Biophotonics
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Lecture No. 57
Controlling the Brain with Light

Welcome back. So, we are continuing our discussion on optogenetics. And today I am going to tell you some of the very basic concepts on how to control the brain with light or the various processes of optogenetics. I will deliberately keep the pace slow so that you have the time to catch up and instead of cramming so many different topics, let us stick to the basic and then expand from there.

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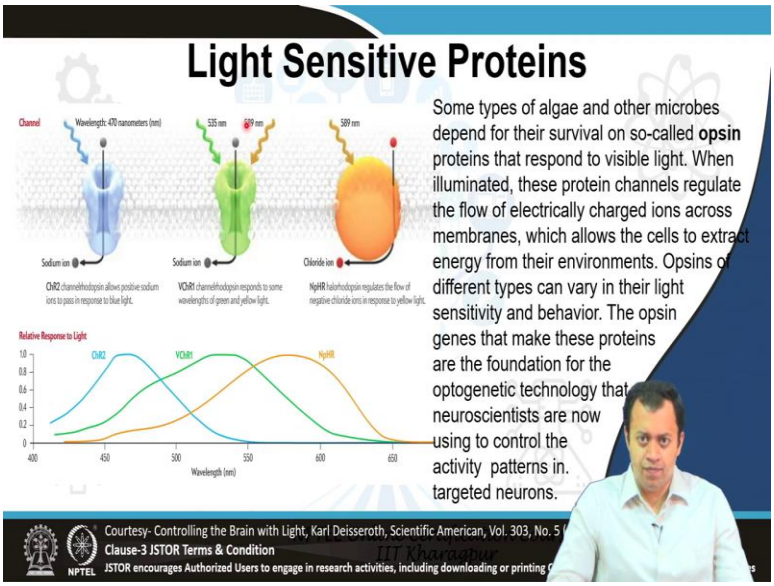

So, how exactly we are going to control the neurons. Well, one very handy technique is if we can utilize light sensitive proteins, proteins which upon being illuminated by certain photons, upon interacting with photons, they change their properties, some sort of electrical signal passes through them, have you heard of any such protein during the course of this topic? Remember, vision, remember the protein opsin that connects with cis-retinal, the chromophore and then when light falls instead of this, it becomes trans-retinal and then it pushes something and then that cause something else, remember.

Do I have to repeat that, the concept of vision? How exactly light ensures that there is a structural change of the protein and this structural change of the protein causes the ion channel to

form and that makes the hyperpolarizability of our rod cells and that results in flowing of neurochemical signal. If you do not then I would ask you to go back to a few lectures before. I think lecture number, module 4 or module 5, where I have discussed this in detail. So, please go and study that and return back. This will make much more sense.

So, the fact of matter remains that there are several types of algae and other microbes that develops this protein opsin, there are several. Remember I said that we have rhodopsin, but different types of algae have several different other kinds of opsins. And when illuminated this protein channel regulates the flow of electricity charged ions across membrane just like the visual cortex, just like the process of vision. We have discussed vision at a length. You have even probably done assignments on that. So, this will make sense if you recall that and remember and come back here.

So, opsins of different types were extracted from different types of algae. So, there was channel rhodopsin, there were VChR1, another channel rhodopsin and then there was halorhodopsin. So, these are the different proteins that attract or that effect or that can be affected by specific wavelengths of light. So, channel rhodopsin when excited by 470 nanometer blue wavelength light, it allows positive sodium ion to pass in response to blue light. This is the property of the protein. This is the light sensitive protein. This protein, this channel rhodopsin ChR2 upon exciting, upon excitement by a blue light of 470 nanometer wavelength, it allows positive ions to pass

Halorhodopsin on the other hand, when it is excited by 589 yellow or orange, yellowish light, it regulates the flow of negative chloride ions in response to yellow light. VChR1, this is another version of channel rhodopsin. It responds to some wavelength of green and yellow light and it also allows sodium ion to pass through it, so 535 and 589. So, VChR1, so if you have a combination of these two and you are exciting by 589, this will allow negative chloride ion, this will allow positive sodium ion to go through. Are you getting? Can you use this?

So, at this present moment you have very little information. There are certain proteins which when excited by a specific wavelength of light allow a specific type of ion to pass through, thereby making some kind of a neurochemical pathway, neurochemical network, an ion passes. And you know how vision works. How human vision works, at least the brightness level, the

shades of grey, how our brain perceives, what sort of light intensity produces, what sort of cis-retinal go to trans-retinal.

