Topic Introduction

Optogenetics: Opsins and Optical Interfaces in Neuroscience

Antoine R. Adamantidis, Feng Zhang, Luis de Lecea, and Karl Deisseroth

Optogenetics is defined as the integration of optics and genetics to control well-defined events within specified cells of living tissue. In this introduction, we focus on the basic techniques necessary for employing microbial opsins as optogenetic tools in mammalian brains. We provide a guide for the fundamentals of optogenetic application—selecting an opsin, implementing expression of opsins based on the neuroscientific experimental requirements, and adapting the corresponding optical hardware for delivery of light into mammalian brains.

INTRODUCTION

The mammalian brain consists of many distinct types of excitatory, inhibitory, and modulatory cells. One of the central challenges of neuroscience has long been to decipher the role of distinct cell types in the organization and function of brain circuits and behavior. We have developed a technology called optogenetics, which leverages microbial opsins and molecular genetics to enable temporally precise control of genetically defined neurons in the mammalian brain in vivo using light. Our previous reports have provided extensive characterization of the opsins and the diversity and structure–function properties of microbial opsin genes described here (Boyden et al. 2005; Zhang et al. 2006, 2007, 2008a; Gradinaru et al. 2008; Zhao et al. 2008; Airan et al. 2009; Berndt et al. 2009; Gunaydin et al. 2010; also see **Microbial Opsins: A Family of Single-Component Tools for Optical Control of Neural Activity** [Yizhar et al. 2011]). Likewise, the technology suitable for freely moving mammals, including optical fiber methodology, has also been described (Aravanis et al. 2007; Zhang et al. 2010). We developed and applied this technology to control specific neural populations during a variety of behavioral tests in rodents, such as sleep-to-wake transition (Adamantidis et al. 2007), conditioned place preference (Airan et al. 2009; Tsai et al. 2009; Zhang et al. 2010), and motor control (Aravanis et al. 2007), and in rodent disease models (Bi et al. 2006; Alilain et al. 2008; Abbott et al. 2009; Gradinaru et al. 2009).

OPSINS IN NEUROSCIENCE

We have engineered a number of naturally occurring and synthetic opsins to enable optical control of electrical and biochemical signaling in neurons. Although all of these opsins require retinal-based cofactors (e.g., all-*trans* retinal for the microbial opsins) to fold properly and respond to light, there are sufficient levels of retinoids present in the mammalian brain so that opsins can be functionally expressed in the intact mammalian brain without supplementation of cofactors (Zhang et al. 2006, 2007). Comparison of the various opsins suitable for use in neuroscience can be found in Table 1, and

Adapted from *Imaging in Neuroscience* (ed. Helmchen and Konnerth). CSHL Press, Cold Spring Harbor, NY, USA, 2011.

Cite this introduction as *Cold Spring Harb Protoc*; doi:10.1101/pdb.top083329

TABLE 1. Comparison of optogenetic tools suitable for fast neural control in vivo in mammals

Opsin	Host organism	Wavelength sensitivity	Mode of control	Modulatory capabilities	Experimental systems tested
ChR2, ChR2(H134R), ChIEF, ChR1/ChR2 chimeras, ChETAs	*Chlamydomonas reinhardtii*	470 nm (maximum activation)	Depolarizing	Rapid on/off; best used for precise activation of neurons on the millisecond timescale. Can be used to evoke single spikes or defined trains of action potentials over a range of frequencies. The H134R mutation yields larger photocurrents relative to wild-type ChR2, but with slower k_{off} kinetics. ChIEF (Lin et al. 2009) and other ChR1/ChR2 chimeras (Wang et al. 2009) provide improved expression and inactivation. ChETA (Gunaydin et al. 2010) provides faster recovery from inactivation, faster deactivation kinetics, improved temporal precision at high spike rates, reduced artifactual plateau potentials and extra action potentials, better temporal stationarity, and faster frequency-following behavior up to at least 200 Hz. Two ChETA variants exist, one involving E123T and one involving E123A. The T variant is 10–15 nm redshifted by comparison with ChR2. This is advantageous in many cases for reduced scattering and deeper penetration of light, but if the experimenter is limited to a ~470 nm laser line or filter, the A variant may be preferable.	In vitro: Dissociated neuron culture (Boyden et al. 2005; Zhang et al. 2006, 2007), acute brain sections from mouse and rat (Ishizuka et al. 2006; Zhang et al. 2006, 2008b; Petreanu et al. 2007, 2009; Wang et al. 2007; Zhang and Oertner 2007), and 293HEK cells (Nagel et al. 2003) In vivo: *Caenorhabditis elegans* (requires supplementation of ATR) (Nagel et al. 2005), *D. melanogaster* (requires supplementation of ATR) (Schroll et al. 2006; Hwang et al. 2007; Pulver et al. 2009) zebrafish (Douglass et al. 2008), chicken (Li et al. 2005), mouse (Bi et al 2006; Adamantidis et al. 2007; Aravanis et al. 2007; Arenkiel et al. 2007; Alilain et al. 2008; Huber et al. 2008; Gradinaru 2009; Tsai et al. 2009; Gunaydin et al. 2010), rat (Aravanis et al. 2007), and primate (Han et al. 2009)
Step-function opsins (SFOs): ChR2(C128A), ChR2(C128S), and ChR2(C128T)	*C. reinhardtii*	470 nm (switching on), 542 nm (switching off for C128A and C128S mutants)	Depolarizing	Point mutants of ChR2 with slow or optically switchable deactivation. C128A and C128S mutants show the most prolonged activation and the highest light sensitivity, whereas C128T retains more temporal precision of activation. SFOs can be switched on and off with blue and green light pulses, respectively.	In vitro: Dissociated neuron culture (Berndt et al. 2009)

