Nanobiophotonics: Touching Our Daily Life

Professor. Basudev Lahiri
Department of Electronics and Electrical Communication Engineering
Indian Institute of Technology, Kharagpur

Lecture No. 41

Introduction to Optogenetics

Hello, and welcome. We are continuing our course of Nanobiophotonics. And today, I am going to discuss a new topic of optogenetics. As the name suggests, it uses optics that is light in order to understand or probe genes. Overall, optogenetics could very well be connected these days with something called neurophotonics. Most of the time, you will see these two terms neurophotonics and optogenetics getting interchanged.

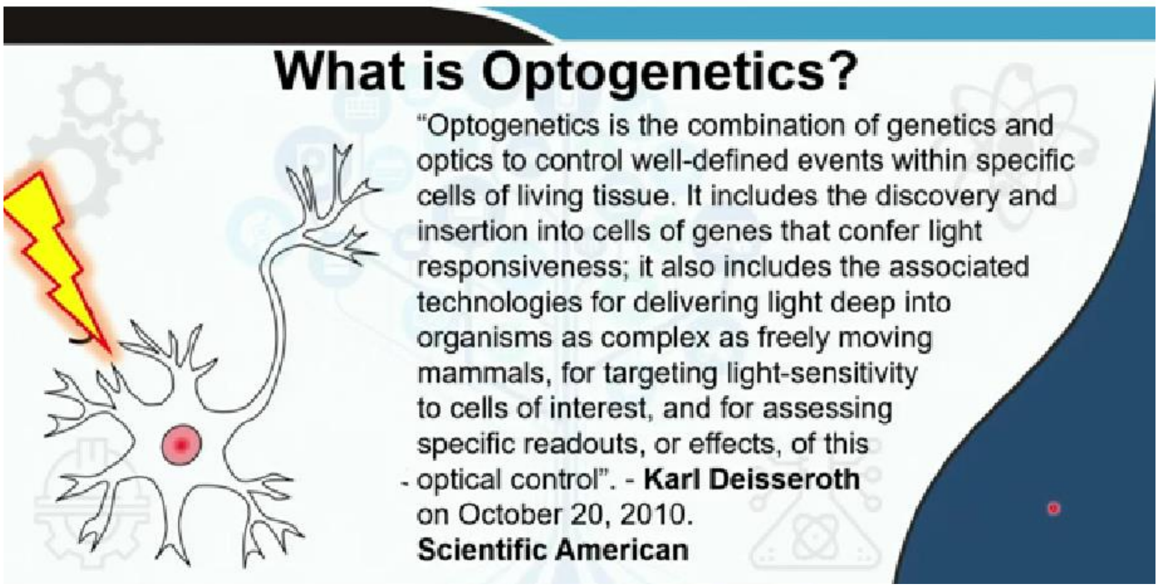
There are subtle differences between them. Frankly speaking, optogenetics is where light is used to insert some gene or manipulate some gene particularly with the brain and neurophotonics as the name suggests, is where light is being sent to probe or understand or image or even manipulate certain areas of the brain. So, as you can understand both of them have huge huge amount of overlap and the difference between them is purely purely academic. Usually, what happens is before the beginning of the course, I give you the overall basis and then take you from that a chapter per say.

Here in optogenetics technology, I have to give you the basis of the technology. At the same time, I have to give you the basis of brain, what brain is and thereby how we can manipulate it. So, here I thought that instead of going directly onto the brain, let me give you the so what part first. i.e. why is brain required to be probed by light? What are the advantages of it? i.e. why optogenetic technologies exist? What is the point of this technology? Life was good already or life was complicated already with optics or light or photonics being probing into individual cells, individual molecules, blood vessels and what not. What is the point or why do we need it? So, in these two lectures, lecture number 41 and the subsequent lecture 42, I will be giving you a detail of why this technology is needed and a glimpse of the preliminary set of technologies that basically started preliminary research that basically started the course of optogenetics. From lecture 43 onwards, I will try to give you an introduction of the brain.

That will be purely from an anatomical perspective, but do not worry if you are not from a medical background, I will try to make it as simple as possible. Obviously, you will not be knowing everything that is there to know about brain, but overall, I will try to give you a basic glimpse of what the function, what are the aspect of the organ brain is and how it controls the entire body of the organism. Now, I would like you to marvel on the delicious irony that when we talk about brains or when we try or when I try to teach you brains, the irony here is a brain is teaching a group of brains about brain. So, understand the irony.

Anyway, let us go into the topic of optogenetics.

