UDC 577.35
DOI – https://doi.org/10.14300/mnnc.2019.14141
ISSN – 2073-8137

PROBLEMS AND OUTLOOKS OF OPTOGENETIC TECHNOLOGIES IN THE 21ST CENTURY

Fomochkina I. I. [1], Sorokina L. E. [1], Petrenko V. I. [1], Halilov S. I. [2], Kubyshkin A. V. [1], Kucherenko A. S. [1], Subbotkin M. V. [1], Kulanova A. A. [1]

[1] Medical Academy named after S. I. Georgievsky of V. I. Vernadsky Crimean Federal University, Simferopol, Russian Federation
[2] Institute of Physics and Technology of V. I. Vernadsky Crimean Federal University, Simferopol, Russian Federation

ПРОБЛЕМЫ И ПЕРСПЕКТИВЫ ИСПОЛЬЗОВАНИЯ ОПТОГЕНЕТИЧЕСКИХ ТЕХНОЛОГИЙ В XXI ВЕКЕ

И. И. Фомочкина [1], Л. Е. Сорокина [1], В. И. Петренко [1], С. И. Халилов [2], А. В. Кубышкин [1], А. С. Кучеренко [1], М. В. Субботкин [1], А. А. Куланова [1]

[1] Медицинская академия им. С. И. Георгиевского Крымского федерального университета им. В. И. Вернадского, Симферополь, Российская Федерация
[2] Физико-технический институт Крымского федерального университета им. В. И. Вернадского, Симферополь, Российская Федерация

Optogenetics is an innovative scientific trend, had developed in the 21st century as the integration of genetic engineering and advanced laser technologies to study the functioning of the human organism, diagnostics, and therapy of socially significant diseases. This work represents modern information and outlooks of optogenetics achievements to solve a wide range of biomedical problems. The review contains a description of the main methods of delivery, incorporation, and control of the expression of photosensitive proteins on the cell membrane. There are described characteristics of the physical and technical side of the optogenetic experiment, are indicated the key advantages and disadvantages of various techniques. The main difficulties of optogenetic technologies, examples of technical solutions for optostimulation and registration of cellular activity synchronically are described.

Keywords: optogenetics, opsins, ion channels, fiber optic systems, photostimulation

В XXI веке на пересечении генной инженерии и передовых лазерных технологий развивается новое перспективное научное направление – оптогенетика, в рамках которой разрабатывается обширный арсенал для изучения механизмов функционирования организма, функциональной диагностики и терапии социально значимых заболеваний человечества. В работе представлены современные данные о перспективах использования достижений оптогенетики для решения широкого круга биомедицинских задач. Обзор содержит описание основных способов доставки, встраивания и контроля экспрессии светочувствительных белков на мембране изучаемых клеток. Дается характеристика физико-технической стороны оптогенетического эксперимента, указываются ключевые преимущества и недостатки различных методик. Описываются основные трудности и нюансы в работе с оптогенетическими технологиями, примеры технических решений для выполнения одновременной оптостимуляции и регистрации клеточной активности.

Ключевые слова: оптогенетика, опсины, ионные каналы, оптоволоконные системы, фотостимуляция

For citation: Fomochkina I. I., Sorokina L. E., Petrenko V. I., Halilov S. I., Kubyshkin A. V., Kucherenko A. S., Subbotkin M. V., Kulanova A. A. PROBLEMS AND OUTLOOKS OF OPTOGENETIC TECHNOLOGIES IN THE 21st CENTURY. *Medical News of North Caucasus*. 2019;14(3):561-566. DOI – https://doi.org/10.14300/mnnc.2019.14141

Для цитирования: Фомочкина И. И., Сорокина Л. Е., Петренко В. И., Халилов С. И., Кубышкин А. В., Кучеренко А. С., Субботкин М. В., Куланова А. А. ПРОБЛЕМЫ И ПЕРСПЕКТИВЫ ИСПОЛЬЗОВАНИЯ ОПТОГЕНЕТИЧЕСКИХ ТЕХНОЛОГИЙ В XXI ВЕКЕ. *Медицинский вестник Северного Кавказа.* 2019;14(3):561-566.
DOI – https://doi.org/10.14300/mnnc.2019.14141

Optogenetics is an innovative and fast-developing scientific trend uniting achievements of molecular biology and laser technologies for the monitoring of various biochemical processes in the cell and the control of cell activity with light. Recent advances in optogenetics are based on the use of genetically encoded photosensitive ion channels, which demonstrate various combinations of photostimulation. It is especially important to ensure the availability of a high-quality fiber-optic

system to deliver the light with minimal losses and then to effectively collect the response signal.