continued

Cite this introduction as *Cold Spring Harb Protoc*; doi:10.1101/pdb.top083329

TABLE 1. *Continued*

VChR1 and higher-conductance variants	*Volvox carteri*	535 nm (maximum activation at 589 nm, in which activation is separable from ChR2)	Depolarizing	Redshifted action spectrum relative to ChR2. Like ChR2, VChR1 can be used to drive reliable action potential firing over a range of frequencies. With 589-nm light, VChR1 can be activated independently of ChR2.	In vitro: Dissociated neuron culture (Zhang et al. 2008a)
NpHR, eNpHR3.0 variants	*Natronomonas pharaonis* (NpHR)	589 nm (maximum activation) 630–680 nm utility 400 nm utility if needed	Hyperpolarizing	Light-activated chloride pump. Can be used to hyperpolarize neurons with high temporal precision; capable of inhibiting single action potentials within high-frequency spike trains. Also can be used to mediate sustained inhibition of neurons over many minutes, with stable currents that recover rapidly. The most potent eNpHR variants (eNpHR3.0) achieve inhibition in the far red, and traffic to neuronal processes. Redshifted/compatible with ChR2 in same experiment.	In vivo: *C. elegans* (requires supplementation of ATR) (Zhang et al. 2007) and mouse (Gradinaru et al. 2008, 2010; Zhao et al. 2008) In vitro: Dissociated neuron culture (Han and Boyden 2007; Zhang et al. 2007; Gradinaru et al. 2008; Zhao et al. 2008; Gradinaru et al. 2010), and acute brain slices from mouse (Zhang et al. 2007; Zhao et al. 2008)
Bacteriorhodopsin and other regulators of H^+ conductance (e.g., eBR/Arch/ Mac/AR/ GtR3 and many others)	Numerous species	Broad range (most 450–550 nm)	Hyperpolarizing	Light-activated proton conductance regulators. May be useful for experiments that are not focused on simply controlling output of a cell, but in which it is important not to alter chloride gradients while allowing for juxta-membranous proton concentration changes. ~10× higher light power required to reach currents comparable with eNpHR3.0 max, and none currently as redshifted as eNpHR3.0, posing challenges for practical in vivo use and integration with ChR2, respectively.	In vivo: Mouse (Chow et al. 2010). In vitro: Dissociated neuron culture (Chow et al. 2010; Gradinaru et al. 2010)
Opto-α1AR	Synthetic protein	500 nm (maximum activation)	Biochemical	Light-activated G-protein-coupled receptor (GPCR), via the G_q pathway.	In vivo: Mouse (Airan et al. 2009) In vitro: 293HEK cell line (Airan et al. 2009)
Opto-β2AR	Synthetic protein	500 nm (maximum activation)	Biochemical	Light-activated GPCR, via the G_s pathway.	In vivo: Mouse (Airan et al. 2009) In vitro: 293HEK cell line (Airan et al. 2009)

Adapted, with permission, from Zhang et al. 2010.