We will slowly take it from here and then we will try to go deeper onto the fantastic world of neuroscience. All of you have heard of neuroscience, the science of the brain. Neuro-photonics or optogenetics is a subfield of it where light is being utilized to understand the aspect of brain. Now, what exactly is optogenetics? Well, Karl Deisseroth, I think Stanford University was, is actually being credited for using the term optogenetics. This gentleman, this professor Deserath coined the term optogenetics and he defined it like this that optogenetics is the combination of genetics and optics to control well-defined events within specific cells of living tissue.



It includes the discovery and insertion into cells of genes that comfort light responsiveness. It also includes the associated technologies for delivering light deep into organism as complex as freely moving mammals for targeting light sensitivity to cells of interest and for assessing specific readouts or effects of optical control. What does this mean? So, there is nothing preventing optogenetics from going to cells which are non-brain or non-nervous system related. However, for all intent and purpose, since Karl Deisseroth's seminal work that got published in 2005 or 2006, I would ask you to check it out. My ears are usually wrong, but usually on that time 2005 or 2006 when this term started coming up optogenetics, I think the research probably was couple of years earlier because usually you do the research and then the term becomes catchy and then it takes over, but anyways the overall the first experiments of this light responsiveness in a particular protein inside a cell in a living organism started in the brain cells, started in the neuronal cells of certain mice, the seminal work that Karl Deisseroth did.

Since then optogenetics is by and large associated with neuroscience, associated with nervous system, associated with some sort of relation with brain. However, technically speaking there is nothing preventing it from going beyond brain. There is nothing to prevent optogenetics to apply to digestive system or the respiratory system. Therefore, I believe there was a sudden link to restrict it to brain, so thereby they came up with the term neuro-photonics. The next module here is neuro-photonics, I will be describing that too and it is up to you to make the difference between optogenetics and neuro-photonics.

Overall there is a huge overlap between those two and many of the times I have seen several books, in fact the book that I am referring to understand it, handbook of neuro-photonics, the first chapter is optogenetics. So, the term neuro-photonics and optogenetics are very commonly interchanged among each other. In both cases you use light and interact it with brain matter, specifically the neurons. You interact light with neurons. What are neurons? Neurons are the unit of nervous system, neurons are the specialized brain cells that have these fingers, these branches like projection.

So, usually you have seen cells, I have shown you pictures of cells or whenever you imagine cell there is a small nucleus, semi-circular kind of a circular type shape, disc like shape with a nucleus in the center or near about the center and various organelles in between I have shown you. Neurons which are the brain cells also have some sort of a nucleus, but it has these branches. Usually one of these branches go very very far away, very very far away and connect with another similar branch of another neuron. They connect by these things, they can be dendrites, they can be exons. Do not worry, I will be describing neurons and the functioning of brain in a later stage, but here for the time being know that neurons are specific cells that are available, that are present not only just brains, but they are associated with the central nervous system.

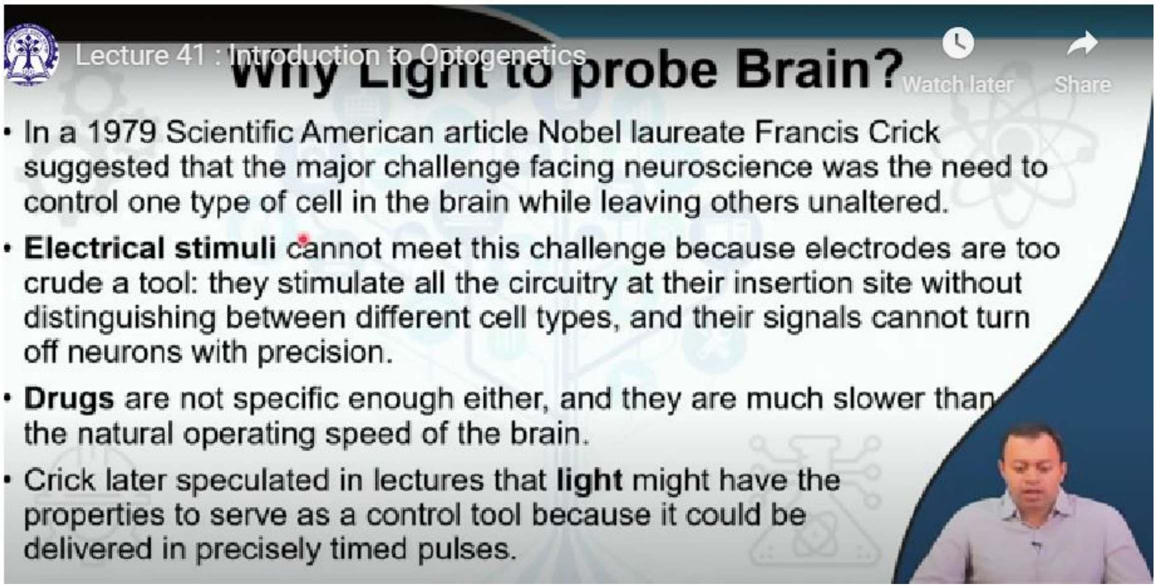
What is central nervous system? Central nervous system is brain and the spinal cord. Along with it you have the peripheral nervous system. The peripheral nervous system is everything else, your overall nerve connection that is connected with every other part of the body. Brain and spinal cord in general, in general medical students you know there is huge depth in whatever I am saying, but for the time being let us cut some slack to the electronics engineers, physics people, etc. Central nervous system, brain and spinal cord, peripheral nervous system, everything else, the nerve centers, etc. all over the body and in both cases, in both cases the prevalent cell type is neurons. Neurons are these kinds of cells that have large large amount of branches, one or two couple of several of these branches go far away. They can be axons, they can be dendrites, I mean I will tell you what they are, how they are. The unique property that makes these brain cells, the neurons brain cell is equal to neuron, different from any other cell, what is the uniqueness, what is the one particular property of these brain cells that make them separate from say lung cell or blood cell or any other cell, is that these brain cells, these neurons have the capacity to transmit electric current. Electronics engineer pay attention, brain cells are those particular type of cells present in your body that are characterized by transmitting, conducting, passing electric current from one neuron, one cell to another cell.

