Bioelectricity
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Lecture - 10

Welcome back to the NP-TEL lecture series on Bioelectricity. So, today we will be starting our tenth lecture. So, in the last lecture, we talked about in details about micro electrode array, the planer micro electrode arrays. So, we talked about how this planer microelectrode arrays could be used in drug discovery, understanding the neuronal circuit, and understanding cardiac action. So, yesterday I showed you a circuit and there where I showed that you know you can make a small circuit with defined number of neurons, similarly you can place there are few other modifications what you can you could recreate. Say for example, we know that the cardiac myocytes or the heart cells are being regulate by the sympathetic and parasympathetic neuron. So, do the muscle cells are been regulated by a moto neuron.

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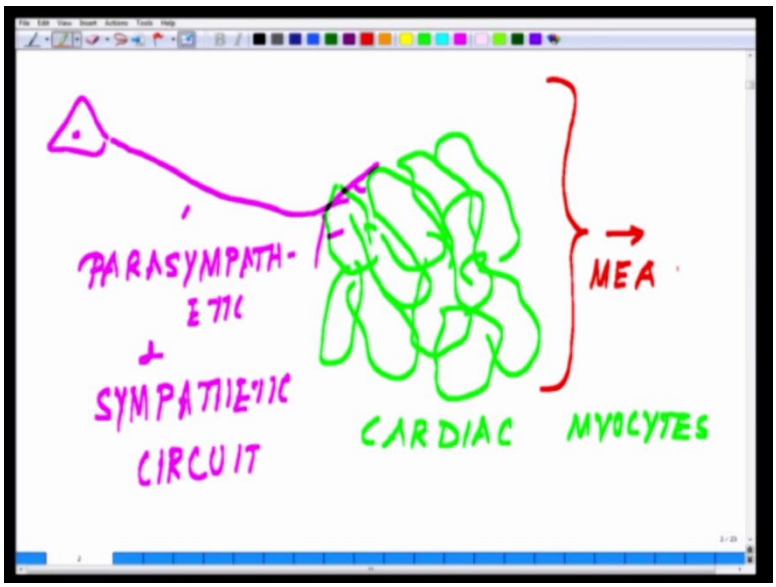

So, what you can do by the same patterning techniques you can rebuild those circuits something like this. Say for example, if these are the cardiac myocytes, the beating cardiac myocytes and we know this cardiac myocytes are being innovated by the sympathetic and parasympathetic neurons. So, what we can essentially do we can really study, so these cardiac myocytes and these are the parasympathetic and sympathetic

circuit. So, these sympathetic parasympathetic actually regulates the cardiac myocytes. So, what essentially do, you can translate this whole thing on in a micro electrode array to study the exact mechanism what is happening.

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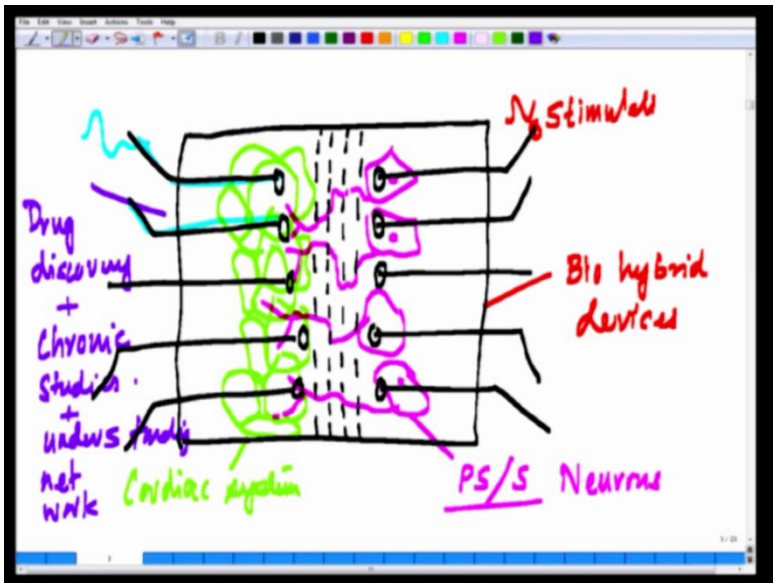

