ISSN 0891-4168, Molecular Genetics, Microbiology and Virology, 2016, Vol. 31, No. 4, pp. 203–207. © Allerton Press, Inc., 2016.
Original Russian Text © E.V. Borisova, E.A. Epifanova, S.A. Tutukova, V.A. Salina, A.A. Babaev, 2016, published in Molekulyarnaya Genetika, Mikrobiologiya i Virusologiya, 2016, No. 4, pp. 128–132.

REVIEWS

Optogenetic Approaches in Neurobiology

E. V. Borisova*, E. A. Epifanova, S. A. Tutukova, V. A. Salina, and A. A. Babaev

Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, 603950 Russia

**e-mail: borisovakaterina17@gmail.com*

Received September 17, 2015

Abstract—Optogenetics is a rapidly developing new technique that combines optical methods with techniques that are used in molecular biology. It can be used for monitoring various optical processes in cells and controlling their activity using light. The technique is based on bacterial opsin expression in mammalian neurons. In this review, the use of optogenetics for controlling the activity of specific neuronal populations in different regions of the human brain is considered in detail. The paper also presents information on light-sensitive proteins, genetically encoded optical instruments, and their use for activation or inhibition of neurons and investigation of the causal relationship between neural networks and pathological symptoms.

Keywords: optogenetics, opsins, channelrhodopsin-2 (ChR2), halorhodopsin (NpHR), archaerhodopsin-3 (Arch), virus vectors, Cre recombinases, loss of motor function, Parkinson's disease, epilepsy

DOI: 10.3103/S0891416816040029

Plants and many microorganisms depend on light, which is the main external signal for their development, morphogenesis, and metabolism. Photons can serve as signals upon their absorption by specialized photoreceptors [4]. Photoreceptors usually contain a chromophore in their structure; upon photon absorption, its chemical bonds are broken and isomerization occurs. This leads to a change in the conformation and/or kinetics of the photoreceptor, which initiates further signaling events. In the course of evolution, these natural photosensors gained maximum effectiveness and specificity in the transformation of light into biochemical signals [37].

Over the last decade, there has been growing interest in these photoreceptors for spatial and temporal control of biological activity by light. Since these proteins are genetically determined, they could be easily introduced into target cells and extracellular structures. It became possible to use light as a switch of activity in certain neuronal populations with accuracy up to milliseconds, which makes it possible to carry out fundamental experiments that elucidate the role of certain neurons in the control of functioning of the nervous system with high accuracy [29].

Light-sensitive proteins that were first found in microorganisms, channelrhodopsin-2 (ChR2) and halorhodopsin (NpHR), which were isolated from the *Chlamydomonas reinhardtii* algae and *Natronomonas pharaonis* archaebacteria, respectively, are the two tools that are most often used in optogenetics [20, 40].

ChR2 is a protein that crosses the membrane seven times and contains a chromophore that is isomerized when exposed to light. ChR2 directly forms ion channels and represents an ionotropic protein. These proteins provide rapid and large cellular depolarization, which make them useful for bioengineering and neuroscience research, including photostimulation [28].

ChR2 is excited upon irradiation with blue light of 470 nm. Upon light absorption by ChR2, pores with a diameter no less than 6 Å are opened. The molecule returns to its initial conformation in the course of milliseconds, with the pores being closed and ion flux being stopped. All natural Ch are nonspecific cation channels that are permeable to H^+, Na^+, K^+, and Ca^{2+} ions [12, 15].

The first experiments have revealed that the ChR2 protein enters into the composition of the cation-selective ion pump in *Xenopus laevis* oocytes and mammal cells [17, 28]. To visualize ChR2-expressing cells, the opsin at the intracellular side is fused to a fluorescent protein that is excited by green light. When exposed to blue light, ChR2 opens, allowing an influx of Na^+ into the cell (Fig. 1) [28].

However, upon continuous irradiation of ChR2, its sensitivity declines. Recovery after desensitization can accelerate a flux of extracellular protons and formation of the negative membrane potential. Thus, ChR2 can be used for cell depolarization [26, 27]. Transgenic mice have been created that synthesize ChR2 and can be used for light-induced activation of neurons and identification of neuronal connections [5].