ATR, all-*trans*-retinal.

additional biophysical details are provided in **Microbial Opsins: A Family of Single-Component Tools for Optical Control of Neural Activity** (Yizhar et al. 2011).

As with all of the microbial opsins, the algal light-sensitive cation-conducting channelrhodopsins (ChRs) (Nagel et al. 2002, 2003; Zhang et al. 2008a) were initially characterized outside of neuroscience in non-neural systems. Two ChRs have been successfully brought to neuroscience, ChR2 from *C. reinhardtii* (Boyden et al. 2005; Zhang et al. 2006) and VChR1 from *V. carteri* (Zhang et al. 2008a). Both of these ChRs have fast kinetics so that defined trains of action potentials can be triggered using brief pulses (1–5 msec duration) of light flashes at a range of frequencies relevant to neural signaling. Although both ChR2 and VChR1 are sensitive to blue light, the activation spectrum of VChR1 is sufficiently redshifted from that of ChR2 so that neurons expressing VChR1 can be activated independently of ChR2 using orange light.

Ultimately, the performance of opsins in neuroscience depends on the precision of modulation of neuronal spiking. Of note, VChR1 is slower to deactivate after "light off," with a time constant of ~120 msec in contrast to the ~12 msec of ChR2. But even the 12-msec deactivation rate of wild-type ChR2 (and its similarly slow transitions to and from the inactivated state) can be problematic, leading to occasional spike doublets, plateau potentials, nonstationary responses in long trains, and impaired frequency response of neurons under optogenetic control (Gunaydin et al. 2010). However, deactivation and inactivation rates are also tunable properties of the microbial opsins. On the one hand, the deactivation rate can be accelerated dramatically with the ChETA point mutations (E123T or E123A), which accelerate channel closing and are associated with faster inactivated-state transitions, and thereby have the effect of reducing or eliminating all of the confounds listed above and allow fast spiking up to at least 200 Hz (Gunaydin et al. 2010). On the other hand, mutations in cysteine 128 of ChR2 (Berndt et al. 2009) have been rationally designed to slow or eliminate deactivation, and allow ChRs to remain open after light has been turned off. These mutant ChRs, known as SFOs, convert a brief pulse of light into a stable step in membrane potential, by virtue of deactivation that is about four orders of magnitude slower. This stable activation can nevertheless be precisely terminated with a distinct, wavelength-shifted (yellow or green) pulse of light. Additional mutants and chimeras generated by shuffling the *trans*-membrane loops of ChR1 and ChR2 have yielded improved expression and inactivation properties (Lin et al. 2009; Wang et al. 2009). Ongoing directed mutagenesis/evolution and genomic approaches will likely yield further enhancements in functionality.

Light-sensitive chloride-pumping halorhodopsins (HRs) have been known for decades to serve as light-activated single-component voltage regulators in reduced systems. We and our colleagues have found that the HR from *Natronomonas pharaonis* can be used to inhibit neural activity in response to redshifted light (useful activation up to 680 nm) in intact tissue and behaving animals (Zhang et al. 2007). As with ChRs, the yellow-light-sensitive NpHR can be activated within milliseconds of illumination, allowing inhibition of individual action potentials within high-frequency spike trains using brief light flashes. NpHR also displays minimal inactivation during chronic illumination, and therefore can be used to optically mimic lesions in targeted brain structures, or to test the functional significance of defined cell types. Although high levels of wild-type NpHR expression can sometimes lead to toxicity because of altered membrane trafficking and accumulation in the endoplasmic reticulum, we have engineered enhanced NpHRs (eNpHR; Gradinaru et al. 2008; Zhao et al. 2008) with appropriate targeting signals to enhance membrane trafficking and folding in mammalian neurons, which implement safe expression at high levels in vivo. At this point, the most potent optogenetic inhibition is achieved with eNpHR3.0 (Gradinaru et al. 2010), which can elicit nanoamperescale hyperpolarizations in response to moderate-intensity (3.5 mW/mm^2) yellow light, and displays step-like currents with fast recovery and stability over behavioral timescales. Other inhibition strategies are also possible. We have found that the classical light-activated proton pump bacteriorhodopsin can also be used to hyperpolarize neurons in response to light (Gradinaru et al. 2010), and other proton conductance can also be used (Chow et al. 2010; Gradinaru et al. 2010). However, the proton conductance at present require ~10× greater light power to achieve currents comparable with eNpHR3.0, which may pose risks for operation in vivo with steady illumination.

