Bioelectricity
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Lecture - 12

Welcome to NP-TEL lecture series on Bioelectricity. So, we are into the twelfth lecture. So, the last lecture, we talked about the patch clamp technique. So, we talked about the current clamp, we talked about the voltage clamp, and I showed you graphically how you could have an access to finite number of channels. And we talked about how we record the action potential that is basically varying the voltage by injecting current which is essentially in technical term it could be called as current clamp. Or voltage clamp where you are clamping the voltage at different level and you are measuring the flow of current across the membrane. And after that I told you that there are several techniques by which you can really manipulate these channels and study their voltage and current or the electrical, over all the electrical properties.

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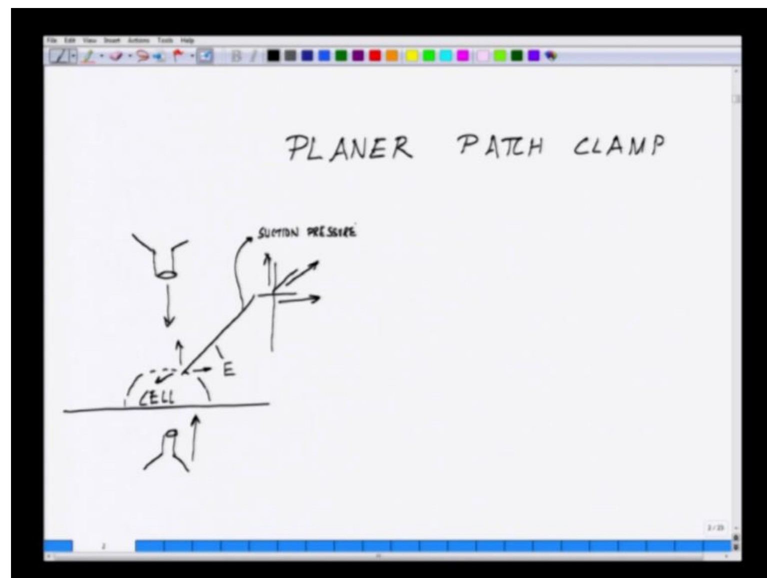

So, today we will be discussing that and we will discuss about one more modification into the existing patch clamp. So, which is also called planer patch clamp array. So, essentially what does that mean. So, before I get into the channel, let us talk about the planer patch clamp array. So, let us draw comparison between the existing patch clamp and the planer patch clamp. So, this is fairly new technique. So, in the existing patch

clamp what is happening that you have this electrode which is coming from the top, and you have this three axis manipulator by which either you can move the electrode like this, like this or like this or up and down. So, three axis by where you can move the electrode. And this all you are doing either seeing the electrode through a microscope from the top. So, essentially this is how it works.

So, if this is your micro manipulator, so this is where electrode is connected. This can move like this, this can move like this, move like this. All the possible movement are possible and here you have the cell, this is your cell and this is your electrode – E, and this electrode can move up and down and likewise. And you are observing all these things using a microscope either from top or from bottom depending on where your sample is. If your sample is in a transparent sheet, you can see it from the bottom or you can see it from the top. And a logistically speaking, this is very cumbersome procedure and it becomes even more cumbersome when you have to out you have to give suction pressure.

So, for any specialized lab in this area, you first of all need an a need a specialized electro physiologist, and on a given day that the best of the best efficiencies. They were very small finite number of patch clamp recording, which could be done by any human individual. So, what are our alternatives? One of the alternative which has been in the mind of neurophysiologist as well as by electronics people could we automatized the whole process somewhere or other. So, how to automatized the process, now that is where comes the whole concept of automated planer patch clamp arrays, just like microelectrode arrays. These are automated patch clam arrays.

