Nanobiophotonics: Touching Our Daily Life
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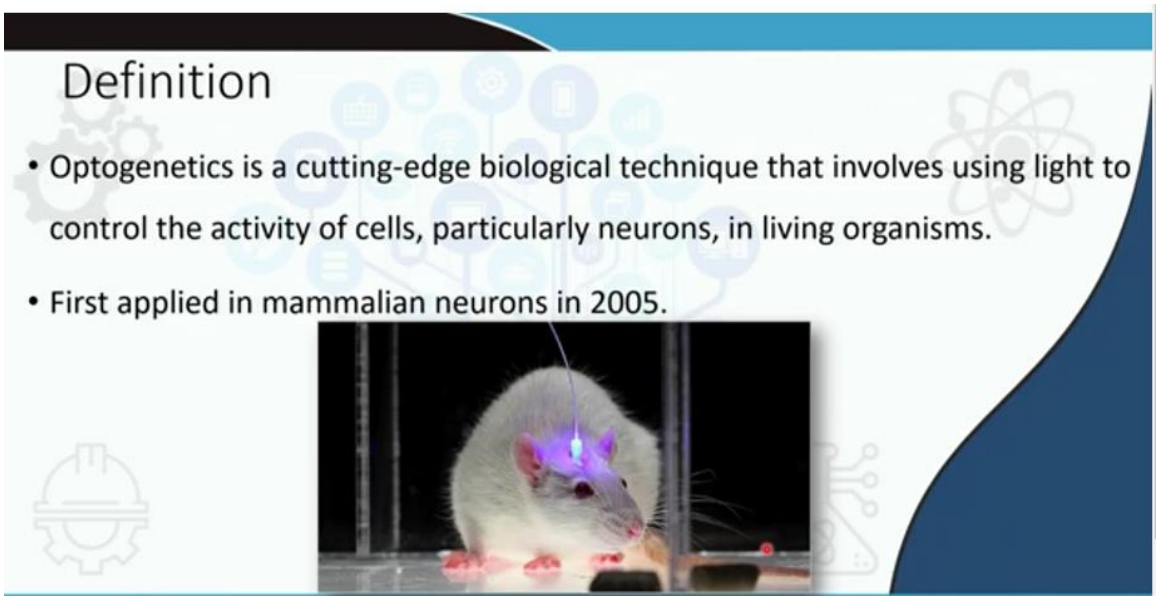
Lecture No. 51
Optogenetic Modulation of Neural Circuits

Hello, and welcome. Today we are going to start ah brand new topic module number 11 and this is quite interesting because here we will be discussing about the application and emerging research area in neuro-photonics. As you know neuro-photonics in itself is quite cutting edge quite new. So, I decided that here let me just give you a glimpse of some of the topics that are you know futuristic that are futuristic in nature in the sense that these things are not yet evolved, but has the potential has the capacity to ah change modify and even challenge our existing understanding on how the brain works. So, several of the topics this is by no means an exhausted list this is by no means to say that these are the only emerging research areas in neuro-photonics. I have chosen of a plethora of different types of cutting-edge present-day research topics on neuro-photonics.

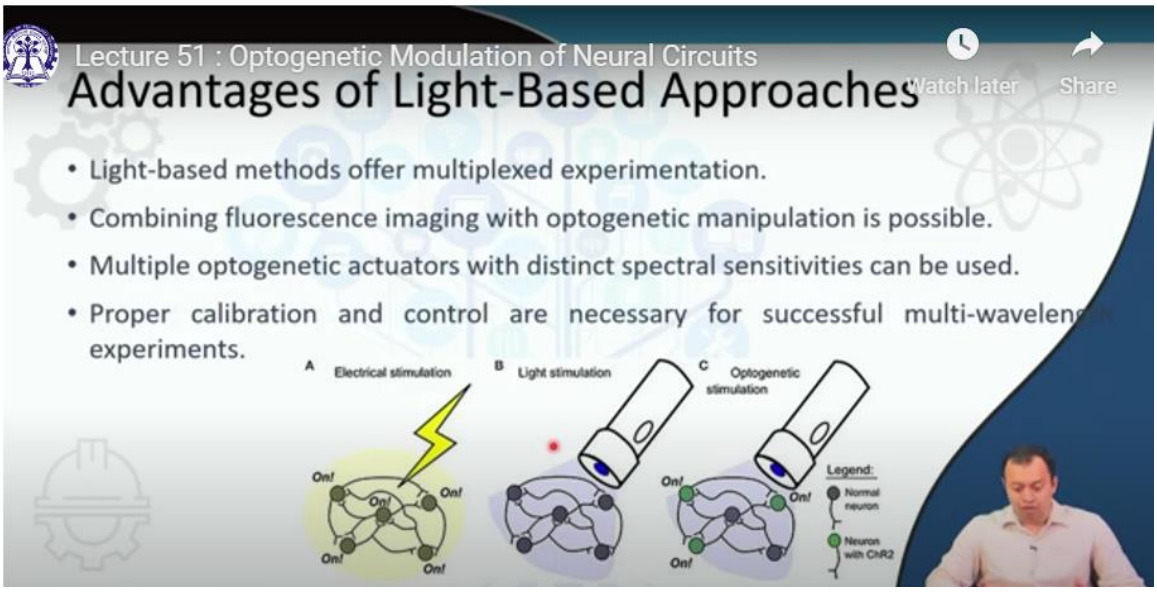
I have chosen just 4 or 5 of them and I will be discussing 5 of many in the in the in the in the session that is going to be module number 11. Remember several of these topics are ah yet unexplored or minimally explored. So, I will be giving you thus far how what we have achieved ok. This is by no means a completed you know research topic where we started with this, this was our objective and this is what we have achieved.