The history of this scientific trend is taken from 1971 when W. Stokenius and D. Osterchelt showed that photons could activate the ion channel of bacteriorhodopsin. A few years later, the English biophysicist Francis Crick proposed the idea of enabling a group of cells with the help of light [1, 2]. However, over the years, the integration of genetic and optical methods has not been effective. In October 2007, the «father of optogenetics,» Carl Disserot and his colleague Luis de Leshea published the results of their studies performed on freely moving mice where recombinant lentivirus with channelrhodopsin-2 gene was stereotactically injected into the lateral hypothalamus and then optical fibers were used to deliver light deep in the brain, directly into the lateral hypothalamus. As a result of the study, the initial hypothesis was confirmed: the light stimulation of hypocretinergic neurons during sleep wakes up animals [3]. And after three years Disserot organized the company Circuit Therapeutics in Silicon Valley (USA), which successfully dealt with the suppression of acute and chronic pain [4].

In response to the appearance of new neurobiological methods, there has also been an improvement in fiber-optic instruments that allow light delivery. Thus, the idea of optical stimulation and recording of electrical pulses synchronically was successfully implemented. Currently, for example, it is possible to directly measure the electrical activity in neurons responsible for motor activity, while controlling them with the help of opsins.

Over time, the field of use of optogenetic techniques has become more extensive. Currently, optogenetic is increasingly being used to study and stimulate not only brain cells but also other organs containing excitable tissues (for example, heart muscle) where various light-responsive systems, delivered via viral vectors or expressed in transgenic animals, are used [5, 6].

Role of light-sensitive proteins in an optogenetic study. The choice of photosensitive proteins – opsins in optogenetic research is due to the possibility of ensuring their expression in almost any cell of the body, including brain neurons.

Currently, two types of channel proteins are distinguished: the first type of opsins – microbial-type opsins type I, which provide regulation of metabolism and ATP synthesis and are expressed on cells in prokaryotes, algae, and fungi. The optimal-type opsins type II is the second type of opsin responsible for vision, smell, regulation of biorhythms, and is found only on membranes of highly organized eukaryotes [7, 8].

It is customary to classify opsins into cationic transmembrane channels, anion channels, chlorine pumps, proton pumps, sensors, and metabolic pathways [9, 10].

Activator proteins that depolarize the membrane and inhibitor proteins hyperpolarize it is poured according to the mechanism of action. These properties can be used in the study, both individually and jointly [11].

Most of these proteins represent a channel consisting of 7 transmembrane domains by structure. In this case, the retinal is a cofactor of these opsins. During the absorption of a light quantum, the retinal isomerizes, contributing to a change in the structure of the protein and a difference in the membrane ions permeability [12].

At present, canalorodopsin-2 (ChR2) is the most popular in optogenetic studies. Back in 2005, a group of scientists at Stanford University under the leadership of Karl Deisserot showed that control of the activity of a group of neurons could be performed by adapting natural channel rhodopsin obtained from green algae of the species *Chlamydomonas reinhardtii.* The maximum activation of ChR2 is achieved with a glow of a wavelength of about 460 nm (blue light) [13]. In this case, the ion current velocity through the channel is only 1.21 ms under the illumination of 20 mW/mm^2.

Several laboratories are still conducting molecular genetic experiments to improve the spectral properties of canalrodopsin. For example, a point mutation in the canalorodopsin-2 (H134R) gene increases the ion current velocity by several times, while the C128S mutation leads to channel deactivation due to luminescence with a wavelength of 542 nm (green light) [14, 15].

In the case of experiments related to the possibility of inhibition of cellular activity, halorodopsin (NpHR), which was studied for the first time from the archaea *Natronomonas pharaonis*, is most often used. This protein is activated by light with a wavelength of 590 nm (yellow light) and induces a flow of chlorine ions into the cell. Another difference between NpHR and ChR2 is the ability to pass only one ion per cycle, and therefore halorodopsin has an even lower speed [16].

Nowadays, Arch and Mac proton pumps are also used to inhibit the cell populations of interest, which pump protons from the cell and are characterized by a higher ion transport rate. Arch proton pump is activated by yellow-green light and causes the most powerful, compared to other proteins, hyperpolarization. The Mac proton pump is activated by blue-green light and is widely used with canal rhodopsin-2 in multimodal lighting strategies [17, 18].

Several years ago, American researchers created artificial constructs between vertebral rhodopsins and G-protein-coupled receptors (opto-XR, where X is a specific receptor). Such constructions make it possible to control intracellular biochemical signaling with the help of light, and also have a certain temporal accuracy and specific cell stimulation. Opto-X-ray images, in particular, open up broad prospects for the realization of the effects of optogenetics in ophthalmology and otorhinolaryngology [19, 20].

As can be seen from these data, the discovery and genetic engineering synthesis of new opsins attract the great interest of scientists, since it is the protein structure that largely determines the possibilities for using optogenetics in studying the principles of functioning of organisms – from single cells to behavior and consciousness.

Methods of delivery of opsins to target cells. There are many optogenetics methods for target photosensitive proteins delivery to cells; in particular, there are the introduction of lentiviral and adeno-associated viral constructs and the use of transgenic animals [21, 22].

The most common opsin delivery method is viral transfection using LV – lentiviral constructs or AAV – adeno-associated virus [23].

Such constructs necessarily contain the gene responsible for the synthesis of rhodopsin, as well as several promoters and regulators that ensure protein expression only in the necessary cells [24].

