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Optogenetics – New Potentials for Electrophysiology

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This review addresses the new potentials opened up by the development of optogenetic methods and the advantages of combining these with conventional electrophysiological approaches in experimental studies to resolve a wide range of neurophysiological tasks. This review includes descriptions of the main difficulties and nuances in studies using optogenetic methods and examples of technical solutions to provide for simultaneous optostimulation and recording of neuron activity.

Keywords: optogenetics, extracellular neuron recording, optrodes, silicon probes, microdiodes, wireless optostimulation and neuron recording systems.

Introduction. Barely more than ten years have passed since the appearance of the first reports demonstrating the capacities of optogenetics in studies with living systems [Zemelman et al., 2002; Zemelman et al., 2003; Boyden et al., 2005; Nagel et al., 2005]. Optogenetic methods have now entered wide use and development in relation to neurophysiological tasks and in the near future optogenetic tools will be further improved, opening up new possibilities for researchers [Deisseroth, 2009; Boyden, 2015; Deisseroth, 2015].

The main advantages of optogenetic methods are their specificity for cells with phenotypes defined by genetic constructs and the ability to control the dynamics of excitatory and inhibitory processes with millisecond precision using light-sensitive membrane channels, i.e., rhodopsins. The first experiments using optogenetics were conducted in invertebrates: flies (*Drosophila melanogaster*) and nematodes (*Caenorhabditis elegans*) [Nagel et al., 2005; Zemelman et al., 2002; Husson et al., 2013]. Limitations have now been overcome and virus constructs have been developed for effective studies on vertebrate brains [Han et al., 2009; Lu et al., 2015; Galvan et al., 2016]. Furthermore, there are several specialized strains of transgenic mice [Madisen et al., 2012] and rats [Witten et al., 2011] for studies using optogenetic methods. The application of optogenetic in combination with other research method has widened the boundaries of our understanding. Interesting results have been obtained using optogenetic methods in combination with other experimental approaches: extracellular and intracellular recording of neuron activity, recording of local potentials, EEG, neuroimaging, fMRI, and behavioral testing [Deisseroth, 2009; Boyden, 2015; Deisseroth, 2015].

In combination with electrophysiological methods and behavioral testing in animals in different experimental conditions, optogenetics allows individual groups of neurons to be identified, manipulated, and recorded in experiments in the living body, with proposals for the functions of these cells, identification of their roles in processes observed in the brain, and studies of interactions in neural networks.

The use of optogenetics in experimental studies requires careful planning of the study with clearly defined tasks. The most effective optogenetic methods are found in situations in which very specific questions are asked and "delicate" tools with actions addressed to target cells are needed. In addition, optogenetic methods, like other experimental approaches, have their limitations and "underwater stones" and, despite the fashion for optogenetics in current neurophysiological research, these methods must be used with caution [Allen et al., 2015; Boyden, 2015].

This review will consider new potentials opened up by the development of optogenetic methods and the advantages of combining them with conventional electrophysiological approaches in experimental studies addressing a wide range of neurophysiological tasks.

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Optogenetic Methods. Selection of Rhodopsin. Thus, we will start from the position that you the researcher have decided to use optogenetic methods in your studies to test a specific hypothesis. Where do you start? Firstly, define what effect you want to obtain on target cells and, therefore, which type of light-sensitive transmembrane protein – rhodopsin – needs to be expressed and inserted into the target cell membrane.

Opsins are classified into two types: microbial-type opsins, type 1, which are found in prokaryotes, fungi, and algae and are responsible in living nature for phototaxis reactions, metabolic regulation, and ATP synthesis [Yizhar et al., 2011b]. Animal-type opsins, type 2, are found only in highly organized eukaryotes and are responsible for vision, the regulation of circadian rhythms, and pigmentation [Sakmar, 2002; Shichida and Yamashita, 203; Yizhar et al., 2011b]. Microbial opsins are used in optogenetics because the synthesis of these protein channels is encoded by a single gene which can be incorporated into virus constructs, thus driving the expression of rhodopsins in virtually any cell of the body, including neurons in the brain.

