Neurobiology

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Lecture 3.4: Action Potentials

In the last video, we looked at the technique of voltage clamp. We saw how it can be used to fix the voltage of a neuron at a particular value. And we also saw how chemicals such as tetrodotoxin can be used to block specific types of ion channels. So, tetrodotoxin, for example, blocks the voltage-gated sodium channels. And similarly, there are other chemicals that can block other types of ion channels like the potassium channels. In this video, we will see how we can use the technique of voltage clamp and these various pharmacological agents to understand how the voltage-gated conductances are changing during an action potential.

Let us do a voltage clamp experiment in which the neuron is first capped at -60 millivolts, the resting membrane potential. And then at some point, it is increased to 0 millivolts and then it is capped there. Now, we already know that when the membrane potential is at the resting membrane potential, then the compensatory currents will be 0. And then at this transition period where it is changed from -60 to 0 millivolts, at that time we will see a large capacitive current.

And then there will be some leakage current depending on this voltage level, but that will be constant. And then we are also seeing this downward deflection in the compensatory current and then it becomes a positive deflection. So, overall in the beginning, we see this large capacitive current, then there is a negative compensatory current, which means that there must be positive ionic current. So, the current is coming into the neuron in the beginning. And then later we see that there is positive compensatory current, which means that there must be negative ionic current or current is flowing out of the neuron later.

So, these are the three main components that we see. And these two later components, this downward deflection and then upward deflection varying over time, this must be because of the voltage-gated conductances, because leakage currents do not change over time, they must be constant. So, this current that is changing with time should be because of the voltage-gated channels. The leakage current and the capacitive currents can be easily removed from this profile, because capacitive current is there in the beginning and leakage current will be constant

and we can easily calculate it how much leakage current will be given the voltage. So, we will have the ionic currents remaining.

And now can we resolve what part of this current is because of sodium channels and what part is because of potassium channels? Well, for that we can use the pharmacological agents as we saw in the last video. So, for sodium, we can block the voltage-gated sodium channels using tetrodotoxin. Similarly, for potassium, there are other chemicals like 4-aminoperidine or tetraethyl ammonium, either of them can be used to block the voltage-gated potassium channels. So, if we repeat the same experiment in the presence of either tetrodotoxin or one of these two chemicals, then we will be able to see what component of this current is caused by sodium and what component is caused by potassium. And this is what we get.

So, once we have removed the capacitive and leakage currents and we have resolved this time varying current into the sodium and potassium currents using TDX or tetraethyl ammonium, this is what we get. So, this is the current that is obtained in presence of tetrodotoxin, which means that sodium currents have been removed. So, this current must be because of potassium. And this is the current that we get in presence of tetraethyl ammonium, which means potassium currents have been removed now. So, this must be because of sodium.

And the sum of these two will give us the original current profile. So, if you add this curve with this curve, this is what we will get. So, this inward current that we were seeing in the beginning that is coming because of sodium channels. And this outward current that we were seeing is coming because of the potassium channels. And we can see that the sodium channels open sooner and then they also start to close.

So, that is why this current is increasing and then decreasing with time. Whereas the potassium currents open more gradually and then they remain open for some time. Now, we can repeat the same experiment for different values of the command potentials. So, every time we start with the resting membrane potential of -60 millivolts and then we take the membrane to different values of command potential. And at each value of command potential, we can observe the potassium current and the sodium currents using the pharmacological agents as we saw on the last slide.

And at each value, we know what the driving force on the potassium ions would be. So, for potassium ions, the equilibrium potential is around -70 or 75 millivolts. So, that is where the driving force on potassium ions will be zero. And then if we take the membrane to higher values of command potential, then there will be higher driving force. The farther we go from -75, the more driving force will be there on the potassium ions, which is the sum of the chemical and electrical driving forces.

