Neurobiology

Dr. Nitin Gupta

Department of Biological Sciences and Bioengineering

IIT Kanpur

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Lecture 3.2: Active conductances

Hi everyone, welcome back to neurobiology. In the last video we looked at ion channels. We saw how the structure of ion channels allows them to become specific to specific ions. We also looked at the idea of gated ion channels. We saw that some channels can open and close in response to the voltage of the neuron or the binding of a ligand or mechanical stimuli like pressure. In particular, the voltage gated ion channels play a very important role in shaping an action potential.

And because these gated ion channels are responding to events happening in the neuron, they can also be called active channels or active conductances. So in this video we will see how these active conductances shape an action potential. We will also look at the electrophysiological methods of patch clamp recording or voltage clamp recording that are used for measuring the ionic currents. How can we measure current through an ion channel? Well, one of the techniques that we commonly use is called patch recording.

This term patch refers to the fact that the recording is done from a small patch of a membrane. And the main component in this technique is a glass capillary. So it's a very narrow glass tube which has an opening less than a micron in diameter. And this glass tube which you can see in this gray conical shaped structure here, this is placed over a piece of membrane as you can see here. So this is a membrane, a lipid bilayer in which ion channels are present.

And this could be the membrane of a neuron or a muscle or any cell in the body that has ion channels. So this glass capillary is placed on the piece of membrane and then some suction is applied through the capillary. So this membrane gets sucked in a little bit into the glass capillary and that helps in forming a tight seal between the capillary and the membrane so that there is no leakage through the capillary. Now let's consider for example that these ion channels are ligand graded ion channels and they are gated by acetylcholine which is one of the common neurotransmitters in neurons or muscles. So if acetylcholine is present these channels can open and then they can close spontaneously.

Now if we want to measure the current through these acetylcholine gated ion channels we can put some acetylcholine in this glass capillary. So there is an electrolyte solution in the capillary that can conduct current and then there is a metal wire at the back of the capillary which goes out and is connected to an oscilloscope or to a computer for storing the data. Now if we have acetylcholine in the electrolyte then it can go and bind to the ion channel and can open the ion channel and whenever the channel opens there will be current through the channel and then we will be able to measure that current on our recording apparatus. So here is what the recording may look like. So there may be zero current at some points which means that the channel is closed and whenever the channel opens we will see some deflection in the current here so there will be some positive or negative current depending on the membrane potential.

So in this particular case whenever the channel opens there is negative deflection in the current and this tells us that the channel is open for this time so it may remain open for a few milliseconds this is the time scale here and then it can close again and then it can open again. So if you record multiple traces you see it opening and closing at different times and what that tells us is that the opening or closing of the ion channels is a probabilistic or a stochastic event. So it can happen whenever the neurotransmitter happens to bind to the ion channel and then the channel can close again after some time spontaneously. But it is also a binary event so it's either in the closed state or in the open state but we don't see the currents at intermediate levels. Here is another example from a certain kind of potassium channels that are sensitive to voltage and now in this case these channels are closed in the beginning and then a voltage change is applied for a certain duration and during that period the channels can potentially open.

So here is one recording trace and we can see that the channel gets in the open state for some time then closes again then again gets in the open state for some time closes again and so on. And here is another recording trace from a different patch of membrane for the same period of stimulation. So we see these different traces and again we can notice that the channels are either closed or open but we don't see intermediate states so we don't see the current at the half level it's either fully there or it's zero. Now if we average over multiple channels and we make a summary of the different channels then we get a trace like this. So this is the total current through many potassium channels in response to the voltage pulse that is given in the membrane and in this average we see a gradual increase in the current.

So the total current through all these channels is zero and then it is gradually increasing and that might give the channels are gradually opening but as we have seen that the individual channels are either in the closed state or open state so they are binary. It's just that the probability of opening is increasing with time. So in the beginning the probability of opening is small so only a few channels can be open and after a few milliseconds the probability of opening is increased so now more channels can be open but any given channel will only be either fully closed or fully open not partially open. So we might get this kind of a recording trace in the patch recording if

we have a relatively larger tip size that includes multiple channels but if the tip size is very small so that there is only one channel contained within the patch that is covered by the tip then we will see a trace that is more like this with binary levels. So here is an example of what a real patch experiment looks like.

So we have a petri dish which contains some neuronal tissue and this petri dish is placed under a microscope so we are looking at the tissue from top. You can see some neurons here these are the cell bodies these oval or circular structures and from these cell bodies we can see these branches of neurons coming out so these various dendrites and exons are coming out from the cell bodies and we can also see this glass pipette that is patching on to this neuron. Now you might be wondering why this glass electrode looks very fuzzy well part of the reason is that it is made up of clear transparent glass but another reason is that only a small region of this image is in focus because we are looking at the image under very high magnification typically 200x to 600x magnification is used. Only a small narrow plane is in focus so this is a horizontal plane that is being visualized from top and this electrode is coming from top and it is patching on the neuron so the tip of the electrode is in the same plane as the neuron and that plane is in focus but as we go higher on the electrode it gets out of focus therefore the farther you go the more out of focus it becomes. Now this tip size is about a micron here and this size may still contain many ion channels within that region so if you want to record from a single ion channel you probably want to have an even smaller tip size.

