Bioelectricity

Prof. Mainak Das

Department of Biological Sciences and Bioengineering

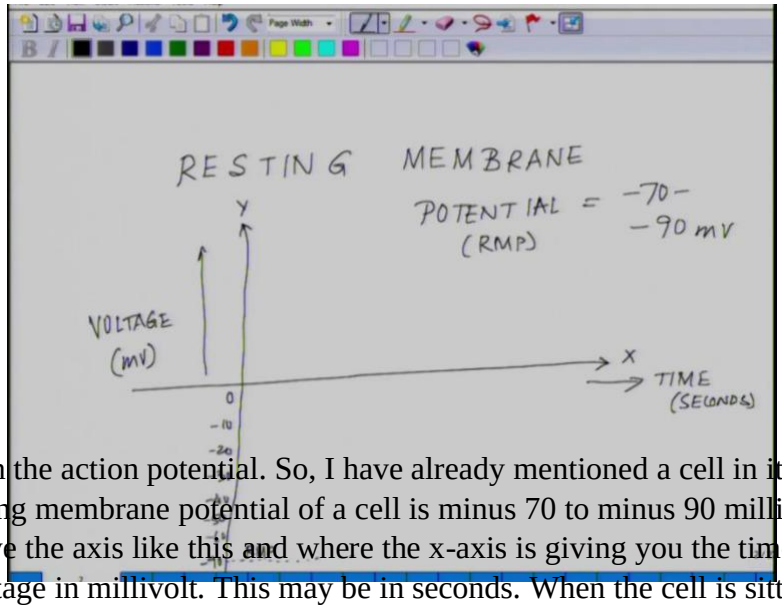
Indian Institute of Technology, Kanpur

Lecture – 7

Welcome back to the NPTEL lecture series on Bioelectricity. So, today we are into the seventh class. So as of now we have talked about the structure of the neuron, and we have talked a little bit about the ion channels, and we have talked about the Nernst equation. And in the last class, I concluded the class showing that potassium ion is kind of slightly leakier as compared to the sodium. So, today, we will talk about the most fundamental unit of electrical activity which is the action potential the most I should see the first electrical event, which leads to the whole plethora of events of neural code is the action potential. What really is action potential and how it is discovered?

So, the story of the action potential is much, much older than the story of understanding of ion channels, proteins, and all these things. It was during the nineteen forties and fifties that or much before that actually, essentially nineteen-thirties and forties, some of the pioneering work by Hodgkin–Huxley - the two British scientists among one of them was a pilot before that to discover this phenomenon. And they were working on Aphasia one of the sea animals because it has a fairly long axon so that you can insert the electrodes into it, and what they essentially discovered at a certain stimulation, they saw a trace something like this.

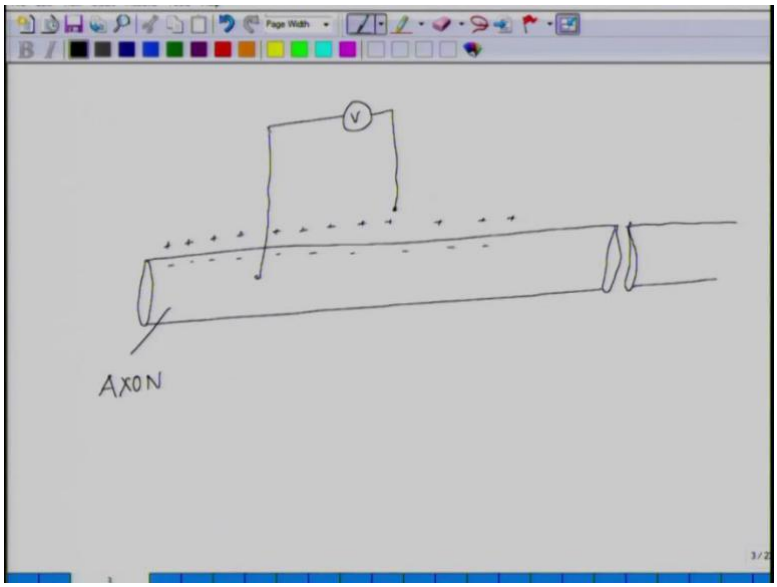
(Refer Slide Time: 02:25)



So, let us start with the action potential. So, I have already mentioned a cell in its normal resting state - resting membrane potential of a cell is minus 70 to minus 90 millivolts. So, essentially, if I have the axis like this and where the x-axis is giving you the time and y-axis is giving you the voltage in millivolt. This may be in seconds. When the cell is sitting

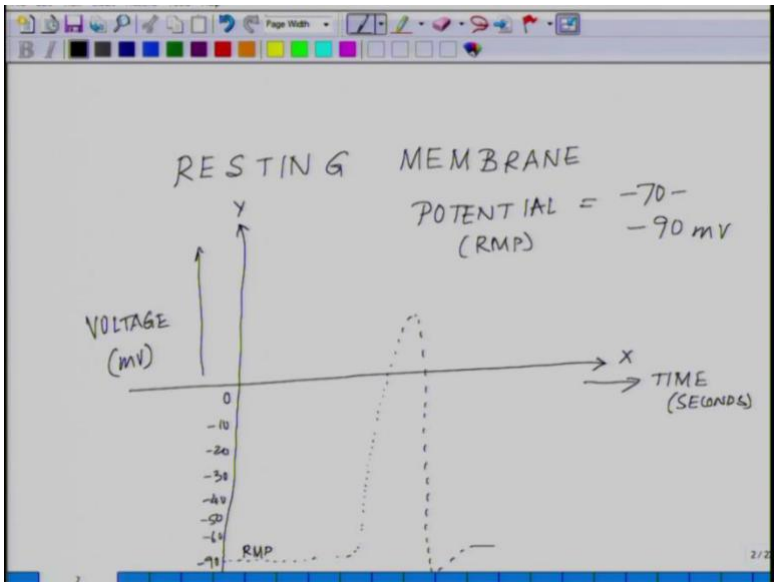
somewhere. So, this is zero, this is minus 10, minus 20, minus 30, minus 40, minus 50, minus 60 likewise. So, the cell is sitting somewhere out here, minus 90. This is the resting membrane potential of the cell, and let us denote it by RMP - resting membrane potential. So, they found a very interesting event if from the resting membrane potential a cell is excited.

(Refer Slide Time: 04:08)



So, say for example, just to visualize what kind of experiment they are trying to do something like this. Say for example, this is the axon out here and part of the axon cell body is out there, and you have an electrode out here, and we have another electrode that is sitting outside. So, with respect to outside, the inside is more negative, we have already discussed this, something like this. This is the axon and this is the voltmeter.

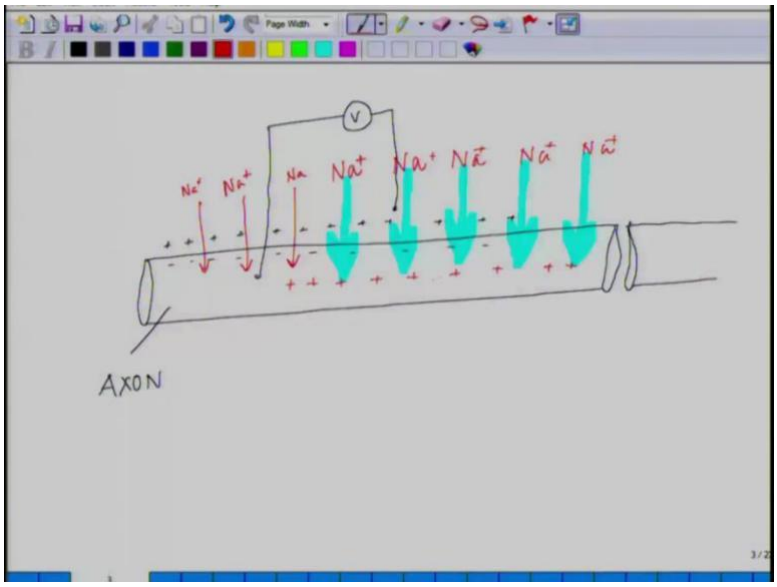
(Refer Slide Time: 04:52)



So, if the cell is excited, it is been observed that if I go back to the trace, there is a cell become more positive, positive, positive and then it overshoots the zero and it comes

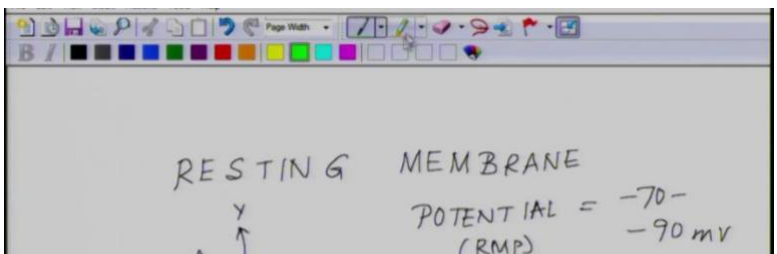
back after some time and something like this. This was the first initial traces which were observed and the way it was observed is something during those days there were no computers, there were no such programs or anything. So, the traces were been seen in oscilloscope and it was imaged. If you see those images, it will be on a black background; you will see the dotted lines like forming like this. So, this was the first seminar discovery made by Hodgkin–Huxley. And based on this trace, at that time, there was no idea of ion channels nothing was really cleared, protein first protein was not crystallized by that time.

(Refer Slide Time: 06:20)



So, what they did they got the stress and they did a curve fitting using different cell equations and based on that the kind of concept which evolved in last soon after that is that initially what is happening inside the cell. So, initially what happens because of excitation, so initially if this is the situation and let me show it using different kinds of ions that are present here sodium is fairly higher outside. So, the first event which takes place is this one; there is an influx of sodium inside like this. So, as the sodium ions are increasing inside, what essentially happening is, inside the cell, it becomes more positive.

(Refer Slide Time: 07:00)



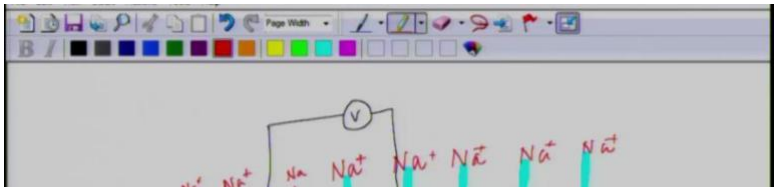
Now go back to this picture. So, this is the zone, where sodium is entering. All the positive ions are getting inside the cell, but then there is a threshold zone that threshold zone gave rise to the concept of odd or none. It means if you could reach a certain millivolt or a certain stage, where these many positive ions have gone and the membrane has become this much degree positive then after that zone there is no stopping. Then if you reach that zone, so that zone lies if you see the picture out here that zone lies somewhere out here something out here this is the zone this is the threshold zone. If the membrane becomes positive almost up to minus after minus 40 from minus 90 from here starts this is the threshold for all or none.

So, what does that essentially means if the membrane reaches this zone by because of the entry of the positive charges, at this stage there is no stopping then this will promote more and more entry of sodium into the system. Essentially if you look at this diagram, so this is followed by more and more sodium entry here. So, much so it overshoots the zero, so here are your zero zones. So, at this stage, in the beginning, while it was sitting, this is called the cell was in a polarised state. Of course, it is negatively polarised as compared to outside then here it becomes depolarised because there is no more polarity of the cell - depolarised state.

After it overshoots the zero out here, this zone it starts to come back to its original baseline level like this. So, what essentially is happening here. So, as of now, we were saying

that there is an entry of sodium going on. So, as a lot of sodium ions get inside the cell, so what happens essentially is because of too many positive charges inside the cells, there is a mutual repulsion between the positive charges.

(Refer Slide Time: 10:24)

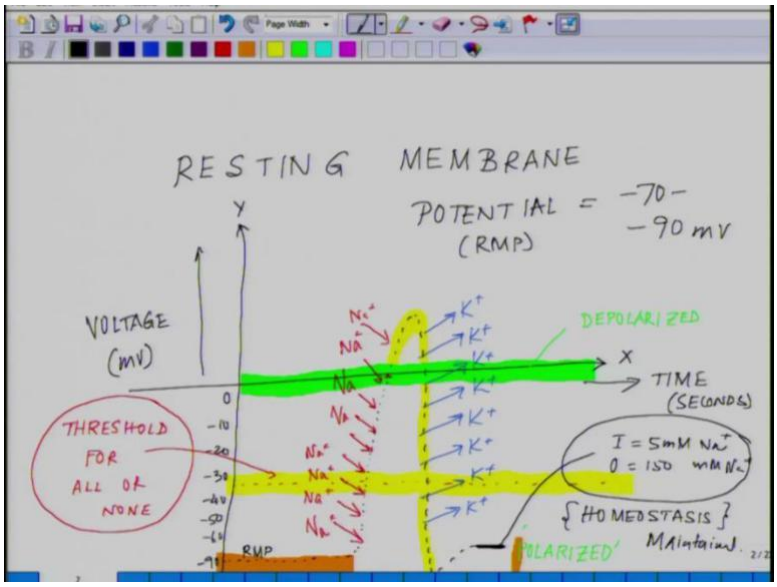


If you look at this picture out here there are already other positive charges of potassium that are present which is fairly high inside the cell. So, there is a positive charge repulsion starts, and this positive positive charge repulsion leads to the next event where the cell starts to allow the potassium ions to get out of the cell. So, the next event that you see essentially is this event two. Now potassium is going out in order to balance the excess sodium, which has entered, and this is the part that you see in this picture out here where this is where from the cell potassium is flowing out till it brings it back to its baseline value. But during this process what essentially is happening as you could see the cell has an excess amount of sodium and less amount of potassium, but the cell has to bring back its homeostasis by maintaining... If you remember in the previous class, we have taken about the inner concentration of sodium should be around 5 millimolar with respect to outside, which is around 150 millimolar. So, how it does?

And there is a third event which comes into play that is the event, there are some very interesting pumps which are sitting on its membrane shown by yellow, and these pump functions in a totally different way. These functions like, so if an individual pump I have to

show it is something like this it is sitting on the membrane-like this and if this is the membrane, this pink one is showing the membrane line. So, what it essentially does is, so this is said for example, if this is outside and this is inside the cell. So, it binds to the sodium from inside the cell, and it binds to the potassium from outside the cell and it binds to the sodium and then this pump flips like this. And this flipping action eventually what happens, all the sodium which are present out inside these ones, these ones, these ones are being essentially thrown out of the cell and all the potassium which are present are restored back inside the cell. So, this is where the sodium-potassium ATPs pump comes into play and this pump is ATP dependent phenomenon. So, it needs a lot of energy to run this pump.

