Review Article

Optogenetics, Tools and Applications in Neurobiology

Abstract

Comprehension of the brain function can be helpful for therapy of neurodegenerative diseases. The brain consists of various types of neuron sets, which organize in three-dimensional complex networks and form neural circuits underlying different behaviors. The circuits act based on the patterns that encode the brain functions. Recognition of the neural patterns requires methods to manipulate the neurons. Electrical stimulation may be the most common method. However, it has significant drawbacks including failure to identify specific neurons in experiments. As an alternative, optical stimulation is a new method that acts in combination with genetic approaches. The novel, optogenetic technology makes it feasible to manipulate either the specific cell types or the neural circuits. This is associated with minimum tissue damages as well as side effects. In this study, a new technology has been introduced, and then its optical and genetical tools have been investigated.

Keywords: *Cell-type specificity, neural circuit, neural probes, opsin proteins, optical manipulation, optogenetics, patterned stimulation*

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Introduction

A compactness disbalance in the chemical substances of the peripheral and/or the central neural system results in psychiatric and neurological disorders. Scientists use appropriate pharmaceuticals together with new, feedback control methods to maintain a balanced concentration.[1] Therefore, many mental disorders can be cured by this idea. Stroke, epilepsy, and Parkinson's disease are the common neurological diseases that are treated by medication. For instance, stroke is one of the foremost reasons for death, but it yet has very limited treatment options available, so that most survivors have long-lasting defects.[2-4]

On one hand, the progress in technology of the brain interface tools has created a modern research field for psychic disease remedy. Interventional psychiatry is a novel approach that manipulates the neuronal interconnections electrically. Deep-brain stimulation (DBS) is an example for this method, which has successfully treated some neurological illnesses. However, it has significant disadvantages. For example, the electrodes implanted in the patients stimulate nontarget cells in their range in addition to the specific cell types. This may lead to additional sensory problems and motor control defects.[5,6]

On the other hand, electrical signaling, which happens among the different and multiple neurons in the brain, determines the brain functions. The generated electrical signals are in the order of milliseconds. These signals are spatiotemporally encoded, because the neurons have been arranged in intricate three-dimensional (3D) circuits in the brain.[7-10] Over time, the electrical signals form activity patterns of the brain. Actually, these patterns encode our thoughts, skills, feelings, and memories, and thereby control the brain function and then its resulting behavior. However, how this process is done is unknown.[11-14] Simulation of the real, neuronal activity patterns helps scientists to control the brain nervous system and, therefore, plays an important role in understanding the brain activities and treatment of its diseases.

Recently, optical stimulation methods exhibited significant capabilities in recognizing the nervous system function. Therefore, the brain stimulation patterns can be generated by optical stimulation techniques based on the light modulation

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methods.[15,16] For this purpose, some new molecules have been identified that target the specific cells using genetic tools and make it possible to investigate the effect of the specific subsets of the cells in complex activities of the brain.[17-22] The result is the well-known optogenetic method that its development depends on either molecular genetics or optics. Optogenetics is a neuromodulation approach that controls the neural activity using light.

For the study of neuroscience, optical techniques are more useful tools than the pharmaceutical and the electrical ones because of their higher speed and accuracy and less damage to tissue. Optogenetics is a very powerful neuroscience method. This method functions on the basis of the bioengineered light-sensitive proteins. It can optionally stimulate or silence particular cell types and neuronal circuits with millisecond temporal accuracy. This method allows more temporal resolution for analyzing a specific neural circuit operation in different diseases.[23-30]

Light-Sensitive Proteins: A Toolbox for Optogenetics

The brain's function is determined by the neuronal accordant activity. The neurons are distributed in the form of 3D networks in the brain. These circuits consist of tens of different cellular subtypes and form functional subnetworks. An external stimulus generates the intricate temporal and spatial function patterns through these neuronal circuits. The generated patterns are responsible for the brain activity and the resulting behavior.[7,8]

Understanding of the nervous physiologic mechanisms requires tools that can control the activity of neurons. Furthermore, difficulties in manipulating the functions of the neurons because of the elaboration and size of their interconnections lead to the demand for more spatiotemporally accurate physiological research tools, because the neurons respond to an external exciter within a few milliseconds.[31]

Advancement of optics and molecular genetics has created fundamental improvements in the study levels of the central nervous system, mostly by the feasibility for detecting and manipulating the neuronal activity and, thereupon, in the understanding of the brain operation.

