Introductory Neuroscience & Neuro-Instrumentation
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Lecture No. 11
The Action Potential (1)

Introductory Neuroscience and Neuro-Instrumentation, the first lecture on the Action Potential

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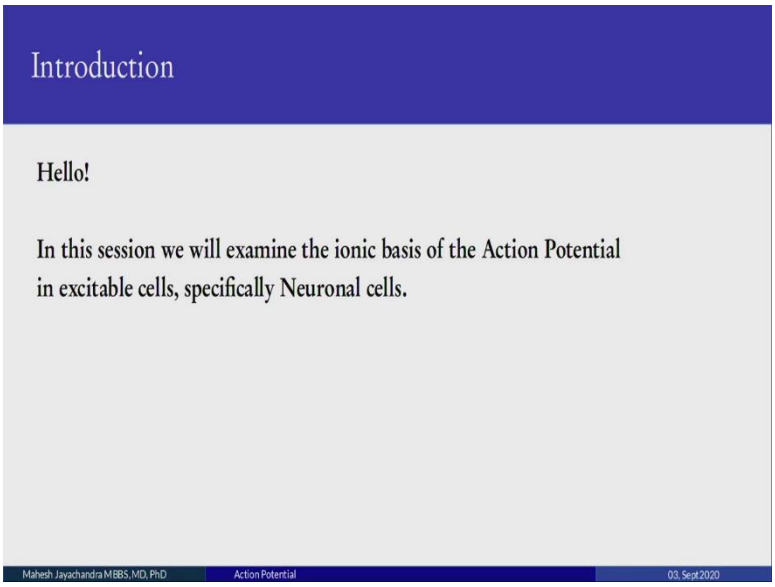

Hello in this session we will examine the ionic basis of the Action Potential in excitable cells, specifically neuronal cells. There are other excitable cells in the body, electrically excitable muscle cells, cardiac cells but we are talking about neuronal cells.

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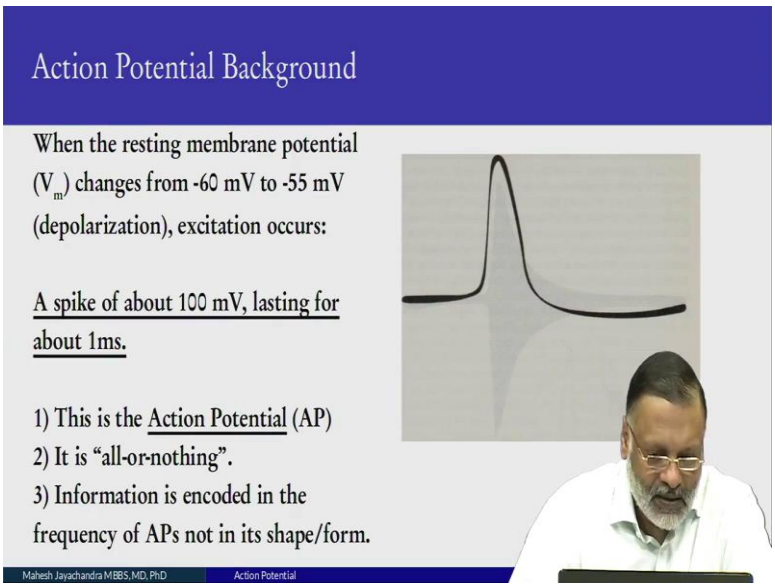

So, the background we learned in the previous lectures that the resting membrane potential of a cell is about minus 60 mill volts in this squid joint axon, and it can be even lower than that in mammalian neurons. So, the resting membrane potential V subscript m when it changes from minus 60 to minus 55 that is depolarization happens then all get excited.

Now, it has to reach a threshold. So, the threshold is about 15 to 20 millivolts and with that, you get a spike of 100 millivolts and at last for about 1 millisecond approximately. So, this is the action potential and the first thing is it is all-or-nothing so when the membrane threshold is reached then you have the spike. It is invariant and the information is coded in the frequency number of spikes that occurred, it's not coded in shape or form.

So, this is the critical point to note that the information of an action potential is coded in its frequency and it is all-or-nothing so it occurs when the membrane potential reaches the threshold.