Cite this introduction as *Cold Spring Harb Protoc;* doi:10.1101/pdb.top083329

Distinct opsins may now be selected from a broad palette to implement control of neuronal output via direct modulation of defined cellular activity (Table 1). The HR approach (eNpHR and variants) is ideal for probing the necessity of defined cellular events with high temporal precision, whereas ChR2 and VChR1 are suitable for testing sufficiency of defined spikes in circuit dynamics and behavior—as when probing the importance of spike timing in neural coding, or testing the spike-frequency dependence of behavioral efficacy in a select population of neurons or other circuit elements (Adamantidis et al. 2007; Gradinaru et al. 2009; Sohal et al. 2009; Tsai et al. 2009). When high photocurrents are needed (as in the case of axonal illumination) with less experimental emphasis on high frequency, the H134R mutant is suitable. In contrast, when a slight decrement in photocurrent magnitude is experimentally acceptable, but overall temporal performance and signal quality is at a premium, the ChETA mutations are ideal (Gunaydin et al. 2010).

Other kinds of modulation are important as well, and in many in vivo experiments it may be advisable to modulate cellular excitability with high temporal precision without driving defined spike trains. SFOs are indicated for use when it is important to modulate the basal activity level of a neuronal population in chronic fashion while minimizing light exposure, and when it is important to allow native asynchronous or sparse neural codes to propagate through the targeted cell type more effectively (Berndt et al. 2009). Finally, as another modulatory strategy, with the aim of studying the causal role of intracellular biochemical signals in circuit and animal behavior, we also have engineered a set of chimeric opsins (optoXRs) by replacing the intracellular loops of bovine rhodopsin with the signal-transducing intracellular loops of GPCRs (Airan et al. 2009). These synthetic optoXRs undergo conformational changes when illuminated with green light and activate specific downstream biochemical cascades, including either G_s or G_q pathways. There is little detectable "dark" activity arising from these probes, in contrast to expression of microbial photoactivated adenylyl cyclases, which elevate cellular cyclic AMP as much as 30-fold even in the absence of light. OptoXRs can be used to recruit and control, with high spatiotemporal precision, receptor-initiated biochemical signaling pathways in genetically defined neural populations (Airan et al. 2009), and thereby optogenetically modulate cellular excitability by biochemical means in behaving mammals. Additional information on the biophysical properties of opsins is presented in **Microbial Opsins: A Family of Single-Component Tools for Optical Control of Neural Activity** (Yizhar et al. 2011).

GENE EXPRESSION AND TARGETING SYSTEMS

As with the genetically encoded anatomical marker green fluorescent protein, each opsin can be selectively expressed in specific subsets of neurons in the rodent brain using a variety of modern genetic targeting strategies (Adamantidis et al. 2007; Aravanis et al. 2007; Arenkiel et al. 2007; Petreanu et al. 2007; Huber et al. 2008; Kuhlman and Huang 2008; Luo et al. 2008; Airan et al. 2009; Gradinaru et al. 2009; Sohal et al. 2009; Tsai et al. 2009). High levels of opsin expression are important for achieving robust optical activation and inhibition and can be readily achieved using viral gene delivery. Both lentiviral and adeno-associated viral (AAV) vectors can be produced at high functional titer and stereotactically injected into target brain regions to mediate high-level opsin expression (Zhang et al. 2006, 2007; Sohal et al. 2009; Tsai et al. 2009). Detailed protocols describing the production of both lentiviral (Kutner et al. 2009) and AAV (Grieger et al. 2006) vectors have been published elsewhere, and protocols optimized for in vivo use in mammalian brains have been made available online at http://www.optogenetics.org and in Zhang et al. (2010). Different lentivirus and AAV serotypes may be selected for different diffusional properties (e.g., we find that AAV5 will transduce a larger brain region than AAV2 or lentivirus) and in some cases cell-type specificity can also arise from specific viral vectors. We have also reported suitable promoter-based cell-type specificity for several small (<4 kb) fragments, such as human synapsin I for targeting neurons, CamKIIα for targeting excitatory neurons, glial fibrillary acidic protein for targeting astroglia, and the ppHcrt promoter for targeting hypocretin neurons in rodents (reviewed in Zhang et al. 2010; http://www.optogenetics.org).

