

Optogenetic methods and technologies in solving applied medical problems

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ABSTRACT

Optogenetics is an innovative and fast-growing field of science combining the advances in molecular biology and laser technologies to monitor various biochemical processes in the cell and to control its activity using light. Therefore, this review is devoted to the implementation of the optogenetic approach to diagnosis and treatment of various socially sensitive diseases at the molecular and genetic level. Furthermore, the article considers different methods of delivery and incorporation of genetic constructs encoding transmembrane proteins. New fiber optic technologies used to develop implantable devices for generating and recording signals in excitable tissues are described. Besides, the most state-of-the-art and popular registration methods are considered in the review.

Key words: optogenetics, opsins, ion channels, fiber optic systems, photostimulation, neurointerface, optogenetic therapy.

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Оптогенетические методы и технологии в решении прикладных медицинских задач

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РЕЗЮМЕ

Оптогенетика – инновационное и быстро развивающееся научное направление, объединяющее достижения молекулярной биологии и лазерных технологий, для решения вопросов мониторинга различных биохимических процессов в клетке и контроля ее активности с помощью света. Данный обзор посвящен вопросам реализации и применения оптогенетического подхода для диагностики и лечения различных социально значимых заболеваний на молекулярно-генетическом уровне. В статье описаны различные способы доставки и встраивания генетических конструкций, кодирующих трансмембранные белки. Рассматриваются новые оптоволоконные технологии, используемые для исполнения имплантируемых устройств генерации и фиксирования сигналов в возбудимых тканях. Приводится анализ современных, наиболее используемых способов регистрации показателей эксперимента, указываются ключевые преимущества и недостатки различных методик.

Ключевые слова: оптогенетика, опсины, ионные каналы, оптоволоконные системы, фотостимуляция, нейроинтерфейс, оптогенетическая терапия.

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INTRODUCTION

Currently, a new interdisciplinary science – optogenetics – is rapidly developing at the intersection of molecular biology and advanced laser technologies. Researchers in this area are developing a wide variety of methods for studying the mechanisms of memory and behavior formation as well as for functional diagnosis and therapy of neurodegenerative, psychogenic and other socially sensitive human diseases. In solving such complex and complicated problems, the knowledge about the functioning of various genetic constructs and implementation of fundamentally new optical devices to study the functioning of excitable tissues is of great importance.

The latest achievements in optogenetics which have been developed and implemented in practical healthcare are based on the use of genetically encoded light-sensitive ion channels which are subsequently exposed to photostimulation in various modes. In such experiments, it is particularly important to ensure the availability of a high-quality fiber optic system which will deliver the light beam with minimal losses and effectively record intracellular changes. These systems represent a unique platform for developing innovative neural interfaces which are used in optogenetics for experiments on freely moving animals.

Currently, optogenetics has profound advantages

over traditional electrophysiological methods due to its selective effect, accuracy and a possibility of both excitation and suppression of the selected cell populations. The latter can be used not only in fundamental neurobiology, but also in applied medicine. For example, it is possible to stop and/or prevent an epileptic seizure by inserting actuators into neurons in the seizure focus and enabling inhibition.

This review article is the first one to comprehensively cover the issues of planning an optogenetic experiment with specifying the key advantages and disadvantages of various methods. It also most fully describes modern advances in optogenetics used in clinical medicine.

OPTOGENETICS: THE HISTORY OF ITS DISCOVERY AND FORMATION

In 1971, W.Stockenius and D.Osterheld discovered that bacteriorhodopsin in the ion channel can be activated by photons. After eight years, Francis Krikvy, an English biophysicist, suggested the idea of activating a group of cells with the use of light [1, 2].

In 2005, a group of scientists from Stanford University led by Karl Deisserot showed that the activity of a group of neurons can be controlled by adapting natural channelrhodopsin (Channelrhodopsin-2), obtained from green algae of the *Chlamydomonas Reinhardtii*

species, using lentiviral vectors for gene delivery [3]. The experiment was performed on fruit flies and mice several times. As a result, it was reliably proved that after incorporation of an opsin in the plasmalemma of a cell and its exposure to the blue spectrum light, the cell membrane was depolarized.

