Chapter 9
Optogenetics

Allison Quach, Nicholas James, and Xue Han

9.1 Introduction

Optogenetics is the use of light and genetically encoded light-sensitive proteins to modulate the activity of specific cells. Here, we focus on the use of optogenetics in neuroscience, where it is used to rapidly activate or inhibit a specific population of neurons in the brain. The basic components required are (1) photosensitive molecules targeted to specific cells, (2) light delivery to the brain, and (3) electrophysiological or behavioral readouts for optogenetic manipulation of a system.

9.2 Microbial Opsins

Opsins were identified in bacteria and algae in the 1970s and have been studied extensively since then [1, 2], but were only recently tested in mammalian neurons for millisecond-scale control of action potentials [3]. Type I (microbial) opsins are seven-transmembrane domain proteins found in archaea, bacteria, and algae. The main classes of microbial opsins that directly convert light to ion transport are channelrhodopsins, halorhodopsins, and archaerhodopsins (Fig. 9.1). Other classes of rhodopsins recruit intracellular and secondary messenger systems that do not directly lead to ion flux.

For opsins to be photosensitive, they require an all-*trans* retinal cofactor bound to a lysine residue in the retinal binding pocket of the opsin [4]. This complex is referred to as rhodopsin. In the presence of light, all-*trans* retinal photoisomerizes to 13-*cis* retinal, resulting in conformational changes in the opsin that allow ion transport.

A. Quach • N. James • X. Han (✉)
Biomedical Engineering Department, Boston University, Boston, MA, USA
e-mail: xuehan@bu.edu

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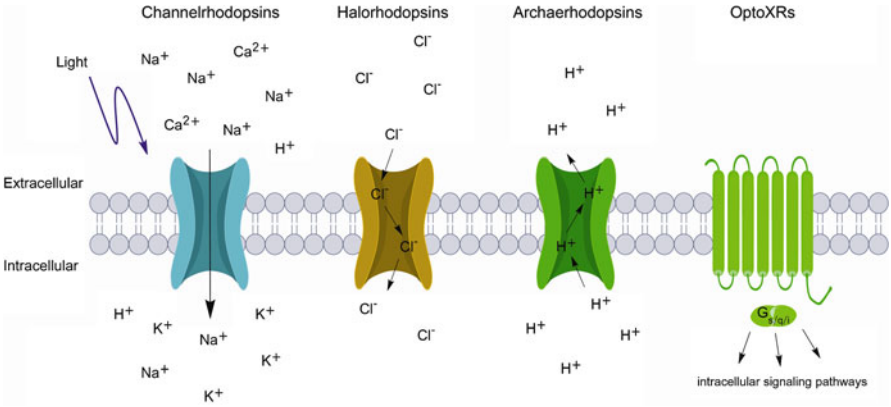

Fig. 9.1 Classes of optogenetic molecules. Channelrhodopsins are ion channels that passively transport cations such as Na^+, K^+, H^+, and Ca^{2+} down their electrochemical gradients to depolarize neurons. Halorhodopsins are chloride pumps that transport ions into the cell, resulting in hyperpolarization. Archaerhodopsins are a second class of inhibitory opsins that pump protons out of the cell. OptoXRs were developed from mammalian opsins and trigger intracellular G-protein cascades

Fortunately, it turns out that mammalian tissue contains sufficient all-trans retinal for microbial opsins to function, and the use of opsin-based optogenetic tools has been demonstrated in various mammalian brains, i.e., rodents and nonhuman primates. In some species, all-*trans* retinal needs to be supplemented for opsins to work, such as in the neural systems of *Caenorhabditis elegans* and zebrafish.

9.2.1 Excitation

Channelrhodopsin2 (ChR2) was identified from the green algae *Chlamydomonas reinhardtii* as a photoactivated ion channel [5] and was the first opsin used to demonstrate optogenetic control of mammalian neurons [3]. At low light intensities, ChR2 functions as a calcium channel [6]; however, at higher light intensities, the flux through ChR2 is predominately cations (including Na^+, K^+, H^+, and Ca^{2+}) flowing down the electrochemical gradient. One characteristics of ChR2 is that ion conduction is uncoupled from photodetection [7]. Rather, the channel closes based on channel kinetics, which makes ChR2 more efficient compared to opsins that are ion pumps.

Since the demonstration of ChR2 as a method of controlling neural activity, many variants have been developed. Mutations have increased photocurrent, varied kinetics, or shifted sensitivity spectrum [8–11].