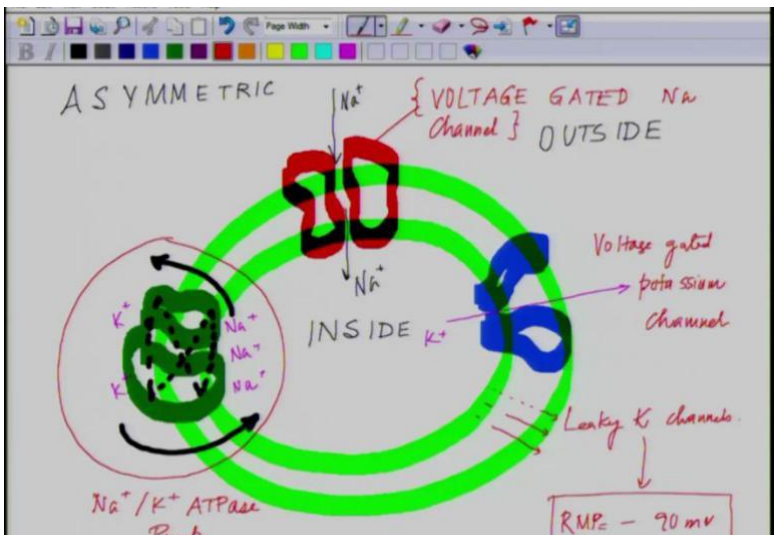
(Refer Slide Time: 14:18)



So now if we summarise the event, so essentially if we go back where I was showing the trace out here, is essentially finally happening is that cell is back into its baseline; and this is where again inside the cell, you have five millimolar of sodium, and outside it is 150 millimolar of sodium. And the homeostasis is being maintained. So, this is one of the key points which has to be understood that there are four events. If I had to summarise this whole thing, the first cell is sitting at minus 90 millivolts or minus 70 millivolts resting membrane potential. And by some x, y, z impulse we will talk about the individual impulse it could be a photon, it could be a ligand, it could be the sound wave for machine-transaction, it could be a small molecule, it could be a odor molecule which comes and binds or it

could be some kind of surface touch which leads to the opening of a bunch of sodium channels or channels which promote the moment of the sodium inside the cell.

(Refer Slide Time: 16:00)



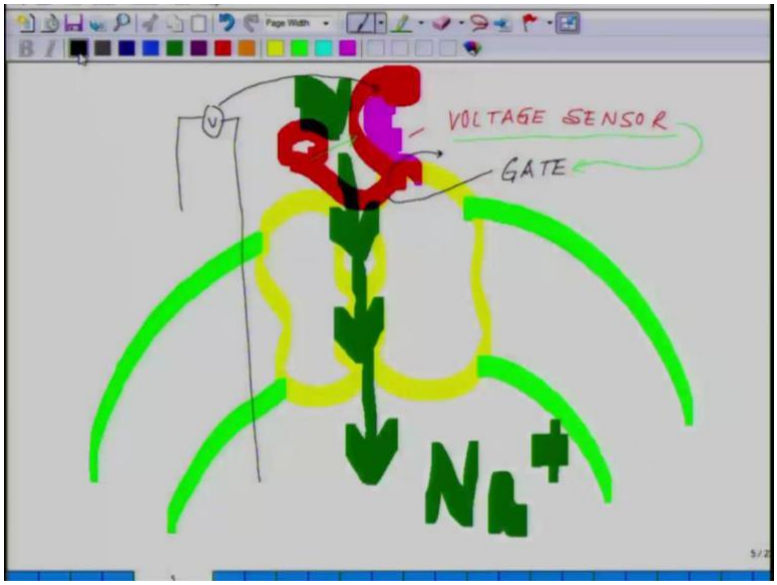
So to tell you here a membrane is asymmetric in nature. What do you mean by is asymmetry is something like this. So, if I draw the membrane-like this if. So, say for example, this is the membrane. So, this is outside and this is inside. What is trying to tell is that the membrane is asymmetric that essentially means the flow of ions is not reversible by the same route. So, say for example, sodium channel through which the sodium enters try to put them on the surface, so they are like this. They are sitting out here in on the membrane-like this and it only allows sodium to move in, but it does not allow it to go out through this route. So, in other words it opens from outside to inside.

Vice versa if you look at the potassium channel which is sitting something like this, this potassium channel only allows potassium to move out from the cell to outside. So, the potassium channel does not allow potassium to flow in through that channel. Then there is a third component which I was trying to describe here, which is another asymmetric component of the membrane that is this component which is essentially wonderful motor which functions to ensure that you know the sodium are bind here on the inner spot and the potassium bys binds on the outer surface and this has a property of you know flipping like this.

So, even this one is asymmetric, because it allows only sodium to bind inside the cell and potassium on the outside, and then it flips back. And if you get analogy for this kind of pumps, if you visit some hotels or some other places, where you have revolving doors when you enter from one side and come out from another side likewise, it moves like this the glass doors with partition like this, it is exactly similar to that. So, the molecules bind on one side, it flips back on the other side. And for this flipping movement, for this movement circular motion of it, it needs energy and this was one of the discoveries by Jane scow and which for which he got a noble prize.

So, if you look at all these three components out which are responsible for executing action potential. You will see all these are asymmetric in nature and that is what came the membrane asymmetry. And then this channel what I have mentioned here, these are voltage-gated sodium channel, these are voltage-gated potassium channel and these are sodium-potassium ATP ase pump. These are the key players other than if you have the leaky potassium channels, which are present, which allows you to maintain the membrane potential at minus 90, leaky potassium channels helps in maintaining RMP at minus 90 milli volts. So, these three are the major component which helps us in our understanding of bioelectrical phenomena at the level of ion channels.

(Refer Slide Time: 21:32)



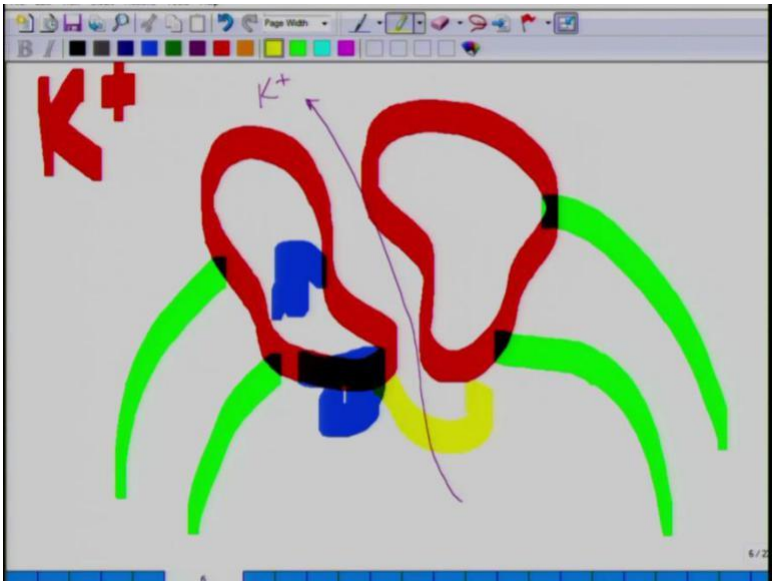
So, if we talk about these voltage-gated sodium channels, so what we are talking about is this if you look at this molecule since once I have introduced the membrane. So, if you

look at this molecule across the cell, again this is the part of the membrane and the molecules are sitting like this, which something like you know this is the port through which the sodium channels move. So, what the essentially have is they have a segment that acts as a voltage sensor. So, they have a voltage sensor that could sense the voltage across the membrane. So, in another word, it can they have a potential by which they can see the change in voltage like this. This voltage change out here influences this voltage sensor; this voltage sensor is connected to another component, which is the gate component like this. So, this gate component is something like this.

If it senses the voltage, it modulates the gate, and the gate moves like this, and the gate attains a new position, which in this position. So, the next position of the gate is like this, and during that event, the gate is actually moving from here to here. So, whenever the voltage sensor senses the necessary voltage, it opens up the gate, and followed by that is the flux of or the stream of sodium starts moving in at that point through this. And these ports are so this is we are talking about the sodium. So, these ports that we are discussing out here are fairly specific, it is just like a filter; a filter which is regulated by a voltage sensor and a gate. And these individual protein molecules are the smallest unit which generates excitability in excitable tissues of the body, which includes our nervous tissue, all the neurons, the muscle, smooth muscle, cardiac muscle, and skeletal muscle, and the neuroendocrine tissues which regulate the secretion of different kind of hormones.

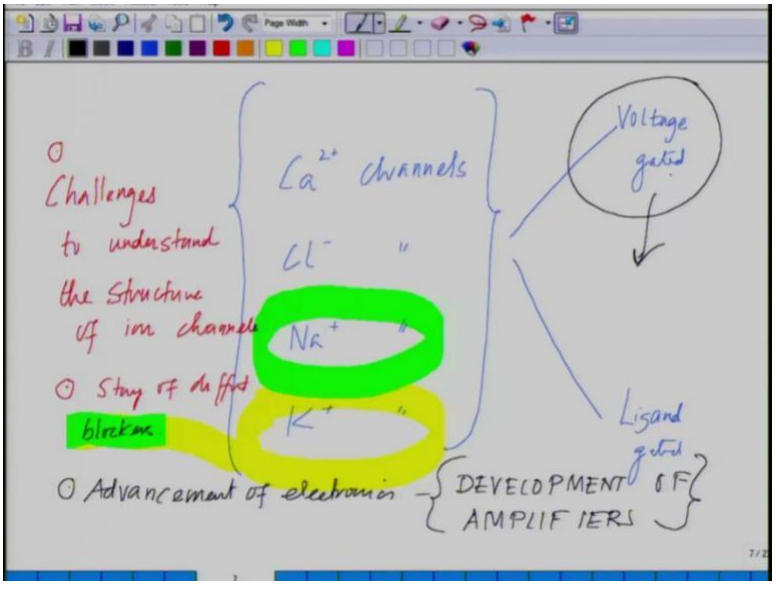
So these complex structures are under intense investigation. To date, we have an idea about the sequence, we have a fair idea about the structure better, but at a very low angstrom resolution of one or two-angstrom resolution. We still we are waiting; scientists are working very hard to figure out the structure because these are some of the most fundamental drug targets for situations like pain, neuropathy whole range of diseases, most of them have their roots in ion channels. So, this is about sodium.

(Refer Slide Time: 25:17)



Same way, if I have to explain to you the potassium it is fairly similar, it is just everything just gets reverse there. If this is your membrane like this and like this. Then you have the potassium channel sitting like this. So, these potassium channels which are, I just put it potassium. So, they are essentially allowing the potassium to flow out. So, automatically they have certain sensor elements which are sitting out there somewhere, and then they have these gate element as I discussed previously, which opens up and allows the flux of potassium outside the cell. So, these are the basic structure before I explain, some of the different variations of the potassium and sodium.

(Refer Slide Time: 26:33)



Similarly, you have the calcium channels, you have chloride channels, likewise, and so on and so forth. So, basically at this level the biology is all about these different ion channels, and they could be voltage-gated, they could be ligand-gated. I have not that was a reason why I told you that I will, first of all, introduce you to the action potential, and then I will come back to the ion channel structure. So, I told you at the beginning of the lecture that when action potential was discovered there was hardly any idea about ion channels. It was shared prediction of those two individual Hodgkin–Huxley through their model that there are ports, which allows specific ions to move in.

And it took in mankind pretty much another thirty to forty years almost three to four decades before the first ion channel recordings took place and that was another breakthrough event. So, those are the stories that we are going to share while we will be talking about the different techniques we will be dealing with because there are two-three things that have to be highlighted here. So, one is what will be discussed in some of these classes, challenges to understand the structure of the ion channel. This is definitely a big challenge and will discuss this is why it is so challenging. And the second problem in the story is, how to what are the different how to the story of different blockers, what I meant by that.

Say for example, if I talk about the theories of the flux of sodium inside the cell. So, well I will draw it if you go back into my, so if you look at it here, I am showing you in the slide that there is a flux of sodium that is taking place. This flux of sodium how I prove that there is indeed a flux of sodium that I can only prove if I have some way to block the block these sodium channels. So that is totally dependent on different kinds of blockers and the same way how could I prove that there are potassium channels. So, I will be needing the blockers to justify my claim that yes indeed, there are potassium channels.

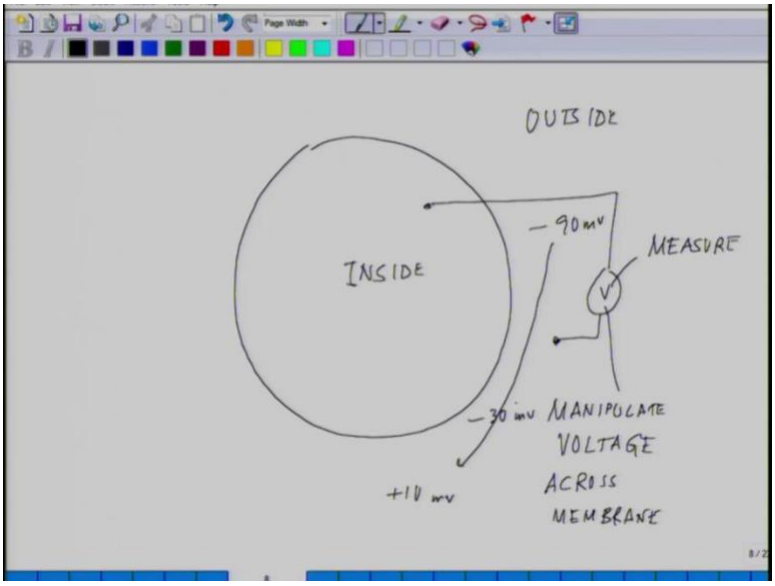
Top of that this field is also very much dependent on the advancement of electronics because the kind of current we are talking about is very minuscule current. We are talking about 10 to the power minus 12 likewise fairly very, very, very small currents, which are because these are ionic current. How to measure those, how even to measure say for example, if you look at this picture, how I could measure the current across a single ion channel, this is the story of bioelectricity where people have been successful in measuring current from single ion channel and that is where you will see the

development of amplifier circuits. So, that is the reason why I was trying to highlight and especially the development of amplifiers.

They go hand in hand, because the better the electronic tools or electrical tools you have, measuring tools you have, better are the chances that you know you can do quality recording; otherwise to measure this kind of electrical phenomena could might as well we ruled out has nothing but you know electrical noise I just measuring noise. So, it has taken mankind, if you go back historical perspective if you look at it, the birth of bioelectricity is much before the organizing subject of electrical engineering during the time of we Galvani and volta long back when they discovered the ionic electricity's or you know biological electricity.

