Bioelectricity
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Lecture – 11

Welcome back to the lecture series on NP-TEL on Bioelectricity. So, in the last lecture, we talked about we initiated the patch clamp in the last half of the lecture, I introduced the patch clamp. So, we talked about how you develop the electrodes and I told you there are the name itself called clamp. So, essentially you are holding clamping a part of the membrane and at one part of the membrane by following the equivalent circuit model, you can clamp two parameters; either you can clamp the current that is why it is called current clamp or you can clamp the voltage, it is called voltage lamp. So, will resume our discussion from that point that what all parameters you can measure.

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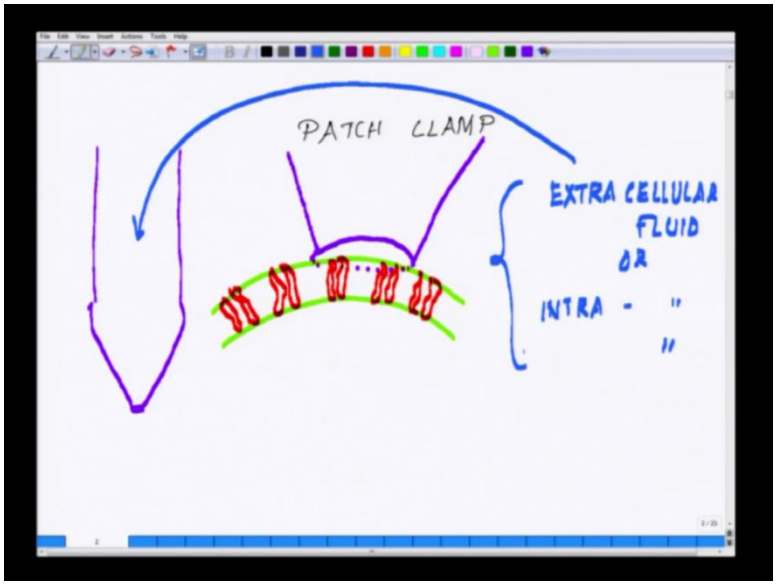

So, once again we are in to lecture eleven, so resuming on patch clamp, so this is where we were. So, in the last lecture, while I was giving you the dimension I told you that this techniques helps you to approach a finite numbers of ion channels. So just to have a recap, what I just trying to tell you, if we call this as a membrane, this is the membrane structure you have and on that you have this embedded channel structure, which are sitting along, this are the embedded channel all along it. Just for the simplicity sake, I am drawing seven or eight channels out here, and then your patch pipette which has a

dimension of one micron tip something like this. You can really hold a finite number of channels under the patch. So, what you do once you, so this is just slightly advance. So, let me come back what you do once have the electrodes ready with you.

So, now, what you are having is essentially you have something like this, you have pulled it and with a tip like this and something like this. Now you have two options, you can fill this electrodes either with extra cellular fluid or intra cellular fluid, extra cellular fluid or depending on the configuration you want to follow intra cellular fluid, these are the two options.

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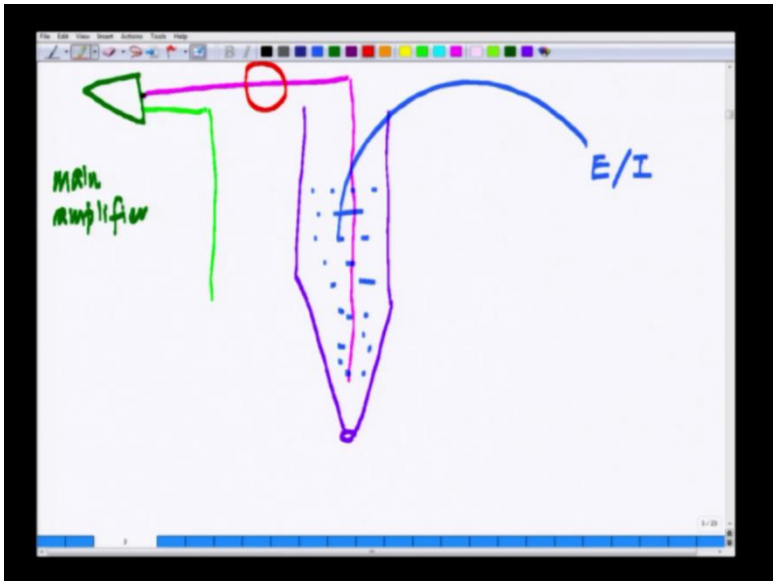

So, now once again lets draw the patch electrode, here you have the patch electrode, and either intra cellular or extra cellular, extra or intra. Then inside this, you put a silver electrode, here is the silver electrode. Silver electrode is first is connected to a something called a head stage amplifier, all the initial amplifier out here, and from here it goes to the main amplifier. And whereas, you have a another electrode which is your ground electrode which is let show it like this, which is essentially there here. So, here you have the main amplifier, here you have the ground electrode, and in between you have something called a head stage amplifier which is out here.