9.2.2 Silencing

Halorhodopsins (HR) are archaebacterial chloride pumps that transport chloride ions into the cell [12]. The first used opsin in this family was Halo/NpHR, from

Natronomonas pharaonis [13–15], a yellow-light-activated opsin. However, it was poorly targeted to membranes in mammalian cells, and therefore, subsequent modifications with membrane targeting sequences were necessary to create variants (e.g., eNpHR, eNpHr3.0) that successfully inhibit mammalian neurons [16].

Unlike ChR2, Halo requires constant light to function as a pump. Halo also has slow recovery due to nonconducting intermediate protein states [17], but the use of blue light can accelerate recovery [14, 18]. Another consideration when using Halo is a potential secondary effect on GABA receptors due to the change in Cl^- concentration. It has been shown that the $GABA_A$ reversal potential can be significantly changed by activating Halo [19], increasing the excitability of modified neurons.

Archaerhodopsins (Arch) are a class of outward-moving proton pumps that are used to silence neural activity [18]. Archaerhodopsin-3, from *Halorubrum sodomense*, is a yellow-green light-activated proton pump that hyperpolarizes neurons. In contrast to Halo, Arch recovers within seconds after inactivation, allowing for finer-timescale control of photoinactivation. Subsequent variants of Arch have increased light sensitivity, allowing silencing of a larger area (ArchT; [20]), or shifted activation spectra (Mac; [18]), sensitive to green-blue light.

It has been shown that the flux of protons out of the cell does not change cellular pH [18]. This is thought to be due to intracellular buffering mechanisms; however, it is possible that the increase of protons extracellularly may have undesired effects on cells in a non-cell-type-specific manner.

9.2.3 Other

Type II (mammalian) opsins are also photosensitive seven-transmembrane domain proteins. However, they are G-protein-coupled receptors that function through second messengers, and light sensitivity is not directly coupled to ion channels. Instead, activation of type II opsins triggers intracellular G-proteins. These so-called "optoXRs" can be used to modulate intracellular signaling and investigate biochemical signaling pathways such as those downstream of alpha-1 and beta-2 adrenergic receptors [21] and the 5-HT1a receptor [22].

9.3 Opsin Targeting

Optogenetics depends on the expression of microbial opsins in a specific subset of cells. The main methods of gene delivery are viral injections and transgenic animal lines (Fig. 9.2). Opsins are targeted to a specific subset of cells using cell-type-specific promoters or anatomical projection targeting. Further specificity can be gained by spatially restricting viral or light delivery [23].