Since then from seventeen hundred probably, you know sixteen to seventeen hundred, we have traveled all the way ion towards the twenty-first century and in that whole course of events, there was this whole field has evolved hand in hand with the electronics industry. With the discovery of semiconductor devices in the nineteen forties and fifties, the discovery of bind in between Shockley Britain, and the whole on slot of development of very high profile electronic devices during the nineteen seventies, amplified technology was really picking up. During that time there were three two individual Ervin nephrin and bird swagman, these are the two individuals who were instrumental in you know recording from ion channels. And they used a technique called patch-clamp and we will be talking about these different patch-clamp current clamp and different techniques in the technique section. But in between, I will kind of will slide it in.

(Refer Slide Time: 33:54)



And another inter interesting thing that we have to discuss is if we talk about this voltage gating, the voltage which is leading to the opening and the closing of the channels. So, what will happen to say for example, if this is a cell sitting out here, and this is the inside milli and this is outside?

And if I have an electro voltammeter sitting here, and say for example, this voltammeter could change also, they not only could measure, it could measure voltage, but it could have the ability to you know manipulate voltage across the membrane. It is an imaginative dual-purpose tool.

If I change, so basically it sets at minus 90 millivolts. So, if systematically or you know in a way I change this to say you know plus zero, or you know plus ten or you know minus thirty, how the ion channels are going to be here, even without using blockers how the voltage gating is going to get influence. So, we will be talking more about this in subsequent classes. So, at this point I wish to introduce the action potential, because that is the key if you look back. So, these are the things, so this is the basic action potential and there is the basic action potential of a neuron.

So, what we are talking about the only basic action potential of neuron out here, and this action potential trace varies from cell type to cell type. And we will come to the other series of action potentials then we talked about what are the different stages of the action potential is taking place, it is stage one - the movement of sodium, followed by stage two - movement of potassium, followed by the pump phenomena. So, these are the three

events that are taking place. Then we talked about the three-component which are involved in the game, we talked about voltage-gated sodium channel voltage-gated potassium channels and sodium-potassium ATP ase pump. Then we talked about basic structure, the voltage sensors, and the gates in sodium and potassium ions. And then we have to talk about the challenges in an understanding of the structure of ion channels and the advancement electronic. And subsequent class, we will talk about how to manipulate the voltage across the membrane which could lead to inside into the understanding of voltage gating. So, I will be closing here for this class, and in the next class will resume further with the propagation of action potential and variation of action potentials in other cell types.

Thank you.

Bioelectricity

Prof. Mainak Das

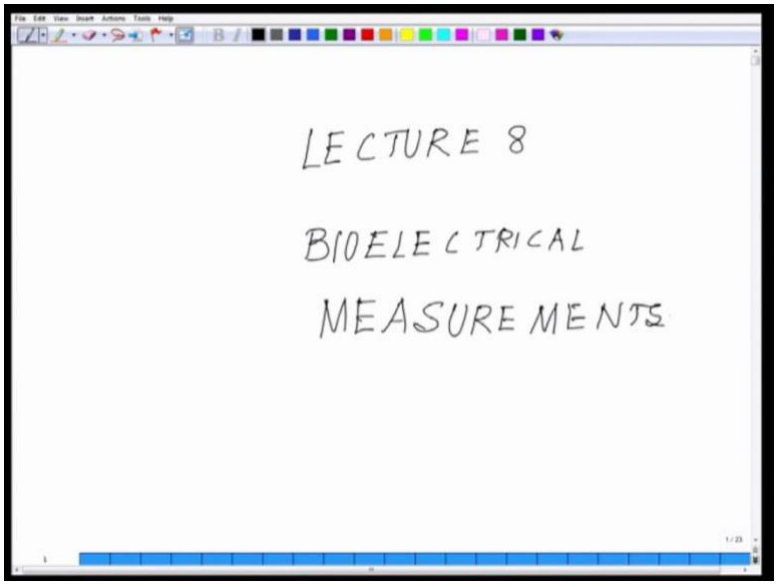
Department of Biological Sciences and Bioengineering

Indian Institute of Technology, Kanpur

Lecture - 08

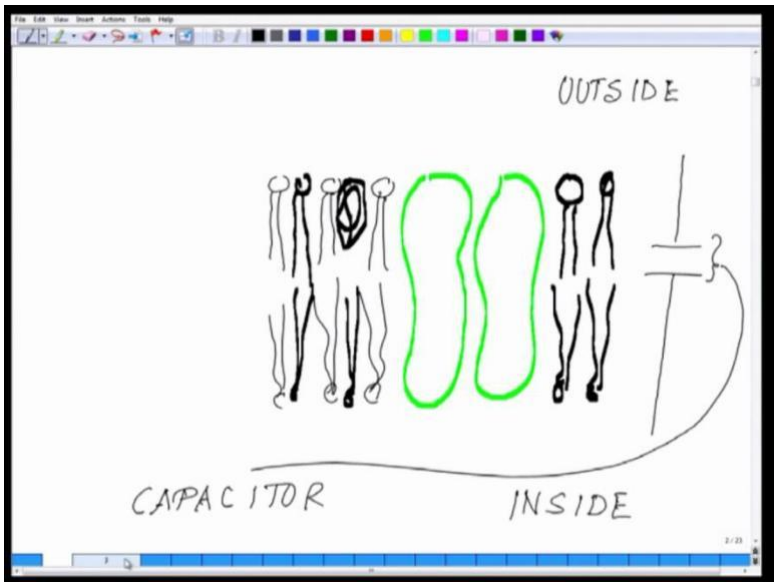
Welcome back to the NP-TEL lecture series on Bioelectricity. So, we are into the eighth lecture, and previously we have talked about the action potentials and the minus 90 millivolt potential difference across the membranes. And we briefly talked about the different ion channels and I promised to you that I will be coming back to the ion channels once I kind of talk about the different techniques which are been used. So, today what we will do, I will introduce you to the basic techniques which are been used for recording the electrical properties of the excitable cells, and from there we will move on to the properties of the ion channels which have been discovered in the whole process.

(Refer Slide Time: 01:40)



Let us start with the historical perspective, how the electrical measurements of the excitable cells have taken place. So, the full kind of move into the electrical measurements, so one of the fundamental things the way bioelectricity person treats a cell is that it treats a cell just like an equivalent circuit model. So, essentially it is like this. So, for example, this is our lecture eight - Bioelectrical Measurements.

(Refer Slide Time: 02:11)



The way a cell is been treated is something like this. So, I have already talked early that a cell is a bi-lipid membrane like this, and then in between, you have the series of membrane proteins sitting like this, channels membrane proteins likewise, and then you have something like this. This is a kind of a and you have cholesterol molecules and all those things which are sitting here. So, essentially this barrier across, so this is indicating the outside the cell, and this is inside the cell. And we have already discussed the different molar concentrations and molar concentrations of the different ions. You can treat this membrane first of all like a capacitor, so the symbol of a capacitor.

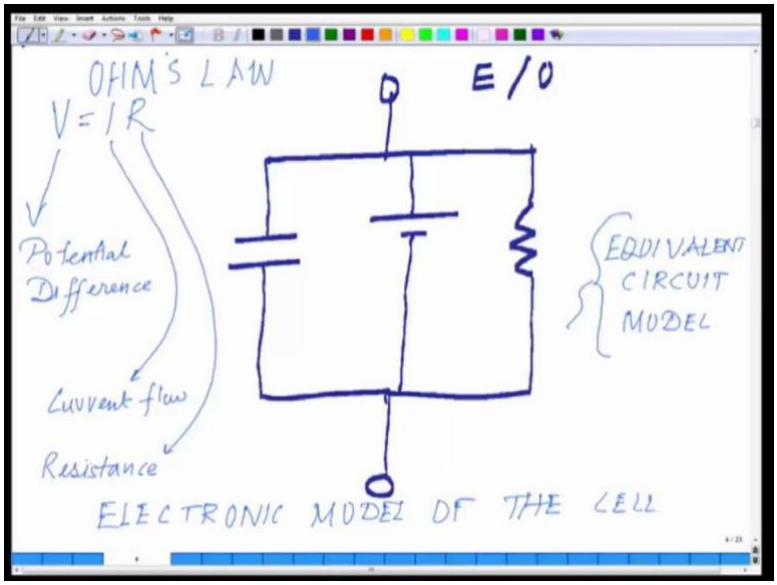
So, essentially the way to treat it as a capacitor arises from the point. So, if you look at the membrane-like this, and if this is part of the membrane either look it bilayer and this is inside shown by I and this is outside shown by O. So inside it is negatively charged with respect to outside. So, these are the negative charges, and outside it is positively charged. So, it is almost similar to a parallel plate capacitor. So, if I connect the wire-like this out here, and I connect the wire-like this out here, so this is one of the ways how you can treat the cell. Similarly, you can treat the cell another way. You can consider this as a battery. So, essentially what you see is across this, if you call this positive and you call this negative terminal. So, if I show you blue this is the negative terminal and right here. This is another

way to treat the cell. Apart from it, the motion across something like from here, things are moving like this is basically things move like this or things moves

like this and also. So, the movement of the ions across this space some form of resistance, you can put as a resistance component.

So now you do, you add all of them together, and there you can call this as plate like this, parallel plates like this. So, essentially what you did, and call it outside or extracellular and this is your intracellular. So, essentially what we did actually, we treated the cell as an electrical circuit, it has all the three basic components. It has a capacitance across its membrane just like when we treat it as a parallel plate capacitor. It has a resistance that ensures or creates an obstruction on occlusion to the flow of ions across this membrane and it functions as the battery. So, if we translate this whole thing into a single diagram it will be something like this.

(Refer Slide Time: 06:19)



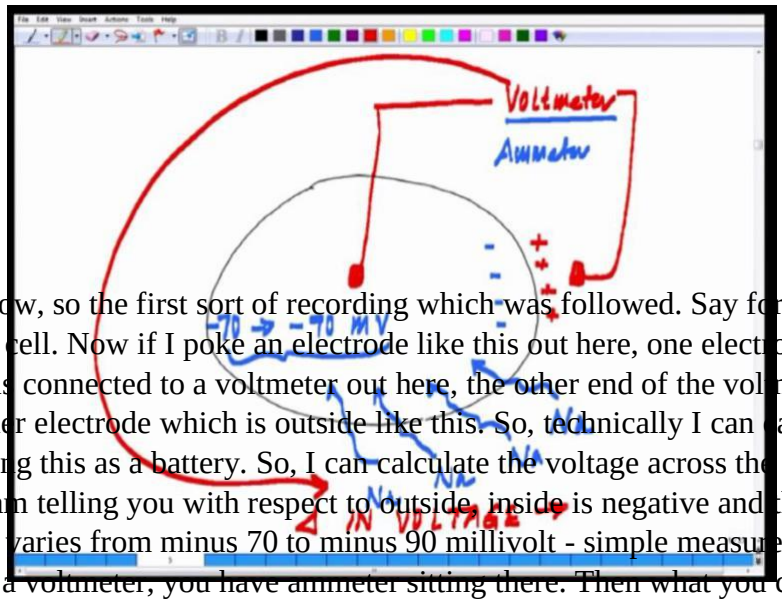
I draw the side, this is extracellular side – E or outside the side, you can you have the capacitors you have the resistance and you have the voltage across it. So, this is called an electronic model of the cell or electrical model of the cell. This is also called the equivalent circuit model. Now this equivalent circuit model, as it is in front of you now you can treat the cell accordingly. You can measure capacitance, you can measure resistance you can measure the voltage and of course the current flow across it.

And the thumb rule in this game is V is equal to I R, where V is equal to ohm's law, where V is equal to potential difference, I is your current flow, and R is your resistance. So, now, just the same way, we draw the equivalent circuit model, now what I will do, I will draw a cell

and I will show how the different kind of measurements which are been followed and the challenges and how far we are by twenty thirteen. So, before I start this let me tell you that these kinds of measurements treating the cell as an equivalent circuit likewise, even much before all these circuit components were discovered, the discovery of evidence of bioelectricity was pretty much there. We have mentioned earlier also from the time of Volta, Galvani all these things were there.

It was just it was never formalized, it took a while it was during the last century that everything kind of got formalized because by the time there is a formal field of electrical engineering which was there. So, everything was kind of structured. So, what we see during last century apart from the development in terms of semiconductor, high-end amplifiers, miniaturization of electrical devices, and very sophisticated good measurement techniques, apart from all these things what you see, the whole field of bioelectricity is slowly getting formalized, because it is kind of discreet even all over the place. And there is no standard wherein one textbook where you can cover all the whole spectrum of bioelectrical phenomena. So, over the period of time and different centuries and a different time, people have a different kinds of experiments, now slowly we trying to understand that these are very very fundamental events which are regulating our day to day events of our life.

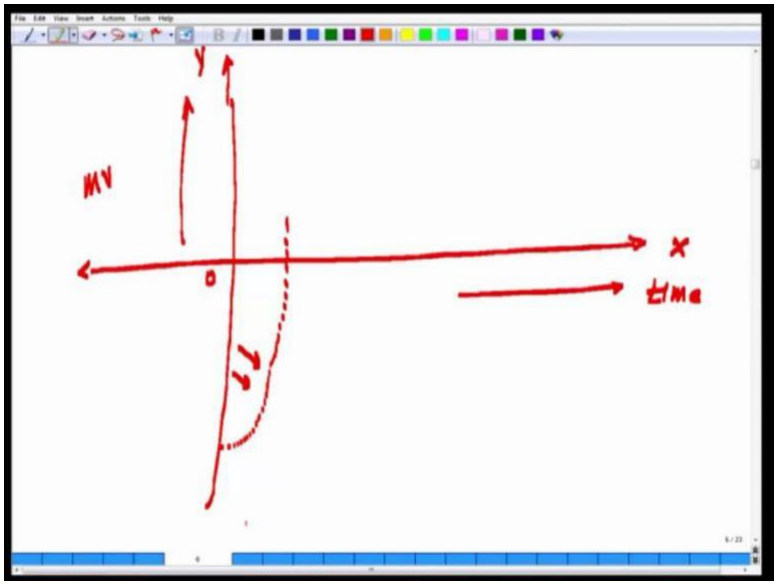
(Refer Slide Time: 10:13)



So, coming back now, so the first sort of recording which was followed. Say for example, I constraint this as a cell. Now if I poke an electrode like this out here, one electrode like this, and this electrode is connected to a voltmeter out here, the other end of the voltmeter is connected to another electrode which is outside like this. So, technically I can calculate because I am treating this as a battery. So, I can calculate the voltage across the cell, so that is what repeatedly I am telling you with respect to outside, inside is negative and this is minus 90 millivolt, and it varies from minus 70 to minus 90 millivolt - simple measurement. Second thing, if instead of a voltmeter, you have ammeter sitting there. Then what you can measure is there is a movement of sodium likewise, it should be able to measure the current across it.