However, many cell-specific promoters have weak transcriptional activity and cannot drive sufficient levels of opsin expression to yield robust photocurrents or do not retain sufficient specificity when used in viral vectors. Therefore, we and others have designed Cre-recombinase-dependent AAV expression systems to enable cell-specific opsin expression using transgenic mice expressing Cre under the control of native cell-specific promoter/enhancer regions (e.g., tyrosine hydroxylase for dopaminergic neurons [Tsai et al. 2009] and parvalbumin for fast-spiking interneurons [Cardin et al. 2009; Sohal et al. 2009]). Although maintaining cell-type specificity, this approach amplifies the transcriptional strength of the cell-specific promoter by allowing opsins to be expressed under the strong EF-1α promoter in Cre-expressing neurons. Given the increasing availability of a wide array of cell-type–specific Cre-transgenic lines (Gong et al. 2007), researchers now have the ability to selectively probe the activity of a large and increasing number of important cell types optogenetically, in a versatile and generalizable fashion.

Cell types may also be targeted by virtue of their projections; this is particularly important for organisms in which genetic and promoter/enhancer information is not readily available. Axons may be selectively depolarized by illuminating away from cell bodies, evoking neurotransmitter release whether through spike-dependent or -independent mechanisms and thereby defining cells for control by virtue of their projections rather than genetic identity. In this setting, strong photocurrents are at a premium and are best achieved with, for example, the H134R ChR2 mutation while sacrificing some temporal precision (Gradinaru et al. 2007). Retrograde-type targeting strategies are also possible, in which axon terminals are transduced with viruses (e.g., herpesviruses or pseudotyped lentiviruses) bearing opsin genes. Finally, transsynaptic retrograde or anterograde targeting is possible with custom viruses that will transport Cre recombinase across synapses (e.g., as a wheat germ agglutinin–Cre fusion protein) to distinct circuits in which the Cre-dependent opsin-expressing viruses (Sohal et al. 2009; Tsai et al. 2009) have been separately injected (Gradinaru et al. 2010; Zhang et al. 2010). These "topological targeting" strategies powerfully complement the genetic strategies, and together make optogenetic targeting versatile and generalizable.

THE OPTICAL NEURAL INTERFACE

Selective expression of opsins in genetically defined neurons makes it possible to control a subset of neurons without affecting nearby cells and processes in the intact brain, but light must still be delivered to the target brain structure. Light scattering limits the delivery of light from the surface of the brain. Indeed, light power density for blue light will drop to ~1% of initial values at a tissue depth of 1 mm (Aravanis et al. 2007). For this reason, we have developed a fiber-optic-based optical neural interface (ONI), which allows optical access to any brain structure in freely moving mammals (Adamantidis et al. 2007; Aravanis et al. 2007; Gradinaru et al. 2007; see also **Establishing a Fiber-Optic-Based Optical Neural Interface** [Adamantidis et al. 2014] and Fig. 1 therein). Spatial resolution chiefly arises from the cellular genetic targeting. However, spatially resolved light delivery can be readily achieved with light-emitting diode arrays or digital micromirror-coupled devices or fibers, but resolution is rapidly lost in tissue because of scattering.

There are several important factors for achieving effective optical stimulation in vivo. First, it is important to choose a light source with sufficient power because ChR2 and NpHR require 1–10 mW/mm^2 of light for reliable activation (the SFOs require ~2–3 orders of magnitude less light power density). For most in vivo applications, solid-state diode lasers can be used to provide sufficient levels of light and are readily coupled to fiber optics. Both virus injection and the delivery of the multimode fiber (50–400 μm diameter) can be guided via the same stereotactically implanted cannula, to ensure registration of light illumination with opsin-expressing cells. The details for setting up the fiber-optic-based ONI (Adamantidis et al. 2007; Aravanis et al. 2007) for in vivo optogenetic control (Zhang et al. 2010) are described in the associated protocol (see **Establishing a Fiber-Optic-Based Optical Neural Interface** [Adamantidis et al. 2014]). Depending on the experiment, either bilateral or unilateral stimulation may be performed; procedures for bilateral and unilateral fibers are similar.

Cite this introduction as *Cold Spring Harb Protoc;* doi:10.1101/pdb.top083329

OUTLOOK

Optogenetics holds promise as a versatile tool for probing the causal role of defined neurons and circuits in freely moving mammals and other animals. There are many important areas for future investigation, ranging from further expansion of the optogenetic control spectrum to enhancing the integration of optogenetics with multiphoton microscopy and integrating complex optogenetic control with increasingly rich and precise readouts to assess circuit behavior. The common theme for ongoing development may be best summarized as "reverse engineering." The more effectively and specifically we can interrogate the input pins and output pins on the "chip" that is the neural circuit, the closer we will be able to come to inferring core computations from the relationship between complex inputs and complex outputs.