Temporal inhibition of different types of cells of the nervous system became possible due to a microbial halorhodopsin, NpHR, which is an electrogenic chlorine pump that is activated by yellow light at 580 nm

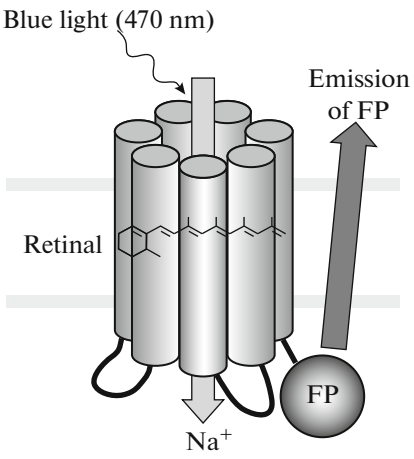

Fig. 1. Chimeric ChR2 with an additional fluorescent protein for visualization of expression.

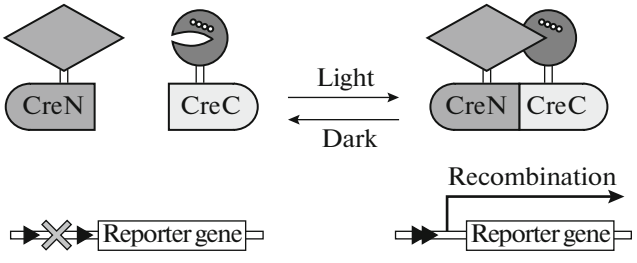

Fig. 2. Regulation of genetic transcription by light using Cre recombinases.

[13]. The use of a specific promoter in neurons leads to a high level of expression of NpHR–YFP, which results in activation of cortical pyramidal neurons with rapid reversible photoinhibition of the action potential. Transgenic mice that express NpHR also have been created [6, 11]. A high level of expression of NpHR leads to formation of aggregates and their accumulation in the endoplasmic reticulum, which is toxic for the cell and manifests by the formation of intercellular vesicles and dendrite swelling [40]. These complications significantly restricted the use of the first generation halorhodopsins as light-sensitive switches. A change in the NpHR localization resulted in a significant enhancement of the inhibitory activity [9, 10, 14].

Another opsin that has been proposed for the purposes of optogenetics is the archaerhodopsin-3 protein (Arch), which has been isolated from the *Halorubrum sodomense* archaebacteria and has the form of a proton pump that is activated by yellow-green light. Arch can also mediate strong and safe shutting down of neuronal activity [16, 33]. Moreover, Arch is spontaneously recovered from light-dependent inactivation in contrast to light-dependent chlorine pumps, which in response to light become inactivated for a long time. These Arch properties can be exploited upon optical shutting down of considerable portions of the brain. The activity of Arch in neurons is well-tolerated, since a shift in pH that is generated by Arch excitation can reduce to the minimum self-limiting mechanisms [7].

The precision of spatiotemporal control of intracellular signaling processes is achieved also due to the use of genetically encoded optical tools that are based on G-protein-coupled receptors (GPCRs). GPCRs provide expression under natural conditions and functioning of several different opsins [18]. Two chimeric GPCRs, optoXRs, which are selective and target certain signaling pathways that are activated in response to light, have been developed and described [2, 29].

To deliver optogenetic tools into an intact neuronal system, adeno-associated virus vectors (AAVs) and lentiviral vectors (LVs) are used [32]. AAVs and LVs have been successfully applied for opsin expression in many animals (mice, rats, guinea pigs, primates) and promote high levels of expression over a prolonged period of time almost without any adverse effects [38]. When viral vectors are used, cellular specificity is achieved using promoters [23].

At present, optogenetic control is often carried out by Cre recombinases (cyclic recombinase), enzymes that are capable of altering the position of nucleotide sequences of DNA in a site-specific manner. Excision of a DNA fragment occurs between two LoxP sites (from lo(cus) (of) X-(over of) P(1)). Cre recombinases carry out specific excision of a DNA fragment that is located between two LoxP sites [35, 39].

Cre recombinases are also attached to proteins that are sensitive to certain drugs. In their absence, Cre recombinase is free in the cytosol and cannot affect DNA. However, when the drug is introduced, the protein that is attached to the Cre recombinase is activated and they are transported into the nucleus. In the nucleus, the Cre recombinase excises genes that are located between LoxP sequences and, as a result, the animal becomes knockout in regards to this gene [3, 14].