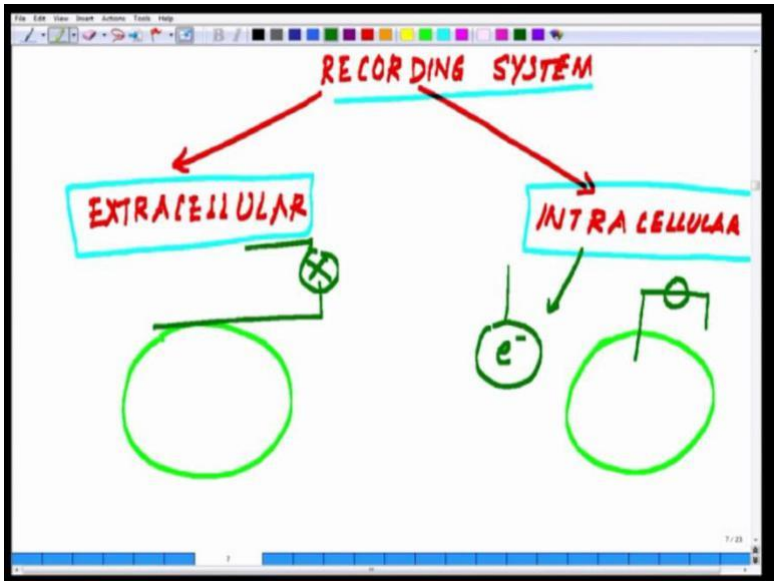
And indirectly what you actually measure is you measure, because of this, you measure the change in voltage that is what you exactly measure.

(Refer Slide Time: 12:03)



So, whenever we talk about an action potential, what you are measuring here is the on the scale and this is zero and y-axis. So, the x is the time, and this the volt in millivolt. So, what you essentially measure is this, this is where the electrode is measuring the influx of sodium likewise. If you compare this, this is what we are measuring. Now this kind of measurement that has been done falls under…

(Refer Slide Time: 12:51)

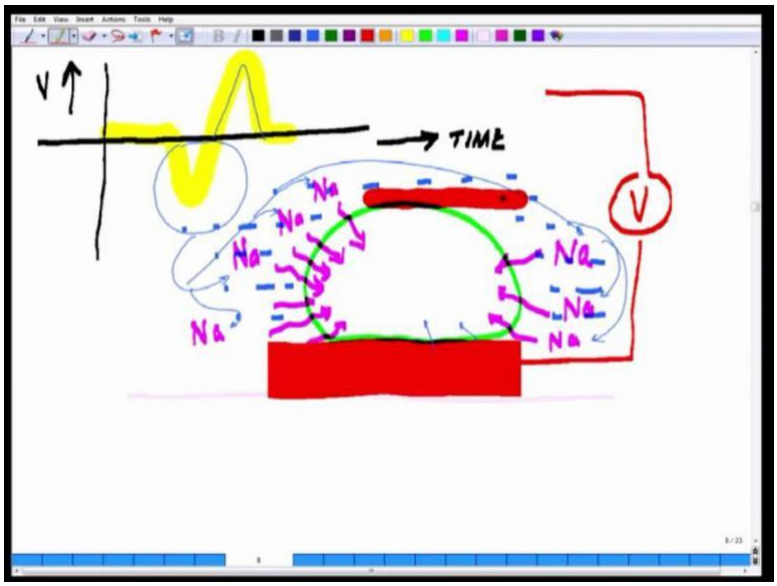


So measurement techniques could be now classified into two groups. The first group is called extracellular recording; the second group is called intracellular recording. So, these are the configuration of the recording system of recording electrodes. Let us first distinguish what is the difference between extracellular recording and what is the difference between intracellular recording. So, the word itself indicates when the electrode is outside the cell that is called extracellular recording; when the electrode is inside the cell, it is called intracellular recording. So, here electrode is if I differentiate the electrode by E like this. So, in this situation, this is the cell, this is the cell and the electrode is something in this position with respect to also here you have the ((Refer Time: 14:08)). So, it is inside the cell. But the second configuration is out here like this, and the electrode is outside the cell with respect to your measuring device you have and there is an electrode at a distance, this is the other configuration.

(Refer Slide Time: 14:31)

So, if I go back on to the previous one, what you see here this kind of action potential pieces and falling down likewise, what you see here is coming from intracellular electrodes, intracellular recording of AP- action potential.

(Refer Slide Time: 15:20)



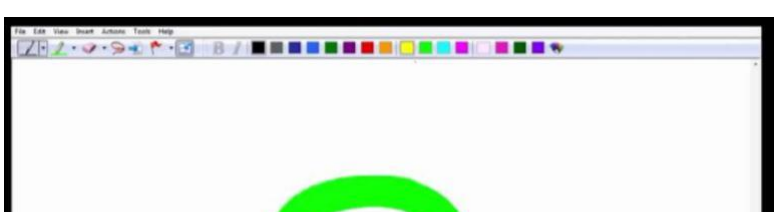
So, went to the previous slide show, this is a classic intracellular recording, but then what will happen in an extracellular recording, coming back. So, now let us see this situation in the next slide what I will do, I will highlight this one in the next slide. So, let us put the cell like this. So, this is the base; on this here, you have a cell sitting on like this. And

now you have two options, imagine an electrode is sitting either sitting underneath like this on the surface or you can have ultra diagrammatic configuration; and another electrode which is an electrode at an if this is an electrode sitting and on top of that this is the cell and this electrode is connected to the voltmeter. And other led off the voltmeter is out at a distance and this cell is in an extracellular field like this it is waved kinda field like this.

When this cell is sheeting an action potential what we essentially see is, there will be an influx of sodium ion from all over the place like this. Sodium is moving in from outside. So, this sodium movement essentially will for a very small fragment of time will meet the electrode which is sitting outside to field as if it is becoming negative because of this surrounding… So, show it like this, if I have a voltage plot sitting here, so y-axis is shown voltage and x-axis shown time. So, the baseline I am showing the baseline like this. So, this is the baseline. Now as soon as the action potential gets initiated, there will be a depth like this, because it will experience at that particular position and it will go up and down. So, this is the zone where the sodium is getting in, this is why the electrode will experience as if locally for a while all the positive charges have moved in.

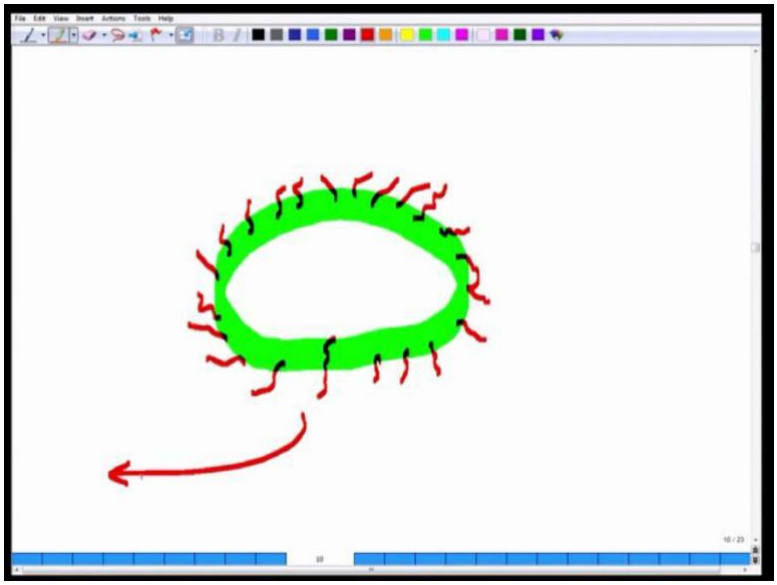
And the electrode on the top of the electrode, you will see a slightly negative and then again ions from the other side will immediately rush in and shoot like this. And this is how you record the excess action potential. In this situation, the advantage and disadvantage, so the advantage is this you are not damaging the cell, your electrode is touching the surface. If you have electrode from the top as I was trying to tell you you could have electrode like this sitting on the top or you could have at the bottom.

(Refer Slide Time: 19:04)



The disadvantage is something else, so the disadvantage is this, so once again draw the situation this is the electrode sitting and this is the cell that is sitting on top of your electrode like this. Now out here, across this gap, there is always a gap out there, we always assume that a cell and an electrode is almost sandwiched over each other, but if you look at the geometry of the cell essentially, so if I keep this cell takes you to a little bit of biology of the cell.

(Refer Slide Time: 19:53)



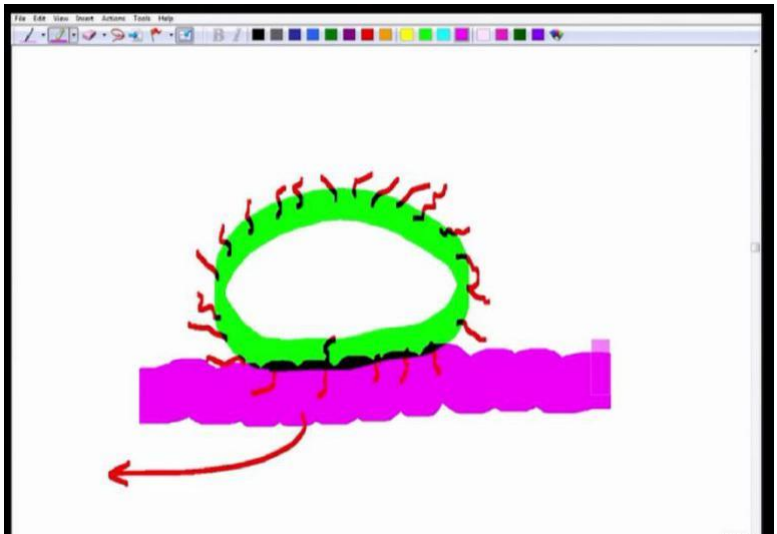
If you look at the cell is something like this is the cell on top of the cell surface you have a whole bunch of different kind of proteins, a whole series of proteins which are like this. It is decorated like this. Any cell is decorated like this. It is not a smooth surface as I am drawing.

(Refer Slide Time: 20:22)

So, when this cell now goes back go back to the previous slide it is in the previous slide now if I add this component. So, I am using a different color just to mark the difference. Let me do something. Let me just change this color and let me introduce all the other components on top of the cell, all the different proteins which are present out there. Now, I introduce the electrode. Now if I put the electrode, now see the problem. The problem is, if you look at it very carefully, you will see a zone which is totally gap. So, that zone I am just putting into yellow now. Now this is the zone, see this gap.

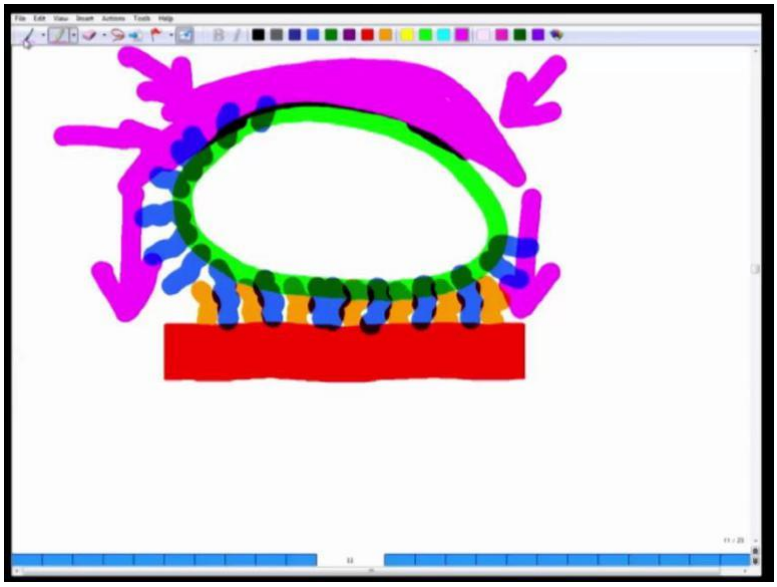
This gap is the cause of a huge amount of leakage, whenever you are making a measurement. And this gap is called technically there is a name for this and this is called cell electrode interface. The cell electrode interface is one of the critical problems of a lot of leakage current which is taking place out here, because this connection or the sandwiching between the cell and the electrode is not perfect, and the cell is almost like a sponge structure. So, the options are either you make the surface of the electrode rough. So, it is something like this.

(Refer Slide Time: 22:54)



So, if this is the rough surface then you made the electrode surface, now let me presume the electrode surface with the pink color. I make the electrode surface something like this instead of making it perfect I make it, so that cell fix on that and kind of you know along with of the topographical feature and along with the ups and downs and bulges of the electrode the cell sets. This is one option by which you can reduce the loss of currents out there, loss of ions, loss of fidelity or improve the fidelity of the signal.

(Refer Slide Time: 23:36)

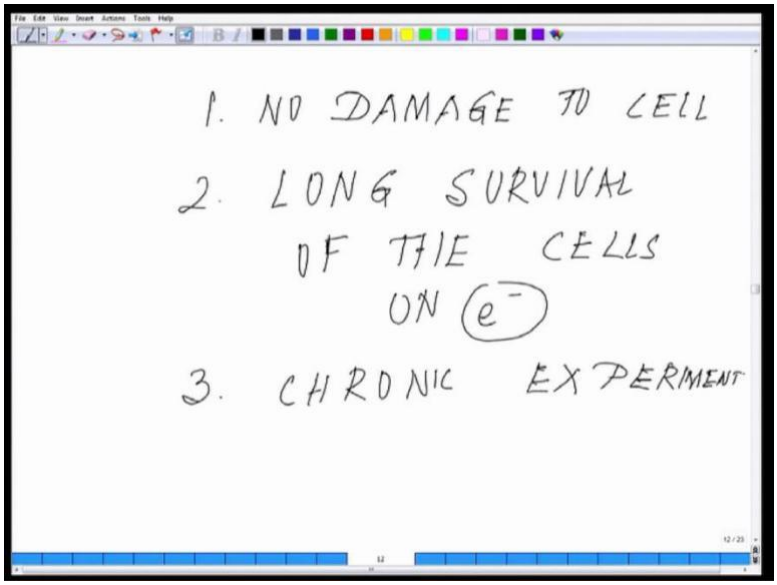


There is another way you can do which is the next technique is you have this electrode, electrode out here, you decorate the electrode with molecules. You decorate it with molecules which will bind to the cell, bind to those, these are the molecules you should decorate the cell and you have a cell out here at the top of it, and now cell has its own a proteins like that which I am showing in blue now. So that way we reduce the gap in the cell electrode interface, this is another way of doing it. So, there are several groups which are trying to develop different kind of antibody light molecules which will bind the cell, hold the cell much tighter on top of the electrode.