It is basically an electrical connection, just like you have wires connecting two different aspects, connecting two different circuital element, connecting two different resistor, two different transistor, two different diode, one diode with a resistor connected with a capacitor. Brain cells are simply that, these dendrites, these branch-like structures could be considered, I know medical people will hate me for this, could be considered as simple wires, simple wire connecting one part of the printed circuit board to another part of the printed circuit board. The printed circuit board becomes a motherboard, the motherboards become the overall central processing unit and the central processing unit is this. The central processing unit is your brain. In neuro-photonics or optogenetics, we try to understand the interaction, we try to understand the interaction of light with such cells, such brain cells, i.

e. neurons, neurons are equal to brain cells and try to see, try to prove, try to image, try to modify, try to control these electrical signals that the neurons will allow to pass from one end to another end. You have nerves all over your body. How do you think you feel pain or touch sensation? How do you think you see something or hear something? All because of the presence of nerves. Yes. And this connection, this feeling, what exactly is feeling, what exactly is touch per se.

All data, all information is being carried from one end, that is the part of your skin which is pricked, which is touched to your brain through nerves and the connection or the information that is being sent is in electrical current format. Your brain is analyzing millions and billions of electrical inputs that constantly your nerve is feeding onto it, processing it, analyzing it and thereby making you, keeping you, making you alive and able to perceive the world around you. Fascinating. The human brain is the most complicated object that a human has encountered in this entire universe. There is no object, not the hadron collider that smashes atoms, not Chandrayaan-3, not you know Artemis probe, not the International Space Station.

The complexity of the machinery of all of those big, big machines fail, fail in front of the human brain. The human brain is the most complicated machines in the entire universe that human civilization has encountered. Irony, irony, I know the most complicated machine exists inside you. Each one of us are gifted with it. Each one of us are gifted with it and it is time we try to probe it with light.
So, optogenetics is basically that trying to control, trying to image, try to understand this passage of information, this passage of electric current using light. So, why light to probe brain? Yeah, why light to probe brain? So, in 1979 Nobel Laureate Francis Crick,



remember the gentleman who co-discovered the double helical structure of DNA along with Watson and Rosalind Franklin, suggested that a major challenge facing neuroscience was the need to control one type of cell in the brain while leaving others unaltered. There are millions and millions of neurons that make up this brain. So, electrical engineers or electronics engineers, how do you try to figure out if one of your printed circuit board, if one of your circuit is not working? You have given a circuit and it is not working. So, what do you do? The easiest thing is you take a multimeter, the two probes of a multimeter, you go on putting at different areas and trying to see which connection is broken.

Yeah, that is the first and the easiest thing. Which connection is broken if the current is flowing, not flowing, etc. We want to understand the brain like that, but there are lies the problem. It is one thing to you know dissect a frog or a mouse, take the brain out and then put it in a petradition, then look into it the electrical connection, but obviously it will not give the same result that a live mouse would give. But how do you understand which particular neuron or which particular set of neuron is responsible for sending the information which is allowing the mice, mice is being an example, which particular set of neurons passing what sort of electrical current, electrical information, which could be utilized, which could be corroborated with the particular action of the mice say eating, say getting frightened, say becoming aggressive, right.