Developing improved two-photon integration will be helpful on both the input and output sides, as two-photon control of opsins will provide improved resolution and penetration within scattering tissues; also two-photon and single-photon imaging of activity in targeted cells with genetically encoded calcium indicators and voltage indicators will enrich the output stream. The output stream will be further enhanced with increasingly advanced multielectrode readouts integrated with multisource light delivery inputs; brain–computer interfaces of this novel hybrid optical/electrical character will help drive the reverse engineering efforts by virtue of greatly enriched control and quantification of the input and output information streams.

Although additional engineering advances will be continue to be made, the essential panel of opsins, targeting methodologies, and optical hardware described here form the core technology required for optogenetics. Together, these methods may allow movement toward a network engineering approach, in which complex excitable-tissue function will come to be understood in terms of system properties emerging from component dynamics.

REFERENCES

Abbott SB, Stornetta RL, Fortuna MG, Depuy SD, West GH, Harris TE, Guyenet PG. 2009. Photostimulation of retrotrapezoid nucleus phox2b-expressing neurons in vivo produces long-lasting activation of breathing in rats. *J Neurosci* **29:** 5806–5819.

Adamantidis AR, Zhang F, Aravanis AM, Deisseroth K, de Lecea L. 2007. Neural substrates of awakening probed with optogenetic control of hypocretin neurons. *Nature* **450:** 420–424.

Adamantidis AR, Zhang F, de Lecea L, Deisseroth K. 2014. Establishing a fiber-optic-based optical neural interface. *Cold Spring Harbor Protoc* doi: 10.1101/pdb.prot083337.

Airan RD, Thompson KR, Fenno LE, Bernstein H, Deisseroth K. 2009. Temporally precise in vivo control of intracellular signalling. *Nature* **458:** 1025–1029.

Alilain WJ, Li X, Horn KP, Dhingra R, Dick TE, Herlitze S, Silver J. 2008. Light-induced rescue of breathing after spinal cord injury. *J Neurosci* **28:** 11862–11870.

Aravanis AM, Wang LP, Zhang F, Meltzer LA, Mogri MZ, Schneider MB, Deisseroth K. 2007. An optical neural interface: In vivo control of rodent motor cortex with integrated fiberoptic and optogenetic technology. *J Neural Eng* **4:** S143–S156.

Arenkiel BR, Peca J, Davison IG, Feliciano C, Deisseroth K, Augustine GJ, Ehlers MD, Feng G. 2007. In vivo light-induced activation of neural circuitry in transgenic mice expressing channelrhodopsin-2. *Neuron* **54:** 205–218.

Berndt A, Yizhar O, Gunaydin LA, Hegemann P, Deisseroth K. 2009. Bi-stable neural state switches. *Nat Neurosci* **12:** 229–234.

Bi A, Cui J, Ma YP, Olshevskaya E, Pu M, Dizhoor AM, Pan ZH. 2006. Ectopic expression of a microbial-type rhodopsin restores visual responses in mice with photoreceptor degeneration. *Neuron* **50:** 23–33.

Boyden ES, Zhang F, Bamberg E, Nagel G, Deisseroth K. 2005. Millisecond-timescale, genetically targeted optical control of neural activity. *Nat Neurosci* **8:** 1263–1268.

Cardin JA, Carlén M, Meletis K, Knoblich U, Zhang F, Deisseroth K, Tsai LH, Moore CI. 2009. Driving fast-spiking cells induces gamma rhythm and controls sensory responses. *Nature* **459:** 663–667.

Chow BY, Han X, Dobry AS, Qian X, Chuong AS, Li M, Henninger MA, Belfort GM, Lin Y, Monahan PE, Boyden ES. 2010. High-performance genetically targetable optical neural silencing by light-driven proton pumps. *Nature* **463:** 98–102.

Douglass AD, Kraves S, Deisseroth K, Schier AF, Engert F. 2008. Escape behavior elicited by single, channelrhodopsin-2-evoked spikes in zebrafish somatosensory neurons. *Curr Biol* **18:** 1133–1137.