There is another technique. Another technique is that you push the cell on top of the electrode with some kind of gel, some kind of a hydrogel or some kind of cryo gel or some kind of a gel you push it down. So, it is something like this, what we are trying to do is let me pick up a fully different color, this is good. So, you are creating some kind of a gel out here which will

put sufficient pressure on the top. So, you are putting pressure like this, and since it is a gel-like structure, it is not going to damage the cell. So, essentially what you are doing is that you are creating a mechanical pressure, which is pushing the cell down. These are the different challenges what I showed you now of doing a recording using an extracellular electrode, but there are several advantages.

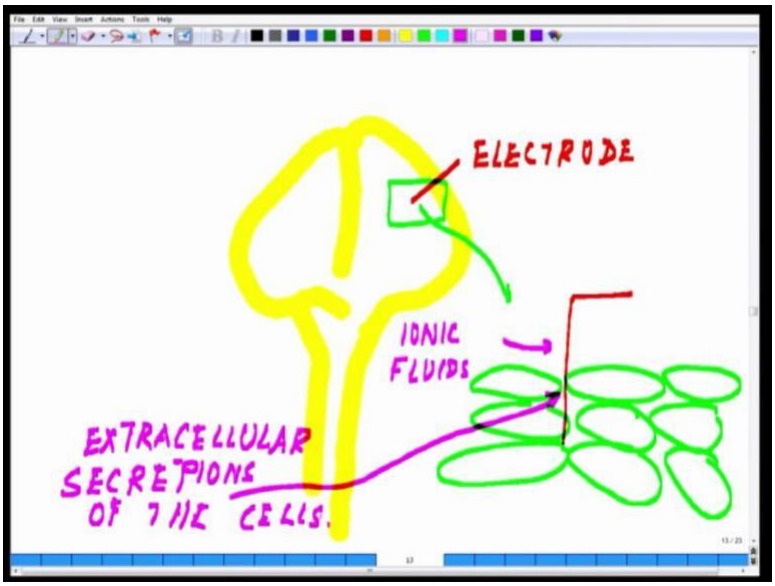
(Refer Slide Time: 25:44)



Now let us enumerate you have talked about the problem of cell electrode interface, talk about let us talk about the advantages. First - no damage to cell; second - long survival of

the cells on electrodes just I am showing thee. Then you can do all the chronic experiments or long term experiments related to drug discovery and all these things. This is the situation when I am talking to you about when you doing the recording outside the human body on a culture dish, but what will happen when you have to implant this extracellular electrode inside the body this complicates it a little further.

(Refer Slide Time: 26:55)

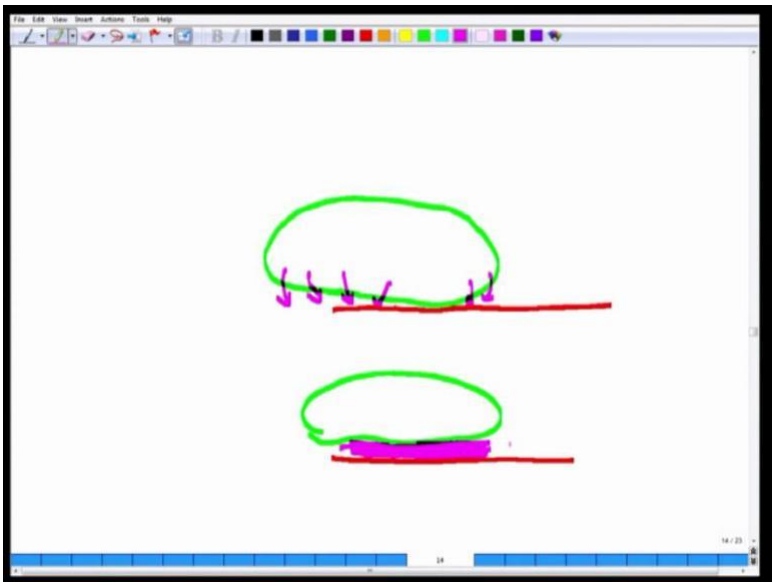


This is a situation, let us imagine say, for example, let us pick up some tissue. let us think we were talking about grain tissue like this. So, this is the now I want to implant an electrode here fine. So, if take the example, this is your electrode which you have implanted, but the problem arises when the electrode is out there is something a real-life problem. Here this electrode is interacting. So, first of all classify what all challenges this electrode are going to face. First of all, this electrode is in a dynamic ionic system, the dynamic ionic system first of all electrode material should be a souvenir that it does not gets corrugated, it does not get damaged because of the ionic material, because there are the whole bunch of sodium-potassium all over place chloride likewise.

The second thing a cell is a dynamic entity. So, a cell continuously secretes certain things in and around it. So, if I have to kind of amplify this image, it will be something like this, at the cellular level what is happening there are a lot of cells like this sitting here and likewise, these are the cells and have an electrode out here like this. So, now, this

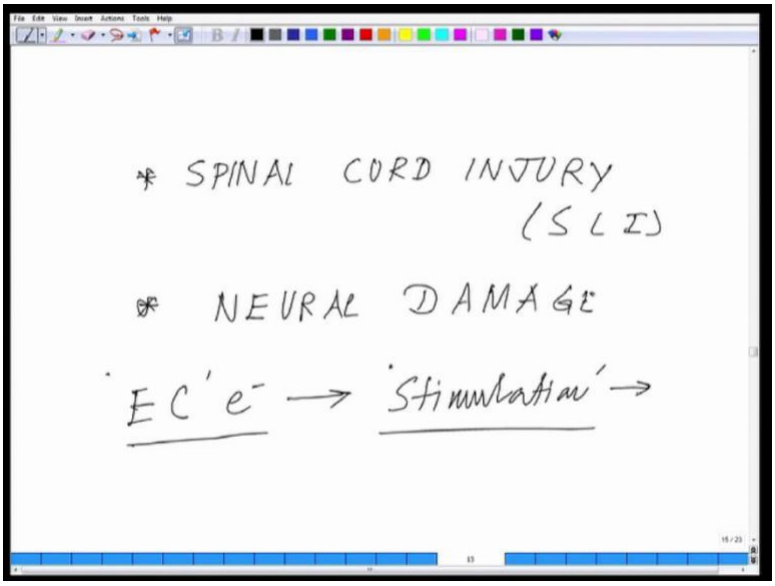
the electrode which is sitting here is experiencing all the ionic fluids and the extracellular secretions of the cells.

(Refer Slide Time: 29:16)



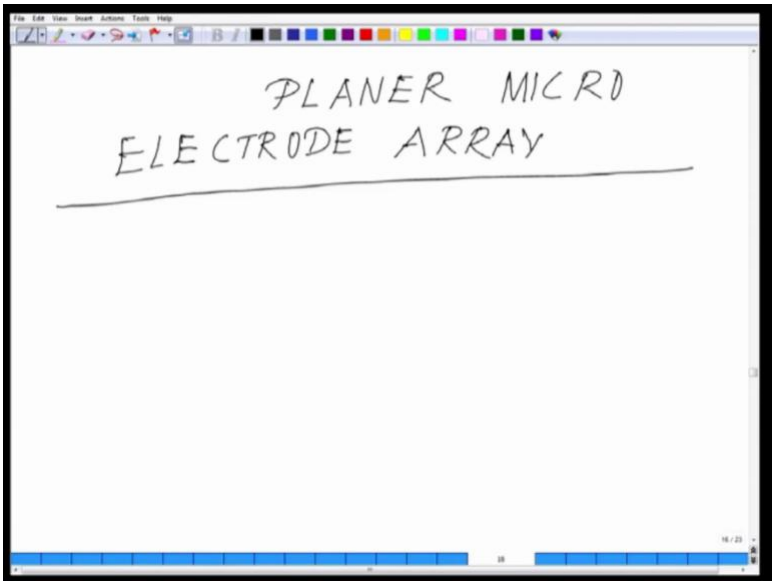
So, what do we really mean by extracellular secretion is something say for example, this is your cell and this is your electrode. Now, this cell is continuously secreting a different kinds of molecules. And these molecules essentially what they do is, this is your electrode and this is your cell, these molecules will eventually plug the connectivity between the electrode and the cell. And thereby you are losing upon the signal what the electrode is supposed to receive all the time. So, you are realizing that extracellular recording or implanting extracellular recording for a stimulation. So, these are multiple purposes in the liver situation, these are implanted into the brain for stimulation.

(Refer Slide Time: 30:24)



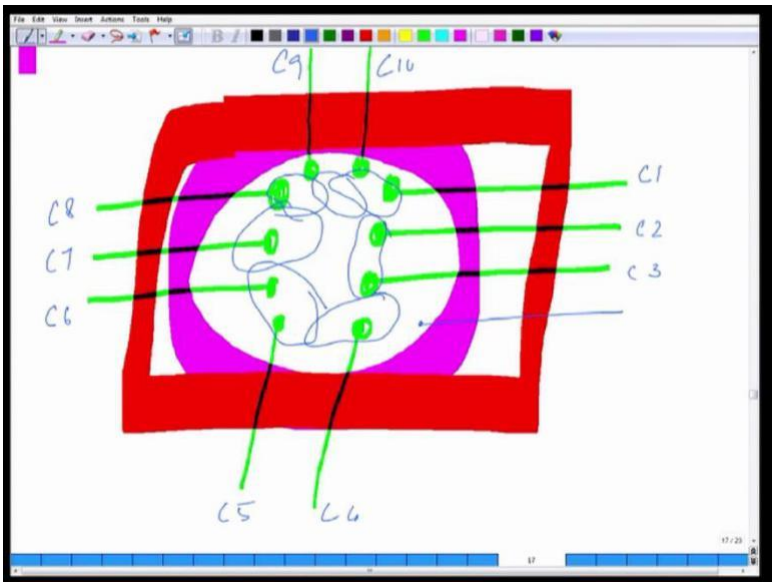
Say for example, for let us enumerate them for spinal cord injury or any kind of just short it is called SCI or any kind of other neural damage. So, these extracellular electrodes, I am just putting EC as extracellular electrodes are used for stimulation electrical stimulation. But they are the problem is that initially they are all fine, but over a period of time, because of all the different situations I told you narrated you their efficiency to transfer the signal reduces the fidelity of signal is been compromised.

(Refer Slide Time: 31:37)



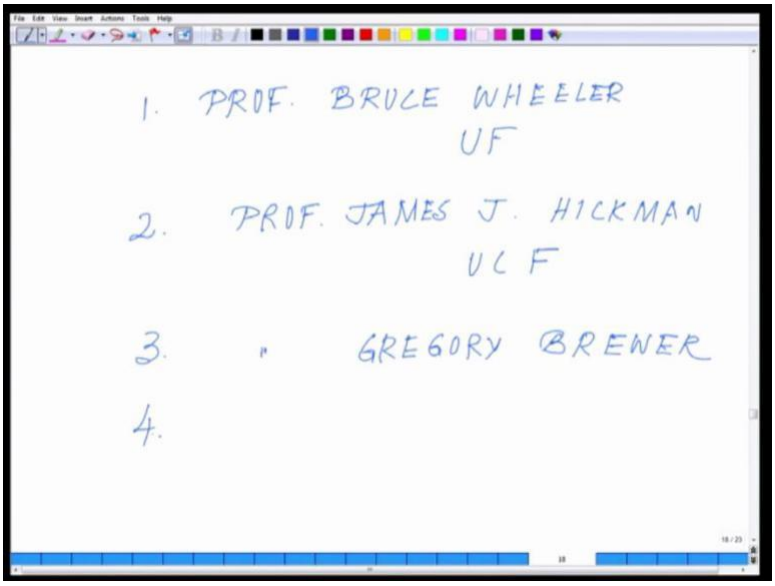
The same thing happens when you grow cells on top of electrodes and some of the devices, which are used by the (( )) industry are called perceptions are called planer microelectrode array. These planer microelectrode arrays are nothing but simply say for example, a backlight sheet on which you have a bunch of electrodes, which are on the surface. And there is a well where you can grow cells on top of the electrodes and you grow the cells. So, I told you there are two situations. So, you can use the (( )) for stimulation and for neuroprosthesis, and there is another situation where you can study the electrical properties of the cell in a culture dish. And it is something like this.

(Refer Slide Time: 32:42)



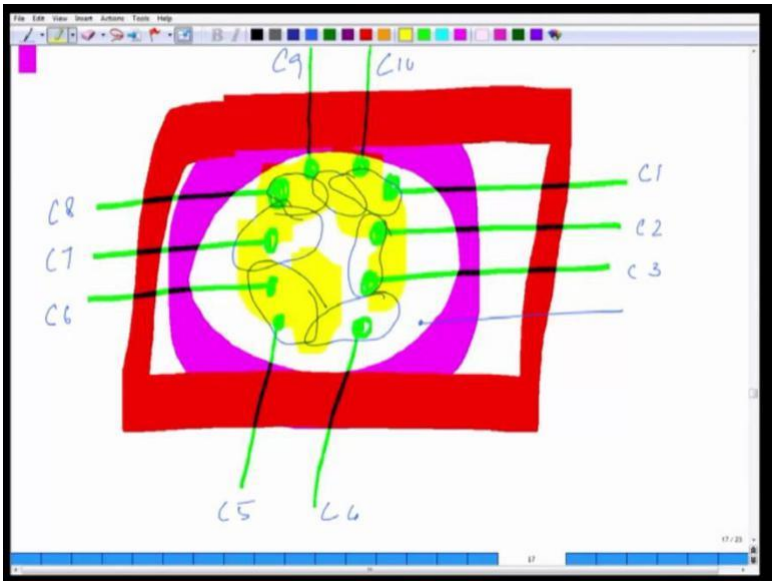
If you look at it the microelectrode arrays will look like this. It is kind of a sheet-like this on which you will see a series of the electrode which are embedded in it like this. And around this, there is a well it is something like this. Inside this well, we can actually grow the cells. So, you have let me look in the cell with blue. So, you really can grow the cells like this on top of this, any kind of cell and these planer and this electrode and there is ground electrode out here. So, each one of these is individual channels, the channel one, channel two, channel three, channel four, channel five, channel six, channel seven, channel eight, channel nine, channel ten. I am just showing it in channels, which I cannot really draw, but there are such 64 channels and 128 channel fantastic my queries which are available across the world.