As aforementioned, optogenetics as a photostimulation technique allows the modulation of the neuronal activity by light. In this manner, a set of light-gated, microbial opsin proteins acts on being stimulated by light. They are expressed in the neurons, which are genetically targeted. In addition, appropriate wavelengths of light are needed to expose these neurons. Bidirectional management of the neural function and also genetical targeting of the specific cell types are possible by optogenetics. Therefore, it is a powerful method for experimental investigations of the brain circuitries related to different disorders.[24,32,33]

Microbial opsins are of different types such as bacteriorhodopsin, channelrhodopsin (ChR),[25] and halorhodopsin (HR).[24] As the primary microbial opsin, channelrhodopsin-2 (ChR2) of *Chlamydomonas reinhardtii* is sensitive to blue light and controls the action potentials with millisecond resolution.[34,35]

Retinal is one of the many forms of vitamin A, and all-trans-retinal is also an essential component of microbial opsins. As shown in Figure 1, in these molecules, light transforms 11-cis-retinal to all-trans-retinal, which cycles back to 11-cis-retinal with the dark condition. 11-cis-Retinal can covalently compound with the protein ChR2 and then alter to all-trans-retinal by a blue photon with a wavelength of 470 nm. This process causes the formation of a 6°A open pore [Figure 2].[36] As the light turns off,

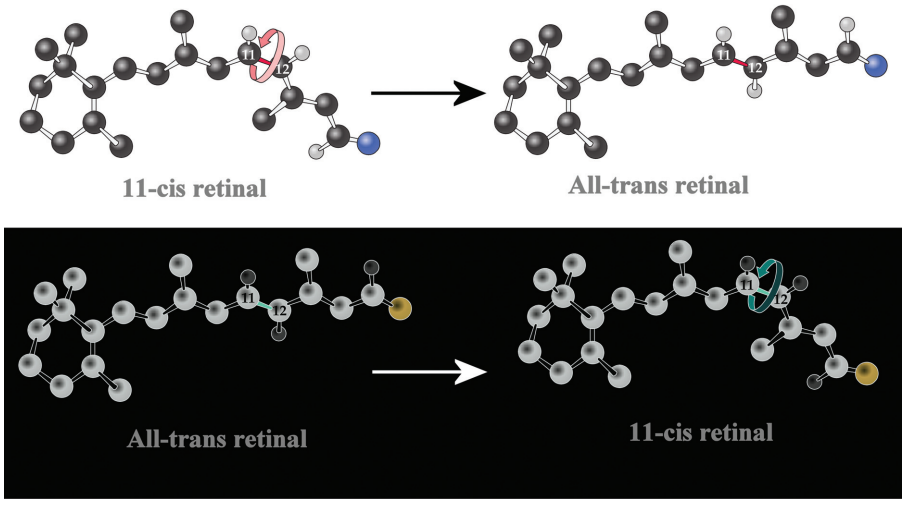

Figure 1: Isomerization of 11-cis-retinal by illumination

retinal returns back to its 11-cis form, and the pore is blocked as short as a few milliseconds.

Actually, blue light stimulation opens ChR2; therefore, cations stream into the neural cell through its membrane. This is cell depolarization that eventually leads to neuronal firing.

However, halorhodopsin obtained from the halophilic bacterium *Natronobacterium pharaonis* (NpHR) is the principal rhodopsin that inhibits the neuronal activity. NpHR acts as a chloride pump. It is sensitive to yellow light with a wavelength of 580 nm; therefore, one chloride ion enters into the cell through its membrane per one yellow photon that the nerve cell receives. This process hyperpolarizes the neuron.[37]

Figure 3 shows the activation and the inhibition of the neural activity by appropriate wavelengths.