No, it would not be like that. It is simply stated that we are trying to understand how the brain works, how we can modify, how can modulate brain, how we can see and this is thus far we have reached. There are as they say poetically miles to go before we sleep. So, ah that gap I am hoping that remaining path you and I will travel together right. So, in today's topic let us talk about optogenetic modulation of neural circuits.
So, we start with something that we already know that we have already discussed and you you know what this is we have discussed in module number 10 that optogenetics is a cutting-edge biological technique that involve using light to control the activities of cells particularly neurons in living organism.



I discussed that there is going to be a difference between ah optogenetics and neuro photonics though optogenetics deals with light to change modify genes. Thus far most of the time light has been utilized or optogenetics term has been utilized for controlling the genes are present in neurons nerve cells. There is nothing preventing of using optogenetics to control the activity of any other cell right. But in 2005 the mammalian neurons were first fired first modified using using these kinds of ah white light source white light source inside inside the ah mammals head mammal being the mice here the specific red eye mice.

And we wanted to try and see how how we can modify genetically how we can modify the different genes and thereby can we modify the behavior can we modify the behavior of the organism. So, obviously, again just to repeat from previous lecture what are the advantages of light-based approach ah we can offer multiplexed experimentation

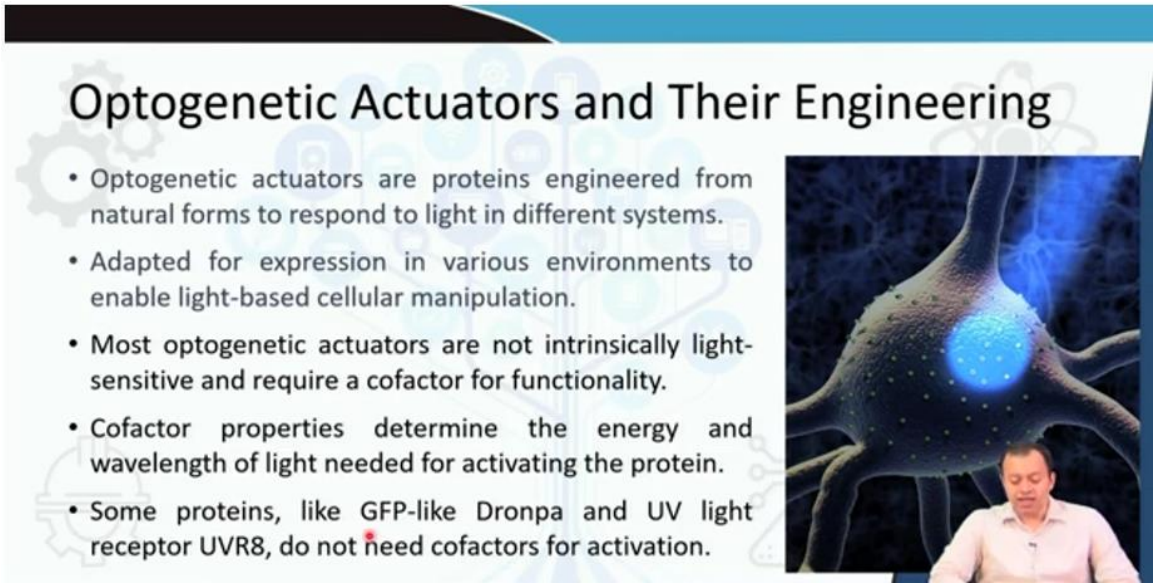


meaning you can have a plethora of different wavelengths of light either being passed to a same optical fiber multimode fiber ah which have different wavelengths. And you can trigger different section of the brain different section of the brain are attached with light activated protein different types of light activated protein a protein A is activated by red light protein B is activated by blue light protein C is activated by green light. And all of those light activated proteins are somehow at different places of the brain some in cerebellum some in cerebrum some in meruloblongata some at you know frontal and you know dorsal lobe by this time you should know what I am talking about we have discussed this in previous lectures if you are ah having difficulty just go back to the previous lecture and you will see what I am what I am discussing. So, you can it is up to you whether you want to ah modulate or fire one single neuron or one single group of neurons or present in one specific part of the brain or you can have ah multiple neurons firing you know simultaneously, but separately and then look into the combine effect that has on the behavior of the organism right.

Problem with electrical signals when you put electrode inside the ah head of ah mice and then ah pass electric current the electric current affects if not the entire brain almost a large portion of the brain. So, it will be very difficult for you to differentiate between which part is conducting what exact sense. In case of light when you have 4 or 5 wavelength simultaneously you can give each one of them individually combination of 2 combination of 3 all 4 and in all combination electronics engineers you know 01010000 like the truth table like the truth table ABCD is the input A being green B means blue ah another is yellow another is orange and then 0 means fire 1 means not fire you have the full truth table 2 to the power n 2 to the power 4 and you have the full truth table and the output just like logic gate just like logic gate you can you can you can do this and thereby try to figure out what is the behavior what is the combined behavior what is the overall behavior of the

organism. We obviously, combine fluorescence imaging with optogenetic manipulation ah multiple optogenetic actuators with distinct spectral sensitivity can be used the sensitivity is a big part ah we can increase or decrease the sensitivity by adding different type of fluorophore light is activating the protein, but how much how much of ah I mean what intensity and how much of the light is going to activate the same neuron will react differently will fire ah the electrical channels will be activated the resting potential and the action potential will be different based on how much intensity of the same light of the same wavelength have been utilized to fire it right we can control it same wavelength of light same protein, but the amount yeah amount is increased 220 volt 400 volt 600 volt they all have different effects. So, combine the same thing utilize the same thing that multiple optogenetic actuators with distinct spectral sensitivity can be used proper calibration and control are necessary for successful multi wavelength ah experiments.