So, we want to probe just like your printed circuit board, you probe one area at a time, one circuital element at a time. We want to probe set of neurons, one neuron at a time, one set of neurons at a time and try to corroborate it, try to match it with the resultant action of the brain. Problem is electrical stimuli when you insert electrodes, previously long long time ago, it used to be a very bad practice to treat mental disorder. The people who had mental diseases were subjected to electrical shock. So, putting electrodes inside the brain, not only it is dangerous, unethical I would say, but they are too crude a tool, they stimulate all the circuitry of the insertion site without distinguishing between different cell types and their signals cannot turn off neurons with precision.

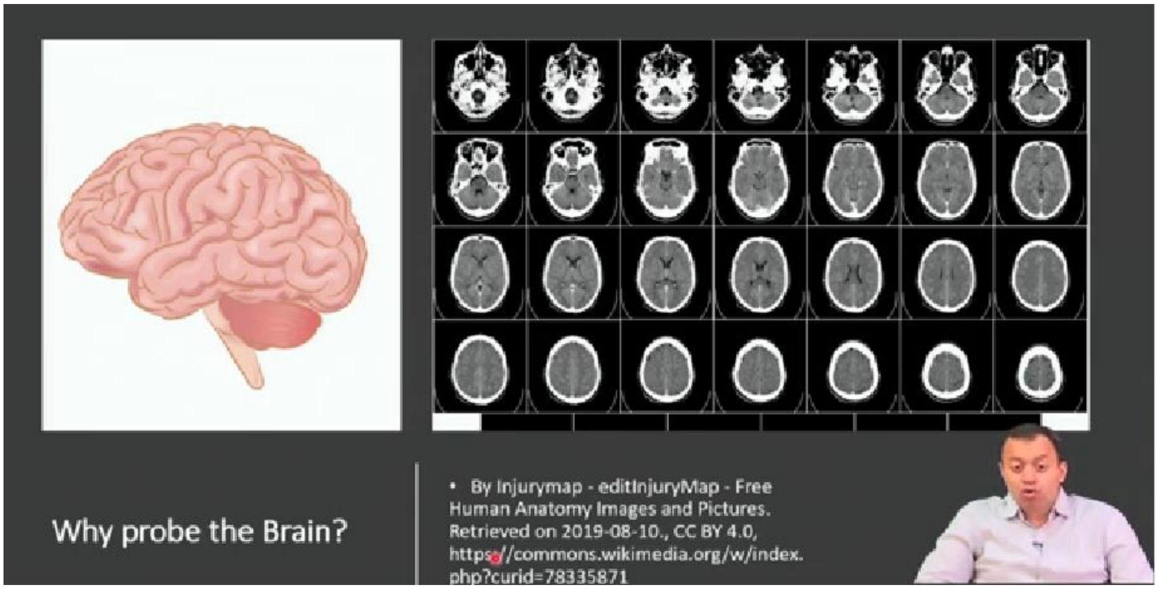
When you are inserting electrodes and passing electric current through the brain, a huge huge portion of the brain is getting zapped, is getting you know voltage is getting electrified. So, how do you know if one single neuron connects talking with another single neuron is able to do a particular action. It is like connecting every single connection of your printed circuit board with a battery source and then try to find out which one is not working, bad analogy, no analogy is perfect, but try to understand. Electrical stimuli are simply too crude a tool, it stimulates all the circuitry at the insertion site without distinguishing between different cell types, even if the amount of current is less. Obviously, you try to not to kill the person or the mice, you give few micro ampere of current, few micro volts current, it is still too crude, you will never be able to know if you want to probe one neuron, one brain cell with another brain cell.

Similarly, you can apply drugs, certain drugs affect certain areas of the brain, but drugs are not specific enough either, how do you distinguish or how do you ensure that the drug is getting connected with one particular neuron and not the neuron nearby. And they are much slower than the natural operating speed of the brain. Your brain is incredibly fast, how fast do you think data travel when a part of your body, a part of your skin is pricked, take a safety pin and prick a part of your body, how soon do you think you will register the pin in few millisecond, few microsecond time, how soon as you open your eyes, you are able to see the world and your brain analyzes, how many seconds does it take for you to, you keep on blinking all the time, how soon your body simply or your brain simply ignores that blinking part and you are constantly being fed with information, your brain is incredibly fast in doing calculations, in doing calculations, multitasking, you are driving a car at the same time you are changing your music in the radio, you are looking for direction, you are looking for the GPS signal while you are talking with somebody, do not do that, bad example, but your brain is incredibly fast. Whereas, if you have given some amount of drugs, which you know anesthetize or which modify certain portion of the brain, the amount of time taken for the drug to take effect is much slower than by the time your brain has done, you know, couple of billion calculations. At the end of the day, this is a supercomputer, this is not a supercomputer, this is a hypercomputer, how many teraflops, how many terabytes of data do you think your brain processes per second, do you think the nearest supercomputer can do it, the supercomputer can do something very crude, very fast, few million addition or subtractions per se, but can a supercomputer look at a beautiful image, look at a beautiful scenery and marvel it, can a supercomputer write poetry, can a supercomputer make music, every single civilization since its inception on this planet, no matter how isolated it is, have some sort of musical connection associated with it.