Currently existing rhodopsins can be classified into nonselective cation transmembrane channels (*channelrhodopsin-1* (ChR1) and *channelrhodopsin-2* (ChR2)), anionic channels (*anion channelrhodopsins* (ACRs)), chloride pumps (*halorhodopsin* (NpHR)), proton pumps (*bacteriorhodopsin* (BR)), sensors (*sensory rhodopsin I* (SR-I) and *phoborhodopsin* (SR-I)), and metabolic channels (*G-protein-coupled receptors optoXRs* (opsin-receptor chimeras)). Detailed descriptions of rhodopsins have been provided in various reviews [Govorunova et al., 2017; Yizhar et al., 2011b; Adamantidis et al., 2014; Klapoetke et al., 2014; Dufour and DeKoninck, 2015; Glock et al., 2015; Cho and Li, 2016; Govorunova and Koppel, 2016; Govorunova et al., 2017].

Inhibition of target cells is obtained using proton or chloride pumps, which on activation by light start to pump H^+ out of cells or Cl^- ions into them, respectively [Han and Boyden, 2007; Zhang et al., 2007a; Chowlet et al., 2010; Gradinaru et al., 2010; Han et al., 2011; Chuong et al., 2014]. Both processes lead to hyperpolarization of target cells.

Selective anionic light-sensitive channels are currently under active development for inhibition of neurons; these include modified rhodopsins – *inhibitory C1C2* (iC1C2) and *slow chloride-conducting channelrhodopsin* (SloChloC) [Berndt et al., 2014; Wietek et al., 2015] or the rhodopsin versions recently discovered in algae – *anion channelrhodopsins* GtACR2, PsACR1, ZipACR [Govorunova et al., 2017; Govorunova et al., 2015; Wietek et al., 2016; Govorunova et al., 2016; Govorunova et al., 2017]. These rhodopsins have chloride permeability and are potentially effective for in vivo experiments [Berndt and Deisseroth, 2015; Dolgikh et al., 2015; Govorunova and Koppel, 2016; Malyshev et al., 2017; Mohammad et al., 2017].

A wide arsenal of modified *ChR2* rhodopsins have been developed for excitation of neurons with different characteristic dynamics of the open/closed states and light spectra for activation/inactivation [Boyden et al., 2005; Nagel et al., 2005; Yizhar et al., 2011b; Klapoetke et al., 2014]. For example, activation of *ChR2134H* channels with brief pulses of blue light (wavelength 450 nm) induces fast cell depolarization, while activation of another modified rhodopsin – *ChR2128 SSFO* (*stable step function opsin*), with slow channel opening kinetics – leads to a prolonged state of elevated arousability of neurons, lasting up to half an hour Yizhar et al., 2011a; Adamantidis et al., 2014]. ChR2128 SSFO can be inactivated with pulses of yellow light (590 nm).

Depending on the kinetics of rhodopsin, i.e., the channel closure time (τ_{off}), different modifications of rhodopsin can effectively provide optostimulation at frequencies ranging from 10–60 Hz and all the way up to 200 Hz [Gunaydin et al., 2010; Yizhar et al., 2011a; Klapoetke et al., 2014]. High-frequency optostimulation can be relevant if your target cells are, for example, interneurons.

If solution of your scientific task requires activation or hyperpolarization of several groups of neurons independently of each other, you can use, along with the "standard" ChR2, which is activated by blue light (450 nm), other modifications of rhodopsin, *ReaChR*, *ChrimsonR*, or *VChR1*, activation of which is shifted to the yellow-red part of the spectrum (630 nm), so-called "red-shifted" rhodopsins [Klapoetke et al., 2014], and *Jaws* halorhodopsin [Chuong et al., 2014].

In addition, red light penetrates further into tissues than blue light, such that larger numbers of target neurons can be influenced [Han, 2012]. This may be one argument in favor of selecting "red" rhodopsins for your experiment.

If influences on intracellular signal cascades are needed, the group of *optoXR* receptors (*G-protein-coupled receptors*) can be used [Airan et al., 2009; Yizhar et al., 2011a, b].

Delivery of Opsins to Target Cells. Delivery of opsin genes to target cells uses a variety of methods: transgenic animal strains [Witten et al., 2011; Madisen et al., 2012; Zeng and Madisen, 2012; Oh et al., 2016], in utero electroporation [Gradinaru et al., 2007; Malyshev et al., 2017], and introduction of lentivirus and adeno-associated virus constructs [Yizhar et al., 2011a; Han, 2012; Carter and Shieh, 2015; Tervo et al., 2016; Sizemore et al., 2016].