Hence, each neuron can be depolarized and also hyperpolarized only with the correct choice of the light wavelength that it receives. In this manner, manipulation of the neuronal activities becomes possible for the researchers.

Furthermore, the cation channels and the anion pumps can express concurrently in one particular cell type, because NpHR and ChR2 have wavelengths of spectral separation.[24]

The optogenetic opsin proteins family is quickly advancing. Newly found opsins are sensitive to higher wavelengths. Yellow/red-shifted ChR types such as channelrhodopsin-1 from Volvox carteri (VChR1), A red- shifted channel-rhodopsin (ReaChR), and Chrimson are some of the recent ones.[38-40] These wavelengths are more suitable because they have less absorption and scattering in the brain tissue; therefore, they penetrate deeper and allow even the activation of the deep neurons optically.[38]

The consecutive high-power laser pulses are the main problem in optogenetics, because the generated heat may damage the neuron. Another family of ChR overcomes this problem and is known as step-function opsin (SFO). It activates the neuron during the light pulse time, and also after the pulse is stopped. Therefore, it is proposed for studies that require longer activation time without constant laser illumination. SFO can also be deactivated by yellow light.[41]

Similar improvements have been made for the inhibition tools of optogenetics. IC1C2 is a new ChR. It is an inhibitory combination from channelrhodopsin-1 (ChR1) and channelrhodopsin-2 (ChR2), acts as a chloride conductor, and is 200 times more light sensitive. It inhibits the neurons using blue light. After stimulation, iC1C2 will open for 1 min and be disabled by red light soon. These properties lead to less light exposure and heat damage of the tissue.[42]

Currently, the opsin toolbox has become so diverse that there is the possibility to choose a tool for different experiments according to its unique properties such as its selectivity of the specific ions, spectrum of absorption, subcellular localization, and sensitivity to light.[43] Table 1 categorizes the mentioned optogenetic proteins with details.

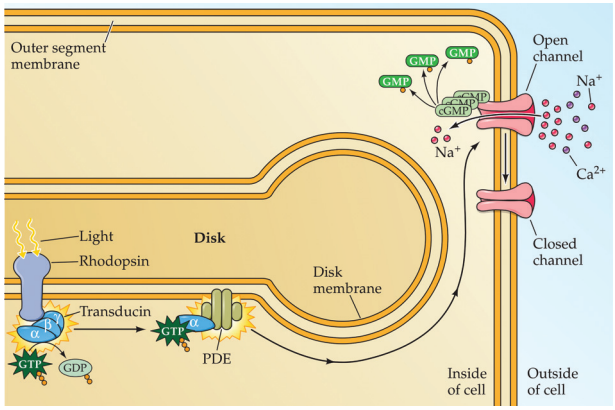

Figure 2: Schematic of channel opening upon illumination

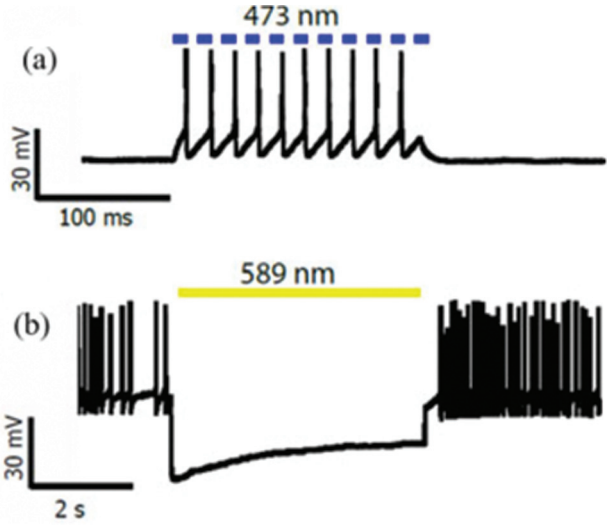

Figure 3: (a) Action potentials triggering by blue light pulse illumination (b) Inhibition of neural activity by yellow light illumination

Table 1: The common optogenetic proteins for neural activity modulation

Opsin tools	CHR2	NpHR	ReaChR	Chrimson	SFO*	VChR1	iC1C2
Action	Activator	Inhibitor	Activator	Activator	Activator	Activator	Inhibitor
Channel/pump	Cation channel	Chloride pump	Cation channel	Cation channel	Cation channel	Cation channel	Chloride channel
Sensitive wavelength spectrum (nm)	400–500	550–620	590–630	Near to 600 (peak)	450–590	500–550	450–500

*Two wavelengths are required for activation, wherein one initiates the current and the second terminates it.