Further studies showed that other natural proteins, such as bacteriorhodopsin, halorhodopsin, and channelrhodopsin, are also capable of regulating the functioning of neurons in response to the light irradiation of different wavelengths. In 2008, Volvox channelrhodopsin-1 (VChR1) was isolated for the first time, and it was already sensitive to the yellow spectrum light [4]. This discovery demonstrated that the use of various modifications of channelrhodopsins with activation peaks shifted to the red spectrum area allows the researcher to stimulate selectively two types of neurons located in the same area of interest.

With updating the research in this area and obtaining new knowledge about the molecular organization of the brain, it became clear that the tissues of the vertebrates already contain trans-retinal which is necessary for the implementation of the method. By absorbing a photon, the retinal isomerizes, thus provoking a change in the protein conformation, which results in a change in the membrane permeability for ions, inducing the current of monovalent (H^+ , Na^+ , K^+) and some divalent (Ca^{2+}) cations, which, in turn, cause depolarization of the neuron membrane. Now researchers can selectively control the activity in certain neurons, as well as predict physiological and behavioral responses of organisms.

Introduction of new research methods in the field of neurobiology enabled to improve fiber optic tools which allow for the light beam delivery. Thus, the idea of simultaneous optical stimulation and registration of electrical impulses has been successfully implemented. For example, currently it is possible to directly measure the electrical activity in neurons which are responsible for motor activity and to simultaneously control them with the use of opsins.

AN OPTOGENETIC STUDY: PRINCIPLES OF PLANNING AND DEVELOPING THE EXPERIMENT DESIGN

The design of any optogenetic study includes the following main elements:

Planning of an experiment

At this stage, the aim and objectives of the experiment are set, the conditions of the experiment are

specified (available or accessible equipment, financial resources, personnel), the input and output parameters are identified on the basis of the collected and analyzed preliminary information (determining the object of the study, the method of delivery of the genetic construct encoding the light-sensitive protein, and the mode of photostimulation), and the plan and timing of the experiment are set as well.

Delivery of the genetic construct encoding the light-sensitive protein

Currently, the method of injecting adeno-associated virus (AAV) and lentiviral (LV) vectors is widely used, which allows to have accurate spatial control over the opsin expression. The specificity of the method is mainly achieved through the use of promoters and enhancers. Only in cells with the corresponding expression pattern for a particular promoter can opsin synthesis be activated [5]. The use of enhancers makes it possible to achieve the strict specificity without overloading the vector design [6].

A set of receptor proteins on the surface of the viral capsid called serotype plays a significant role in the effectiveness of the implementation of viral particles, since the serotype determines in which way the target cells will be infected (for example, in the area of the neuron body or at its processes). It has already been experimentally proved that the AAV 2.1 serotype is suitable for infecting rodent brain cells, and the serotypes 8 and 9 are suitable for infecting neurons in primate brain.

Another strategy that ensures the presence of a specific gene in the experiment is the use of transgenic animals, i.e. living organisms in whose genome foreign genes were introduced. Currently, cre / loxP animal lines are widely used, that express cre recombinase, which excises the exon surrounded by LoxP sequences. As a result, an animal line is created which misses a certain gene that is of interest for researchers [7].

To create transgenic animals, viral and non-viral technologies are used. The latter include approaches based on physical and chemical effects that allow for cell transfection *in vitro* [8]. One of the methods of creating such transgenic research objects is associated with embryonic stem cells. First, the cloned DNA is integrated into the embryonic stem cell culture, then the selected transgenic embryonic stem cells are cultured and used to create the necessary lines [9, 10].

To obtain transgenic mice, the method of intraportal electroporation is also used. In this case, a solution with DNA encoding opsin is injected *in utero* on

certain days of embryonic development, and then it is exposed to a short high-voltage electric discharge, which allows to impact selectively on specific cell types and brain regions [11]. This method requires exact knowledge of the time and migration trajectory of certain neuron groups during embryonic development. This technique allows to provide high quality targeted gene delivery to the II and III cortical layers, striatum and hippocampal neurons [12]. Unlike viral methods, electroporation can introduce more gene copies and deliver DNA of any size with large promoter segments to achieve higher cell specificity.

The most well-known chemical method of genetic construct delivery is liposome transport: the genetic material is placed in a liposome, which binds to the cell membrane and promotes its intracellular release. The specificity of this method is achieved by binding a specific ligand to the liposome surface.