So, you have light electrical simulation everything is on light simulation and you have optogenetic simulation where normal neuron, neuron with CH2 channel rhodopsin some of them are on ah some of them are off green means on grey means off. So, you can selectively you can selectively ah describe which portion of the neurons are on as in case this will only happen when we have ah light activated protein channel rhodopsin as compared to when none of the proteins are light activated or all of the proteins are electrically stimulated everything is on right. So, what exactly are optogenetic actuators and their engineering? So, optogenetic actuators are basically proteins engineered from natural form to respond to light in different system I told you there are several jelly



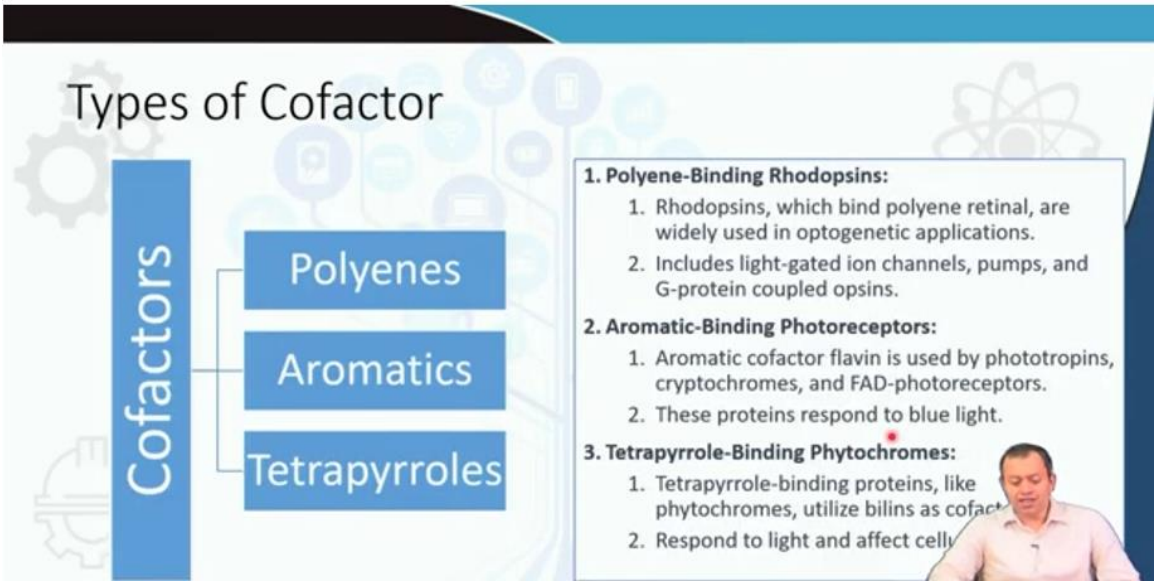
fishes you know which show bioluminescence you must have seen it in Instagram reels and places that people go to ah some specific beaches and at the night the the the because of the presence of jelly fish or some kind of ah plankton basically called phytoplankton

they glow you must have seen it in in in in different ah you know Instagram reels and social media posts etcetera. So, there are these jelly fishes there are this ah sea creatures that themselves contain proteins they themselves contain genes to manufacture these proteins express these proteins inside their body which glow when excited by specific form of light right and this glow although faint is usually not seen during ah sunlight, but when the low light conditions especially during evenings and at the night they simply glow up. If you have not seen this happening at different beaches ah just you know do a quick internet search ah glowing beach in some place in I think southeast Asia or I think Latin America ah Caribbean and you will see.

So, what we did what the scientist did that they were able to extract that particular gene the light activated protein manufacturing gene were simply extracted and that was placed inside the brain or inside ah a mammal right. So, optogenetic actuators are engineered proteins from natural forms to response to light in different system they do not exist naturally in mammals most mammals to most normal mammals if there are substantial variety of mammals something might be ah coming up in days, but usually mice do not glow in dark yeah usually mice do not glow in dark. So, these actuators that are engineered proteins engineered proteins are adapted for expression in various environments to enable light based cellular manipulation most ah engineered proteins are not intrinsically light sensitive and require a cofactor for functionality cofactor properties determine the energy and wavelength of light needed some protein like GFP green fluorescence protein like drop 9 UV light receptor do not need cofactor for activation. So, what exactly is a cofactor? Cofactor is a helping molecule cofactor could be an organic or inorganic molecule which is usually not protein cofactor considered it some kind of like a fluorophore that is connected with a large protein and which itself is you know getting activated by the light and which then creates a biochemical reaction into the overall protein into the overall protein and the process starts right. So, there are certain proteins which are directly activated by light and then there are certain proteins that need some sort of a catalyst some sort of a extra additional molecule associated with it this extra additional molecule helping molecule are called cofactors.

So, actuators are the engineered proteins and cofactors are those helping molecules that are attached with the actuators to make them react in a specific way when light falls onto them the actual photochemical reaction happens because of the cofactor because of the cofactor this cofactor triggers a particular effect on to the activator. Now you might ask why this is necessary or why do not we use all proteins which are light activated by themselves yeah there are certain proteins just as I saying, but you do understand that it is simply not that easy to extract a gene from jellyfish and simply insert into a mammal and think that the behavior of the mammal will be exactly the same yeah. You do have to bring into some sort of a compromise you do have to bring in some sort of compromise you