Table 2: The basic mechanisms for light delivery in the optogenetic tests

Light delivery mechanism	Benefits	Challenges	Prototype
Implanted optical fibers	Easy to construct	Coupling efficiency between the light source and the fiber, with light delivery only near to the fiber tip	Figure 4
2D multichannel waveguides	Design simplicity, high light delivery output, and stimulation of multiple sites in different depths of the brain	Limited spatial resolution	Figure 5
3D multichannel waveguides	Light distribution into the cortex	Large size of the device and complex optics for light coupling into the waveguides of the array	Figure 6

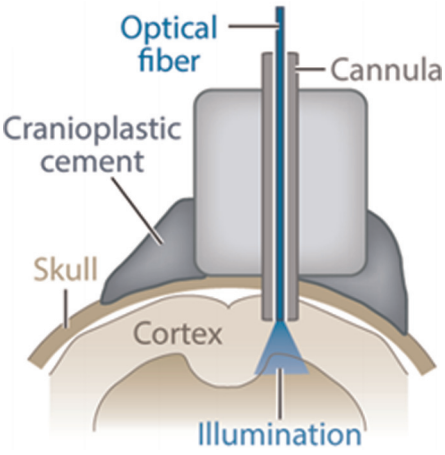

Figure 4: An optical fiber implanted into the brain for light delivery

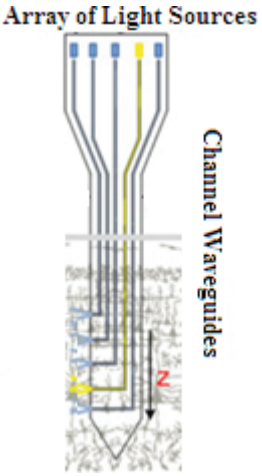

Figure 5: 2D multichannel waveguides

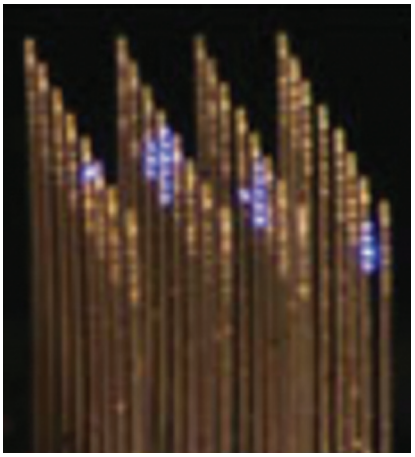

Figure 6: 3D multichannel waveguides

The result of an optogenetic manipulation of the neurons especially in most *in vivo* experiments depends on several factors. First, the number of photons that reach the opsin-expressing cells in the tissue, which is difficult to know because of light scattering and absorption in the tissue. Second, the protein expression extent into the cell, which is not necessarily the same for all cells. Third, the biophysical characteristics of the neuronal cells; for instance, the neurons with little input resistance are stimulated with much light-induced current.[44] Hence, the number of cells optically involved and modification processes of their properties are really unknown.

Light Delivery Mechanisms

In optogenetic brain studies, scientists aim to plan efficient mechanisms to give light to the brain surface and also inside its tissue. For example, the optical fibers implanted into the brain transfer laser pulses to the deep depths in the brain for stimulation.[45,46]

There are some drawbacks with optical fibers. First, their delivery light is limited to around the tip. Second, because the brain structure is 3D, the simple fibers cannot distribute the light into it. In addition, all the neurons containing opsin, which are placed near to the fiber point, will activate simultaneously. Therefore, the fibers cannot control the neurons spatially. Finally, more effective setups are needed, which may be more complex as well.[47-50] Table 2 contains a summary of the main methods used so far.[45,51]

Existing methods have not the ability for showing spike timing through the various neurons and cannot repeat the

spatiotemporal activation patterns related to the stimulated neuronal groups.