(Refer Slide Time: 34:40)



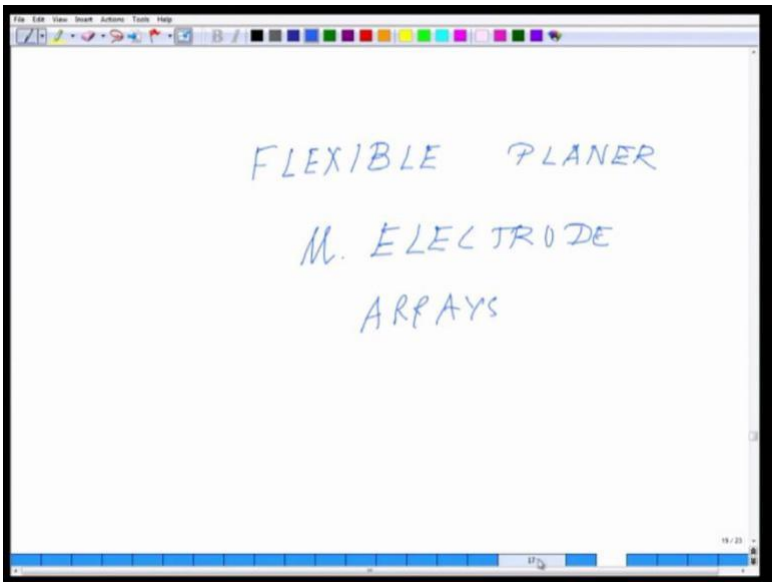
Here just before I proceed there is some individual's work which I want you guys to look online, I will be please to go through the work of these references, please go through Professor Bruce Wheller in the university of Florida James well. Then go through the work of Professor Bruce Wheller and Professor James J Hickman should go through some of his work on his microelectrode arrays and university of central Florida – UCF. Then you should go through the work of Professor Gregory Brener, and then there is one more group (( ))who's work I will in the next lecture, I will tell you there is another very fantastic group in Germany who are doing very nice work in this field. Please go through these some of their work they are really nice pieces of work which you guys will be really interested to look at how the world is moving in this direction.

(Refer Slide Time: 36:24)



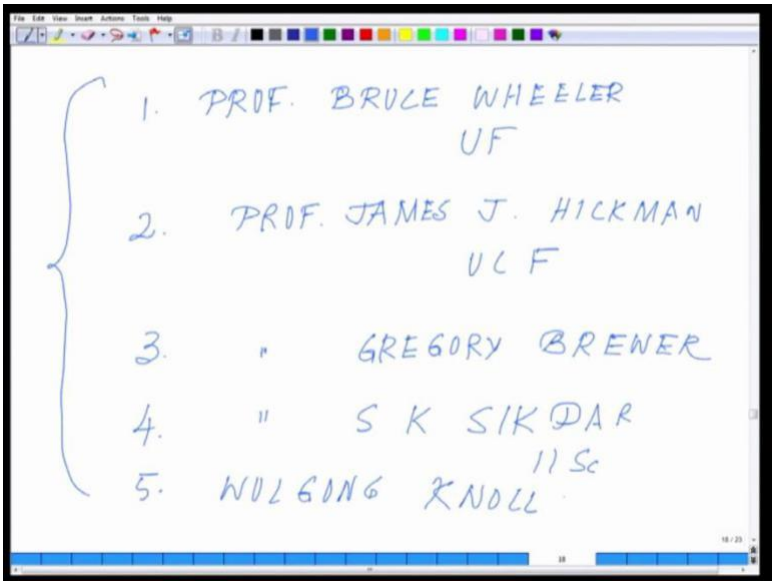
So, coming back, so you actually can on these kinds of planer microelectrode array and do one more exercise go to Google image and look for planer microelectrode arrays images of the planer microelectrode arrays, this will help you. So, pupils are developing a neural network on top of it. So, they are making something like you know like this. The network you are forming and actually, you can monitor the signal propagations within such networks. So, the whole field is really moving at a very interesting pace.

(Refer Slide Time: 36:59)



So, apart from it, there is the second generation modification that is taking place which is basically you are making these arrays which are flexible planer microelectrode arrays. What you are essentially doing the base what you are making out here, what I will show you you are making this base as a flexible base. So, they are much more easily, they are not a rigid structure, you can really do a lot of measurements on them. And out here all the recordings what you are getting are recordings what I explained out here from here this all the extracellular recording. Now correlate this structure what you have in front now which I showed in one of the previous slides with the structure that I showed you. So, this is one single electrode, and here what I showed you is ten different electrodes, and it could be sixty-four it could be one twenty-eight likewise and so on and so forth.

(Refer Slide Time: 38:21)



And those of you are in the Indian context, you may refer to the work or of Professor S K Sikdar who is working in the Indian Institute of Science in Bangalore. He also has a very active group out there. Then a few other people I will cross check for other people who are there in the field whose work I really will appreciate if you guys go through it, go online, give search and see the kind of things they are doing. So, what I will do now at this stage, I will be closing the lecture today, and we will continue again a little bit of a microelectrode array and then we will move onto the next technique which will be the intracellular techniques.

Thanks a lot.

Bioelectricity

Prof. Mainak Das

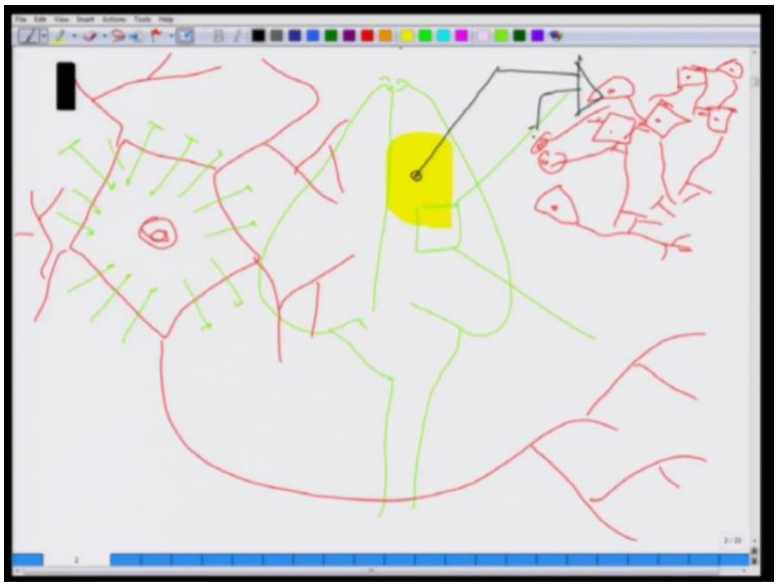
Department of Biological Sciences and Bioengineering

Indian Institute of Technology, Kanpur

Lecture - 9

Welcome back to the NPTEL lecture series on Bioelectricity. So, we have finished the eight lectures, now we are into the ninth lecture. In the last lecture, we talked about the microelectrode array and I requested you people please go online and check the real image of the microelectrode array. So, one of the things that people are currently trying to do, so we will briefly talk about the applications of microelectrode array what people are trying to do. So and then we will move on to the intracellular recording. So, one of the things, what needs a bit of visualization is that think of this whole brain its thousands and thousands and thousands of neuron making multiple circuits out there something like that.

(Refer Slide Time: 01:10)



If you have to visualize the brain, it will be something like this. So for example, this is your brain, and this is the brain stem and here you have the brain. Now at any point, if you pick up any particular point and if you magnify what essentially you will see the series of neurons like this sitting out there functionally dynamic state. It is a complex network something like this. And in between, you have the glial cell and likewise series of supporting cells something like this. So, at one point in time, in this network, it has

been estimated that one neuron receives could receive the ability to receive signals from ten thousand other neurons. In other words, what that translates down into at one point of time a neuron on it is surface has ten thousand synopsis something like this.

Say for example, if this is a single neuron and this is the axon, and here you have the dendritic tree something like this. This is the nucleus. So, this neuron at one point can receive a signal from say ten thousand different sources like this. I could only draw certain feasible space I have; I mean I have limited space; I cannot draw all of them to just to show you. So, one of the critical challenges of modern neuroscience as a whole is how really to understand, how a network functions because, in a complex brain, it is exceptionally challenging. Most of the time, when you insert an electrode what you record is a field potential. What I meant by field potential is that say, for example, I have an electrode cell, I am showing like like this, I have this electrode out here. This electrode will only measure something like this, and this region all the activities which are taking place out here in a broad region that is all it does.

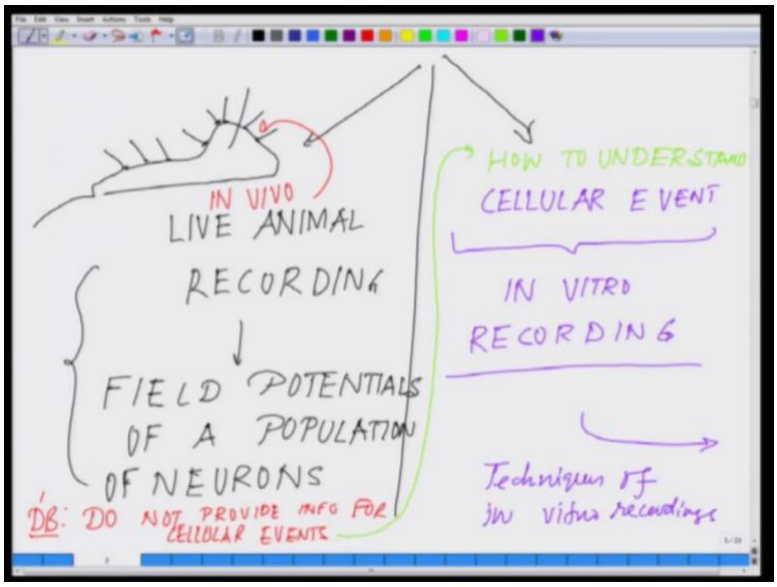
So, an electrode recording like that cannot really pinpoint what is happening at a small will show like this or it cannot really pinpoint the cellular events which are taking place. What you are getting is something an aggregation of say ten thousand neurons at one spot or like you know five thousand neurons, of course, it depends on how smaller is the size of your electrode, how finer the electrode is you get a field study. The summation of the electrical activities of a population of a neuron that is essentially is helpful for several to understand the rhythms and several, circadian activities all though there are several things leap and all those things, that is not really the way you can figure out what exactly is happening at individual cells.

Say, for example, I have a drug which is targeted to specific kind of neuron or say, for example, I have a drug which is getting into the brain, I have no idea what it is doing to the individual cell, for that you need a different approach. So, most of these approaches depend on the individual culture model. What does that mean, there are two ways how you can approach use the electrical power or the bioelectrical techniques for understanding the biological phenomenon. One is that you insert an electrode or you poke an electrode in a live animal this is one way. Where the live animal, the animal is moving around and you are recording, in real-time you are recording the events, which are taking place that is one way which is nevertheless one of the most powerful profound ways to do it. But, that

would not give you any ideas I was mentioning to you about individual cell what is happening.

In order to understand an individual cell, you have to go down to at the cellular level and that you cannot do in a live system then you have to either take out the part of the tissue outside the system you can make a slice you can do a slice. On the slice, you can keep the slice alive for six to ten hours; and on that slice, you can do recordings. There is another way where you place the slice on a microelectrode array that I have shown last time. So, in that situation what you are essentially doing is, you are keeping the site to architecture intact.

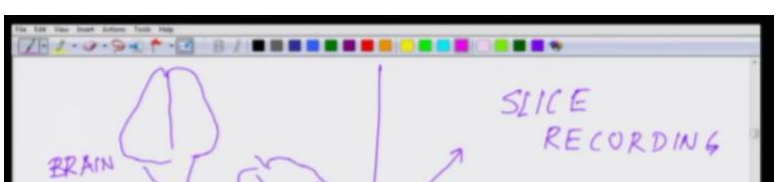
(Refer Slide Time: 06:26)



So, for graphical representation what I am trying to tell you, so these are the different techniques. So, this is the live animal. Say for example, if this is, these are recording techniques - live animal recording. So, you have electrode either implanted like this or you have a surface electrode like this, and the animal is alive. This is the spinal cord likewise you know and essentially what you are recording are the field potentials. The other set of recording out here, which is in you cannot do it in the live animals, so this will give you field potential of a population of neurons. So, the drawback as DB. Do not provide information for a cellular event then this takes us to the next level, how to understand the cellular event. For the cellular event, you need to have what we call in vitro recording and what we essentially call this technique as in vivo or in

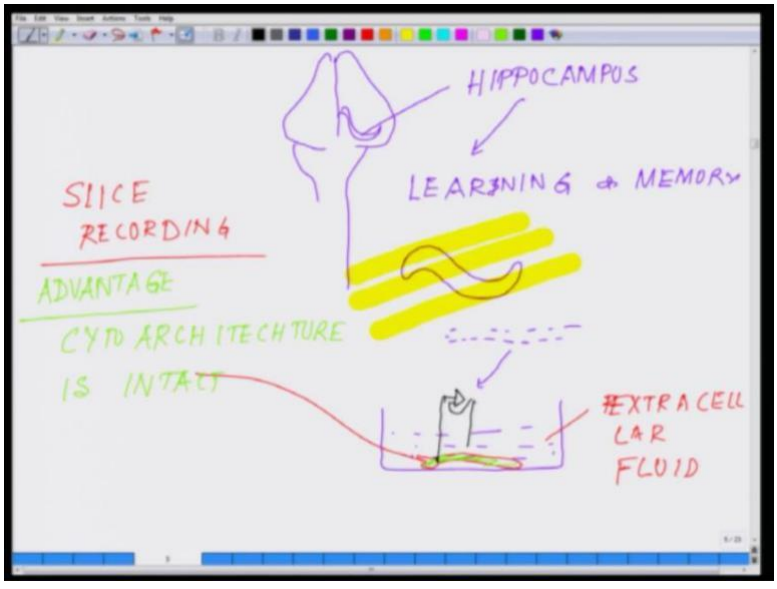
animal recording. So, to in vitro recording, so in the next slide, we are moving different techniques of in vitro recording.

(Refer Slide Time: 08:57)



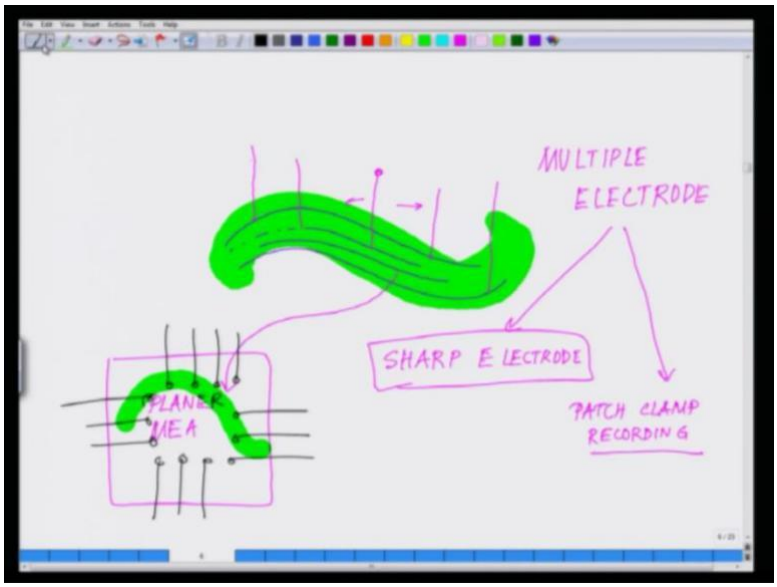
So, say for example, if I look at all the excitable tissues, so one of them is your brain and the spinal cord then you have the heart, here is the brain and SC. So, essentially you have two major techniques, one is slice recording, the other one is disassociated cell culture recording. What did you mean by slice recording? So, in the next slide, we will talk about what meant by slice recording.