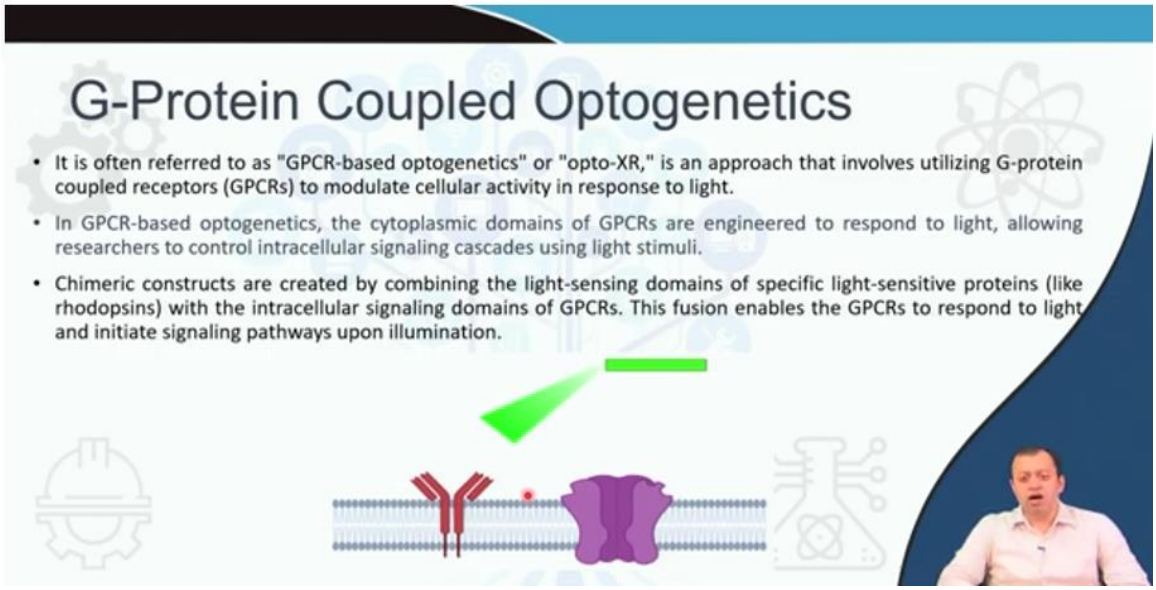
cannot simply start inserting XYZ gene from any XYZ living organism into any other organism yeah it will simply not biologically viable. So, there are certain you know limitations that you have to follow certain proteins like GFP etcetera you can directly insert using human body bigger part and mammal body we still have to get the ethical permission to do these kinds of thing in human body especially in their brain, but then we can put some normal protein existing protein non harmful protein tag it with another molecule which is itself photosensitive right. So two ways you extract you engineer proteins that are photosensitive either you can engineer proteins that are directly photosensitive, but have limited value how much photosensitive how much energy how much electrical field how much ions they are going to trigger what kind of biochemical reaction that is going to happen or you can simply tag you can simply level certain existing proteins with a cofactor with a cofactor the cofactor is the one which is getting attracted which is getting stimulated by the presence of light and it creates some sort of a some sort of a biochemical reaction which starts sending signals biochemical reactions etcetera into the overall protein which in turns affect the overall neuron cell which is basically made up of that.



So, what are the different types of cofactors that we can use remember these are non protein these are non protein molecules they can be polyenes aromatics or tetrapyrrols sometimes metallic nanoparticles have also been used we will be discussing metallic nanoparticle pretty soon in brain. So, polyene binding rhodopsin I we have discussed rhodopsin before. So, rhodopsins which binds polyene in retinal are widely used in optogenetic applications especially something related to when you are trying to understand and the property of vision visual cortex remember visual cortex were at the back of your head this includes light gated ion channels pumps and g protein coupled opsins g protein are guanine nucleotides proteins that are some sort of a you know enzyme type things that binds the nucleotides aromatic binding photoreceptors aromatic cofactor flavin is used by phototropins and FAD photoreceptors these proteins responds to blue light this aromatic cofactor aromatic molecule they are specifically for blue light and tetrapyrrols. Pyrols

probably you know are very common these days pyrols are this long ligand molecules long chain molecules used in enzymes to solar cells to super capacitor chemist will probably know. So, tetrapyrrols these are long chain molecules type of things they are binding proteins like phytochromes ah utilize blintzert cofactors and response to light and affect cellular processes.

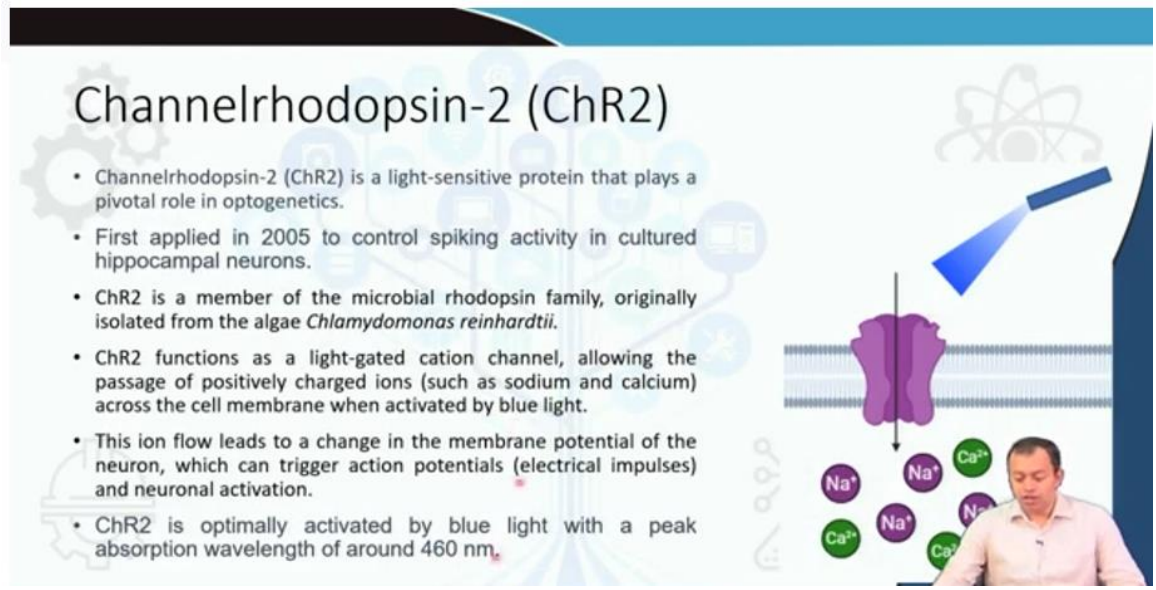
So, if you are not you do not need to go into too much of chemistry into this, but understand that there are usually three different types of cofactors. Cofactors are non-protein molecules that attach themselves into the protein and they are the one which are directly directly photosensitive they are directly affected when light falls into them when light falls into them they start some sort of a modification some sort of a change which results in the overall chain of the molecule of the protein that they are attached with. You know about domino right you know several cards or several you know match boxes all you know lined together you just drop one and it starts every other match box starts falling you have seen this you know numerous times in social media. So, it is like that you affect one part of the molecule which is attached with a long complex protein chain which is then connected with the cell and you just hit one with a pulse laser source and the biochemical reactions get triggered it results in you know action potential resting potential all of that firing and you can clearly map it either behaviorally or by doing all those microscopic techniques. So, cofactors are the one that starts this domino chain reaction.