The use of small virus volumes or the tapered optical fibers can provide more accurate manipulation and spatial control for the optogenetic tests. Therefore, the number of stimulated cells can be fewer. In addition, the cell's properties such as its level of electrical activity are used to express opsin precisely.[43] However, the relation of spiking activity with ChR2 expression is yet unknown.

One complete approach is patterned stimulation that controls the light spatiotemporally. Figure 7 displays the difference between the types of illumination.

Patterned Stimulation Methods

Deflecting a light beam randomly was the first idea for pattern generation in laser-based neurostimulation systems [Figure 8]. The beam scanned the cell body alternatively. Therefore, the neuron was stimulated more effectively. A major drawback of this system was its sequential manner.[52,53] However, the real activity patterns in the brain were spatiotemporally complicated, because tasks were done in parallel using its neural networks. Special methods that can control numerous, photostimulation locations in parallel implement these patterns.[54]

Photostimulation systems using spatial light modulators (SLMs) such as liquid crystal and microelectromechanical (MEMS) ones allow thousands of parallel photostimulation beams. Therefore, they are able to generate the brain activity patterns.[51]

The liquid crystal SLMs just manipulate the phase of the incoming light. Using this method, the laser beam can be concentrated on different depths simultaneously in the 3D tissue. Therefore, the desired stimulation patterns are used in the brain.[51,54-56]

As a MEMS SLM, the digital micromirror device (DMD) from Texas Instrument is a large array with micromirror elements. Each element is individually addressable. Then it can be tilted. It reflects the light toward the object or an absorber according to the angle of the tilt. Massively parallel patterns can be generated by timing the mirrors to be on or off. Therefore, DMD modulates the amplitude of the incident light beam.[57,58]

Two sample optogenetic setups with mentioned SLMs are shown in Figure 9.[51,59]

Table 3 shows a comparison of the light modulation techniques.[31]

Probes in Optogenetics

Detailed understanding of neural circuits structures underlying behaviors and also their dynamics need to direct electronic recordings of neural activity.[29] It is possible by using neural probes. The simplest probe available is a single metallic microwire (~50–100 μm in diameter). Gold, tungsten, platinum, and steel are the fundamental materials used for the electrodes. An array of multiple microwires can also be designed to record more neuronal sites.[60,61]

These invasive electrodes are probes that penetrate within the brain tissue. Any stimulation leads to the movement of ions through the membrane of a neuron and changes their concentrations around the electrode consequently. In this manner, single spikes are generated and then recorded by these individual electrodes.[62]

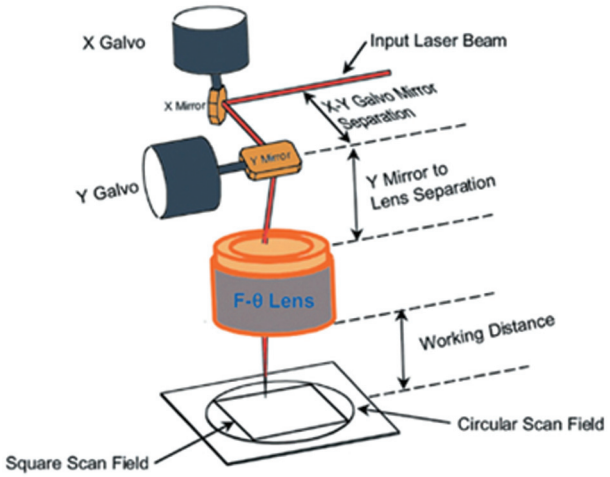

Figure 8: A laser scanning system prototype [8]

