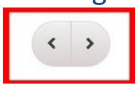
Chapter 1 – Ion gradients

Welcome to Chapter One of this cellular neurophysiology MOOC. If you have not done it already, take a look at the videos filmed in the lab and the supplements offered last week.

After this Chapter 1 introductory video, we will offer you 4 course videos:
- Observations and Hypotheses
- Ion Transport and the Nernst Equation
- Ion Current
- Patch Clamp Technique

The questions (not provided) asked in between some videos should help you test your knowledge.
We remind you that there are several sections to go through. You can navigate between sections by clicking the icons above similar to these here:

or using the arrows at the bottom of the page that are identical to these here:



Enjoy the course!

CHAPTER 1 INTRO (2:43)
In this first chapter, I will explain to you the basic knowledge required to understand the electrical signals given off by neurons, and I will also explain to you the only two equation which you will need afterwards. What are the objectives of this first chapter? At the end of this chapter, you will know about electrochemical gradients. This is an extremely important gradient because it allows the passage of ions thereby creating neuronal electrical signals. Thus, you will also find out that it is a membrane potential, and you will be able to predict in which direction the ions will cross the membrane at a given potential, you will know about ion currents and that an ion current changes the potential of a membrane. What are the prerequisites for understanding this course well? You should already know about cells, cell membranes, that is to say, about lipids and proteins and also about ions. It is even better if you are familiar with a few basic notions in physics, such as resistance, current, potential. Using an example from everyday life, can you explain the difference in potential and currents? Undoubtedly, the simplest analogy is that between electrical current and water flow. Let us imagine a water tower. It is filled with potable water thanks to a pump that pumps water to the very top of the tower. Thereafter, this water can flow down under the action of gravity and into every home that is connected to running water. The difference in height between the top of the water tower and the plumbing inside the houses enable water to flow on its own from the tower to the houses. And the water flows at a certain flow rate which is expressed in terms of volume per minute which will depend on the diameter of the pipes: the smaller

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the diameter of the plumbing, the lower the water flow rate. In biology, we are not talking about water flow but about the flow of ions that are charged molecules. The difference in height becomes the difference in potential on either side of the membrane. Current intensity depends on the flow of ions, i. e. the number of +/- charges per second while resistance to the flow of water corresponds to the resistance to the passage of ions across the membrane. This analogy is not perfect since ions are charged molecules which complicates things somewhat. But at least this has helped grasp the basic concepts, such as resistance, current, and potential. What resources are made available for this first chapter? There are course videos, quizzes, practical exercises, supplements to revisit certain key concepts and a glossary to explain the research terminology.

CH.1-1 : OBSERVATIONS AND HYPOTHESES (9:04)

To some people, this first course may seem a little arduous, because there are two mathematical equations but these are the only two equations that you will subsequently use for all the courses in this MOOC. Let us start with these much vaunted ions. Which ones are they? The intracellular medium, i. e. the neuronal cytoplasm, and the extracellular medium contain ions. What are these ions that cross the neuronal membrane? We find them in the intracellular medium, i. e. in the neuronal cytoplasm, and in the extracellular medium which is found around neurons. Specifically, these ions are sodium Na+ ions, potassium K+ ions, calcium Ca2+ ions, and ions of chloride Cl-. Here, these first three ions are positively charged and the last one is negatively charged. It turns out that these ions are also located on different sides of the neuronal membrane.