Gong S, Doughty M, Harbaugh CR, Cummins A, Hatten ME, Heintz N, Gerfen CR. 2007. Targeting Cre recombinase to specific neuron populations with bacterial artificial chromosome constructs. *J Neurosci* **27:** 9817–9823.

Gradinaru V, Thompson KR, Zhang F, Mogri M, Kay K, Schneider MB, Deisseroth K. 2007. Targeting and readout strategies for fast optical neural control in vitro and in vivo. *J Neurosci* **27:** 14231–14238.

Gradinaru V, Thompson KR, Deisseroth K. 2008. eNpHR: A *Natronomonas* halorhodopsin enhanced for optogenetic applications. *Brain Cell Biol* **36:** 129–139.

Gradinaru V, Mogri M, Thompson KR, Henderson JM, Deisseroth K. 2009. Optical deconstruction of Parkinsonian neural circuitry. *Science* **324:** 354–359.

Gradinaru V, Zhang F, Ramakrishnan C, Mattis J, Prakash R, Diester I, Goshen I, Thompson K, Deisseroth K. 2010. Molecular and cellular principles for diversifying and extending optogenetics. *Cell* **141:** 1–12.

Grieger JC, Choi VW, Samulski RJ. 2006. Production and characterization of adeno-associated viral vectors. *Nat Protoc* **1:** 1412–1428.

Gunaydin L, Yizhar O, Berndt A, Sohal V, Deisseroth K, Hegemann P. 2010. Ultrafast optogenetic excitation. *Nat Neurosci* **13:** 387–392.

Han X, Boyden ES. 2007. Multiple-color optical activation, silencing, and desynchronization of neural activity, with single-spike temporal resolution. *PLoS One* **2:** e299.

Han X, Qian X, Bernstein JG, Zhou HH, Franzesi GT, Stern P, Bronson RT, Graybiel AM, Desimone R, Boyden ES. 2009. Millisecond-timescale optical control of neural dynamics in the nonhuman primate brain. *Neuron* **62:** 191–198.

Huber D, Petreanu L, Ghitani N, Ranade S, Hromadka T, Mainen Z, Svoboda K. 2008. Sparse optical microstimulation in barrel cortex drives learned behaviour in freely moving mice. *Nature* **451:** 61–64.

Hwang RY, Zhong L, Xu Y, Johnson T, Zhang F, Deisseroth K, Tracey WD. 2007. Nociceptive neurons protect *Drosophila* larvae from parasitoid wasps. *Curr Biol* **17:** 2105–2116.

Ishizuka T, Kakuda M, Araki R, Yawo H. 2006. Kinetic evaluation of photosensitivity in genetically engineered neurons expressing green algae light-gated channels. *Neurosci Res* **54:** 85–94.

Kuhlman SJ, Huang ZJ. 2008. High-resolution labeling and functional manipulation of specific neuron types in mouse brain by Cre-activated viral gene expression. *PLoS One* **3:** e2005.

Kutner RH, Zhang XY, Reiser J. 2009. Production, concentration and titration of pseudotyped HIV-1-based lentiviral vectors. *Nat Protoc* **4:** 495–505.

Li X, Gutierrez DV, Hanson MG, Han J, Mark MD, Chiel H, Hegemann P, Landmesser LT, Herlitze S. 2005. Fast noninvasive activation and inhibition of neural and network activity by vertebrate rhodopsin and green algae channelrhodopsin. *Proc Natl Acad Sci* **102:** 17816– 17821.

Lin JY, Lin MZ, Steinbach P, Tsien RY. 2009. Characterization of engineered channelrhodopsin variants with improved properties and kinetics. *Biophys J* **96:** 1803–1814.

Luo L, Callaway EM, Svoboda K. 2008. Genetic dissection of neural circuits. *Neuron* **57:** 634–660.

Nagel G, Ollig D, Fuhrmann M, Kateriya S, Musti AM, Bamberg E, Hegemann P. 2002. Channelrhodopsin-1: A light-gated proton channel in green algae. *Science* **296:** 2395–2398.

Nagel G, Szellas T, Huhn W, Kateriya S, Adeishvili N, Berthold P, Ollig D, Hegemann P, Bamberg E. 2003. Channelrhodopsin-2, a directly light-gated cation-selective membrane channel. *Proc Natl Acad Sci* **100:** 13940–13945.

