Biophotonics
Professor. Basudev Lahiri
Department of Electronics & Electrical Communication Engineering
Indian Institute of Technology, Kharagpur
Lecture No. 56
Introduction to Optogenetics

Hello, and welcome. We are at the last lec the so-called homerun of our journey in biophotonics. I hope several of you were able to understand or enjoy the topic. Some of the concepts were completely new, some of the concepts were quite common, quite unknown to you. So, you might have had gone through mixed feeling while going through this course. So, have had I. But overall, I have got a very positive feeling about this particular concept of biophotonics. And I hope that several of the concepts that were unknown to you, I was able to convey at least some part of that to all of you.

Several of you complained that there are not enough reading material in the presentation, in the slides. Well, the fact of matter in this case remains that the slides are not the presentation. I am the presentation. The slides are simply visual medium that helps you imagine, understand, conceptualize, visualize, the lecture that I am giving. So, the idea here is, you will hear what I am saying and take notes like you do in a classroom. If you think that all the information will be available in the slides and you will at your leisure time memorize those and try to crack an exam, like in any coaching center this unfortunately is not that thing. I mean, I am not saying there is anything wrong in coaching centers. It is just that I am not that.

This, what I am talking is the presentation and you have now the capacity to pause, rewind, rewrite, go back and forth. And thereby you take notes and tally it with the presentation, the, tally it with the slides, tally it with the pictures, tally it with the images, tally it with the animations that I have done, so that the concepts become bit more clear. I am here to provide you concept not to help you memorize something and then crack an exam.

So, based on that, let us continue and try to conclude our, this topic of biophotonics, which is a very, very new and emerging topic, which is optogenetics and neurophotonics. In fact, the very fast experiments that could be truly called optogenetics were performed only in 2005 and 2006 in Stanford University, United States, and 2010, the prestigious Science Magazine called optogenetics as the technique of the decade.

Since then optogenetics is going further and further but it is still emerging. And every single year we try and we get to see new concepts where light is used to reveal the activities of the brain. And continuation of optogenetics is neurophotonics, where we actually try to modify the overall properties of the brain, try to understand, try to ascertain, try to modify and maybe try to cure some sort of disease that is affecting the brain.

So, let us get started. Today's class, I will simply give you the introduction to optogenetics, because remember, this is a very new, very emerging, very heavy and incredibly complicated topic. So, let us go through this topic in an easy pace, in a slow manner, where instead of trying, me trying to cram as many concepts together into this, let us try to go through some of the basic concepts of what optogenetics is and why is it required, what is the rationale behind studying optogenetics.

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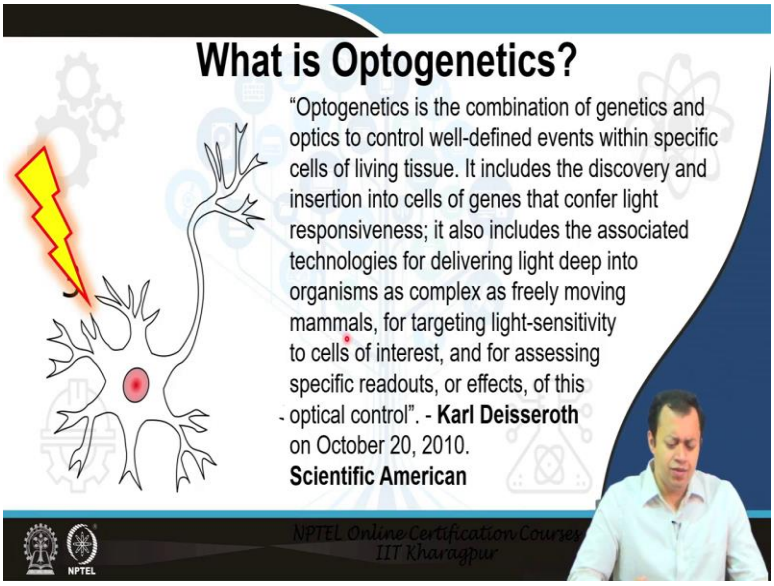

So, first question first, what exactly is optogenetics? Well, optogenetics from the name suggests, it is the combination of genetics and optics to control well defined events within specific cells of living tissue. So, you use some sort of a light to either control or understand the functioning of a particular nerve cell or a particular neural activity. Now, let me be clear, yes, up to an extent visualization or imaging of neurons can fall under optogenetics, but that is not exactly what optogenetics is all about.