So in order to do that what you have to do say for example, you have a array like this, out here and we have these electrode sitting all over the place like this. You can make these arrays custom made you can. So, I am just drawing a limited number of arrays in order to and say for example, you have a physical barrier like this, so that the cells on the other side cannot move to this side; whereas, the only the processes can move something like a physical barrier like this. So, what you do, so you take the cardiac myocytes and you start growing the cardiac myocytes on this other side. So, these are the cardiac myocytes which are growing.

These cardiac myocytes cannot move on to the other side, because you have created the barrier, so that these cells cannot move; whereas, you put your sympathetic parasympathetic neuron likewise, but the process can really travel to this site. Similarly, you have. Essentially, what you are doing, you are recreating that circuit of control of sympathetic parasympathetic to the cardiac myocytes or in other word, you can study the heart physiology, cardio muscular physiology on the small chip or a microelectrode chip and these are the power of these kinds of simple system where you can. So, what you essentially you can do you can say for example, you can stimulate this say you can

stimulate this and stimulate this electrode out here, and you could see the response out here you will be able to see the kind of response which is taking place on this on this electrode or where ever.

So, you could have a one-to-one connection between it. So, this side, they showing just for you the parasympathetic I am showing by or sympathetic or parasympathetic neurons. And on the other side, you have the cardiac system. And these kind of devices are called bio hybrid devices; and this bio hybrid devices are nothing but where there is electronic component are been integrated with the biological component and that leads to a marriage of these two diverse system in order to extract meaning full information out of it which could be used for several purpose. I mean look at this system this could be used for I mean wide array, this could be used for drug discovery one of the major areas; this could be used for chronic studies a long term affects of drugs or something like that. This could be used for understanding network, which is a very challenging task in a real life situation, you really cannot.

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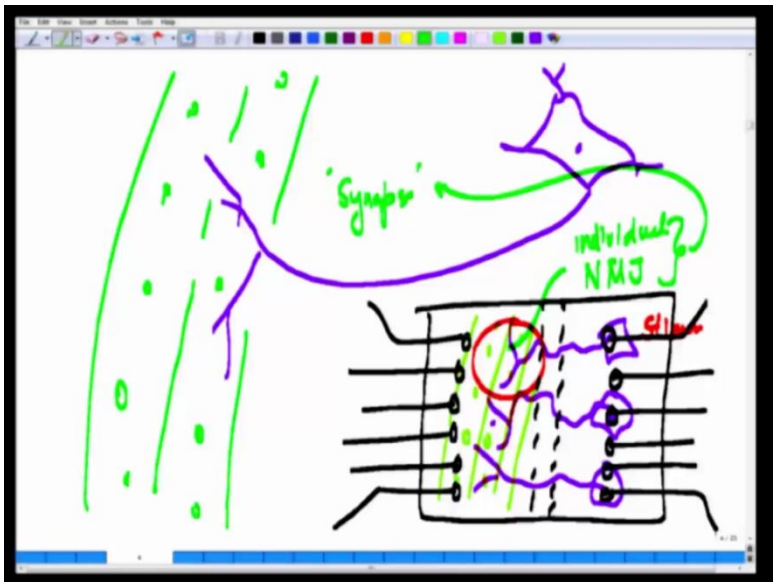

And basically what you can do you can component by component into these kind of circuits to do. Similarly you can build another circuit like, you could have, so we know that most of this motor neuron from the spinal cord are the ones which are innervating all your skeleton muscle an these are you skeleton muscle which are sitting out here like this with you know. So, how to build these kinds of circuits. So, again the similar way, you

can translate them on a micro electrode array the way I was showing you. This is the array and again you create partition, you can really custom made all these things and you have the electrodes sitting like this, like this. The electrodes are sitting all over the place, and here you are going growing the muscle cells like this. And the gap is so much that it would not allow really and you can actually pattern these substrates in such a way that they will only grow in a certain fashion as I have already discussed with you people about the pattern and here the cells will and here they are forming their neuromuscular junction something like this.