So, the way it works you have to understands the logic, the way it works, there are two amplifiers here one is a small head stage amplifier which is picking up all the signals then it transmit the signals to the main amplifier. In the main amplifier, all this analog

signals are being processed. So what is an amplifier? Amplifier is essentially, is a device inside it contains very powerful electrometer that electrometer is essentially measuring the charges and they amplifying that current, because since you are receiving current at a very small regime like you know nano ampere, pico ampere, so you have to really amplify that in order to distinguish it from the surrounding noise.

So, those current eventually these are all analog currents you are receiving. These analog currents are now send to a deck card which is a digital to analog to or a d card analog to digital converter – adc. From analog to digital converter, now these digitize signals, so what is happening essentially like this.

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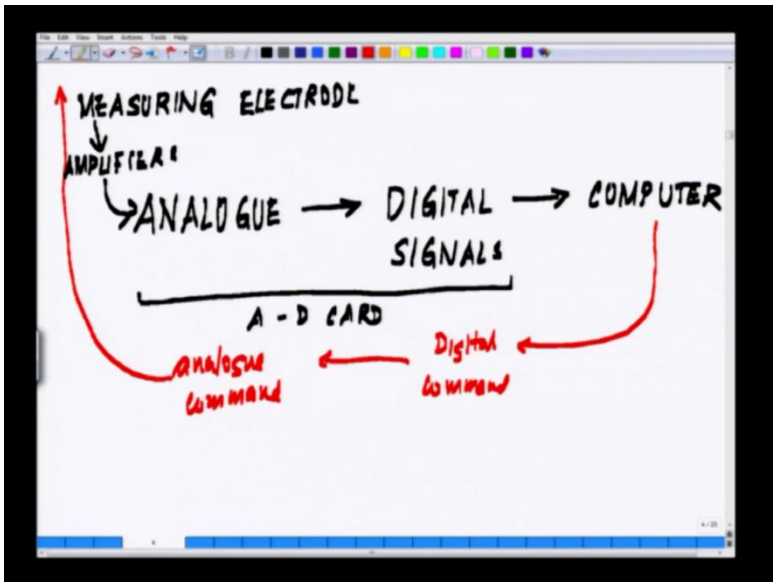

So, what you are you receiving from the pipette is analog signals, these analog signals are converted into digital signal. These digital signals are then sent to the computer for analysis. You can really see it in the computer screen, but nowadays with the modern electronics what you essentially can do you can use the computer to give the command also. So, what you need for the signal, which is coming from the pipette like this. This is coming from the from the measuring electrode, if I just make it slightly more precise from measuring electrode to the amplifiers from amplifiers, it goes to the analog the ad card and similarly the same computer now can give a command which will be the reverse root which it gives a digital command again a digital command.

This digital command is converted into analog command because that what a cell will understand this analog command is eventually send to the cell. So, it is pretty much two way traffic, you can use a computer both for recording as well as giving commands. Though initially when patch clamp discovered computer was slowly like you there not many computer pc's, there this is back in nineteen seventy, but the modern electronics has kept us with all this features that this whole process has become much more smooth. You really can acquire other lot of data.

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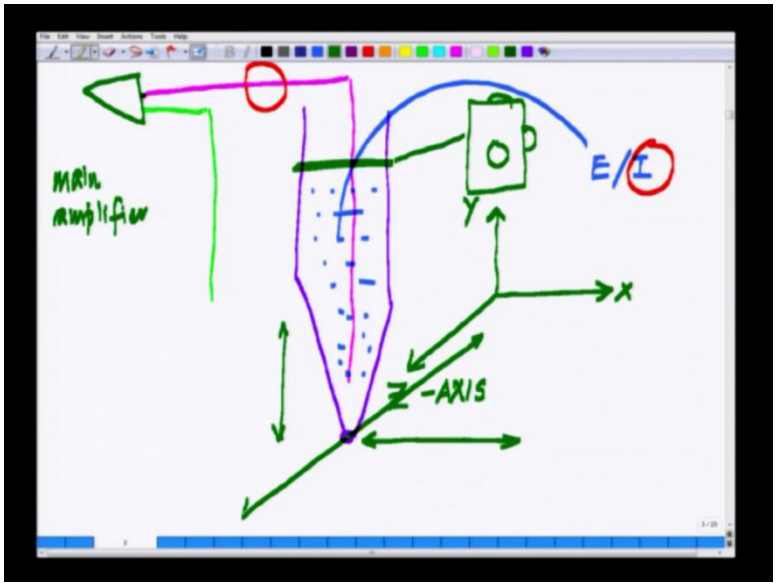