Optogenetics is more or less this term control and understanding. Understanding how neurons work at a cellular or sub-cellular level in a living tissue if possible. So, it is one thing to take out an organism, say for example, a mouse's brain, dissect it, take some neurons out and fix it in a particular petri dish or a slide and then look it under a microscope. That technically is not optogenetics that is simply microscope. You go for very high-resolution microscopy, super resolution microscopy and try to see. But here we are trying to understand what kind of neural response result in what sort of a behavior of a living organism, try to understand that.

Optogenetics try to understand the functioning of brain cells, brain tissue in a living organism to understand the overall behavior, to understand the overall nature. It includes the discovery of insertion into cells of genes that confer light responsiveness, it is all, it also includes the associated technologies for delivering light deep into organism as complex as freely moving mammals. Thus far, we have mostly done on rats and mice's of targeting light sensitivity to cells of interest and for assessing specific readout of this optical control. This was one of the major articles which was written in Scientific American by Karl Deisseroth.

He I think was the pioneer in optogenetics in the 2005, the experiments, the optogenetically experiment that I was talking about in University of Stanford, Stanford University, was done by Professor Karl Deisseroth. He was trying to measure the neuronal activity by shining light. So, that somewhat is optogenetics. So, optogenetics is combining light along with genetics to understand or study a particular neural response in complicated animals like a freely moving mammal.

We have not sedated it. We have not killed the mice. We have not put it under heavy dose of chloroform. We are trying to ascertain what specific behavior of the mice is result of what particular neural network firing in its brain. So, obviously, the question comes that, why do this? Why, what is the point?

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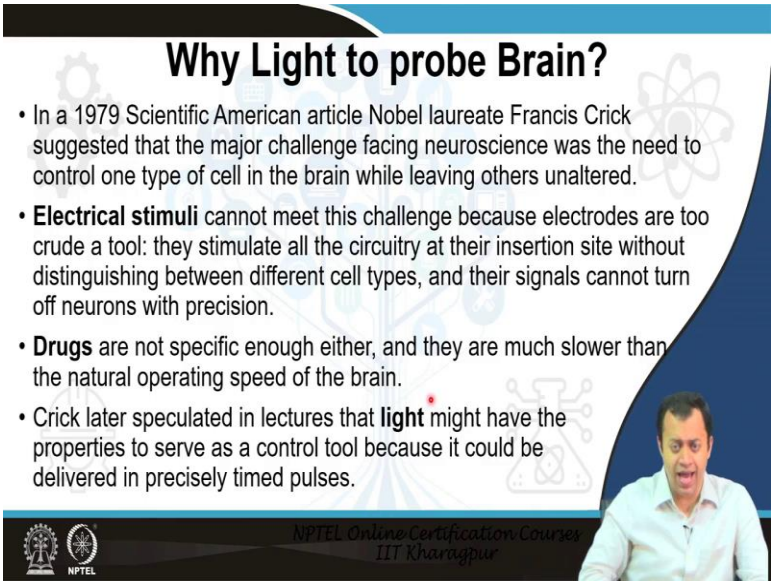

Well, the fact of matter remains that let us try to understand the rationale behind it. So, the Nobel laureate Francis Crick, the same Crick, who was co-discoverer the DNA, suggested that the major challenge, he wrote it in 1979 in Scientific American as well, that facing neuroscience was the need to control one type of cell in the brain while leaving the others unaltered. This was the challenge. This was the biggest challenge in neuroscience that Francis Crick, the guy who discovered, co-discovered DNA, the double helix of DNA 1979 thought that we need to understand the brain's functioning one cell at a time. We need to understand the functioning of the brain one cell at a time.

However, at that time, in 1969, no technology was developed that could try to ascertain that the function, a huge complicated mass of tissue is our brain. What is the function of individual cells? Yes, you will say up to a level we understand which part of the brain does what, left brain, right brain, the hippocampus, the optical cortex, but we are talking about cellular level. We are talking in a cellular level; what sort of cell individually perform what specific function. So, that was the challenge that was facing neuroscience at that time. And this was something that was ascertained or asked by Francis Crick.