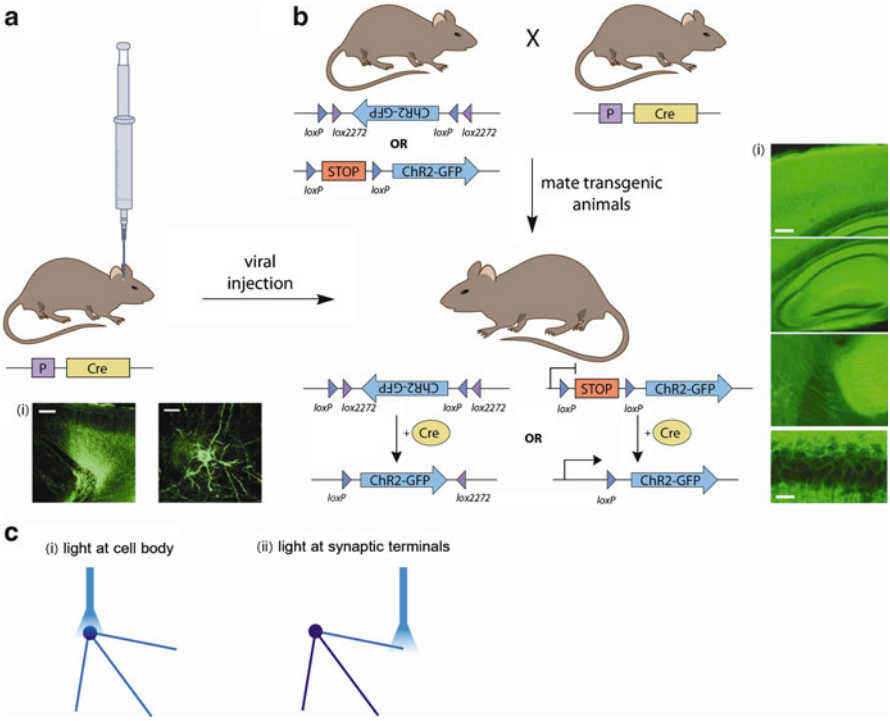

Fig. 9.2 Cell-specific targeting. (**a**, **b**) Cell-specific targeting of opsins with Cre-LoxP recombination [28]. Genes may be delivered virally into Cre-driver line animals (**a**), and Cre-dependent opsin lines and Cre-driver lines can be mated (**b**). **a**-*i* Fluorescence image of Arch-GFP expression in mouse cortex after lentiviral injection. Scale bars, 200 μm (*left*) and 20 μm (*right*). **b**-*i* Native EYFP fluorescence in transgenic ChR2(H134R)-EYFP mice. Scale bars, 200 μm (*top*) and 20 μm (*bottom*). Fluorescence images from [18], © (2010) The Author(s), and from [31], © (2012) The Author(s). (**c**) Anatomical specificity can also be achieved with targeted light delivery to either the cell bodies (*i*) or synaptic terminals (*ii*) of opsin-expressing neurons

9.3.1 Viral Gene Delivery

Lentiviral (LV) and adeno-associated viral (AAV) vectors are commonly used to deliver opsin genes to neurons. Viral methods are frequently used in species where transgenic animals are not readily available, such as in rats [24] and primates [25]. Another benefit of using viruses is high copy numbers and, therefore, high expression of opsins. Strong opsin expression reduces the possibility of tissue damage from higher laser powers.

Viral tropisms are another method by which cell-type-specific expression can be manipulated [26]. Different viruses infect different cell types with varying efficacy due to membrane proteins that are necessary for infection. This can be used to better target opsins to specific cell types. However, viral tropisms also make it difficult to target certain cell types, limiting the full use of optogenetics.

A major limitation of viral methods is the maximum genetic payload that can be packaged into virus. For example, AAV can package up to 4.7 kb of DNA, while lentivirus can package up to ~8 kb. This size cap limits the promoters that can be used with viral vectors; this presents an issue because short promoters may non-specifically target cells [27]. This can be ameliorated using the Cre-LoxP recombinase system of expression in conjunction with viral delivery (discussed below; Fig. 9.2a [28]).

9.3.2 Transgenic Animals

In animals, where a transgenic approach is available, it is a powerful method of targeting specific cell types. Transgenic mouse lines are crossed using the Cre-LoxP recombination to control the expression of transgenes in specific cells (Fig. 9.2b; [29, 30]). For optogenetics, two basic approaches are used: (1) LoxP sites flank a stop codon that is removed in the presence of Cre and (2) the opsin of interest is flanked by the recombination sites and flipped into the coding orientation by Cre. In both cases, the gene is transcribed in the presence of Cre and introduced by viral delivery or in transgenic mice expressing Cre in target cells. ChR2, eNpHR3.0, and Arch Cre-dependent lines have been created that, when crossed with Cre reporter lines, demonstrate long-term expression at suitable copy numbers for optogenetic excitation or silencing [31].

Many transgenic mouse lines have been created [31]. This has made optogenetics accessible; all that is needed is for researchers to breed Cre-dependent opsin lines with Cre-driver lines to create cell-specific opsin expression in neurons of interest.

9.3.3 Anatomical Specificity

In addition to using cell-type-specific expression of opsins, spatial specificity can be achieved through several methods: virus injected at a specific anatomical area, anterograde or retrograde labeling of neurons, and restricted light delivery.

Labeling projections to or from a brain area of interest could be possible with anterograde or retrograde viruses. Lentivirus EIAV pseudotyped with rabies glycoprotein was found to allow retrograde labeling of neurons [32], but has not yet been used for optogenetics. Additionally, a glycoprotein-deficient pseudotyped rabies virus limits the number of synapses the virus can cross [33], allowing for cleaner optogenetic control of the neurons of interest. Herpes simplex virus (HSV) and certain serotypes of AAV may also be used to target projections [34].

Light can be delivered to target either the cell bodies (affecting all postsynaptic targets) or the synaptic terminals of neurons alone (Fig. 9.2c). This requires sufficient opsin expression at the terminals, but the concept has been demonstrated in vivo

with ChR2 [35, 36]. Another potential concern is antidromic stimulation of the targeted fibers, which may lead to unintentional activation of other areas that the neurons project to.