Nagel G, Brauner M, Liewald JF, Adeishvili N, Bamberg E, Gottschalk A. 2005. Light activation of channelrhodopsin-2 in excitable cells of *Caenorhabditis elegans* triggers rapid behavioral responses. *Curr Biol* **15:** 2279–2284.

Petreanu L, Huber D, Sobczyk A, Svoboda K. 2007. Channelrhodopsin-2-assisted circuit mapping of long-range callosal projections. *Nat Neurosci* **10:** 663–668.

Petreanu L, Mao T, Sternson SM, Svoboda K. 2009. The subcellular organization of neocortical excitatory connections. *Nature* **457:** 1142–1145.

Pulver SR, Pashkovski SL, Hornstein NJ, Garrity PA, Griffith LC. 2009. Temporal dynamics of neuronal activation by Channelrhodopsin-2 and TRPA1 determine behavioral output in *Drosophila* larvae. *J Neurophysiol* **101:** 3075–3088.

Schroll C, Riemensperger T, Bucher D, Ehmer J, Voller T, Erbguth K, Gerber B, Hendel T, Nagel G, Buchner E, et al. 2006. Light-induced activation of distinct modulatory neurons triggers appetitive or aversive learning in *Drosophila* larvae. *Curr Biol* **16:** 1741–1747.

Sohal VS, Zhang F, Yizhar O, Deisseroth K. 2009. Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. *Nature* **459:** 698–702.

Tsai HC, Zhang F, Adamantidis A, Stuber GD, Bonci A, de Lecea L, Deisseroth K. 2009. Phasic firing in dopaminergic neurons is sufficient for behavioral conditioning. *Science* **324:** 1080–1084.

Wang H, Peca J, Matsuzaki M, Matsuzaki K, Noguchi J, Qiu L, Wang D, Zhang F, Boyden E, Deisseroth K, et al. 2007. High-speed mapping of synaptic connectivity using photostimulation in Channelrhodopsin-2 transgenic mice. *Proc Natl Acad Sci* **104:** 8143–8148.

Wang H, Sugiyama Y, Hikima T, Sugano E, Tomita H, Takahashi T, Ishizuka T, Yawo H. 2009. Molecular determinants differentiating photocurrent properties of two channelrhodopsins from chlamydomonas. *J Biol Chem* **284:** 5685–5696.

Yizhar O, Fenno L, Zhang F, Hegeman P, Deisseroth K. 2011. Microbial opsins: A family of single-component tools for optical control of neural activity. *Cold Spring Harbor Protoc* doi: 10.1101/pdb.top102.

Zhang YP, Oertner TG. 2007. Optical induction of synaptic plasticity using a light-sensitive channel. *Nat Methods* **4:** 139–141.

Zhang F, Wang LP, Boyden ES, Deisseroth K. 2006. Channelrhodopsin-2 and optical control of excitable cells. *Nat Methods* **3:** 785–792.

Zhang F, Wang LP, Brauner M, Liewald JF, Kay K, Watzke N, Wood PG, Bamberg E, Nagel G, Gottschalk A, et al. 2007. Multimodal fast optical interrogation of neural circuitry. *Nature* **446:** 633–639.

Zhang F, Prigge M, Beyriere F, Tsunoda SP, Mattis J, Yizhar O, Hegemann P, Deisseroth K. 2008a. Red-shifted optogenetic excitation: A tool for fast neural control derived from *Volvox carteri*. *Nat Neurosci* **11:** 631–633.

Zhang YP, Holbro N, Oertner TG. 2008b. Optical induction of plasticity at single synapses reveals input-specific accumulation of αCaMKII. *Proc Natl Acad Sci* **105:** 12039–12044.

Zhang F, Gradinaru V, Adamantidis AR, Durand R, Airan RD, de Lecea L, Deisseroth K. 2010. Optogenetic interrogation of neural circuits: Technology for probing mammalian brain structures. *Nat Protoc* **5:** 439–456.

Zhao S, Cunha C, Zhang F, Liu Q, Gloss B, Deisseroth K, Augustine GJ, Feng G. 2008. Improved expression of halorhodopsin for light-induced silencing of neuronal activity. *Brain Cell Biol* **36:** 141–154.

Cite this introduction as *Cold Spring Harb Protoc;* doi:10.1101/pdb.top083329

Optogenetics: Opsins and Optical Interfaces in Neuroscience

Antoine R. Adamantidis, Feng Zhang, Luis de Lecea and Karl Deisseroth

Cold Spring Harb Protoc; doi: 10.1101/pdb.top083329