Speaking about adenoviral vectors, it should be emphasized that such constructs have a small capacity – only about 4700 nucleotide pairs. However, the small size of the virus itself, its high concentration per unit volume, and the weak immune response of tissues allow scientists to infect [25] effectively.

Lentiviral constructs have a large capacity and can include up to 9–10 kb, while the level of expression of the opsin itself is much lower [26]. In this connection, the use of lentiviruses is limited to more local tasks.

An essential role in the effectiveness of infection has the set of receptor proteins on the surface of the viral

capsid – the serotype. It determines how the infection of target cells will occur (for example, neuron body or neuron processes). It has already been experimentally confirmed that serotype AAV 2.1 is suitable for infection of rodent brain cells, and serotypes 8 and 9 – for infection of primates neurons [27].

The modern technologies in optogenetic researches are Cre-LoxP (Cre-LoxP recombination technology), FLP-FRT, and DIO (double-inverse orientation or FLEX construct). Cre-recombinases (cyclic recombinase) – enzymes with the ability to change the location of the nucleotide sequences of DNA. These enzymes are capable of cutting a specific DNA fragment. Once in the nucleus, cre-recombinase cuts out the genes located between the LoxP sequences (from the English – the crossing-over locus in the P1 phage), as a result of which the animal becomes knockout by this gene.

FLP recombinase (Flippase) recognizes the similar sites of FRT (Flippase recognition targets) and inverts the DNA region between them [28].

DIO-systems (FLEX-system) is more complicated. It includes double LoxP sites on regulatory genes or on the encoding opsin gene itself, which makes it possible to regulate the expression of rhodopsins under conditions when the target cells have particular properties or the promoter is weak and does not provide the desired level of rhodopsin expression [29].

Another strategy ensuring the presence of a specific gene in an experiment is the use of transgenic animal lines (living organisms into whose genomes foreign genes have been transferred). However, nowadays, only transgenic mouse lines are widely used in laboratories. The limiting factors for using other types of research objects are ethical issues and high maintenance costs.

To obtain transgenic animals, viral and non-viral technologies are used. That included approaches based on physical and chemical effects, allowing cells transfection *in vitro* [30].

One way of creating such transgenic objects is associated with embryonic stem cells. First, cloned DNA integrates into embryonic stem cells culture, then selected transgenic embryonic stem cells are cultured and used to obtain the necessary lines [31].

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 embryo development, and then it is exposed to a high-voltage electric discharge for short duration, which allows to selective effect on specific cell types and brain regions. Unlike viral methods, electroporation can introduce more copies of a gene and deliver DNA of any size with large promoter segments to achieve higher cell specificity [32].

The most well-known method of chemical delivery of a genetic structure is liposomal transport for transfection cells and DNA cloned embryos. Liposomes, which are membrane-like vesicles, protect foreign DNA from enzymes. When the necessary conditions are created, cationic liposomes can interact with DNA molecules to form the «lipid-DNA» complex [33].

Optical stimulation. When planning an experiment with stimulation of the selected cells, an important step is the correct selection of the fiber, light source, and devices with which all components are connected.

For example, for stimulation of nerve cells in brain tissue, we've used dielectric waveguides, which are optical fibers that can transmit electromagnetic energy [34]. The real waveguides used in FOCLs are flexible fibers made of transparent dielectric materials, which consist of two areas: the central core and the shell, which is coated with a protective coating. In the core domain, the refractive index *n* can be constant or vary over the cross-section, and the refractive index of the cladding is constant. Two of these phases correspond to stepwise and gradient profiles of the refractive index, respectively [35]. To create guiding properties, the index of core refraction must be higher than the refractive index of the cladding, where the bulk of the transmitted radiation propagates through the core, and only a small fraction propagates through the cladding.

Optical fibers are divided by the number of modes which are characterized by the waveguide parameter (V):

$$V = \frac{2\pi\rho}{\lambda}\sqrt{n_{co}^2 - n_{cl}^2}, \quad (1)$$

where ρ is the radius of the core, λ is the free space wavelength, n_{co} is the refractive index of the core, n_{cl} is the refractive index of the shell [36]. When V<2.4 a fundamental mode HE_{11} with orthogonal polarizations HE_{11}^{+} and HE_{11}^{-} is excited in a fiber, which is called single-mode fiber. For waveguides with V>2.4 a multimode state is implemented with a minimum number of modes equal to six: HE_{11}^{+}, HE_{11}^{-}, HE_{21}^{ev}, HE_{21}^{od}, TM_{01}, TE_{01} [37].

An important characteristic of a light-stimulated optical fiber is the numerical aperture $NA = \sqrt{n_{co}^2 - n_{cl}^2}$, which characterizes the angle between the aperture beam and the optical axis, which is called the aperture angle $\Theta_A = \arcsin(NA)$ [38]. The choice of the magnitude of the aperture angle is based on the goal of the stimulation. For example, to stimulate nearby neurons in volume 0.1 mm^3 – 0.2 mm^3 it is convenient to use a single-mode fiber with $NA \approx 0.15$. A multimode fiber, in which the fiber diameter is more extensive and has $NA \approx 0.45$, is successfully used in optical stimulation of neurons in the volume 0.5 mm^3 – 1 mm^3 from the end of the fiber [39].