9.4 Light Delivery

Lasers and LEDs are the primary sources of light used in optogenetic experiments. These light sources emit light within a narrow band of wavelengths and also allow for precise temporal control of the light. They either shine light directly on the tissue or are coupled with fiberoptics for insertion into the brain to target deeper tissues. As described above, the method of light delivery to the brain is a crucial element of optogenetics. The amount and wavelength of light used determines how many neurons are affected by the light and how responsive those neurons are to the light. Additionally, optimal light conditions are determined by the type of opsin used and the experimental conditions desired (in vivo vs. in vitro, prolonged vs. rapid perturbations, etc.).

9.4.1 Optical Properties of the Brain

The amount of light that reaches a neuron depends on factors such as distance from the light source, absorption and scattering, and wavelength; factors that are taken account into Monte Carlo simulations of light propagation [37]. These models predict that light decreases nonlinearly to a mere 1 % at locations 1 mm away from the light source [18, 38], numbers consistent with experimental results [39]. The major factor contributing to this steep falloff is that most opsins are activated by light in the visible wavelengths (450–600 nm), which falls into the range where hemoglobins absorb the majority of light. This is the impetus behind efforts to develop red- and far-red (>650 nm)-shifted opsins that can be activated with far lower light intensities [8].

9.4.2 Tissue Damage from Device Insertion and Heat

Device insertion as well as heat generated by light can damage tissue. To reduce mechanical damage from device insertion, ideally one would use devices as small as possible. For example, multiple thin fibers or fiber arrays are helpful in reducing mechanical tissue damage [40]. In a fiber array, multiple thin fibers are used to illuminate an entire area, especially in larger rodents such as rats, and in primates, where the light from one fiber might only reach a fraction of the brain area of interest.

Heat-generated damage is difficult to evaluate. Sharp temperature increases, especially at the tip of the fiber, could damage tissues. Even if the heating is not sufficient to cause a lesion, the diffusion of heat could affect cellular metabolism [41]. In addition, heating alone has been shown to influence neurons' responses, as demonstrated by pulsed infrared light stimulation [41] or possibly by pulsed ultrasound stimulation [40, 42]. Optogenetics using light of a few mW of total light power in vivo seems to be safe. It is important to perform controls experiments that address these issues.

9.4.3 Electrode Improvement to Reduce Optical Artifact

A major advantage of optogenetics is that it is orthogonal to many readout technologies, such as electrophysiology, fMRI, behavior, and cellular imaging. However, it was found that laser light produces a strong voltage deflection artifact when directed to the tip of metal electrode [20, 39, 43], but not when directed to glass microelectrodes where the silver/silver chloride electrode in the glass filled with solution was not exposed to light. The light-induced artifact is slow evolving and thus corrupts local field potential (LFP) but not spike waveforms. Thus, cautions have to be made when interpreting LFP results in optogenetic experiments. Optimizing electrode tips, such as coating with conducting polymer indium tin oxide (ITO), may prove useful in reducing this artifact on LFP recordings [44].

9.4.4 Other Side Effects

As discussed briefly above, the extent of nonspecific effects of introducing exogenous proteins at such high concentration in neurons is not known. Activation of Halo changes the $GABA_A$ reversal potential [19], and the acidification of the extracellular space due to Arch activation may have unintended consequences. Simply overexpressing membrane proteins may increase the membrane capacitance, changing neuronal excitability [45].

9.4.5 Hardware for Optogenetics

Specialized hardware for various optogenetic experiments have been well developed in many laboratories, and neurophysiology companies, and are continuously being optimized. For example, thin optical fibers with a size comparable to a recording electrode can be easily handled with conventional methods. Optical fibers can be easily positioned or stereotactically targeted to a brain region of interest or incorporated in a tetrode design in freeing moving rodents. Fiber arrays have been designed to target multiple areas of interest or large areas of interest [40] and may be powered

wirelessly [46]. Optical fiber rotary joints are useful devices easily obtained from vendors to prevent tangling of optical fibers, electrophysiological recording cables, or LED power cables.