And then the cascade reaction forms and then the ion channel opens and they, or closes and there is a hyperpolarizability and then there is a potential difference between outside the cell and inside the cell. As a result, there is a current flowing. So, can you envision how can you utilize this information to understand how probably a neuron will work. Professor Deisseroth thought that. He had this information. He knew how vision work. And he has now these kinds of proteins that has been isolated from algae, which upon excitement by specific wavelength of light produces a specific electrical signal.

Some sort of sodium ion will pass through, and you can measure the concentration of the sodium ion and thereby measure the electrical signal, measure the frequency response, measure the voltage measure, measure the current etc. These are the two disjoint, apparently disjoint information that was present in front of Karl Deisseroth and his group of students in the year 2005.

So, I am asking you, what would you have done to understand, using this two disjoint apparently disjoint information, to probe the brain? Think about it. This is how scientists work. And if Karl Deisseroth can think, so can you. Do not question you believe, do not question your capacity. You can think of that as well. Now, tell me in the comment section how many of you can think of it or what was your idea before pause it and write it down. And then I am going to tell it to you anyways. That is what it is about. But if you want or if you can, if you are willing, just pause my video for a second and think about this two disjoint information.

The goal, the aim of yours is to understand brain patterns, neuron by neuron. You have this two information. Opsin can do visual information. So, opsin is a proved protein. And you have developed or you have isolated three separate opsins, which produces different type of chemical signals upon excitement by different type of wavelength of light. With this information, can you go any further and go closer to your goal of ascertaining neural signals. Think about it.

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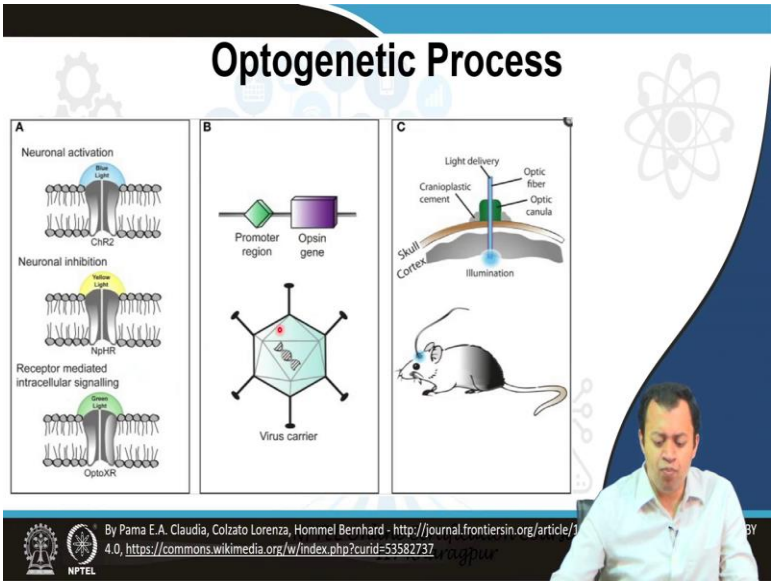

So, what Professor Karl Deisseroth did was he tried to encode or make sure that there are certain genes that can create opsin. Opsin is a protein. The protein gets made by genes or genes contain the information. The genetic expression is a protein. The genes contain information how to code a protein. So, he modified certain genes which have the capacity to produce opsin proteins. Not just any opsin, these 3 or these 2 kinds of proteins.

He then put it in a virus as a carrier and this virus was made to affect the brain of a particular mouse. Now, he also ensures that the virus carrier contains this opsin gene along with a specific promoter. Remember promoter this was the start code that our soldier needs to or spy in that gene lecture that I said this promoter is the start message AUG the start codon. The start codon only after which the gene will start express itself. This is the start codon or this is the beginning. So, the promoter was made very, very specific.

The promoter will only attach to specific neurons. The promoter will attach to specific neurons, so that though the virus was affecting the entire brain, only certain neurons were attaching with the opsin gene. Rest of them, since there was no promoter, remember no message. So, it was not getting encoded. So, those neurons which matched with the promoter, which matched with the starting part of the gene, they got modified, mutated you can say and those certain nerve cells, neural cells, brain cells could synthesize, could express opsin.

And then it was only a matter of fact by putting some current of a probe, optical fiber probe that was connected with a laser light of these specific frequencies, which was illuminating the brain. You ensure that only specific neurons are attached using the promoter. You can be very, very specific with this. You remember this. You can be very, very specific. So, although the virus, you are using the virus as a carrier, the virus, some kind of a virus which the, which will not kill the mouse obviously, the mouse with the mouse will slowly get rid of the virus.