The second important principle in the experimental setup is the Laser-Light Amplification by stimulated emission of radiation, which is connected with the optical fiber. Given its size, the most practical and convenient way to work *in vivo* is the laser diode. The power of laser radiation depends on the current intensity applied to the diode. By adjusting the current strength, the intensity of the laser radiation can be quickly changed, as well as the frequency and duration of the impulse.

The primary sources of leaks are laser-fiber and fiber-fiber connection nodes [41]. The optimal solution to this problem is the coaxial connection of all-optical elements with minimal gaps. The USA company Thorlabs has a separate line for the production of visual components for optogenetic stimulation *in vivo*, where the light source has an adapter SMA905 for a fiber-optic cable with a metal casing, which is tightly fixed with a nut. Using LedDriver it is possible to adjust the radiation power with the maximum value 8–21.8 mW by changing the current strength supplied to the laser. The optical cable at the one end has a connector SMA905 or FC/PC with length 0.5–1 m. A ceramic ferrule with a diameter of 1.25 mm or 2.5 mm and a numerical aperture of 0.22, 0.39, or 0.50 is placed at the second end of the fiber [42]. An interconnector tightly concatenates optical cable with cannula placed in the cerebral cortex.

The output radiation is measured on a fully assembled installation, including a cannula, with a power-measuring sensor. For example, the power of 1–10 mW/mm^2 is required to stimulate rhodopsin, and the power of<0.01 mW/mm^2 is required for ChR2128 SSFO activation, depending on the objectives of the experiment [43].

The possibilities of optogenetics in solving biomedical tasks. Selectively influencing excitable tissues, optogenetics opens up great opportunities, especially for neurophysiology, the main task of which is to study the pathology of the brain.

For example, in an experiment with a modeled Alzheimer's disease, it was possible using optogenetic stimulation not only to establish the therapeutic role of slow corticothalamic oscillations induced by light but also to study the pathogenic effect of double-frequency oscillations on the development of this pathology. These results determine the perspectives for the construction of a treatment and prevention complex based on optogenetics achievements [44, 45].

Another common pathology is Parkinson's disease, which has a morbidity rate of 70 to 145 people per 100 000 population and currently has only an alternative to drug therapy. Since 2010 scientists have been using stereotactic introduction of tiny electrodes to stimulate the hypothalamic nucleus of the brain. The results after such stimulation were several times greater than the efficacy of drug therapy [46].

The functional optogenetic approach is also considered as a rehabilitation method, particularly after cerebral infarction. This may have a multicomponent effect: increase of neuronal activity in ischemic tissues in combination with the reorganization of afferent and efferent neuronal chains [47].

Through optogenetic research on sleep and awakening, pleasure, fear, aggression, and other basic psychiatric processes, a significant contribution has been made to the modern understanding of the pathogenesis of several mental diseases [48, 49]. It is expected that by optogenetic normalization of biochemical processes and selective stimulation of brain regions pathogenically associated with a particular disease, it will be possible to treat diseases related to depressive syndrome, anxiety, addiction, as well as schizophrenia and autism spectrum disorders [50].

Optogenetic methods also offer a wide range of opportunities for vision restoration in case of degenerative retinal diseases. For example, in the experiment with «switching-in» halorhodopsin eNpHR in light-insensitive rods, it was possible to restore the natural behavioral responses of laboratory animals to light [51, 52]. Additionally, there is a possibility for creating artificial photoreceptors after incorporation of ChR2 into bipolar cells or ganglion cells [53, 54].

Since 2011 new approaches related to the use of optogenetics in cardiology have been developed as an alternative to current electrical stimulation of the heart tissue. The main advantage of these methods is the selective excitation of only the inner layer – the endocardium [55, 56]. In general, nowadays, the optogenetic approach is ready to offer a replacement to such devices as pacemakers and defibrillators, which allow electrical signals to be supplied at a certain rhythm, however, carrying certain risks (damage to heart tissue, battery failure, etc.) [57].

Another option for the application of optogenetic stimulation may be the correction of the main components of endocrine disease pathogenesis, in particular, the development of a sugar-lowering system. The possibility of achieving normoglycemia on the model of type II diabetes mellitus has been experimentally established. During the study, a secreting glucagon-like peptide-1 (GLP-1) and alkaline phosphatase cell culture were transplanted intraperitoneally and subcutaneously; in the first case, the light beam was supplied using optical fiber, in the second – transdermally, while in both cases there was a significant decrease of blood glucose [58]. A similar logic of the experiment was also preserved in the study with wireless control over the process of supplying the light beam using the smartphone application. The cell culture also synthesized GLP-1 and insulin, and a LED was used as a light source, subcutaneously implanted with the culture in a hydrogel capsule [59].

