REVIEW

Optogenetics: The Key to Deciphering and Curing Neurological Diseases

Fuzhou Wang
Group of Neuropharmacology and Neurophysiology, Division of Neuroscience, The Bonoi Academy of Science and Education, Chapel Hill, NC 27510, USA
Correspondence to: Fuzhou Wang, Email: fred.wang@basehq.org
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Optogenetics is an emerging branch of biology that combines genetics and optics to achieve precise light control of specific cells in organisms. It is a method of studying excitable cells that uses proteins that are embedded in the cell membrane and are activated by light (i.e. "opto"). Such proteins (opsins) are found in most animals in the retina of the eyes, as well as in some plants, such as green algae. In order to integrate photoactivated proteins into neuronal membranes, it is necessary to introduce rhodopsin genes obtained from other organisms into neurons, hence the "genetics". Optogenetics is widely used in the field of modern neurobiology, and plays an essential role in the study of the mechanism of neural circuits, behaviors, central nervous system diseases, and mental disorders. Based on the development of optogenetics technology, this paper introduces its optimization and localization expression, which not only provides references for the research and development of optogenetics, but also provides the possibility for in-depth research and treatment of neurological diseases.

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OPTOGENETICS technology is an emerging biotechnology that combines optics and genetics to achieve precise control of certain events in specific cells, tissues, and loops (1). The core components of optogenetics include photosensitive elements that can respond to light stimuli and produce effects and related technologies for recording and analyzing this activity. It mainly covers (i) expressing light control elements in specific cells or tissues; (ii) delivering light as a stimulus source to target cells or tissues; (iii) recording the output and analyzing it (2). As early as 1971, studies have found that there are photosensitive proteins in bacteria that can be photosensitive, but they were not used to develop light control elements at that time (3). In 2005, the microbial rhodopsin protein was proved to be successfully expressed in mammalian specific neurons and conferred photosensitivity on them, which caused activation effects (4). In the following decade, with the demand for single-component light control components in the field of neurobiology, optogenetic technology developed rapidly and was widely used in neurobiology research. Many diseases, such as Alzheimer's disease, Parkinson's disease, depression, epilepsy, autism and other neuropsychiatric diseases has been becoming the foci of optogenetics research and potential therapeutic strategies (5, 6).

The application of optogenetics technology needs to follow the pathway below: find a suitable light-sensitive protein → genetic information transmission (transfection of light-sensitive protein genetic information to target cells through methods such as transfection, viral transduction, and establishment of trans-

genic animal lines) → controllable demonstration (control the specificity of the demonstration light in time and space to achieve precise regulation of cell activities) → read the research results (detect the voltage inside and outside the cell membrane) → evaluate the impact of cell activities on the entire animal.

The Brain and Neuronal Study Choices

Consciousness, personality, and intelligence all are created by neurons. This means that if we want to study these aspects of the human being, we must definitely understand what is happening with the neurons. The main problem is that there are too many neurons and it is impossible to keep track of them all at once. In addition, neurons tend to form clusters, so it is problematic to separate the action of one neuron from another, and to act on each cell separately in most cases. So, two ways exist to study them: to take the average activity of groups of neurons, or to investigate one single neuron if possible. The first is often used in studies of the mammalian brain; the latter is generally on simple nervous systems, such as the sea *gastropod alysia* (7).

Many methods exist for studying the nervous system, whereas they are almost always not that accurate. For example, functional magnetic resonance imaging does not allow individual cells to be seen and recognizes only relatively slow processes, i.e. changes in the blood filling of cerebral vessels (8). Electroencephalography is faster, but it also does not distinguish between individual neurons (9). Finally, fluorescent dyes can be used that change color in response to changes in the concentration of certain ions in the cell or to their total charge expressing as the membrane potential of the neuron, but these dyes act rather slowly. Their time on/off the resolution (response time) is not high enough to detect a separate action potential in a neuron (10). More precisely, this was considered until the recent publication, the authors of which were still able to do this (11).

It was extremely difficult to observe the activity of a single neuron without affecting neighboring cells. It was even more difficult to change this activity particularly. We can inject target pharmacological drugs into the brain that act only on cells with certain properties, then look at these cells under a microscope or cut a part of the brain and record its electrical activity with microelectrodes (12). However, such experiments are extremely time-consuming. In order to find out how the signals of a small number of cells have changed, we need to grow a whole brain, feed it and take care of it, and after preparing the drug it can be used for an hour and a half, mostly longer.

Another option is to stimulate individual neurons with artificial electrical signals, driving these signals along the corresponding nerve, or watering cells with neurotransmitters in the framework of an artificial synapse model (13). But for this, you first need to find suitable cells, and this is not a trivial task.

