·Review·

Optogenetics: a novel optical manipulation tool for medical investigation

Jun-Ping Yao[1,3], *Wen-Sheng Hou*[1,3], *Zheng-Qin Yin*[2,3]

Foundation items: National Natural Sciences Foundation of China (No. 81070749); Chongqing Science and Technology Project, China (No. CSTC, 2010AB5118)

[1]College of Biology Engineering, Chongqing University, Chongqing 400044, China
[2]Department of Ophthalmology, the Third Military Medical University, Chongqing 400038, China
[3]Chongqing Key Lab of Visual Damage and Regeneration and Restoration, Chongqing 400038, China
Correspondence to: Wen-Sheng Hou. College of Biology Engineering, Chongqing University, Chongqing 400044, China. hwscqu@yahoo.com.cn

Received: 2012-04-18 Accepted: 2012-07-25

Abstract

• Optogenetics is a new and rapidly evolving gene and neuroengineering technology that allows optical control of specific populations of neurons without affecting other neurons in the brain at high temporal and spatial resolution. By heterologous expression of the light-sensitive membrane proteins, cell type-specific depolarization or hyperpolarization can be optically induced on a millisecond time scale. Optogenetics has the higher selectivity and specificity compared to traditional electrophysiological techniques and pharmaceutical methods. It has been a novel promising tool for medical research. Because of easy handling, high temporal and spatial precision, optogenetics has been applied to many aspects of nervous system research, such as tactual neural circuit, visual neural circuit, auditory neural circuit and olfactory neural circuit, as well as research of some neurological diseases. The review highlights the recent advances of optogenetics in medical study.

• KEYWORDS: light-sensitive proteins; optogenetics; optical manipulation; medical
DOI: 10.3980/j.issn.2222-3959.2012.04.22

Yao JP, Hou WS, Yin ZQ. Optogenetics: a novel optical manipulation tool for medical investigation. *Int J Ophthalmol*/2012;5(4):517-522

INTRODUCTION

The precise control of neuronal activity in space and time is a major goal in neuroscience and medical research. Electrical [1-3], magnetic ultrasound [4,5] and pharmacological [6] stimulation have been used to activate neural tissue. Although successful to some degree, these techniques either simultaneously affect surrounding cells and their processes in addition to the target population or have slow kinetics and poor reversibility. Recently, genes that produce light-sensitive proteins have been used to control the electrical and biochemical activity within defined cell populations in order to overcome these spatial and temporal limitations. This strategy is used to genetically modify neurons so that they produce light-sensitive molecules, an approach known as optogenetics[7].

Optogenetics combines optics, genetics and bioengineering to either stimulate or inhibit cellular activity via light-sensitive proteins. By genetic targeting, optogenetic stimulation and inhibition of heterogeneous brain tissue can be achieved in a cell type-specific manner. In contrast, electrical stimulation unselectively interferes with all present cell types. As light of moderate intensity does not interfere with neuronal function, optogenetics uniquely combines cell type-specific control with millisecond time scale temporal resolution in a fully reversible manner. Once channelrhodopsin 2 (ChR2) had been recognized as optical interrogation tools in neuroscience, significant efforts have been made to lift the optogenetic approach to a level of broader applicability [8-11]. Bioengineering of existing light-sensitive proteins genes from different microorganisms generated a variety of chimeric light-sensitive proteins with modified properties regarding trafficking, kinetics and light responsivity[12-20]. Optogenetics has been intensively used for the analysis of complex biological mechanisms *in vitro*[8,9] and *in vivo* [21-24]. With an optical neural interface, optogenetic can be used to studied the animal behavior *in vivo* for freely moving mammals[22,25-27]. Beside neuroscience, optogenetics also can be used in other research fields such as cardio-myocytes and embryonic stem cells [28,29]. Optogenetics will be an excellent experimental tool and therapeutic applications in the future with the continuous progress in optical technologies and light-sensitive proteins engineering[19,30-32].

OPTOGENETIC TOOLS

Light Sensitive Proteins The field of optogenetics has advanced rapidly since 2005 when the technology was first described by a group of scientists from Stanford University[9,10].