(Refer Slide Time: 10:05)



So, say for example, this part - this is the brain, you anesthetize the animal, you remove the brain, and you kill the animal. And we know that this is one of the areas which is called the hippocampus which is involved in learning and memory campus. We will talk more about it when we will talk about learning and memory involved in learning and memory. So, what you do essentially you take out that organ like this, and then you make slices something like, you make slices like this. So, you essentially get you can make slices in different ways. So, you essentially get a very thin tissue-like this, and then you put them in a chamber immolating the condition of the brain either tissue is sitting like this. So, here is the extracellular fluid with a different energy source to make this tissue survive and when you approach already the cytoarchitecture is all maintained. Just to mention slice recording and advantage, cytoarchitecture is intact. Then on that what you are getting you are putting the electrode, portion black like this and you start doing the recording.

(Refer Slide Time: 12:53)



So, this is essentially is talking about the slice recording, and slice recording is very popular because your architecture remains intact, you can really poke there, so the circuits remain intact, you can really poke the electrode at a specific zone of the circuit and stimulate one circuit. And say for example, in this diagram if I just highlight this further. So, it will be

something like this, if this is the part of the tissue, so for example, if this is the hippocampus is almost like this and it is known that the hippocampus has something like this. So, the hippocampus has different circuits on its system something like this c a 1, c a 2,

c a 3 - these are the circuits within the hippocampus. We are coming in-depth on this one after the ward. So, now, what you can do now to study this different circuits and connectivity you can poke electrode here, you can stimulate here, you can do a recording from here or you can poke an electrode here, you can do a simultaneous recording from here or you can do it from here you can floe here likewise. Or you can stimulate here and you see how it is distributing on both sides likewise, and you can put multiple electrodes, so this is another advantage.

And you can do two kinds of the recording here, you can do sharp electrode recording which I have already talked and you can also do something called which I have not discussed yet patch-clamp recording. We have not discussed it, but I definitely discussed it with people, and some people even tried to take this whole circuit and take this whole slice and put it on top of a microelectrode array on a planer meant. This whole circuit is placed on a planer meant. If you guys have seen it, it is something that looks like this. Please again I request you kindly go online and check the structure that will help you, and the circuits sit like this something like this.

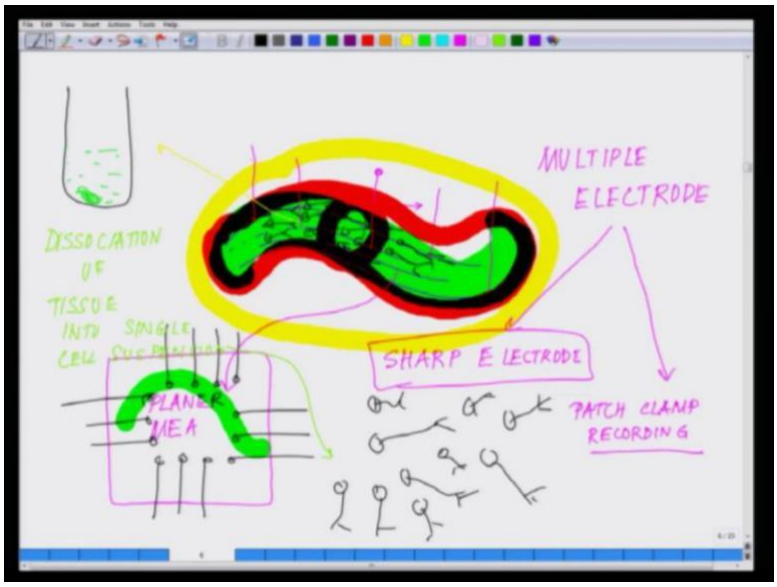
So, you see these are different techniques that are being used to understand the functioning of the brain by extracellular. So, this is what I showed you in planer microelectrode array is an extracellular pattern of recording then you could have intracellular electrode and you could have patch clamp. Patch-clamp, I have not discussed with you people what I am going to discuss after this. So, this is one way, but there is another way which is the third way and which is so you have to realize the drawback of slice recording is this. Slice recording cannot last a slice cannot last more than six to ten hours. It is really tough, because it is three-dimensional tissue out there, which is already taken out from the system, it is not really adapted.

So, for maybe you can make it last of eighteen hours maybe, maybe a day if you are exceptionally good, but the problem is that whenever we talk about the drug trials, chronic situations, chronic experiments the story changes. Why the story changes, because it will realize that say, for example, I put a drug, and the drug will be acting over a period of months and maybe sometimes years. And most of the animal trials are really costly, whenever an on long term effects at the cellular level at times get missed.

Say for example, if you go back to the slide where I showing you the real animal situation. Say for example, I injected a drug into this animal. Now, this is circulating all over the body, all over the place. So, it is really tough to know exactly what is happening at the individual cellular level; and over a period of time, how it works. Chronic experiments are really tricky and really tough to do. And on top of that with animal ethics and the cost of animals, every drug discovery really takes a huge amount of funding, huge and that is how when the drug comes to market it becomes so costly, it is not costly because this drug is out of the world. It is costly because it has to go through all the different channels of screening and those screening takes enormous money, an enormous amount of funding is required, so that capital investment essentially jack up the price of a drug when it comes to the market.

So, now coming back, what is the other technique, and especially these kinds of drugs are exceptionally costly when you talk about the nervous system or the cardiac system which are kind of you know pretty much your lifeline a cardiac drug. It is not easy, I mean it is really tricky, you have to go through all possible channels of hoops before there is drug kind of gets in the system. So, coming back where we aware of, what is the third technique, so we talked about the slice recording. Now we talk about the third set of recordings which is fairly old, yet fairly new also, there are two aspects of it.

(Refer Slide Time: 19:19)

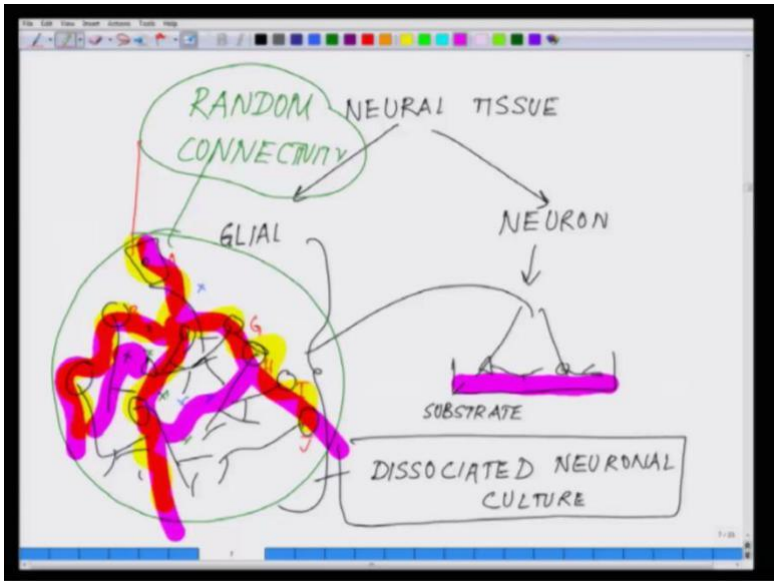


What you do essentially here, so from this diagram itself, let us start to draw. What you do here, say for example, I wanted to have understood about the hippocampus. What I will do is that I will pull out the hippocampus I will break the hippocampus in the sense I will disassociate the cells of the hippocampus by doing by using different enzymes or different mechanical ways. So, if you look at this circuit now, so at the cellular level if you try to look at this circuit, the circuit is essentially nothing but series of neurons sitting like this, like this, thousands and thousands of neurons sitting like this. And they are making circuits at a

different level and this is just the top layer I am showing in multiple layers and likewise. They are arranged in a specific array, specific circuit, and everything.

Now what we do is that you take this hippocampus take this out, take this whole thing out or you can take any part of it if you are very good at dissection or something. Then take this out and say for example, you have collected that part of the tissue out here likewise in extracellular fluid, then you break the tissue, and this breaking of the tissue is called disassociation, disassociation of tissue into a single-cell suspension. What we mean by single-cell suspension, means now you have all these individual components that I was drawing are separated out something like this. In that process of course, the tissue undergoes a lot of damage or something, but those, which survive are important for you.

(Refer Slide Time: 21:17)



Now you take these neurons and the accessory cells depending on you have the different mode of that you can purify at this stage you can, so if you have the neural tissue in case

of say neural tissue, you can purify the neural tissue, you can have the glial cells separated which are the supporting cells. You have the neurons separated out. And then you put them in a dish to grow, of course, they would not grow just in the thin layer, you need do you a bit of homework, you have to coat this dish with something on with they prefer to grow some kind of substrate on which these neurons will grow.

So, this is the substrate; and on top of the substrate, you have the neurons growing like this. So, if you get a top view of this something like a top view little look like this, neurons are all over the place likewise. It will be a random connection between different neurons, this is a dissociated neuronal culture. So, these dissociated neuronal cultures, now you can approach the individual cell with individual electrodes. You can have a sharp electrode like this. You can approach the individual cell, and you can monitor several events you could put x, y, z compounds out here say, for example, compound a compound b likewise and there is another compound out here or a third compound out here likewise, and we can figure out their figure out what they do.

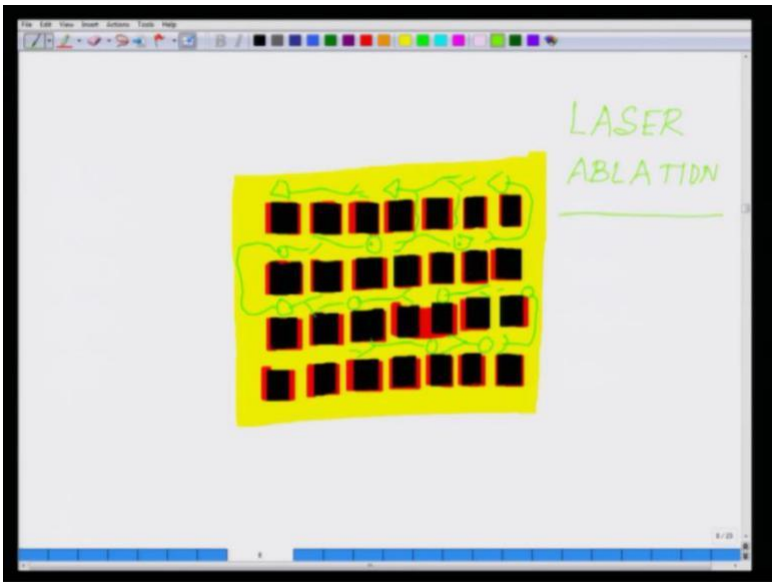
So, this technique gives you access to the individual cells in disassociated culture this is what it does, but it comes with a drawback. The drawback is that I told you in the previous slice preparation the cytoarchitecture is maintained. In other words, if you go back, so the circuit is all maintained out here circuit is not getting destroyed, but out here what you did, once you disassociate everything, then there is random connectivity; you do not have real control over their connectivity. So, there is random connectivity.

So, this network is forming in a very very random manner, you really cannot dictate that how many synapses are forming, you really cannot have any control, but eventually, it becomes really cumbersome to detect. Say, for example, think of a practical situation if this is I level at this as A, and I level this as B, this as C, this is D, this is E, this is F, this is G, this is H, this is I, and J likewise. Now say, for example, a signal is getting originated from here, and I am seeing the signal is all over the place. Now I really do not know how the signal has moved, I really cannot trace it, because the signal may move like this, the signal may move like this, the signal may move like this, the signal may take back turn and likewise signal may have connectivity like this. So, there is no way I can figure out how the network exactly functions.

So, network behavior is really tricky, it is almost the same situation as when you do a field potential measurement. You really have no control over the number of cells which will be involved in generating that signal. So, you really do not know, and moreover, you really cannot in a random circuit, it is really difficult to keep a tab at the changes at individual synopsis because, at an individual synopsis, it is again getting connectivity from multiple sources, because it is random. There is no way that you can control that connectivity, because anything gets from connection with anybody, so that makes the story very complex and that is something you do not wish to happen.

But if any technology, now if you need to a directed or you know completely patterned growth, you need some different kind of technology then starts within this disassociate culture the current technology which most of the people in the area of bioelectricity or bioelectrical accordance are following. So, they are trying to develop build circuits out of this disassociated cell how they are doing so…

(Refer Slide Time: 26:51)



So coming back to the basics again, so I drew that I told you that there is a substrate. Say for example, let us try to understand it say for example, you have a culture substrate like this. Imagine this is the culture substrate; all the cells will grow on it everywhere. On these culture substrate, say for example, if you have a way that you could introduce some pattern, say for example you do something like this, you ablate this part of the circuit. What I meant by ablation means I am removing that particular yellow color compound from here. So, if

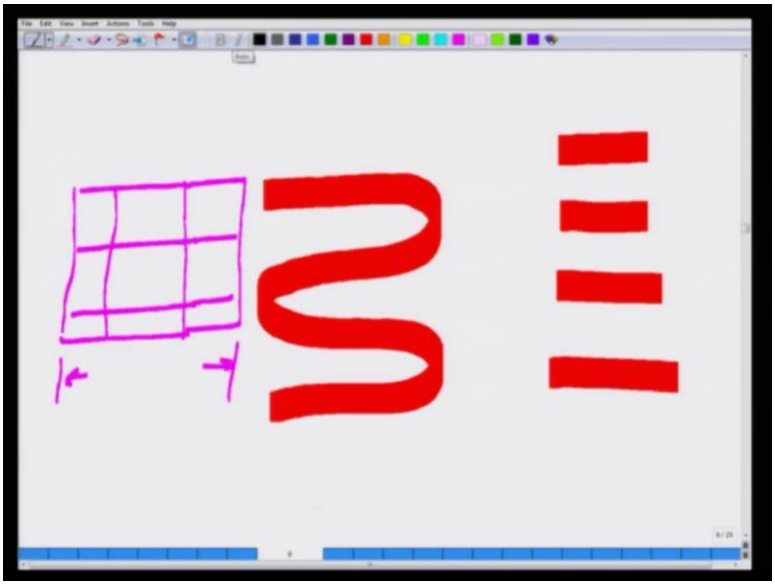
you remove the yellow nothing will grow there provided. You backfill it with something which one promotes. So, essentially what you are getting now look at it. So, essentially now cells so say for example, I backfill it what I meant by that on these zones you fill it with another something.