So, even the most, you know, isolated human civilizations have been able to achieve with their crude tools, something that our latest supercomputers are unable to perform, right. You will argue that these days artificial intelligence has gone, you know, leaps and bounds, chat, GPT can do whatever you feel like, but I think you are, you have to accept that till now, I do not know what about the future is, what future holds, the artificial intelligence, the capacity of the artificial intelligence is nowhere closer to what a human brain can achieve, right. So, drugs are too crude and too slow, electrical stimuli are too crude, drugs are too slow and they are not specific either as compared to the functioning of the brain. Crick later speculated in lectures that too in 1979 that light might have the properties to serve as control tool because it could be delivered in precisely timed pulses. If your brain, if your brain calculates at millisecond speed, microsecond speed, light, a laser light for that example, can provide pulses at the same speed as well.

A light pulse, a laser pulse can exist, a pulse can exist, a pulse can exist for few microseconds, few nanoseconds. You have heard this, right. We were discussing about microsecond, nanosecond, picosecond, femtosecond pulses. So, light can have the pulse with the bandwidth of the pulse matching that of the calculator speed of the brain. So, thereby, thereby by sending high frequency pulses into certain areas of the brain which can be localized using various imaging techniques, various nanotechnology based techniques, breaking various diffraction law techniques, maybe you can simply probe one cell, one brain cell, one neuron at a time.

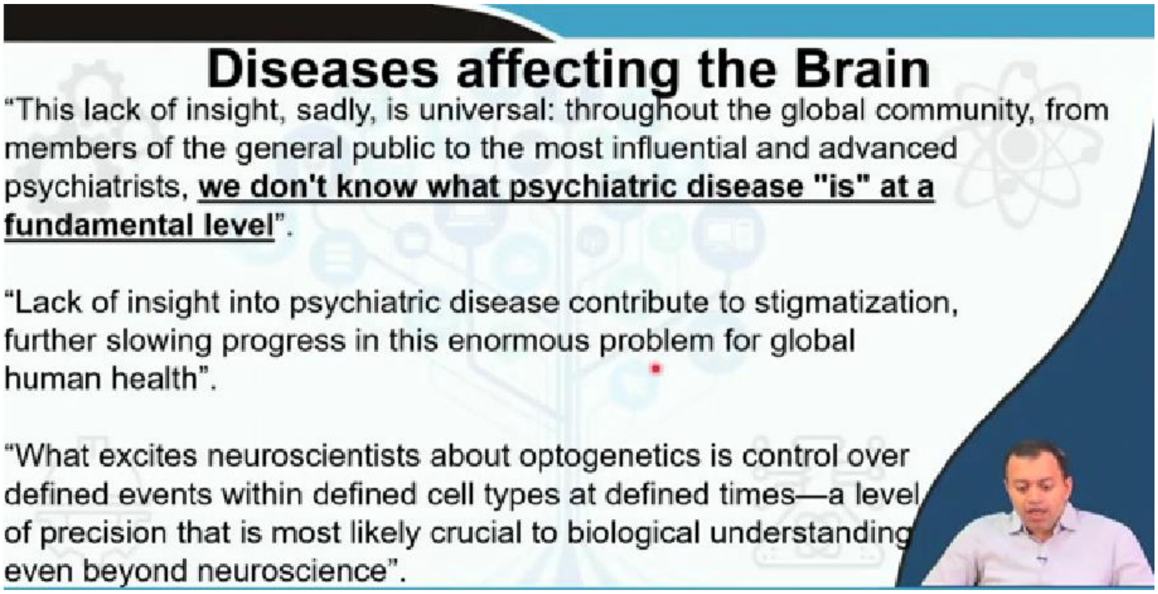
So, the probe of the multimeter with which you manage to see the problem in your circuit, that probe is now two sets of lasers. Think about it. And they are being put into single neurons or group of, small group of clusters of neurons one at a time and map the entire brain. So, see, why probe the brain? Well, the answer is obvious. The brain is more or less the organism, it is the central processing unit.



Of course, without a display monitor, without mouse, without cue board, it will be very difficult to run the computer or the laptop. But at the end of the day, let us face it, the most important part of the computer is the central processing unit. The most important part of the organism is also the brain, the central processing unit. I fully agree without heart, without lungs, without kidney, you will not survive. But all of them, despite having all of them, if there is no brain, the organism does not exist.