In one system, light-regulated Cre recombinase was made so that its N-terminus was connected with the yeast protein (CRY2, a cryptochrome protein that carries a light-activated domain) and C-terminus with another yeast protein (CIB1, calcium- and integrin-binding protein) that interact with each other. In the dark, Cre lacks recombinase activity (Fig. 2). Upon irradiation of CRY2–CIB1, the N-terminus and C-terminus interact and the recombinase activity is restored [21].

In some light-regulated systems, a light-sensitive module and a partner that is connected with it are attached to a module that binds DNA and activates transcription. This system functions as a transcription factor. When the photosensor and a partner that is connected to it are exposed, the regulating components interact and initiate transcription (Fig. 3) [19, 36].

Moreover, the strategy is employed of the axon being irradiated distantly from the cell body of a trans-

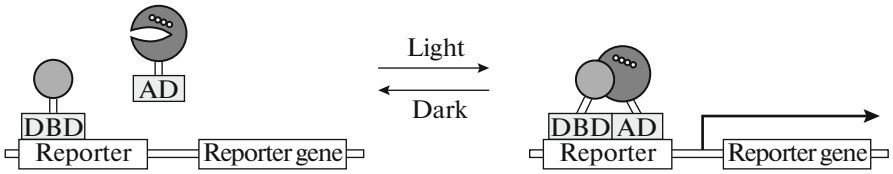

Fig. 3. A system for regulation of transcription. DBD is DNA-binding domain. AD is activation domain.

duced cell (thus using a mechanism of transport of light-sensitive molecules down the axon). The light source is concentrated not on the cell body, but on the axon, which makes it possible to modulate neuronal activity depending on its projections and not the scheme of gene expression. It has been experimentally proven that ChR2 is transported into axons, where it is able to effectively transform photostimuli into action potentials. Axons can be stimulated even if they are detached from the parental soma [31].

Optogenetic tools can be used on live laboratory animals that are responsive to stimuli, such as rats and primates, which further will make it possible to study the effects of switching neural networks upon pathological brain symptoms.

Optogenetics can be applied for studying the neuronal basis of sleep and its disorders. ChR2-mediated activation of orexin/hypocretin-expressing neurons in the lateral hypothalamic area in mice increases the time of transition from sleep to wakefulness, which is in accordance with the data that the dysfunction of these neurons is connected with sleep disorders and narcolepsy [1].

Attempts have been made to apply optogenetics for studying the mechanisms that underlie learning and memory [24].

In the laboratory of K. Deisseroth, the etiology of schizophrenia and Parkinson's disease is being studied. Viral vectors were used to infect mouse brain, which resulted in target cells expressing light-sensitive proteins on their surface. Light was supplied by a fiber-optic cable that was implanted directly into the mouse motor cortex that is responsible for movement to the left. Mice that were exposed to blue light ran to the left and stopped when the light was absent.

Deisseroth in collaboration with J. Henderson applied optogenetics to control symptoms of Parkinsons disease in laboratory mice with fiber-optic implants. By exposing different neurons, it was found that the greatest therapeutic effect was achieved not by stimulation of cells of a certain cell type, but by affecting the activity of the axons that connect them. When the axons were stimulated by light with high blink frequencies, the mice behaved normally; when blinking was stopped, symptoms resumed [9].

Researchers from another laboratory are developing methods for helping patients with paralysis and traumatic brain damage and believe that optogenetic tools will find practical application, from restoration of limb movements that have been paralyzed due to stroke, craniocerebral injuries, or spinal-cord trauma to fighting spasticity that has been induced by cerebral paralysis [27].

By using optical stimulation, the natural order of contraction of fibers of motor nerves was restored. Earlier, to restore lost motor functions electrical stimulation was used. This method made it possible for paralyzed people to move during several minutes. However, it should be taken into account that large nerve fibers are more sensitive to electrical stimulation and, thus, muscle contraction can occur in the wrong order—first, large fast twitch fibers and, then, small slow twitch fibers, which results in convulsive movements that cause rapid fatigue. Then, an optical cuff that was covered by tiny LEDs, which were directed inward, was made and located around the sciatic nerve of bioengineering animals. Optical stimulation lead to the right order of contraction of muscle fibers, including contractions that are similar to those that occur under normal conditions. Under optical stimulation, muscles retained about one-third of their initial maximum strength for 20 min and remained at this level for a rather long time, whereas the same muscles were exhausted in 4 min upon electrical stimulation. Today, this approach is mainly used in different studies, but in the future it may find clinical application if a way to safely introduce genes that encode light-sensitive proteins into the human genome is found [27].