So, on black, I really cannot draw anything, because everything will, let us see if we could do it with red, this is the backfilling agent. So, that is ensuring that those ablated surface. So, what we those who are not understanding ablation, let me just explain. What you do essentially do is say, for example, you have the substrate like this, you take a mask, and if you imagine this is the substrate and I have the mask and I keep the mask here. If I keep the mask here and I put a laser beam or something. So, at this part, where the mask is covering it nothing will get ablated; rest of the places will all get ablated; does that make sense? So, that is exactly what I am trying to tell you.

Say, for example, I put a mask, the zone in which you are exposed to the laser beam is the one which is blank. So, it turns out those spots. What is left with all the yellow that things so grow, and now where you have the black spots, there what you do is that you backfill it? You did it in such a solution, which will only sit on top of those ablated regions that compound would not sit on the top of the yellow, so that is what I trying to do. So, this is that next compound by which you ensure this black compound is the one which will not allow any cellular growth at that particular black surface likewise.

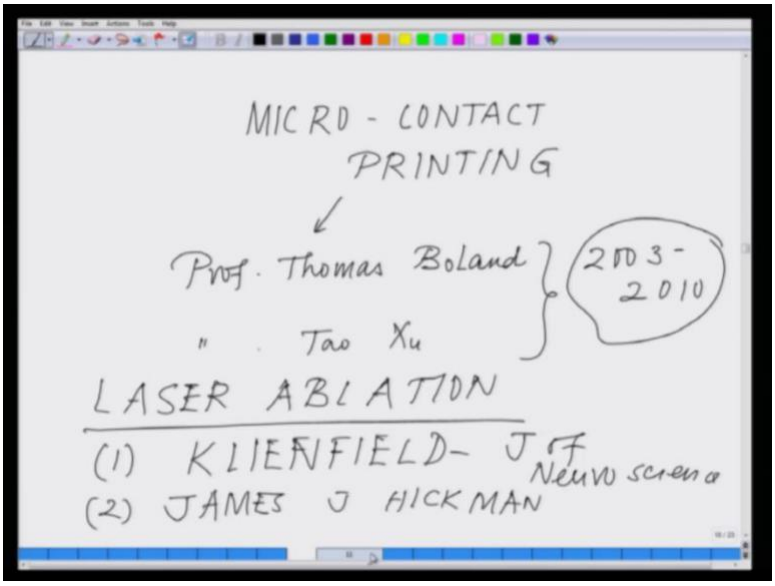
Now you have a pattern situation; this pattern situation will allow the neurons to grow like this. The neurons could grow if their thickness is like this, something like this. If this thickness is good enough for a neuron to grow then there may be connectivity coming out like this, possibilities are there, but there are ways to you know to control that. Now what you are seeing essentially is, you are trying to control the position of the neuron, and you really can do it in a very interesting way. There are several geometries, which you can follow. So, this is one geometry I showed you. So, this is done by a technique which is called laser ablation. You ablated using a laser. There is another way you can do it. You can make these circuits using your old-style inject printers what you do is that you print the circuit.

(Refer Slide Time: 32:06)



Say for example, you on your word document, you draw the circuit, narrow it down and on the cartridge, where you fill the old inject printer, you fill the ink. You throw away the ink sterilize it, and on top of that you put this either the substrate you want to do. So, what will you do the jet printer will make, say for example, a circuit like this say for example, I want a circuit like this, I want the cells to grow like this. So, make a circuit like this or it can even make a circuit like only lines or it can make a circuit like or it can make a circuit like this. So, there are several ways you can make circuits, and these are some of the different ways that I am trying to highlight, and you can control the dimension.

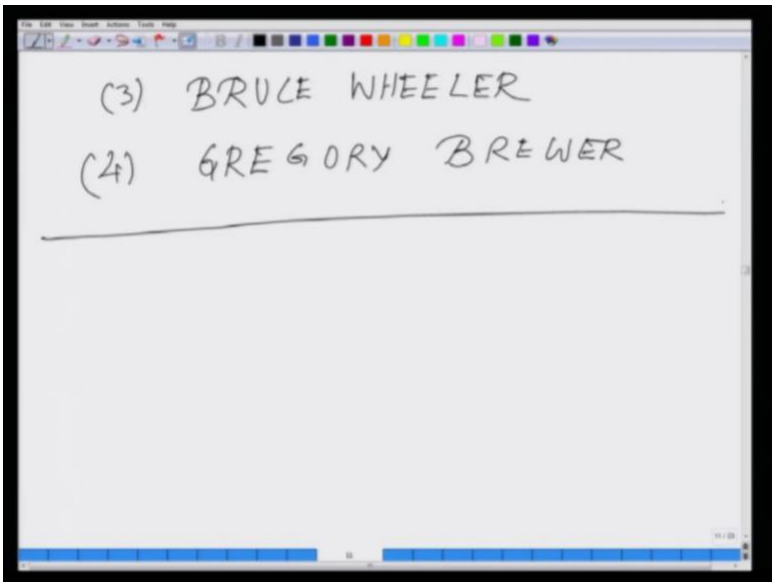
(Refer Slide Time: 32:39)



So, for this, I will recommend you, kindly go through some of the extra materials which I expect you to see the papers for microcontact printing one of the pioneering people you should refer to the work of Prof. Thomas Boland; currently, he is in the University of Texas at Alpaso; Prof. Tao Xu, these people have done very significant amount of work on microcontact printing. It is worth reading some of their work; how they have done it using very very simple most of this work was published from 2003 to 2010. Now, also some of the work they are publishing and they are absolutely phenomenal I mean the way they have done all these things just with very crude techniques around them. They could really do very nice microcontact printing and some very well documented papers are there from their side. So, this is one group paper I like you, people, to look at it.

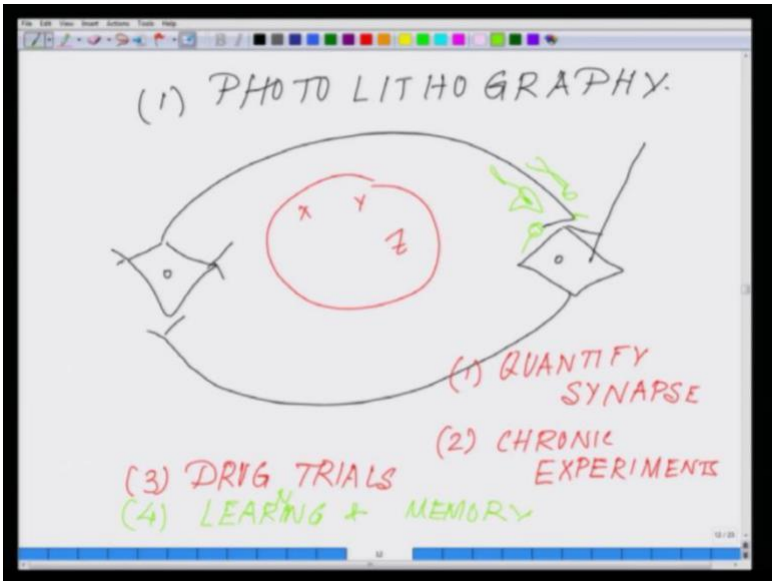
Laser ablation and all this work I expect you please go through the work of there are few people whose work will be really looking one will be, this is one of the very old paper Kline Field in the journal of neuroscience. It is a very seminal paper Kline Field; and you should go through the work of James J Hickman, he has done extensively extensive work in that area.

(Refer Slide Time: 34:40)



You should look through the work of Bruce Wheeler who also has done a very significant amount of work in this area and you have Gregory Brewer. These are the people who have done a significant amount of research in this area, and it is defiantly I will recommend you people please go through some of their work, they have worked in wide areas, but definitely, they have made some seminal contribution in these kinds of printing circuits. So, the current status is like this.

(Refer Slide Time: 35:25)



I mean you can go to the other end of the world. So, there are techniques that are being used in laser ablation and then there is something called photolithography and this will get a lot of references in Prof. Hickman's work photolithography and professor Gregory's work and Prof. Kline Fields to work and of course, Prof. Bruce Wheeler work. So, we talked about the microcontact printing where you should look through the work of Prof. Thomas Boland, and there are few other people who have done very significant work. I will come back to that in the next lecture. So, these people have shown that they actually can guide the neuron in a specific trajectory a single neuron.

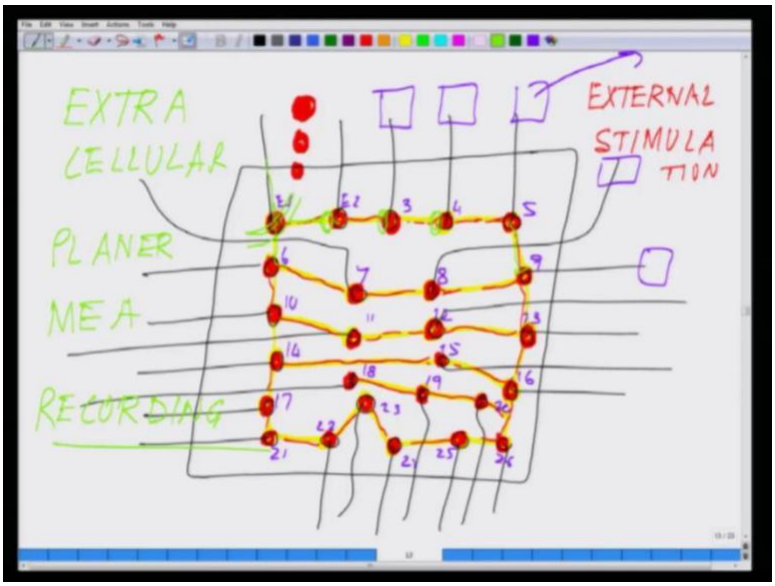
So, you will see some of these circuits like you know you can nowadays, you can develop like two neuron circuits like this. These are you will see these kinds of circuits are being developed single two neurons. So, now in these two neurons, you can really approach with a single electrode, you can have x y or z compounds all over the place, you can really quantify the synapse. So, what all you can do, you can quantify synapse – one, you can do chronic experiments for a long period of time and this chronic experiment could be these circuits could survive for more than a month or so if you are really good at it.

So, they may chronic experiments you can quantify the synapse, you can do cheap drug trials they reduced on the cost of drug trials. On top of that you can introduce the supporting cells like you know the glial cells, you can study the dynamics of the glial cell and on top of that here is

the control model where you can study learning and memory. So, these kinds of control circuits you can make series of them. I mean as you at the authors whose papers I have mentioned you or the those who have made some kind of contribution if you will read these papers, you will realize you can make series of such circuits to approach a single cell in a very elegant way. And you can really understand the network behavior in a very simplistic reductionist approach; of course, it comes with its drawback, because you rebuilding the circuit. So, you know there will be some error here and there, but the way biology works are that you start from the whole animal, you come at the single-cell level and then it all has to merge.

So, there is no one technique that is perfect and there can never be on technique which is perfect. So, the whole idea is you know having multiple techniques trying to tell you or trying to unrevealed truth of nature this is what we are always trying all throughout like. We are trying different techniques, so this is one approach.

(Refer Slide Time: 39:15)



So, another approach in the same line which is you know hybrid approach which is been followed, I am going to draw is I introduce you to the microelectrode arrays. Let me draw a microelectrode array and tell you what is that approach, it is a very interesting approach. Say for example, you have these microelectrode arrays sitting out here like this, now you may have electrodes like this. So, if you can pattern this, say for example, I have a pattern like this. The cells will follow this trajectory something like this. Now I connect this like this. So, now the rest of the places where you see yellow are the only places where the cells will grow; the rest of the

place cells will not grow. So, I modify the surface of this planer microelectrode array in such a way that cells will grow all along those electrodes they are connecting the electrodes.

The dimension of the electrode is said twenty to thirty microns, and those lines say for example, they were an aspect ratio of 10 to 20 microns or maybe 10 microns. And specifically, except the places where the electrodes are their aspect is slightly more maybe this is 20 micron and the lines are said 5 microns thick. So, on a five-micron surface, it is really tough for a cell to sit, but the cells will sit preferentially will sit on top of the electrode, because this electrode regions have more surface area, it is around 30 microns or 20 to 25 microns. So, when you put the disassociate cells in this chamber what will happen?

So, for example, so if I represent the cells with red, so now, I am putting the cells into it. So, cells will preferentially try to sit here, because these are the zones where they will try to migrate to on top of the electrodes because that is where they will get the maximum surface area to grow. These are all disassociated cells. So, once they will sit like this, what they will try to do, they will try to send out processes like this. They will try to send out processes like this to connect with each other. They can do it in like this, like this, in several ways you can do. You can even simulate this circuit, in order for this whole process to take place, and they will form a very controlled network. A network that you can monitor in real life something like this. So, they will start forming network inside you keep this whole system inside in incubator and you monitor it, as they are forming the network

As they are forming the network, so what you can do, you can give an external stimulation for network formation and stimulation. And you can register the electrodes say for example, I registered them as E 1, E 2, 3, 4, 5,6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26. So, I can register the electrodes and you really can monitor that activity at the individual channels. At the individual channels of the amplifier, you can monitor the activity of what is happening in which electrode. Now once a network is formed, say for example, I give a signal out here, I give a stimulation out here now how this stimulation is moving along this circuit, I can monitor in real-time. How the synopsis is forming out here, how the synopsis forming here, how it is forming here, how which circuit is getting more strengthen, how it is getting more strengthened I can study all these things now. What and then based on this I can back-calculate what is probably happening in the brain.

So, if you look at it there are profound scopes of, which is open up with the advancement of modern microelectronics, we are able to access a single neuron on top of an electrode. And this is all could be done using an extracellular recording, these are all extracellular planer MEA or

microelectrode array recording. So, this is the advantage that the microelectrode array offers in order to study the circuit from a very reductionist approach. It is not a holistic approach, it is a very very reductionist approach; you are building the system from the base again from the grass root, brick by brick you are building the system.

So, I will close in here for this class; and in the next class, we will talk about the other end of the intracellular recording, where we will be approaching a single ion channel because once I will introduce the ion channel then I will talk to you about the structures and the details of the ion channels.

Thanks a lot.