So, why probe the brain? See, at this present moment, there are several of these techniques like CT scan, MRI, etc, that can image the entire brain and is able to tell you which are the, you know, injured part, if there is any damage, if there is any tumor, these days, brain tumors are diagnosed mostly by either CT scan or MRI or a combination of both and then a group of people decides what needs to be done. But again, those are for a lack of better word, I am sure I will get a lot of slack from medical students. They are pretty crude. Come on, they are pretty crude. If we have to understand disease at a cellular level, say one of the cells, one of the dot cells here or a group of cells here are showing malignancy, somewhere like that, do you think your CT scan or your MRI, magnetic resonance, magnetic field will be able to resolve? Why do you think it will not be able to resolve if you think so? Remember the diffraction limit, diffraction limit is available everywhere.

Your probing material is much smaller in size than the wave or than the probe station that you are trying to measure it with. So, overall the same principles exist if you are using sound wave, if you are using magnetic fields, if you are using x-rays, it is quite difficult, sometimes harmful, but that is arguable. At a cellular level, at a cellular level without doing dissection, without taking it out, it is very very difficult to understand if a disease or an injury have had taken part. So, that is why optogenetics have started. Now, problem is just like our body is affected by different types of diseases and the physical diseases are all different, malaria is different from COVID, COVID is different from dengue, dengue is different from influenza, influenza is different from cancer.



There are several different types of diseases that affect the brain i.e. the nervous system. They are all different in nature. Just like no human being can say that she or he has never fallen ill in their life, no matter how they claim, I know old people claim that I have never fallen ill in my entire life, but let us be absolutely honest, no immunity like that exist.

Usually, just like all of us has one time or other have fallen ill either by common cold or something severe than common cold, we have encountered physical diseases. Similarly, every single human being at certain period of time have had contracted some sort of a mental disease. Problem is the stigmatization. If you go to the hospital or if you go to the doctor stating that you are suffering from fever and please prescribe me some paracetamols or antibiotic, no one blinks an eye. But if you go to the doctor and ask for some sort of a mental help, because my nervous system is simply affected, which happens to every single human being at some period of time, there is immediate stigmatization.

Not only we fail to understand the different categories of mental diseases, but going for help for mental disease is ridiculed or stigmatized to a large section of our society. And this is the problem. No one should be making fun of anybody going and asking for help. No one laughs at you when you say that you need antibiotic.

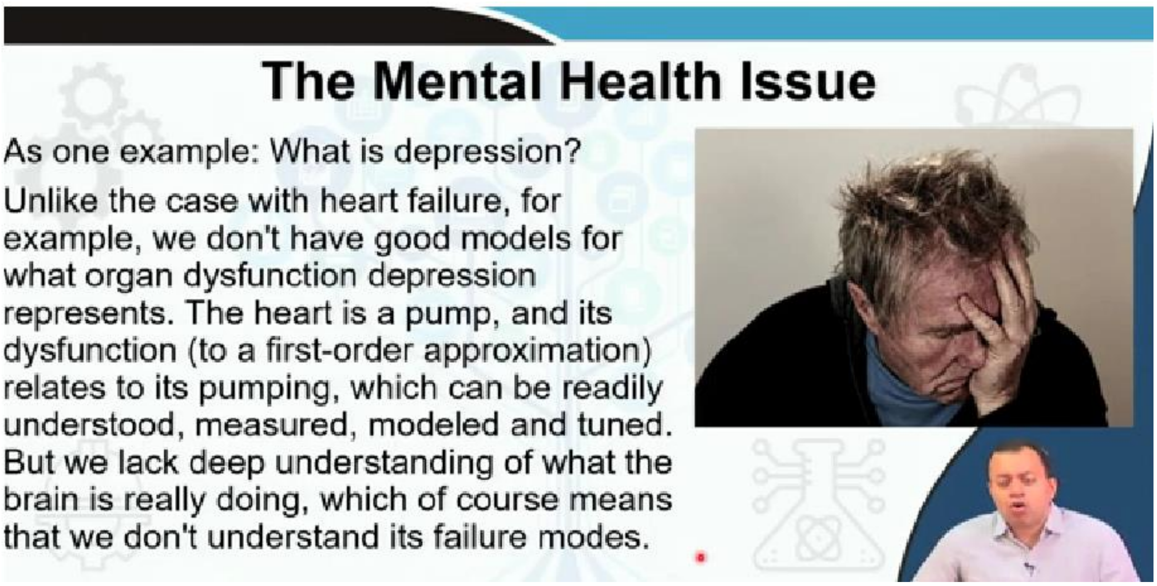
No one laughs that if you have influenza or COVID. So, what is the difference here? Problem is, because of the stigmatization to a large extent, the research in this aspect is also not as importantly viewed or not as extensive as that of the physical disease. Also, another significantly most important reason is probing brain is difficult. So, as it says the lack of insight sadly is universal throughout the global community from members of the general public to the most influential and advanced psychiatrists. We do not know what psychiatric disease is at a fundamental level. We do not know when we talk about this term like depression or attention deficit ADHD, attention deficit hyper disorder or schizophrenia or any other kind of the psychiatric diseases, what exactly it is at a molecular level.