So, G proteins have recently got quite some traction G protein I actually forgot it is I think guanine nucleotide protein, but do correct me if I am missing something in between G proteins are guanine nucleotide protein. So, G protein coupled optogenetics they have come up with a very interesting name opto XR GPCR G protein coupled optogenetics R stands for I do not know what it stands for it often referred to a GPCR based optogenetics is an approach that involves utilizing G proteins coupled receptors OR for receptors to

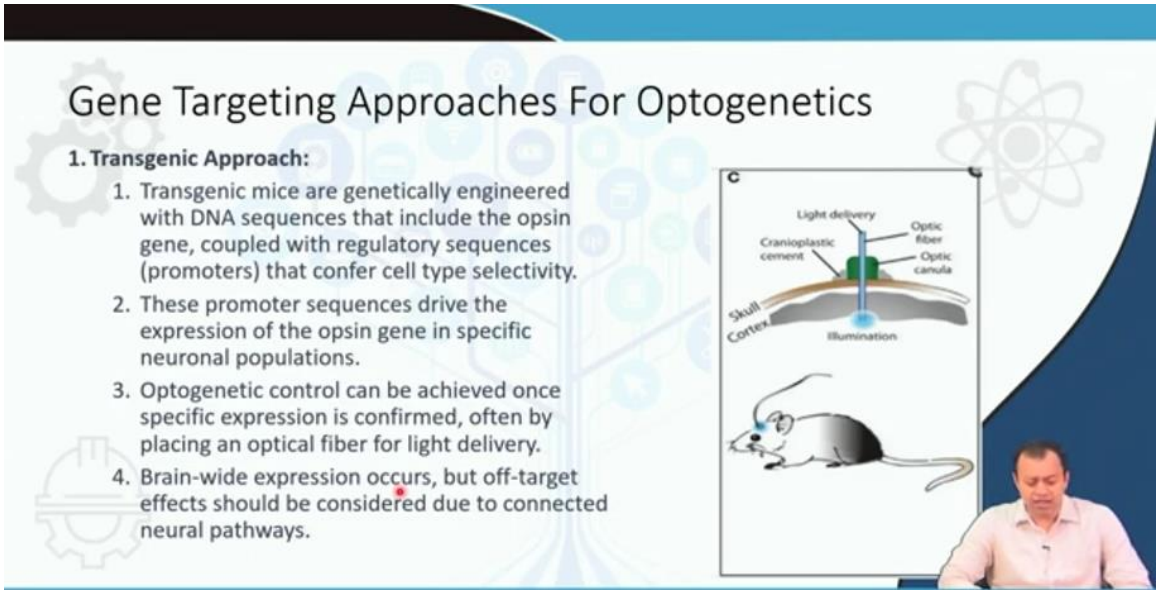
modulate cellular activity in response to light. So, as I said not only it will respond to light and do the biochemical reaction that biochemical reaction will result in some sort of modification in the overall cellular process right there are plethora of you know cellular processes that keeps on happening. So, GPCR it is interesting that the cytoplasmic domain of GPCR are engineered to response to light allowing researchers to control intracellular signaling cascade using light stimuli. So, let us try to understand this.

So, you have produced or you have created some sort of a protein into the cell wall right into the cytoplasmic membrane or to into the cell wall and depending on whether this protein is activated or not activated you can open or close certain membrane certain pores certain areas certain doors of the overall cell membrane cell wall is for plant. So, chimeric constructs are created by combining the light sensing domains of specific light sensitive proteins rhodopsins with their interacting signaling domains of GPCR this fusion enables the GPCR to response to light and initiate signaling pathway upon illumination. So, a door into the cell membrane which is which is which is connected with a specific protein you can upon your choice upon shining light onto it open or close open or close and thereby allow certain molecules to pass through or not pass through thereby affect the overall environment overall cellular environment change the pH increase the temperature change the polarizability remember the change of polarizability between outside and inside results in flow of electric charge. So, how much amount of electric charge will be flowed can be controlled can be simply modified by opening and closing the channel gate opening and closing the cell membrane gates right you know about this action potential outside is n a inside is potassium ions and then you are opening the channel you are closing the channel and thereby the polarizability its hyper polarized or unpolarized this is exactly how vision keeps on happening hyper polarization etcetera. So, you now have in your hand the tool to simply manipulate it according to your own will depending on the type of laser source that you are using depending on the pulse duration depending on the intensity depending on the wattage you can open or close these pores in the neuronal cells in the neurons and thereby allow the overall polarizability of the neuron resulting in either triggering action potential or creating resting potential this is extremely cutting edge think about how far we have we have come.