So, currently, there is a wide range of methods that provide the presence of opsin-encoding genes in experimental models. The priority characteristics of the above mentioned methods are precision and selectivity of action, relative accessibility, and reproducibility. The most common method for optogenetic studies is the use of transgenic animals and viral vectors. However, development and implementation of new methods for genetic construct delivery are also relevant, since they will significantly expand the scope of optogenetics and accelerate implementation of the method into practice, including its use for human treatment.

Light beam delivery

In general, the functions of the optical fiber complex for light delivery are as follows: generation of and control over light emission, modification of light emission and its filtering, and transport of light to tissues and cell cultures [13]. Today, even the simplest optogenetic experiments require programmable pulse generators to modulate LED or laser emission and create a high-quality controlled light impulse. Studying the brain functions requires neural interfaces that can record the brain activity and stimulate it with high precision in space and time. Among researchers, the most popular method is *in vivo* optogenetic technique in the laboratory, for which implantable optical fiber is permanently inserted and guided through the cannula. Fiber implantation overcomes such limitations as brain tissue damage during second insertion of fiber, potential fiber defects within the implant, and accuracy of cannula positioning inside the animal brain. One of the advantages of the permanent optical fiber im-

plantation in *in vivo* experiments is the possibility of combining the optogenetic method with other types of investigation. The main parameters that must be determined before starting any optogenetic experiment are the wavelength, the intensity and mode of stimulation, which determine the success of the study and intracellular activity management.

Registration of the results of the optogenetic experiment

To visualize changes in the parameters of cellular activity in freely moving animals, a large number of different biosensors can be used, including genetically encoded ones based on the fluorescence effect. These methods make it possible to evaluate not only cellular activity, but also a change of the cell signal status. For example, the family of GCh acetylcholine sensors based on G-protein receptors that selectively respond to a specific mediator with fluorescence recorded by epifluorescence, confocal, and two-photon microscopy is widely used [14]. Scientists are interested in the possibility to manipulate a certain neuron population and simultaneously register the results, which will allow to study the relationship between cellular activity and cellular functions in more detail. The latest development in this area is a miniscope, which allows to visualize fluorescence and make simultaneous optogenetic manipulations, changing the activity of the neurons [15].

One of the most common methods for assessing the electrophysiological changes in the membrane potential of excitable cells is the patch clamp technique. The use of this method *in vitro* in combination with optogenetics makes it possible to investigate the synaptic activity of isolated neurons and establish their role in activating and inhibitory effects on the cerebral cortex.

Behavioral tests are used to assess neurological status and monitor animal behavior

Thus, the diversity of modern optogenetic technologies determines the possibility of their application as a main or additional method in various fields of science. However, further development and improvement of optogenetic technologies remain relevant. The development of biotechnology, genetics, optics, and biochemistry allows to fully realize the potential of these research areas. In particular, at each stage of the optogenetic study, several challenges can be distinguished. At the first stage, improvement of methods of gene delivery and expression is relevant, as well as a targeted study of genes encoding photosensitive pro-

teins, which is especially important for future use of optogenetics for treatment of human diseases. At the second stage, it is important to further study the well-known photosensitive proteins: not only their physicochemical properties, but also their functions in the cells of the organisms in which they were found. This will allow to further consider them in the pharmacological aspect. Of particular interest is the possibility of modifying photosensitive proteins, due to which it will be possible to finely regulate their functions in the optogenetic experiment. Studies at the third stage are aimed, firstly, at investigating modified light irradiation for developing new methods using light of different wavelengths, focused, scattered, and multipath irradiation. Secondly, they are aimed at improving the hardware complex, which is especially important in order to standardize the method. Finally, at the fourth stage, the problem of an objective evaluation of the experimental results arises, to overcome which a comprehensive analysis is required, including functional tests, registration with electrodes and probes, and fluorescence biosensors. It is also essential to develop non-invasive methods for recording results, especially in the field of medicine.

APPLICATION PROSPECTS: OPTOGENETIC APPROACH IN THE SOLUTION OF BIOMEDICAL TASKS

Neurophysiology

Optogenetic selectively impacts on excitable tissues and has a number of advantages in comparison with other methods studying this fundamental area (in particular, in comparison with classical neurostimulation). The advantages of optogenetics include the ability to influence certain types of neurons of interest and their individual intracellular structures with precise spatiotemporal control. This determines the prospects for expanding modern ideas about the functional structure of the brain. Thus, the possibility of activating locomotion movements by light stimulation of glutamatergic neurons of the spinal cord in transgenic mice was experimentally demonstrated, which showed the key role of these cells in the process of movement [16].