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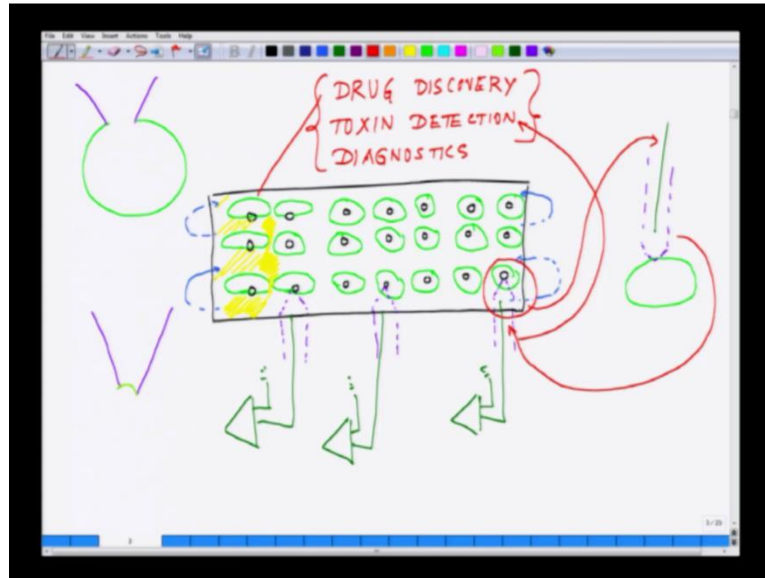

So, let us visualize situation what is happening, here is a cell, you are approaching the electrode and you are creating a small hole on the top of it, when you are going in to the holes cell mode. If you do not go in holes, so are the other ways, how you can recreate that situation without approaching from the top. So, one option is say for example, I have a substrate something like imagine a substrate like this, where I have say one micron holes like this, and I ensures that something like this, I ensure my individual cells are sitting on top of it like this. So, the cell is sitting in three dimension on top of this small hole. So, where is small hole and ensuring that the medium is not really flowing out because there is continuous supply of medium, which is replenishing it or recalculating it.

So, say for example, any medium which is present there is getting which is coming out from there is kind of getting re-circulated into the system back. And of course, fresh medium could be put here now underneath those small holes you have imagine something like exactly like a patch pipette, you have already existing structure which could be put and replaced something like that for simplicity saying I will be showing only three or four, so that you understand. This is come from underneath all are from underneath.

So, say for example, if this is the sheet and top of that, imagine this watch is the cell what I have, this is what I am trying to draw from underneath. So, such multiple things

are underneath and exactly you follow the same configuration inside that you have this electrode like this and you have the ground electrode. So, this is connected to the amplifier. Now if you look at this configuration, what I have drawn here, and if I translate it in terms of patch clamp, you just reverse this on the top. Imagine, it is coming from the top, it will be the again same configuration, here is the cell and here is the electrode. I just reverse the configuration to this, so either you are here, it is the same configuration, it is just the upside down. Now you are approaching the cell from the bottom. You really do not have to approach the cell it is already the set up is already made.

Now as soon as the cell touches on top of this electrode, what you essentially you do is you follow the same protocol, but it is completely automatized. So, at one point of time, you can provide it of course you could ensure that the all the individual cells are sitting on top of those small holes, and that could be done using modern lithography - part portal lithography where you can ensure that you can ensure that only the cells sitting there. Say for example, so I put Yolo out here, ensuring that the cells are not going to grow in these places. If I could ensure that something like this, the cells will only sit on top of those small one micron volt and if one could ensure that that essentially what you are what is happening is that now you have a high through put planer patch clamp array just like high through put planer microelectrode array.

So, this is one approach which is currently underway specially in Germany, and some of the university in Germany, and some of the companies has taken to over and there are fairly successful for cell lines where the size of the cells are uniform and they could be put much more easily. But in terms of real primary neurons directly taken out from that animal stilt it is possible, but it is not a stilt full stream line there is enormous amount of work which is going on to ensure even that is feasible.

So, what we are essentially seeing while summarizing this and of course again in this situation, you could have all the three different modes you could have a hole cell mode where you have the electrode like this which is the easiest one would you could see or you could have. Of course, one more thing, here I will add. You may not be able to so easily study this kind of thing for individual channel, where I showed you that the inside up out and all those things, where you have only the membrane out there and you can study the membrane that may not be so easy enough. But again at least you could do

high through put screening at least you do not have to you know spend so much time for drug screening.