Methods of optogenetics have also been applied for studying epilepsy, in which extreme synchronous activity occurs in one or more regions of the brain, which can lead to seizures. Epileptic seizures may occur due to the loss of inhibitory interneurons or as a result of rearrangement or improper strengthening of excitation pathways. In any case, the use of opsin alleviates aberrant rhythmic activity of neurons during seizures, which may have a positive therapeutic effect. For example, activation of NpHR in organotypic hippocampal cultures, in which convulsive activity was induced by electrical stimulation, reduced epileptiform peaks [34]. Moreover, in mice with modeled temporal-lobe epilepsy, the activation of subpopulations of GABAergic interneurons or inhibition of excitatory pyramidal cells under natural conditions resulted in rapid cessation of seizures [25]. Sometimes, epilepsy can occur due to rearrangement of long-distance connections within the brain as a result

of a stroke. In rats, an optogenetic orientation of thalamus neurons that are connected with cortex areas that are prone to epilepsy also reduces seizures [30].

Using optogenetics, gene combinations that together can lead to the occurrence of psychiatric disorders were identified. The investigation of unifying hypotheses that might help to explain how discrete psychiatric symptoms can result from diverse multigene effects is promising. For example, research on the genetics of schizophrenia and autism sequentially pointed to the presence of genes that are involved in the regulation of the balance of excitation in the brain. These data coincide with optogenetically obtained conclusions that the alterations of excitability in certain cells or projections can specifically modulate social behavior in mice. Preclinical optogenetic experiments have also revealed certain cells and pathways that can modulate anxiety, depression, and eating and behavioral disorders, along with many other matters, which is of great significance for psychiatry [8, 20].

These results can be applied in clinics differently. First, understanding of psychiatric symptoms at this level makes it possible to make more complex pathophysiological hypotheses and give a better understanding of the etiology. Second, by combining interviews with patients, testing data, and personalized genomic studies, the diagnostics of psychiatric disorders may be significantly balanced, which would improve both prophylaxis and treatment. Third, knowledge of cells and their projections that are involved in the development of psychiatric symptoms promotes identification of clinically significant biomarkers, which can change not only the diagnosis, but also the prognosis of the results of the treatment. Fourth, it can be used for rapid identification of novel targets for drugs or brain stimulation as a treatment. This may lead to significant improvement in the understanding of causal relationships in pathophysiology [8].

CONCLUSIONS

Thanks to optogenetics, it has become possible to reduce or enhance the activity of certain neuronal populations in different brain regions. Thus, finding cause-and-effect relationships between neuronal networks and symptoms of pathologies using optogenetic tools may help in the development of effective therapy and lead to an increase in the effectiveness of drugs and occurrence of new less invasive surgeries for treatment of neurodegeneration disorders, as well as make it possible to broaden neurological, biological, and psychiatric studies.

ACKNOWLEDGMENTS

This study was supported by the Federal Targeted Program "Research and Development in High-Priority Areas of Development of the Scientific and Technological Complex of Russia (2014–2020)" on the topic "The Development of Methods, Technologies, and Platforms to Study the Functioning of the Nervous System Based on Creation of a High-Resolution Informational Model of Cortical Brain Structures," agreement no. 14.581.21.0016, unique identifier RFMEFI58115X0016.