He figured out or scientists since then figured out that electrical stimuli cannot meet this challenge because electrodes are too crude a tool. They simulate all the circuitry at the insertion site without distinguishing between different cell types and their signals cannot turn off neurons

with precision. So, you have a complex tissue which is a part of the brain. You want to simulate certain section of the tissue, i.e. a group of cells you want to fire them, you want to switch them on and you want to switch the other part of. Thereby, try to see what is the precise function of that specific type of cells within that specific tissue, within that living brain of an organism that is, what was required.

Electrical stimuli by sending electrical electrodes was too crude. Most of the time you are going to kill the animal. If somehow you are managing to do that, because electrode basically send electrical pulses, electrical current, it will simulate all the circuitry at their insertion site. It cannot distinguish between different types of cell if it is conductive light, current will flow through and their signals cannot be simply turned off. You cannot pass current through a group of cells and in the middle portion, you will say okay, every alternate cell needs to be switched off.

Can you do that? Can you do that in a wire? You are sending light through a wire or sending current through a wire and every alternate centimeter square, millimeter square you need to switch them off, no current should flow through. How are you going to do that? Maybe with complicated circuitry, maybe some kind of a thing you can do, but what inside a brain of a particular animal without killing it. How do you plan to do that?

Drugs are not specific enough either. You can send drugs and try to ascertain its position, but they are much slower than the natural operating speed of the brain. You remember I asked you a question that how fast our visual cortex work, I mean, you close your eye and then you open your eyes immediately, how soon will the light signal travel from your eye, the cornea, the lens through the retina, through the optical nerve, through your visual cortex, how fast this thing happened, 10 to the power minus 12 second, 10 to the minus 9 second or 10 to the power minus 6 second, which drug work this fast so that you will be ascertain.

So, drugs are not suitable either. This is slower than the natural operating speed of brain, your brain, or your neurons fire very, very fast for certain neural activity, vision, for example. So, drugs would not be able to give you that specific information that what neuron is doing what at what particular position, at what timing, so that we are able to ascertain various neural activities of the brain. Crick later speculated in his lectures that light might have properties to serve as control tool. We can probably control the neural functions using light because it could be delivered precisely. It could deliver in precise time pulses.

Light pulses can be at nanosecond, picoseconds, attosecond level. It has the capacity to ascertain or excite specific cell, specific cellular functions, several areas, several organelles, several sub-cellular components, absorbed a specific light, specific wavelength of light and then their activity changes, maybe electrons jump up, maybe the molecule starts rotating, starts vibrating, and thereby perhaps, Crick said that light might have properties to utilize it, but this was in 1979.

We have not developed that much of technology at that age. It was only in 2005 when in Stanford University, several experiments were done, which showed that it is indeed possible to use light or laser pulses to understand, to control, to measure various activities of the brain, various neuronal activities of the brain, in a cellular level, in a neuron by neuron level. You do not have to activate or illuminate the entire brain surface to understand what is going on. You can activate single neurons or group of neurons at a time and ascertain that the rest of the neurons around it are switched off. You can actually do that.

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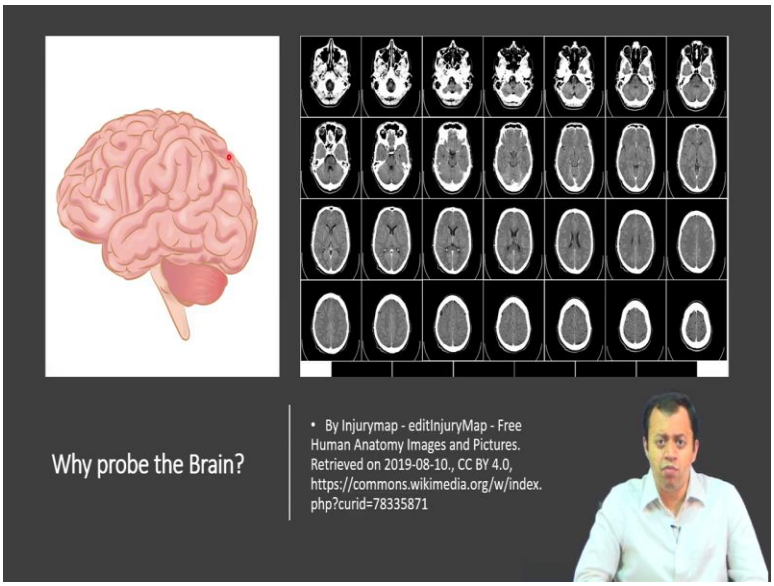

But then again, the question will come that why do that. Why probes the brain? Well, couple of things. First, the brain or the human brain is the most complicated matter that we have encountered in this entire universe. Nothing in this universe, so, thus, so far that we know is more complicated than the human brain. And we know very, very little of it. Yes, there are several methods available CT scan, MRI, which images the brain, but frankly speaking and I

know my colleagues will hate me for saying this, these are incredibly crude techniques when it comes to ascertaining at a neuronal level what is going on inside a human brain.