The advantages of transgenic animal strains are that your desired rhodopsin is expressed in the whole population of target neurons, for example, *ChR2* is expressed in cortical pyramidal cells and excitatory hippocampal, thalamic, and midbrain cells in the transgenic mouse strain *Thy1-ChR2-YFP* [Arenkiel et al., 2007; Wang et al., 2007]. The zone for potential implantation of recording electrodes and optic fibers for stimulation in these animals is restricted only by the sizes of the areas of interest (a zone of the neocortex or a small nucleus) and the experimental tasks. More local expression of rhodopsin in a cell population can be achieved by intracerebral administration of virus constructs.

Viral transfection is the most widely used method for delivering opsins and regulatory genes to target cells. Lentivirus (LV) or adeno-associated virus (AAV) constructs are generally used [Carter and Shieh, 2015]. Along with the gene responsible for rhodopsin synthesis, the virus vector contains a series of regulatory promoters driving the expression of rhodopsin only in certain cells, as well as a gene for a fluorescent protein operating as a marker for infection, e.g., *Green fluorescent peptide* (GFP) or *Td tomato*. Thus, the *Ca2/calmodulin-dependent kinase II* (CaMKIIa) promoter drives rhodopsin expression in pyramidal glutamatergic neurons and the *Tyrosine hydroxylase* (TH) promoter leads to expression only in dopaminergic and noradrenergic neurons [Yizhar et al., 2011a; Sizemore et al., 2016]. Promoters have nucleotide sequences of different lengths, creating limitations for their inclusion into, for example, adeno-associated virus vectors.

AAV has a capacity of about 4700 b.p., so only small regulatory genes can be included in AAV vectors. However, AAV has a number of advantages, namely: small virus particles, high concentration of virus particles per unit volume (high titer), and milder immune responses in tissues than lentiviruses; these allow AAV virus particles to travel further from the injection site and produce effective infections [Yizhar et al., 2011a; Carter and Shieh, 2015].

More capacious lentivirus constructs can include 9–16 kbp and ready virus particles are large in size. However, the areas of infection is smaller than with AAV and the level of rhodopsin expression is lower [Yizhar et al., 2011a; Sizemore et al., 2016]. Lentivirus constructs are more suitable for tasks requiring limited local expression of rhodopsin in small brain structures. Lentivirus and AAV vectors insert into the genomes of target cells and support stable, long-term expression of rhodopsins [Carter and Shieh, 2015].

The serotype – the overall set of receptor proteins on the surface of the viral capsid – plays a very important role in producing specific infection [Castle et al., 2016; El-Shamayleh et al., 2016]. Thus, the AAV serotype 2.1 has been shown to be effective in infecting neurons in the brains of rats and mice, while AAV serotypes 8 and 9 are better at infecting neurons in the brains of primates [Masazimu et al., 2011] and serotype AAV5 also infects glial cells [Castle et al., 2016].

Receptor proteins on the surfaces of virus particles determine how target cell infection will occur: via the "anterograde" or "retrograde" routes, i.e., where the "entry gate" for the virus will be located – in the cell body or via the distal parts of the neuron. Transsynaptic anterograde and retrograde infection can also be identified [Beier et al., 2011]. For example, populations of neurons sending projections to particular brain structures can be identified using *rAAV2-retro* [Tervo et al., 2016] or lentivirus (*pseudotype lentiviral vector*) constructs. The composition of the lentivirus envelope should include fragments of the glycoprotein coat of the rabies virus and vesicular stomatitis virus, which promote infection and subsequent retrograde transport of the genetic construct to the cell body and nucleus [Carpentier et al., 2012; Beier et al., 2016]. In this situation, expression of rhodopsin and fluorescent marker proteins (if not additionally restricted by specific promoters) will be seen not only at the virus suspension injection site, abut also in target neurons projecting to this area.

If the scientific task requires good expression of rhodopsin not only in neuron bodies, but also in the terminals (for example, if you are planning optostimulation of terminals), a careful approach to selecting rhodopsins is needed, as not all rhodopsins are well transported to the distal parts of neurons. After injection of virus, an average of 2–3 weeks are needed for synthesis and insertion of the required quantity of rhodopsin channels into neuron cell membranes to obtain a response to optostimulation.