Difficulties and limitations in optogenetic studies. Despite the greater variety of models for conducting an optogenetic experiment, there is still a number of unresolved problems that do not allow to fully realize the full potential of this scientific field [60]. In this regard, modern technologies are aimed at overcoming difficulties at every stage of the optogenetic methodology, in particular:

– lack of reliable information about the complex state of the organism after establishing the ability to express optogenetic molecules;

– violation of the bioethical rules while performing optogenetic studies in humans, in particular, human genome intervention [61];

– the difficulty in choosing the optimal photostimulation program taking into account the sensitivity threshold of opsins, specific qualities of the cellular ionic balance, etc.;

– high-intensity photostimulation, which can cause cell death and lead to ambiguous conclusions;

– uneven illumination intensity, light scattering in tissues [62].

Conclusions. A new promising scientific field – optogenetics, arisen at the intersection of genetics, molecular biology, chemical synthesis, and optics, makes it possible today to selectively control the functional activity of different types of cells, organs, and whole organisms. The development of research in this field not only lays a solid foundation for an in-depth understanding of the mechanisms of functioning of biological systems but also gives the key to the development of new approaches in the diagnosis and treatment of socially significant diseases. Given that modern optogenetic technologies have a full arsenal of tools, it becomes possible to identify the main areas for optogenetics application in future research directions: 1) development of new drugs for effective treatment of brain diseases, visual impairment, etc.; 2) obtaining innovative prostheses controlled by light; 3) therapy of cardiovascular diseases by controlling the motor activity of the smooth muscles of the heart by light, as well as obtaining the new type of pacemaker; 4) creating maps of the interconnections between neurons and individual parts of the brain in order to get unique data for development of artificial intelligence.

The main problems that are yet to be resolved are a violation of the bioethical rules while performing optogenetic studies in humans, in particular, human genome intervention and development of an optimal program for photostimulation of human neurons with minimally invasive interventions.

Disclosures:
The authors declare no conflict of interest.

Acknowledgments:
The studies were partially supported by the Development Program of the V. I. Vernadsky Crimean Federal University for 2015–2024.