So, coming back where I was stopping? So, now, you have this main amplifier here. So, with this setup let me introduce let us assume to start of first that we fill it with intra cellular solution fine, now this electrode is fit on a micro manipulator. So, this micro manipulator where it is fit it has the potential to make this electrode move in all the three axis. So, let us introduce micro manipulator come up front here. So, it is connect to the micro manipulator where like this knob you have this knob then you have this knob. So, that ensures that you can move it in x y and z axis in all the axis you should be able to move the micro move this electrode. So, essentially this electrode can move like this, this electrode can go up and down and this electrode can travel a distance something like this also.

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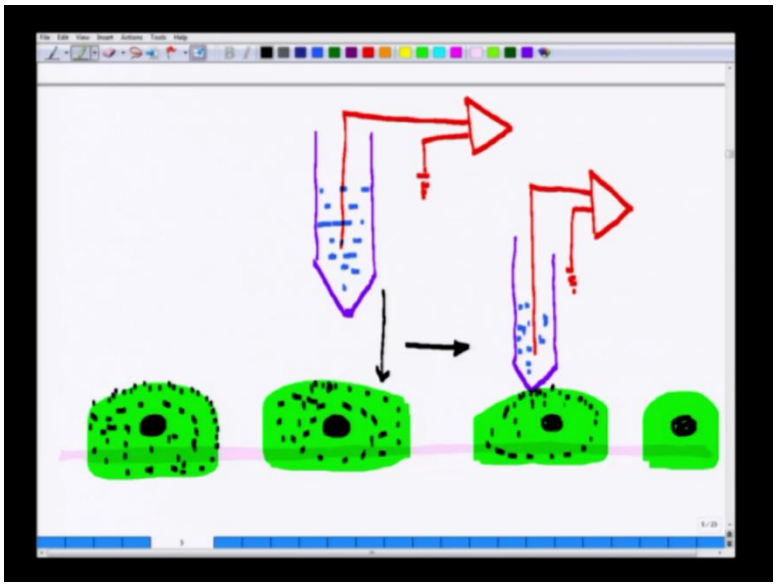

So, the practical application, say for example, now I let us see practical situation where we have the culture dish, but this is the cell culture dish out here, and this is the matrix on which the cells grow on this matrix. Now, let us put the cells. So, let`s represent the cell by this are the individual cell sitting. So, now we would not to study, this study the electrical properties on the individual cells. So, let us be more realistic to look through. So, this the nucleus this also sitting and here we have a patch electrode with us let us put the patch electrode in place and inside the patch electrode you have one of this silver and it is filled with the intra cellular fluid to start off with. So, now using the micro electro micromanipulator you are approaching the cell.

So, basically going down on the cell eventually what will happen? If I this share the stage two you will have the tip of the electrode touching on top of the cell and this is the amplifier. So, this exactly the situation. So, if I represent the entire ion channel on top of this, as leg something like this if you construct this black dots are the ion channels on on the surface of the cell. So, now we have finite number of ion channels in a very close proximity of the electrode, this is very important because this is the diagram which I was trying to show in this slide.

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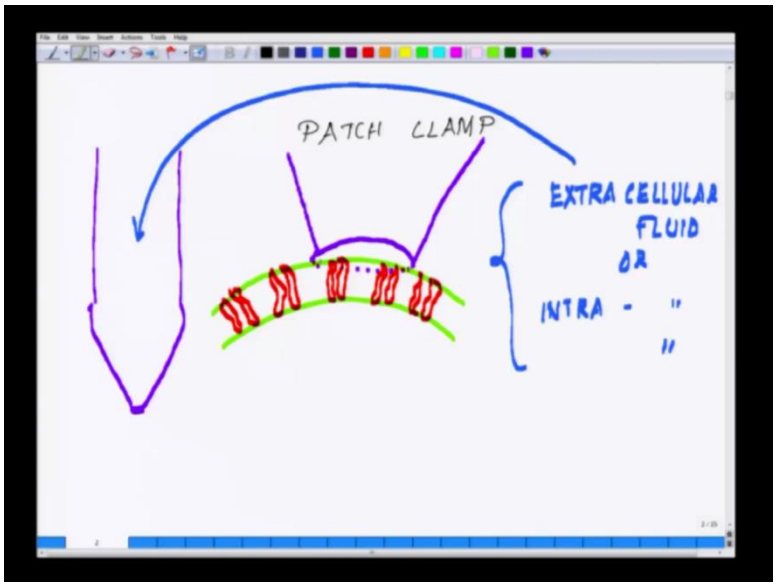

