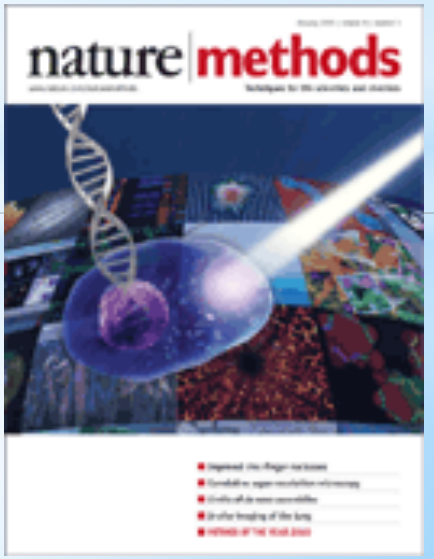
Genetic approaches to control living cells: OPTOGENETIC, CHEMOGENETIC and MAGNETOGENETIC

*Optogenetic: use of light to control living cells

* OPTOGENETICS is a technique that combines complex principles derived from physics with genetics, cell biology, and neuroscience
* It refers to the integration of genetic and optical control to achieve gain or loss of function of precisely defined events within specified cells such as neurons.
* The field of optogenetics has been named and pioneered by K. Deisseroth and E. S. Boyden whose leading contributions in using light to control animal behavior are now widely recognized. In recognition of its outstanding scientific value, **optogenetics has been named the method of the year by *Nature Methods* in 2010**



http://www.youtube.com/watch?v=I64X7vHSHOE

http://web.stanford.edu/group/dlab/optogenetics/

To decipher a complex biological process, one needs tools to perturb the various actors involved to gain information about the important parameters. In this context, light seems like a very attractive actuator to perturb a system with high spatiotemporal resolution

* The advantages of using light as an effector are obvious:
* it is noninvasive,
* can be precisely targeted with exquisite spatial and temporal precision,
* can be used simultaneously at multiple wavelengths and locations,
* can report the presence or activity of specific molecules

Optogenetic, as employed today to study the neural circuit underpinnings of behavior, most commonly involves three core features:

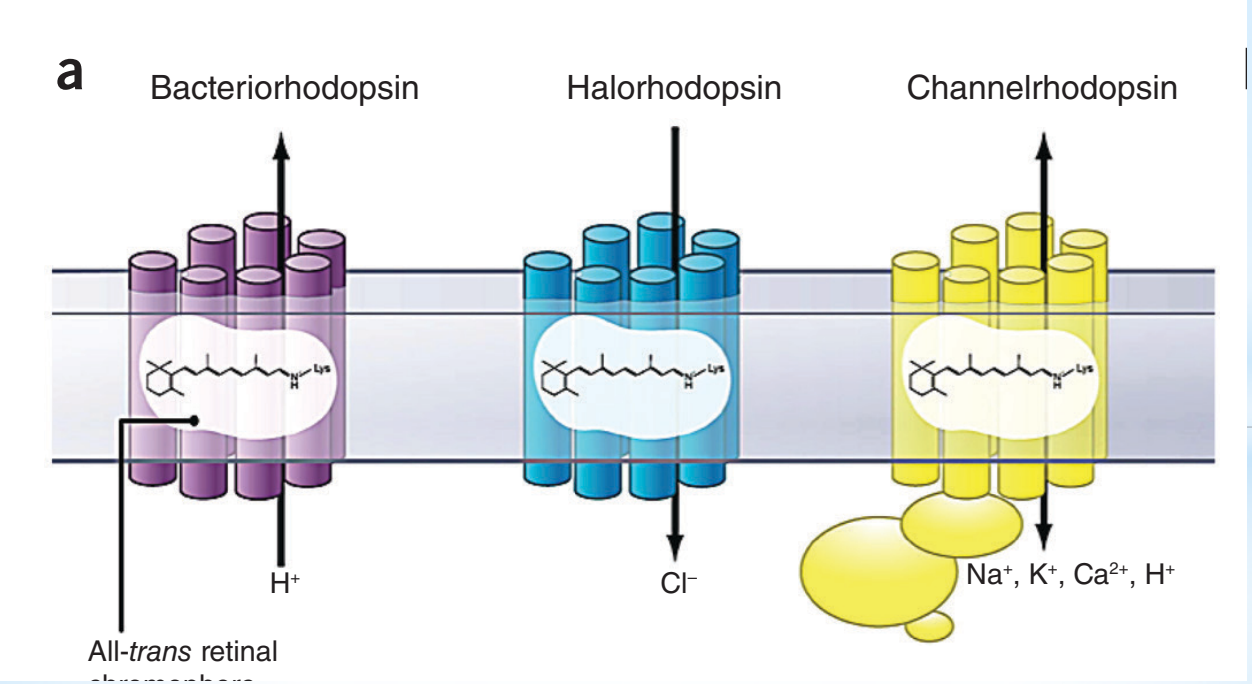
(i) **Microbial opsins**, members of an ancient, but uniquely well-suited, gene family adapted from evolutionarily distant organisms such as algae and archaebacteria, with each gene encoding a distinct protein that directly elicits electrical current across cellular membranes in response to light,

(ii) general **methods** for targeting sufficiently strong and specific **opsin gene expression** to well-defined cellular elements in the brain

(iii) general **methods** for guiding sufficiently strong and precisely **timed light to specific brain regions**, cells or parts of cells while the experimental subject carries out behaviors of interest.

MICROBIAL OPSINS

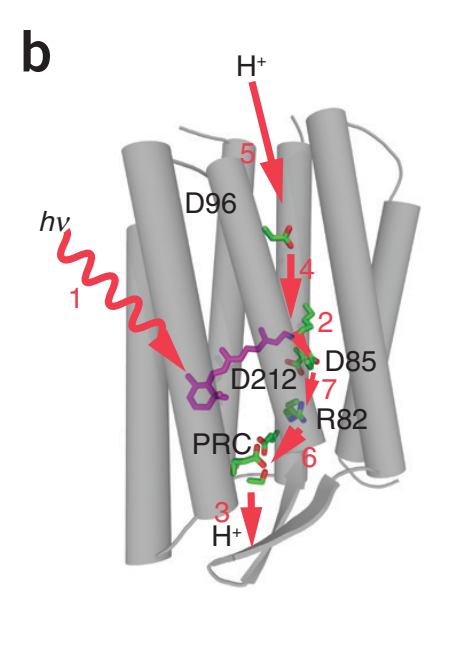
The microbial opsin genes and the microbial rhodopsin proteins encode, a family of molecules (Fig. 1a) functionally completely distinct from (and unrelated in primary sequence to) the better- known rhodopsins that mediate phototransduction in the vertebrate eye.



Deisserhot, Nat Neurosc, 2015

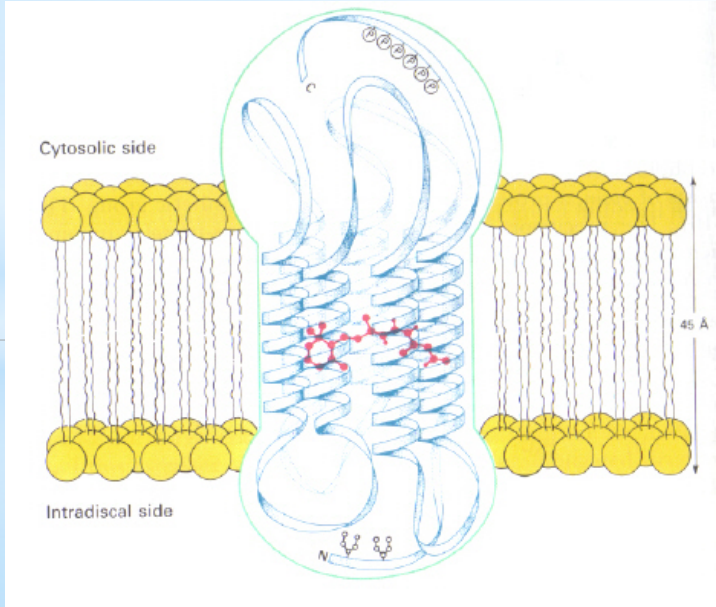
MICROBIAL OPSINS

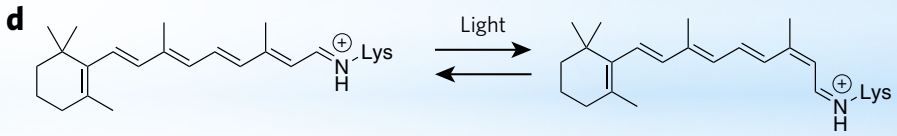
Instead of coupling to intracellular second-messenger cascades to indirectly influence ion channels, like their vertebrate counterparts, these microbial proteins for the most part directly transduce photons into electrical current



Deisserhot, Nat Neurosc, 2015

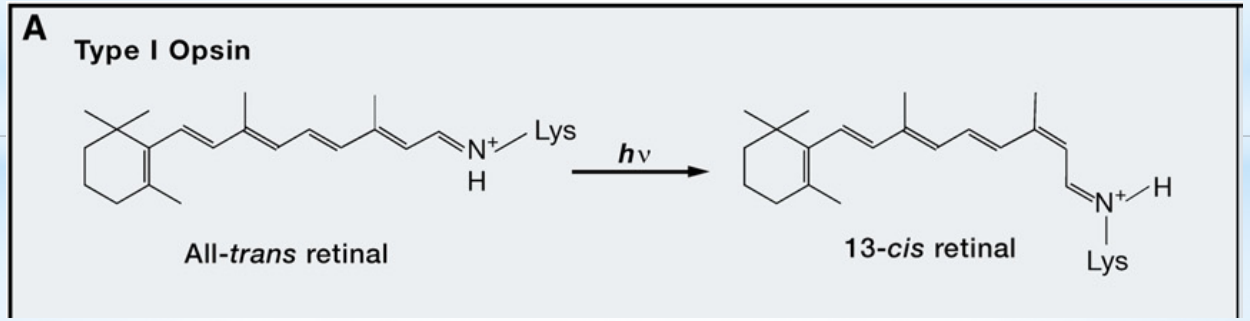
* The **retinal** molecule is covalently **fixed in the binding pocket** within the 7-TM helices and **forms a protonated retinal Schiff base (RSBH+)** with a conserved lysine residue located on TM helix seven (TM7). The ionic environment of the RSBH+, heavily influenced by the residues lining the binding pocket, dictates the spectral characteristics of each individual protein; upon **absorption of a photon**, the retinal **chromophore isomerizes and triggers a series of structural changes leading to ion transport or channel opening**





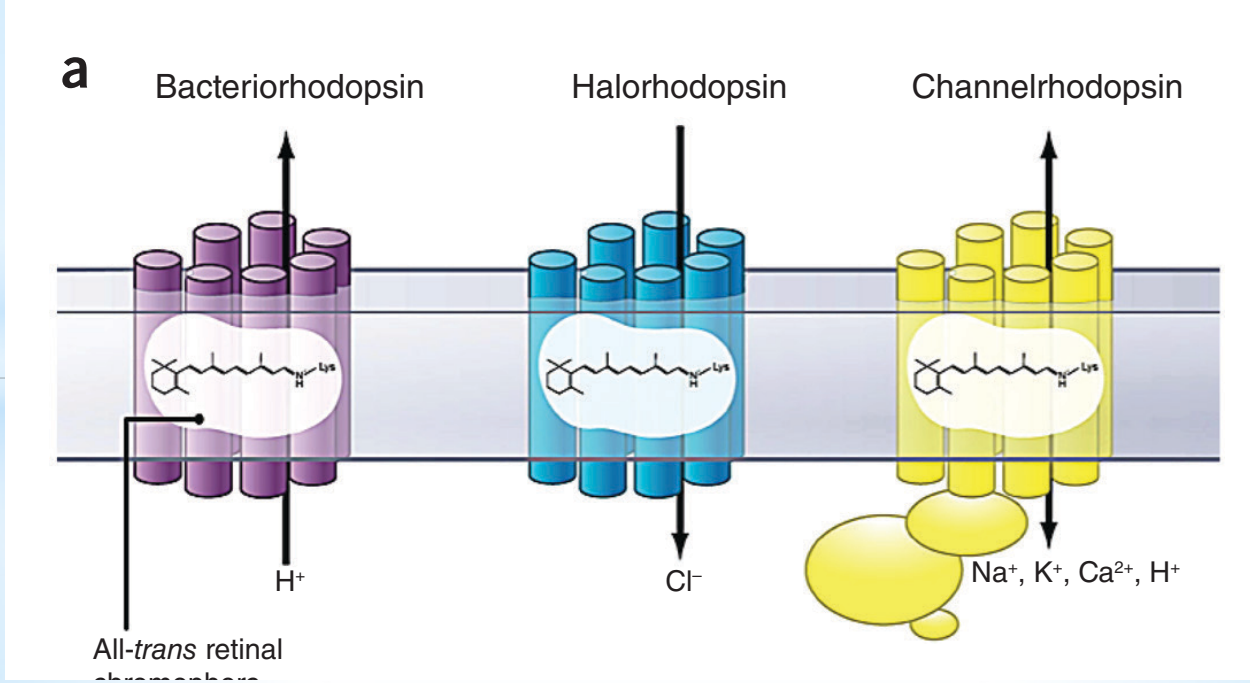
MICROBIAL OPSINS

* the chromophore is covalently linked to the protein, which allows for very rapid thermal resetting following photoactivation.
* They commonly contain an all-*trans* retinal isomer at the ground state that isomerizes to 13-*cis* upon light activation, also known as microbial-type chromophore
* The activated retinal molecule in type I rhodopsins remains associated with its opsin protein partner and thermally reverts to the all-trans state while maintaining a covalent bond to its protein partner (Haupts et al., 1997). This reversible reaction occurs rapidly and is critical for allowing microbial rhodopsins to modulate neuronal activity at high frequencies when used as optogenetic tools.