So, these are the power of these kinds of systems. So, basically what you can do out here, you can stimulate individual motor neuron and you can see the response out here in the muscle. So, you have in your control now, the individual synopsis. So, you can really access individual neuron muscular junction or n m j or neuron muscular junctions synopses. So, these are the reductionist approach to study bioelectrical events, which are responsible for our very core of our survival. And these not only to help you to understand circuit as I was mentioning, there are whole method of other application this is this is a upcoming rising area by hybrid devices which can change the way we think.

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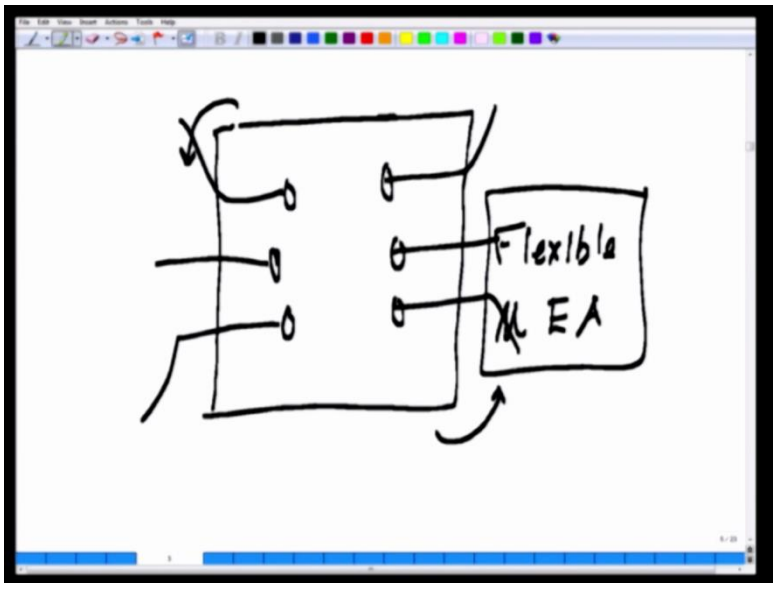

Another approach is now what is a next level of development, which is taking place whenever you look at these kind of arrays these are flexible arrays. So, these are flexible arrays. So, now, what you do essentially is, these flexible arrays now there are materials

by which you make these non flexible arrays you can make them flexible arrays, flexible micro electrode arrays - MEA(s). These flexible MEA(s) could be implanted direct to the into nervous system or any other excitable systems of the body. So, you can really role them up, you can put it there and they have they are finding in future, they will rather they will find applications in terms of prostheses, in terms of wide range of applications which is kind of like, it is tough to comprehend at this time, but these are areas which are going to change the way we study biological systems.

Though these are very reductionist approach, but they give very profound information, very clean information with least amount of noise in them. So, with this understanding of microelectrode arrays, I will move on to the patch clamp technique. So, before I start the patch clamp technique, so let us get some of the time line right, what happen when during last.