So, at each value of command potential, we know this driving force and at each value of command potential, we can also observe the potassium currents. And now the current will be a function of the driving force as well as the conductance. So, if we know the driving force and we know the conductance, then multiplying those two should tell us what the current is. And here we know the current and the driving force. So, we can divide the current by the driving force, which is simply $V - E_K$, then we will get the conductance at different values of these voltages.

And similarly for sodium, we know that the equilibrium potential for sodium is around +55 millivolts. So, that is where the driving force on sodium ions is zero. And then as we go farther from it, we will have more driving force on the sodium ions. So, we would expect the currents to increase as we go farther, but the current again is a function of both the driving force as well as the conductance. So, as you go farther from +55 millivolts here, we see that currents increase, but then after some time the currents start to decrease, which means that the conductance at these lower levels of voltages must be low.

That is why we are not seeing current, even though there is a large driving force on sodium ions here. So, again by dividing the current values by the driving force, which is $V - E_{Na}$, we will be able to know what the conductance is as a function of time as well as a function of voltage of the membrane. So, here is the exact relationship between the current, the conductance and the driving force. For sodium, the current is equal to the sodium conductance times the driving force on the sodium ions. This driving force depends only on the equilibrium potential of sodium and the voltage.

The difference between them tells us what the driving force is and the current is measured experimentally as we saw in the last slide. And if we divide this current by the driving force, we get the conductance as a function of time and similarly for potassium. So, in these experiments where we start at -60 and then we take the membrane potential to different values of common potential, we can calculate the conductance as a function of time for different values of these common potentials. So, this is what we see. At -40, we see the conductance increasing.

At -20, we see it increasing even more, 0 even higher and +20 even higher. And for sodium also, we see something similar. So, at -40, there is a smaller increase in sodium conductance, the voltage-gated sodium conductance. At -20, it's more, at 0, it's even more and +20, it's even higher. So, in general, both these voltage-gated potassium conductance and voltage-gated sodium conductance, they increase if the membrane potential is increased.

And that's why they are voltage-gated conductances because they depend on the voltage. If we were looking at passive ion channels, then the conductance would have been the same regardless of what the membrane potential is. And similarly, for ligand-gated ion channels, their conductance would not depend on the voltage. It will only depend on the presence of the

corresponding ligand. Now, another interesting thing we observe here is that the potassium conductance increases with time if the higher membrane voltage is maintained.

So, if we maintain the membrane voltage at -40 millivolts, the potassium channels open gradually and then they remain open. And at higher membrane potentials, they open even more. And we must remember that the state of an individual channel is binary. So, it's either conducting current or not conducting current. But the reason we see this gradual change is because we are looking at the aggregate conductance of many channels.

So, what this tells us is that the probability of opening of channels is increasing with time. The channels don't open all of a sudden. They start to open gradually. And the more membrane potential we have, the higher is the probability of opening. For sodium also, the more membrane potential we have, the higher is the probability of opening.

But we see one difference here compared to the potassium channels, which is that the sodium channels open and then they seem to close again. And they are not opening again, which means that they are actually going into that third state, which is the inactive state. A channel can be in open state, closed state, or inactive state. And the sodium channels seem to be going into that inactive state after they have opened. Whereas, the potassium channels do not go in the inactive state.

They just open gradually and remain open. And the sodium channels also open faster than the potassium channels. So, in 1950s and 60s, Hodgkin and Huxley performed these voltage clamp experiments using the skid giant axon. And they calculated the sodium conductance and voltage-gated potassium conductance as a function of voltage. So, they were able to draw those curves and then they fitted equation to those curves.

So, they were able to get mathematical descriptions of these conductances. Now, in these voltage clamp experiments, the voltage is kept constant and is not allowed to change. But in a real neuron, we do not have clamping. Once some channels open and the currents flow in, then the voltage will change. And in response to the voltage changes, then the conductances will change further.