Optogenetics may also be the key to solving more complex, integrated research tasks. For example, by supplementing the experiment with functional data using the optogenetic method, it was found that neurons of the rostral ventromedial medulla oblongata innervate functionally heterogeneous tissues (myocardi-

um, skeletal muscle). Previously it was suggested that this brain region is associated with the regulation of the tone of smooth myocytes alone [17].

In addition, the possibility of studying the cerebellum using the optogenetic approach is discussed, which is especially relevant considering the poorly studied functional connections of the cerebellum with the cerebral cortex and subcortical structures [18].

Neurology

The mentioned advantages of optogenetics determine its wide range of possibilities not only in the field of neurophysiology, but also in clinical disciplines, such as neurology and psychiatry, making it possible to consider this approach as a promising treatment method. One of the areas of optogenetic research is the possibility of treating neuropathic pain. The exact pathogenesis of this condition is unknown. However, the possibility of effecting its peripheral and central components was experimentally proved. In the first case, nociceptors were considered a therapeutic target, in the second – a gelatinous substance [19].

In an experiment with a model of Alzheimer's disease it was possible to establish the therapeutic role of restoring slow oscillations (0.6 Hz) in the corticothalamic networks by optogenetically increasing the activity of pyramidal neurons. Moreover, the pathogenic effect of slow oscillations in the corticothalamic networks with optogenetically doubled frequency (1.2 Hz) on the development of this pathology was identified. This creates the prerequisites for the development of medical and preventive care based on the achievements in optogenetics [20].

Since 2010, scientists have begun to propose methods of treatment for Parkinson's disease through deep brain stimulation. The method is based on the stereotactic use of miniature electrodes that stimulate the subthalamic nucleus of the brain. The effectiveness of using this treatment option far exceeds the effectiveness of drug therapy.

A functional optogenetic approach is also considered as a rehabilitation method, in particular, after cerebral infarction. In this case, a multicomponent action is possible: an increase in neuronal activity in ischemic tissues in combination with the reorganization of afferent and efferent neural pathways [21].

Psychiatry

The prospect of functional reorganization of afferent and efferent neural pathways, and, in particular, a targeted study of cellular and subcellular interactions in the nervous tissue determine a great interest of psy-

chiatrists and narcologists in optogenetics.

It is assumed that by optogenetic normalization of biochemical processes and selective stimulation of brain regions pathogenetically associated with a particular disease it will be possible to treat diseases with depressive syndrome, anxiety, addiction, as well as schizophrenia and autism spectrum disorders [22, 23]. In particular, in one of the experiments, American scientists identified a significant decrease in depressive symptoms in animals after optogenetic stimulation of dopaminergic neurons associated with the nucleus accumbens, responsible for the formation of various behavioral reactions [24].

In 2014, scientists from the University of Buffalo managed to control addictive dependence in rats habituated to alcohol using light. In this group of animals, genetic modification of dopamine release systems was carried out, thereafter, with the help of light it was possible to stimulate selected groups of neurons and achieve prolonged release of the neurotransmitter [25].

In another experiment using optogenetic stimulation of the orbitofrontal cortex in experimental animals, inhibition of compulsive symptoms was detected, which creates the prerequisites for the development of treatment for a number of disorders associated with impaired interaction between the orbitofrontal cortex and striatum [26].

Ophthalmology

The ability to restore and regulate photosensitive cells using optogenetic stimulation determines the prospects for using this method in the field of ophthalmology, including treatment of retinal diseases, which is extremely important given the disappointing WHO statistics on morbidity of retinal degenerative diseases.

It should be noted that optogenetic studies conducted in the field of ophthalmology have a number of differences compared to other areas of medical research. First of all, opsins with a retinoid cofactor are most preferred, which is more physiologically reasonable; and, secondly, genetic material is usually delivered with viral vector injections in the intraretinal and subretinal space using an adeno-associated virus [27]. The main problems of application of the optogenetic method in this area include the occurrence of retinal remodeling with impairment of cytoarchitectonics and functional relationships between its layers, problems with precise determination of the site for the required injection to obtain an optimal result, and some mismatch between physiological ranges of light percep-

tion and ranges of light to which opsins react [28]. However, it is assumed that continuous improvement of the optogenetic experiment technique will overcome the difficulties encountered. Thus, nowadays, optogenetic engineering technologies allow to sensitize to light not degraded photoreceptor cells, but ganglion cells, which reduces the risk of developing complete loss of vision in patients with retinal degenerative diseases [29].