References

1. Deisseroth K., Feng G., Majewska A. K., Miesenböck G., Ting A. [et al.] Next-generation optical technologies for illuminating genetically targeted brain circuits. *Journal of Neuroscience*. 2006;26(41):10380-10386. https://doi.org/10.1523/JNEUROSCI.3863-06.2006
2. Nagel G., Szellas T., Huhn W., Kateriya S., Adeishvili N. [et al.] Channelrhodopsin-2, a directly light-gated cation-selective membrane channel. PNAS. 2003;100(24):13940-13945. https://doi.org/10.1073/pnas.1936192100
3. Boyden E. S., Zhang F., Bamberg E., Nagel G., Deisseroth K. Millisecond-timescale, genetically targeted optical control of neural activity. *Nature Neuroscience*. 2005;8(9):1263-1268. https://doi.org/10.1038/nn1525
4. Imamura H., Nhat K. P. H., Togawa H., Saito K., Iino R. [et al.] Visualization of ATP levels inside single living cells with fluorescence resonance energy transfer-based genetically encoded indicators. *PNAS*. 2009;106(37):15651-15656. https://doi.org/10.1073/pnas.0904764106
5. Zaglia T., Di Bona A., Mongillo M. A. Light Wand to Untangle the Myocardial Cell Network. *Methods & Protocols*. 2019;2(2):34-36. https://doi.org/10.3390/mps2020034
6. Yizhar O., Fenno L., Zhang F., Hegemann P., Diesseroth K. Microbial opsins: a family of single-component tools for optical control of neural activity. *Cold Spring Harbor Protocols*. 2011;3:102. https://doi.org/10.1101/pdb.top102
7. Sakmar T. P. Structure of rhodopsin and the superfamily of seven-helical receptors: the same and not the same. *Current Opinion in Cell Biology*. 2002;14(2):189-195. https://doi.org/10.1016/S0955-0674(02)00306-X
8. Shichida Y., Yamashita T. Diversity of visual pigments from the viewpoint of G protein activation – comparison with other G protein-coupled receptors. *Photochemical and Photobiological Sciences: Official Journal of the European Photochemistry Association and the European Society for Photobiology*. 2003;2(12):1237-1246. https://doi.org/10.1039/B300434A
9. Adamantidis A. R., Zhang F., de Lecea L., Deisseroth K. Optogenetics: opsins and optical interfaces in neuroscience. *Cold Spring Harbor Protocols*. 2014;(8):815-822. https://doi.org/10.1101/pdb.top083329
10. Klapoetke N. C., Murata Y., Kim S. S., Pulver S. R., Birdsey B. [et al.] Independent optical excitation of distinct neural populations. *Nature Methods*. 2014;11(3):338-346. https://doi.org/10.1038/nmeth.2836
11. Glock C., Nagpal J., Gottschalk A. Microbial rhodopsin optogenetic tools: Application for analyses of synaptic transmission and of neuronal network activity in behavior. *Methods in molecular biology*. 2015;1327:87-103. https://doi.org/10.1007/978-1-4939-2842-2_8
12. Gradinaru V., Thompson K. R., Zhang F., Mogri M., Kay K. [et al.] Targeting and readout strategies for fast optical neural control in vitro and in vivo. *Journal of Neuroscience*. 2007;27(52):14231-14238. https://doi.org/10.1523/JNEUROSCI.3578-07.2007
13. Lin J. Y. A user's guide to channelrhodopsin variants: features, limitations and future developments. *Experimental physiology*. 2011;96(1):19-25, https://doi.org/10.1113/expphysiol.2009.051961
14. Adamantidis A. R., Zhang F., Aravanis A. M., Deisseroth K., de Lecea L. Neural substrates of awakening probed with optogenetic control of hypocretin neurons. *Nature*. 2007;450(7168):420-424. https://doi.org/10.1038/nature06310
15. Yizhar O., Fenno L. E., Davidson T. J., Mogri M., Deisseroth K. Optogenetics in neural systems. *Neuron*. 2011;71(1):9-34. https://doi.org/10.1016/j.neuron.2011.06.004
16. Zhang F., Wang L. P., Brauner M., Liewald J. F., Kay K. [et al.] Multimodal fast optical interrogation of neural circuitry. *Nature*. 2007;446(7136):633-639. https://doi.org/10.1038/nature05744
17. Husson S. J., Liewald J. F., Schultheis C., Stirman J. N., Lu H. [et al.] Microbial light-activatable proton pumps as neuronal inhibitors to functionally dissect neuronal networks in *C. elegans*. *PloS One*. 2012;7(7):e40937. https://doi.org/10.1371/journal.pone.0040937
18. Han X., Qian X., Bernstein J. G., Zhou H.-H., Franzes G. T. [et al.] Millisecond-Timescale Optical Control of Neural Dynamics in the Nonhuman Primate Brain. *Neuron*. 2009;62(2):191-198. https://doi.org/10.1016/j.neuron.2009.03.011
19. Govorunova E. G., Koppel L. A. The road to optogenetics: microbial rhodopsins. *Biochemistry (Mosc)*. 2016;81(9):928-940. https://doi.org/10.1134/S0006297916090029
20. Airan R. D., Thompson K. R., Fenno L. E. Temporally precise *in vivo* control of intracellular signalling. *Nature*. 2009;458:1025-1029. https://doi.org/10.1038/nature07926
21. Witten I. B., Steinberg E. E., Lee S. Y., Davidson T. J., Zalocusky K. [et al.] Recombinase-Driver Rat Lines: Tools, Techniques, and Optogenetic Application to Dopamine-Mediated Reinforcement. *Neuron*. 2011;72(5):721-733. https://doi.org/10.1016/j.neuron.2011.10.028
22. Madisen L., Mao T., Koch H., Zhuo J.-M., Berenyi A. [et al.] A toolbox of Cre-dependent optogenetic transgenic mice for light-induced activation and silencing. *Nature Neuroscience*. 2012;15(5):793–802. https://doi.org/10.1038/nn.3078
23. Carter M. E., de Lecea L. Optogenetic investigation of neural circuits *in vivo*. *Trends in molecular medicine*. 2011;17(4):197–206. https://doi.org/10.1016/j.molmed.2010.12.005
24. Sizemore R. J., Seeger-Armbruster S., Hughes S. M., ParrBrownlie L. C. Viral vector-based tools advance knowledge of basal ganglia anatomy and physiology. *Journal of Neurophysiology*. 2016;115(4):2124-2146. https://doi.org/10.1152/jn.01131.2015
25. Masamizu Y., Okada T., Kawasaki K., Ishibashi H., Yuasa S. [et al.] Local and retrograde gene transfer into primate neuronal pathways via adeno-associated virus serotype 8 and 9. *Neuroscience*. 2011;193:249-258. https://doi.org/10.1016/j.neuroscience.2011.06.080
26. Tervo D. G. R., Hwang B.-Y., Viswanathan S., Gaj T., Lavzin M. [et al.] Variant Permits Efficient Retrograde Access to Projection Neurons. *Neuron*. 2016;92(2):372-382. https://doi.org/10.1016/j.neuron.2016.09.021
27. Kühn R., Torres R. M. Cre/loxP Recombination system and gene targeting. *Methods in molecular biology*. 2002;180:175-204. https://doi.org/10.1385/1-59259-178-7:175
28. Dittgen T., Nimmerjahn A., Komai S., Licznerski P., Waters J. [et al.] Lentivirus-based genetic manipulations of cortical neurons and their optical and electrophysiological monitoring *in vivo*. *PNAS*. 2004;101(52):18206-18211. https://doi.org/10.1073/pnas.0407976101
29. Kim J. Y., Ash R. T., Ceballos-Diaz C., Levites Y., Golde T. E. [et al.] Viral transduction of the neonatal brain delivers controllable genetic mosaicism for visualising and manipulating neuronal circuits *in vivo*. *The European journal of neuroscience*. 2013;37(8):1203-1220. https://doi.org/10.1111/ejn.12126
30. Arenkiel B. R., Peca J., Davison I. G. In vivo light-induced activation of neural circuitry in transgenic mice expressing channelrhodopsin-2. *Neuron*. 2007;54(2):205-218. https://doi.org/10.1016/j.neuron.2007.03.005
31. Zeng H., Madisen L. Mouse transgenic approaches in optogenetics. *Progress in brain research*. 2012;196:193-213. https://doi.org/10.1016/B978-0-444-59426-6.00010-0
32. Park J. E., Silva A. C. Generation of genetically engineered non-human primate models of brain function and neurological disorders. *American journal of primatology*. 2019;81(2):e22931. https://doi.org/10.1002/ajp.22931
33. Bitzenhofer S. H., Ahlbeck J., Wolff A. Layer-specific optogenetic activation of pyramidal neurons causes beta-gamma entrainment of neonatal networks. *Nature communications*. 2017;8:14563. https://doi.org/10.1038/ncomms14563
34. Warden M. R., Cardin J. A., Deisseroth K. Optical neural interfaces. *Annual Review of Biomedical Engineering*. 2014;16:103-129. https://doi.org/10.1146/annurev-bioeng-071813-104733
35. Bartic C., Battaglia F. P., Wang L., Nguyen T. T., Cabral H. [et al.] A multichannel recording system with optical stimulation for closed-loop optogenetic experiments. *Methods in Molecular Biology*. 2016;1408:333-344. https://doi.org/10.1007/978-1-4939-3512-3_23
36. Aravanis A. M., Wang L.-P., Zhang F., Meltzer L. A., Mogri M. Z. [et al.] An optical neural interface: in vivo control of rodent motor cortex with integrated fiberoptic and optogenetic technology. *Journal of Neural Engineering*. 2007;4(3):43-56. https://doi.org/10.1088/1741-2560/4/3/S02
37. Kwon K. Y., Lee H.-M., Ghovanloo M., Weber A., Li W. A wireless slanted optrode array with integrated micro leds for optogenetics. In 2014 IEEE27th International conference on micro electro mechanical systems (MEMS). 2014. https://doi.org/10.1109/memsys.2014.6765765
38. Shin Y., Yoo M., Kim H.-S., Nam S.-K., Kim H.-I. [et al.] Characterization of fiber-optic light delivery and light-induced temperature changes in a rodent brain for