We see that sodium ions here are in a much higher concentration on the outside than on the inside. It is the same for chloride ions, and the same for calcium ions that are in a much higher concentration on the outside than on the inside of a neuron. The situation for potassium is totally different because it is much more concentrated on the inside than on the outside of a neuron. Despite these differences in concentration, each section is electrically neutral. So, you can stop the video for a few seconds and calculate the concentration of positive ions on the outside as compared to the negative ones, and the same on the inside. If we make the calculation, we see that on the outside, there is only 146 millimolars of + charged ions and that it is the same for - charged ions. My writing style is somewhat ... unorthodox. And on the inside, on the other hand, we see a large difference, i. e. there are 154 millimolars of + charged ions whereas there are only 14 millimolars of - charged ions here (chlorides). And that is because, in fact, on the inside, there are anions that are not among the ions listed here. They are organic anions, for instance, such as negatively charged amino acids, negatively charged proteins, nucleic acids, or else ions like HCO3- or HPO4--. And then, the interior of the neurons is also electrically neutral. The only differences in concentrations between the inside and the outside of the membrane, as we just saw, exist in an adult and are held constant. What are the mechanisms that keep these concentrations constant? First hypothesis: possibly, it is because ions do not cross the membrane; thus, there is nothing to regulate. Or ions do cross the membrane and there is a system that keeps the concentrations constant. So, we are going to analyze these hypotheses one by one. To find out whether ions cross the membrane, we stage the following experiment: we take the giant axon of a squid which is an axon that has a very large diameter; so, it is easy to study, and we place it in a bath containing radioactive sodium (Na*) that we see here. The idea is as follows: if ions do cross the membrane, we should find radioactive sodium inside the axon. Thus, what do we do? We stimulate the axon and what we see is that after a moment, there is radioactive sodium appearing on the inside which we can measure. This means that some sodium crossed the membrane and entered the axon. This transport of sodium ions into the interior of the axon is impervious to energy blockage that we will see a little later; so, it is enough to block ATP production to see that this is completely impervious. Therefore, this is passive ion transport into an axon. If there is passive ion transport into an axon, concentrations will change. So, what is the system that keeps the concentrations constant?

Now, we stage the opposite experiment by taking the giant axon of a squid loaded with radioactive sodium ions and we put it in regular sea water. We look to see if any sodium exits into this regular sea water and calculate this sodium efflux over the surface of the axon and per unit of time. And we observe that, in effect, radioactive sodium ions appear in the extracellular medium. This means that sodium ions exited. This efflux of sodium ions, is it a passive efflux, same as a little while ago, that does not require energy, or is there, in fact, a different system?

To find this out, we add an ATP production blocker which is called dinitrophenol. We see it here. We add it and observe that as soon as it is added, this sodium efflux ceases and drops off to 0. And as we remove it, this returns to its normal slope. So, the sodium efflux that we observed in this experiment is dependent on ATP production, so it uses ATP to make energy, ADP and inorganic phosphorus, and this energy helps transport sodium to the outside. More specifically, it is what is known as a sodium- potassium pump, that is to say that here it exchanges, sodium exits and potassium enters at the same time. This helps maintain sodium and potassium concentrations continuously. Sodium entered in a passive manner but exists through active transport. As for potassium, we will see that it exists passively and re- enters through active transport. And in fact, this is true for all the ions, be it sodium, potassium, or calcium... There are also calcium pumps and also pumps for chloride ions. So, if ion concentrations are maintained constant on either side of the membrane, it is not because ions do not cross the membrane but because they cross the membrane passively and are then actively ejected back where they came from thanks to an energy source.

In summary, if we consider sodium ions, we have seen that these sodium ions cross to the inside of the membrane passively without needing energy and re-exit in an active manner thanks to pumps, and here they enter through channels. We are going to see all of this again later. Thus, they enter, they re-exit, and the concentrations remain constant. It is the same for all the ions although not necessarily in the same direction but it is the same for all the ions. So, in a direction opposite to that of passive crossing, there is always active transport that requires energy.

Sodium, potassium, chloride, or calcium ions cross the membrane. How do they cross the membrane? Do they diffuse through the membrane lipids or are there proteins that have to be in that membrane for them to get across it?

We synthesize a lipid bilayer without any channels or proteins, and we place it in a container with a compartment on the left containing radioactive sodium and a compartment on the right which is totally empty. And same as in the previous experiment we will see whether radioactive sodium travels from the left towards the right. Now, we realize that it absolutely does not. Here, we find no radioactive sodium in the right-hand compartment.