Finally, there is a method of artificial release of glutamate from synaptic vesicles under the influence of ultraviolet radiation (this method is also called glutamate uncaging). In fact, the light in this case mimics the action of an excitatory signal that arrives at the cell, triggering the release of a neurotransmitter at the synapse. This is a very accurate and effective tool, but it also has a drawback. By the action of ultraviolet radiation, they are now able to release only glutamate, but not all neurons secrete it, and not some other signal carriers. In addition, the artificial release of glutamate does not activate the target neuron as strongly as electrical stimulation along the nerve, and it is difficult to get the neurons to emit action potentials in this case (14).

From Algae to Neuron: A Big Step Forward of Optogenetics

Research in the field of neuroscience has made considerable progress, but it seems that it is still far away from the precise and accurate regulation of physiological and electrical activities at the level of individual neuron. However, all this became possible with the discovery of a light-sensitive protein in 2005 (4). Although such light-sensitive proteins were discovered as early as the 1970s (3), they really attracted attention and were applied to the field of neuroscience until the 2000s.

Oesterhelt and Stokenius (3) proved in 1971 that bacterial rhodopsin protein can be activated by visible light and act as a channel protein to transport ions across the membrane. Subsequently, in 1977, Matsuno-Yagi and Mukohata (15) discovered more members of this family. In 2002, Hegeman et al. further discovered channel rhodopsin (channelhodopsin, ChR) from the unicellular alga Chlamydomonas reinhardtii (16). Like the eye pigment, chanelrodopsin reacts to light irradiation, only in a slightly different way: it enhances the influx of positive ions into the algae cell. This affects its membrane resting potential: within thousandths of a second, it approaches zero from a deliberately negative one to realize depolarization. In this case, the *Chlamydomonas* cell does not generate action potentials, but this would be theoretically possible: electrical signals similar to action potentials that also arise in plant cells (17). However, because the photoactivation of microbial rhodopsin protein requires the participation of a chemical cofactor (cis-retinal), researchers believe that using microbial rhodopsin protein for optical control in mammalian neurons is not a feasible strategy (18).

In 2002, Zemelman et al. used Drosophila photoreceptor-related genes, including rhodopsin (rhodopsin), α-arrestin-2 and G protein α subunits to develop multi-component photoactivation (19). It was named "chARGe", and with the help of gene expression technology, for the first time realized the use of light to activate a small group of specific neurons in a group of mixed neurons (20). Similarly, Banghart et al. realized the use of light to accurately and reversibly control neuronal activity in rats by coupling light-sensitive chemical molecules to potassium ion channels (21). However, technical limitations such as multi-component protein expression, chemical modification of proteins, and tissue penetration of chemical small molecules have hindered the further application of multi-component optogenetics.

Until 2005, Boyden et al. successfully expressed microbial rhodopsin protein in mammalian neurons for the first time, and successfully achieved the light activation of neurons without the need for other cofactors or components (4). Subsequent studies confirmed that mammalian cells naturally contain *cis*-retinal, a cofactor necessary to activate bacterial rhodopsin protein (22). By 2010, a variety of microbial rhodopsin proteins, including channel rhodopsin (channelrhodopsin, ChR), bacterial rhodopsin protein (bacterial rhodopsin), and halorhodopsin (halorhodopsin) have all been proved to be able to affect mammalian nerves

through the optical activation or inhibition of the element. This realizes the optogenetic control of neurons in intact mammalian brain tissues and even free-moving mammals (22, 23).

With the deepening of research, the discovery of new light-sensitive ion channels, such as Novel cation channel rhodopsins from Guillardia theta, namely, Gt_CCR1-4 (24), provides the possibility for more in-depth research and application of optogenetics. As thus, the door to single-component optogenetics was opened totally.

How the Light Activate Neurons?

The basic idea to check the presence of a gene in a cell is viral transfection (25). Ideally, the gene for chanelrodopsin-2 is attached to a promoter, inserted into the virus, and the virus itself is injected with a fine needle into the mouse brain, through which we just need to check where exactly the viral particles located or whether we missed the injection site. To understand this process, in addition to the gene for the light-sensitive protein, it is necessary to introduce into the neuron the gene of the reporter substance, of which will indicate the presence of chanelrodopsin. A convenient reporter is a fluorescent protein, because fluorescence in cells is visible both on brain slices and, if sufficiently concentrated, even outside the body. The ChR2 gene and the reporter gene (e.g., yellow fluorescent protein, YFP) are under the same promoter, so they are expressed together, and both proteins are produced in the cell at the same time (26). Therefore, if there is chanelrodopsin in the neuron, it fluoresces.

So far, it seems that all conditions are in place, all that remains is to build a light source into the brain, which will activate the neurons carrying chanelrodopsin. This source is typically a miniature fiber-optic LED that produces light at a wavelength of about 480 nm (blue). The fiber is inserted into the desired area of the brain and fixed with a special cannula on the surface of the skull (**Figure 1**) (See the detailed description of light transmission below). An animal can wear such a device for a long time to record the real-time activity of the target neurons. This also determines that the animals undergoing optogenetic experiment can move freely, and their behaviors are closer to natural.