We now have the means to hyperpolarize neurons by activating chloride pumps with yellow light [33-36], or conversely, ways to depolarize neurons by activating channelrhodopsins (ChRs) [18,37] or engineered step-function opsins [14,17] with blue or green light[38,39].

Channelrhodopsins (ChRs) ChR-2 was the first ChRs used to excite neurons with light. It is a cation channel isolated from the eyespot of *Chlamydomonas* algae and maximally excited by blue 470nm light [8]. Subsequent chimeragenesis, mutagenesis and bioinformatic approaches have introduced additional ChRs variants, such as ChR-2 with a H134R mutation (ChR-2/H134R), ChR-2 with an E123T mutation (ChETA), Volvox carteri ChR-1 and -2 (VChR-1, VChR-2), ChR-2 with C128 or D156A mutations (ChR-2/C128X, ChR-2/D156A), chimera D and EF (ChD, ChEF), and chimera EF with an I170V mutation (ChIEF). Each of these ChR variants has unique features and limitations [40]. Recently, a ChR-2 (T159C) mutant has been developed by a joint three-laboratory team composed of the labs of Peter Hegemann at Humboldt University, Karl Deisseroth at Stanford University and Thomas Oertner at the Friedrich Miescher Institute. When expressed in neurons, the T159 C mutant elicits photocurrents almost twofold larger than those of wild-type ChR-2 [24]. Researchers can use the T159 C mutant to activate neurons with dimmer light pulses, which will be handy when performing experiments *in vivo*. As with previous higher-current ChR-2 mutants, however, the closure of the TC mutant's ion channel after a light stimulus is slightly longer. To tackle this, the group combines the TC mutation with the E123T mutation or 'ChETA'. The double E123T,T159C (ET-TC) mutant exhibits both increased photocurrents and faster kinetics compared to wild-type ChR-2 [41]. By modifying one residue in wild-type ChR-2, the Kleinlogel group generated a mutant with higher calcium permeability, called ‘CatCh’ [19]. After expressed in nonneuronal cells, CatCh's modest preference for calciumions elicits approximately three times higher currents and a slight slowdown of its kinetics compared to wild-type ChR-2. But when expressed in neurons, the cell's light sensitivity increased nearly 70-fold and its membrane produced a surprisingly rapid and complete repolarization after each spike. CatCh can act as a light-gated membrane-bound calcium source.

Opto –XRs Opto-XRs (opto-β_2AR, opto-α_1AR) are synthetic chimaeric transmembrane proteins that have an extracellular component derived from a light-sensitive rhodopsin, and an intracellular component that is derived from other G protein coupled receptors, such as various specific adrenergic receptors. In cells expressing these ‘Opto-XRs’, distinct second messenger cascades can be induced by illumination[13].

Halorhodopsin Halorhodopsins are light-sensitive chloride pumps isolated from two strains of archaea, *Halobacterium salinarum* (HsHR) and *Natronomonas pharaonis* (NpHR). Illumination of HsHR- or NpHR-expressing oocytes leads to rapid outward currents and both have excitation maxima near 580nm. HsHR was found to have a lower extracellular Cl^- affinity than NpHR and measured currents showed rapid rundown at low extracellular Cl^- concentrations that did not fully recover in the dark. Halorhodopsin pumps are thought to have a very low affinity for Cl^- on the cytoplasmic side of the membrane where Cl^- is released, because halorhodopsin-mediated chloride pumping can achieve molar concentrations of cytoplasmic Cl^-. The pump currents exhibited a more or less linear voltage dependence, and the Cl^- current was robust for both halorhodopsins across all physiological voltage regimes. NpHR was chosen for neuronal applications on the basis of its higher extracellular Cl^- affinity and stability[35].

Expression Systems Delivering Genes That Produce Light Sensitive Proteins in the Cell A major aspect of optogenetics is the delivery of genes that produce light-sensitive proteins in the neurons. Different genes can be selectively expressed in defined subsets of neurons *in vivo* using a variety of expression targeting strategies.