So, now what you have finite number of ion channels under that small pipette which is approximately zero point five to say one micron at this stage there is a very simple technique is being done along this. In this I did not introduced that now I am going to introduce it you have small tubing which is connected to it for giving a gentle suction and we will see what happens when you give a gentle suction at this stage. So, let us move on to the next slide lets magnifies this confirm this configuration. So, so that it becomes make more sense.

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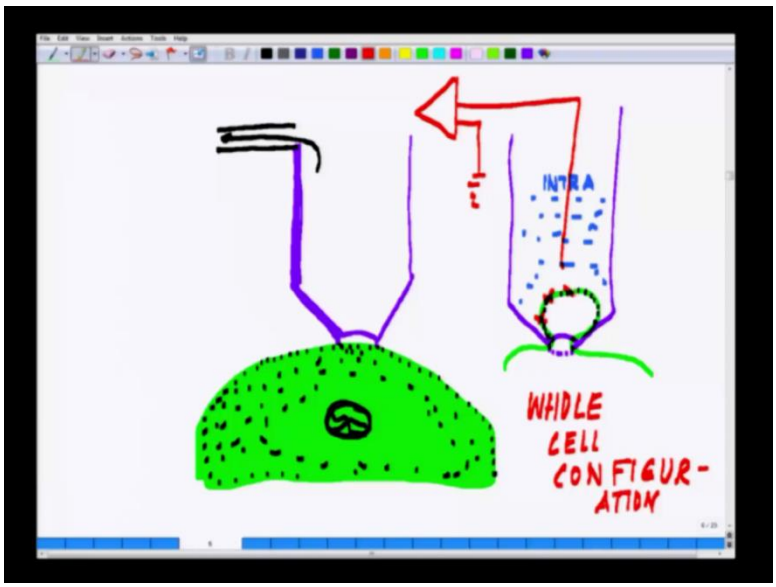

So, here is our cell sitting here something like this individual cell and we want to patch this cell and what we have our a series of ion channels on its surface. This black dots are the ion channels the I was showing in the previous slide they are all over the place all scattered around on the surface off course and here you have the nucleus of a cell. So, now you are patch electrode configuration let us look at it at the patch electrode is sitting much more magnified zone your patch electrode is sitting something like this almost touching on the surface now at this point you give a gentle suction that is what I was trying to show in the previous slide.

You just give a slide suction what will happen this particular part of the cell will get inside the patch pipette essentially this is what is going to happen. So, now, if I introduce the ion channels ion channels are sitting on top of this. Now we have started when you stared I told you let us assume that this is filled with intracellular fluid intra cellular fluid and here you have the silver electrode which is moving to the amplifier now at this stage there are two options first option is that the first configuration is that called the whole cell configuration. When you have the whole cells within your control in the whole cells what you do you send small impulse or a current pulse out here which is good enough to damage this membrane.

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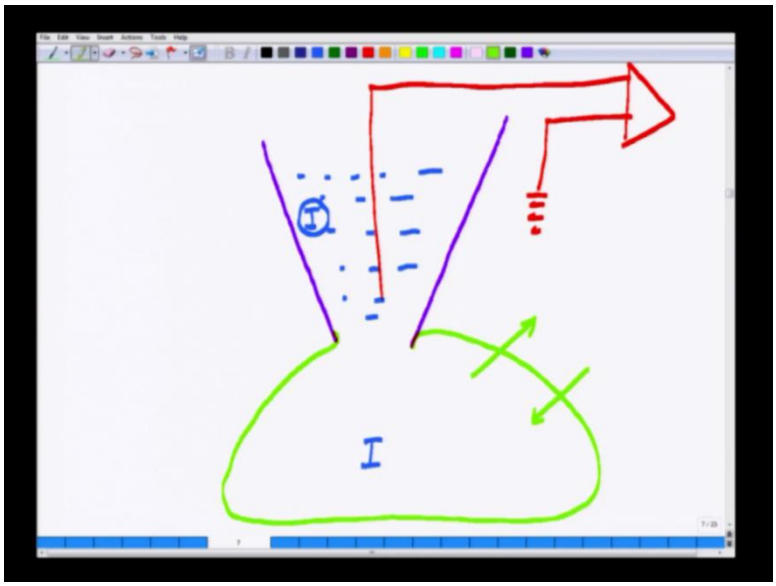