However, it is not enough to conduct a full-fledged experiment if we only have the light source alone because we cannot tell how the "experimental" neurons reacted to photostimulation. To realize this, the electrical signals in response to exposure to light need to be recorded simultaneously. Therefore, in parallel with the light instrument, a recording microelectrode is needed to be implanted into the brain together with the light source for activating the neuron (**Figure 1**). Practically, Deisserot and colleagues exactly used these recording microelectrodes and confirmed with the help of chanelrodopsin that the membrane potential of the cell could be altered strongly and precisely by light and until to the generation of action potentials (4).

How the Light Is Transmitted?

Light transmission technologies mainly include light sources and transmission technologies that deliver light sources to tissues. Laser (diodeordiode-pumped solidstate, DPSS) and LED (light-emitting diodes) are two commonly used light sources (27). The main advantage of laser is that the spectral range is very narrow (< 1 nm), which reduces cross-interference in polychromatic optogenetics and imaging. Another advantage is low divergence, which greatly improves the efficiency of coupling optical fiber for transmission. But the main disadvantages are the high price and the limited stimulation frequency in some bands (28). The LED light source does not require complex electronic control components, is low in cost, has great potential for the development of wireless transmission, and can easily achieve high-frequency stimulation. But the main disadvantage is a wider wavelength range and greater divergence, the latter restricts high-intensity light transmission (27).

Currently, the most commonly used method of transmission technology is optical fiber transmission. The diameter of the optical fiber used in optical fiber transmission is usually tens to hundreds of microns. By implanting it in the target brain area and coupling it with a light source, light input in a freely moving animal can be realized. When the input light intensity (depending on the type of opsin used and its expression) exceeds the threshold required for opsin activation, the opsin can be activated to cause neuron activation or inhibition, triggering the light required for the ChR2 action potential The strength is generally 5 mW/mm2 (29). When the light intensity required to implant the end of the optical fiber is known, the light intensity around the end of the optical fiber will gradually decrease as the distance increases due to the influence of scattering and absorption by the surrounding brain tissue, so the optical fiber implantation technology can only activate a certain amount The opsin in the area, and the light intensity of the surrounding area cannot be uniform. Although the stronger the light intensity, the greater the effective activation range, but the tissue heating caused by light can cause damage to neurons (30, 31). Therefore, in addition to appropriate light intensity and control experiments, reducing light intensity attenuation and tissue thermal damage is the future development direction of optical fiber transmission.

Optical fiber technology has been widely used in the experiments of deep brain stimulation in free-moving animals, but the implantation of optical fiber will still inevitably cause a certain degree of brain damage and local hemorrhage, especially when using large diameter optical fiber for high strength when the light is input (32). Therefore, for light stimulation of more brain areas and multi-point stimulation of special spatial locations, researchers have developed miniaturized micro-optical fibers and tapered fibers (33), thereby reducing mechanical damage to tissues. At the same time, fiber bundles containing hundreds or thousands of micro-fibers can be wrapped in an insulating sleeve and implanted in the target brain area, so as to achieve simultaneous stimulation of a larger brain area or sequential stimulation of different brain areas (34). Connecting a silicon chip to one end of the implanted optical fiber and coupling it to a light source array is another experimental scheme for multi-point stimulation of the brain (35). In short, these diversified optical transmission strategies make optical input in multiple brain regions and multiple spatial locations more convenient and effective.

Switch Proteins in Optogenetics

After the microbial rhodopsin protein was proven to be used to activate neurons, the researchers further discovered a light-sensitive protein with different characteristics naturally