What exactly is changing chemically or molecularly in a person's brain when the person is claiming that she or he is depressed? Yes, we go for counseling and then psychiatric analysis, this that previously they used to give people electric shocks and those barbaric practices. But till now, till date, despite having all those technological advances, we are still nowhere clear that what exactly is the disease, some amount of glucose variation in your blood, you call it diabetes, some amount of change of oxygen saturation in your blood, you can call it some kind of lung-based infection i.e. COVID. What exactly is then the effect of depression? All of us have been depressed at certain period of our life, we all receive setback, we all receive failure, we all have had depression at some time in their life, what is the change that is happening? What is the chemical change that is happening? Cholesterol, too much Cholesterol arteries are clocked, you are getting heart attack.

Great, understood. What about the brain? What exactly is changing at a physiological level, at a molecular level, at a biological level, at a chemical level inside the brain so that you are either depressed, you are suicidal, you have an attention deficit, hyper attention deficit, hyperactivity disorders, you have schizophrenia, you have epileptic behavior, all of those things. Lack of insight into psychiatric diseases contribute to stigmatization, further slowing process in the enormous problem for global human health. What excites neuroscientists about optogenetics is controlled over defined events within the derived cell types at defined times. A level of precision that is most likely crucial to biological understanding even beyond neuroscience. You have heard or seen people, they are afraid of, they have phobia, certain people have certain phobia like ironically enough, I have a phobia for mouse yet I have to work with them.

So, I used to be very much afraid of mouse. Some people have irrational fear towards snakes or spiders or darkness or so many things, you know about these phobias. What exactly is it physiologically? What exactly are the molecules, molecular change that is making you afraid of something? Some people get tremendously afraid before a particular exam. All of you know there is something a phenomenon called exam fever. Your body gets into temperature, high temperature just before the exam, few days before the exam, too much pressure, too much thing. All of that is accepted stress and pressure and all those terms.

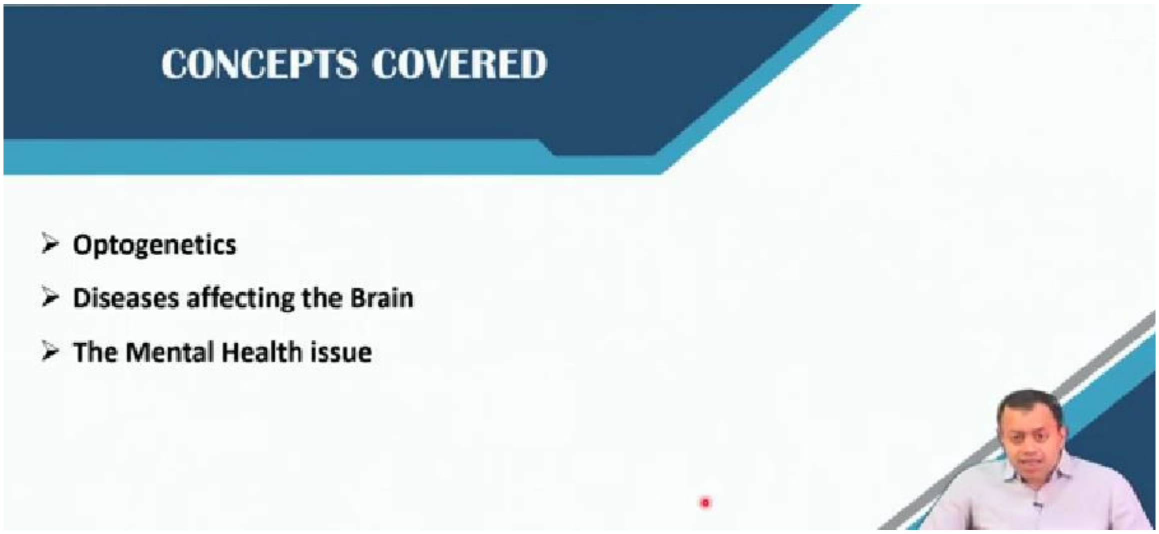
But what does it actually mean? What am I measuring? What chemical composition change in my body when I am under too much stress? What chemical composition changes in my body when I am in too much stress? When I am studying too much? Do not say blood pressure, blood pressure does not change when you are in front of an exam. Anyways, so the mental health issue is what is depression? Unlike the cases with heart failure for example, we do not have a good model for what organ dysfunction depression represents. Which particular organ is affected due to depression? You will immediately say brain, but brain is affected for almost all cases. What exactly is changing when you are depressed? The heart is a pump and its dysfunction to a first order approximation relates to its pumping, which can be readily understood, measured, modeled and tuned. We have lots of models for heart and immediately heart failure resulting in pumping of blood can be easily understood.



