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.

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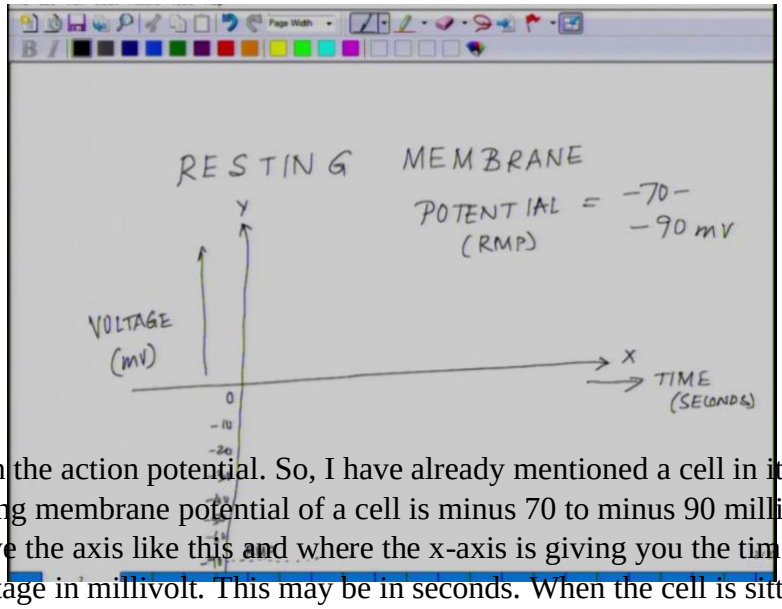

So, let us start with the action potential. So, I have already mentioned a cell in its normal resting state - resting membrane potential of a cell is minus 70 to minus 90 millivolts. So, essentially, if I have the axis like this and where the x-axis is giving you the time and y-axis is giving you the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

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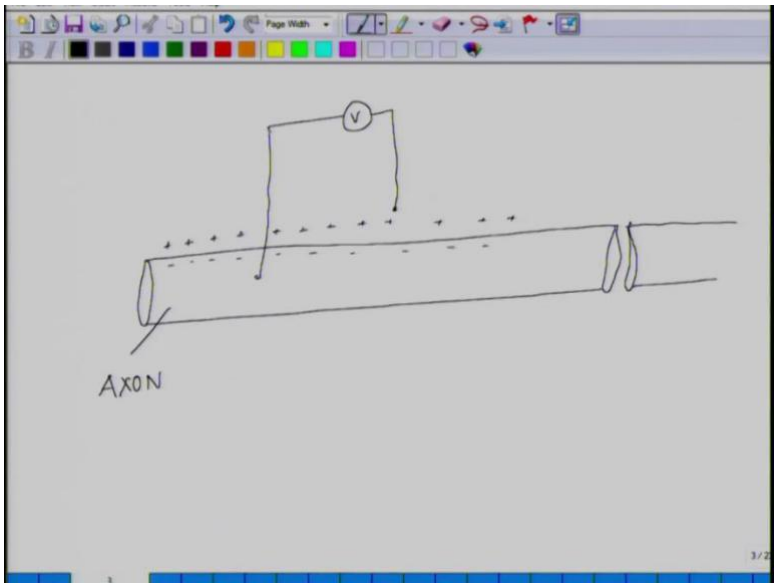

So, say for example, just to visualize what kind of experiment they are trying to do something like this. Say for example, this is the axon out here and part of the axon cell body is out there, and you have an electrode out here, and we have another electrode that is sitting outside. So, with respect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

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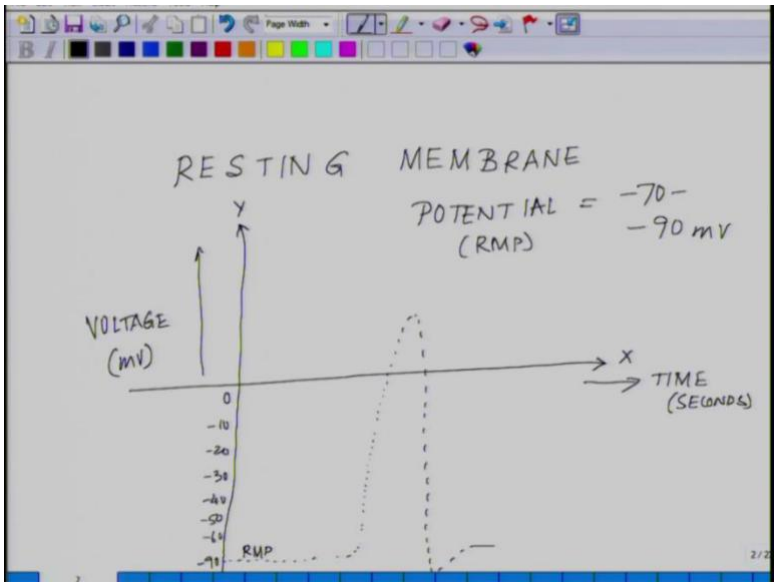

So, if the cell is excited, it is been observed that if I go back to the trace, there is a cell become more positive, positive, positive and then it overshoots the zero and it comes

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.