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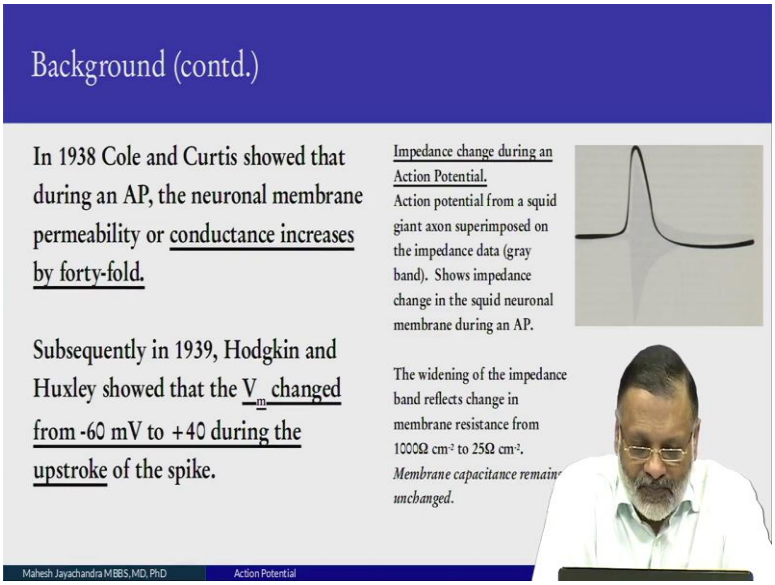

So, in 1938, Cole and Curtis showed this is in marine biological laboratory Woods Hole Massachusetts, they showed that during the action potential the membrane permeability or its conductance to various ions increases 40 times from normal. So, subsequently the next year Hodgins and Huxley showed that the membrane potential changes from minus 60 millivolts to plus 40 nearly 100 millivolt change during the upstroke of the potential.

So, on the right, you see the impedance change during an action potential. So, the action potential is the thick black line and it is superimposed on the impedance data which is the grey band and you see during the time course of the action potential, the grey band increases

substantially, and then it comes back to normal. This widening of this impedance band reflects a change in the membrane resistance from 1000 ohms per square centimeter, it drops to 25 ohms per square centimeter.

Please note that the membrane capacitance does not change, it is a passive property depends on the phospholipid layer so that does not change. Now, how do you measure impedance? Now, if you remember from your high school that is a concept of Weston bridge where you have four resistors and they are all balanced and then they are balanced the central galvanometer is at 0 so one of the arms of the Weston bridge is the impedance measurement from the squid so that tells us what the impedance changes are.

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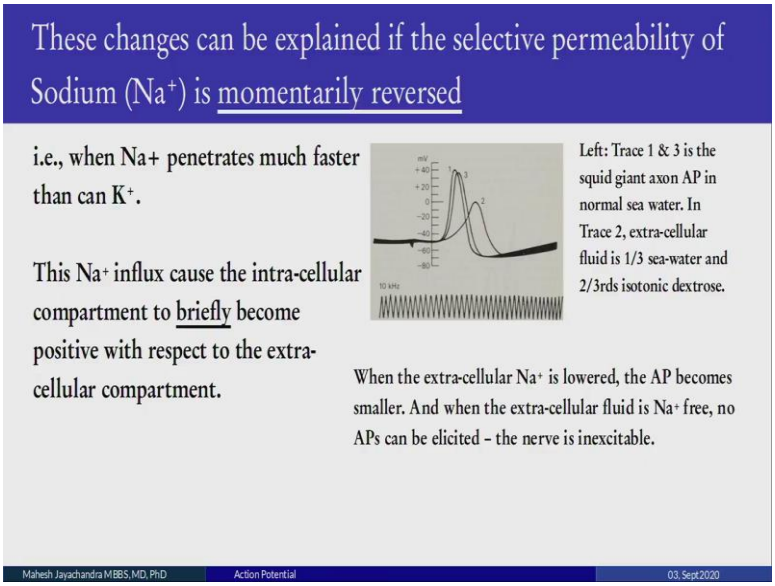

So, how do we explain these impedance changes? I mean what happens? Because it comes back to the baseline. So, these can be explained if the selective permeability to sodium is reversed for a brief period that is sodium penetrates much faster than potassium, this sodium influx causes the intercellular compartment to become positive briefly with respect to the extracellular environment.

So, looking at their actual data and some of their data still unrivaled in its clarity and clearness you see traces so trace 1 and 3 is the action potential of the squid giant axon in normal seawater. In 2, the sodium outside one-third of it has been replaced with isotonic dextrose and you see the action potential is much smaller. 3 is when you replace it back and then it comes back to normal.