For specific infection of target cells, optogenetics makes active use of *Cre-LoxP* (*Cre-LoxP recombination technology*), *FLP-FRT*, and *DIO* (*double-inverse orientation or FLEX construct*) methods [Kühn and Torres, 2002; Carter and Shieh, 2015]. The defined nucleotide sequence of the *LoxP* site is introduced into the DNA. The genes located between these *LoxP* sites are manipulated using the *Cre* enzyme. *Cre* enzyme is a recombinase which recognizes *LoxP* sites in DNA. Depending on the orientation of the *LoxP* sequence, the recombinase either removes a block of nucleotides from the DNA between the *LoxP* sites or inverts this region.

The *FLP-FRT* system is analogous to the *Cre-LoxP* system. *FLP* recombinase (*Flippase*) recognizes identical *FRT* (*Flippase recognition targets*) sites and inverts the DNA region between them.

The *DIO* (*double-inverse orientation*) or *FLEX* system is based on *Cre-LoxP* technology and is used in creating AAV vectors for makig *Cre*-dependent systems to control rhodopsin expression in target cells [Saunders et al., 2012]. This technology can be used to regulate rhodopsin expression in cells in conditions in which the target cells have very specific properties and it is difficult to control rhodopsin expression with a single regulatory gene promoter or the promoter has too large a nucleotide sequence for construction of an AAV vector, or the promoter is weak and does not drive the required level of rhodopsin expression.

In is most general form, the *FLEX* system (*DIO* system) can be described as follows: the AAV vector contains double *LoxP* sites on the regulatory genes or the opsin gene itself and it is only when the infected cell contains *Cre* recombinase, which transforms the virus vector DNA after transfection, that the opsin gene becomes available for subsequent transcription and translation and, thus, for synthesis of rhodopsin. This scheme is used in transgenic animals in which *Cre* recombinase is synthesized in defined neuron populations. For example, in rats of the transgenic strain *PV::Cre*, parvalbumin (*PV*)-expressing interneurons also express *Cre* recombinase. When an AAV vector is introduced using the *ChR2* and *FLEX* system, *ChR2* expression

will only be seen in these rats in *PV* interneurons in the area into which the virus suspension is injected.

Another strategy consists of coinfection of target cells using an AAV vector containing the *Cre* recombinase gene under control of a promoter operating specifically in the target cells and an AAV vector with the FLEX system and opsin.

Thus, virus constructs can be used not only to introduce genes for the desired rhodopsin into cells, but also specific combinations of different methods can be used to control the expression of this rhodopsin in target cells.

Optostimulation. An important stage in planning experiments using optostimulation is selection of the type of optic fiber, light source, and optostimulation parameters to create specific experimental conditions.

Delivery of light to brain tissue is via glass or plastic optic waveguides – optic fibers [Warden et al., 2014; Bartic et al., 2016]. The basic construction of the optic fiber (for example, made by Doric Lenses, Thorlabs, Plexon Inc.) with round cross sections consists of glass or polymer cores with a refractive index of about 1.5. The core is surrounded by a sheath (cladding) with lower refractive index to ensure complete internal refraction. Thus, light waves propagate quickly through the core of the optic fiber with minimal losses. The optic fiber is also coated with a protective plastic sheath.

The optic fiber may be unimodal with a core diameter of up to 10 μm or multimodal with a core of 50–400 μm. The main difference between them is that light in monomodal optic fibers is propagated virtually parallel to the central axis of the core, while light rays in multimodal fibers can simultaneously pass through different angles of refraction.

Another important feature of optic fibers is the numerical aperture (NA), which characterizes the maximum angle of divergence of light rays from the axis of the optic fiber at the output, i.e., the angular size of the area of propagation of light from the end of the optic fiber. The smaller the diameter of the core and the lower the numerical aperture, the narrower the light beam when it exits the optic fiber. Monomodal optic fibers are suitable for tasks requiring small areas of stimulation of the order of 0.1–0.2 mm^3 from the tip of the optic fiber, for example within the recording sites of silicon probes or for stimulation of a small number of close-lying neurons [Bartic et al., 2016]. Monomodal optic fibers with large diameters and numerical apertures NA of 0.3–0.5 have been used successfully for optostimulation of neurons in volumes of 0.5–1 mm^3 from the tip of the optic fiber [Aravanis et al., 2007; Yizhar et al., 2011a].