precise optogenetic neuromodulation. *Biomedical Optics Express*. 2016;7(11):4450-4471. https://doi.org/10.1364/OL.37.004841
39. Cardin J. A., Carlén M., Meletis K., Knoblich U., Zhang F. [et al.] Targeted optogenetic stimulation and recording of neurons in vivo using cell-type-specific expression of Channelrhodopsin-2. *Nature Protocols*. 2010;5(2):247-254, https://doi.org/10.1038/nprot.2009.228
40. Wu F., Stark E., Im M., Cho I. J., Yoon E. S. [et al.] An implantable neural probe with monolithically integrated dielectric waveguide and recording electrodes for optogenetics applications. *Journal of Neural Engineering*. 2013;10(5):056012. https://doi.org/10.1088/1741-2560/10/5/056012
41. Royer S., Zemelman B. V., Barbic M., Losonczy A., Buzsáki G. [et al.] Multi-array silicon probes with integrated optical fibers: light assisted perturbation and recording of local neural circuits in the behaving animal. *The European Journal of Neuroscience*. 2010;31(12):2279-2291. https://doi.org/10.1111/j.1460-9568.2010.07250.x
42. Abaya T. V. F., Blair S., Tathireddy P., Rieth L., Solzbacher F. A 3D glass optrode array for optical neural stimulation. *Biomedical Optics Express*. 2012;3(12):3087-3104. https://doi.org/10.1364/BOE.3.003087
43. Pisanello F., Sileo L., De Vittorio M. Micro- and Nano-technologies for Optical Neural Interfaces. *Frontiers in Neuroscience*. 2016;10:70. https://doi.org/10.3389/fnins.2016.00070
44. Airan R. D., Thompson K. R., Fenno L. E., Bernstein H., Deisseroth K. Temporally precise *in vivo* control of intracellular signalling. *Nature*. 2009;458(7241):1025-1029. https://doi.org/10.1038/nature07926
45. Kastanenka K. V., Calvo-Rodriguez M., Hou S. S. Frequency-dependent exacerbation of Alzheimer's disease neuropathophysiology. *Scientific reports*. 2019;9(1):8964. https://doi.org/10.1038/s41598-019-44964-z
46. Chen Y., Xiong M., Zhang S. C. Illuminating Parkinson's therapy with optogenetics. *Nature Biotechnology*. 2015;33(2):149-150. https://doi.org/10.1038/nbt.3140
47. Krook-Magnuson E., Armstrong C., Oijala M., Soltesz I. On-demand optogenetic control of spontaneous seizures in temporal lobe epilepsy. *Nature communications*. 2013;4:1376. https://doi.org/10.1038/ncomms2376
48. Touriño C., Eban-Rothschild A., de Lecea L. Optogenetics in psychiatric diseases. *Current opinion in neurobiology*. 2013;23(3):430-435. https://doi.org/10.1016/j.conb.2013.03.007
49. Deisseroth K. Optogenetics and psychiatry: applications, challenges, and opportunities. *Biological psychiatry*. 2012;71(12):1030-2. https://doi.org/10.1016/j.biopsych.2011.12.021
50. Tye K. M., Mirzabekov J. J., Warden M. R. Dopamine neurons modulate neural encoding and expression of depression-related behaviour. *Nature*. 2013; 493(7433): 537–541. https://doi.org/10.1038/nature11740
51. Henriksen B. S., Marc R. E., Bernstein P. S. Optogenetics for retinal disorders. *Journal of ophthalmic and vision research*. 2014;9(3):374-38, https://doi.org/10.4103/2008-322X.143379
52. Berry M. H., Holt A., Salari A. Restoration of high-sensitivity and adapting vision with a cone opsin. *Nature communications*. 2019;10(1):1221. https://doi.org/10.1038/s41467-019-09124-x
53. Ganjawala T. H., Lu Q., Fenner M. D., Abrams G. W., Pan Z. H. Improved CoChR Variants Restore Visual Acuity and Contrast Sensitivity in a Mouse Model of Blindness under Ambient Light Conditions. *Molecular therapy: the journal of the American Society of Gene Therapy*. 2019;27(6):1195-1205. https://doi.org/10.1016/j.ymthe.2019.04.002
54. Berry M. H., Holt A., Salari A. Restoration of high-sensitivity and adapting vision with a cone opsin. *Nature communications*. 2019;10(1):1221. https://doi.org/10.1038/s41467-019-09124-x
55. Yu L., Zhou L., Cao G. Optogenetic Modulation of Cardiac Sympathetic Nerve Activity to Prevent Ventricular Arrhythmias. *Journal of the American College of Cardiology*. 2017;70(22):2778-2790. https://doi.org/10.1016/j.jacc.2017.09.1107
56. Majumder R., Feola I., Teplenin A. S., de Vries A. A., Panfilov A. V. [et al.] Optogenetics enables real-time spatiotemporal control over spiral wave dynamics in an excitable cardiac system. *Elife*. 2018;7:e41076. https://doi.org/10.7554/eLife.41076
57. Cheng Y., Li H., Lei H. Flexible and precise control of cardiac rhythm with blue light. *Biochemical and biophysical research communications*. 2019;514(3):759-764. https://doi.org/10.1016/j.bbrc.2019.05.035
58. Ye H., Daoud E., Baba M., Peng R. W., Fussenegger M. A synthetic optogenetic transcription device enhances blood-glucose homeostasis in mice. *Science*. 2011;332(6037):1565-8. https://doi.org/10.1126/science.1203535
59. Shao J., Xue S., Yu G. Smartphone-controlled optogenetically engineered cells enable semiautomatic glucose homeostasis in diabetic mice. *Science translational medicine*. 2017;9(387):eaal2298. https://doi.org/10.1126/scitranslmed.aal2298
60. Mohanty S. K., Lakshminarayananan V. Optical Techniques in Optogenetics. *Journal of modern optics*. 2015;62(12):949–970. https://doi.org/10.1080/09500340.2015.1010620
61. Kravitz A. V., Bonci A. Optogenetics, physiology, and emotions. *Frontiers in behavioral neuroscience*. 2013;7:169. https://doi.org/10.3389/fnbeh.2013.00169.89
62. Packer A. M., Roska B., Häusser M. Targeting neurons and photons for optogenetics. *Nature neuroscience*. 2013;16(7):805-815. https://doi.org/10.1038/nn.3427