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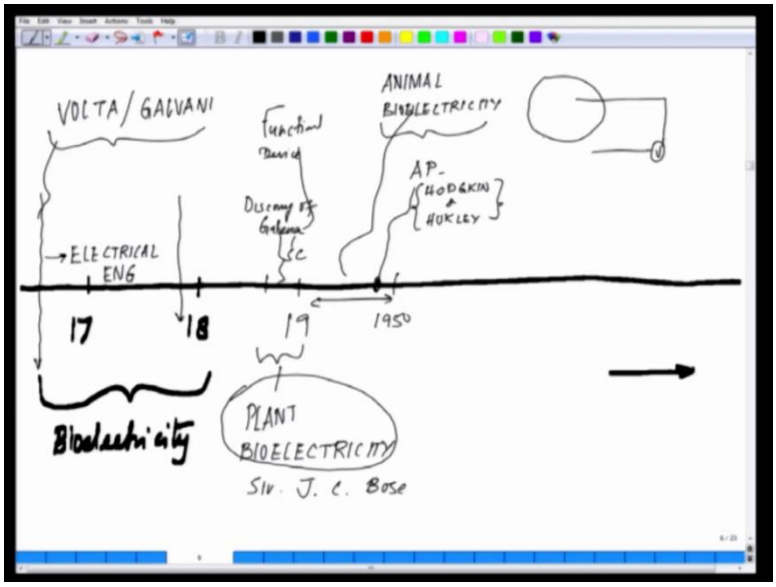

So, if you go back and if you look the way this is the I m drawing the x axis and I am showing you the time. So, somewhere 1700 or 1800, this is the time when there were preliminary understanding. So, people have understood bioelectricity was pretty much there, all over the place. And somewhere down out here, somewhere out here bioelectricity was discovered followed by that people were starting to do a whole bunch of started doing sharp and during this time of course, this gives to birth whole field of electrical engineering. If you go back to the time of Volta Galvani during the time well,

there was no formalized discipline called electrical engineering, and people who are discovering charges and all these kind of things. And it is during that time that the bioelectricity was discovered as a matter of fact some of the bioelectrical phenomena were discovered much earlier that formalizing the whole field of electrical engineering.

So, after that during 1800, there were people doing sharp electrode, they were trying you know pushing electrode and with voltammeter they were trying to make recordings and everything. During early 1900, so this is the time when late 1800 and 1900, this is the time plant bioelectricity discovered and plant we will be of course, will be dealing with this whole section this is the whole section in this course, we will be dealing with it and the pioneer in this was Sir J. C. Bose. He worked extensively on mimosa - touch me not plant and several other related plants, which show electrical activity and published his seminal contribution and pretty much proved beyond doubt, these plants have plants exhibit bioelectrical phenomena.

So, in one way, it can be said those where the systematic beginning of understanding bioelectricity. Early half or later half of 18th century, and as a matter of fact the that is the same time the pretty much out here just before later half of 18th century, when the discovery of Galena as semiconductor. And as a matter of fact semiconductor concept of semiconductor came even earlier than that by the first such evidence which could not be described was shown by Michael Faraday, he really could not explain. He saw some deviation from the known ohms law, there was a unusual deviation non-linear relation started picking up, he could not explain it. It was after that the semiconductor was been discovered and I am forgetting the name of the individual, I will get back to you with that and then it was the discovery of galena as a functional device.

So, this is basically what he showed is, what Bose showed is a functional device, but after this he moved on to the plant and maximinal contribution in understanding plant bioelectricity. As a matter of fact he is the first one to propose that plant have life actually that was the time it was kind of you know out of the world and how it could be said that plants have life. You are pretty much sure that they generate action potential like impulses and those are recorded and that was a pretty much a systematic beginning. Then on during this part 1900 to 1950 I should say, it is the time when there was enormous work happened across Europe on animal bioelectricity - a systematic study.

Mind it, these studies were going on it is not that systematic studies were even then. Some of the i should say some of the very hallmarked was the discovery of action potential by Hodgkin and Hukley, there is several other people whose name I am not mentioning here, but that does not mean their contribution were any less. It is just I am just trying to build up the story how the patch clamp came in to being. But even Hodgkin and Hukley, they used sharp electrodes which I have shown you before earlier. So, you have this cell, and you have sharp electrode and you are making recording, this is the voltammeter on whatsoever.