Of course, if some of the drug really work then you may go over and verified further using patch clamp, the regular patch clamp arrays, where you know pull out the channels and you know study the channel dynamics and everything. So, this is one of the most recent advancement of last five to ten years, I would say slightly more may be you know of translating the traditional patch clamp into a high through put screening system for especially this kind of things find application in the drug discovery industry. There it is being really one of the favorite candidate drug discovery and toxin detection and and in the diagnostics; this is where this innovative technology or designing problem or designing accomplishment finds a lot of applications.

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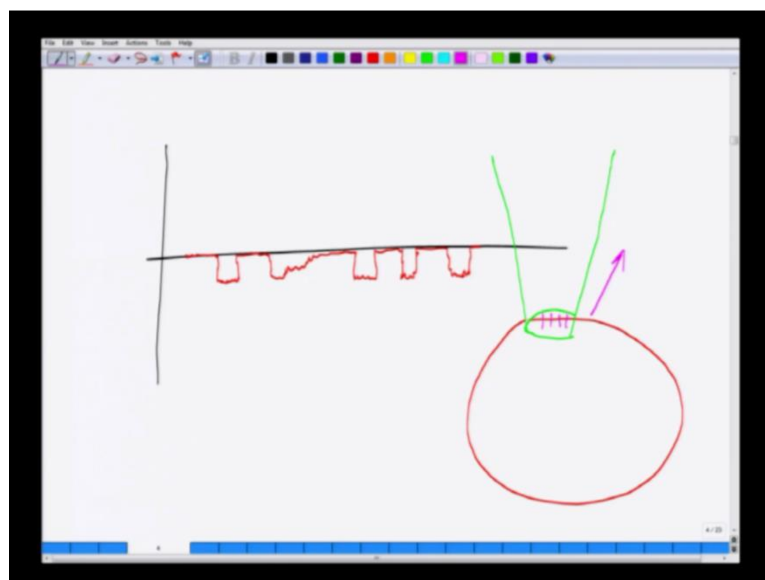

So, as of now we have talked about that we could approach the individual channels. So, next what we will be talking about is these individual channels, how those channels structure could be manipulated. Say for example, so just before going that so whenever we are measuring individual channel in terms of its something like this, what you see essentially is the channel opening and closing, you will see something like this. These are single channel opening, what you are seeing. So, these are the situation where you have this cell, and there is a patch out here, and you have the finite number of channels out here something like this, and you are measuring the conductance of individual

channel. You can pull this out inside out or outside out whichever ways you can measure the conductance of the individual channel.

What you essentially do, if really know the total number of channels on a you know within this much area, you have this many then you back calculate and tell that for how many channels you are getting it, form that you can back calculate and say single channel how much will be the conductance. So, with this back ground of approaching the channel, so I told you I am not getting the structure of the channels as of now, first of all I want to introduce you to the channel how to measure channel electricity. Since now I have introduced you the channel electricity, I will introduce you to one more technique which will help you to appreciate the research of last 30, 40 years since the time patch clamp has been discovered that how molecular biology techniques have helped in understanding bioelectrical phenomena at the cellular level.

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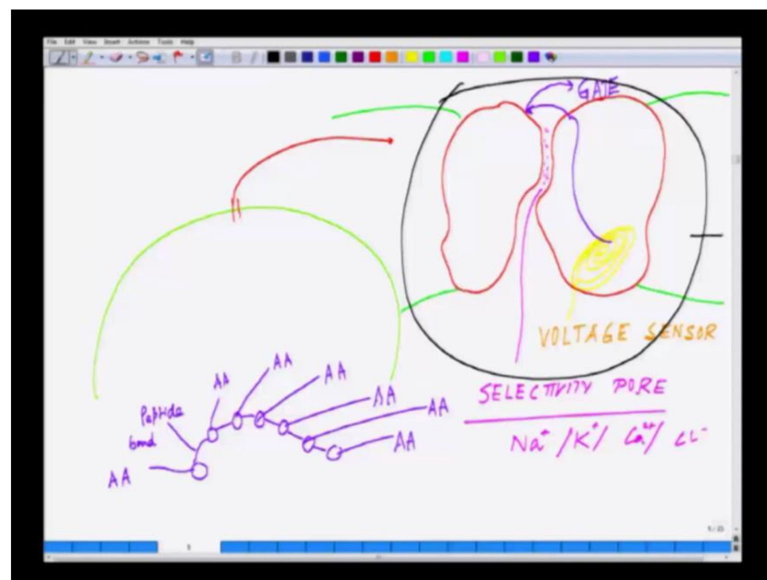