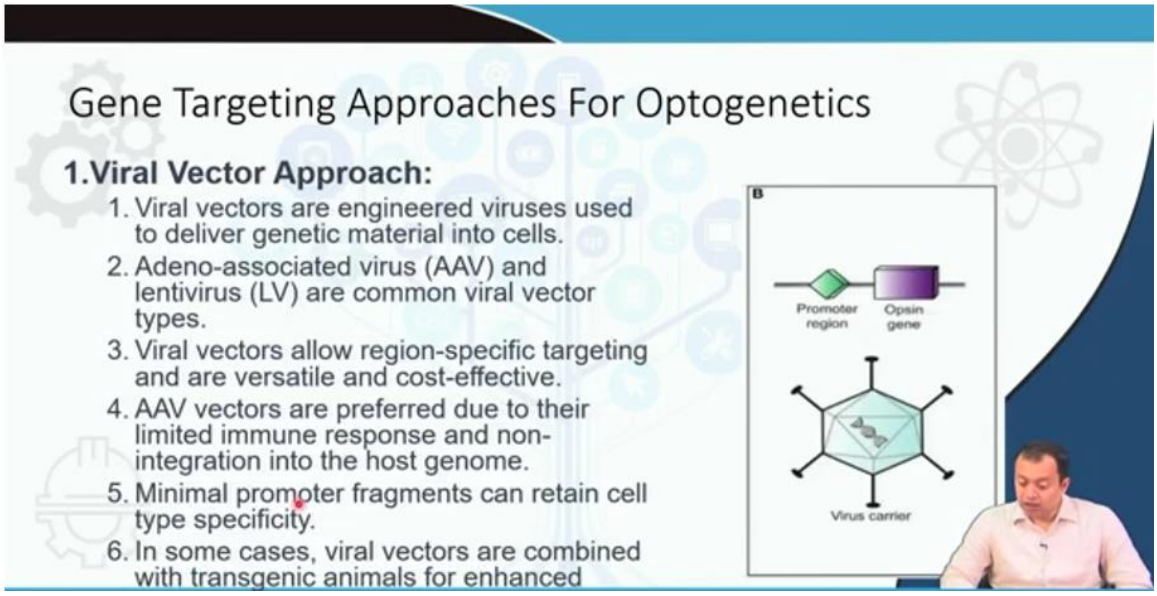
So, this is exactly what I was talking about. So, this is channel rhodopsin is a light sensitive protein that plays a pivotal role in optogenetics this applied in 2005 and this comes from this. So, ChR2 functions as a light gated cation channel allowing the passage of positively charged ions such as sodium and calcium across the cell membrane when activated by blue light. So, this is the gate you have activated it by blue light and it allows the flow of positive ions. So, if this was negative before by adding positive you are neutralizing it or if was positive before by adding far more positive ions you are hyper polarizing it you are making it much more polar much more positive this ion flow leads to change in the membrane potential of the neuron which can trigger action potentials electrical impulses of neuronal activity.

ChR2 channel rhodopsin is optimally activated by blue light with a peak absorption wavelength of around 460 nanometer. So, by switching on and off you can make this go inside or simply close it off and thereby trigger action potentials or not trigger.

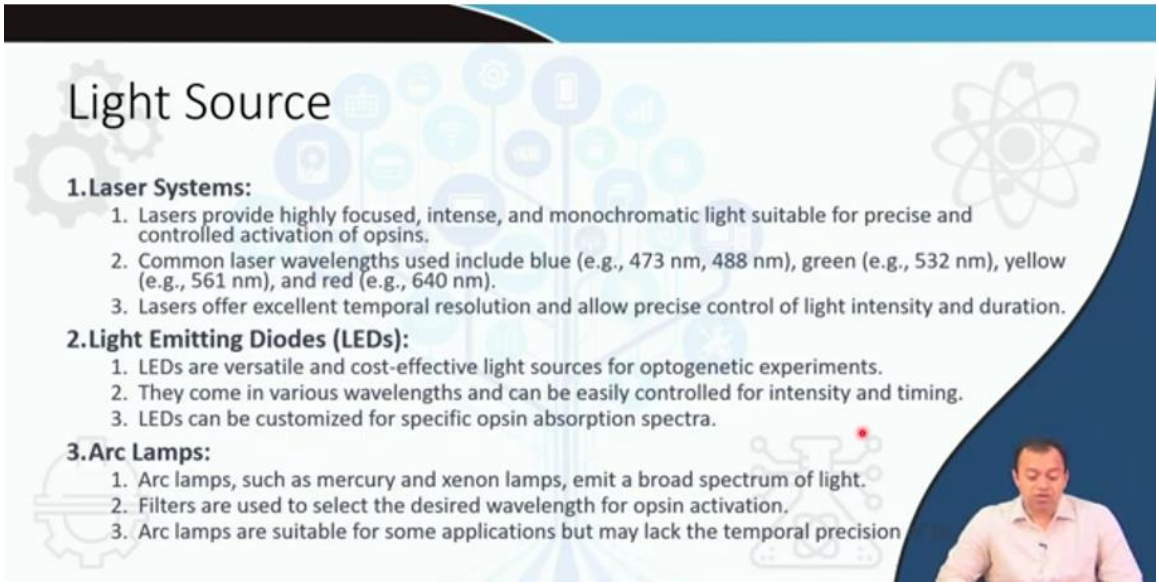


We also have gene targeting approaches this I think you have ah we have discussed we produce transgenic mice's these are the transgenic terms means a mouse usually have ah ah particular set of genome sequence ah you transfer certain genes from other ah species onto it making it transgenic which is genetically modified. You have heard of GMO ah foods genetically modified organism food brinjal there was a controversy about GMO brinjal coming up. So, transgenic mice are genetically modified mice are genetically engineered with DNA sequences that includes the opsin gene coupled with regulatory sequence.

This promoter sequence promoter is the gene that starts the start codon sequence drive the expression of the opsin gene. So, if you have an already existing gene inside the mouse which is getting affected by light upon light delivery then obviously, you have you have better control and there is no point of adding cofactors etcetera you still might need cofactors, but depending to increase or decrease the intensity intensity. Optogenetic control can be achieved once specific expression is confirmed brain wide expression occurs, but off target effects should be considered due to connected neural pathways.