And what you are left with this this configuration in whole cell this is it then this is the configuration. You are left with that it is your electrode tip like this. And here is the cell

here is the electrode going out is the ground electrode and this is the intra cellular fluid filled in it mind it this fairly thin I am just for your understanding I am showing it slightly bigger. So, this is since this is intracellular fluid inside the cell you also have intracellular fluid.

So, these fluids are the same similarity. So, essentially now your electrode becomes part of the cell it becomes almost it is in continuous cell. So, what is ever current which are either moving out or inside the cell out here through this ion channels now could be recorded. So, this is the first and first and most I should say most important configuration to understand the whole cell electrophysiology.

Now what all you can do, let us see the power of this technique. At this stage you can hold the membrane at different voltages, you can hold the membrane at different voltage and you can measure the current or what you can do you can hold. You can inject finite amount of current inside this cell and you can measure the change in voltage. So, first of all let us try to do that step one.

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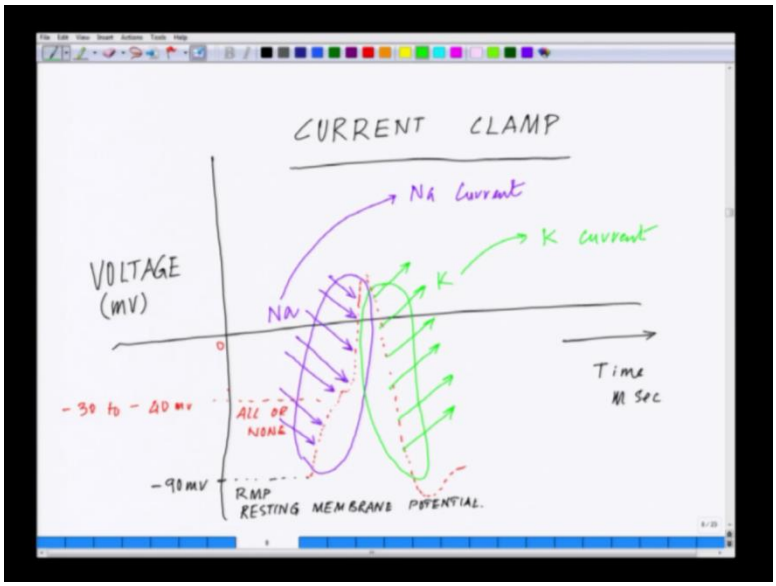

So, first of all do the current clamp and then we will do the voltage clamp current clamp is essentially mean you are injecting finite amount of current inside the cell and changing its polarity. So, if you go back here let us see how the current clamp is going to work. So, once now in a current clamp mode. So, whenever we are doing a current clamp what you are measuring out here you are measuring the change in voltage. So, your y axis is

voltage in milli volt and your x axis is time milli seconds and the cell we know sitting at minus ninety milli volt. So, this is the resting membrane potential RMP -resting membrane potential.

Now as you are injecting positive charges is into inside the cell let us see here you are introducing say for example, imagine like this you are introducing positive charges inside the cell. So, what will happen is it will a very similar situation as that of sodium getting inside the cell. So, this will immediately you will open all voltage gated sodium channel and all the voltage gated sodium channel which lead to enormous flux of sodium ions inside the cell. So, as sodium ion is getting inside the cell this is what you are going to observe the membrane voltage is start to go toward the positive side. So, this is zero this is going to go positive like this and if it reaches something like say minus forty or minus thirty.

So, if you call this minus forty and here is slightly above minus thirty then this shoot something called phenomena called all or none its almost the membrane as if it looks like it collapses because then there is no way to cannot look back. It over shoots zero like this that is call or none, but it has to reach to that of minus forty and between minus thirty and minus forty milli volt. and if that happens all the surrounding voltage gated channel start opening up and they will shoot an action potential and then off course it again comes back because this the time when all the potassium channels starts opening and the rest is we have already studied.

So, this is how you do a real current clamp measuring where you are seeing action potential, but now the challenge is how we know at this part of the curve where there is influx of sodium there was enormous flux of sodium how I can measure the influx of this sodium. So, in other words this sodium is nothing but sodium current how I could measure the sodium current and how I could measure the other current which is the out here, the potassium current which is going out of the cell in order to do that.