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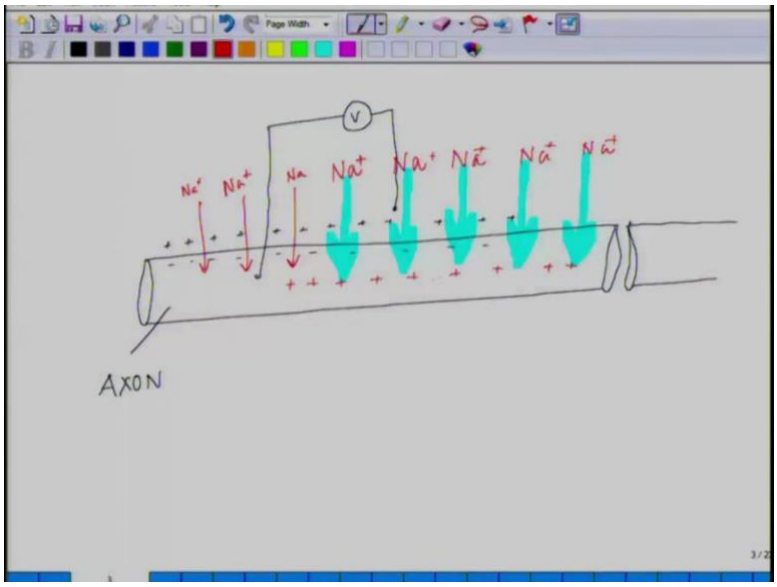

So, what they did they got the stress and they did a curve fitting using different cell equations and based on that the kind of concept which evolved in last soon after that is that initially what is happening inside the cell. So, initially what happens because of excitation, so initially if this is 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.

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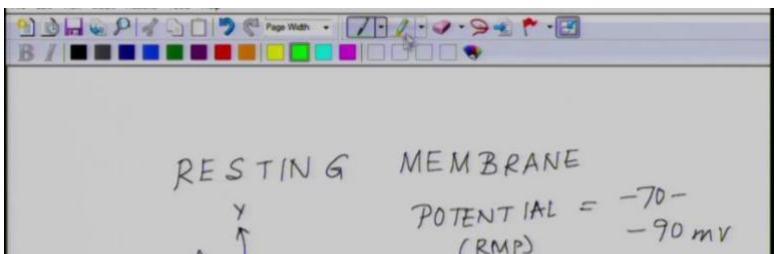

Now go back to this picture. So, this is the zone, where sodium is entering. All the positive ions are getting inside the cell, but then there is a threshold zone that threshold zone gave rise to the concept of odd or none. It means if you could reach a certain millivolt or a certain stage, where these 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.

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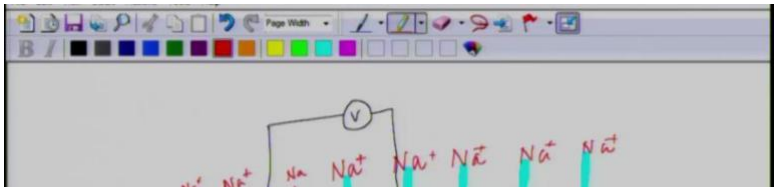

If you look at this picture out here there are already other positive charges of potassium that are present which is fairly high inside the cell. So, there is a positive charge repulsion starts, and this positive positive charge repulsion leads to the next event where the cell starts to allow the potassium 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.

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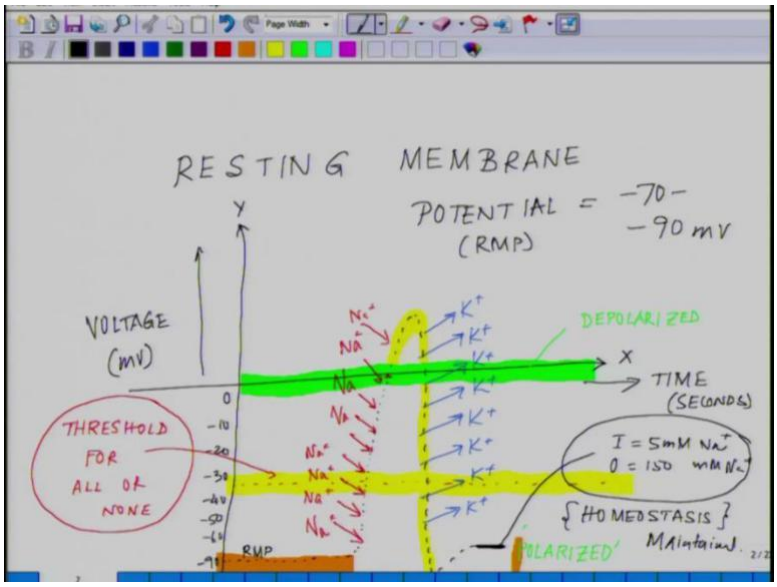