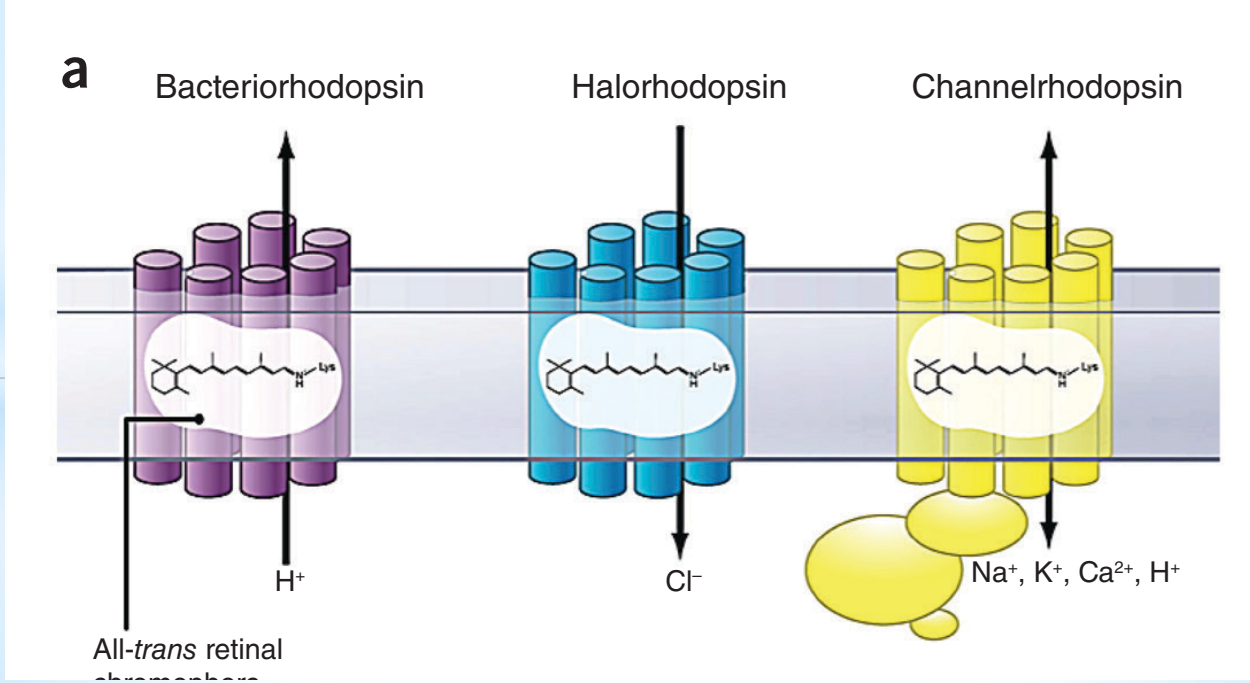
Three branches of this family tree have found utility in optogenetics: the bacteriorhodopsins, the halorhodopsins and the channelrhodopsins.

* The naturally occurring **BACTERIORHODOPSINS** (the first-discovered members of this family, which pump protons out of the cell) and **HALORHODOPSINS** (which pump chloride ions into the cell) are typically **inhibitory** in neural systems, as both of these types of hyperpolarizing current make it harder for neurons to fire action potentials



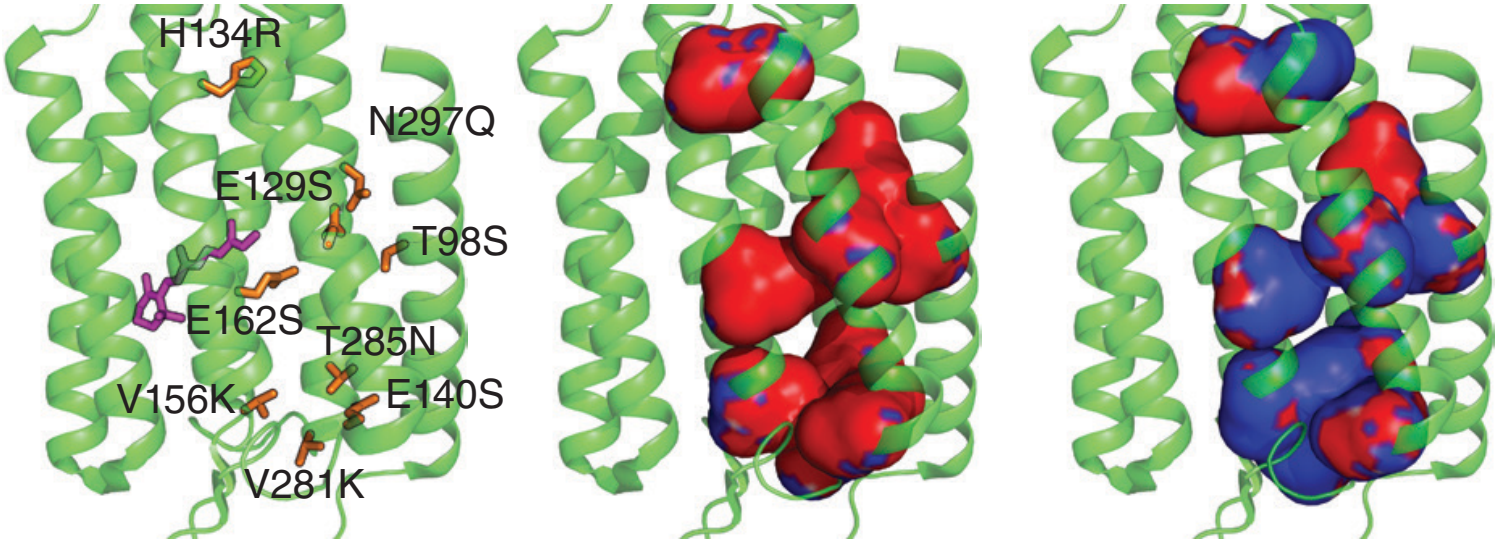
Deisserhot, Nat Neurosc, 2015

* in contrast, the naturally occurring **CHANNELRHODOPSINS** for the most part allow positively charged ions to flow freely through the opsin pore and so tend to be **depolarizing and excitatory**



Deisserhot, Nat Neurosc, 2015

* The channelrhodopsin crystal structure revealed positioning of transmembrane helices (green), the binding pocket of all-*trans* retinal (purple), and angstrom-scale positioning of residues lining the pore (left). In the course of testing the pore model, structure-guided mutagenesis of the residues in orange (left) shifted expected pore electrostatics from largely negative (red, center) to largely positive (blue, right) and switched ion selectivity from cation to anion (chloride) conductance.



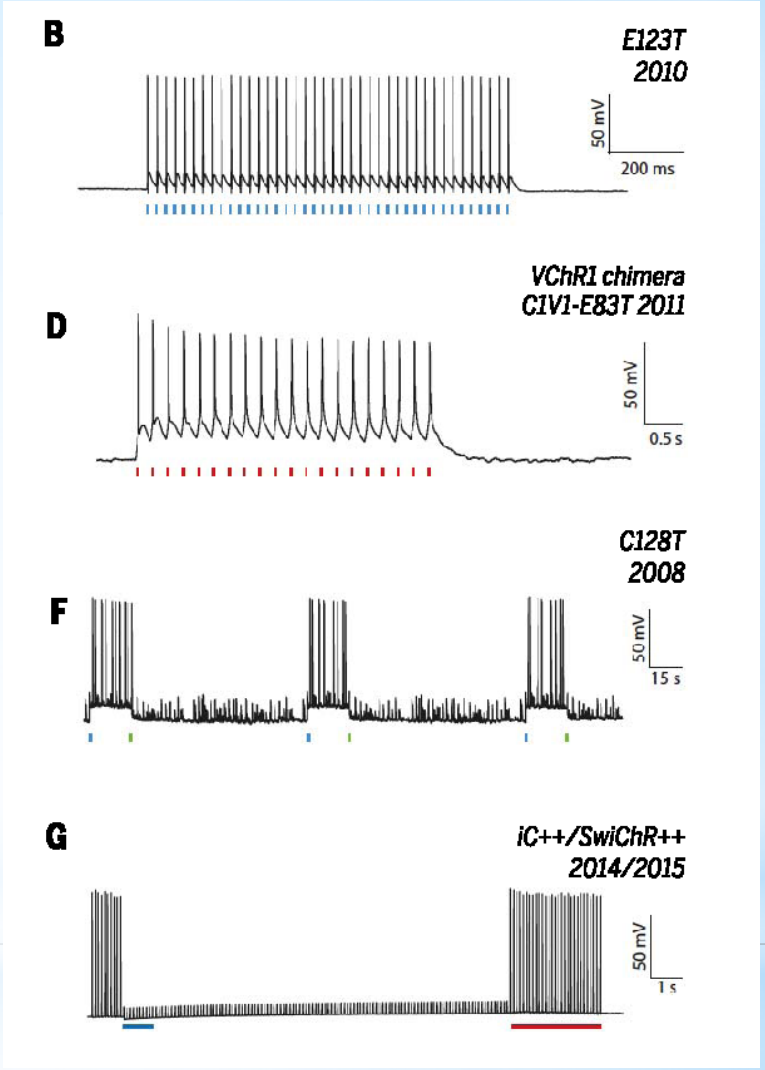
Deisserhot, Nat Neurosc, 2015

* This pattern held for many years until the high-resolution crystal structure of channelrhodopsin allowed structure guided engineering of the opsin channel pore to create inhibitory chloride-conducting channels in 2014, followed by identification of a natural chloride-conducting channelrhodopsin in 2015.
* Over the years, more variants in these protein families have been discovered in nature (or engineered in the laboratory) to have faster kinetics, bistable properties, altered ion conductances and shifted color-response properties

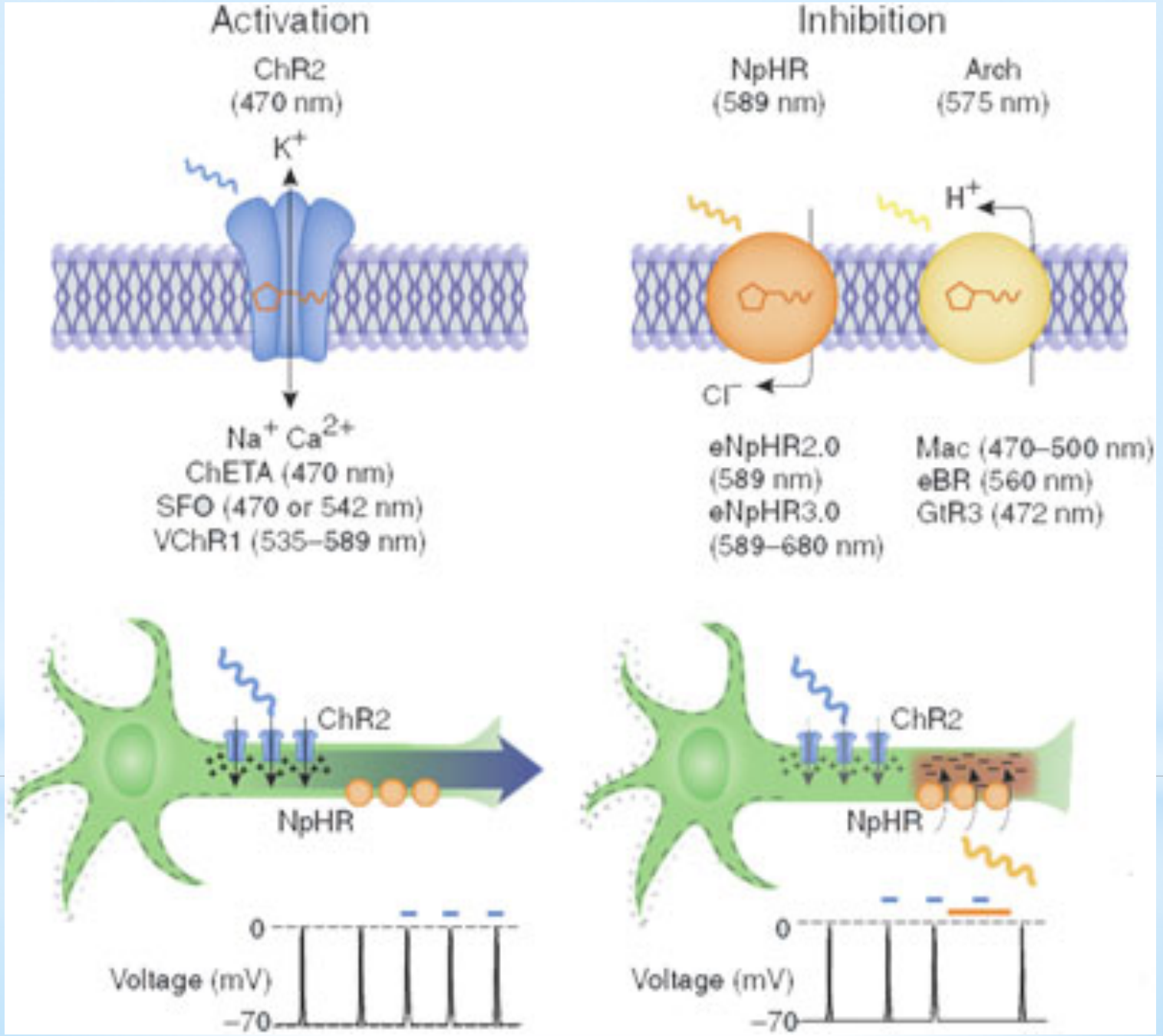
* Likewise, discovery of the red-shifted channelrhodopsin VChR1, which gave rise to the initial red light-activated channelrhodopsin C1V1, led to both deeper understanding of the spectral diversity of channelrhodopsins and new technological capability for *in vivo* single-cell two-photon control and integration with genetically encoded activity-imaging readouts.
* Although VChR1 photocurrents were small (<100 pA), several modifications in combination—including provision of membrane-trafficking/endoplasmic reticulum export motifs identified earlier for enabling halorhodopsin optogenetics, chimerization with ChR1 elements, and in some cases mutations reducing the blue shoulder and/or ChETA mutation—resulted in diverse members of the new C1V1 ChR family.