But based on their action potential traces, they did a complete mathematical formalism of what they believed is something like when the membrane is becoming short, but then the suspicion that there are entities like ion channels. So, there was no ion channels which were know and mind it this is time when not a single protein was kind of crystallize, it was the time when Maxparudes was trying to crystallize hemoglobin. Today you see, so many protein which have been crystallize and all those things, and forget about membrane protein. Those are not even clear. And it is the time when the most famous celebrity model of very called the fluid mosaic membrane in model that was not pause I mean it was a kind of it was not clear at that time, what is structure of membrane.

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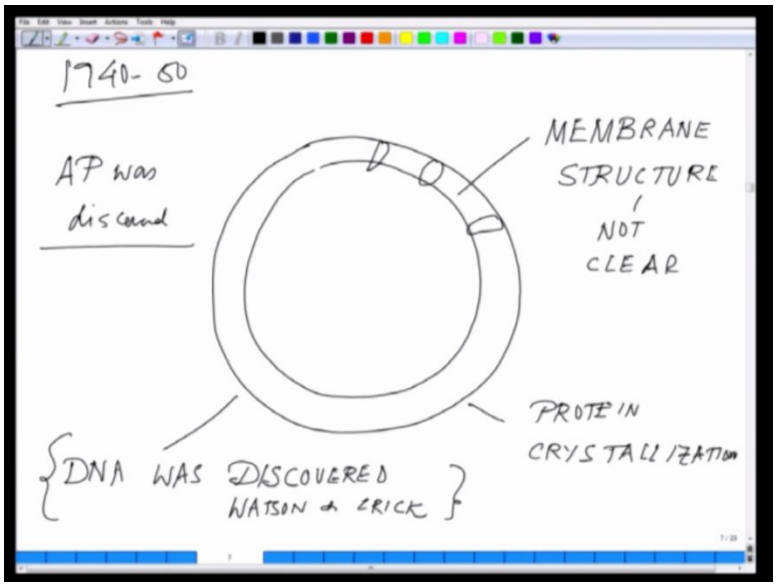

Essentially, what we did not know at that time was, if this is a cell, the structure of membrane was not clear. So, this is I am talking about nineteen forties membrane of

structure was not clear, the ultra structure was not know, proteins crystallization has not taken place. So, really we have no idea much much idea about protein just recently the DNA was discovered. So, this is I am talking about nineteen forties and fifties. DNA was discovered by Watson and Crick, and it was the time when action potential was discovered, but nothing was clear that the array channel and all those things which is talk. So, easily nowadays was was like you could be at that time could be like you know a daring dream that was pretty much Hodgkin and Hukley did in there mathematical formulation came up with an possibilities there is specific channel through vegan passes likewise and so on and so forth.

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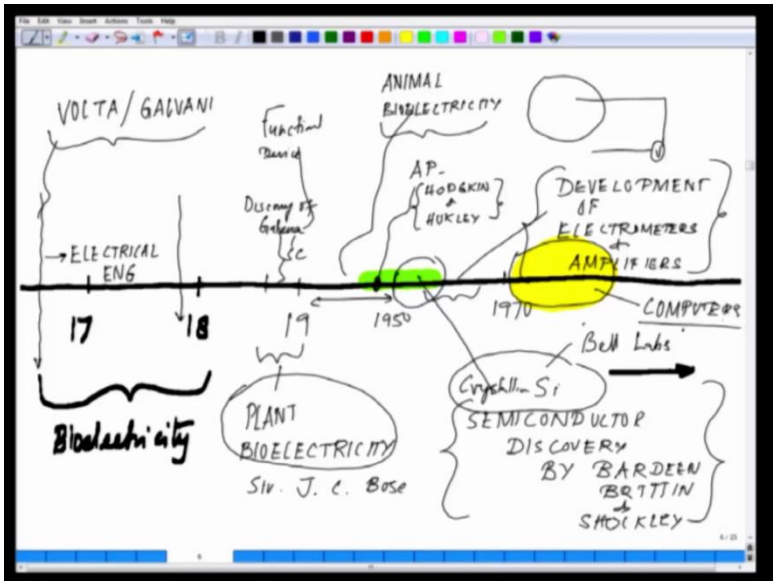