Granted that these CT scan and MRI has increased or has enhanced neuroscience with a quantum leap, but still for all intent and purpose, if there is something substantial like a tumor, like some kind of an injury, some kind of a blood clot, the MRI machines or the CT scans are able to detect, no problem. But can MRI or CT scan go in a cellular level, identify a particular neuron and figure out its response?

These are the challenges that Crick actually envisioned that these are some of the challenges that neuroscience is facing. We need to ascertain or we need to understand the brain neuron by neuron, cell by cell, what are the functions, what are the chemical changes, what are the neurochemical changes that is taking place, which results in our thought. We think. Even children of few years start thinking their processes. So, what exactly is the thought process, what exactly is thinking?

We all think. We all recall something especially an exam, that is the formula, so I will write it down. We feel good. We feel bad. We have different emotions. We have different moods. What exactly that, does that physically mean? When you are calculating, when you are doing some kind of mathematical calculation in your head, while purchasing something in a market, what chemical changes take place in your brain. When you are thinking a good thought, you are recalling a funny incident, what exactly chemical changes take place. When you are emoting, when you have a particular mood, when you are laughing, when you are crying, what exactly take place at a neuronal scale in your brain.

When you are thinking a formula, when you are thinking a mathematical calculation or when you are recalling a particular say passage from a poem, when you are thinking what to say before giving a speech, when you are thinking that I need to do that, tomorrow I have a work I will do this way, this entire thought process the millions of different thoughts that come to your head, what is physically changing. How is one thought different from another, a physical point of view, what kind of neurochemical changes take place in your brain that allows you to do mathematical calculations or recall a memory from last time or look for directions.

You have gone to a place like couple of years ago, now you remember I have to go right and then left and then straight ahead and then right again. These are memory, but what exactly. One memory is different from another memory. This is not simply the memory; this memory of direction is not equal to you remembering a mathematical formula or remembering a poem or a song. We do not know.

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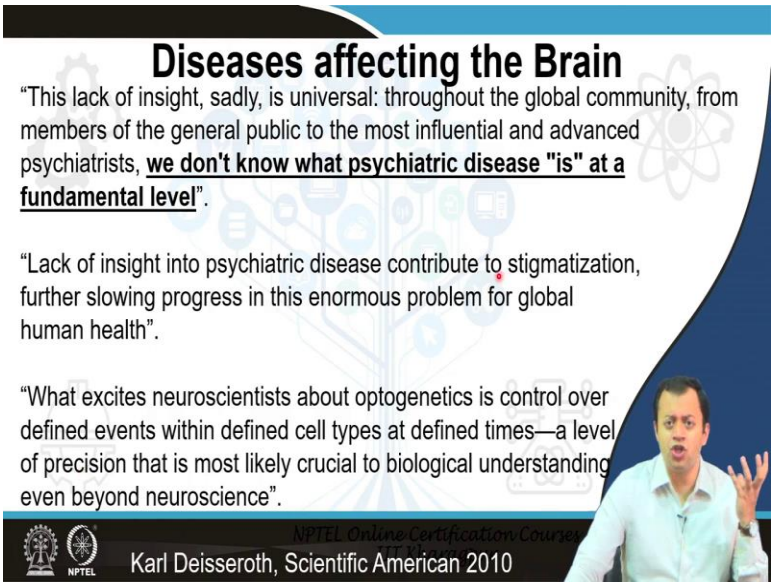

And this is what is causing us to consider mental diseases, the diseases that affects the brain. Not only we understand what a normal brain should be, we do not also understand what diseases affect the brain to prevent it from behaving normally, understand this. You need to have a reference. My reference for my body is that it has a temperature range of say 96, 97-degree Fahrenheit. That is my normal body temperature. So, when it rises 98, 99, 100, 102 Fahrenheit that is, I understand, it is a fever. So, my reference is 97 degrees Fahrenheit. That is my normal thing. That is my normalization.