Deisserhot, Nat Neurosc, 2015

* In 2011, C1V1 enabled the first red light-driven spiking as well as in vivo combinatorial optogenetics [two populations separably controlled with red and blue light], which allowed the long sought demonstration in mouse experiments a causal role for excitation-inhibition balance in governing gamma oscillations and social behavior.
* Another application emerged with (i) the discovery of high responsivity of red light-driven opsins to two-photon illumination, enabling single cell resolution optogenetics in brain tissue, and
* (ii) integration of red light-excited control with blue light-excited readout (via genetically encoded activity sensors such as GCaMP Ca2+ reporters).



Deisserhot and Hegemann, Science, 2017


Activation
ChR2
(470 nm)
K+
Na+ Ca2+
ChETA (470 nm)
SFO (470 or 542 nm)
VChR1 (535–589 nm)
Inhibition
NpHR
(589 nm)
Arch
(575 nm)
H+
Cl−
eNpHR2.0
(589 nm)
eNpHR3.0
(589–680 nm)
Mac (470–500 nm)
eBR (560 nm)
GtR3 (472 nm)
ChR2
NpHR
0
Voltage (mV)
−70
ChR2
NpHR
0
Voltage (mV)
−70

The key functional properties of these proteins were widely known for decades, and many investigators had sought to create strategies for controlling neurons with light. So why did it take time to develop and apply methods for placing these proteins into different classes of neurons in behaving animals?

Deisserhot, Nat Neurosc, 2015

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The development of optogenetics was a biological three-body problem in which it was hard to resolve (or, even more importantly, to motivate attempts to resolve) any one of the three challenges without first addressing the other components.

For example, microbial rhodopsin **photocurrents** were predicted to be **exceedingly small**, suggesting a difficult path forward even if efficient delivery and incorporation of the all-trans retinal chromophore were possible in adult non-retinal brain tissue, **and even in the event of safe and correct trafficking** of these evolutionarily remote proteins to the surface membrane of complex metazoan neurons.

Deisserhot, Nat Neurosc, 2015

For these weak membrane conductance regulators to work, **high gene expression and light-intensity levels would have to be obtained in living nervous systems while simultaneously attaining cell-type specificity and minimizing cellular toxicity**

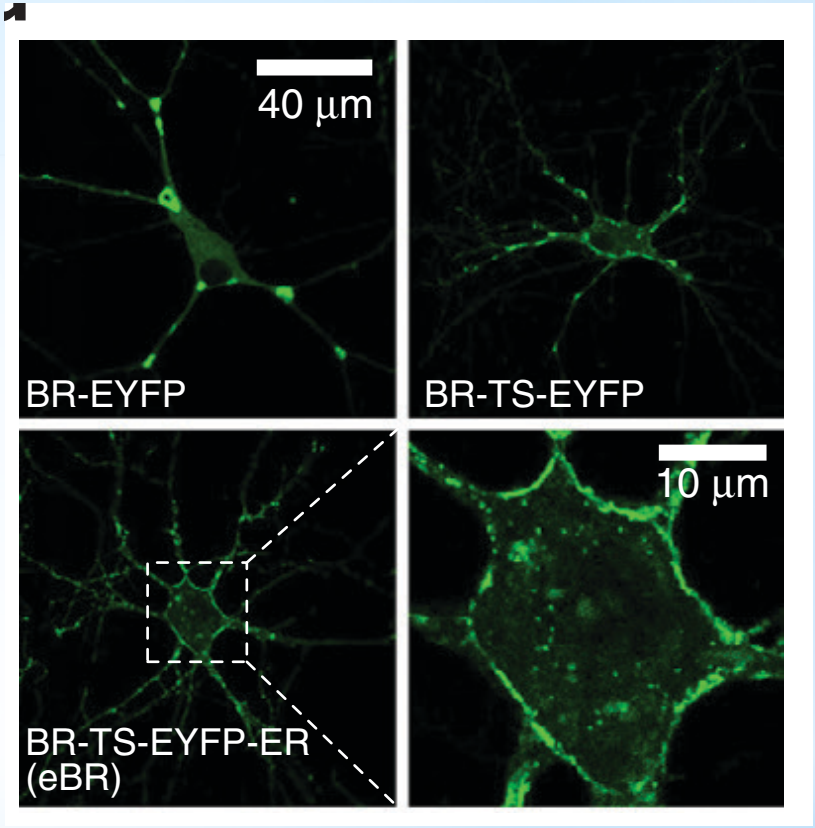
All of this would have to be achieved even though neurons were well known to be highly vulnerable to (and often damaged or destroyed by) overexpression of membrane proteins, as well as sensitive to side effects of heat and light.

All three classes of microbial opsin-derived proteins suffer to some degree from formation of aggregations within metazoan host cells but in all cases this can be addressed **with membrane trafficking motifs** borrowed from mammalian channels

Shown: original BR fused to enhanced yellow fluorescent protein (EYFP); upper left depicts accumulations seen with wild-type BR expression in mammalian neurons,

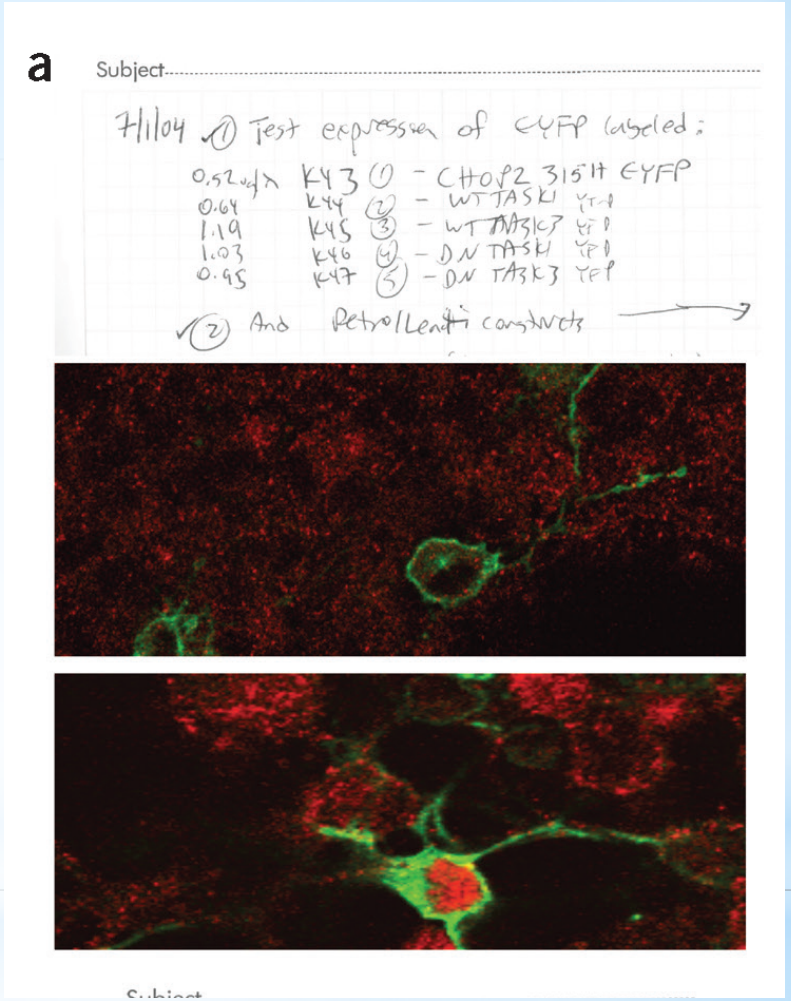
upper right shows the effect on surface membrane expression of adding a neurite targeting motif (TS),

and the lower row shows the effect of combined TS and ER (endoplasmic reticulum export) motif provision



Deisserhot, Nat Neurosc, 2015

However, once neural membrane expression with appreciable lightactivated functionality of a microbial opsin had been seen (Fig. 2a) and it first became possible to report “I think it worked,” the landscape changed from speculation to action.

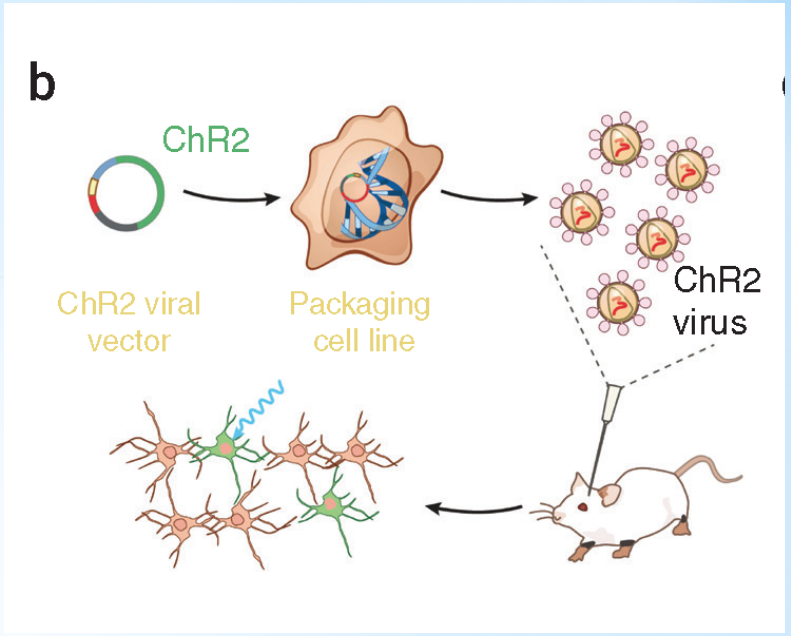


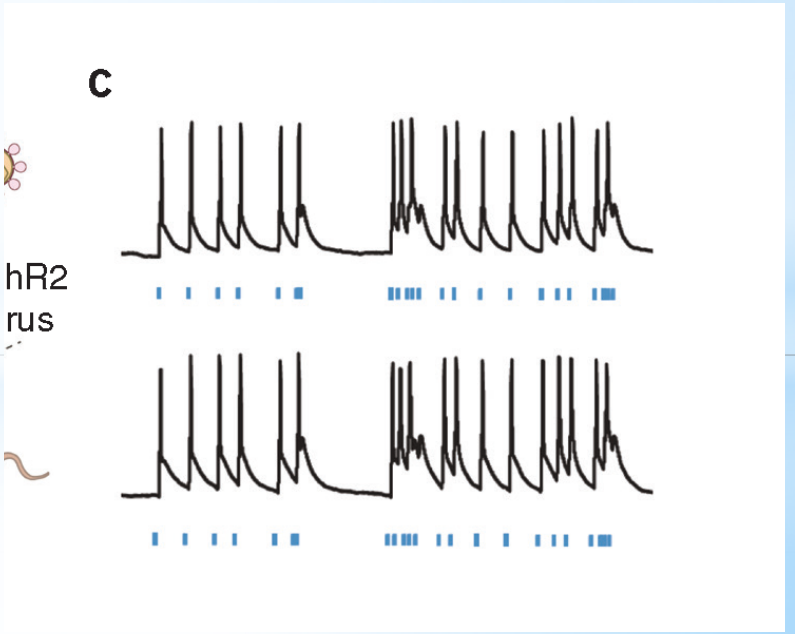
Deisserhot, Nat Neurosc, 2015

The ensuing 2 years indeed saw action on many levels:

constructing and concentrating the crucial expression vectors for stable, well-tolerated expression (Fig. 2b),