So, when the extra-cellular sodium is lowered the action potential becomes smaller when its totally sodium free then there are no action potentials. So, the action potential, the initial part of it is completely sodium ion-dependent.

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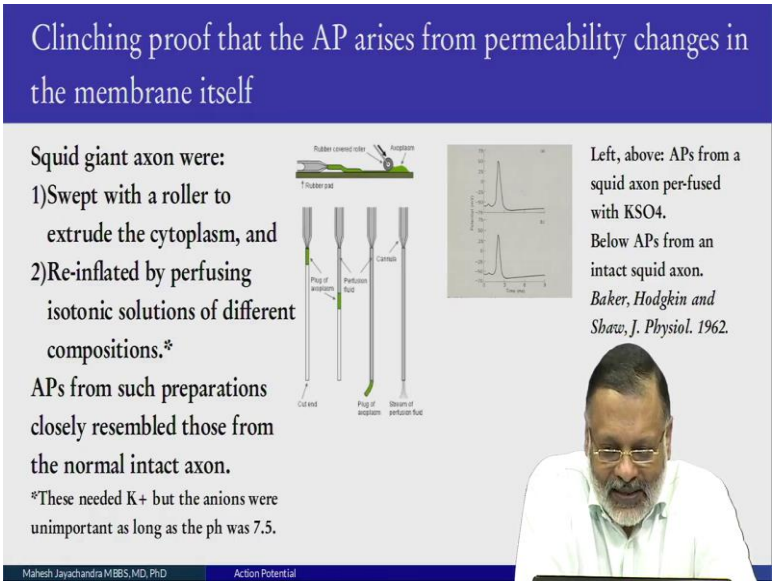

So, how do they prove it? That are actually from the membrane? So, this was referred to an earlier lecture so you take this squid giant axon and you actually have a small roller and you roll out the cytoplasm inside and then you re-perfuse it with whatever solution you want, it has your potassium solution and it does not really matter it could be any potassium solution as long as the ph is around 7.5.

So, you can replace the intercellular fluid with solution potassium sulphate action potentials look practically the same as it were with regular intercellular contents. So, on the right you see that area where the top figure is action potentials from a squid axon per-fused with potassium sulphate, and below are action potentials from the intact squid giant axon.

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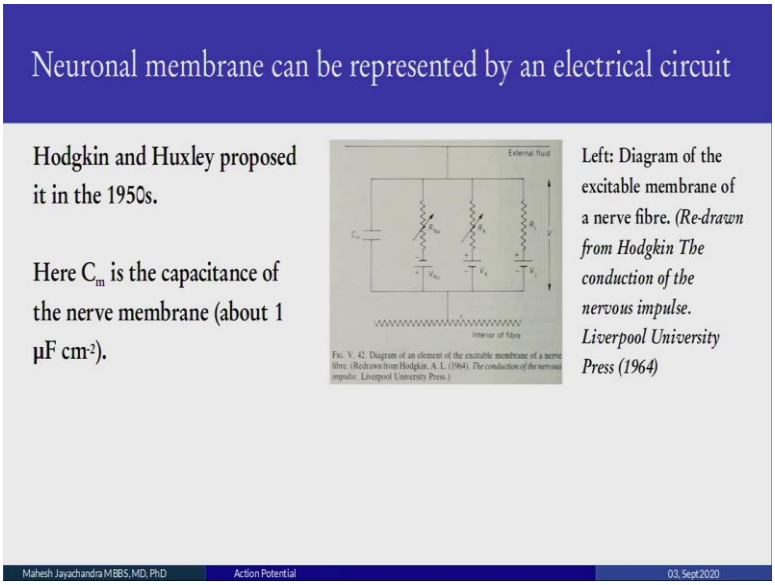

Neuronal membranes can be represented as an electrical circuit, all you engineers would understand this. It was proposed by Hodgkin and Huxley in the 50s and here C subscript m is the capacitance of the nerve membrane usually it is put at one micro ferret per square centimetre. In the middle is the diagram of an excitable membrane of a nerve fibre.

So, cm is the capacitance R_{Na} is with its battery below is the sodium channel R_k the resistance subscript potassium is the potassium channel and these are the main conductances And you also have an r leak which is small conductance where things it is not a perfect compartment so things keep leaking out and there will be a small potential due to that.