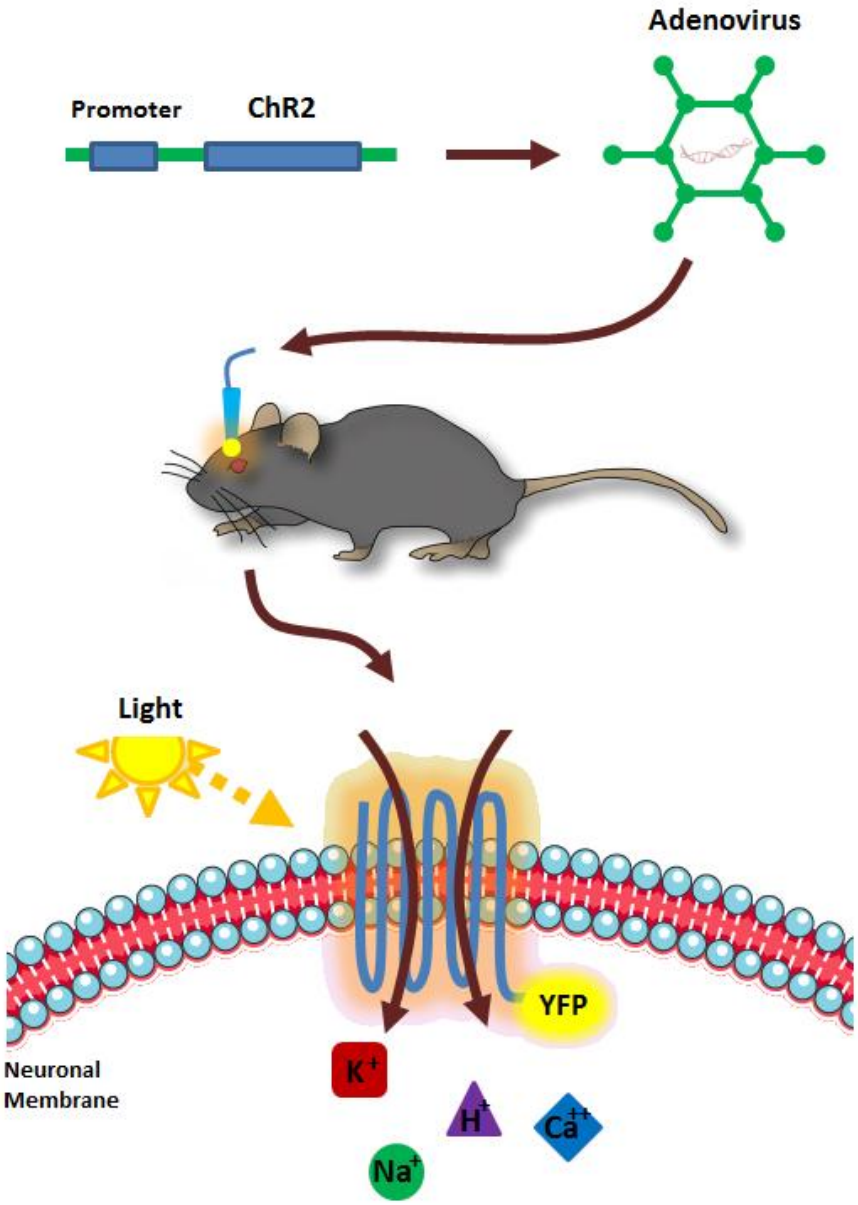

Figure 1. Schematic for the Introduction of a Photo-Activated Channel into Neurons.
The adenovirus carrying ChR2 is introduced into a specific location in the mouse brain, and stimulated by light of different wavelengths to activate the introduced and carrying ChR2 neurons, thereby producing specific neurological functional changes.

occurring in the organism, named opsin. Among them, the type I opsin that exists in microorganisms is mostly ion channels and ion pumps, and most of the type II in vertebrates is G protein-coupled receptors (36, 37).

Beside channelrodopsin, there are other photoactivated channels in the service of optogenetics. Halorhodopsin, an archaeal protein that, when activated by yellow light, lets in negatively charged chlorine ions (**Figure 2**). This will lead to hyperpolarization of the cell membrane, i.e., the potential difference on it becomes more negative than at rest, and the cell becomes silent when halorhodopsin is activated (38).

The activation of both bacteriorhodopsin (BR) and protorhodopsin (PR), like channelrodopsin, will open the positively charged protons producing an opposite effect with channelrodopsin through pouring protons out the cell causing membrane hyperpolarization (39) (**Figure 3**).

Some exotic animal opsins were named as Opto-XR functioning as hybrids of rhodopsin of the retinal cones of mammals