9.5 Applications

Traditionally, pharmacological manipulations in neuroscience experimentation have been performed with receptor-binding agents. However, the spatial spread and time course of infusion methods have proven problematic in many instances, leading to inconsistent experimental control and ambiguity in the interpretation of results [47]. Similarly, electrical stimulation of cortical sites and subcortical nuclei is difficult to interpret as gross electrical stimulation activates neurons near the electrode indiscriminately [48]. Optogenetics combines consistent and temporally precise activation of spatially aggregated neurons with specific activation of cell types, delivering a high standard of experimental control over neural circuits [11, 49]. Optogenetics research has proven most powerful when performed with dynamic and varied experimental approaches. Utilized in tandem with electrophysiology, microscopy, and behavioral paradigms, targeted light delivery has allowed researchers to manipulate distinct classes of cortical GABAergic interneurons, distinct nuclei and processes of neuromodulator pathways, and neuroanatomically defined cell classes in order to investigate their functional roles in visual processing, memory formation, behavioral learning, stress, mood disorders, and other processes. Here, we discuss a few examples in which optogenetics has been used to reveal the contribution of specific cells in neural computation in transgenic and virally injected animal models.

9.5.1 Parsing the Function of Interneuron Subtypes in Cortical Processing Circuits

Electrophysiological configurations are crucial for understanding the local and large-scale neural behaviors produced by optogenetic manipulations because they are used to characterize and visualize activity at specific brain sites and timescales relevant to experimental hypotheses. For example, single unit recordings, multi-unit recordings, and local field potentials—spiking activity of a single neuron, firing of a small group of neurons, and lower-frequency fluctuations in extracellular field potentials, respectively—are often used to determine the relative activation of neural ensembles in order to quantify the effects of optogenetic manipulations and assess their neurophysiological contribution to behavioral expression.

Several studies published recently highlighted the potential for optogenetics research to effectively glean cortical circuit properties underlying perception and contextual memory. With the use of optogenetic-electrophysiology configurations, researchers have revealed defined processing roles for GABAergic interneuron

classes in cortex. Lee et al. [50] used Cre-induced expression of ChR2 to show that activation of parvalbumin (PV)-positive interneurons heightened (PV-Cre) mice's ability to discriminate between oriented spatial gratings concordant with sharpened orientation tuning—measured as a function of firing rate—in neurons of the primary visual cortex (V1). A further work investigated interneuron function in V1 by utilizing multichannel in vivo recording in conjunction with viral induction of halorhodopsin (Halo) and electroporation delivery of ChR2 in somatostatin (SOM)-Cre mice [51]. Since mice were electroporated in utero with ChR2, to be expressed in superficial layers of the maturated cortex, Adesnik et al. [51] were able to show that SOM-positive interneurons suppress surround excitation of pyramidal neurons, suggesting that SOM-positive interneurons receive converging input from nearby cortical neurons and regulate spatial summation of visual input. Finally, while investigating hippocampal circuitry, Royer et al. [52] used Halo to silence PV and SOM interneurons within region CA1 and observed that each cell type plays a specific role in place cell firing with respect to theta-frequency local field potentials, indicating that together, SOM and PV interneurons coordinate temporal aspects of spatial context processing in hippocampus. As discussed in these studies, light delivery methods used in tandem with electrophysiological recording allow researchers to control cortical circuits while observing simultaneous alterations in single cell and neuron population excitability in order to investigate diverse neuronal functions.

9.5.2 Activity-Dependent Expression of Opsins in Behaviorally Relevant Cell Ensembles

A promising aspect of optogenetics research is its capacity to predictably alter animal behavior, allowing the investigation of neural circuits related to behaviors. When used to perturb defined neural populations, optogenetic stimulation and silencing can be used to infer relationships between states of neural activation and underlying cognitive functions. A profoundly useful strategy in identifying and modulating mammalian cell ensembles has been in targeting neural populations by their activity levels during experimental paradigms. This work has largely been pioneered in the laboratory of Susumu Tonegawa at the Massachusetts Institute of Technology [53]. Using the doxycycline system to trigger co-expression of ChR2 with c-fos (an immediate early gene) in mouse dentate gyrus, Liu et al. [53] optogenetically reactivated neurons recruited during contextual fear conditioning, causing mice to reexpress a conditioned response, i.e., freezing. They found that this occurred even when mice were housed in a previously habituated context, indicating that optogenetic reactivation of c-fos-labeled neurons elicits retrieval of contextual memories. In this way, researchers are designing flexible molecular strategies to target and control previously inaccessible neuronal ensembles and opening the door for dynamic, innovative approaches to the examination of *modularity* and cell sparsity within complex neural systems.