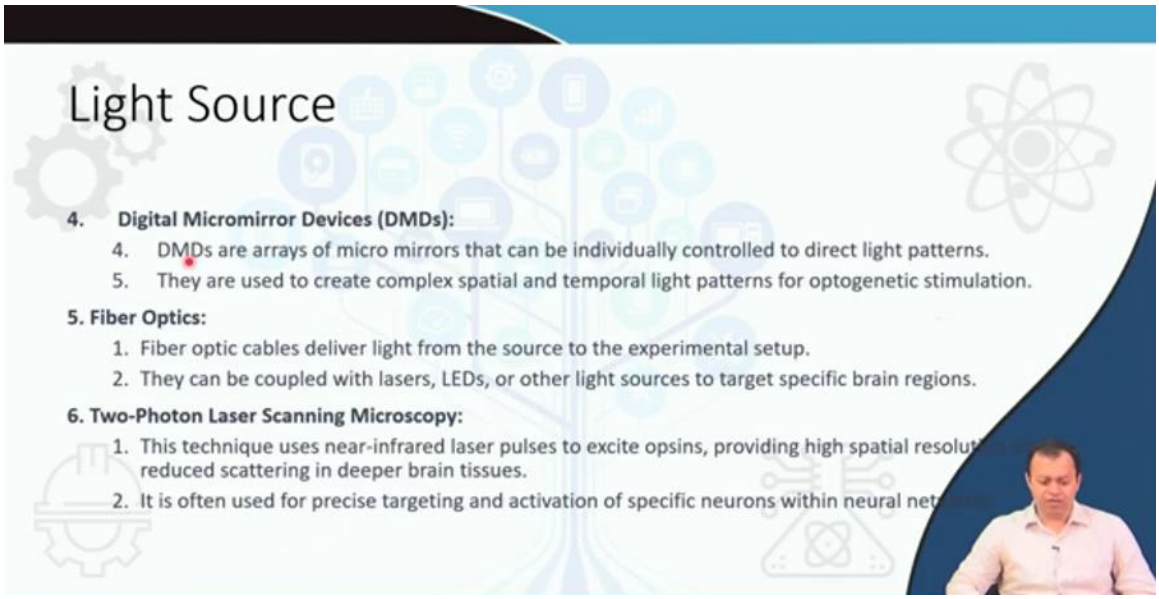


You use basically a virus to carry the particular gene which then ah attaches itself to the nucleus of a neuron into the nucleus the virus inserts its own viral DNA viral protein ah sorry viral nucleic acids DNA or RNA that nucleic acid is the nucleic acid which produces opsin you have engineered this virus as well you have engineered this virus do not you think that virus could be engineered ah think about the pandemic that we just suffered. Anyways ah viral vectors are engineered virus using to used to deliver genetic materials into cell ah several different types of virus adenovirus lentivirus are common viral types ah minimal promoter fragments are required in some cases viral vectors are combined with transgenic animal for enhanced specificity.

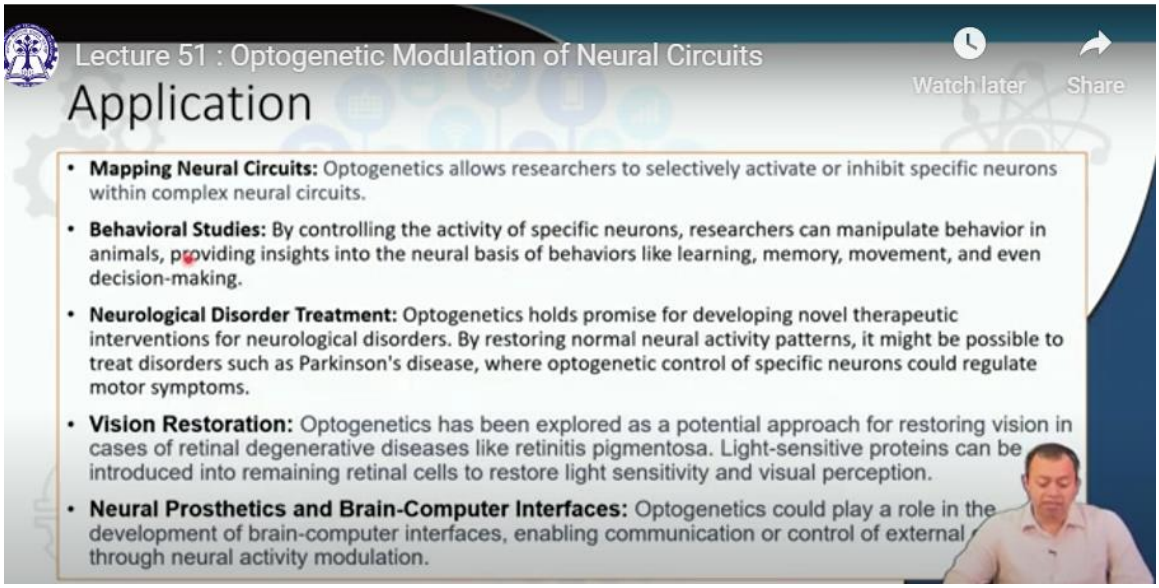


What type of laser source do you need well light source do you need obviously, there is a laser laser source that you need to trigger actually this this this firing of action potential through neurons lasers are the best one laser provide highly focused intense

monochromatic light common laser wave guides are blue basically to ah affect channel rhodopsin etcetera blue green etcetera lasers over excellent the main advantage of laser is its highly focused intense and the polarization is properly done as compared to light emitting diodes and arc lamps, but they are far more cheaper than the laser system.