What is the normal condition of a particular brain? We do not know. And because of that, ascertaining the diseases that affects the brain is tremendously difficult. So, Professor Karl Deisseroth said that we do not know what psychiatric disease is at a fundamental level. The diseases that is associated with the brain, we simply, the psychiatric diseases that is associated with the brain, we simply do not know. And since we do not know, there is a huge amount of

stigmatization in a global level. We just paint everything with the same brush stroke that person is mad.

Just like our body has gone through several physical ailments, we have malaria, we have diphtheria, we have whooping cough, we have cancer, we have Corona virus, COVID-19. Similarly, there are several diseases that affect the brain. And just like every human being, no matter what they say, at one time in their life, I know our grandfather say they had never been ill in their entire life, you know how true is that, every human being at one time or other their body have been affected by some sort of pathogen, some sort of disease. The person has to go through some sort of disease in the body.

Similarly, the mind is also equally affected and every single human being has gone through several different types of mental diseases at one time or another. It simply that we do not know, we do not ascertain, we do not understand that what is disease condition, what is normal condition. And thereby, we simply do not know what psychiatric diseases at a fundamental level is. What excite neuroscientist about optogenetics is that we can now understand, maybe we can now understand, we can control over a defined event which define cell types at defined times at a very precise level. There is an enormous stigmatization with psychiatric health, psychiatric diseases, mental diseases.

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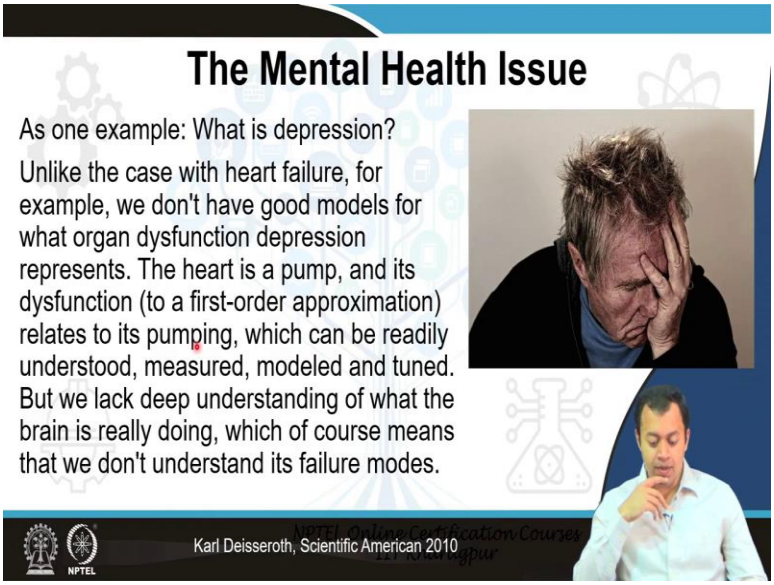

Trust me, I work in this institute of higher learning academia and constantly I see students suffering from depression. All academics at one time or other perhaps, perhaps, I do not know for certain there is no survey have probably gone through depression and depression is more common than you think it is. But what is physically happening, what physically causes depression, especially among students. You must have heard someone very close or some of your friends while studying have gone through depression. Herein lies the problem with psychiatric or mental diseases.

When it comes to any other disease, heart failure or cancer, we have very good model. A heart is a pump at the end of the day, crudely speaking, it is a pump contains several valves and it is pumping blood all over the body. So, you know that this particular valve if it malfunctions, what needs to be done. You can have a first order approximation relates to the pumping, what speed, what Reynolds number, what needs to be done, so that this is the normal blood which is flowing at this particular rate, the fluid will go through at this particular rate per second per minute, and that is the normalized reference point of a healthy living heart. And when there is something deviation from the reference, you immediately refer to it and try to correct it.

Similar thing with cancer, a physical change takes place, some sort of mutation has gone through and as a result, there is bad protein formation, some sort of tumor formation, the cell is proliferating at a much, much higher rate and the result is cancer, result is tumor formation, result is metastasis. What is depression? From a physical point of view, what is depression? What neurochemical changes are taking place in our brain? In order to understand what changes are taking place in your brain, you have to ascertain what is the normal condition? We do not know.

So, if we do not know what is the normal condition of the brain, how are we able to understand what is the abnormal condition of the brain. For the first time, we have the capacity using optogenetics to ascertain neural by neural, neuron by neuron, different functions of the brain. This is what optogenetics promised. And we are quite far away from reaching or going there. It is still at a very, very nascent stage, but emerging rapidly.