So now if we summarise the event, so essentially if we go back where I was showing the trace out here, is essentially finally happening is that cell is back into its baseline; and this is where again inside the cell, you have five millimolar of sodium, and outside it is 150 millimolar of sodium. And 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.

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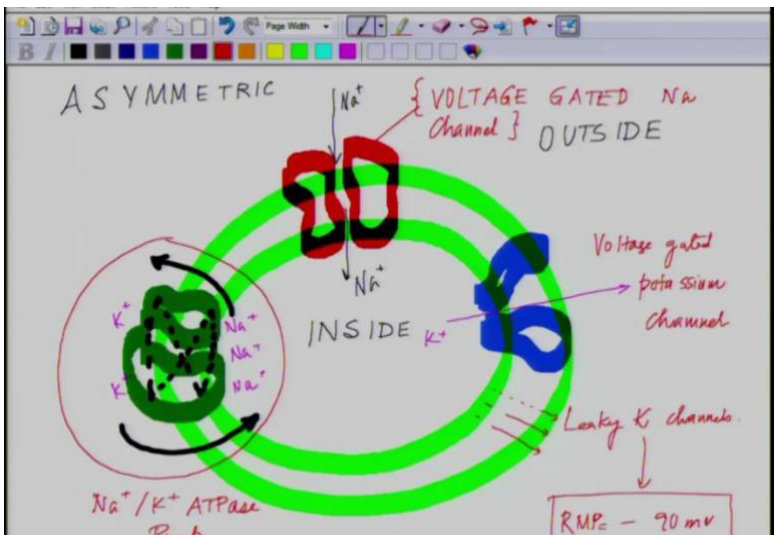

So to tell you here a membrane is asymmetric in nature. What do you mean by is asymmetry is something like this. So, if I draw the membrane-like this if. So, say for example, this is the membrane. So, this is outside and this is inside. What is trying to tell is that the membrane is asymmetric that essentially 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.

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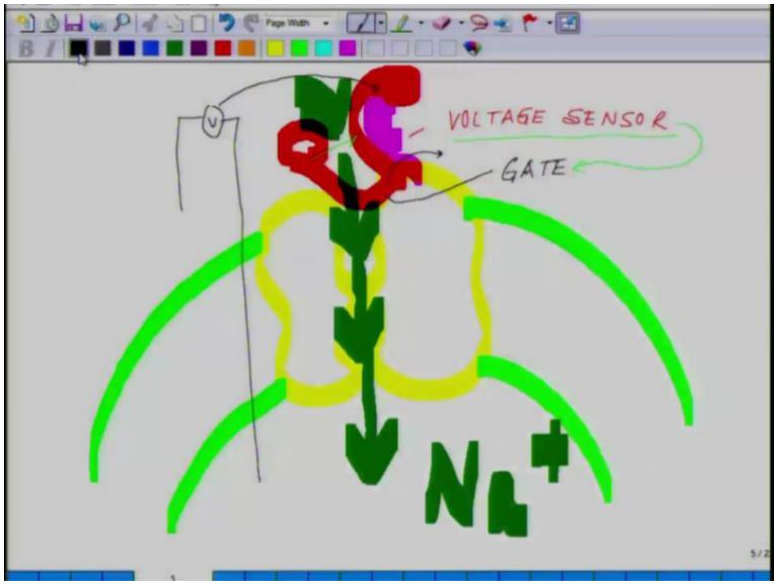

So, if we talk about these voltage-gated sodium channels, so what we are talking about is this if you look at this molecule since once I have introduced the membrane. So, if you

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.