There is purely mathematical based on certain experimental data. Then came the next breakthrough in ninety seventies with the discovery of technique called patch clamp, so if I go back in the timeline chart. So, the next discovery was here 1970 and in between these there is one discovery out here which is very significant though nothing to do with biology though, but it played significant role out here that is this is the one, this is the time when semiconductor was discovered. And they go hand in hand semiconductor discovery by Bardein Brittin and Shockley.

It was during that time that open up the flat gate of developing silicon base devices mind it semiconductor I am wrong here. Let me correct it crystal semiconductor. So, semiconductor was known much earlier than that was, but what was the breakthrough

was here was crystalline silicon. And these was the discovery made in Bell Labs, New Jersey. This discovery change the way we perceive the world there are certain discovery totally change everything and this is one discovery, but how this discovery has to do with what we know today about ion channels or bioelectricity.

So, the connection is really straight forward, because of the discovery of semiconductor, this leads to development of electronic devices which were much more performed as compare to previously when pivolory, germanium and all those kind of you know where the signal acquiring fatality is very low and all those. But here there a scope that it could really acquire very performed able signal. So, that leads to the development of very high precision during this period, it open up the fled gate for the development of very high end elector meters and amplifiers.

So, those of you have forgotten electrometers, if you remember that you could measure charges using the gold leaf foil that is why they use electrometers. So, this was the time which was a really very amazing time, when like you know some of the for breaking discoveries took place and whole field was kind of rocking, and with the discovery of very high gain amplifier. So, one of the challenge, you have to realize that you majoring current at nano ampere, pico ampere dimensions, it is not easy it is very noisy you really have to have very good whole or understanding about the phenomena; otherwise you will be recording noise.

So, it is during that time this discovery of these high end amplifiers and simultaneously it was during that time petty much around seventies on this is the time which saw the next development, I will come back to this and all have played very significant role to what is people see today is with computers. It is the time when computers were slowly coming into existence. So, with the discovery of crystalline silicon as a material semiconductor material for device development followed a development very high end amplifiers for these kind of recording. The stage was set for the next discovery, and the next discovery was started when we start is that this discovery of patch clamp.

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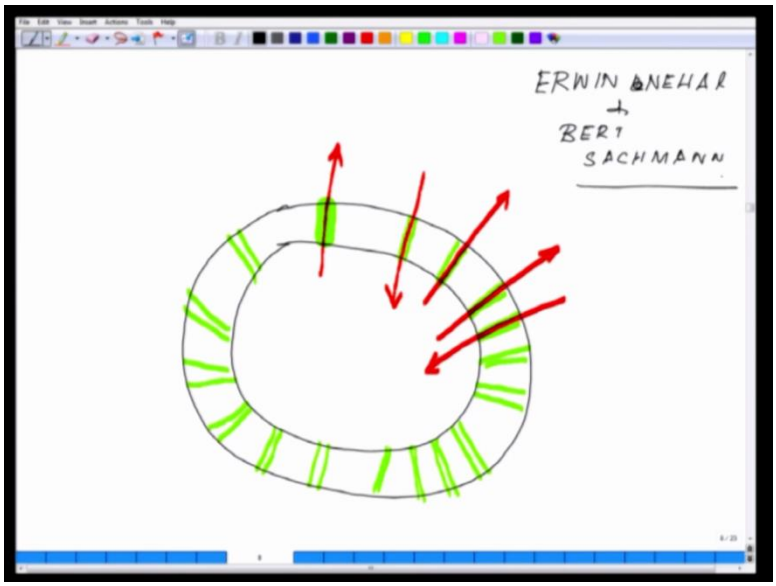