So, whenever we talk about channels now I will pick up the again let us see. So, this is the cell and we are talking about an individual channel. So, this channel when you look at the molecular level, it is something like a structure like, you know which we have already discussed in depth in detail something like and you have the membrane both side of course running through like this. Now you have three features, here you have a something called this zone, which is the selectivity pore. So, this selectivity pore decides whether it will be sodium or it will be potassium or it will be calcium or it will be you

know chloride or whatever, then you have voltage sensing element somewhere in this structure, which could sense the voltage. Voltage sensor and then this voltage sensor is somewhere rather is connected to a gate, which ensures the opening and closing, there is a movement in this. So, this is basically your gate. So, this is the overall channel architecture.

Now in terms of the molecular structure of this whole thing, this is the gross molecules. In terms of the integrity details of the molecular structure if you look at it, so this is nothing but this is a simple protein, which is occurred a shape like this. It could be a monomeric protein, it could be a dimeric protein, it could be trimeric protein, it could be a tetrameric protein, it could be a pentameric protein, it could be a hexameric protein likewise. So, whenever we talk about protein, so what essentially this structure if it bussed down, so it is basically there are amino acids like this. This individual circles are the amino acids AA; and this is the peptide bond, which is attaching individual amino acid.

So, these amino acids join together and form this three dimensional geometries of large large huge proteins, which are ten thousand, fifteen thousand amino acids structures. These are fairly huge. So, how will you understand which part of this structure, so whenever I am writing gate, voltage sensor, selectivity port which part of it is really involved in gating or which part involved in voltage sensing, which part is involved in selectivity for the specific ions, so how it is being determined. So, let us break down this problem - complete problem into a array of amino acids first.

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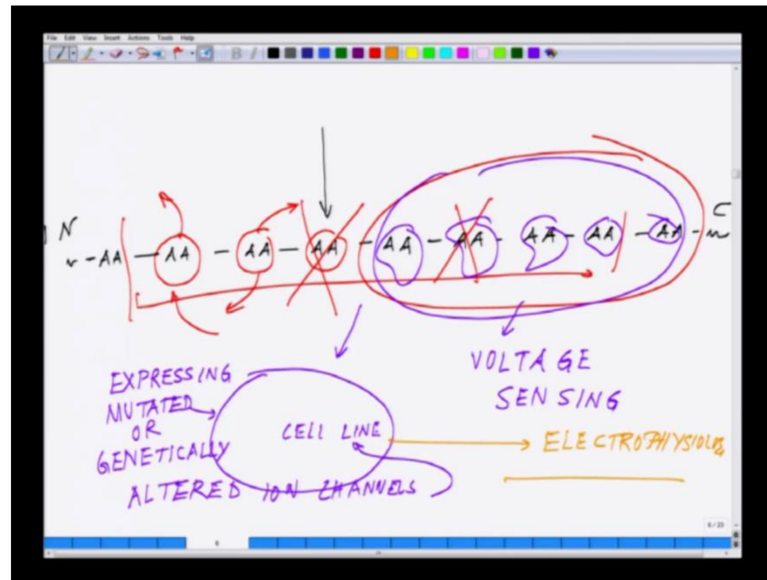

So, it will be something like this. So, you can break it down this AA stands for amino acids, and this is the protein. So, that there is a N terminus of that protein, and there is a C terminus of protein. Now the way it is being done is most tedious way. So, whenever we talk about this amino acids, they are coded by the specific cordons. So, this structures are regulated by specific nucleotide sequences from the nucleus from the DNA. So, now, the way it is being done individually replace each one of them at a time or a chunk of them at a time or you remove them, delete them, mutate them. So, mutate them means you replace it with something else, something like this or you delete them, you delete a sequence.