Now we will move on to the next technique which is called voltage clamp now we have to measure the current in order to measure the current, we have to fix the voltage at different level.

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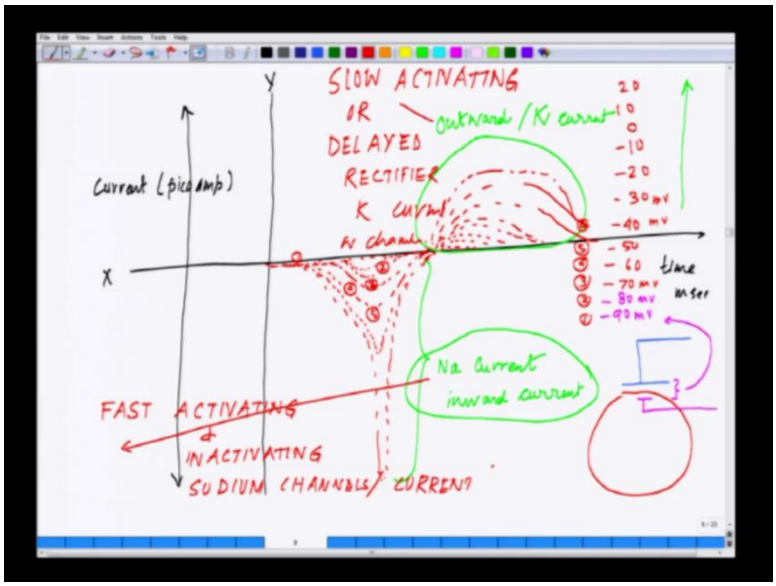

So, our now the way the recording will work something like this you are measuring the current and this axis and the y axis and the x axis you have the time current in pico ampere or nano ampere and on this if the time in milli seconds assume the cell is sitting out here the base line. So, this is we are assuming at the zero current and there is no current at this point now let us again go back to the configuration of the cell. So, this where we injecting current now expose this membrane to different voltages. So, it is sitting at minus ninety I make it from minus ninety I stared holding holding it across.

So, let me draw this. So, if you have this cell out here and you have two electrodes like this say for example, one electrode like this and there is another electrode here like this. Now you are changing across it the voltage minus ninety milli volt this is where the base line value is at this point then I make it minus eighty milli meter. I am holding the membrane of minus eighty across it once its minus eighty what I will see may be some of the base line, I will see a small dip. So, then I move to minus. So, so this is your it is it is the corresponding number one trace one this is your trace one, this is your trace two then I put it to minus seventy milli volt. Then I saw trace very similar three then one two three then I move to minus sixty, I saw another trace coming like this four minus fifty five minus forty this are all milli volt ha something like this minus thirty.

This is where I will see this is where all the potassium channels starts to open thirty milli volt then simultaneously. So, what is happening out here where like this now as i am

going further say minus twenty minus ten zero ten milli volt twenty milli volt what will essentially happen this sodium current which gets activated in narrow window of out here they would not a new for they will get close down. So, now, what we will be recording this side of the circle. So, axiom it is being followed that this current you can show this current on both on the upper side of the axiom, but it is generally people follow it to show it like this. So, this part of the circuit what you have to seeing out here of the current basically your sodium current or any form of inward current which is in entering inside the cell.

And out here what you see are the outward current or one of the major outward current is the potassium current. Now what you see eventually as you are going up with you voltage clamp traces it stars to see potassium traces like this and off course they have a range and this currents are much more delayed. So, this the sodium current generally this kind of sodium currents are called fast activating and in activating sodium channel or current which are ensuring the flow of this current and this currents are called slow activating or delayed rectifier potassium current or channels.

So, a just a word of caution there are several types where do have this fast activating or inactivating sodium current will come those. At this stage, we are not getting into those. We will come those later at this point, we are talking about most of the neurons, what they have they have this fast activating and inactivating sodium current out there. Now how to ensure that these are sodium current this is the challenging question. So, how you can ensure is this say for example, one second you have this cell out here with lets. So, the sodium channels in red and show the potassium channel in any other color.