The patch recording can be performed in various configurations. The simplest configuration is known as the cell attached configuration that we have already seen in which the glass pipette is put over the membrane and a tight seal is formed between the pipette and the membrane and then we can measure the currents that are flowing through the ion channels that are covered in this part of the membrane. A slightly different configuration is known as the whole cell configuration in which a stronger suction is applied once you have the seal over the membrane and the result of that strong suction is that this membrane can break at this point and what you get is a hole here so that the inside of the neuron the cytoplasm becomes continuous with the inside of the pipette and what that allows is that now we can measure the membrane voltage because whatever change happens in the voltage of the neuron that can be detected by the pipette and if there are any currents flowing through the ion channels not just the ion channel within this part but throughout the membrane of the whole neuron those can be measured by this pipette now. So the whole cell configuration is good if we want to see the overall changes in the neuron. There is another advantage of whole cell configuration which is that it allows us to inject things various chemicals from the electrode into the neuron if we want to manipulate the activity of the neuron.

So for example we may want to inject some ligands that can bind to certain ion channels and can block them or various chemicals that have different effects on the neuron. Another thing we could do is to inject some dyes through this electrode. So if the electrode contains a fluorescent

dye and we let the electrode be connected to the neuron for some time then this dye will diffuse out and it will go in different branches of the neuron and that can allow us to visualize the anatomy of the neuron. So this is a common technique that is also used in my lab here. We do recordings from the neurons in the olfactory systems of insects and after the recording we inject a dye in the neuron so that the dye can fill the neuron and after the experiment we take the drain out and then we can visualize it under a microscope and then we can get images like this.

So we can see the whole structure of one particular neuron from which we had done the recording and we also know what kind of neuron it is by looking at the anatomy of the neuron. So there are some advantages of whole cell recording but an obvious disadvantage is that it is more invasive. So we are actually rupturing the neuron at a small point here and that may cause harm to the neuron and one way of reducing that harm is to ensure that this hole is as small as possible and the seal is as tight as possible. And the second thing to ensure is that the electrolyte that is present in the pipette is very similar in its composition to the cytoplasmic fluid. So at least the concentrations of some basic ions are maintained at the same level.

There is one more technique that is commonly used for recording the membrane potential and that is called the sharp intracellular recording. So in that case an even sharper electrode is used which is typically nanometers in tip size and electrodes of that sharpness can be directly inserted within the neuron without having to form a seal on the membrane. So now that we have seen the voltage grated ion channels and we have also seen how the currents through these ion channels can be measured, we are in a good position to understand how action potentials are generated. If we look closely we can observe four specific properties of action potentials. The first property is that there is a threshold for generation of action potentials.

So if you recall if we inject a small current that causes a small depolarization in the membrane we do not see action potentials. Only when a sufficiently large current is injected that causes a sufficiently large depolarization then only we see an action potential generated in the neuron. So there is a threshold for generation of action potentials. The second property is that action potentials are all or none events which means that the size of the action potential does not depend on the size of the input. Once the input crosses a threshold a full-sized action potential is generated.

It doesn't matter whether the input was larger or smaller. The size of the action potential is more or less constant. So if we do not see half action potentials for example or we do not see double the size of action potentials if we give double the input. The third property is that the action potentials can be conducted without loss of amplitude. So if you measure the size of action potentials at two different locations in an axon you will find approximately the same amplitude of action potentials.

And fourth property is that there is a refractory period for generation of action potentials. So this term refractory period basically says that there is a small time after an action potential is generated in which another action potential cannot be generated. So there must be some minimum time gap before the next action potential can be generated at the same location in the neuron. All of these properties cannot be explained by the basic passive membrane properties like the capacitance or the ion channels with constant conductance. These properties actually depend on the voltage-graded ion channels which have active conductances that can change over time in response to changes in the voltage of the neuron.

Let's look at some of the initial observations that were made by scientists that helped us in understanding what happens during an action potential. So one thing that Hodgkin and Katz noticed is that if you reduce the amount of extracellular sodium then the amplitude of the action potential is reduced. And that tells us that perhaps the sodium influx is responsible for determining the amplitude of the action potentials. So it may be that when sodium ions come in that causes this increase in the membrane potential during the rising phase in the action potential. Later it was also found that if you have a depolarization that is larger than a threshold then sodium conductances increase.

And therefore if the sodium conductance becomes larger than potassium conductance then the membrane potential could go towards the equilibrium potential of sodium which is generally high around +55 millivolts or so. So that might cause increase in the membrane potential. And similarly it was also noted that during this falling phase of the action potential there is a rise in the potassium conductance and that can explain why the membrane potential is falling. Because if potassium conductance increases then potassium ions will try to take the membrane potential towards their equilibrium potential which is around -70 millivolts. But these descriptions are qualitative in nature.

They tell us what kinds of ions are involved. So sodium and potassium ion channels are involved here. But we would like to know exactly why we have this shape of the action potential because this is a very standard shape. And how exactly the conductances change during the course of an action potential in a quantitative manner. And for that a new technique was required known as the voltage clamp.

So we will understand how voltage clamp method was used to get a detailed understanding of the action potential in the next video. Thank you.