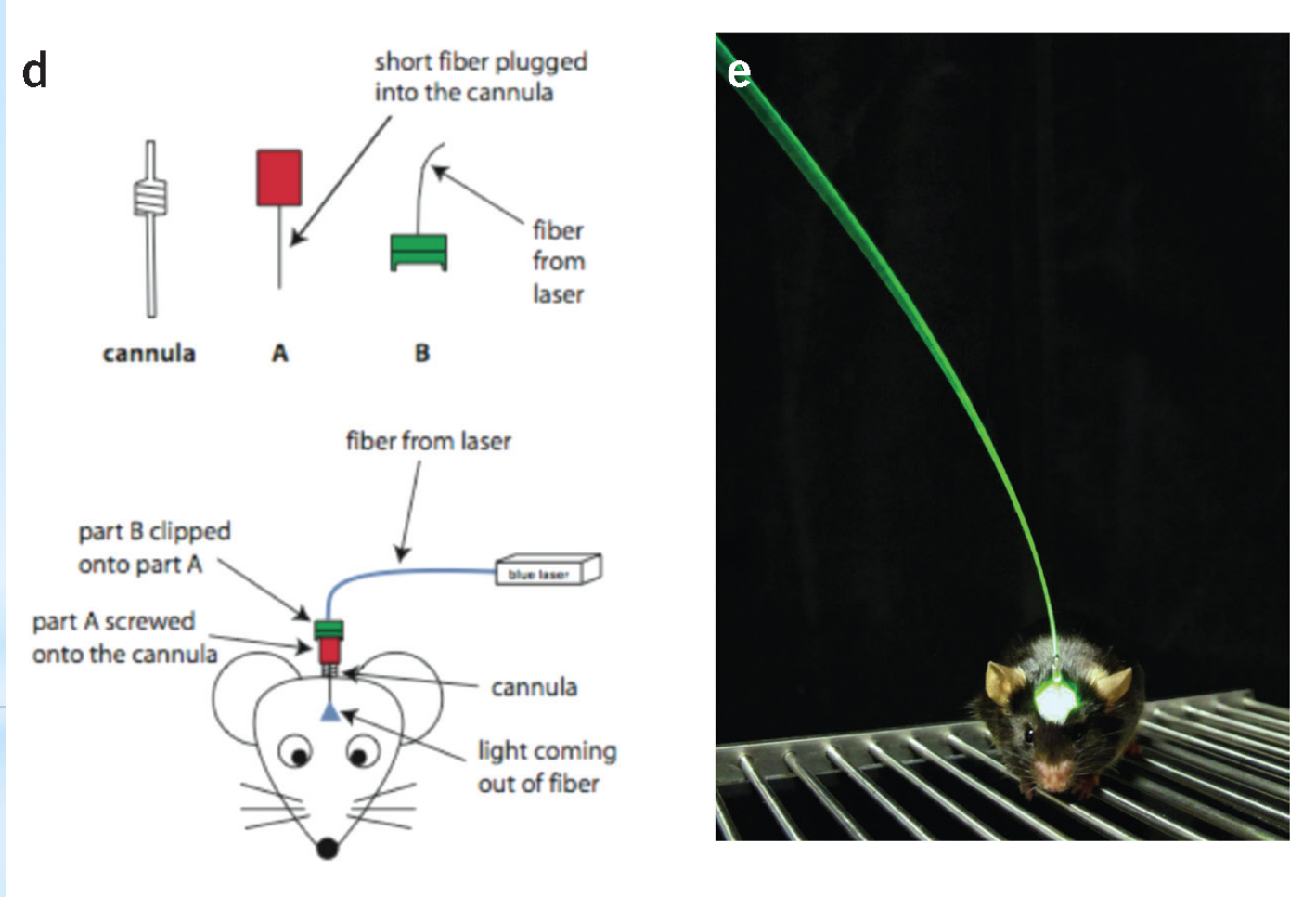
testing real-time read outs using electrophysiology and behavior in vitro (Fig. 2b,c)





Deisserhot, Nat Neurosc, 2015

and then, crucially, in vivo; and design (Fig. 2d) and implementation (Fig. 2e) of neural interfaces for in vivo light delivery and behavior

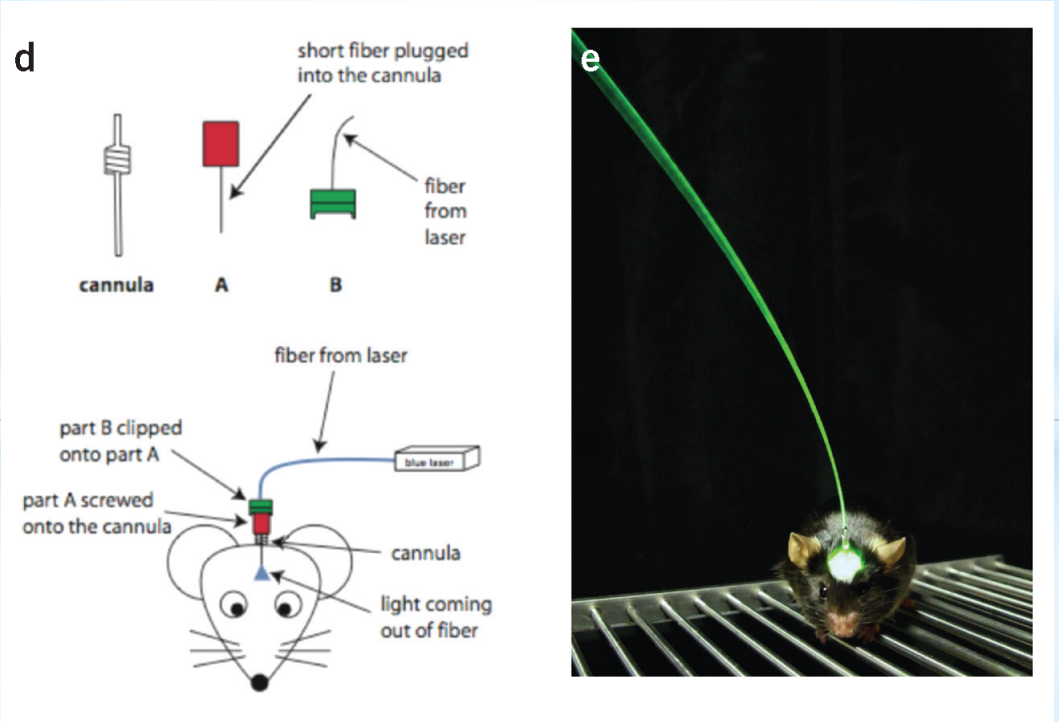


Deisserhot, Nat Neurosc, 2015

There was therefore a compelling need to safely, focally and flexibly deliver visible light via a neural interface deep into the brain of a freely behaving mammal at a high-intensity: ~100 mW mm^2 at the interface output, ~100× greater than needed at the opsin-expressing cells themselves because of expected scattering losses over the effective brain volume and much more intense than needed for imaging.

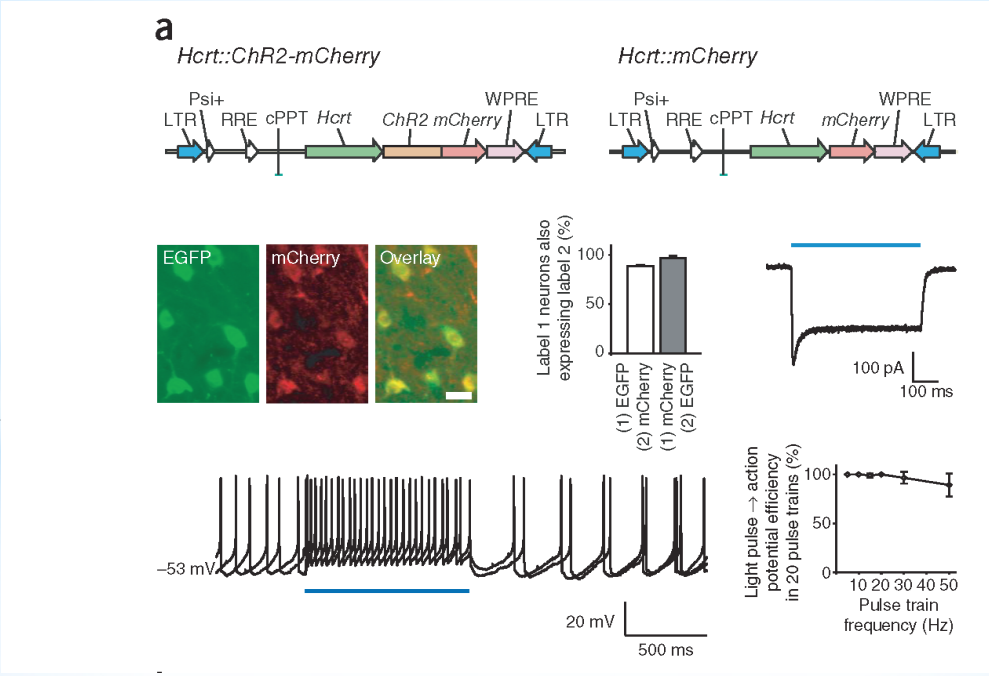
LEDs at the time were underpowered for coupling to optical fibers, and so necessity rapidly drove development of optogenetic interfaces based on **laser diode-coupled fiber optics**.

Among other features, including **heat isolation and activity feedback**, these interfaces crucially also registered virus injection to illumination site (Fig. 2d,e), which opened a new realm of experimental possibilities for targeted control and readout during behavior.

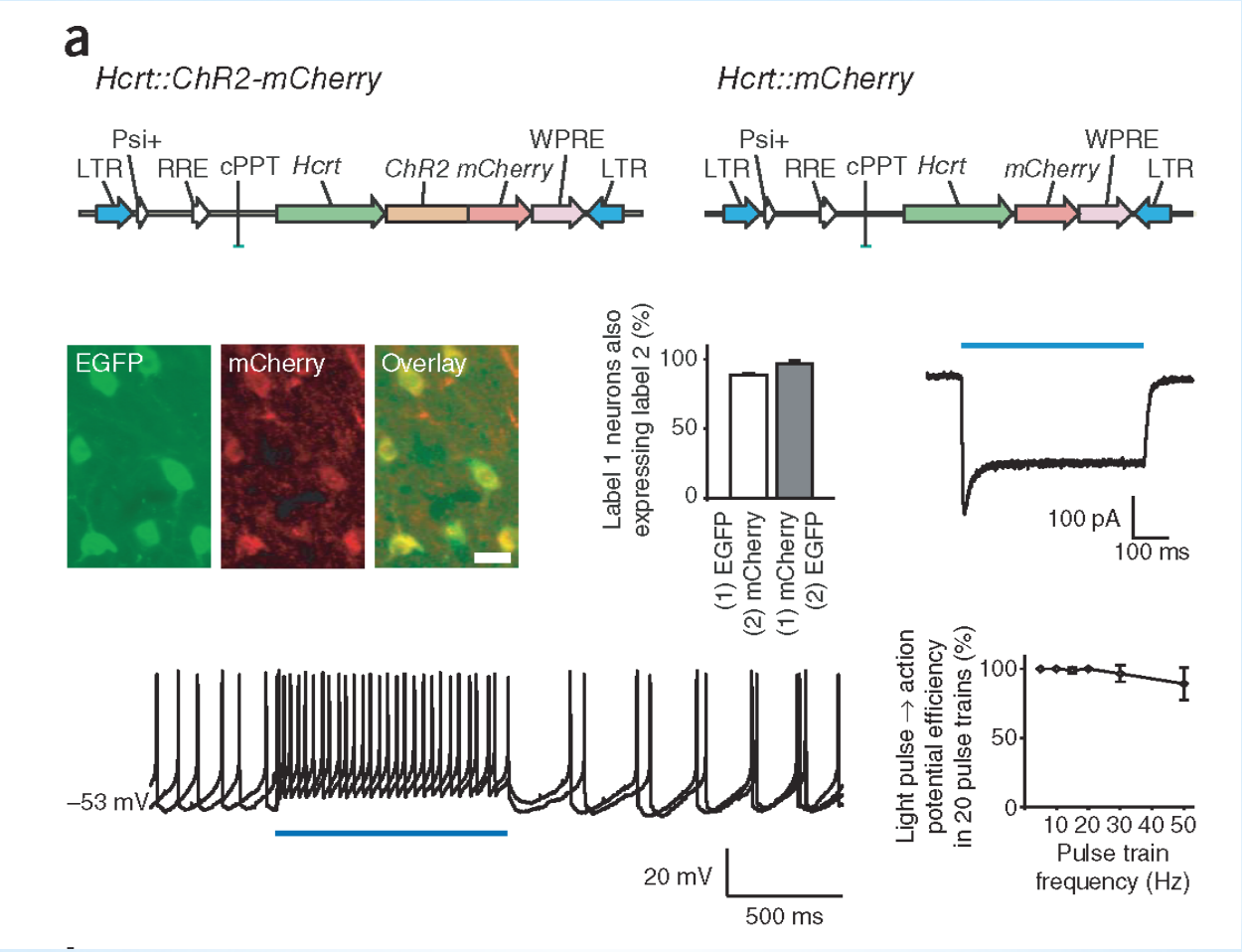


Deisserhot, Nat Neurosc, 2015

By mid-2007 (Fig. 3a,b) it was possible to selectively target a microbial opsin gene with high specificity and penetrance to a defined population of neurons deep in the brain of adult mice (in this case, hypocretin/orexin neurons in the hypothalamus), to play in a broad range of spike patterns through an optical fiber to those cells, to collect simultaneous multimodal system readouts during freely moving behavior (in this case, describing sleep/wake status via electroencephalography (EEG) and electromyography), and to demonstrate a causal role for defined activity patterns in specific brain cells in a natural behavior (in this case, sleep-wake transitions).