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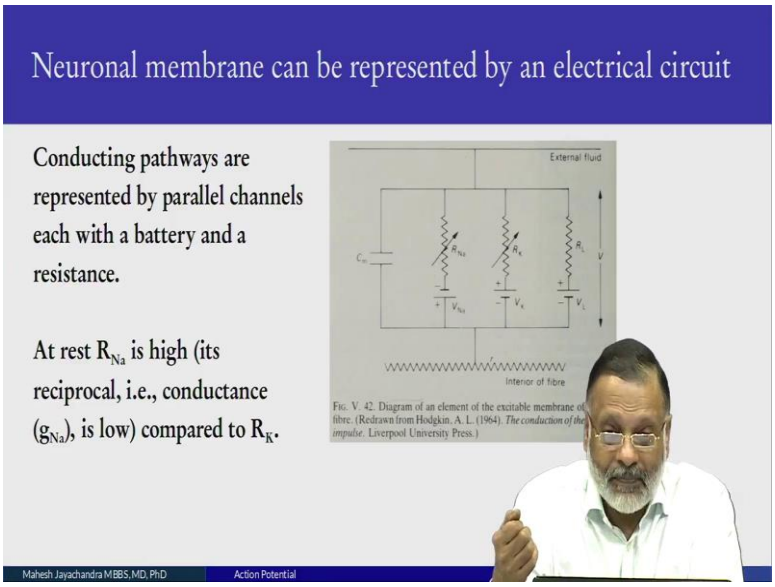

So, the conducting pathways besides the capacitance they are represented by parallel channels because in the membrane with the phospholipids membrane, you have sodium channels, you have potassium channels, so each of these channels has a battery and a resistance, the battery is the electromotive force which pushes the ion into or outside the cell.

So, at rest, the resistance for sodium is high which means, its reciprocal which we actually used rather than resistance which is g that is denoted by g which is the conductance is low compared to potassium.

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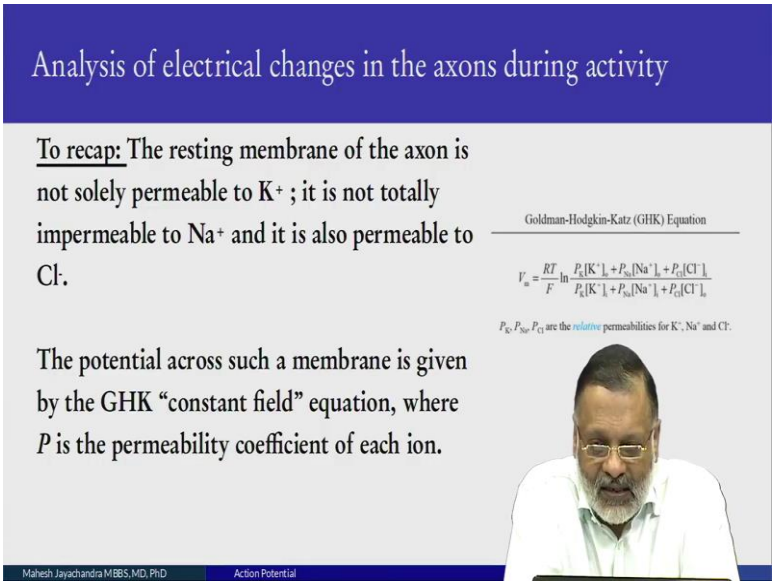

So, let us analyse some electrical changes in axons during activity. First to recap the resting membrane potential of an axon is permeable to sodium but not solely permeable to, I am sorry, the resting membrane potential to the axon is solely permeable to potassium, it is not impermeable to sodium and is also permeable to chloride. So, the potential across such a membrane is given by the GHK field equation, where P is the permeability coefficient of each ion or the conductance.

So, if we look at the equation it was we went through it in an earlier lecture. Instead of just potassium which gives us only the potassium ion affects, we also add in sodium and chloride and we call it a constant field equation because we assume that within the phospholipid membrane the potential is constant. So, when you put all these things aside then you get a realistic value of the resting membrane potential.