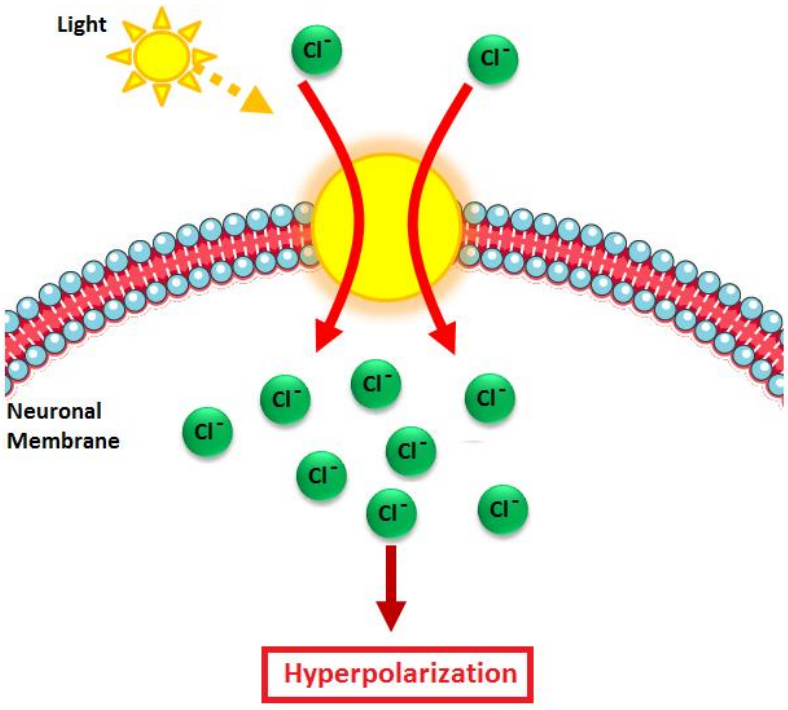

Figure 2. Schematic of Halorhodopsin Operation.
While this channel is active, it is more difficult for the cell to generate action potentials.

and receptors coupled with G-proteins (40, 41) (**Figure 3**). In particular, the Opto-XR series carry fragments of adrenergic receptors, and one unusual variety consists of a portion of rat rhodopsin and a portion of a serotonin type 1A receptor (18, 42). Of course, without being channels, Opto-XR cannot provide an action potential, but their activation mimics the activation of receptors. In other words, after exposure to Rh-CT (5-HT1A), the cell will function as if serotonin arrived at its synapses. It has been shown that such hybrid proteins are located on neuronal membranes approximately in the same place, where are the real receptors for similar neurotransmitters (43). Therefore, they can be used to study various signal transmission systems in the brain without fear of obtaining results that are far from reality.

Optogenetics and Behavior

Behavior depends on the sequences of neural signals that appear at the right time in the right place. It turns out that, knowing the time, place and sequence of these signals, we can recreate the desired form of behavior and obtain the missing information about the structures through which this behavior manifests.

Drosophila, like people, is affected by a long stay in close clusters of their own kind. It is known that carbon dioxide (CO_2) has a very unpleasant odor for fruit flies. If a fly smells CO_2, it will fly to where the air is fresh. If this insect was in a flock, others quickly retreat behind it. Even if, among a hundred flies, only half of the flies smell, and the rest is blocked by the sense of smell, the entire hundred will move away from the source of the stench. This synchronicity is not achieved through the sense of smell. The avoidance response in this case is: some flies send signals to others about the need to move away from the source of CO_2. Insects transmit the call to escape by touching each other. For flies, they receive signal through the mechanosensory neurons located at the tips of their paws. To prove this, optogenetics was used. Flies in an atmosphere with a normal content of CO_2, optogenetically activated the mechanosensory neurons of the tips of the paws with built-in ChR2 cells, and the insects showed an avoidance response, as if there was a strong smell of carbon dioxide next to them (44).

With the help of photoactivation, information can be introduced into neurons that they did not receive in reality. That is, optogenetics allows creating false memories. The researchers inserted channelrodopsin-2 into neurons in two regions of the hippocampus responsible for translating information from short-term memory into long-term memory: the dentate gyrus and the CA1. Initially the animal remembered that it was worth being afraid of sound in one setting, but not necessary in another. After that, the neurons with channeledopsin were specially activated when the animal was in other "scenery" and, in theory, should not have shown fear. However, during this procedure, the mouse showed the same fear of sound as experienced in prior setting (45).

Since it turned out to be implemented in the head that the animal never remembered, then it is definitely possible to return the lost information. First, the rodents were taught a certain skill,

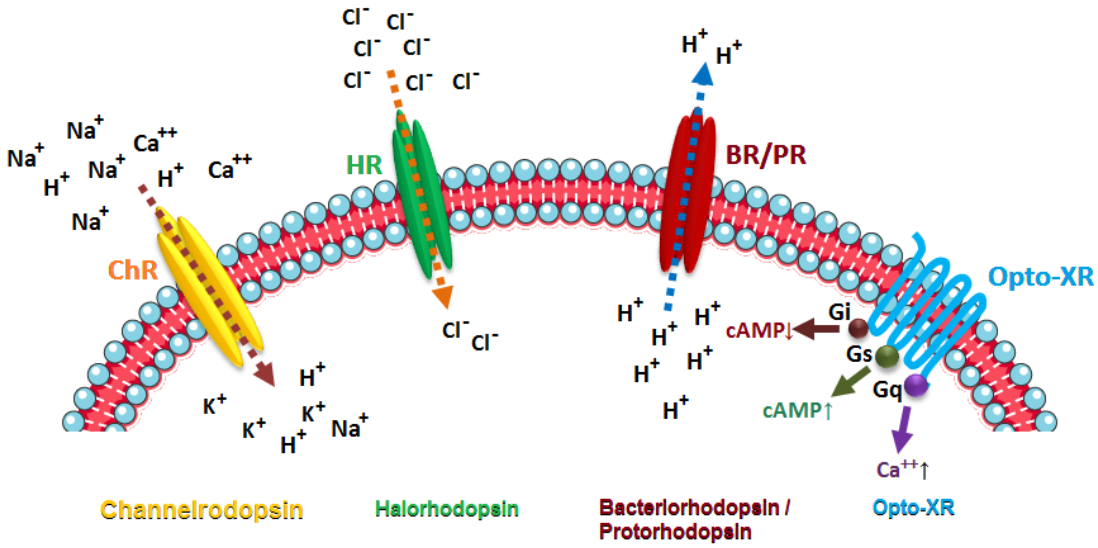

Figure 3. Photoactivated Channels in Optogenetics.

