Neurobiology

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Week - 02

Lecture 2.4: Membrane potential

In the last few videos, we have been looking at electrical properties of neurons. We have seen different ions that are present commonly in neurons and we have also looked at properties like resistance, capacitance and current and voltage in neurons. Now, we will try to use these properties to understand how neurons function and we will start by looking at one very important property of neurons which is the membrane potential. Can you guess what organism is shown in this picture? This is a squid. It is a kind of a sea animal and it is about couple of feet long. The reason that I am showing you this organism here is that many interesting lessons about the electrical properties of neurons were learned by studying this organism.

Thus, it has been a very useful model organism in neuroscience and the things that we have learned from squids are also true in humans. Of course, that is because many aspects of the neuronal structure and function have been conserved throughout evolution. For doing different kinds of experiments, different organisms may be suitable. Now, what could be the advantage of using squids, say over mice or monkeys? Can you guess? The main reason that researchers found squid to be a useful model organism is because it has some neurons whose axons are very wide.

In humans and mice, the axon diameter is typically about a micron, but in a squid, there is one axon which is known as the giant axon has diameter about 1 mm, that is about 1000 times more than the diameter of our axons and that makes it very easy to work with having this large diameter. So, one can easily probe this axon. Researchers have measured the concentrations of different ions inside and outside the squid axon and the concentrations of potassium, sodium and chloride are shown here. Now, these exact values are somewhat different from what we have seen for mammalian cells in a previous video, but you can appreciate the fact that the ions that are more abundant inside and the ions that are more abundant outside are the same between humans and squids. So, we have more sodium and chloride outside compared to inside and we have more potassium inside compared to outside, even though these exact values are different from the values that we see in humans, but the relative trend remains the same.

In fact, the concentrations of sodium and chloride that you see here, these are more abundant outside, these concentrations are basically the concentrations of these ions in seawater because it is a sea organism and the space outside the neurons is filled with a medium which is basically like seawater. So, it is not surprising that sodium and chloride are more abundant outside. When researchers measured the voltage inside this giant axon, the voltage or the membrane potential of this axon is about -65 millivolts. Now, of course, this value is going to depend on whether the neuron is at rest or is it actually active. If the neuron is active and it is showing action potentials, then this value will change and it will fluctuate over time.

So, this is the value when the neuron is not active and so it is sometimes called the resting membrane potential that is -65 millivolts. So, we have seen that the value of the resting membrane potential is around -65 millivolts and the reason this value is negative is because there is more negative charge inside the neuron compared to outside. There are actually both positively and negatively charged ions inside and outside. It is not that there are only negative ions here and only positive ions here. It is just that the number of negative ions inside is slightly more than outside and you can imagine that the negative charges would repel each other and they would try to move as far as they can.

So, they go and accumulate on the membrane and an equal amount of positive charge accumulates on the other side of the membrane. We have already seen in the last video that the membrane can act like a capacitor. So, it can store charge and these negative and positive charges are stabilized by each other. So, if you have a membrane that is thinner, the stabilization would be more and the capacitance would be more. So, a thinner membrane would be able to store more charge.

Now, why do we have more negative charge on the inner side to start with? You might be tempted to say that maybe when the neuron was formed, it had more negatively charged particles inside and that is why it remains negative throughout its life. That explanation would have been satisfactory if the membrane was completely sealed and there was no exchange of charged particles between inside and outside. But we know that that is not the case. There are in fact ion channels present throughout the membrane and ions can move in and out through these ion channels. Even if you were to actually push out some ions from inside to outside or outside to inside, that will disturb the balance but soon the same balance will again be achieved and the membrane potential will again become -65.

This resting membrane potential is achieved by an active process that tries to maintain the value at a certain point by moving the ions. And why that happens that we will try to understand as we go along. Let us start by thinking about how do we actually measure the membrane potential. We typically do this by the approach of electrophysiology in which we put electrodes inside the neurons. So, a typical setting is something like this in the very simplest form.

So, say you have a neuron and you have an electrode that is placed in the outside medium, the extracellular medium and you have another electrode that can go inside the neuron and then the reading that you will get will tell you what is the potential difference that is read by the electrode inside relative to outside. If the value is small, it can also be amplified and then it can be stored in a computer or it can be visualized on an oscilloscope. Now, let us consider an experiment in which we first start by placing the electrodes, both the reference electrode and the actual electrode, both are placed outside and then at certain time point we push this electrode inside the neuron. So, let us guess what we would see on the oscilloscope. So, of course, before we have put the electrode in, both the reference electrode as well as the recording electrode are both outside and they are in the same medium.

So, the recording will be 0, the value that is observed will be 0 and once we push it in, then the voltage that we will see will be around -65 millivolts. So, it is 0, before insertion and after inserting the electrode, it will be a negative value, same as the resting membrane potential. Electro physiology allows us to measure the membrane potential and at the same time it also allows us to stimulate the neurons and we can do that by basically passing current into the neurons. For that, we have to modify the setup a little bit. So, if you want to do both recording from a neuron as well as stimulate the neuron, then a simple setup could look something like this.