So, anyone of you who has interest in the brain, interest to know how brain works or if you want to know how the neural networks, the real neural network, not your artificial neural network, the natural neural network works neuron by neuron, this is something I strongly suggest you should

be looking up to. This is the future of biophotonics, optogenetics and neurophotonics where we use light to ascertain neurons. So, I will be describing in detail some of the techniques and, in the next class.

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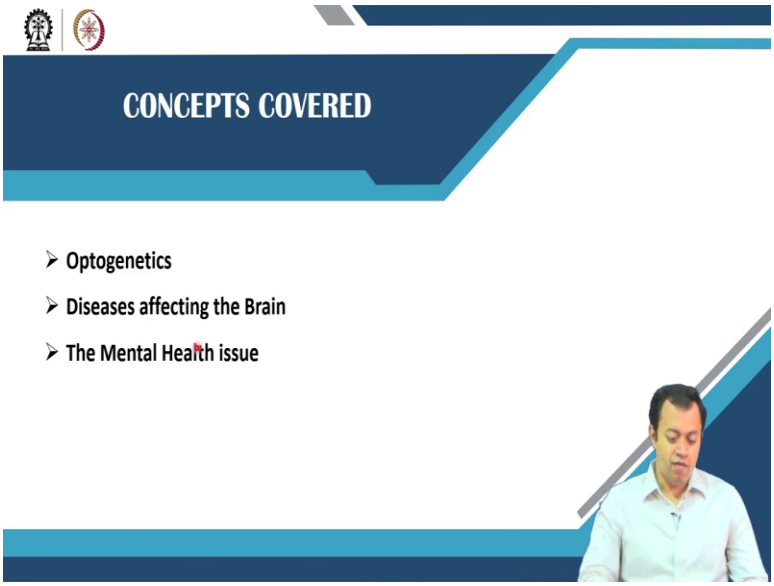

So, these are the topics that I covered today, diseases affecting the brain. So, why do we need to probe the brain? Why do we need to ascertain? What is a normal case is? What the standardized case is? And what kind of deviation from that standard case produces? What sort of disorder? And can that be reversed, i.e., can that be killed? I do not know. I do not think anyone has the answer. But it is important to ask questions. The survivability of our species depends on asking questions. So, we wanted to ask this question depending on the technology at hand, depending on the capacity we have, we need to ascertain it.

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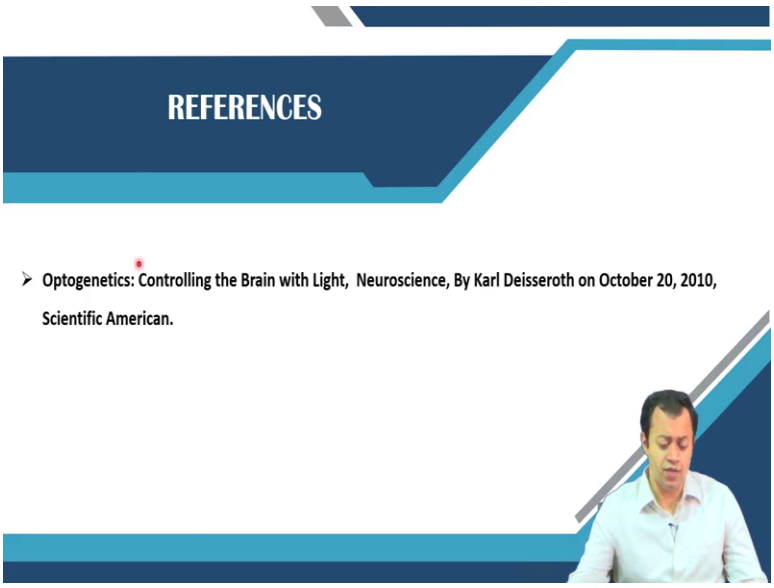

So, the, just go through this Scientific American Journal on Optogenetics. This is written very, very nicely. There are very little scientific jargons especially neuroscience is filled with jargon. So, it is difficult for any person from not a neuroscience background to just look into a neuroscience paper and understand, but hats off to Professor Karl Deisseroth, who wrote this Controlling the Brain with Light using, in 2010 in Scientific American. Please I ask you, all of you to go through this reference that I have, I am quoting and try to see how wonderfully our world is going to change with optogenetics. So, thank you. Thank you very much.