But by that time the virus has interjected into the brain nucleus, brain cells nucleus, this sort of foreign DNA, this foreign DNA was getting attached to only specific nerve cells depending on the promoter, depending on which promoter it was being genetically modified, genetically engineered to attach to. So, those cells where the promoter got attached to could synthesize opsin.

So, you, when you send specific blue light and then measure the electrical conductivity, you can be absolutely, absolutely certain that this electrical conductivity, this electrical signal that I am getting is only associated with this specific neuron that is affected by the virus carrying the channel rhodopsin gene. Understand this part, understand. This is the genius of Karl Deisseroth. This is the genius of this team. They had the same information that I just said you. They ensured, because this is a protein. Protein cannot, opsin is protein. Our body have opsin anyways. Our body synthesize opsin. This connects with cis-retinol, trans-retinol and thereby we have vision.

So, our body can synthesize opsin if we have the specific gene. We do not have the specific gene or the mouse do not have the specific gene that these algae probably have from which halorhodopsin, channel rhodopsin etc. are isolated. So, he figured out that from this protein that this gene creates this protein, this gene was injected, transfected into the cell, certain cells of the mouse only certain neurons were affected. He made ensure of a specific promoter. Specific promoter was attaching to specific cells.

And that area, that cell, that neuron was producing this sort of electrical conductivity upon excitement by blue light. You have a neural activation. This is the positive sodium channel. This is the, I think, the negative chloride channel. And you can have an intracellular signaling. You can have something in between by adding blue and yellow to get some kind of an orange light, green light. This VChR through where both can actually fire or maybe little bit of sodium can

only fire with two. So, you have the proper control and thereby you can observe neural activity. Understand this.

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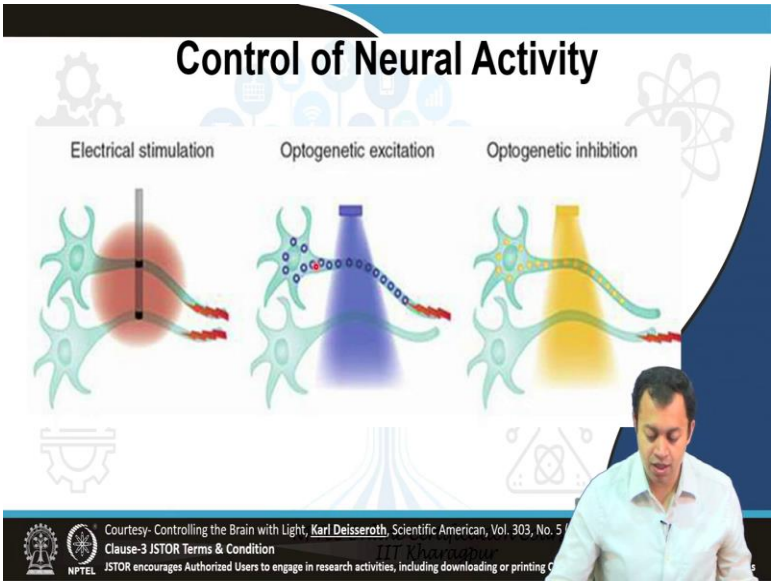

Electric simulation, if you have two different types of neurons side by side, electrical simulation will affect both of them. Whereas in optogenetic excitation if this contains the channel rhodopsin ChR2, I will come to the slide later, only this neuron is firing. This neuron will not fire by blue light. And this produces positive electric signal, positive sodium ions. Whereas, you can also have nearby signal or you can have, so you can affect or you can transfect this neuron with both channel rhodopsin as well as halorhodopsin.

When you are shining blue light positive ions will go. When you are shining yellow light, negative ions will go. The nearby neuron is completely unaffected because it has no opsin of any type whatsoever.

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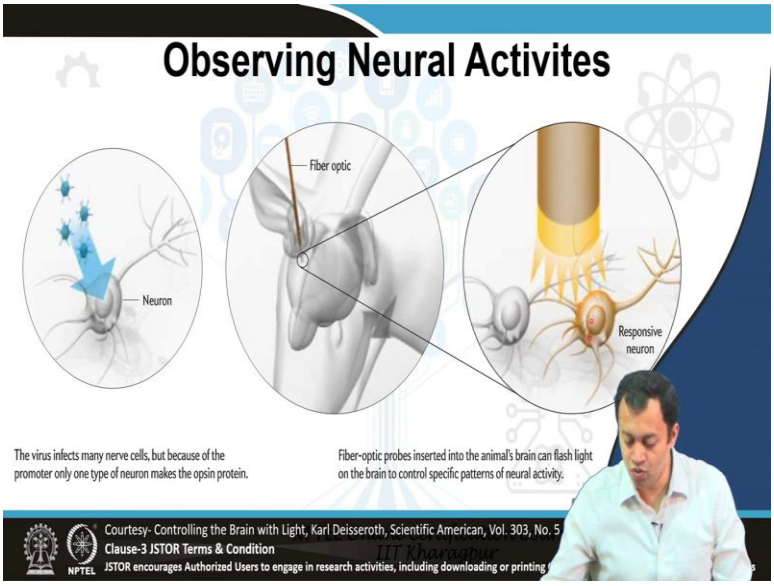