About authors:

Fomochkina Iryna Ivanovna, MD, DMedSc, Professor of the Department of general and clinical pathophysiology;
tel.: +73652554940; e-mail: fomochkina_i@mail.ru; ORCID – 0000-0003-3065-5748

Sorokina Leya Evgenyevna, student;
tel.: +73652554940; e-mail: leya.sorokina@mail.ru; ORCID – 0000-0002-1862-6816

Petrenko Vitalina Igorevna, postgraduate student;
tel.: +73652554940; e-mail: petrenko-vitalina@mail.ru; ORCID – 0000-0001-9451-1757

Halilov Server Iskanderovich, postgraduate student; tel.: +7652608194; e-mail: server.khalilov.94@mail.ru;
ORCID – 0000-0002-1682-9441

Kubyshkin Anatoliy Vladimirovich, MD, DMedSc, Professor,
Head of the Department of general and clinical pathophysiology;
tel.: +73652554940; e-mail: kubyshkin_av@mail.ru; ORCID – 0000-0002-1309-4005

Kucherenko Aleksandr Sergeevich, postgraduate student;
tel.: +73652554940; e-mail: aleksandr-kucherenko@bk.ru; ORCID – 0000-0002-5861-190X

Subbotkin Mikhail Vladimirovich, student;
tel.: +73652554940; e-mail: msgerm@yandex.ru; ORCID – 0000-0002-0521-0619

Kulanova Alina Alekseevna, student;
tel.: +73652554940; e-mail: kulanovalina@gmail.com; ORCID – 0000-0001-7553-5382