Selection of the type of optic fiber also depends on the light source which you intend to use in the experiment. The light source can be a laser (Dream lasers) or LED diodes (e.g., Thorlabs, DC2100), which are connected to the optic fiber implanted into the brain via an adapter and rotary contact (fiber optic rotary joint) [Adamantidis et al., 2007; Aravanis et al., 2007; Yishar et al., 2011a; Warden et al., 2014]. Apart from miniature LED diodes (high power micro light-emitting diodes (μ-LED)) [Kim et al., 2013; Kwon et al., 2014; Warden et al., 2014; Iseri and Kuzum, 2017] or laser diodes (for example, Osram) [Stark et al., 2012] can also be used.

The transmission effectiveness of the light flux when the optic fiber is connected to the light source and at all connection points between the optic fiber and the adapters and rotary contacts and at the silicon probe is one of the main challenges of contemporary optic interfaces. As soon as the cladding is damaged, light leakage and loss occur and the output intensity (power) of the light flux decreases.

Lasers are powerful coherent monochromatic light sources; when connected with monomodal optic fibers of diameter 10 μm, only around 25% of the light beam enters the optic fiber. The efficiency of light transmission increases to 70–80% when multimodal light fibers of diameter 50 μm and more are used [Bartic et al., 2016]. LEDs and laser diodes are noncoherent light sources and their use is more effective using multimodal optic fibers.

The power of the light flux in LEDs and laser diodes depends on the current passed through the diode. Controlling current strength provides for relatively fast changes in optostimulation parameters: the intensity (power) of the light stimulus, as well as its frequency and duration. In the case of lasers, control of optostimulation parameters requires tuning of special gates – shutters – which introduces an additional complication [Warden et al., 2014].

The advantages of laser and LEDs are their availability and low cost as compared with lasers. The drawback as compared with lasers is the occurrence of oscillations in light output intensity (power).

The intensity of the light flux at the output of the optic fiber is a very important parameter for optostimulation. This depends on the power of the light source, the diameter of the optic fiber, and the effectiveness of the optic fiber coupling at all the junctions in the apparatus: from the light source to the rotary joint and the adapter on the animal's head. The power of the output light flux is measured on a fully assembled system including the part of the optic fiber which will be implanted into the brain, using light power meters [Yizhar et al., 2011a]. The resulting intensity can be taken as the ratio of the light output power to the cross-sectional area of the core of the optic fiber and is measured in mW/mm^2. Effective stimulation of rhodopsins requires power levels of 1–10 mW/mm^2 depending on the type of rhodopsin or the experimental task [Boyden et al., 2005; Aravanis et al., 2007; Adamantidis et al., 2014; Dufour and De Koninck, 2015]. For example, activation of *ChR21218 SSFO* requires a light intensity 2–3 orders of magnitude less (<0.01 mW/mm^2) [Adamantidis et al., 2014].

Zhang et al. identified a limit to light intensity at 300 mW/mm^2 [Zhang et al., 2011]; data have been obtained showing that brain tissue damage is produced by levels of 100 mW/mm^2 [Cardin et al., 2010].

This damage is linked with local increases in temperature [Shin et al., 2016].