So, this was how to observe the neural activities. You implant optical fiber on to the brain of your mouse. This is the mouse's head and this is the brain, a very, very small keyhole type surgery and you place some sort of a crown on top of the mouse's head. The crown is very sensitive, but very, very thin. So, that the mouse is not agitated too much. And that crown, some kind of a hat type thing crown or hat measures any electrical changes taking place and this hat needs to be very sensitive enough. So, that few micro volts, few nano volt it can measure.

So, it is like a crown connected with a fiber optic tip. The fiber optic tip is extracting light. So, this was the first part. The virus infects many nerve cells, but because of the promoter only one type of neuron makes the opsin protein. Fiber optic probes inserted into the animal's brain can flash light onto the brain to control specific pattern of neural activity. This was the genius of Karl Deisseroth and his team who were able to utilize, who were able to take two from here, two from here and make four.

And thereby, only one type of neuron was getting fired. This is the responsive neuron. This is the unresponsive neuron. And you can have both excitation as well as inhibition by shining different types of light. And depending whether this is channel rhodopsin or halorhodopsin, it can produce positive ions, positive sodium ions or negative chloride ions.

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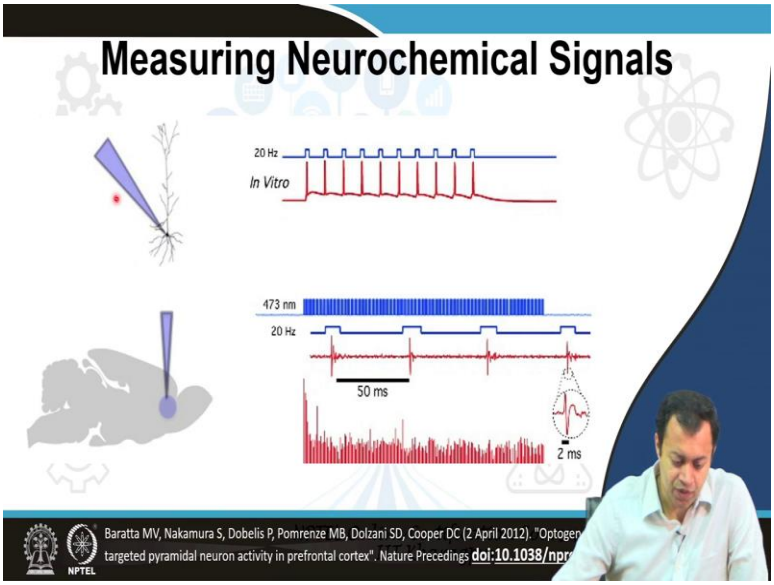

And this was what they measured. Several new topics came up. So, they were actually shining blue light here. This is the neuron. This is the network neuron. And this is the zoomed in version. And they were able to measure the signal the so-called signal. Every time they were firing 473 nanometers. They were every time firing at a 50 millisecond apart. They were able to get this sort of a neurochemical signal. And thereby, later days, they were able to prevent pain from traveling. So, I think a piece of whisker or something else of the mouse was cut off.

But using some kind of changing the neural pathway or changing the chlorine ion going to the nerve, brain cortex which results in the sensation of pain, they were able to prevent it. So, although some part of the mouse's body was cut or injured by sending light and by blocking, sending different types of light, they were able to block the specific signal from going to the brain.

So, basically getting some sort of a control on the neurochemical signal that passes from one neuron to another neuron, this network, you are controlling the information that is being passed through different networks, different nodes of the network, and thereby prevent some sort of neural activity, some sort of a sensation.

If we can prevent, we can bring it back, we can engineer it. That is what all it about. Everything is about control. If we can control the various functions of the brain, your pain, your mood, your

depression, may be just maybe we would be able to understand and cure. And that is where, that is the main topic of biophotonics is.