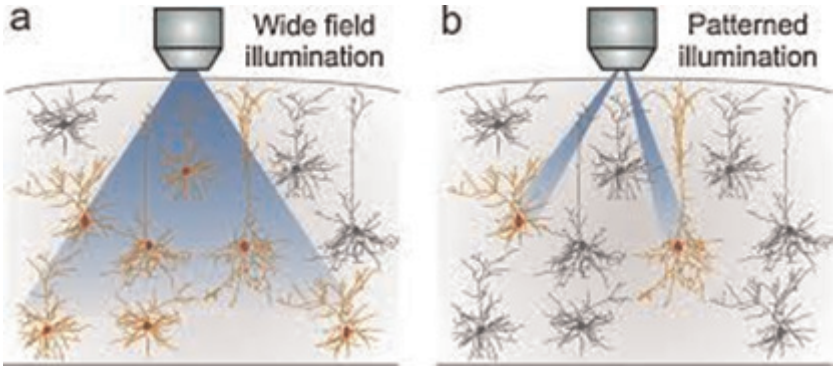

Figure 7: (a) usual illumination (b) patterned illumination [43]

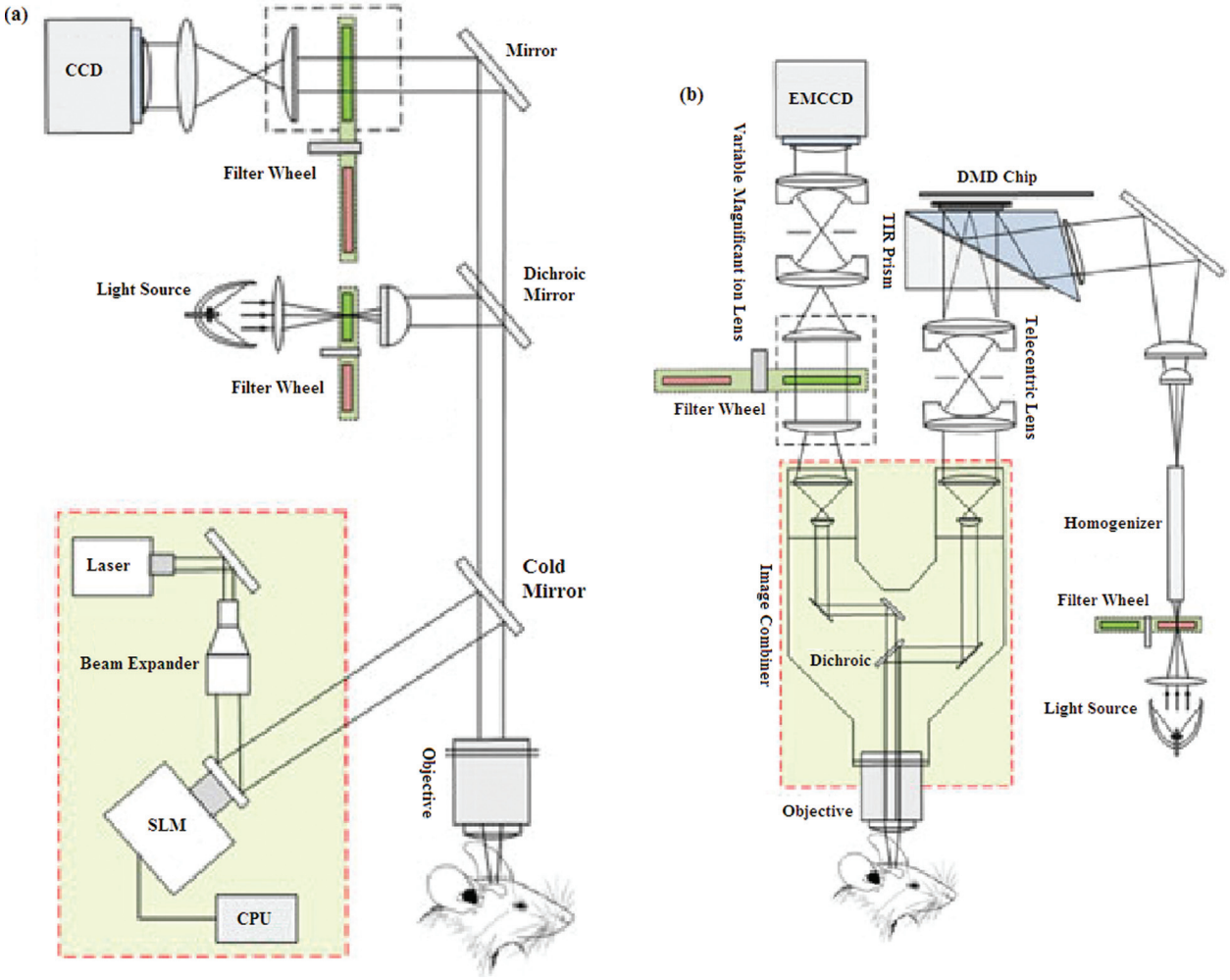

Figure 9: (a) A light delivery system based on liquid crystal modulator and holographic microscopy (b) A DMD based optical system design for optogenetic stimulation and imaging [51]

Table 3: The light modulation methods

Technique	Spatial resolution	Penetration depth into the brain	Ability for spatiotemporal patterns generation
Scanning lasers	50–100 μm	<300 μm	No
Liquid crystal SLMs	5–20 μm	<300 μm	Yes
DMD^{TI} SLMs	50–100 μm	<300 μm	Yes
Optical fibers	100 μm–1 mm	Limitless	No

The optical fibers are most common in the optogenetic tests; therefore, it is possible to integrate them with electrophysiological probes. The new integrated device is called an optrode, and comprises of a metal electrode that externally sticks to an optical fiber.[32,63-65]

Optogenetics: Advantages and Applications in Neuroscience

As aforementioned, optogenetics is a potent experimental method for analysis of the neural circuitries, which are involved in the psychiatric and neurological disorders. In this method, expression of the light-sensitive proteins in the interested cell type in the brain tissue can be controlled by a gene delivery mechanism. Therefore, specific cell type targeting becomes feasible.

Another advantage of optogenetics is its bidirectional control of the neural activities simultaneously. This makes it possible to manipulate activities of the neurons even in large networks such as the cortex.

There are many different applications of optogenetic neuromodulation in literature.[26-28,46] However, we can classify them into three major classes.

Control of the neural activity in optogenetics includes both its activation and inhibition. Temporal resolution is