So, you still have your recording electrode as before, this is a recording electrode, this is the reference electrode and in between you have some voltage measurement apparatus and this will do the recording as normal. Now, you have a second pair of electrodes where one is placed outside the reference and one goes inside the neuron and this second electrode can be used for stimulating the neuron. So, if this electrode is connected to some current generator, then whatever current you generate here will be passed into the neuron and you can generate both positive currents or negative currents, those currents will be passed into the neuron. By positive current, I mean that the positive charge is flowing from the electrode into the neuron and negative current means that positive charge is flowing from the neuron into the electrode or you can imagine negative charge flowing from the electrode into the neuron and if you pass negative current into the neuron, then what would happen is that the, so either positive charge is flowing out or negative charge is coming in, in either case the membrane will become more negative. So, let us look at the outcome on an oscilloscope.

So, this is the reading of membrane potential that we are getting from the recording electrode and we are visualizing it on an oscilloscope here and this trace here, dark blue trace here shows the stimulation that we are providing using the stimulation electrode. So, at t is equal to zero starting time point, there is no current and the reading on the oscilloscope is around -65 millivolts, which is the resting membrane potential. Now, at certain time point, we start applying

negative current. So, we apply a pulse of negative current from this time point to this time point and as you can expect, the value of the membrane potential becomes even more negative at that time and when you release it, when the stimulation is removed and the current becomes zero again, then the value of membrane potential again comes back to -65. So, it does not remain negative, it comes back to -65.

When you apply a stronger inhibitory pulse of current, the membrane potential again becomes negative, this time even more negative than before and then when you remove the current, it again comes back to resting membrane potential. That tells us that the resting membrane potential is actively being maintained and if you apply an even stronger negative current, then you see an even stronger lowering of the membrane potential to a larger negative value. This trend of membrane potential going down is sometimes described as hyperpolarization. So, let me explain this term in slightly more detail. So, the part of polarization here refers to the fact that the membrane is already negatively charged.

So, membrane is not at zero but it is at a negative voltage. So, we say that the membrane is polarized and when it becomes even more negative, so it is becoming even more polarized. That is why we call it hyperpolarized. So, just like we use the stimulation electrode to pass negative current into the cell, we can also use it to pass positive current using the same setup. So, we have a recording electrode and the stimulation electrode but now instead of giving negative current pulses, we are going to give positive current pulses and what that means is that positive charge will flow through the electrode into the neuron.

Now, our neuron was negatively charged to start with, the membrane was negatively charged. As positive ions come in, it will become less negative or it will move towards a more positive value. So, again using the terminology of polarization, so my membrane was polarized or negatively charged and now it is becoming less negatively charged. So, we can call it, it is becoming depolarized or less polarized and let us see how what we would observe for different levels of current pulses. So, in the case of hyperpolarization, we had seen that when the pulse was small, the hyperpolarization was small and when the pulse was large, the hyperpolarization was large.

Now, here is the data for depolarization or positive current pulses. When there is no current, we see the resting membrane potential around -65 millivolt. When you give a positive current pulse, you see an increase in the membrane potential and if the current pulse were to last long time, then the increase will also last. So, the membrane potential will remain high for that duration. So, because these values are stable, they are called electrotonic potentials.

Tonic refers to something that lasts longer. When the current pulse is removed, then it comes back. If you give a larger current pulse, you see a larger depolarization or a larger increase in the

membrane potential. But if you give an even larger current pulse, then we see something very different happening. So, the membrane potential does increase as we expect, but then it goes through a phase of very rapid increase and a fall and then it comes back.

So, this is different from the electrotonic potential and this is called actually an action potential and this is the currency of neural activity. So, this we will try to understand later in the course, but currently let's focus on what is happening here. One thing that I want to point out is that the current pulses are very sharp. So, we increase the current here and it goes to the full value. So, it's a square pulse, then it suddenly stopped and it comes back to zero.

But the change in membrane potential is not as sharp. So, it rises gradually and falls gradually. Here also it rises gradually and falls gradually and even here it rose gradually before the action potential kicked in. So, no matter how sharp your current pulse is, the change in the membrane potential is somewhat gradual. So, in this video, we have looked at the concept of membrane potential.

We have seen how it can be measured and also how it can be manipulated by injecting positive current or negative current inside a neuron. Now, based on the recordings that we saw and the outcome of these manipulations, you may have several questions. One obvious question is why do we see these action potentials in certain situations and that we will look at slightly later in the course. Another question that comes out is when we apply a square current pulse, why do we see a gradual rise in the membrane potential instead of seeing a sharp rise, just like the current? So, what gives rise to this gradual increase rather than a sharp increase? A second question that also comes up is why is the membrane potential around -65 millivolts? So, why is it not -20 or not -100? What defines this level? So, that we will try to understand in the next few videos. Thank you.