and then protein synthesis inhibitors that interfere with memory recently formed into the brain. The interesting thing, however, was the forgotten memory could be brought back by optogenetically activating certain neurons in the hippocampus (46). Therefore, optogenetic technology is a crucial step to realize the external control of expected behavior and recovery of the missing memory.

Researchers have found the area in the brain that controls hunting behavior and successfully found a way to control its switch (47). There are two groups of neurons in the brain that control the hunting behavior of mice: one group coordinates to chase the prey, and the other group controls the muscles of the neck and jaw. Both groups of neurons are located in the amygdala that controls the body's actions, feelings, and fear. These neurons are activated by laser to control the switching of these pathways at any time. When the stimulating laser was turned off, the mice walked around the cage normally; but once the laser was turned on, the mice frantically attacked everything on their way: live crickets, fake insects, even branches or bottle caps. They will pounce on the prey, grab the prey with their paws and bite repeatedly. Subsequently, the researchers tried to inhibit the function of each group of nerves separately. When they inhibited the neurons responsible for hunting prey, the mice chased more slowly, but they still bite; conversely, if the nerves responsible for biting were inhibited, the rats would hunt down the prey, but don't bite.

Therefore, optogenetics technology has extremely pivotal significance in behavior control, whether it is positive or negative.

Optogenetics and Sleep

Optogenetics has played a great role in the study of sleep mechanism and its function regulation. It was known that in the lateral regions of the hypothalamus there are neurons that secrete the substance orexin, aka hypocretin. Reduced level of hypocretin in the hypothalamus is associated with unstable wakefulness (48, 49). If the neurons that release hypocretin are not active enough, the subject will develop narcolepsy. The patient either completely falls asleep, or shows some signs of sleep like a sharp drop in muscle tone or loss of consciousness. Narcoleptic seizures are uncontrollable and can happen at the most inopportune moment - say, when a person is driving.

But this could be a coincidence, and without additional experiments it could not be argued that it is orexin neurons that support wakefulness. It was necessary to check what happens to the sleep during their activation. Researchers inserted channelelrodopsin-2 into the hypocretin neurons of the hypothalamus of mice, and then periodically stimulated the rodents' brains with light while they slept (28). Mice were awakened from this effect both at the stage of slow-wave sleep and at the stage of fast-wave sleep. At the same time, orexin neurons were activated with different strengths depending on how often they were shone. All this taken together gives the right to assert that the release of orexin (hypocretin) in sufficient quantities really ensures the maintenance of wakefulness.

Optogenetics and Psychiatry

It can be said that the application of the method of optogenetics in psychiatry is one of the main final goals. It would be great to first learn how neurons in the brain are connected to each other, and then stimulate or suppress the transmission of signals between the desired groups of neurons. The task is extremely laborious, but theoretically completely feasible. Some of them involve dopamine-secreting cells. This neurotransmitter plays a huge role in maintaining a good mood and gives a sense of reward for what you have done. Drugs such as cocaine mimic the

effects of dopamine on neurons, causing a feeling of satisfaction. The body remembers this sensation and strives to get the drug, psychological and physiological dependence is formed.

Optogenetics technology allows researchers to study the function of neural circuit components more thoroughly, and plays an essential role in identifying new neural pathways that are dysregulated in neuropsychiatric diseases such as depression. Due to the heterogeneous populations of various neurons such as the bed nucleus of the stria terminalis (BNST) and their large projection areas, the study of how neural circuits change after stimulation is limited. Using optogenetic tools, the different neural circuits that contribute to and disrupt these diseases in the body can be selectively isolated.

The nucleus accumbens is part of the brain's reward system. If its dopamine-secreting cells are supplied with halorhodopsin and then stimulated with light, the nucleus accumbens is "turned off," and at the same time, the previously developed cravings for cocaine temporarily disappear (50). It would be possible to do similar manipulations on drug addicts, but for this it will be necessary to change the genome of their neurons, which is not yet allowed to do.

Adequate dopamine protects against some forms of depression as well as Parkinson's disease. And if the contribution of optogenetics to the study of parkinsonism is not yet very large, then the depressive behavior of rodents has been successfully tried to eliminate using photoactivated channels (51).