This means that the lipid bilayer is impermeable to sodium, and if we repeat this with other ions, we will realize that it is impermeable to all ions. So, for ions to cross the membrane, it looks like proteins are required in this lipid bilayer.

Thus, now we redo the same experiment but we have a lipid bilayer wherein we have integrated some proteins, a lot of proteins, and specifically those we call ion channels.

And then, we realize that radioactive sodium crosses to the other side, and that we find radioactive sodium molecules on the right. Thus, thanks to certain transmembrane proteins that are called ion channels, the lipid bilayer is able to transport ions. There is no doubt that this is passive ion transport. This does not require any energy. These ion channels are proteins delimiting an aqueous pore at their center. This pore that we see here is, in fact, a hole, if you will that can be open or closed and that is hydrophilic and lets ions through that are also hydrophilic.

CH.1-2 : ION TRANSPORT AND THE NERNST EQUATION (7:07)

Ions cross the neurons' cell membranes. Even if this transport, referred to as passive, requires no energy, forces are required to move the ions. What are these forces that make the ions move across a cell membrane? On of the forces that makes the ions move is a concentration gradient because molecules always move from an environment in which their concentration is highest towards one where their concentration is lowest. Thus, a concentration gradient has a tendency to make potassium ions, for instance, exit an environment where their concentration is highest towards an environment where it is lowest. Sodium ions, on the other hand, have a tendency to enter, under the action of a concentration gradient from an environment where their concentration is highest from am environment where it is lowest. The same is true for the calcium ions which also have a tendency to enter as their are much more concentrated outside. And the chloride ions also have a tendency to enter. Thus, it can be seen that almost all the ions have a tendency to enter through the concentration gradient with the exception of potassium because its concentration gradient is reversed. But it turns out that the cell membrane carries a charge, that is to say, there is potential difference between its two surfaces. The interior carries a greater negative charge than the exterior, and we can see -60 mV which is supposed to be the value while the neurons are at rest, i. e. when they are not excited. This difference in the membrane potentials is written as Vm = Vi Ve by convention; therefore, the membrane potential is equal to the intracellular potential less the extracellular potential and is dimensioned in millivolts. Given that ions are charged molecules, they are attracted by the charges on either side of the membrane. Potassium ions, for example, will be attracted by the charges that are more numerous on the interior surface of the membrane. Thus, by the action of the electrical gradient, they will have a tendency to enter through the membrane. Similarly for the sodium ions that, much like the potassium ions, are positively charged: through the action of the electrical gradient, they will have a tendency to enter through the membrane. It is the same for the calcium ions that are going to have a tendency to enter through the membrane. And quite the opposite for the chloride ions because they are attracted to the + charges on the outside of the membrane. Now, how do these two forces, the concentration gradient and the electrical gradient, combine? This is what we call the electrochemical gradient. Now, we have the two forces simultaneously, the one that results from the concentration gradient and the one that is caused by the electrical gradient. Both these forces act on the ions which are charge molecules. For the sodium ions, it is simple because both the forces have the same direction; therefore, the resulting force will make sodium enter. For the calcium ions, it is also very simple because both the forces have the same direction; therefore, in this case as well, the calcium ions will enter. It is much more complicated for the chloride and the potassium ions because the two forces have opposing directions. Which is the one that wins? Do the ions enter or exit? And at what potential? Is it the same for the chloride ions? To find out, the moment when those two forces balance each other must be identified. Thus, the ions are subject to two forces: the concentration gradient and the electrical gradient. We saw previously that the concentration gradients remain constant. Hence, the force created by the concentration gradient is constant. The electrical gradient is the force that changes depending on membrane potential. This is a little complicated, finding out which direction an ion will go. This can only be discovered only based on certain membrane potentials. To find out which way an ion will go, we use the ion's equilibrium potential as a reference. An ion's equilibrium potential is the membrane potential for which the concentration force is equal and opposite to the electrical force. Hence, this ion's net flow is zero. The Nernst Equation is used to calculate an ion's equilibrium potential. The Nernst Equation is as follows: Eion = RT/zF ln exterior ion concentration over interior ion concentration. Eion is the ion's equilibrium potential expressed in millivolts. RT/zF is a constant, R is the ideal gas constant, T is the absolute temperature in degrees Kelvin, z is the ion's valency, and F is the Faraday constant. And all this is under a natural log. If we change and make all the calculations and convert to log base 10, the equation will have the form Eion = 58/z log of exterior ion concentration divided by interior ion concentration. Thus, z depends of the ion and may be equal to + 1, + 2, 1, in general, for the ions we have seen. If we were to calculate for the concentrations provided at the start of the course, we would see that sodium's reversal potential is +58 mV. This means that the membrane must be at +58 mV for an equal number of sodium ions to enter and exit. On the other hand, given that the potassium gradient is the inverse of that for sodium, we see here that potassium's equilibrium potential is very negative: -97 mV. This ion is in equilibrium when the membrane is at -97 mV. For calcium, the value is +128 mV. For chloride, it is -59 mV. There are values that the membrane never reaches, such as +58 and +121. Thus, these ions will always cross to the inside. They will never reverse direction. It is the same for potassium: it will always exit. It is just for chloride that this is very complicated. Let apply this Nernst Equation to the membrane, and specifically, to potassium ions. The equation tells us that if potassium channels open, and only the potassium channels, the potassium ions will exit until the membrane potential is at -97 mV. At that moment the potassium ions will be in equilibrium, and there will be an equal number entering and exiting. Why does the outward migration of potassium ions cause the membrane's potential to change to -97? Because since potassium ions are exiting, there is a loss of positive charge from the membrane's interior surface. This loss of positive charge causes the membrane's interior surface to become increasingly negative with respect to the exterior surface until ion transport stops at -97 mV. Throughout the range of potentials that a neuron may assume which normally lies between -80 and +20, potassium ions exit. As we have seen, it is much more complex for the chloride ions but we will look at them later, in Chapter 5.