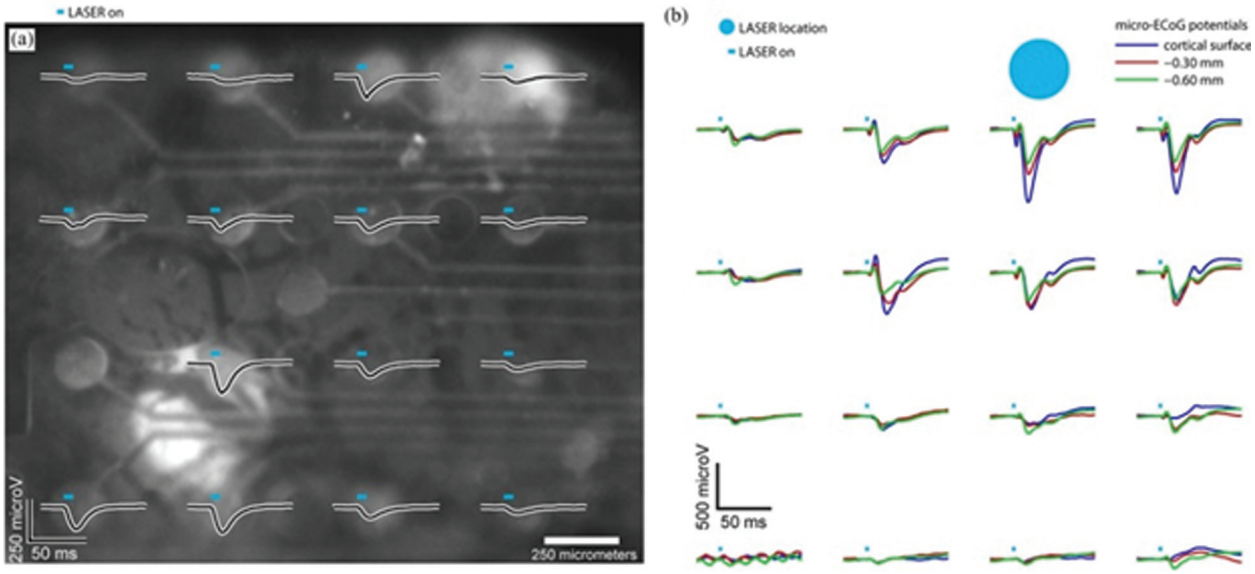

Figure 10: (a) Optogenetically evoked cortical potentials driven with a SLM and recorded by a micro-ECoG array (b) Potentials recorded on the cortical surface with micro-ECoG in response to photostimulation at three depths into the cortex with a fiber coupled to the laser [51]

in the order of milliseconds. In addition, any firing pattern can be produced by a sequence of light pulses, which represents a neural code. Thereafter, the effect of this code can be studied on the postsynaptic neurons. This process is known as cracking neural codes. We need to know the neural code, because we need to measure the activity in the neural circuits and understand how that activity draws the behind behavior.

Neural circuits interrogation is the next important application. Analysis of mental disease circuitries and perception of their effect in the disease are all provided by the activity modulation of the different specific cell types of the neurons.

Bidirectional control of the neural circuits is also used for pattern generation of neural diseases reversibly. Therefore, new models of neurological disorders are produced, which can be studied to find new treatments. Generally, because the neuronal cells can be controlled selectively, efficient mental treatments with minimum side effects can be achieved.

A complex network of cortical and subcortical connections governs both the motor and sensory functions of the brain. Herein, there are some case studies of optogenetics applications in neuroscience.

Injury to the brain such as by stroke affects not only the damaged area but also parts linked to it remotely. If remapping of the brain connections arises immediately after stroke, no new structural connections will be formed at first. Structural changes will occur over time after stroke. Therefore, understanding the progression of the neural circuit dynamics is important to improve the poststroke recovery strategies.Recently, Lim research group used the optogenetic method combined with voltage-sensitive dye imaging to study remapping of the functional cortical connections.[66]

Cortical excitability is one of the main problems after stroke that has been studied by the optogenetic approaches. Anenberg group did a complete research in this field using a stroke model that they generated by blocking arterioles of the motor cortex.[66]

Optogenetic microelectrocorticography (micro-ECoG) is a new plan that combines optical stimulation using optogenetics with electrical recording of produced action potentials of the stimulated neurons. ECoG array is implanted on the cortex surface. Previously, micro-ECoG arrays were used only for electrophysiological recordings. In addition, desired illumination pattern sequences can be generated using optogenetic micro-ECoG setups equipped to SLMs. In this method, the light patterns are projected onto the cortical surface, the neurons are stimulated, and their potentials mapped simultaneously by the micro-ECoG array [Figure 10].[51,65]

Conclusion

The existing brain stimulation techniques such as the DBS, the transcranial direct current stimulation, and the transcranial magnetic stimulation lack the ability of specific cell type and neural circuit targeting. Optogenetics has overcome this problem and made manipulations with temporal precision to study the mechanisms of neurological disorders. However, for its application in humans, other involved sciences including gene therapy, opsin engineering, and optoelectronics must develop as well.

Recent developments in the brain interface systems have shown satisfactory results for human researches.

The main challenge for optogenetic implementation in humans is the expression of adequate amount of opsins, which need to be activated for neuronal stimulation and specific behavior extraction, without heat damage. A high photocurrent opsin or an infrared one may be suitable for the purpose. In addition, a low-heat light source needs to be developed.

Despite the aforementioned obstacles, optogenetics provides greater advantages and fewer side effects compared to the other brain stimulation techniques.

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Conflicts of interest

There are no conflicts of interest.

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