9.5.3 Modulating Neuromodulator-Producing Neurons Within Heterogeneous Nuclei

Optogenetics has also proven particularly useful in experimentally accessing neurotransmitter pathways because neuromodulatory neurons are often embedded in heterogeneous nuclei such as the nucleus accumbens (NAc) and the ventral tegmental area (VTA). Researchers often co-express opsins with the metabolic enzymes of neuromodulators, allowing localized activation and silencing within subcortical, midbrain, and brainstem nuclei—inducing or suppressing neuromodulator release. Witten et al. [54] utilized viral expression of Halo in choline acetyltransferase (ChAT)-Cre transgenic mice in order to silence cholinergic interneurons of the NAc. By silencing this sparse population of interneurons bilaterally, they were able to eliminate cocaine conditioning behavior—measured as preference for the location of cocaine delivery. Similarly, Tsai et al. [55] used AAV delivery to express ChR2 in tyrosine-hydroxilase (TH)-Cre transgenic mice, expressing in dopaminergic neurons of the VTA. They observed that light-activated, phasic release of dopamine from VTA, sufficient to induce increased dopamine concentrations in NAc, elicits learned reinforcement responses without delivery of an external reward. In this way optogenetic applications effectively combine spatial specificity—afforded by localized light delivery—and targeted neurotransmitter activation, allowing researchers to unravel the causal roles of anatomically distinct branches of the cholinergic and dopaminergic neurotransmitter cascades in producing animal behaviors.

9.5.4 Interrogation of Long-Range Neuromodulatory Interactions

Finally, an important application of optogenetics has been in the investigation of large-scale circuit interactions. Subcortical and midbrain nuclei engage in complex interactions that determine aspects of volitional behavior and reinforcement conditioning. By taking advantage of neuronal projections between nuclei and cortical regions, researchers can simulate native, large-scale brain activities. In one such study, Chaudhury et al. [56] used retrograde virus tracers to express ChR2 and Halo in NAc and prefrontal cortex (PFC) projections to VTA dopaminergic neurons in order to examine the relationship between reinforcement circuitry and social defeat. They found that the activity of dopamine projections from VTA to NAc and PFC have opposing roles in eliciting susceptibility and resilience to social defeat—indicated by the mouse's capacity for effective reinforcement learning. Utilizing a similar paradigm to deconstruct long-range neuromodulatory interactions during volitional behavior, Warden et al. [57] used viral ChR2 expression in $Ca2^{+}$/calmodulin-dependent protein kinase-α-Cre (CaMKIIα) transgenic mice in order to stimulate projection afferents from PFC within the raphe nucleus of the brainstem. They observed that PFC enhances effortful action under stressful conditions by directly accessing the serotonergic pathway, indicating that PFC interacts with 5-HT to elicit action selection during stress scenarios.

In this way, viral and retroviral expression of opsins can be used to experimentally access the origins and eccentricities of large-scale neurotransmitter systems as they project to and interact between nuclei over extended distances. Indeed, future work with optogenetic co-expression of opsins under CaMKIIα or SERT (a serotonin transporter protein) promoters and dopamine-related promoter regions could be useful in elucidating an integrated neuromodulatory cascade by which PFC interacts with dopamine and 5-HT projecting nuclei, perhaps granting us further insight into a brain-based perspective of reinforcement learning and mood disorder [58].

9.6 Concluding Remarks

Optogenetics, an exciting novel neurotechnology, offers new ways to modify and control neural activity rapidly and reversibly. Three major classes of light-activated opsins, channelrhodopsins (for neural activation), and halorhodopsins, and archaerhodopsins (for neural silencing), when expressed in mammalian neurons, generate sufficient photocurrents to alter neural activities when irradiated with light. By targeting these opsins to specific cells of interest, it is now possible to investigate the casual roles of these neurons in neural computation and animal behaviors. We have described several advances in the application of optogenetics owing to varied experimental approaches: hybridized light delivery and electrophysiological recording configurations, dynamic methods of molecularly induced opsin expression, specific cell targeting within heterogeneous nuclei, and experimental control of neuronal projection systems. Much of the research performed with optogenetics has focused on neocortical, striatal, and hippocampal networks, revealing new windows into the neurophysiology of memory, executive processing, learning, and perception. In this regard, optogenetic applications have displayed extraordinary potential for unraveling neural systems behavior and underlying cognitive functions, as well as furthering our understanding of pathological brain states related to psychiatric disorders and disease. While optogenetics has revolutionized the investigation of specific cells in neural computation with high spatial, temporal, and cell-type specificity, it is important to properly interpret results from optogenetic perturbation studies. Continued progress in generating novel opsins with improved functions or power spectra, along with increasingly available transgenic animal models and associating hardware, will facilitate the continued wide spread use of optogenetics in mapping neural circuits.

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