Researchers already know that there are many types of neurons related to depression. At present, it has been clarified through optogenetic technology: dopaminergic neurons in the ventral tegmentalarea (VTA) of the brain are related to depressive behavior (52). At the same time, studies have shown that VTA non-dopaminergic neurons such as endoglutamatergic neurons and gamma-aminobutyric neurons may also have a regulatory effect on depressive behavior (53, 54).

Using optogenetic methods to discover the neurons related to depression in the nucleus accumbens (NAe): D1 hype medium spiny neurons (D1-MSNs), D2 medium spiny neurons (D2) hype medium spiny neurons, D2-MSNs) and cholinergic interneurons (55, 56).

As we all know, serotonergic neurons in the dorsal raphe nucleus (DRN) are closely related to depressive behavior (57). The recent application of genetic technology has found that γ-cyanobutyric acid neurons in DRN are also related to the production of depressive behavior (58).

Research has found that the neural pathways related to depression mainly include the following:

- VTA-VAc dopamine nerve pathway, activation of this pathway leads to social avoidance behavior and weakened sugar water preference behavior in mice (59, 60).
- VTA-Medial prefrontal cortex (mPFC) dopamine neural pathway: This pathway excites makes mice have antidepressant effects and inhibits social escape (61).
- mPFC-DRN neural pathway: Optogenetics technology shows that when this pathway is excited, mice are depressed, and when this pathway is inhibited, mice are antidepressant (62, 63).
- The BNST-VTA neural pathway has an impact on reward behavior and exploratory behavior. BNST is related to the treatment of stress and pathological anxiety (64). In addition, BNST has been used as a key structure for drugs to regulate stress induction (65). Light stimulation of the BNST area produces anxiety-like behaviors. In a series of experiments, it was found that light stimulation of BNST-VTA's glutamate leads to anorexia and anxiety-like behaviors, while light stimulation of BNST-VTA terminals promotes reward-related behaviors and buffers anxiety caused by stress (66, 67). These two findings indicate that the effects of BNST on stress, anxiety, and rewards are more complex than previous studies.

Optogenetics and Memory

Optogenetics helps to solve many questions concerning the functioning of the brain. For example, high-precision targeting activation or deactivation of brain areas made it possible to map areas responsible for long-term and short-term memory. In addition, it became possible to approach the study of memory from a new angle.

Our perception of our surroundings is represented in the brain by a combination of active and inactive neurons. Remembering is the reproduction of that combination of excited neurons that once arose. A study using light evoked memories in mice and give them new meaning (68). This study is based on the classical approach to the study of memory using the mouse as a model object. The focus is on the fear response to an electrical shock that occurs in the room where the animal once experienced it. Let's imagine a mouse in room A, here it behaves normally. Move the rodent to room B with a different environment and subject it to a mild electrical shock. Now, every time it is brought to room B, the mouse showed up fear even without shock. Assessing fear in this case is quite simple: usually a very mobile rodent groups up and freezes. The memory of room B is associated with pain in the animal. Neuroscientists have succeeded in making light-sensitive only those neurons that were activated while the mouse was in room A. The combination of light-sensitive neurons in this case is a recorded memory of this room. Further, the experimenters evoked this memory with the help of light during an electric shock in room B. Did the mouse after that become afraid of a shock in room A, where have you never experienced it before? The correct answer is yes. The beauty of this work is based on earlier studies of the brain, which identified a region associated with memory. This is the hippocampus. It was his neurons that were manipulated in the mentioned experiment.

Another study had used optogenetic methods to restore the memory of Alzheimer's disease mice (69). First, by optogenetic modification of mice, they emit yellow fluorescence when they store memories and red fluorescence when they regain memories. After that, the author gave the genetically modified wild-type mice and Alzheimer's mice lemon scent stimulation, and then applied electrical stimulation to link the two memories. A week later, the author again gave these mice lemon scent stimulation. The results showed that the wild-type mice were able to show both yellow and red fluorescence at the same time,

and also appeared fearful performance, which shows that while forming a memory, the memory is also recalled. However, the light-emitting areas of the brains of Alzheimer's mice are significantly different, indicating that their brains are disordered in the process of regaining memories. After that, the researchers used a beam of blue light to stimulate the brains of the mice, thereby reactivating the mice's memory of the lemon smell and electrical stimulation, so that the mice shivered when they smelled the smell again.

A recent study introduced the *CHR2* gene of Adeno-associated virus carrying the CaMK promoter into bilateral dentate gyri, followed by repeated intrahippocampal injections of soluble low-molecular-weight amyloid-β1-42 peptide and was stimulated with a 473 nm laser. Optogenetic stimulation improved working and short-term memory in mice with Alzheimer's disease through increased expression of NR1, glutamate receptor 2 and mGluR-5 in the hippocampus, and decreased expression of glial fibrillary acidic protein and interleukin-6 indicating that optogenetics can be used to regulate the neuronal-glial network to ameliorate memory functions in mice with Alzheimer's disease (70). Another study revealed how serotonin affects the pathogenesis of Alzheimer's disease in a comprehensive perspective and suggested that the optogenetics manipulation of serotonin nuclei retrieve the lost memory by closing the inward-rectifier potassium channel Kir2 on the memory engram cells (71).