So, likewise you using mutation technique, using deletion, using different kind of point mutation, replacing the amino acids. Over forty years of research, now today we know at least for some of you handful of channels, so in the mean time there are couples of things happens cloning as I was telling you discovery by Cary Moilis which changed the way molecular biology is being done, the modern current molecular biology. Then came the whole sequencing the first time it was Sishumunuma and all these people who could you know sequence the whole channel. Once you this sequence then you go back using genetic tools that you know that exactly how to mutate specific amino acids.

So, that way what you do now you have a control on the structure. You can you can ensure, if this is a sequence of amino acids like this, you can really ensure the this part is

replaced or likewise or say for example, this is involved. Let us take a single example, you know this sequence say for example, this four amino acids one to the four amino acids are involved in same voltage sensing, just for the hypothesis sake. So, what I do essentially is I kept on replacing each one of them one at a time, and I express those mutated ion channels on some cell line, expressing those mutated ion channels on some cell lines. And use those cell line in the mean time, cell line technology was fairly straight forward now with that development over last forty years and the cloning and everything is fairly straight forward. So, I expressing mutated or genetically altered ion channels on cell lines.

So, now, you have a cell lines which has genetically altered ion channels. When you take this and you perform the electrophysiology, so that where you will be able to figure out that how a specific change in a sequence or a mutation at a particular part could influence its voltage sensing, could influence the selectivity port, could influence the gate likewise. And it is a very very tedious process; as a matter of fact, I mean think of it with in sodium channel there are so many subtypes, fast activated inactivating sodium types; within them, there are types then you have slow activating channels. Then you have so many potassium channels, then you have calcium, then you have chloride, and even you have water channels, aquaperins.

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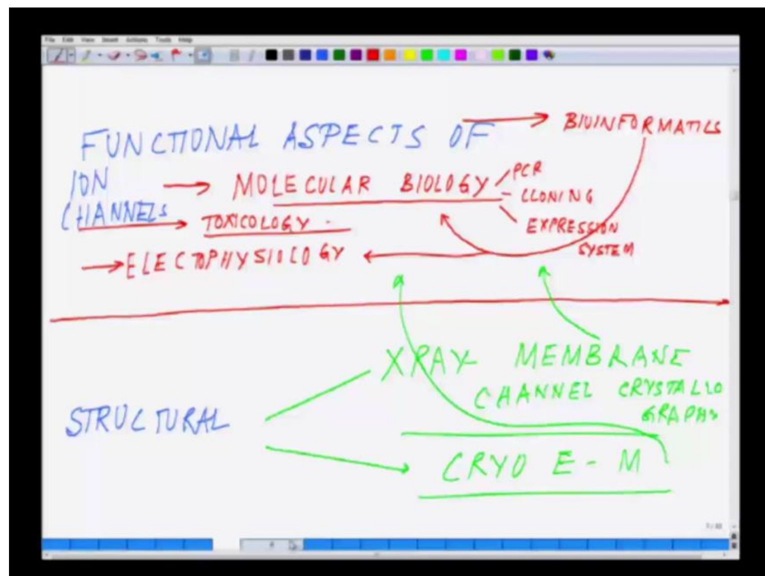

So, really to do it like this, and there is not much other tool. Only the other tool which is available is bioinformatics tools where. So, this is another thing which happen in middle mean time. So, you have electrophysiology going moving on. So, electrophysiology techniques where getting kind of electrophysiology and going hand in hand with molecular biology tools; simultaneously came fairly late slightly late in the game is something called bioinformatics, where you can start predicting the structure function relationship by theoretical modeling. So, essentially you can tell the molecular biologist that which particular sequence may should be or you can share with this both of them which particular path may be involved in voltage sensing or gating or selectivity port likewise and so on and so forth.