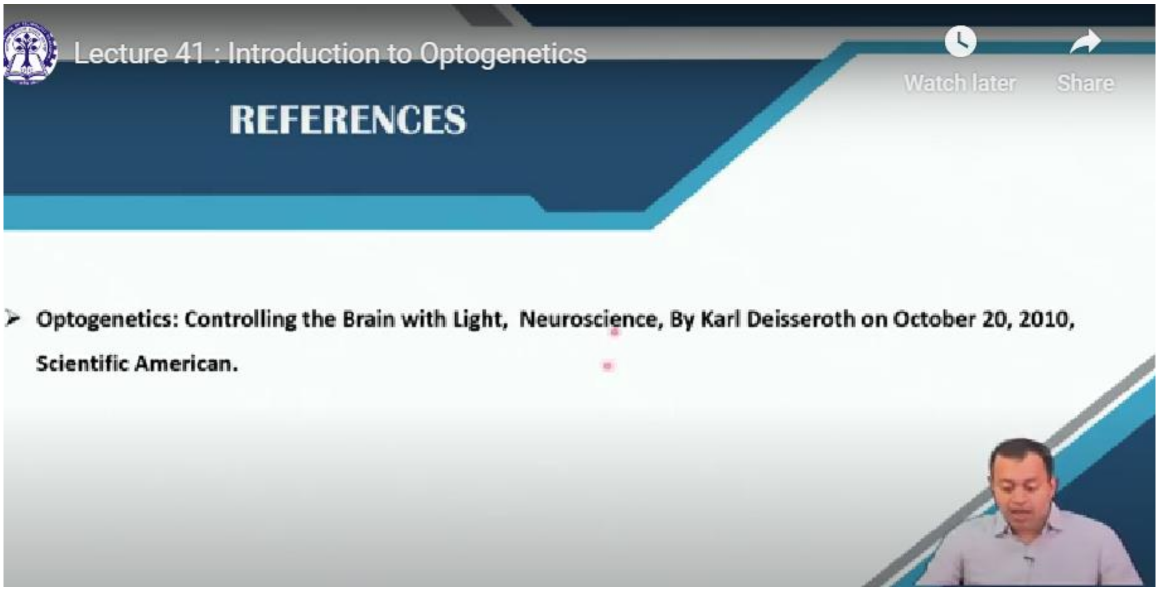
But till now, we lack deep understanding of what the brain is really doing, which of course means that we do not understand it failure modes. You understand a proper functioning of the brain, thereby you understand what is the problem with the heart, which has resulted in a heart attack. You do not understand the brain. You do not understand the functioning of the brain to a fuller extent. So, how do you plan to know what is wrong with it? If I have given you half part of the circuit of a total motherboard, just one fourth of it and you are being asked to understand what is wrong with the circuit, what is wrong with the motherboard, would you be able to do it? Or suppose I have given you knowledge only about resistors, only resistors.

How resistor works? You know and then I have given you a motherboard containing thyristors, capacitors, transistors, HEMT, IC, this, that, what not and you are asked to prove it. With your knowledge, with your limited knowledge on how Ohm's law-based resistors work, do you think you will be able to explain how diode or transistor or complicated IC etc is working in a complete motherboard? These are analogies that I am talking about. So, this is the problem statement that I made today. This is the problem statement that why we need light to prove the brain. A, we do not understand the brain and

B, not only we need to understand it, we need to understand what is wrong with the brain and C, if we can cure what is wrong.



All three can be combined together in this process of optogenetics. So, please go through this fantastic paper written in Scientific American for those of us who are not from neuroscience background. Scientific American usually publishes scientific work for the general public rather than very very specialized people. So, if you have to write for Scientific American, you have to write it for a large audience and non-scientific audience.



So, usually those articles are incredibly interesting and easy to understand. So, please please go through this particular Scientific American article written by Professor Desiroth in 2010.

I quite enjoyed it and I will see you in the next class. Thank you very much.