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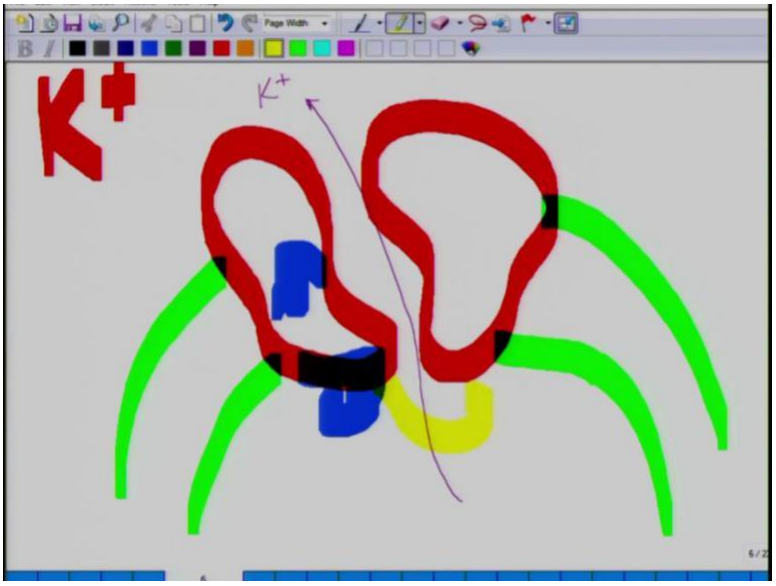

Same way, if I have to explain to you the potassium it is fairly similar, it is just everything just gets reverse there. If this is your membrane like this and like this. Then you have the potassium channel sitting like this. So, these potassium channels which are, I just put it potassium. So, they are 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.

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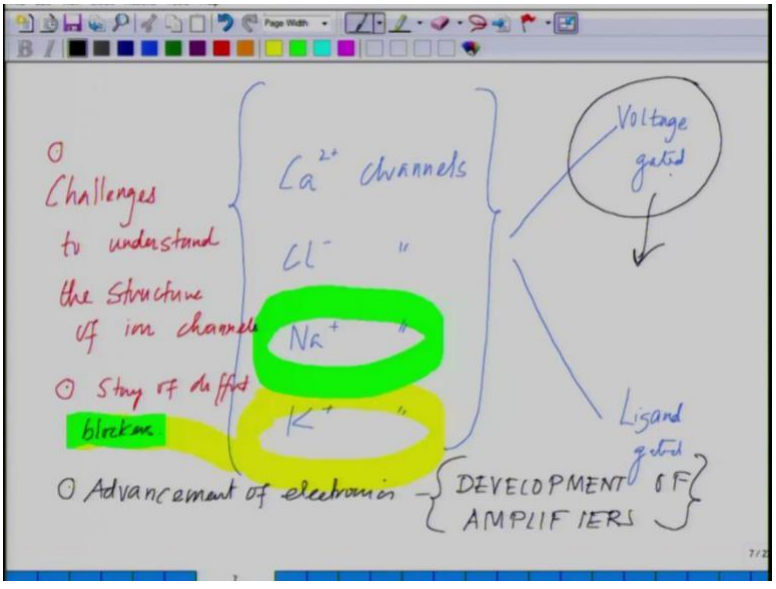

Similarly, you have the calcium channels, you have chloride channels, likewise, and so on and so forth. So, basically at this level the biology is all about these different ion channels, and they could be voltage-gated, they could be ligand-gated. I have not that was a reason why I told you that I will, 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.

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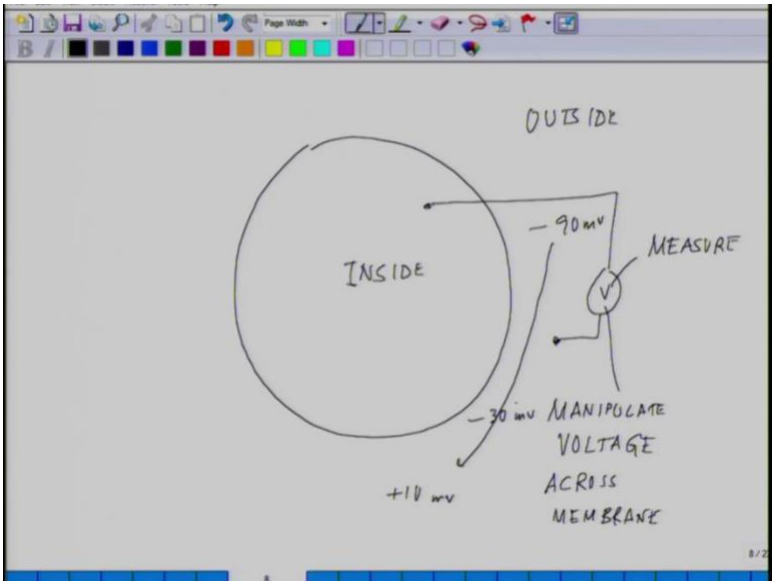

And another inter interesting thing that we have to discuss is if we talk about this voltage gating, the voltage which is leading to the opening and the closing of the channels. So, what will happen to say for example, if this is a cell sitting out here, and this is the inside milli and this is outside?

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.