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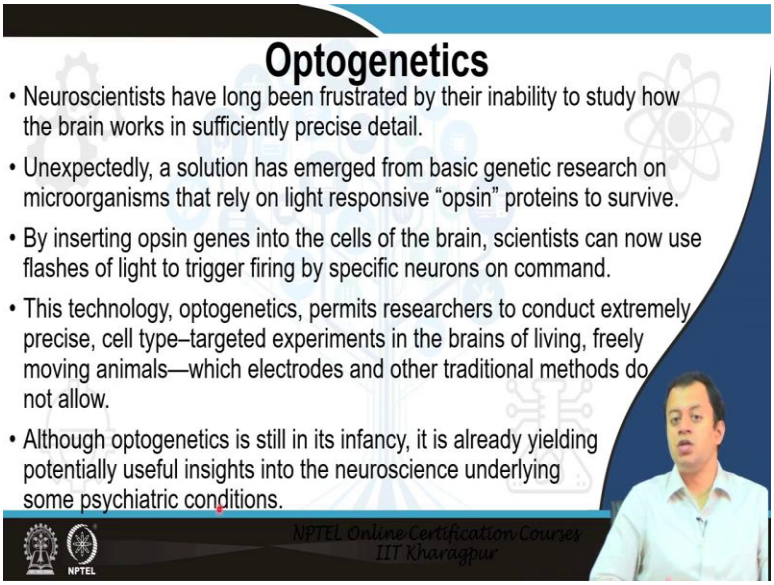

So, to summarize, neuroscientists have long been frustrated by their inability to study how the brain works in a sufficiently precise detail, cell by cell, neuron by neuron. Unexpectedly a solution has emerged from basic genetic research with microorganism, mostly algae on light responsive opsin proteins. They require these opsin proteins, these algae, these jellyfish type things who send certain light to communicate or to, for different purpose of survival.

By inserting obscene genes into the cell of the brain, scientists can now use flashes of light to trigger by specific neurons on command. And this technology optogenetics permits the researcher to conduct extremely precise cell type targeted experiments in the brain of living, freely moving animals, which electrodes and other traditional methods do not allow.

Although, optogenetics is still in its infancy, it is already yielding potentially useful insights into the neuroscience underlying some psychiatric conditions. Was not this your goal to begin with? So, think about the genius of some of these people who come up with apparently disjoint information and bring them together in some sort of a solution, some sort of a solution for the betterment of human life.

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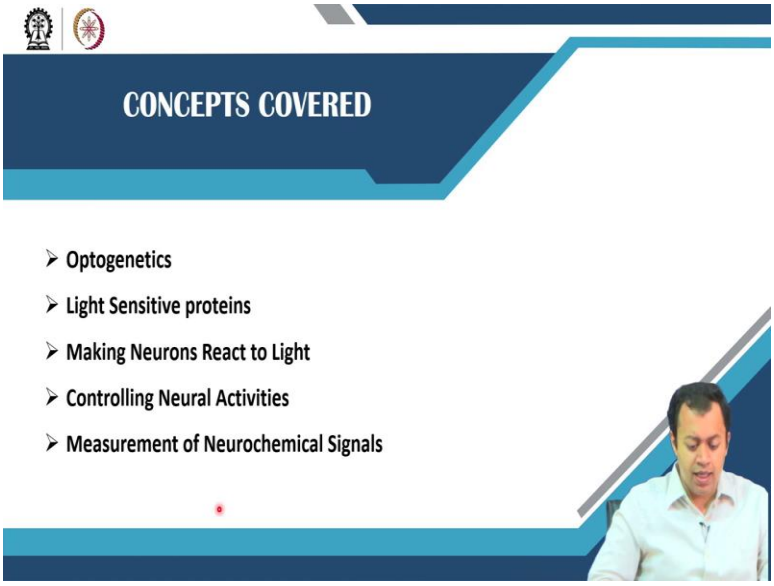

So, these are some of the topics that I discussed today. I know the pace was quite slow. And this is how it is going to be for the next three classes till we finish this entire course. This is a very interesting topic. There would not be any advantage in cramming so much different concepts of optogenetics in a two-hour lecture. So, let us try to understand the bare minimum so that we can expand on it in future. This is a very, very new topic, very, very hot topic. And I want several of you to work on these areas, this research and come up with novel research.

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So, please go through some of the references that I have cited. I have given a couple of DOIs as well. This will help you locate them and read it yourself and you will understand how, in what particular direction this topic of optogenetics is going. In the next three classes we will try to look at certain specific instances, maybe imaging, maybe some sort of a surgery we will see and this is what we are going to see in the next couple of classes. Thank you. Thank you very much.