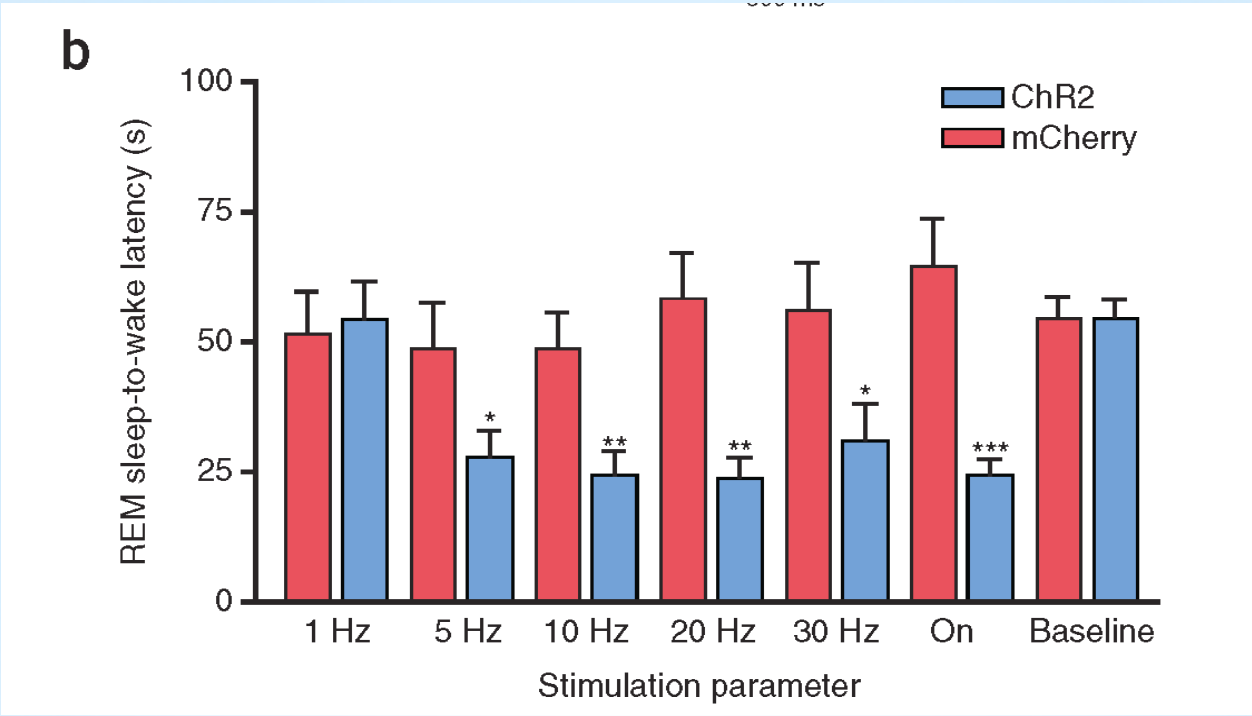
Deisserhot, Nat Neurosc, 2015



Top, initial cell-type targeting for optogenetics in behaving mammals, based on a 3.1-kb hypocretin (Hcrt) promoter fragment in lentivirus; control vector without opsin gene at right.

LTR, long terminal repeats; RRE, Rev-responsive element; WPRE, woodchuck post-transcriptional regulatory element; ChR2, channelrhodopsin-2; cPPT, central polypurine tract; Psi+, cis-acting packaging sequence; mCherry, a red fluorescent protein. Middle, specificity, penetrance and efficacy of expression in Hcrt neurons (green); ChR2–mCherry fusion (red) shown in mouse lateral hypothalamus (scale bar, 20 μm); right, photocurrent in hypothalamic slice. Bottom, neurons firing action potentials upon illumination; two sweeps superimposed. Error bars, s.e.m.

Adamantidis, A.R…. de Lecea, L., Nature, 2007



Dose-response of light flash effects; in experiment corresponding to a, latencies of wake transitions are shown from rapid eye motion (REM) sleep after a single 10-s photostimulation bout at different frequencies (15-ms light pulses). Error bars, s.e.m.

Adamantidis, A.R…. de Lecea, L., Nature, 2007

A broad adoption of optogenetics with microbial opsins did not occur until 2009.

The transition that followed was enabled only with the convergence of optics and genetics components of optogenetics, the second and third fields that would have to come together with microbial opsin genes.

Between 2004 and 2009, discovery along these key dimensions proceeded rapidly.

In these 5 years, the field accordingly saw development of versatile, high-titer cell-targeting opsin viruses and the creation of the initial broadly expressed and specific transgenic opsin mouse lines.

Cell-type targeting of opsin genes was not only achieved with genetics, however. It was **the fiber-optic hardware method** that enabled what is now the one of most widely used and generalizable approaches for targeting cells in behaving animals on the basis of anatomy or wiring.

Deisserhot, Nat Neurosc, 2015

Discoveries with optogenetics

Although the full scope of the findings that have resulted in the field can no longer be reviewed in detail, it is interesting to take note of key examples in the different categories of investigation that have emerged.

Broadly speaking, optogenetic methods have now illuminated the causal role of defined cell types and projections in natural as well as disease- related physiology and behaviors, ranging from the most basic homeostasis to advanced cognitive functions.

Discoveries with optogenetics

Exmples:

* optogenetic methods have now been used to illuminate the causal neuronal **underpinnings of movement regulation**, including the identification of surprising bottom-up circuit mechanisms by which the spinal cord and cerebellum regulate forebrain control of skilled and voluntary movements.
* the **transmission of primary sensory information** to the brain has also been studied extensively with optogenetics, including in the domains of olfactory, auditory, visual and tactile processing.

Discoveries with optogenetics

Exmples:

* Many studies have employed optogenetics to discover and map the **pathways along which such information flows in the brain,** including analysis of physical circuit connectivity itself, tagging cells defined by type or connectivity for use in other analyses, and integrating with fMRI or PET imaging to generate brain-wide maps of activity patterns recruited by defined neural cells or projections

Discoveries with optogenetics

Exmples:

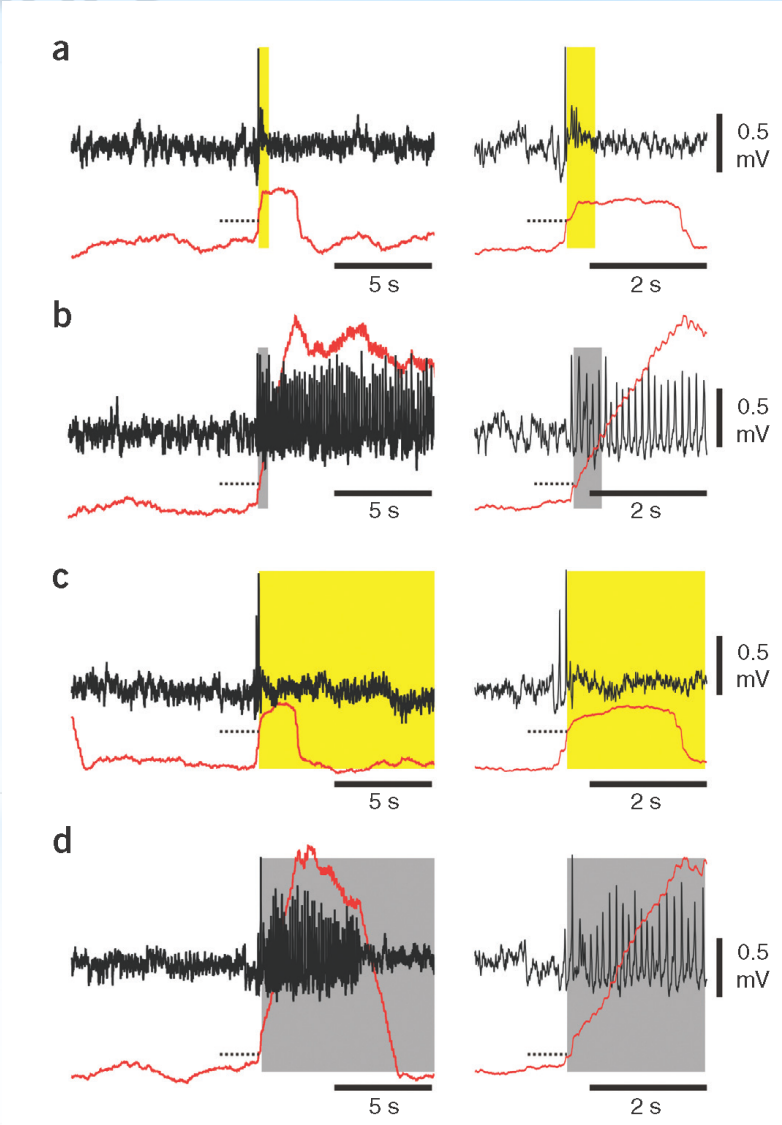
* Circuit activity patterns have also been identified, using microbial opsin genes expressed in conditional viruses and targeted with the fiber-optic interface, that control and modulate **many motivated behaviors**.

Indeed, insights have been derived into the causal circuit underpinnings of **reward** itself, as well as into the circuit implementations of **fear and anxiety**. Optogenetics in these cases has enabled the delineation of different cell types that, even though juxtaposed and intertwined, can have fundamentally oppositional roles in these complex behaviors.

Discoveries with optogenetics

Exmples:

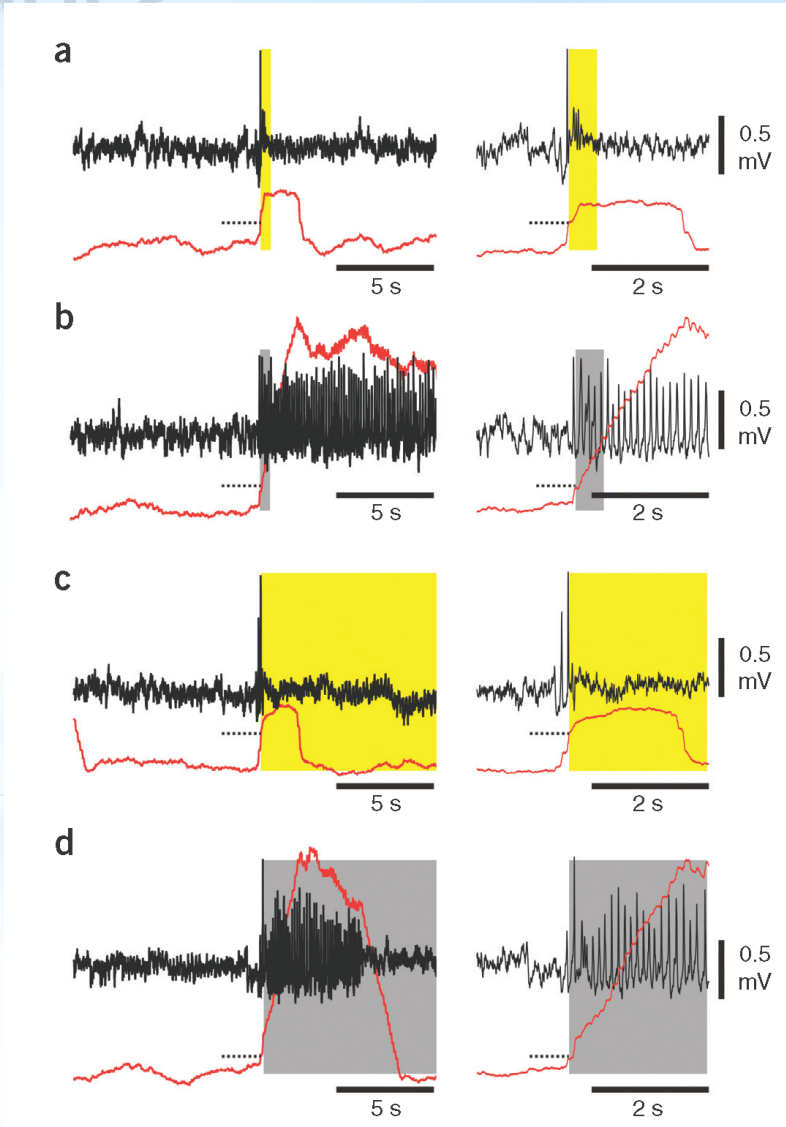
* Many discoveries have also emerged regarding the neural circuitry of symptoms related to **disease states.**
* Optogenetic methods have been applied to study the cellular activity underpinnings of **seizure propagation and termination**



Paz, J.T. et al. Nat. Neurosci. 16, 64–70 (2013)

Discoveries with optogenetics

* Progress in activity-guided intervention for optogenetics. (a-d) Closed-loop **targeting of thalamocortical neurons in epileptic cortex.** Yellow light terminates seizures defined by EEG and behavior, detected and interrupted in real time with closed-loop optogenetic inhibition using eNpHR3.0, an engineered inhibitory halorhodopsin (a,c). Without yellow light, native epileptic events follow an unmodified time course (b,d). **Thalamocortical activity** was thus, surprisingly, **identified as necessary for poststroke epileptic events in this context.**



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Discoveries with optogenetics

Exmples:

* Also on the neurological front, optogenetic investigations have led to the determination of the cells and pathways that promote or inhibit normal and parkinsonian movement patterns.

Technical challenges and outstanding problems

* If one attempts to selectively photocontrol a subcellular component (with typical 200-nm dimensions, e.g., a synapse, a pseudopod or a mitochondria), a focused laser beam using **two-photon photoactivation should preferentially be used.** This contrasts with one-photon excitation, which activates all of the photosensitive molecules along the illumination path, thereby increasing photodamage of the sample.

Technical challenges and outstanding problems

* Another major issue related to multiplexing is the **control of the expression level** of the two (or more) photoactivable engineered proteins and their stoichiometry
* Regarding the opsin gene expression itself, as with any foreign or native gene (especially those encoding membrane proteins), genes for optical actuation and for readout of structure and function bring a risk of possible toxic effects in the setting of high, long-term expression.
* This was addressable, and now every major class of overexpressed microbial opsin (proton pumps, chloride pumps and channels) in mammals has been shown to benefit from the provision of mammalian membrane trafficking signals to facilitate efficient movement through protein production pathways to the cell surface membrane.

