal volumes so their concentration changes were of equal magnitude but opposite sign. Suppose the outside compartment were much larger than the inside compartment. How would this affect the concentration changes?

2 Exercise: The Resting Potential

The resting potential across the cell membrane of a neuron is usually in the range of -30 to $-80mV$. However, the cell membrane is a dynamic structure with selective permissions for what can and cannot pass through it. Goldman, Hodgkin and Katz examined how membrane permeability to different ionic species affects membrane potential. In this simulation we will replicate some of their experiments and thought processes.

Experimental Set-Up You have a cell with a membrane that is permeable to K^+, Na^+ and Cl^-. The potential V_m that develops across the membrane is monitored by a sensitive electrometer. You can change the intracellular and extracellular concentrations of these ions. With (virtual) pharmacological agents you can also control the relative permeability of the membrane to each of these ionic species from 0.0001 to 1.

2.1 Resting Membrane Potential Simulation

Following the instructions above for the Nernst potential simulation, open the `VREST.hoc` file included with this document on the project's GitHub repository. Click the link to retrieve the file if you accidentally lost or deleted it. Again don't forget to take record of the parameters and results you get for each question below.

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

Table 2.1: Typical ionic concentrations in mammalian nerve cells.

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

A Set P_{Cl} to 0.0001.
1. When P_K is 0.01 and P_{Na} is 0.001, what happens to the resting potential?
(Hint: what does the Nernst potential for these two ions tell you?)

2. What if P_{Na} is 10 times larger than P_K

3. What is the maximum hyperpolarizing or depolarizing effect for each of these two ionic species?

B Set P_K 10 times larger than P_{Na}.
1. Increase P_{Cl} from 0.0001 (by clicking the up arrow next to P_{Cl}). How does this effect V_m?
2. What is the effect of increasing P_{Cl} if P_{Na} is much larger than P_K?

3. Why does P_{Cl} affect V_m in this way?

C Set P_K to 0.01, P_{Na} to 0.001, P_{Cl} to 0.0001 and reduce the intracellular chloride concentration to $10mM$.
1. Record the resting potential.

2. Predict what will happen if P_{Cl} increases to 0.01 and then check your prediction.

3. Suppose the intracellular chloride concentration was much larger, say 60mM – Now what do you think would happen if P_{Cl} increases from 0.0001 to 0.01? Test your prediction.

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

3 Exercise: Modulators of Membrane Potential Equilibrium

How can the intracellular concentration of potassium reach such high levels if the extracellular concentration is so low? One mechanism is co-active transport, which shuttles $3Na^+$ from the cytoplasm in exchange for 2 K^+ at the expense of a single molecule of ATP. These-active transport mechanisms constitute a large proportion of the energy requirements for a single neuron and, along with large impermeant ions (i.e., membrane proteins), contribute to a specific form of equilibrium called the Gibbs-Donnan effect.

Experimental Set-Up In this next simulation, both compartments contain K^+ and Cl^- ions, which can pass through the semipermeable membrane. The intracellular compartment also contains proteins with negative charges (A^-). These anions are too large to go through the membrane pores. The presence of these impermeant ions affects the distribution of ions that can pass through the membrane. A virtual voltmeter is used to measure the potential across the membrane in millivolts.

3.1 The Gibbs-Donnan Effect

At the start of each simulation run, $[K^+]$ is identical on the inside and outside

$$[K^+]_i = [K^+]_o \text{ at } t = 0$$

However, because of the internal anions, charge balance dictates that initial $[Cl^-]_i$ will be different.

$$[K^+]_o = [Cl^-]_o$$
$$[K^+]_i = [Cl^-]_i + [A^-]$$
$$\text{thus} \Rightarrow [Cl^-]_i < [Cl^-]_o \text{ at } t = 0$$

This concentration gradient causes an electrical potential difference and drives a redistribution of KCL across the membrane. In turn, this movement of solute is accompanied by a change of osmotic pressure that could cause a movement of water from one compartment to the other. Here we assume that the two compartments and the membrane between them are rigid so this cannot occur.

Open the simulation `DONNAN.HOC`

A Start with the default values for P_K(0.01), P_{Cl} (0.1), $[A^-]$ (60mM) and $[K^+]_o$ (100mM).
1. Run the simulation. Sketch (or take a screenshot) of the time courses of V_m and the concentrations of K^+ and Cl^-. Record the steady-state values of these variables.

2. Cut $[A^-]$ in half and repeat the simulation. How does this affect the time course and steady-state distributions of K^+ and Cl^-? What about V_m?

3. What do you think would happen if you made the anion ($[A^-]$) concentration as small as possible? Test your prediction.

4. Now make the anion concentration as large as possible and see what happens.

5. Summarize and explain the effects of changing $[A^-]$.

For some of the slower time courses you may need to increase the simulation time to make sure steady-state is achieved.

B Return $[A^-]$ to its original value and try changing P_{Cl} over the range 0.01 - 1.0. Hint: try 0.01, 0.02, 0.05, 0.1, 0.2, 0.5 and 1.0.

1. What happens to the steady-state ion concentrations? Why?

2. What happens to the steady state V_m?

3. How does P_{Cl} affect the time course of these changes and why?

C Return P_{Cl} to its original value and vary P_K over the range 0.01 - 1.0. How does this affect:

1. The steady-state ion concentrations. Why does it have this effect?

2. Steady state V_m.

3. The time course of these changes. Why does it have this effect?

D What do you suppose would happen if both P_K and P_{Cl} were increased to 1 and why? Test your prediction and explain the result.

E Based on your observations A-D, complete the following table using the key that follows it:

Perturbation	Time course	Steady state *Vm*	$[K]_o$	$[Cl]_o$	$[K]_i$	$[Cl]_i$
↑ $[A]_i$						
↑ initial $[K]_o$						
↑ P_K alone						
↑ P_{Cl} alone						
↑ both P_K and P_{Cl}						

Symbol	Effect of increasing the parameter
+	accelerates time course or increases steady-state value (*Vm* less negative)
–	retards time course or decreases steady-state value (*Vm* more negative)
+/–	mixed effect on time course or steady-state value
0	no effect

4 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, rather than Gibbs-Donnan equilibrium alone?