Of course, the use of optogenetics can achieve the deletion of specific memories. A study used light to successfully eliminate special memories in the mouse brain, revealing how different parts of the brain work together to restore episodic memory. In the application of optogenetics to research on memory recovery, scientists hypothesized that recovering episodic memory involves coordinated activities between the cerebral cortex and the hippocampus. The theory is to study the pattern of reactivation of brain activity involving the cerebral cortex and hippocampus in the process of episodic memory recovery, so that individuals can experience those events again. The researchers used genetically modified mice to conduct research. When the mouse nerve cells are activated, they can all fluoresce and express special proteins to promote the nerve cells to be turned off by light. Place the mouse in a cage for training. In the cage, the mouse will experience electric shock. Normally, the mouse in the new environment will use the sense of smell to adapt to the environment, and they will be in a fear response (72).

Therefore, optogenetic technology is not only a tool for the study of memory mechanisms, but also a potential targeted treatment method for discovering and improving memory impairment.

Optogenetics and Retina

The rhodopsins of microbes were recognized as a successful tool for creating light-sensitive cells. But can microbial rhodopsins be used to treat blindness? There is a hereditary disease - retinitis pigmentosa that is associated with degeneration of cells in the retina. It causes progressive loss of vision. No effective treatment for retinitis pigmentosa exists today (73). A significant proportion of cases of this disease are associated with abnormalities in the gene encoding rhodopsin. This gene is important for the functioning of two types of receptor cells in the retina. These are rods that have good light sensitivity, but are not capable of providing color vision, and cones that are allowed to distinguish color, but are less sensitive to light intensity. In patients with retinitis pigmentosa, the rod cells die rather quickly, but the cones, having lost the ability to perceive light, they live for a long time. Is it possible to replace non-working rhodopsin in the remaining cells with a light-sensitive protein from bacteria? A group of Swiss researchers led by Botond Rosca used channel rhodopsin from archaebacteria to answer this question.

In a murine model of retinitis pigmentosa, if microbial rhodopsin is introduced into the retinal cones with the help of viruses, partial restoration of vision is observed in rodents. In standard tests, these mice showed improved spatial orientation compared to diseased rodents without therapy. Interestingly, the microbial protein used in the study was called eNpHR 3.0, an improved halorhodopsin from Natronomonas pharaonis version 3.0. Microbial rhodopsins are constantly being improved by genetic engineering methods to improve their work in mammalian cells (74).

Transferring this method of treatment to humans is not an easy step because it uses viruses. Many additional control experiments are required to show that this technology is completely harmless to humans. Nevertheless, the authors demonstrated the efficiency of the eNpHR 3.0 protein in isolated cells of the human retina. Such work is a serious advance in the treatment of diseases associated with the loss of neuronal activity. For example, if cells damaged by a neurodegenerative disease are made light-sensitive, they can be restarted by irradiating them with light.

Optogenetics technology brings unprecedented hope for retinal-related diseases. With the continuous upgrading and improvement of this technology, vision restoration due to various reasons will become possible.

Concluding Remarks

Optogenetics is a rapidly developing bioengineering technology that integrates optics, software control, gene manipulation technology, electrophysiology and other multidisciplinary technologies. The main principle is to use gene manipulation technology to transfer light-sensitive genes (such as ChR2, eBR, NaHR3.0, Arch or OptoXR, etc.) into specific types of cells in the nervous system for the expression of special ion channels or GPCRs. The light-sensitive ion channel will selectively activate or inhibit the passage of cations or anions under different wavelengths of light stimulation, thereby causing changes in the membrane potential on both sides of the cell membrane to achieve the purpose of selectively exciting or inhibiting cells.

Optogenetic technology has two unique characteristics: high temporal and spatial resolution and cell type specificity. It overcomes many shortcomings of traditional methods of controlling the activities of cells or organisms, and can perform non-invasive precise positioning and stimulating operations on neurons, thus completely changing the research situation in the field of neuroscience and providing unprecedented revolution for the development of neuroscience means. It provides the possibility of new treatments for possible central nervous system diseases in the future.

The application field of optogenetics technology covers many classical experimental animal species, such as: fruit flies, nematodes, mice, rats, marmosets and cynomolgus monkeys. It involves many aspects of neuroscience research, including basic research on neural circuits, learning and memory research, addiction research, movement disorders, sleep disorders, Parkinson's disease models, depression and anxiety disorders, etc. So optogenetics will be an effective key to deciphering the mechanism of neurological diseases and discovering its therapeutic strategies.

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