Technical challenges and outstanding problems

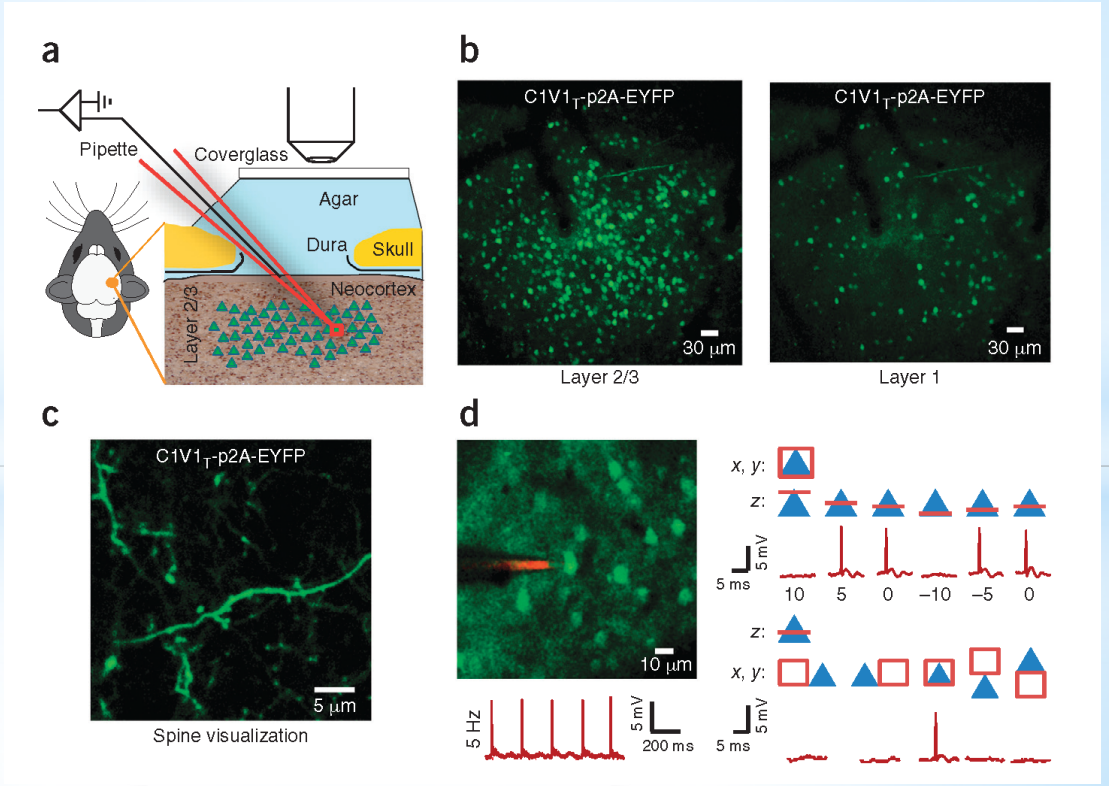
* Avoiding long-term expression with certain viruses (for example, rabies and herpesviruses) or with certain promoter/enhancer combinations that are too strong (such as CMV-based promoters in many settings) is also important, and optogenetics experiments should include, where practical, histological and/or electrophysiological validation of cell health, as well as baseline comparisons with nonopsin-expressing cells or animals. Of course, in most cases, each animal can also serve as its own light-off/light-on/light-off control to verify that the experiment is truly reading out an effect of actuation rather than of preparation or changes over time—controls that are harder to achieve in other approaches such as lesion studies.

Technical challenges and outstanding problems

* As capabilities in the field move toward **independent control of multiple single cells in a population** and to **optogenetic control of sparse**, distributed ensembles of cells defined by activity history, relative intrapopulation timing will become an increasingly interesting avenue of experimentation.

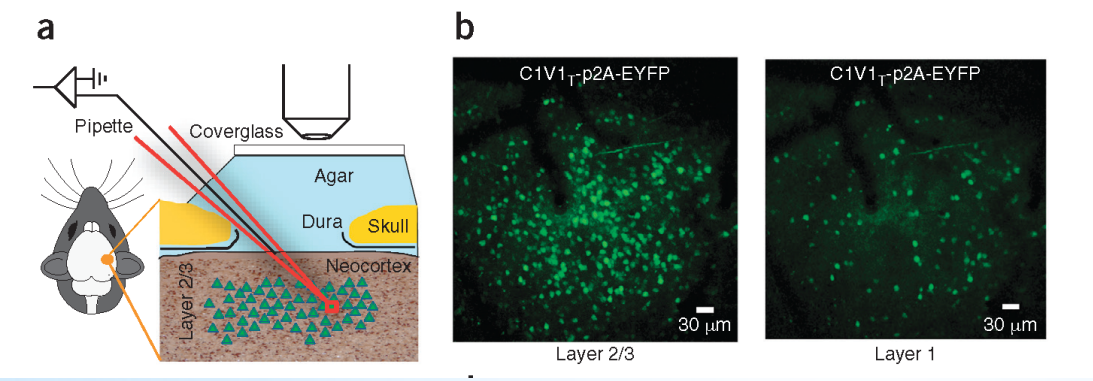
Technical challenges and outstanding problems

- Recently achieved with two-photon illumination-optimized opsins and spatial light targeting during behavior)



Deisserhot, Nat Neurosc, 2015

Technical challenges and outstanding problems



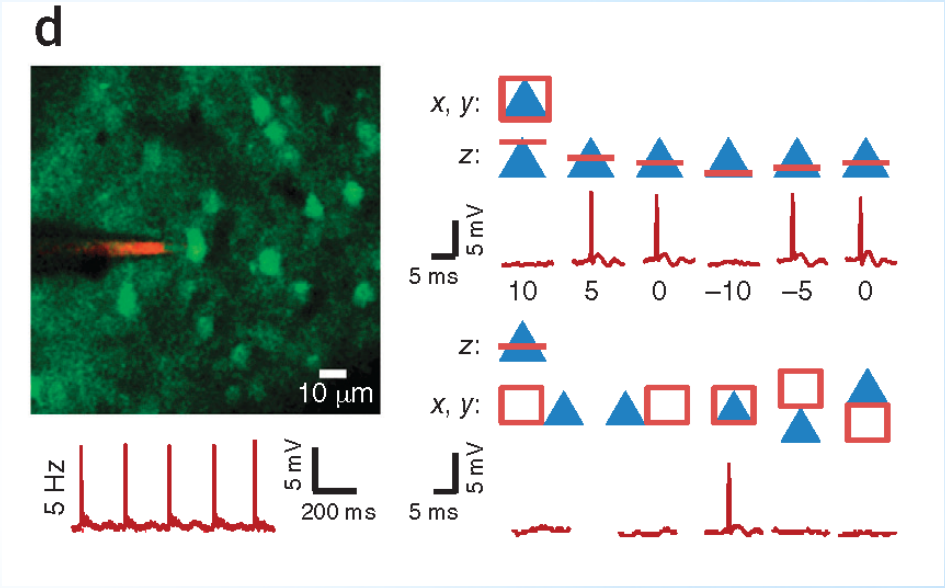
* Progress in spatially guided intervention for optogenetics: beyond cell subpopulation or projection targeting. (a–d) Initial *in vivo* twophoton, single-cell-resolution optogenetics with guided light: optogenetic control of spiking in adult mice. (a) Experimental setup targeting superficial layer 2/3 somatosensory neurons with C1V1. (b,c) Transduced neurons in somatosensory cortex shown at low (b) and high (c) magnification with cell-filling fluorophore version of the opsin virus used to facilitate cell identification, imaging and control.

Prakash, R. *et al. Nat. Methods* 9, 1171–1179 (2012).

Technical challenges and outstanding problems

d) Left, layer 2/3 pyramidal cells transduced with C1V1 under loose patch conditions (note red dye-filled patch electrode). Lower left, trace showing **5-Hz control of spiking with 1,040-nm raster-scanning illumination.**

Right, axial (upper) and lateral (lower) single-cell resolution of two-photon optogenetic **spiking control *in vivo***. Blue triangles indicate pyramidal neurons and red boxes illustrate region of- interest raster-scan positioning; traces show spiking occurring only while scanning within the cell.



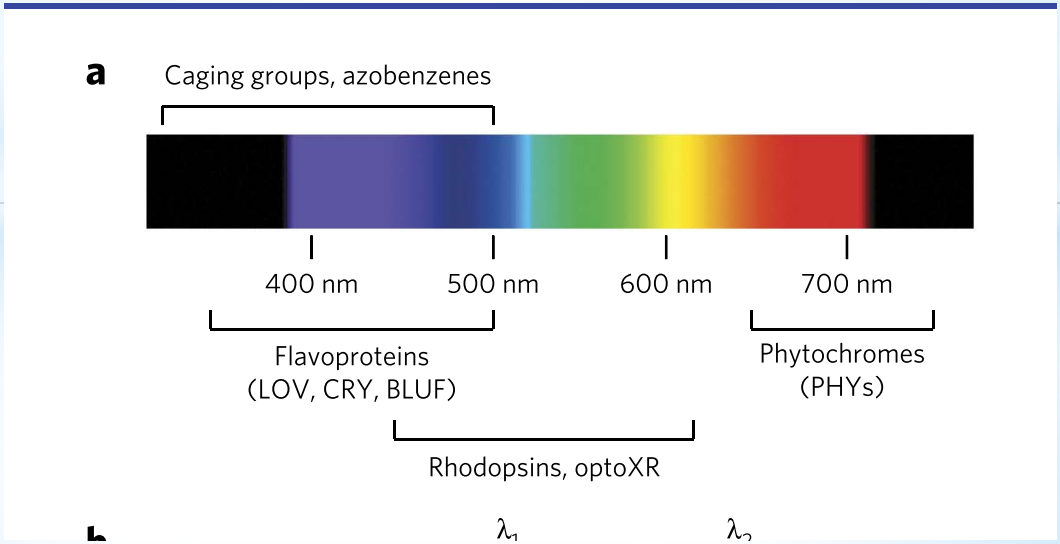
Prakash, R. *et al*. *Nat*. *Methods* 9, 1171–1179 (2012).

Technical challenges and outstanding problems

* The various methods developed to control protein with light, besides presenting different advantages and drawbacks, offer the **possibility to independently control the activity of several proteins within the same cell or at different locations in a living organism.** This feature will be particularly useful when analyzing feedback in both time and space.

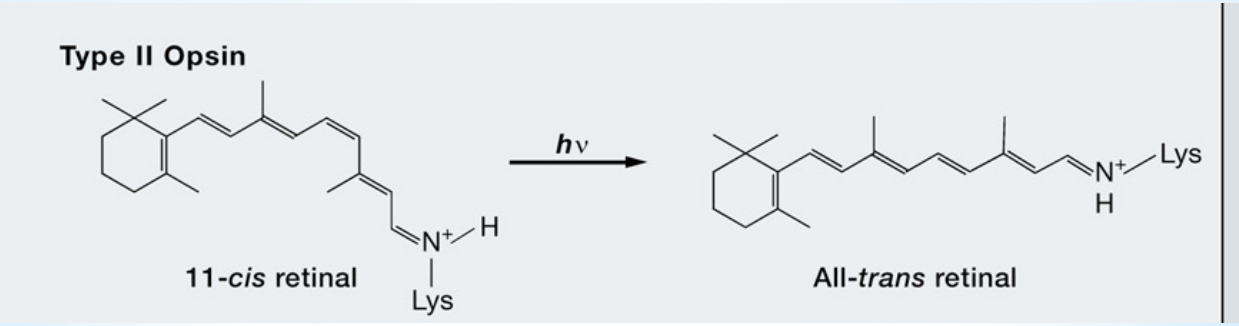
Technical challenges and outstanding problems

* Such multiplexing **requires independent activation of different photochemical systems**. The simplest way to do so relies upon **using light sources at sufficiently different wavelengths to control orthogonal photoactivable systems**. Photoactivatable proteins with light absorption spanning the whole visible range are already available. In contrast, despite recent progress, most caging groups and photochromic molecules are still restricted to the blue

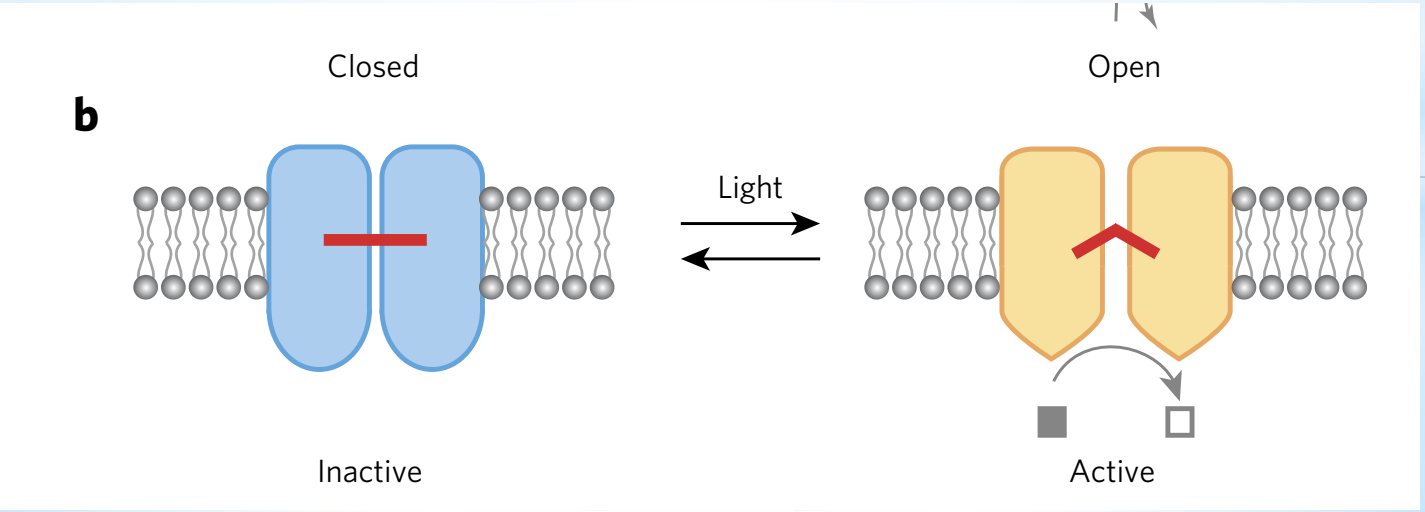


TYPE II OPSIN

* **type II opsin** genes are present only in higher eukaryotes and are mainly responsible for vision (Sakmar, 2002). A small fraction of type II opsins also play roles in circadian rhythm and pigment regulation (Sakmar, 2002; Shichida and Yamashita, 2003).
* Type II opsins primarily function as **G protein-coupled receptors (GPCRs)** and appear to all use the 11-cis isomer of retinal (or derivatives) for photon absorption. Upon illumination, 11-cis retinal isomerizes into the all-trans configuration and initiates protein-protein interactions (not ion flux) that trigger the visual phototransduction second messenger cascade.
* Unlike the situation in type I rhodopsins, here the retinal dissociates from its opsin partner after isomerization into the all-trans configuration, and a new 11-cis retinal must be recruited. Due to these chromophore turnover reactions and the requirement for interaction with downstream biochemical signal transduction partners, type II opsins effect cellular changes with slower kinetics compared to type I opsins.



