Neurobiology

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Lecture 3.3: Voltage Clamp

Hi everyone, welcome back to Neurobiology. In this series of videos we have been trying to understand how action potentials are generated. We have discussed that passive conductances cannot explain the action potentials. We have to look at the active conductances also. And in particular we need to look at what happens to voltage-gated ion channels. We have looked at the technique of patch recording in the last video that allows us to measure the current through ion channels.

But we need something more. We need to modify this technique a little bit more so that we can see how the voltage-gated ion channels are opening and closing in response to changes in voltage and to derive a quantitative relationship between voltage and the conductance. So in this video we will see this how the technique of voltage clamp works and how it can be used to understand how action potentials work. As we discussed in the last video, the initial experiments by Hodgkin, Huxley and Katz showed that the conductances that are important for action potentials could be a function of voltage.

So if there is change in the voltage of the neuron then we can see a change in these voltage-gated conductances. We would like to measure how exactly these conductances are changing for different values of voltage. So if you could keep the voltage at a particular value and then if we can measure conductances at that value of the voltage then we could understand the dependence of the conductance on voltage. But the problem is that as soon as the conductance changes it in turn affects the voltage. So it is very difficult to maintain a steady voltage while the conductances are changing.

To take a concrete example, we have discussed that a sufficiently large depolarization can cause opening of sodium channels and when the sodium channels open they allow sodium currents to flow inwards. So sodium ions come in and they make the membrane potential even more positive. So there is further depolarization and that causes further opening of sodium channels. So membrane potential keeps on rising. What would be nice if the membrane potential rises and then stays steady at a particular value then we can measure the sodium conductance at that value.

But because the membrane potential is continuously changing it is difficult for us to measure the sodium conductance as a function of voltage. So solution to this problem is obtained by voltage clamp which allows us to fix the voltage at a particular value. Now let us see how voltage clamp actually works. So let us say we have a neuron in a petri dish. So this is an axon whose voltage we are trying to clamp or fix at a particular value.

We put a recording electrode inside the axon and there is a reference electrode outside. This recording electrode can tell us what is the voltage of the neuron relative to outside and this difference can be fed to an amplifier to stabilize the recording or enhance it. But for now let us just assume that there is no amplification happening here. So we are measuring the actual voltage of the neuron. This voltage is fed to the negative terminal of a feedback amplifier.

This feedback amplifier is very important for voltage clamp and here the amplification vector will be quite large. So the recording of the neuron, the voltage of the neuron is coming at the negative terminal and at the positive terminal we apply the command voltage also called the command potential or the holding potential. So this is the voltage at which we want to clamp the neuron. So if we want to set the voltage of the neuron to +20 millivolts then we will apply the value of +20 millivolts here and this signal can be generated using a standard signal generator. Now this amplifier will look at the difference between the command voltage and the actual voltage of the neuron and it will multiply this difference by the amplification factor which in this case will be a very large value.

So if there is any small difference it will be amplified and the clever thing here is that this output of the amplifier is connected back to the neuron. So here we have a stimulating electrode that is going into the neuron and if there is any difference in the actual voltage and the command voltage then that difference will be amplified and the result will be fed back into the neuron. So now let us see how the amplifier would function. So if the voltage of the neuron is same as the command voltage then this difference will be zero so there will be no feedback output and the neuron will continue to be at the command voltage and if the voltage of the neuron is less than the command voltage so if it's a more negative value or less positive value compared to the command voltage then this difference becomes positive and this positive difference is multiplied by the large amplification factor of this amplifier. So it will result in a positive value and that very large positive value because it is connected back to the neuron it will quickly make the neuron more positive.

So if the neuron was less positive to start with it will quickly become more positive and reach the same value as the command potential and similarly if the neuron's voltage was on the other side so if it were more positive than the command potential then this difference will become negative and a large negative value will be generated and fed back into the neuron so the neuron

will become less positive and again will become the same as command potential very quickly. So this feedback ensures that if there is any slight disturbance of the membrane potential relative to the command potential then the membrane potential is brought back to the command potential very quickly so the neuron will remain at the command potential. Now let's consider the ionic currents so if there is any current flowing through the ion channels that current will try to change the membrane potential but this feedback current through the amplifier which is also known as the compensatory current so this compensatory current will balance out the ionic currents to ensure that the membrane remains at the command potential. So if there is current flowing in through the ion channels then the compensatory current will flow out and if there is current flowing out through the ion channels then the compensatory current will flow in. So by measuring the compensatory current we will know what is the amount of ionic currents coming into the neuron.

If ionic currents are flowing in then the compensatory current will be negative so a negative value of compensatory current tells us that ionic currents are flowing into the neuron. So broadly the electrophysiological recordings from neurons can be put into these two classes current clamp and voltage clamp. In the current clamp recordings as it's clear from the name we are clamping or controlling the current so this is the default older method of recording. Before the current lecture all the previous recordings we saw were current clamp recordings so we are simply measuring the voltage and we can control the amount of current injected into the neuron. This can be zero or we can inject some positive or negative current in the neuron and these recordings are useful to see how the voltage of the neuron is varying over time in response to either the current injections or in response to various stimuli that is given to the animal or to the tissue.

On the other hand in the voltage clamp recordings we are controlling the voltage so we fix the voltage of the neuron to a particular value specified by the command potential. What we measure over time is the current so we are measuring the compensatory current which tells us how much is the ionic current flowing through the membrane and these voltage gram recordings are useful if we want to measure how the conductance depends on the voltage. So by setting the voltage at different values and measuring the currents at those values we can figure out what are the conductances at different values of v. In the last slide we saw that there was one recording electrode and one electrode for passing current in the voltage clamp but there are ways in which both these functions can be performed by the same electrode. So one can do patch recordings in the voltage clamp mode also with just a single electrode that requires some fancy electronic circuit design but now there are commercial amplifiers that can achieve that.