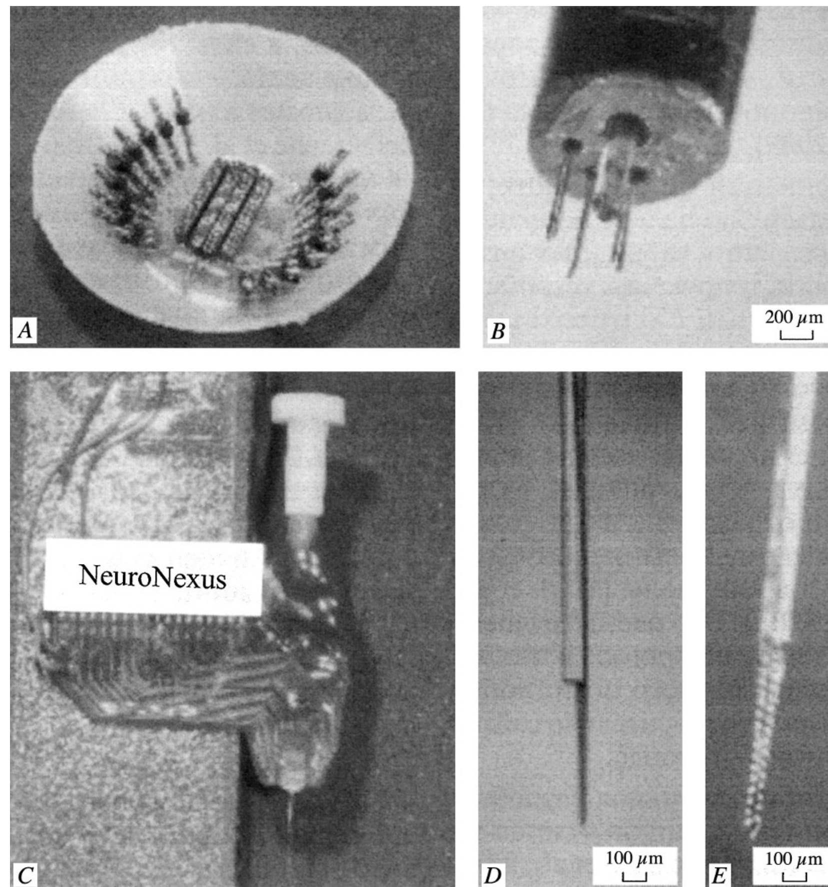

Fig. 1. Devices for optostimulation and recording of neuron activity. Optodrive with 16 microdrives for tetrodes and two microdrives for optic fibers, plan view (*A*). Implanted part of the optodrive with six tetrodes and one optic fiber (*B*). Silicon probe, external view (*C*); tip of silicon probe with 32 neuron activity recording sites and one optic fiber (*D*, *E*).

Light intensity decreases with increases in the distance from the tip of the optic fiber, which is associated with scattering and absorption of light by brain tissue. Experimental data indicate that the light intensity is adequate for activation of rhodopsins in brain tissue volumes of the order of 0.5 mm^3 from the tip of the optic fiber using multimodal optic fibers with large numerical apertures; the volume is even smaller for monomodal optic fibers [Aravanis et al., 2007; Han et al., 2009; Wu et al., 2013].

Various methods have been developed for manipulating the volume of propagation light in brain tissue, for example, the optic fiber can be cut at an angle or the tip can be made thinner by etching with hydrofluoric acid [Royer et al., 2010; Pisanello et al., 2014; Hanks et al., 2015; Dufour and De Koninck, 2015].

Abaya et al. described an interesting development for "volumic 3D" optostimulation – a glass matrix (3D glass optrode array), use of which can also increase the volume of brain tissue involved in optostimulation [Abaya et al., 2012].

The optostimulation frequency parameter varies for different types of rhodopsins and are defined by the channel conditions and experimental tasks. For example, stimulation of *ChR2* uses frequencies of 10–20 Hz [Aravanis et al., 2007], while stimulation of halorhodopsin *NpHR* uses single stimuli lasting up to several milliseconds [Adamantidis et al., 2014]. As a rule, each experimental task involves the optostimulation frequency and the light intensity in each pulse to be selected.

Optogenetics and Electrophysiology. A variety of instruments have been under active development in neurophysiology in the last few years, providing for simultaneous optostimulation and electrophysiological recording of neuron activity in chronic conditions.

The optrode is a relatively simple and accessible device for these purposes – this is a "hybrid" electrode and optic fiber. A simple optrode is an electrode or tetrode glued to an optic fiber [Gradinaru et al., 2007; Stark et al., 2012]. Electrode tips should be positioned 0.1–0.5 mm beneath the optic fiber tip, i.e., within the cone of light propagating from the tip of the optic fiber.

The tip of the optic fiber is usually subjected to additional processing – etching with hydrofluoric acid (HF) to

make the optic fiber thinner and, thus, decreasing traumatic effects in brain tissue [Hanks et al., 2015; Royer et al., 2010]. In addition, in the treated part of the optic fiber, the reflecting layer (cladding), which usually prevents release of light to the outside of the optic fiber, is removed by treatment and lateral scattering of light increases (http://brodywiki.princeton.edu/wiki/index.php/Etcning_Fiber_Optics). Thus, the thickness of the optic fiber is decreased, as is the extent of tissue damage. In this case, the tip of the recording electrode can be positioned within the etched part of the optic fiber and neuron activity can be recorded in the light propagation zone.

One example of optimization of the basic construction of an optrode is the model developed in the laboratory of Anikeeva [Anikeeva et al., 2011; Canales et al., 2015; Park et al., 2017]. The optetrode created using new technologies, biocompatible composite materials, and polymers, is an optic fiber surrounded by electrodes (tetrodes) for electrophysiological recording and additional channels for introducing viruses or pharmacological agents (microfluidic channels) [Canales et al., 2015; Park et al., 2017]. Electrodes, optic fibers, and fluidic channels are joined, Heating and subsequent stretching, to form a multifunctional flexible polymer fiber of diameter no greater than 200 μm, which can be used to introduce viruses and for subsequent optostimulation and recording of neurons from the virus administration zone.