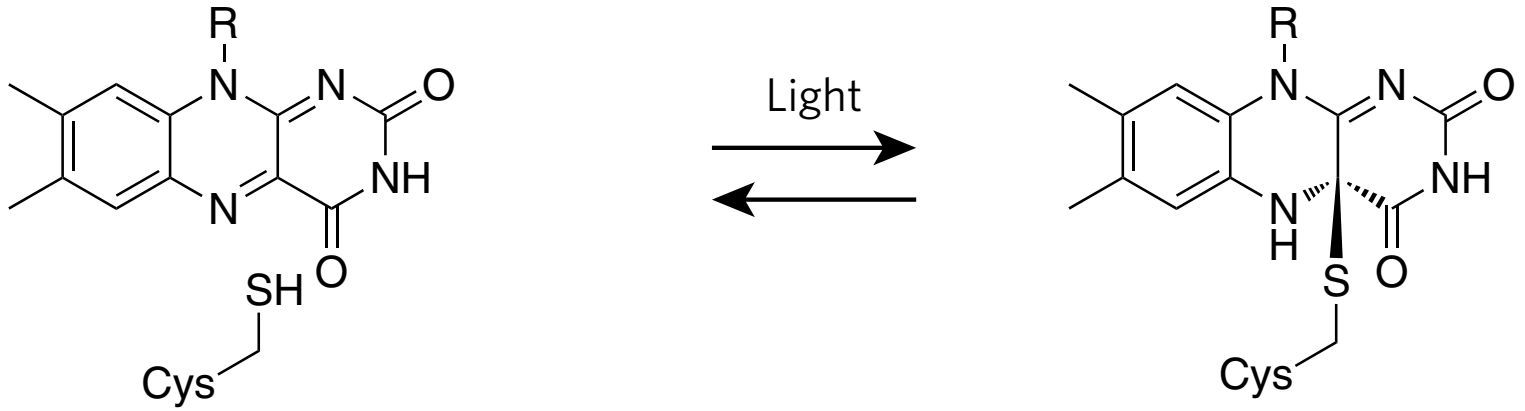
* However, structure-function work in type II vertebrate opsins from many laboratories (such as Kim et al., 2005 inspired the design of synthetic opsins (collectively termed **optoXRs**) allowing the control by light of signal transduction via G proteins and thus the photocontrol of second messengers (such as cyclic AMP and InsP3) or ion channels.
* By replacing the intracellular loops of bovine rhodopsin with the intracellular loops from GPCRs, an expanding family of optoXRs has enabled optical control of Gs, Gq, or Gi signaling in neuronal settings



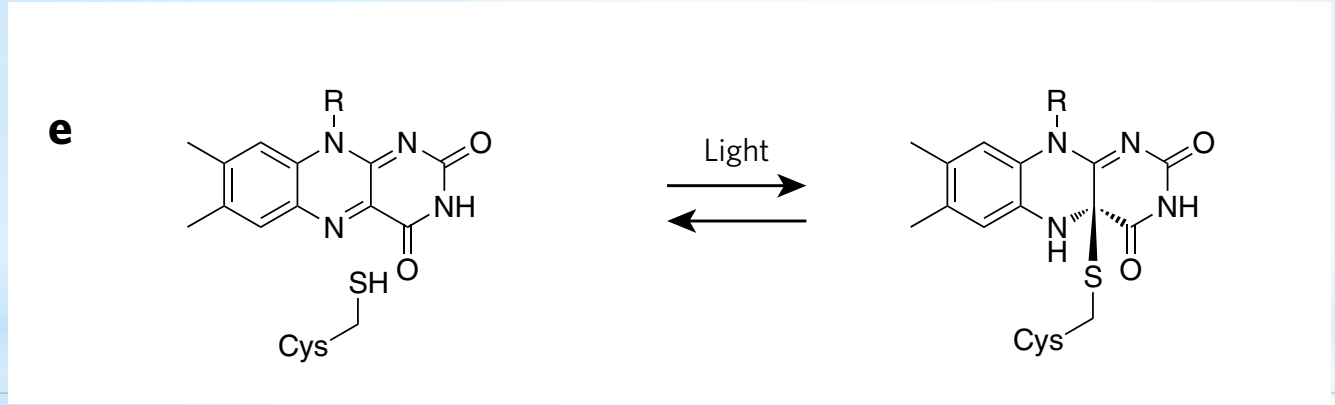
FLAVOPROTEINS

* **Flavoproteins** attracted particular interest because of their riboflavin-based chromophore, either flavin adenine dinucleotide (FAD) or flavin mononucleotide (FMN), which is naturally present in most cells.

e



* For some of these enzymes, light activation induces the formation of a thiol adduct between the chromophore and a conserved cystein residue (**Fig. 2e**), which triggers a marked modification of the protein structure (**Fig. 2c**). Three major flavoproteins were used: light-, oxygen- or voltage-sensing (LOV) proteins; blue light-utilizing flavin (BLUF) proteins; and the plant light-sensitive cryptochrome (CRY2).



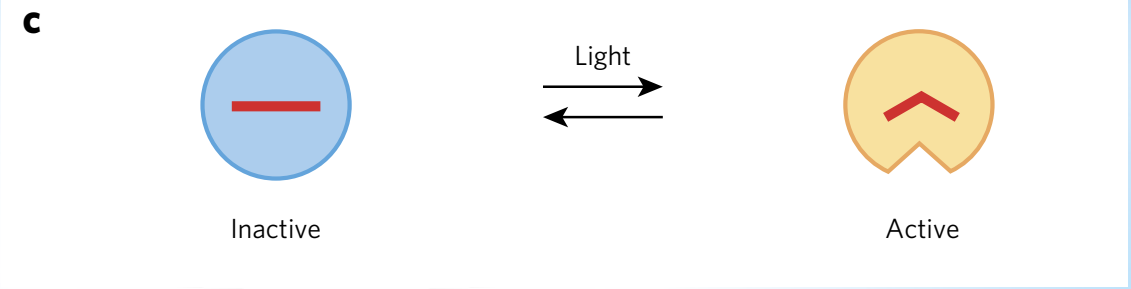
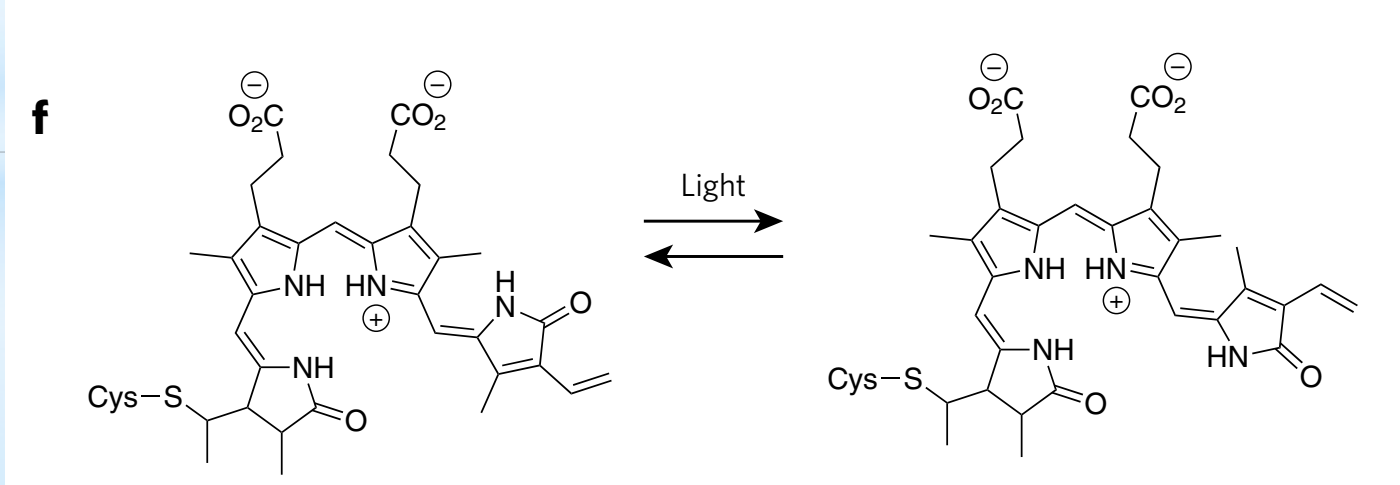


Table 1 | Examples of cellular features controlled by genetically encoded photoactuators

Light target	Light-gated module	Biochemical output	Targeted feature	λ_1 (λ_2) (nm)	ALI_1 (ALI_2) (mW cm^{-2})	ID (s)	TR (s)	Ref no.
FAD CRY	CRY2-Gal4BD transcription factor	CIB1-Gal4AD binding	Transcription initiation	461–488	–	0.1	1,000	23
FAD CRY	CRY2-CreN recombinase	CIBN-CreC binding	Recombination	461–488	–	2	–	23
FAD CRY	Tale-CRY DNA binding	CIB1-VP64 binding	Transcription initiation	466	5	0.5	1,000	25
FAD CRY	Tale-CRY DNA binding	CIB1-Sin3IDx4 binding	Histone acetylation	466	5	0.5	1,000	25
FMN LOV2	DHFR(x2)-LOV2 DHRF	TH-folate synthesis	Nucleotide biosynthesis	White light	–	300	50	29
FMN LOV2	LOV2-TAP Trp repressor	DNA binding	Gene expression	470	20	30	40	21
FMN LOV2	Lov-Rac1 small GTPase	GTPase	Actin dynamics	458	–	300	43	94
FMN FKT1	FKT1-VP16AD transcriptional activator	GI-Gal4BD binding	Transcription initiation	450	0.3	300	>5,000	22
FMN FKT1	FKT1-Rac1 small GTPase	G1Cher-CAAX binding	Actin dynamics	450	0.3	300	>5,000	22
FMN LOV2	LOV2-degron-targeted protein	Ubiquitination	Protein degradation	465	0.8	15,000	100	33
FMN LOV	mPAC adenylate cyclase[a]	cAMP	Signaling	460	0.6	100	16	31
FAD VVD-LOV	Gal4-vivid transcription factor	DNA binding	Gene expression	460	0.1–1	80,000	8,000	24,26
FAD BLUF	bPAC adenylate cyclase[a]	cAMP	Signaling	455	0.5	1–10	12	30
Bilin PHY	PHY-Gal4BD transcript. factor	PIF-Gal4AD binding	Transcription initiation	664 (748)	0.02–0.2 (0.02–0.2)	<1	$>10^3$	20
Bilin PHY	PHY-Cdc42 small GTPase	PIF-WASP binding	Actin dynamics	656 (766)	–	–	–	32
Bilin PHY	PHY-mCherry-CAAX mb. anchor	PIF -YFP binding	Protein trafficking	650 (750)	0.4 (5)	<1/<4	–	19
Bilin PHY	PHY-mCherry-CAAX mb anchor	iSH-YFP-PIF PI3K activation	Signaling	650 (750)	–	30	–	27

PLANT PHYTOCHROMES

* Among the other photoreceptors exploited to develop optogenetic tools, one can cite the **plant phytochromes (PHYs)**, which make it possible to control with light the heterodimerization of proteins.
* The photochemical behavior of phytochromes depends on the light-catalyzed *cis-trans* isomerization of a bilin chromophore (**Fig. 2f**). A major attractive feature of this class of proteins is their activation with red light, which enables easier multiplexing. Another advantage is that infrared light can be used to regenerate the inactive state. By alternating red and infrared illumination, the active state can thus be turned on and off at will.