The Nernst equation:

$$E_{ion} = \frac{RT}{zF} \ln\left(\frac{[ion]_e}{[ion]_i}\right)$$

$$E_{ion} = \frac{58}{z} \log\left(\frac{[ion]_e}{[ion]_i}\right)$$

CH.1-3 : ION CURRENT (5:00)

We know that there are two forces that carry ions across a neuron's cell membrane: that of the concentration gradient and that of the electrical gradient. We also know that we can predict the direction of ion transport at a given membrane potential thanks to the Nernst equation. Now, what we are going to see is that this transport of ions across the membrane creates an ion current because a current is a movement of charges. I = QT, Q is the transported charge in coulombs per t, a unit of time, here in seconds. For ionic channels, current is expressed in picoamps, i. e. 10-12 ampere. These are very low currents and require and amplifier to measure. Where there is current, there are also resistance and potential. As for potential, we have seen membrane potential, resistance we have not yet seen, and here is the current. Here is Ohm's Law which is a law in physics. Among neurons, in a channel, U is the driving force which in a regular current transports electrons, and in neurons, it transports ions. We have seen that it is an electrochemical gradient, a driving force in English, and that it is equal to the resistance to the passage of ions in a channel times the unit ion current. And again, it is Ohm's Law here. It is the potential RI. We transform Ohm's Law because for neurons, we will mostly want to see current. Thus, we write it a different way. The unit current across a single channel is equal to gamma times the electrochemical gradient. What, then, is gamma? It is the inverse of resistance, or the unit conductivity, and it is dimensioned in siemens. It is only a little calculation, very simple, that transforms 1/r into gamma. Thus, we will speak of conductivity much more than resistance. It is the inverse of resistance. It is the ease with which ions pass through a channel. This is the equation to calculate a unit current through a single channel. Now, what happens when multiple channels of the same type open in a membrane? Thus, through all the membrane's potassium channel, for instance, we would have a total potassium current equal to the conductivity, or the ease with which potassium ions cross the membrane, times the electrochemical force. What is this total current? In fact, total current is related to the unit current through the number of channels. Thus, if it is sodium current we are interested in, total sodium current depends on the unit sodium current, the number of sodium channels in a membrane, and the probability that they will open. This means, that here Npo is, in fact, the number of open channels at the time of the current measurement. It is the same for conductivity. It is related to the unit conductivity through the number of open channels. It is obvious that if this number of open channels is very large, the conductivity will be high because ions will be passed through with great ease. If there are few open channels, the conductivity will be low, and if there is only one, well, the conductivity will be equal to the unit conductivity. This is Ohm's Law as it applies to neurons. What is the role of ion currents? The primary function of ion currents is to change membrane potential. If potassium ions exit the neuron, for example, the membrane potential will become hyperpolarized because the interior surface will lose ions with a + charge. If ions with a charge enter, then the membrane potential will also hyperpolarize. If ions with a + charge enter, then the membrane potential will depolarize. And these changes in polarity, depolarization, hyperpolarization, are neurons' typical signals. We will see a very important signal, referred to as action potential, in Chapter 2. Ion current also has other functions. Depolarizing and hyperpolarizing the membrane, they can open or close other channels that are sensitive to voltages and open and close if the membrane is more or less depolarized or more or less hyperpolarized. And finally and very locally, the currents can change the concentration of an ion. For example, if there are many calcium channels in a small volume, entry of calcium ions will result in a local increase in calcium concentration. This increase will be temporary because there are pump systems that will recover the concentrations but this small increase could have an important physiological role to play, and we will see in Chapter 3, for instance, that it is responsible for releasing neurotransmitters.