The current through the membrane can be divided into various subcategories. Broadly we have the capacitive current which is the current that charges the lipid bilayer or charges the membrane and this current comes into play when the membrane potential is changing. If the membrane potential remains fixed at a particular value then the capacitive current will be zero. The ionic

current is the current that flows through the ion channels and this can be further subdivided. So the ionic currents can be divided into leakage currents which are the currents that are flowing through the passive ion channels.

So the passive potassium channels or sodium channels or chloride channels these are the channels that are always open and any current flowing through these ion channels can be called passive currents or leakage currents and then we have the active conductances which can include the voltage-gated sodium conductances, voltage-gated potassium conductances and other active conductances but these two are more important for understanding action potentials. Now as we just discussed the capacitive currents are there only when the membrane potential is changing and in the case of voltage clamp because we keep the membrane potential fixed at a particular value at the command potential so once the voltage has reached the command potential then the capacitive current will be zero. Only when the voltage is changing from one command potential to another in that brief transition period there will be some capacitive current but once the command potential is reached and it is maintained for some time then the capacitive current will be zero in that duration. The leakage current is present whenever the membrane potential of the neuron is different from the equilibrium potential of the passive ion channels and the currents depend only on the difference between the current membrane potential and the equilibrium potential of these ion channels. So once the membrane potential is fixed at a command voltage the difference from the equilibrium potential will also be fixed so the leakage currents will remain constant throughout the duration of the voltage clamp.

On the other hand the voltage gated ion channels if these conductances can change over time even if the membrane potential is fixed at a particular value but these conductances can change over time and so the current through these ion channels can also change over time. So if we are measuring compensatory current in a voltage clamp experiment and we see some component of it that is changing over time then we can know that it is not because of capacitive current or leakage currents but it must be through some active conductances. So imagine we are doing a voltage clamp experiment on a neuron whose resting membrane potential is around -60 millivolts. So in this experiment we let the neuron be at -60 millivolts in the beginning and then at some point we clamp the voltage at -50. So the membrane potential suddenly goes from -60 to -50 here and then we keep the command potential at -50 for a certain duration and then after some time we bring it back to -60 and then leave it there.

So this is the profile of the command voltage. It's a step from -60 to -50 for a certain duration and then it is brought back to -60. Now let us consider what the currents will be that we can measure through the compensatory current. So in the beginning the compensatory current is zero because the membrane was at its resting membrane potential. Now at this point where the membrane potential goes from -60 to -50 millivolts in a very short span of time the membrane is increasing in its voltage.

Membrane is getting charged so there must be a large compensatory current flowing in to charge the membrane from -60 to -50. So we will see a sharp pulse of compensatory current and then this will quickly become zero again. Compensatory current is there in the beginning and similarly there is a opposite compensatory current when the membrane potential goes from -50 to -60. The membrane is becoming more negative in a short span of time so there is negative compensatory current at that time and then it becomes zero later and in between the capacitive current will be zero. Also at this resting membrane potential the leakage currents were zero but when the membrane potential becomes more positive the difference between the membrane potential and the equilibrium potential of leakage channels becomes large.

So the leakage currents will flow out of the neuron to make the neuron more negative which means that the compensatory current will flow in to compensate for the leakage currents. So we will see a positive value of the compensatory current and this value will correspond to the magnitude of the leakage currents. So if we ignore the voltage gated conductances for now then the capacitive current and the leakage currents will look something like this. So we will see the sharp pulse here and then a constant value that corresponds to the leakage current and then a negative sharp pulse here. But once we consider the voltage gated conductances then the picture becomes more complicated because it is not easy to figure out whether the observed current is through voltage gated sodium channels or voltage gated potassium channels and that's where some chemicals become very useful and one such chemical is called tetrodotoxin or TTX.

Tetrodotoxin is actually a poison that is derived from puffer fish or some other animals. This is what a puffer fish looks like. It's actually a pretty interesting creature. The reason it is called puffer fish will become obvious if I show you another picture here.

So it's the same fish. This is actually a defense mechanism. So if it sees a predator then it puffs a lot of water in its body and it becomes this giant large ball filled with water that is too large for most animals to eat it. So it's a nice defense mechanism but sometimes this may not be adequate because this takes time and in the meanwhile some fish can come and eat the puffer fish. So there is another defense mechanism that this creature has and that is tetrodotoxin which is present in its body and this can act as a deterrent for other fish to eat it because it's really poisonous. It's in fact thousand times more poisonous than cyanide.

It's also very poisonous for humans and the way it works is by blocking the voltage gated sodium channels. Now this might seem like a very simple thing and it might seem strange that how this blockage of sodium channels has this strange effect but if you think about it the voltage gated sodium channels are involved in the generation of action potentials and because action potentials are important for the functioning of most neurons, TTX can basically paralyze most of the neurons in the body and because neurons control so many aspects of body functioning it can

have a really drastic effect on the body. In fact this is how most natural poisons function. So even many poisons derived from snakes also affect some part of the neural activity in the body. They may affect other channels or other receptors in the brain and because the neural activity is controlling various muscles in the body any disruption can lead to heart attack or breathing problems or other such severe problems which can easily result in that.

For our purpose, tetrodotoxin comes as a handy tool because now we can block the voltage gated sodium channels selectively and that can help us in resolving whether the observed current is through sodium channels or potassium channels. Thank you.