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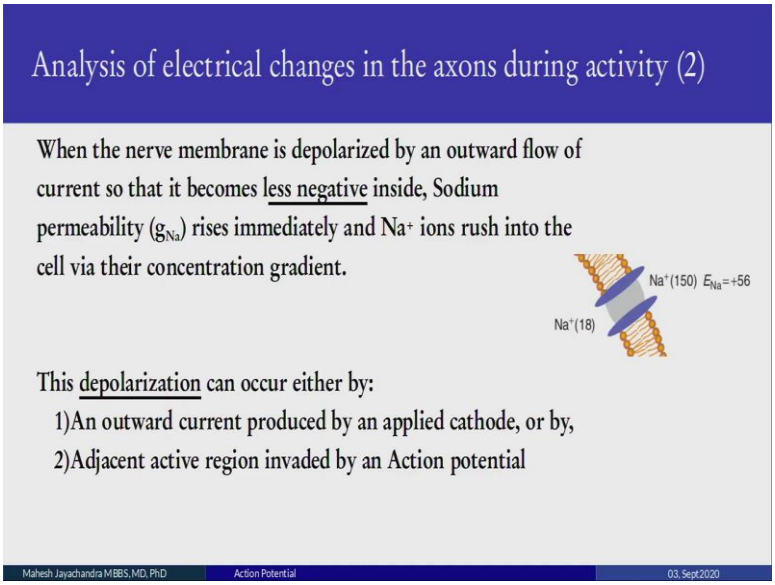

So, when the nerve membrane is depolarized by an outward flow of current so it becomes less negative inside, sodium permeability or g subscript Na rises immediately and sodium ions rush into the cell because of the concentration gradient, if you remember outside is seawater sodium is about 150, inside is about 18 to 20. So, it is huge rush of water rushing in our concentration gradient since water falling from a height you think of sodium ions. So, how does this occur, how does it depolarize?

So, it can occurs in two ways, you can cause an outward current by applying a cathode or the adjacent segment or area has an action potential that action potential invades this segment and causes depolarization.

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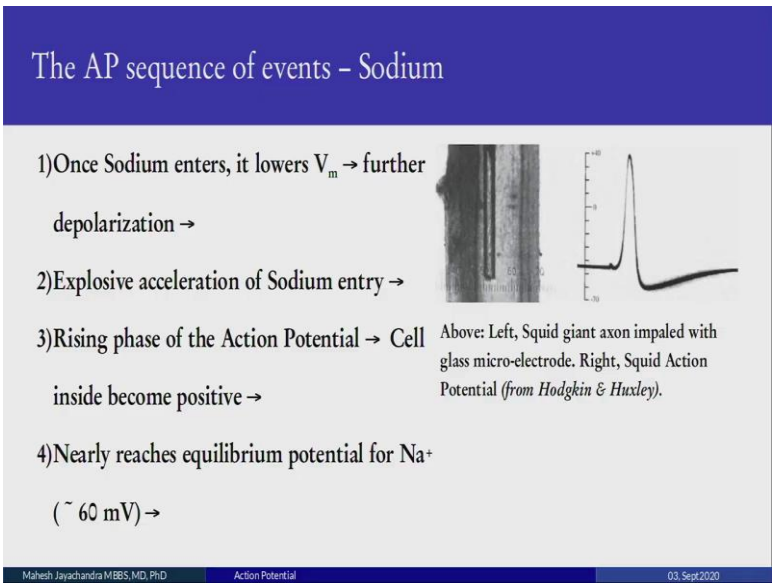

So, once sodium enters it lowers the membrane potential and causes further depolarization and then there is an explosive acceleration of sodium entry and that is the rising phase of the action potential, the cell inside becomes positive. It really nature reaches the equilibrium potential for sodium if you remember it is about plus 60 millivolts and if you see on the right you have the action potential, you have the intercellular electrode and that is in this squid giant axon.