CH1-4 : PATCH-CLAMP TECHNIQUE (3:28)

How does one go about measuring an ion current? We take a technique called voltage clamp. Here is Ohm's Law. If one induces a potential in the membrane, this potential becomes constant like the equilibrium of an ion is constant. We have here a constant; therefore, the electrochemical force is constant. Gamma is a variable and the i of an ion is a variable; thus, we can measure the i of an ion which will now only depend of the unit conductivity of a channel. Now, it is the same thing when we want to measure total current. We maintain the membrane potential to have this entire factor as a constant and we measure the total current which will depend on the number of open channels in the membrane. Now, if we wanted to measure a change in potential. In the same equation, we have Vm which is the variable we want to measure and we have the E of the ion which is a constant. What do we do, then? We set the current that we inject through an electrode. This enables us to measure changes in potential. You must understand well that current clamp means that the current sent through an electrode is held at a known level by the researchers. But it does not mean that the current going through the membrane is constant. And we let the membrane potential we are measuring evolve. How do we measure? We use electrodes that we also call pipettes. Pipettes and electrodes are the same thing. Here they are shown in cross-section. These are glass pipettes filled with a fluid which conducts electricity, and we see here a metal wire which will help us record the signal. Thus, here, we have a pipette that is moss green and this other one a a rather light green. Here, we attach the pipette to a neuron which is represented by a round cell. If we leave it like this, we will measure the current going through the very small piece of membrane under the pipette. There may be one or more channels under the pipette. Now, right now, the pipette is full of extracellular fluid because it is outside, and the intracellular fluid remains the neuron's native fluid. Also, to record a very small piece of membrane and only a few channels or even a single channel, we are in the outside-out mode. We will leave this in English because it is a little bizarre and it means "outside to outside". This means that the exterior of the membrane remains outside. What did we do? We pulled and excised a very small piece of the membrane and we are hoping there are channels in it. And then, when total current needs to be measured, we go to the whole-cell mode, that is to say that we started by attaching to a cell, then the researcher breather through the pipette making this characteristic little noise. The little piece of membrane here was out, was removed, and the fluid inside the pipette passed into the neuron, and thus, the pipette is electrically connected to the entire neuron. Then, to achieve this, we fill the pipette with an initiation intracellular fluid because the fluid will find its way inside. Well, all throughout the course we will see these three configurations. The first two to record only a few channels suing a unit current, and this to register total current, or changes in potential.