So, if you see that timeline the way it is moving, and if this is electrophysiology was there long time back, then came the molecular biology specifically with PCR, cloning, expression systems. And in the mean time, it is going on hand with is toxicology, because as I was telling in one of the previous classes, you need certain specific compound which can block this channels. So, you have to have those kinds of toxins like tetra detoxins, four a p trithyl-amonium likewise. So, toxicology was also is which fairly old science moving with electrophysiology then you have the molecular biology then you have bioinformatics coming into play.

Simultaneously there is another technique which people are on the structural set, this is all about the functional aspect of ion channel. So, this functional aspect of ion channels could be correlated with the structural aspect; and the structural side, simultaneously, there are you are moving with technique like you know x-ray. So, one of the very hot area is membrane channel crystallography then you have cryo electron microscopy - cryo E-M. So, all this are adding more and more information about the smallest filter system or smallest filtering machine of the biological world.

If we look back from very historical perspective the way things are moved electrophysiology or studying bioelectricity was there for a long period of time; within the biological system, it was known since the time of Luge Galwani and Alsandro Volta that this techniques are existing. The techniques started getting finer and finer and finer, and one of the important breakthroughs came in nineteen seventies, when with the discovery of the patch clamp, where you really can access the smallest unit which is

involved in mobilizing the charge or mobilizing the ion channels which is the ion channels.

Then simultaneously with the discovery of PCR sequencing, cloning, the whole molecular biology world open up a total new vista, then came happen the first marriage between the molecular biologist and the electro physiologist along with the toxicologist who were putting the helping hand, you know blocking channels as this was moving simultaneously there was enormous understanding about crystallography. So, people started attempting could we crystallize this structures, really could we see those filters which are so precise that they could only allow a specific form of ions to move through. As crystallography was proceeding the discovery of cryo E -M or very low temperature electron microscopy, where basically what we do is something like freeze fracture to do.

If this is the membrane then imagine this is the ion channel sitting on the membrane or you just using a cryo ion, if you cut it at a very very very low temperature. So, essentially you can dissect out, you can separate out that cross section of that membrane and you can really study the whole topology or the whole topographic feature of the membrane channel. While this was all happening, simultaneously the cyber world was really flourishing; PCS were pretty much ruling the market and everything, and that is the time when PCS were about to come nineteen eighties, the whole field of informatics or bioinformatics were all the known sequences of different proteins where all getting database.

Now peoples are started predicting you know what like this; so already the data, which are feeding through from those who are doing this mutation and then electrophysiology. So, people started predicting you know these residues may be helpful. So, instead of think of it, you can do random mutation out here, you can keep on doing mutation forever, but if a bioinformatics or ion for theoretical biologist with bioinformatics specialization comes into play, there will tell you you know what these are say for example, these are meaningful ones or try this ones. So, they could actually reuse your time for discovery instead of you know having a random walk all throughout like you know mutated, this mutated, this mutated, this mutated, this and there is no end to that.

And then you do the electrophysiology and then you say yes, you mutate this, this is how the voltage sensing kind of got hampered or the ion selectivity got hampered or

conductance reduces or the gating becomes little; obviously, conductance reduces or something when the gating is not working. So, if you look at it, the way the modern world is moving to solve one problem you need basic understanding of all the different tools which are available at your disposal. You need really big team effort to understand these different bioelectrical phenomena at the molecular level and could we translate them to make a device those are even bigger challenges.

So, as of now what all I have talked to you people is all about ionic electricity; and there is one technique which is left which I have not talked to you where solid state electronic device, especially the semiconductor devices like field effective transistor are being used to measure these kind of ionic event. So, those are some of the pioneering discovery by Peterframhers from Max Banks institute, Biochemistry. So, we will talk about that in the next lecture. So, what I expect from you people, just kind of you know open up your windows of looking at a problem from multitude of an angle, and that will really help you people to appreciate this subject, and try to you know have a very broader vision to solve a very fundamental problem.

Thanks a lot.