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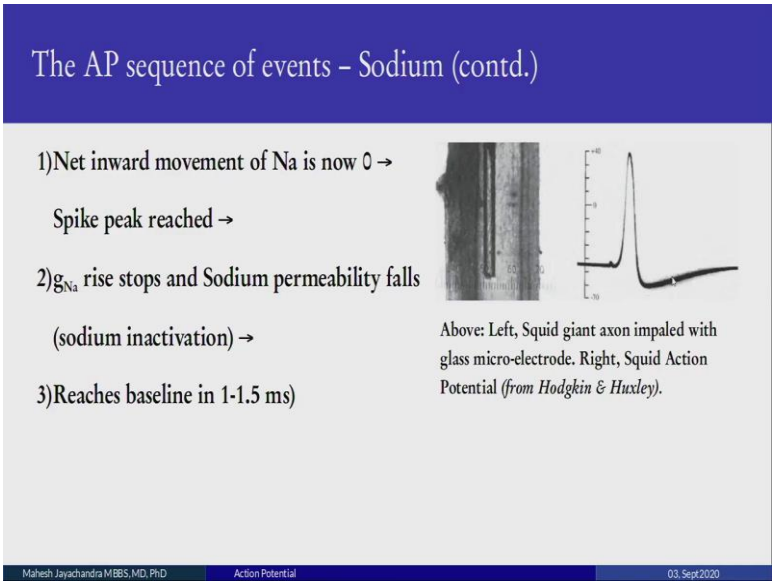

So, then what happens is once it reaches the peak the net inward movement of sodium becomes 0 and that is the peak. So, the conductance of sodium stops, and sodium permeability falls. This is called sodium inactivation. And it reaches baseline in about 1 to 1.5 milliseconds.

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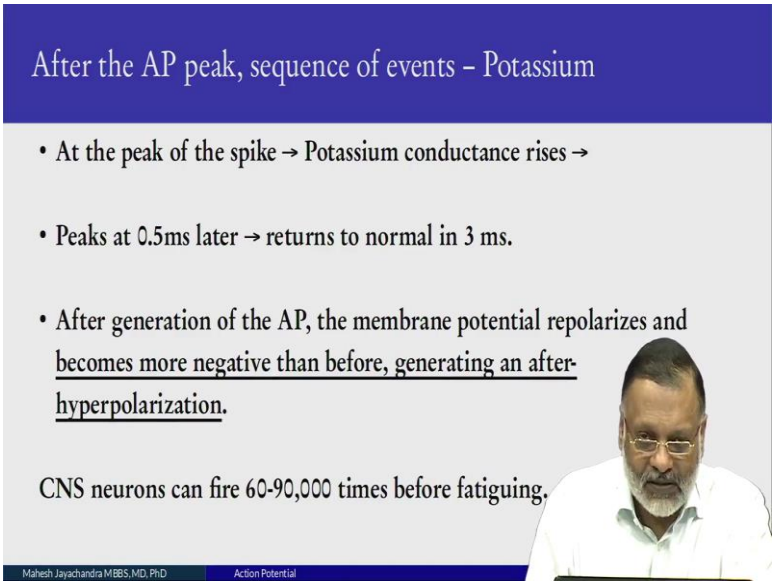

So, what happens with potassium? So, at the peak of the spike, the potassium conductance rises, not with the sodium but a little delayed. It peaks about half a millisecond later and returns to normal in 3 milliseconds. After the generation of the action potential, the membrane potential repolarizes, becomes more negative and it goes below 0. So, here it comes down, and then it goes below 0. So, this is called hypopolarozation.

It is gone below 0. This is depolarization and this is coming back to normal and this is hyperpolarization. And you know it is also called an after-hyperpolarization because it occurs after the action potential. Now, please bear in mind that a neuron can fire 60,000 to 90,000 times before it fatigues. So, it is very very efficient compared to all electronic devices and there is a lot of redundancy build in, these are huge safety margins. It fires because it losses energy and the energy has to be made up with the ATP phosphate transfer. But 60 to 90,000 times, is good to go.

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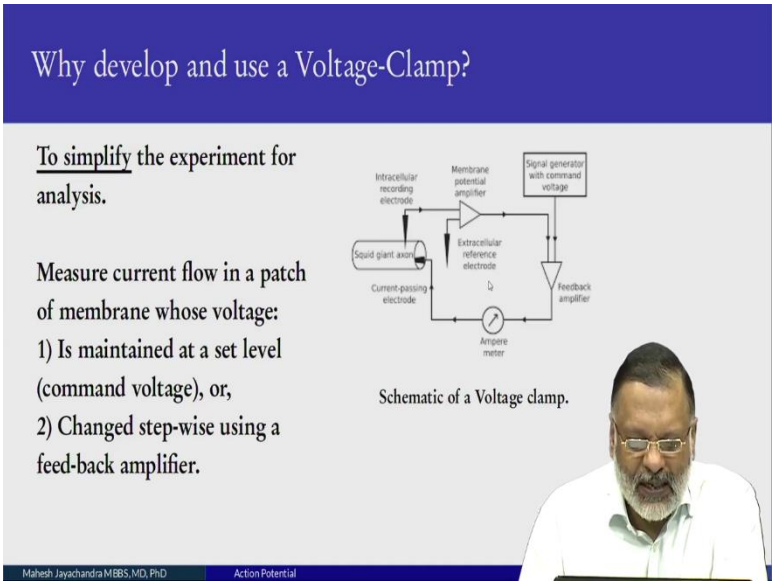