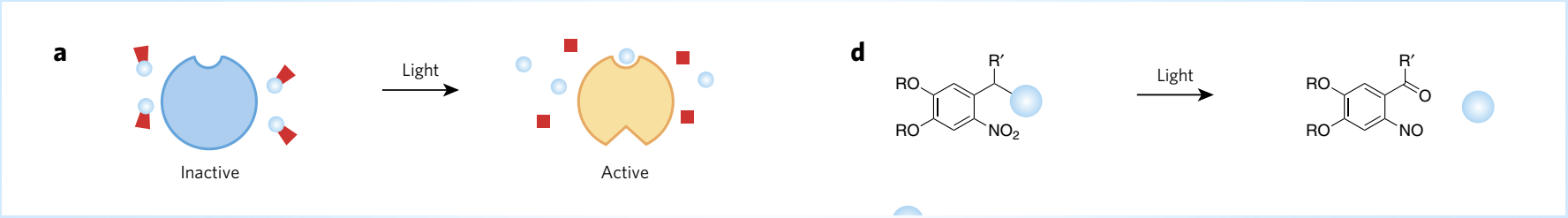


* In all of the developed systems (LOV, BLUF, CRY2 and PHY), photoisomerization of the bound chromophore offers the possibility to photocontrol a variety of cellular protein conformational change of the protein, which can be used to control the activity of a fusion protein either directly by unmasking a protein function through a conformational change or indirectly through the control of protein-protein interactions.
* This toolbox functions from chromatin modification to DNA transcription or recombination, protein translocation, enzymatic activity, cell morphology, signaling pathways and protein degradation.

HYBRID APPROACHES

* In parallel to the development of the toolbox described above, hybrid approaches have been developed that combine genetic modifications of the protein of interest and the use of exogenous photoactive synthetic molecules.
* The interest of relying on synthetic light-sensitive molecules is that a large collection of photochemistries is available. Various photolabile protecting groups can turn a biomolecule into a photoactivable entity.
* These so-called **caging groups** temporarily block the interactions with biological partners and can be photolysed with very high spatiotemporal resolution to locally release the biologically active molecule. Photoswitchable synthetic platforms that can interconvert between two functional conformations upon illumination are useful alternatives when reversibility is needed.

* The caging concept (**Fig. 4a,d**) was first used to increase the spatial resolution of inducible gene expression platforms to photocontrol protein concentrations by acting at the transcriptional level.

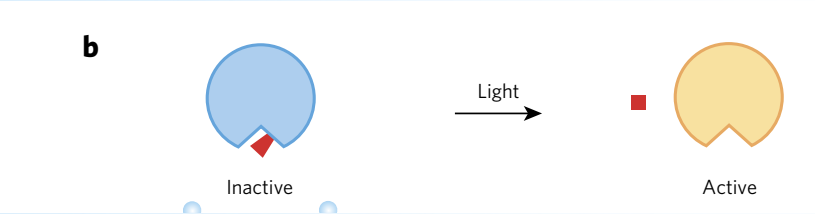


(a) Photolysis of caged compounds releases actuators interacting with a biological target, which becomes active after switching conformation and/or compartment and/or partner.
(**d**) Light activation irreversibly breaks a bond in caged compounds.

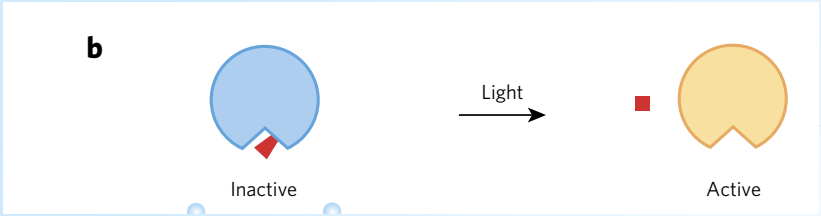
* A caged ecdysone was developed to create a photoactivable ecdysone-inducible gene expression system. Upon light illumination, the caged ecdysteroid is rapidly converted into active ecdysone, which binds and activates the ecdysone receptor, promoting its association to a responsive element and inducing the expression of the gene under its control.

* Caged selective estrogen-receptor modulators were used to control with light both gene expression and gene repression mediated by ERα and ERß. Photoactivable doxycycline derivatives were designed to activate with light transgenes on the basis of the tetracycline *trans*-activator ‘Tet-on’ system.
* This technique allows gene expression to be turned on in various organisms (mouse embryos and *Xenopus laevis* tadpoles) with very high spatiotemporal resolution by local illumination with ultraviolet light or by two-photon uncaging.

* To control proteins at the post-translational level, the caging concept has also been extended to the direct caging of proteins to photocontrol their activity at the single-residue level. However, although caging groups can be introduced easily within small molecules by chemical synthesis, the insertion of a caging group within a protein sequence is a much more challenging task.
* To circumvent the issue of chemical derivatization and cell delivery, methods to genetically encode caged amino acids (lysine, tyrosine and cysteine) in mammalian cells were developed for the site-specific introduction of caging groups into protein sequence (**Fig. 4b**).



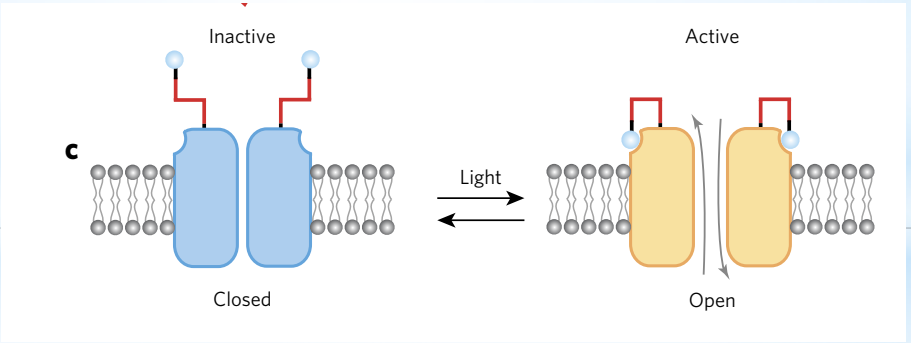
(a) Proteins can be caged by site-directed insertion of caged unnatural amino acids.



(a) Proteins can be caged by site-directed insertion of caged unnatural amino acids.

* This technology made it possible to photocontrol protein localization, signal transduction and gene expression. This approach, demonstrated initially in mammalian cells, should rapidly benefit from the recent upgrade of the unnatural mutagenesis strategy to multicellular organisms.

* The use of photoswitchable platforms for reversibly controlling protein function has been almost exclusively used so far to design light-gated ion channels and ionotropic receptors for applications in neuroscience.
* The light gate consists of a pore blocker (in the case of light-gated ion channels) or a ligand (in the case of ionotropic receptors) attached to the protein (via a nucleophilic amino acid side chain) with a photoswitchable azobenzene moiety, which acts as optical switch (**Fig. 4c**)



* A light-gated K+ channel was generated using a gate containing a quaternary ammonium as pore blocker.
* Illumination with long-wavelength light converts the azobenzene into its *trans* configuration, enabling the blocker off in rat neurons.
* Similarly, a light-activated ionotropic glutamate receptor was obtained by covalently tethering a glutamate analog to the receptor with an azobenzene linker. Photoisomerization provides the ability to reversibly control ligand binding, initiating allosteric domain closure and channel opening.
* This strategy, initially shown in non-neuronal cells, enabled the remote control of neuronal activity in culture and *in vivo* on the millisecond timescale.
* Recently, this concept was extended to the light-gated potassium-selective glutamate receptor.

* The power of using microbial opsins to modulate neuronal electrical activity has also stimulated strong interest in using light to control biochemical events in cells.

Table 1 | Examples of cellular features controlled by genetically encoded photoactuators

Light target	Light-gated module	Biochemical output	Targeted feature	λ_1 (λ_2) (nm)	ALI_1 (ALI_2) (mW cm^{-2})	ID (s)	TR (s)	Ref no.
UVR8	VSVG-YFP-UVR8-UVR8	ER retention	Protein secretion	312	0.3	7	>3 × 10^4	28
Retinal animal opsin	NinaE* 'chARGe' GPCR	IP_3, DAG	Neuron activation	White light	–	22	–	89
Retinal micr. opsin	ChR2 channel (Na^+, K^+)[a]	Ion flux	Neuron activation	442	10^3	–	–	2,4
Retinal animal opsin	Rh-5HT1A GPCR	G-gated channel	Neuron activation	485	–	3	–	8
Retinal animal opsin	Rh-β2AR GPCR	cAMP	Signaling	504	500	6	–	7
Retinal animal opsin	Rh-α_1AR GPCR	IP_3, DAG	Signaling	504	500	6	–	7
Retinal micr. opsin	MChR1 channel (Na^+, K^+)[a]	Ion flux	Neuron activation	531	–	2	–	90
Retinal micr. opsin	ChR1 channel (H^+)[a]	Ion flux	Neuron activation	500	400	0.2	–	91
Retinal micr. opsin	VchR1 channel (Na^+, K^+)[a]	Ion flux	Neuron activation	589	1.4	1	–	92
Retinal micr. opsin	eNpHR3.0 pump (Cl^-)[a]	Ion flux	Neuron inhibition	590–660	0.4	–	–	93
Retinal micr. opsin	NpHR pump (Cl^-)[a]	Ion flux	Neuron inhibition	593	2,200	0.05	–	72

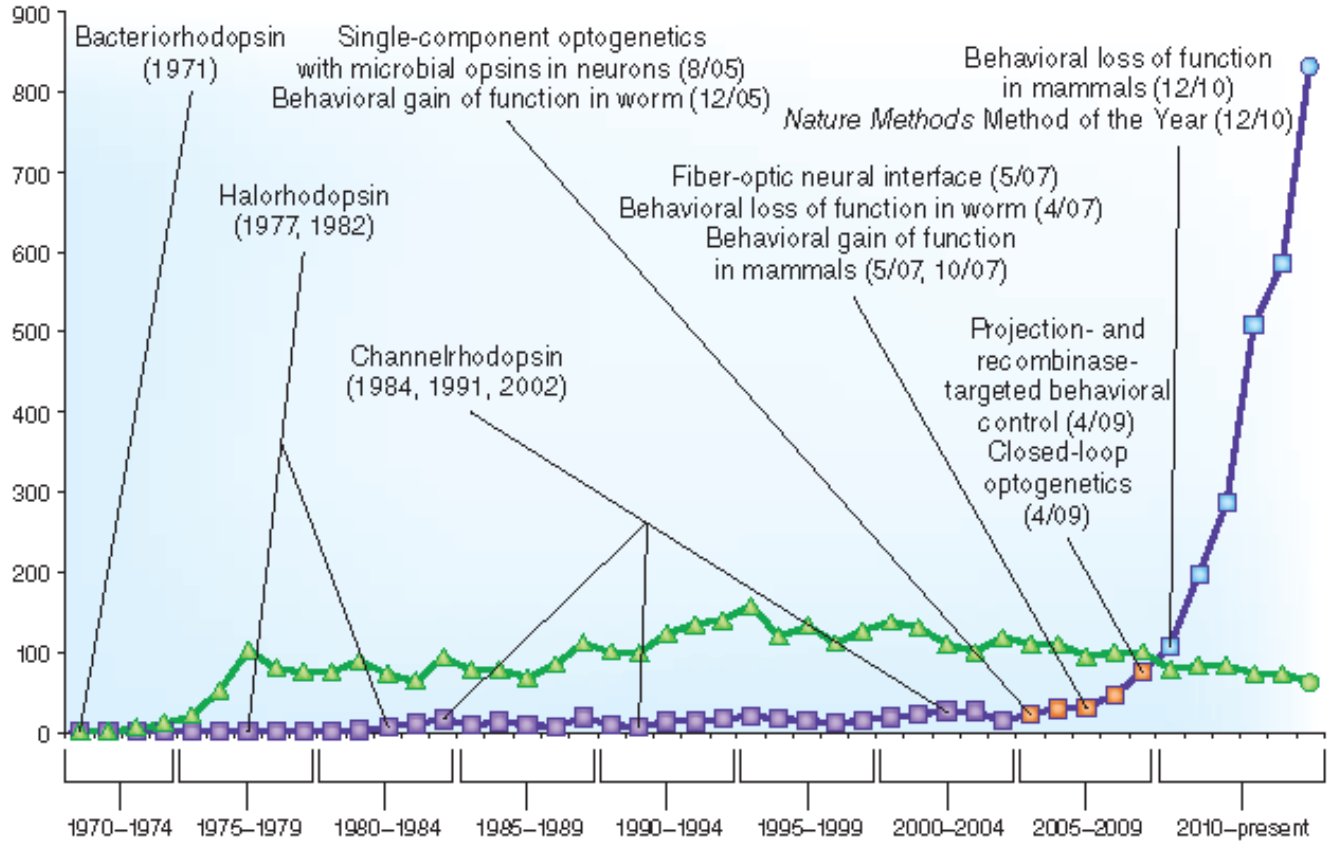


Figure 7 Publication timeline for microbial opsins and optogenetics over 45 years. Trajectory of the number of papers per year searchable in PubMed by bacteriorhodopsin (triangles); the second trajectory (squares) shows papers searchable by keywords encompassing all other related efforts: halorhodopsin, channelrhodopsin or variations of optogenetics. Note steady progress of the groundbreaking bacteriorhodopsin literature, not surpassed by the rest of the field until 2010. Key pioneering papers relevant to bacteriorhodopsin[7], halorhodopsin[198,199] and channelrhodopsin[15,46,200] are indicated. Publication counts: PubMed search on 1 July 2015. The first 5 years of single-component optogenetics are shown in orange, during which time few papers were published, and the second 5 years (to the present) shown in blue. Circular symbols for 2015 represent linear extrapolation based on the first 6 months.

Deisserhot, Nat Neurosc, 2015