REFERENCES

1. Adamantidis, A.R., Zhang, F., Aravanis, A.M., Deisseroth, K., et al., Neural substrates of awakening probed with optogenetic control of hypocretin neurons, *Nature,* 2007, vol. 450, pp. 420–424.
2. Airan, R.D., Thompson, K.R., Fenno, L.E., Bernstein, H., and Deisseroth, K., Temporally precise in vivo control of intracellular signaling, *Nature,* 2009, vol. 458, pp. 1025–1029.
3. Amos, W.B. and White, J.G., How the confocal laser scanning microscope entered biological research, *Biol. Cell,* 2003, vol. 85, pp. 335–342.
4. Arenkiel, B.R., Peca, J., Davison, I.G., Feliciano, C., et al., In vivo light-induced activation of neural circuitry in transgenic mice expressing channelrhodopsin-2, *Neuron,* 2007, vol. 54, pp. 205–218.
5. Bendtsen, J.D., Nielsen, H., von Heijne, G., and Brunak, S., Improved prediction of signal peptides, *J. Mol. Biol.,* 2004, vol. 340, pp. 783–795.
6. Bi, A., Cui, J., Ma, Y.P., Olshevskaya, E., et al., Ectopic expression of a microbial-type rhodopsin restores visual responses in mice with photoreceptor degeneration, *Neuron,* 2006, vol. 50, pp. 23–33.
7. Chow, B.Y., Han, X., Dobry, A.S., Qian, X., et al., High-performance genetically targetable optical neural silencing by light-driven proton pumps, *Nature,* 2009, vol. 463, pp. 98–102.
8. Deisseroth, K., Etkin, A., and Malenka, R.C., Optogenetics and the circuit dynamics of psychiatric disease, *JAMA,* 2015, vol. 313, no. 20, pp. 2019–2020.
9. Deisseroth, K., Optogenetics, *Nat. Methods,* 2011, vol. 8, pp. 26–29.
10. Derby, M.C. and Gleeson, P.A., New insights into membrane trafficking and protein sorting, *Int. Rev. Cytol.,* 2007, vol. 261, pp. 47–116.
11. Duschl, A., Lanyi, J.K., and Zimanyi, L., Anion binding to the chloride pump, halorhodopsin, and its implications for the transport mechanism, *J. Biol. Chem.,* 1990, vol. 265, pp. 1261–1267.
12. Evans, C.L., Potma, E.O., Puoris'haag, M., Cote, D., et al., Chemical imaging of tissue in vivo with video-rate coherent anti-Stokes Raman scattering microscopy, *Proc. Natl. Acad. Sci. U. S. A.,* 2005, vol. 102, pp. 16807–16812.
13. Figueiredo, M., Lane, S., Tang, F., Liu, B.-H., Hewinson, J., et al., Optogenetic experimentation on astrocytes, *Exp. Physiol.,* 2010, vol. 96, pp. 40–50.
14. Gradinaru, V., Thompson, K.R., Zhang, F., Mogri, M., et al., Targeting and readout strategies for fast optical neural control in vitro and in vivo, *J. Neurosci.,* 2007, vol. 27, no. 52, pp. 14231–14238.
15. Gradinaru, V., Thompson, K.R., and Deisseroth, K., eNpHR: A *Natronomnas* halorhodopsin enhanced for optogenetic applications, *Brain Cell Biol.,* 2008, vol. 36, nos. 1–4, pp. 129–139.