Another optetrode version consists of tetrodes surrounded by a light-conducting sheath [Lin et al., 2012; Canales et al., 2015]. Optodrives – microdrives with several electrodes/tetrodes and a large-diameter (of the order of 200 μm) multimodal optic fiber – are quite widely used as a solution to the challenge of recording large numbers of neurons [Voigts et al., 2013; Bartic et al., 2016; Freedman et al., 2016; Liang et al., 2017] (Fig. 1, *A*, *B*). The microdrive includes a mechanism providing for independent fine adjustment of electrode tip and optic fiber positions for optimal recording of activity during experiments. Unfortunately, this advantage hides the "weak points" of use of microdrives for positioning implants for optostimulation and recording. In this construction, it is very difficult to control the distance between the optic fiber tip and the electrode tips in the brain. The experimenter cannot be sure that the cell activity recording sites are within the area of propagation of light from the optic fiber and, thus, that the changes seen in neuron activity are due to the direct actions of light on rhodopsins in the membranes of these cells and not to the network effects of optostimulation [Wu et al., 2013].

Microdrives therefore use large-diameter optic fibers with numerical aperture NA = 0.3–0.5, such that the closest electrodes are within the 0.5-mm^3 volume of light propagation and the power of the light is sufficient to excite rhodopsins in the membranes of neurons close to the recording site.

The light propagation area can be enlarged by increasing the intensity (power) of the light flux. An increase in light intensity to more than 100 mW/mm^2, as shown above, can also lead to local increases in temperature and damage to brain tissue and induce electrophysiological artifacts [Cardin et al., 2010]. However, large-diameter optic fibers (200 μm and more) can also produce damage to brain tissue in the neuron recording zone [Wu et al., 2013].

Chen et al. developed a multi-optrode array starting with an optic fiber partially coated with a metal – gold – conducting layer and a polymer – Parylene – insulating layer [Chen et al., 2013]. The optrode tips formed a tapered fiber. Thus, the recording site was located immediately in the area in which the light beam exited the optic fiber. In this configuration, low-power light can be used, sufficient for stimulation of neurons around the optrode tip [Zhang et al., 2009].

One optrode modification which gave rise to the development of a whole new direction in the technologies of making hybrid optostimulating and recording devices was that of integrating silicon probes with the optic fiber (silicon probes with integrated optic fibers) (Fig. 1, *C–E*). The silicon probe is a multichannel microelectrode on a silicon support [Kim et al., 2013; Buzsáki et al., 2015; Iseri and Kuzum, 2017]. Studies in Buzsáki's laboratory initially glued an optic fiber with an etched tip to each silicon probe using epoxy resin [Stark et al., 2012; Pisanello et al., 2016]. This was very laborious and did not guarantee accurate microscale positioning of the optrode tip relative to the recording sites on the silicon support.

The intense development of hybrid silicon probes is currently proceeding in a number of directions [Buzsáki et al., 2015; Fan and Li, 20156; Pisanello et al., 2016]; there are also manufactured commercial devices (for example, Neuro Nexus, Cambridge NeuroTech).

Fundamental to experimental tasks involving identification and investigation of specific neurons in a population (optogenetic tagging) or the structure of local networks based on identified neurons [Stark et al., 2012; Buzsáki et al., 2015; Grosenick et al., 2015] is minimization of the network effects of optostimulation [Han et al., 2009; Wu et al., 2013] and electrophysiological artifacts occurring in response to light [Fan and Li, 2005]. These conditions can be fulfilled if the size of the light propagation area is decreased and light intensity is reduced to the level required for stimulating neurons locally in several probe recording sites.

For this purpose, hybrid silicon probes for conducting light to recording sites at probe tips use optic waveguides with rectangular cross sections and small apertures. Waveguides are integrated with large-diameter optic fibers which are in turn connected to the light source – a laser, LED, laser diode, or μ-LED [Wu et al., 2013; Schwaerzle et al., 2017]. Waveguides are made from commercial materials such as SU8 or dielectrics such as oxynitride. One disadvantage of these materials which limits their use for chronic implantation is their ability to adsorb water, which leads to changes in their optical properties [Fan and Li, 2015].