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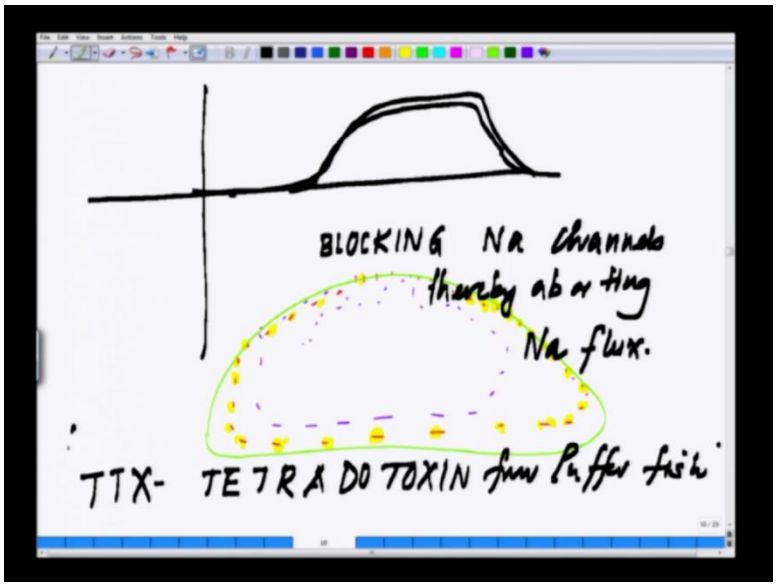

The way you can show this is say for example, you have a specific a very specific toxin which bind to one again which bind to sodium channels. So, say for example, it comes and it binds to this sodium channel. So, what you will essentially see is say for example, this is yellow is the toxin. What I am putting now and it is coming and binding to this sodium channels what I will see out here in this trace is this part of the current will be abolished, this is gone. This will not be there instead the trace will look something like this, what I will be essentially seeing is nothing and only the potassium current. So, what you have done is that you have block blocking sodium channels there by aborting sodium flux and this could be done using toxins like tetra do toxin, which is obtain from puffer fish which in short it also called t t x tetra do toxin from puffer fish.

This t t x could block the sodium channels. Similarly if I have another blocker which blocks the potassium channels, what I will see going back to this on sec. Let me go back to yeah, then what I can do is that I can abort this part of the cell this part of the current this will be lost.

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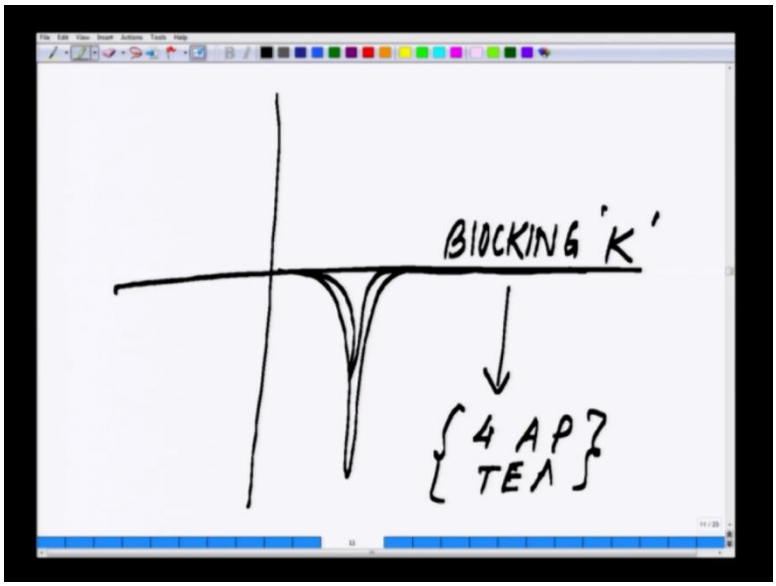

So, the way the trace will come is something like this the way it will come you have the sodium current like this, and that is it will have all the sodium current like this there would not be any. So, this is where you are blocking potassium and this you do using compounds like four a p tetra diethyl ammonium there is a series of compound which can block the potassium current.

So, you essentially see now we are able to access the ion channels which was absolutely not feasible with the sharp electrodes or even with extra cellular electrodes or any other known thing that was the reason why this discovery in nineteen seventies change the way we look and it was the same time when pretty much the same time when the p c r was discovered by Carry Malus. So, that open up a flood gate of cloning. So, what happens is that was the time when molecular biology was got a huge boost and electro physiology got a very huge boost in terms of the discovery by Erwin and Neehard on the patch clamp. So, all this techniques started you know marrying. So, people where developing cells where they could really specifically expressed sodium channels they could express potassium channels.