16. Gradinaru, V., Mogri, M., Thompson, K.R., Henderson, J.M., and Deisseroth, K., Optical deconstruction of parkinsonian neural circuitry, *Science,* 2009, vol. 324, pp. 354–359.
17. Hardie, R.C. and Raghu, P., Visual transduction in *Drosophila*, *Nature,* 2001, vol. 413, pp. 186–193.
18. Hägglund, M., Borgius, L., Dougherty, K.J., and Kiehn, O., Activation of groups of excitatory neurons in the mammalian spinal cord or hindbrain evokes locomotion, *Nat. Neurosci.,* 2010, vol. 13, pp. 246–252.
19. Hong, M., Fitzgerald, M.X., Harper, S., Luo, C., Speicher, D.W., and Marmorstein, R., Structural basis for dimerization in DNA recognition by Gal4, *Structure,* 2008, vol. 16, no. 7, pp. 1019–1026.
20. Iossifov, I., O'Roak, B.J., Sanders, S.J., Ronemus, M., Krumm, N., et al., The contribution of de novo coding mutations to autism spectrum disorder, *Nature,* 2014, vol. 515, no. 7526, pp. 216–221.
21. Kato, H.E., Zhang, F., Yizhar, O., Ramakrishnan, C., Nishizawa, T., et al., Crystal structure of the channelrhodopsin light-gated cation channel, *Nature,* 2012, vol. 482, pp. 369–374.
22. Kennedy, M.J., Hughes, R.M., Peteya, L.A., Schwartz, J.W., Ehlers, M.D., and Tucker, C.L., Rapid blue-light-mediated induction of protein interactions in living cells, *Nat. Methods,* 2010, vol. 7, no. 12, pp. 973–975.
23. Kienle, E., et al., A toolbox of Cre-dependent optogenetic transgenic mice for light-induced activation and silencing, *Nat. Neurosci.,* 2012, vol. 15, pp. 793–802.
24. Kohl, M., Shipton, O., Deacon, R., Rawlins, J., Deisseroth, K., and Paulsen, O., Hemisphere-specific optogenetic stimulation reveals left-right asymmetry of hippocampal plasticity, *Nat. Neurosci.,* 2011, vol. 14, pp. 1413–1415.
25. Krook-Magnuson, E., Armstrong, C., Oijala, M., and Soltesz, I., On-demand optogenetic control of spontaneous seizures in temporal lobe epilepsy, *Nat. Commun.,* 2013, vol. 4, p. 1376.
26. Liu, X. and Davis, R.L., Insect olfactory memory in time and space, *Curr. Opin. Neurobiol.,* 2006, vol. 16, pp. 679–685.
27. Llewellyn, M.E., Thompson, K.R., Deisseroth, K., and Delp, S.L., Orderly recruitment of motor units under optical control in vivo, *Nat. Med.,* 2010, vol. 16, pp. 1161–1165.
28. Nagel, G., Szellas, T., Huhn, W., Kateriya, S., Adeishvili, N., et al., Channelrhodopsin-2 a directly light-gated cation-selective membrane channel, *Proc. Natl. Acad. Sci. U.S.A.,* 2003, vol. 100, no. 24, pp. 13940–13945.
29. Palczewski, K., G protein-coupled receptor rhodopsin, *Annu. Rev. Biochem.,* 2006, vol. 75, pp. 743–767.
30. Paz, J., Davidson, T., Frechette, E., Delord, B., Parada, I., Peng, K., et al., Closed-loop optogenetic control of thalamus as a tool for interrupting seizures after cortical injury, *Nat. Neurosci.,* 2013, vol. 16, pp. 64–70.
31. Petreanu, L., Huber, D., Sobczyk, A., and Svoboda, K., Channelrhodopsin-2–assisted circuit mapping of long-range callosal projections, *Nat. Neurosci.,* 2007, vol. 10, pp. 663–668.
32. Richter, C.-P. and Tan, X., Photons and neurons, *Hear. Res.,* 2014, vol. 311, pp. 72–88.
33. Shimizu-Sato, S., Huq, E., Tepperman, J.M., and Quail, P.H., A lightswitchable gene promoter system, *Nat. Biotechnol.,* 2002, vol. 20, no. 10, pp. 1041–1044.
34. Tønnesen, J., Sørensen, A., Deisseroth, K., Lundberg, C., and Kokaia, M., Optogenetic control of epileptiform activity, *Proc. Natl. Acad. Sci. U.S.A.,* 2009, vol. 106, pp. 12162–12167.
35. Tsai, H.C., Zhang, F., Adamantidis, A., Stuber, D.G., et al., Phasic firing in dopaminergic neurons is sufficient for behavioral condition, *Science,* 2009, vol. 324, no. 5930, pp. 1080–1084.
36. Wang, X., Chen, X., and Yang, Y., Spatiotemporal control of gene expression by a light-switchable transgene system, *Nat. Methods,* 2012, vol. 9, no. 3, pp. 266–269.
37. Yin, T. and Wu, Y., Guiding lights: recent developments in optogenetic control of biochemical signals, *Pfluegers Arch.,* 2013, vol. 465, pp. 397–408.
38. Yizhar, O., Fenno, L.E., Davidson, T.J., Morgi, M., and Deisseroth, K., Optogenetics in neuronal systems, *Neuron,* 2011, vol. 71, pp. 9–34.
39. Zeng, H. and Madisen, L., Mouse transgenic approaches in optogenetics, *Prog. Brain Res.,* 2012, vol. 196, pp. 193–213.
40. Zhao, S., Cunha, G., Zhang, F., Liu, Q., et al., Improved expression of halorhodopsin for light-induced silencing of neuronal activity, *Brain Cell Biol.,* 2008, vol. 36, nos. 1–4, pp. 141–154.

Translated by E. Berezhnaya