So, now coming to the voltage clamp, we can measure from inside the cell, no problem. Why do we need to clamp the voltage? So, we do it to simplify the analysis. With the voltage clamp, we can measure current flow in a patch of membrane whose voltage 1 is maintained at a set level. This is called the command voltage or it can be changed stepwise using a feedback amplifier.

So, you see the circuit on the right, you have a squid, you have a current passing electrode and then you have a signal generator with a command voltage and this is the kind of circuit that is used. We will get into details subsequently.

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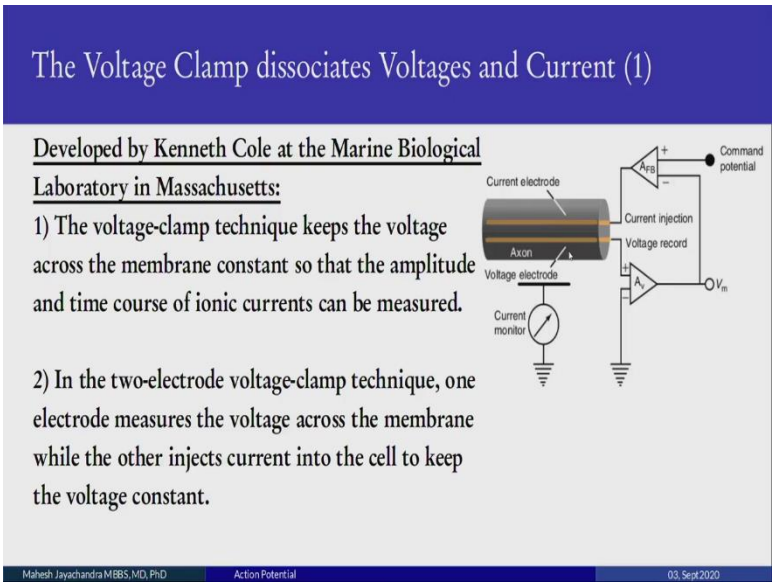

So, what is the advantage of the voltage clamp? So, remember potassium things are happening, the sodium things happening, voltages are happening and current is happening. So, this technique dissociates voltages and currents. So, we can hold the voltage constant and see what the current is or we can current clamp it and see what the voltage is. So, these techniques were developed by Kenneth Cole at MBLM, Massachusetts.

And quickly, they were used by Hodgkin and Hugely in Plummet in Cambridge and they figure out the action potential with this technique. So, consider this circuit on the right, you have the axon, you have a current electrode and you have a voltage electrode. So, the idea is you keep the voltage constant and you see how much current passes at that particular voltage. And not only the amplitude but also the time course. So, in the two electrodes, the classic voltage-clamp technique, one electrode measures the voltage across the membrane while the other injects the current to keep the voltage constant.

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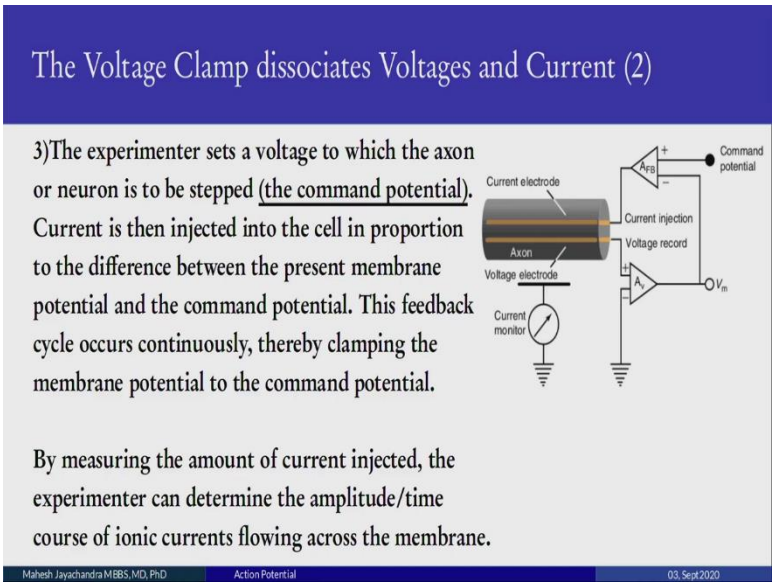