Otorhinolaryngology

Optogenetics also opens up wide opportunities in the field of otorhinolaryngology. In particular, this approach can be used to restore hearing impairment at the receptor level when acting on physiological mechanisms of sound perception, as was shown in experiments with optogenetic stimulation of the auditory nerve, as a result of which excitation of the corresponding nuclei of the brain stem was recorded [30, 31]. Obvious advantages distinguish optogenetic constructs from cochlear implants, allowing to impact selectively on cells in a certain part of the cochlea. This experiment was carried out by researchers from the University of Massachusetts, and they achieved partial restoration of hearing using low-intensity light [32].

Endocrinology

Optogenetic stimulation may be used for correcting the pathogenesis of endocrine diseases and developing a blood glucose lowering system in the long run. The possibility of achieving normoglycemia in a model of type II diabetes mellitus has been experimentally established. During the study, a cell culture secreting glucagon-like peptide-1 (GLP-1) and alkaline phosphatase was transplanted into LepRdb/db mice intraperitoneally and subcutaneously. In the first case, the light beam was supplied using optical fiber, in the second case, it was delivered transdermally; and in both cases, a significant decrease in blood glucose was registered [33]. A similar logic of the experiment was also preserved in a study with wireless control over the process of supplying a light beam using a smartphone app. Moreover, the cell culture also synthesized GLP-1 and insulin, and a subcutaneously implanted LED with the culture in a hydrogel capsule was used as a light source [34].

Cardiology

Due to the fact that the functions of the heart are inextricably linked with electrochemical processes, optogenetics opens up great opportunities for recovery of patients with cardiovascular diseases, since it

targets more physiological and subtle mechanisms than currently used medical and surgical methods of treatment.

The advances in optogenetics can be used to obtain a new type of pacemaker in which the function of cardiac pacemaker cells will be controlled by light instead of electrical impulses. For example, in the United States, a team of Stanford scientists led by Oscar Abilez is working on a project to develop a new biological pacemaker controlled by light. The results of the studies suggest that there are reliable ways to restore the healthy functioning of the heart muscle using light. The most important advantage of optogenetics in cardiology is selective excitation of only the inner layer – the endocardium [35]. This method of treatment of atrial fibrillation will improve the condition of patients and reduce side effects compared to the use of existing electric pacemakers, which eliminate arrhythmia, but cause severe pain due to excitation of skeletal muscles [36, 37]. The optogenetic method can currently replace devices, such as pacemakers and defibrillators, which allow to deliver electrical signals at a certain rhythm, but carry certain risks (damage to the heart tissue, battery failure, etc.) [38].

Pharmacy and pharmacology

No less relevant is the use of optogenetics in the field of pharmaceutical sciences. First of all, optogenetics will optimize the pipeline at the stage of research and development of drugs. This method will provide such opportunities as search for new therapeutic targets for drugs through a comprehensive study of the etiopathogenesis of diseases, optogenetic screening, functional optogenetic analysis, and stratification of patients, which is an important step towards personalized medicine [39]. The action mechanisms of the optogenetic component of therapy can encompass regulation of intracellular signaling pathways, increase in permeability of cell membranes, control over cell proliferation and differentiation, upregulation of the active substances of the drug after delivery to the cell, etc.

In addition, optogenetics is considered a promising method for studying the toxicity of drugs at the stage of their development, which is economically feasible: currently used methods are not effective enough – about one third of drugs do not pass clinical trials in phases II-III [40].

Finally, optogenetics can be used as a highly specific tool for drug delivery, allowing to control the speed, rhythm, and dose of the substance released, as well as to overcome histohematic barriers [41].

CONCLUSION

Optogenetics is a promising field of science for solving various biomedical problems. Advances in molecular biology and laser technologies open up new possibilities and allow to solve complex biomedical problems. Improvement and application of this method expand treatment opportunities for a wide range of diseases with minimization of pharmacological effects and considerable efficiency and selectivity of action, which may allow to actively use this method not only in fundamental medicine, but also in practical health care in the future.

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