So, how the conductance depends on voltage is already described now by these equations. And how the voltage will change in response to conductance change or in response to the currents coming in that we can determine using the equivalent circuit of a neuron. So, if we know the capacitance of the neuron and we know what currents are coming in, then we can predict how the voltage will change. So, that is what Hodgkin and Huxley did. They described the change in voltage mathematically using this equivalent circuit of a neuron and the knowledge of how the conductance depends on the voltage and time.

So, they were able to drive these kinds of equations where the capacitance times the rate of change of voltage C (dV/dt) is equal to the total currents coming into the neuron, which includes the sodium current, the potassium current and the leakage current and any external current that might be injected. And these conductances were described by the equations that they were able to fit on the observed experimental data. So, with these, they were able to describe exactly how the membrane potential of a neuron would change once you give it some depolarization. And that is what we see. So, if you start with some depolarization, then the conductances increase and in response to conductance, the voltages further change.

So, in the beginning, sodium conductance is increasing. So, membrane becomes more positive because sodium ions flow in. But later as potassium conductance increases, the membrane potential starts to fall. So, this action potential is nothing but simply the change of voltage in response to the changes in the voltage-gated sodium and potassium conductance. And Hodgkin and Huxley's equations allow us to describe the shape of an action potential with very good accuracy in mathematical terms.

So, this is one of the first examples where a biological phenomenon was described very well by a bunch of very simple equations. And for this work, Hodgkin and Huxley received Nobel Prize in 1963. So, let's summarize what happens during an action potential as described by the Hodgkin-Huxley model. So, in the beginning, if we give a sufficiently large depolarization to the neuron, then that causes some sodium channels to open. The voltage-gated sodium channels open quickly and that opening causes some sodium current to flow in the neuron.

And as the sodium current flows in, it makes the membrane potential more positive, increases the membrane potential and that causes further opening of the sodium channels. So, as more sodium channels open, the membrane potential becomes even more positive. So, there is a positive feedback loop that results in a very sharp rise of the membrane potential. And it will try to take the membrane potential towards the equilibrium potential of sodium ions, which is around +55 millivolts. So, that is the maximum possible value that it can reach.

But before that happens, two things start to happen. One is that the sodium channels, although they open quickly, they also start to inactivate as we have seen earlier. And secondly, the voltage-gated potassium channels also start to open. So, the inactivation of sodium channels reduces the inward current and the opening of potassium channels now produces an outward current because potassium ions will flow out and will try to make the membrane potential more negative towards the air equilibrium potential of -75 millivolts. So, as this happens, the membrane potential stops increasing and then eventually starts to fall and then we see a very rapid fall in the membrane potential.

So, this is what causes this sharp rise and then a sharp fall in the membrane potential. The rising phase is because of the sodium ions and the falling phase is because of the potassium ions. So, this model nicely describes the shape of an action potential, the rising phase and then the falling phase. But if we look carefully at the shape, we notice one more interesting phenomenon here which is that the membrane potential actually goes below from where it had started. So, the value of the voltage here is lower than the starting point and this is called the after hyper polarization.

So, this additional hyper polarization that we are seeing after an action potential is called the after hyper polarization. Can you guess what the reason for this after hyper polarization might be? If we recall, the membrane potential is basically determined by the balance between different ions. The sodium ions try to take the membrane potential towards the air equilibrium potential and potassium ions try to take the membrane potential towards the air equilibrium potential here. And the resting membrane potential is dependent on how much sodium conductance and potassium conductance there is in the neuron. During an action potential, the sodium conductance had increased but then it also inactivated.

While the potassium conductance increased a little bit later but then it did not inactivate. So, these potassium channels are still open at this point and therefore the potassium ions keep on going out and the membrane keeps on becoming more negative. So, because we have additional potassium conductance here, the membrane potential will go towards the equilibrium potential of potassium ions. So, it will go towards -75 millivolts but not below that. And then as these voltage-graded potassium channels gradually close, then the membrane potential can return back to the default baseline value. Thank you.