So, the experimenter sets up the voltage where the axons should be held. This is the command potential. So, the current is then injected into the cell in proportion to the difference, the delta between the present membrane potential and the command potential. This occurs continuously, therefore, clamping the membrane potential to the command potential. So, by measuring the amount of current injected to hold it at the command potential, we can determine the amplitude as well as the time course of ionic currents following across the membrane.

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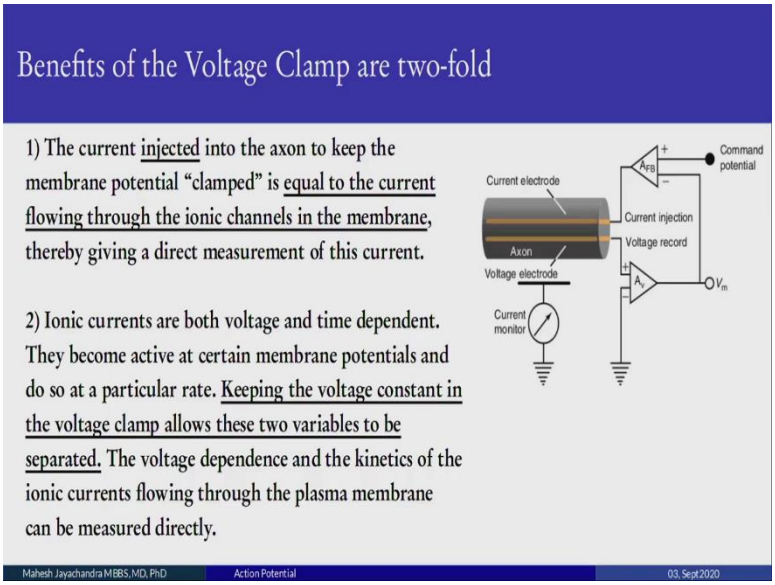

So, the advantages. The current injected into the axon is equal to the current following through the iconic channels of the membrane. Remember, current in membranes is not electrons but ions, and therefore you get a direct measurement of this current at the particular voltage. So, ion currents, ionic currents, sodium, potassium are both voltage and time-dependent. They become active at certain membrane potentials and do so at a particular rate.

So, keeping the voltage constant at the voltage clamp, allows these two variables to be separated. The voltage dependence and kinetics of these ionic currents can therefore be measured directly. This was not possible before, this technique.

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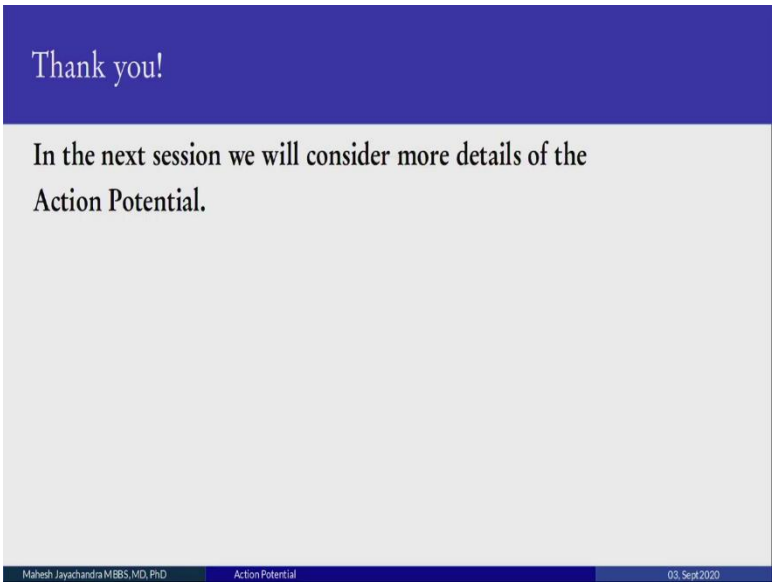

So, thank you and in the next session, we will consider more details of the action potential.