Then they could really access the individual cells with a profound sorority that yes, that is what is happening all the mechanism stated to come inside the play, and simultaneously that was the time when people started using molecular biology technique to let us see how all this things are being done. Say for example, now one more thing

which I have forgotten to tell you people is out here. So, when this cell comes in contact out here, this is where it form something called when you pull the cell, and go to this configuration out here it forms something called a giga ohm seal giga ohm seal giga ohm seal is very important. Because this seal ensures that there is no leakage along this. All the leakage are being prevented it has been really measured.

This was the ingenuity of the discoverer of the technique that you know, because between the glass and the cell there is a really a very strong seal, which is formed and with the modern software you really can see physically whether the giga ohm seal is formed or not something like this. This figure on mine is electrode and if this is the cell it form something like that a fantastic strong seal out there, which would not allow any leakage to take place. So, this is one thing which I just missed out while I was showing this while coming back what are the technique which are being used we talked about different toxin. So, this whole field depends enormously on different kind of toxins and a some of this toxins are also have a strategic important because they could be use by the terrorist.

So, there are lot of restriction in using in this toxins, but this kind of toxins have open up whole plethora of a approach to understanding ion channels, how they are binding? They are competitively binding or non competitively binding permanent blocker there, like wise this is the whole field in its own merits, where all this toxicology merges with ion channels with ion channel physiology.

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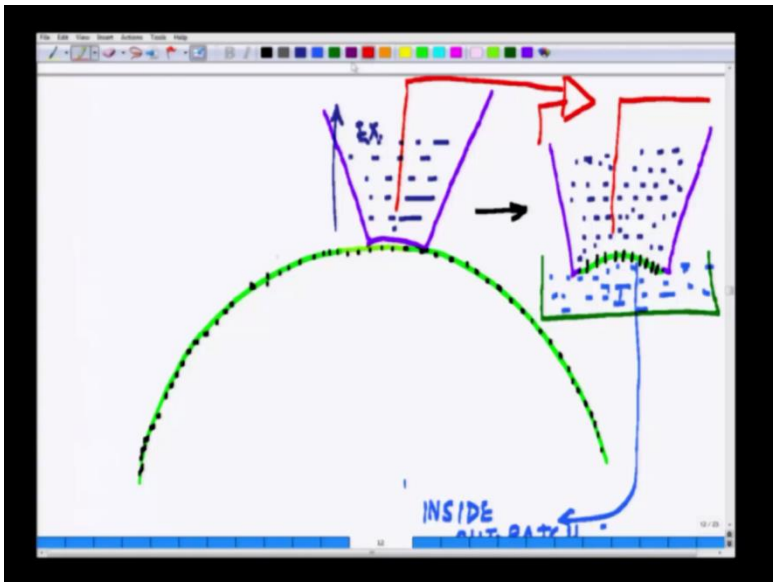

So, coming back how most of this, so whenever we talk about the ion channels, so I told you one configuration which is a whole wholesale configuration there could be several other configuration. So, say example I have this membrane out in front of me with the channels like this.

Let me complete the membrane, and all these channels are sitting here on its surface. Now, I take a take patch pipette the patch pipette comes here my patch pipette on top of a bunch of channels, it is my silver electrode and instead of filling this electrode with intracellular fluid, now I fill it with extra cellular fluid . So, now, in the fluid here is extra cellular, this is extra cellular fine at this stage instead of completely blowing out the membrane part of the membrane patch of that membrane instead of going into the whole cell mode. What I did now is that I chop of I just pull the electrode, because now it is in under the control of the we should see the micro manipulator I just gently pull the membrane.

So, what I will do essentially get is this configuration, you have the electrode inside the electrode you have this is the electrode what you have is part of the cell like this part of the membrane like this. And your finite number of ion channel in t i and inside this you have the extra cellular fluid which is filling it here, it is and then what you do you put it in a chamber or in a small dish which has intracellular fluid in it. So, here you have the intracellular fluid now what is this configuration essentially tells you, this you have a

complete control on manipulating this ion channels a finite number of ion channels now this is called here basically insight out patch inside of the membrane is now exposed.

So, this are especial configuration hence which are being done to you know. So, this are special different kind of configuration which are being followed to you know to understand the ion channels behavior. So, what I will do? Now I will talk little bit more about this one's this individual channels how they were studied. I will close in here, I accept you people really to go through this slides very carefully because there lot of information here go through the patch clamp understand the concept understand current clamp and the voltage clamp. And you only can access the different ion channels from here, what we will do? We will talk about how do the molecular biology techniques helped us in understanding the different ion channel gates ports voltage sensors, and all other things. So, I will close in here in the next class will discuss about all those things.