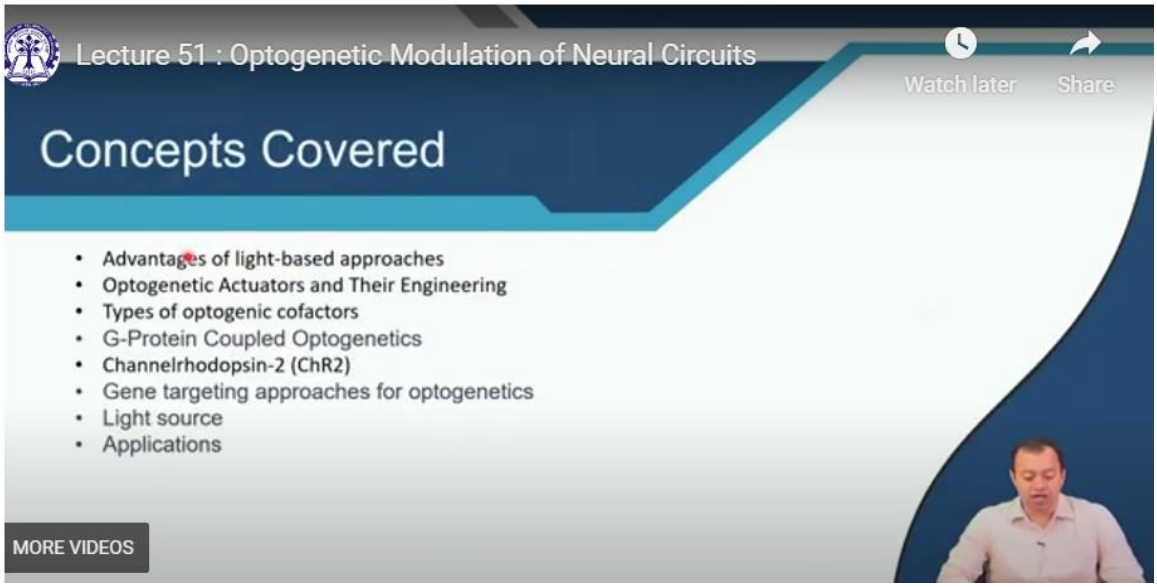
Obviously, fiber optics and two photon laser scanning microscopies has also been used we have discussed two photon laser scanning microscopies before and fiber optics as you know we can insert the fibre sensor and what are the applications we map neural circuits



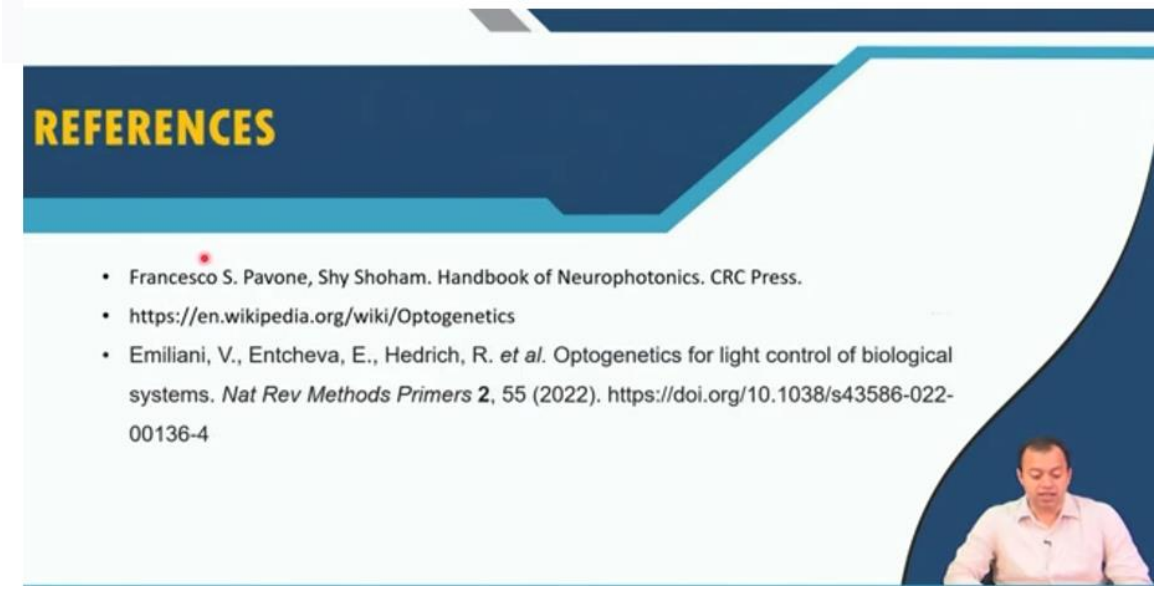
this is the main and significant ah applications the entire brain neuron by neuron could be mapped in a mammal and each neuron could be identified with its specific functions specific group of function instead of thinking that this part of the lobe of the brain is

associated with sensors and this part is associated with language and this part is associated with ah attention and concentration we now can potentially map neuron by neuron what function is working. Neurological disorder treatment is of course, there behavioral studies triggering a particular set of neurons allows a particular behaviour to be ah happening there is a very famous YouTube video in which there is ah optical fibre connected with the head of an ah mice and as soon as the light is triggered laser is on the mice is ah behaving in a predatorial manner it becoming violent and attacking anything that is nearby. So, they have put ah you know plastic caps or pieces of wood which are not food for the mice, but as soon as the light is triggered the mice is attacking it as soon as the light is switched off the mice is calm. So, you can you know try to see the behavioural response see that in ah if you if you get a chance ah search for it in YouTube video and you will see optogenetics mice just type that and you will get it I have not added it here because of course, copyright issues, but I can ask people to visit a particular YouTube video. Vision restoration neural prosthetics and brain computer interfaces all are the different applications.

So, in conclusion there are several ways in which optogenetics can help us and show immense potential to map the brain.



So, these are the concepts that I have covered today and this is the reference and I will



see you in next class. Thank you.