Another solution to the task of local light propagation consists of using μ-LEDs positioned in the immediate vicinity of recording sites on the part of the silicon probe im-

planted in the brain [McAlinden et al., 2013; Kim et al., 2013; Kwon et al., 2014].

One problem with the first approach is that of large losses of light at the junctions of optic fibers with different cross-sectional areas and shapes. The main problem with using μ-LEDs for direct optostimulation of neurons is the increase in diode temperature during operation and, thus, damage to brain tissue in conditions of long-term stimulation [McAlinden et al., 2013].

Kim et al. developed a multifunctional microelectrode probe with μ-LEDs on a flexible polymer base [Kim et al., 2013]. The probe has a multilayer construction where, apart from the layers with platinum electrodes and μ-LEDs, there is also a layer with temperature microsensors for real-time monitoring of local temperature changes and a layer containing light sensors to measure light intensity during the operation of the μ-LEDs. All the layers are joined together with epoxy resin and the overall rigidity of the construct during probe implantation into the brain is ensured using a matrix made of biodegradable silk, which dissolves in brain tissue during the few minutes after implantation. Thus, a flexible polymer multilayered construct is left in the brain. The power supply to the diodes and control of optostimulation can be wireless. In addition, μ-LEDs with different wavelengths, e.g., 675 nm (GaAs LED) or 450 nm (GaN LED) can be placed on the silicon probe and optostimulation of different types of rhodopsins in neurons in the probe implantation area can be delivered.

Apart from optodrives and silicon probes for optogenetic studies, matrixes made from various polymer materials have been actively developed. These can be chronically implanted on the brain surface and allow optostimulation and recording of neuron activity over larger volumes of brain tissue than silicon probes or optodrives [Zhang et al., 2009; Abaya et al., 2012; Wang et al., 2012; Kwon et al., 2014]. Matrixes can also be combined with wireless control systems [Kwon et al., 2014]. Several rows of optrodes are placed on a polymer support and are integrated with μ-LEDs. Thus, each neuron activity recording site – each electrode tip – is located within the area of light propagation from its μ-LED. Because of the dense distribution of μ-LEDs on the matrix, the areas of propagation of the emitted light overlap, giving rise to a larger area of optostimulation as compared with single optic fibers.

The further development and improvement of optrodes is running in several directions: decreases in the weight and size of constructs, flexible implanted parts, miniaturization of light sources, increases in the number of light sources, increases in the effectiveness of the combination of light sources and optic fibers without loss of power, the ability to pass light of different wavelengths through a single optic fiber, increases in the number of recording electrodes, monitoring of electrode positions close to the light source, the ability to alter the positions of electrodes and light sources using microdrives, wireless control of optostimulation and recording of neuron activity, and control of optostimulation in a closed-loop stimulation system [Grosenick et al., 2015; Bartic et al., 2016].

Guided by the tasks of our experiments and exploiting the latest advances in optogenetics, you can select viral constructs, rhodopsins, and hybrid devices for optostimulation and recording, which allows you to carry out electrophysiological studies to a new level.

Particular care is needed in relation to interpreting the effects of optostimulation on neuron activity. This is especially so in experiments whose aim is to identify neurons and the structure of local networks.

The first response of a neuron with rhodopsins inserted into the membrane to optostimulation occurs with a delay of several milliseconds, which is determined to a large extent by dynamic activation of the rhodopsin channel itself [Cardin et al., 2010]. And, as a rule, changes in neuron activity develop throughout the stimulation period. The effects seen with delays of tens of milliseconds or after the end of optostimulation, for example, so-called "rebound," may result from the network interactions of neurons [Han et al., 2009; Wu et al., 2013].

The occurrence of photoelectric artifacts in optostimulation also needs to be considered, as these can distort the electrophysiological signal. These are induced by a photoelectrochemical phenomenon, known as the Becquerel effect [Cardin et al., 2010; Fan and Li, 2015].

Conclusions. Optogenetics is currently undergoing a technological boom. New composite materials, the development of microelectronics to create wireless systems, closed-loop systems, and the development of biotechnology, genetics, and nanotechnology – all allow experimental studies of basic scientific problems to be taken to a new technical level. Optogenetic methods are helping us proceed to the next level of understanding of neurophysiological challenges and address new scientific questions. This opens up new potentials for detailed investigation of the neural networks of the brain, studies of different types of behavior, and the development of new approaches to the treatment of neurodegenerative diseases.

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