And then the venue shifted all the way to Germany, the two gentlemen, two Germens Ervin Nihar and Bert Sachmann, they were instrumental in developing patch clamp technique, what essential patch clamp is all about. So, if this is a cell, we are talking about fine, this is a cell. And these are the ion channels on the cells this green millions of such ion channels all over the cell. So, what do you wanted to understand is you want to access or you want to measure the current following through the these ion channels. It could be a potassium channel, it could be a sodium channel, it could be a calcium channel, it could be a chloride channel. So, your goal is this, you onto see the movement like this or you want to measure like this or cell like this or this, so to measure these ionic fluxes.

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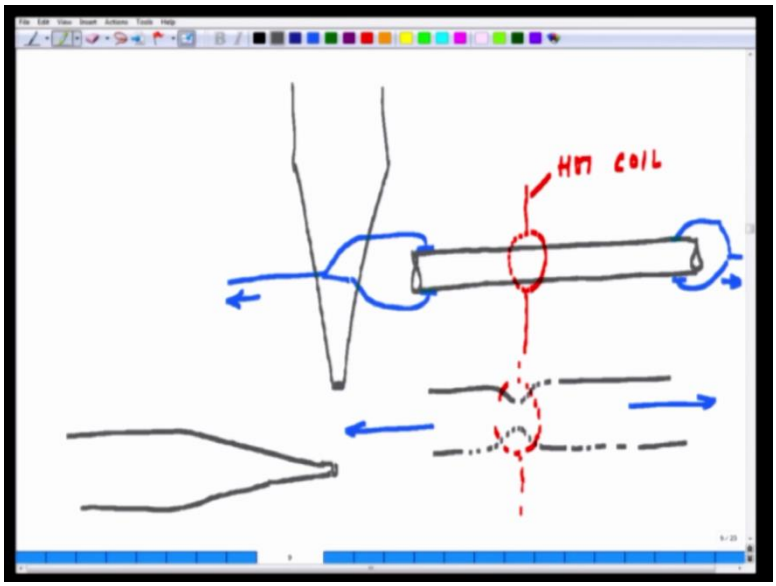

In order to understand that you have to really come close, so that you can access an individual the ion channel how to do so. So what was been done by Nehar and Sachmann. Essentially do is with develop this nice glass piped. So, how they did it, let us understand first. So, you have this, you all have seen capillary. So, you have capillary tube, now you take the capillary tube and keep it in without touching, and keeping it in a very hot coil keep it in an hot coil. So this which temperature you can control, and on these two sides you have two clamps by which say for example, these are clamps by which you can pull this on two direction on either direction.

So, now what will happen, when you expose it to very high temperature, this will start to melt like this, and then you give a very strong pull on either side. This is very understanding sets, so you have this coil ok. If you go for a very strong pull what you be essential landing up is with this. Depending on this strength of the pull will be landing up with a narrow bore or a thick bore glass piped like this. This is what I was trying to draw here which is essential like this. This is what we are going to land up with.

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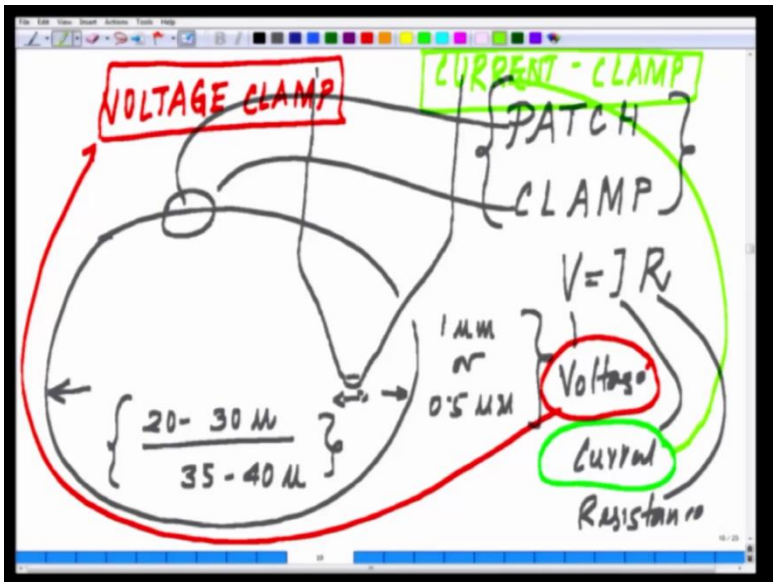

Now if you look at the diameter of these kind of glass piped, there will be approximately around one micron or micrometer or may be 0.5 micrometer likewise. And mind it if you look at the cell is compare to this, which is 20 to 30 micron or even sometimes may be 35 to 40 micron, the bigger the better of you are in terms of the picking up cell. So, know. What is essential you can do with that small tip, you can access at least the one-micron patch of a cell, and mind it what is name, the name is patch clamp. In other word, what we are trying to do by this word, what this word says, you are talking a small path of the cell like this, and you are clamping it or you are in a holding it. So, you are holding a small patch of a cell, and in that small patch of cell, you are manipulating the membrane properties long back, small patch and that is why this called patch clamp.

And what all you can hold there are only two parameter and I will come to the whole electronic configuration, but let understand it. There are two things you can hold here, if you go by the fundamental V is equal to IR, you can either so where V is your voltage, I is your current and R is your resistance. There are only two things hold here either you can hold voltage or you can hold the current. When you clamp in that zone hold the voltage, this is called voltage clamp, because your holding the voltage or the voltage is in your control, you can the change the voltage, you can clamp it at different level minus 80 minus 70, minus 50, minus 40, minus 30, minus 20, minus 10, 0 likewise, you can hold the voltage.

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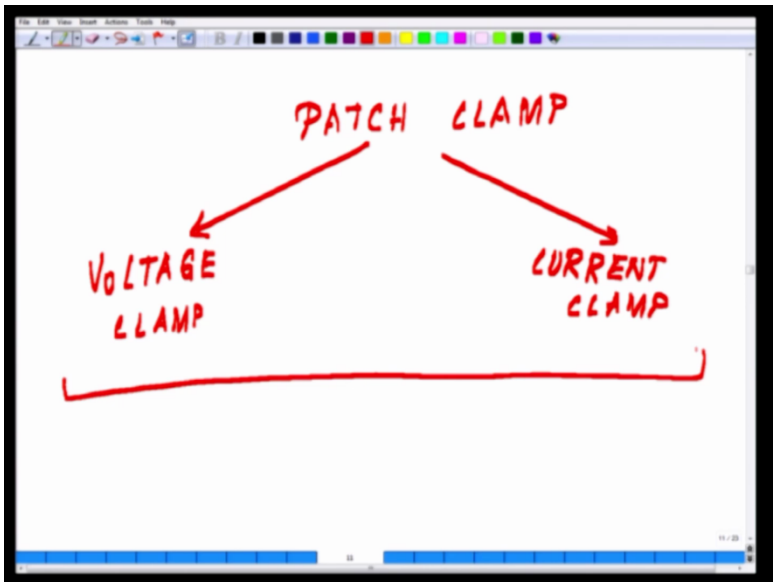

You can clamp the current, which is called current clamp. So, the current clamp is essentially you can eject finite amount of current thought the preparation thought that electrode. Now what will we do we will, so essentially would see patch clamp with, so five kind of give a graphical summary of this. So, you have patch clamp and under patch clamp you have voltage clamp or you have current clamp.

Now with this background, I will move on to the next phase, where we will be talking about how this circuits have been built, how these have been dealt and all other details. So, today we will close in here, and in the next class, we will move on we will talk about in depth about the circuits.

Thanks a lot.