Viral Vectors Lentivirus and adeno-associated virus (AAV) can be used to transfect genes that will produce light-sensitive protein expression in a wide range of experimental subjects ranging from rodents to primates [42]. Most of the common AAV and lentivirus vectors carry strong ubiquitous or pan-neuronal promoters, while others have more specific promoter fragments that retain cell type-specificity, allowing selective targeting in animals that are not accessible to transgenic technology. For example, adding the myosin-Va binding domain (MBD) of melanophilin to the C-terminus of ChR-2-yellow fluorescent protein (YFP) produces somato-dendritic localization but is largely excluded from the axon [43]. Fusing the II-III loop from Nav1.2 to the extracellular domain of neurofascin [44], green fluorescent protein (GFP)[45] or YFP[46] produces reliable localization in initial segment of the axon in dissociated hippocampal cultures.

Although cell-specific promoters are effective at restricting gene expression to subsets of genetically defined neurons, some promoters have weak transcriptional activity. To amplify the transcriptional activity in a cell specific manner, conditional AAV vectors [47,48] have been developed recently to capitalize on the numerous cell-specific Cre-driver transgenic mouse lines that have been made available by individual labs and collective projects such as GENSAT. These conditional AAV expression vectors carry transgene cassettes that are activated only in the presence of Cre. The use of strong ubiquitous promoters to drive the Cre-activated transgene selectively amplifies light-sensitive protein gene

expression levels only in the cells of interest.

Electroporation Specific cell types can also be developmentally targeted by *in utero* electroporation[49,50] of precisely timed embryos. *In utero* electroporation has been used to express light-sensitive proteins in inhibitory neurons of the striatum or in the hippocampus[50,51]. In addition, unlike viral delivery methods, *in utero* electroporation can be used to deliver DNA fragments of any size, therefore permitting the use of larger promoter segments to achieve higher cell-type specificity. Electroporation also allows a high copy number of genes to be introduced into the target cells.

Transgenic animal Transgenic technology can be used to restrict gene expression to specific subsets of neurons in mice or rats. Light-sensitive protein genes can be functionally expressed in subsets of neurons in intact circuits using either short transgene cassettes carrying recombinant promoters or bacterial-artificial-chromosome based transgenic constructs. Several transgenic mouse lines carrying ChR-2 under the Thy-1 promoter, which have been proven to be useful for a wide array of experiments, have been generated without any noticeable behavioral or reproductive defects[12,52,53].

Light Sources Activation of neural circuits containing light-sensitive proteins can be obtained by several means. Xenon arc lamps, light emitting diodes (LEDs) and lasers can provide the proper wavelengths for *in vitro* situations, such as brain slice preparations and cell cultures. LEDs can be used for illuminating superficial brain areas and implantable laser-coupled optic fibers have been used to illuminate deeper brain structures in living animals[54].

Readout Several classes of circuit readout are compatible with optogenetic control. Voltage-sensitive dye (VSD) imaging can be combined with optogenetic techniques to conduct all-optical stimulation and imaging of evoked circuit responses in brain slices. VSDs are lipophilic molecules whose optical absorbance or emission properties vary depending on the electrical potential across the membrane. High-speed cameras that capture changes in the optical signal during VSD imaging allows researchers to measure electrical activity changes in neurons on a millisecond timescale and with micro-scale spatial resolution[55-58]. Optically compatible VSDs, such as RH-155 (absorption -700nm), are sufficiently separated from the excitation peaks of ChR-2, VChR-1 and NpHR to permit multimodal all-optical stimulation and imaging [52,53].

A second class of readout involves *in vivo* control integrated with either or both electrical recordings and behavioral measures. Targeting light-stimulation and electrical-recording devices so that they reach the delivery site of light-sensitive protein genes poses an experimental challenge that can be readily overcome with the appropriate protocol. A fiber-optic based optical neural interface has been developed to meet this challenge [25] and uses stereotactically implanted cannula guides to achieve targeted virus infusion and light delivery into the desired brain structure.

OPTOGENETIG APPROACHES IN MEDICAL SCIENCE

Up to now, optogenetic approaches have been shown to be superior to any other technology for selective stimulation of defined cell populations[59]. Traditional metal electrodes have limited cell selectivity, primarily due to their low spatial resolution and inability to selectively activate different neuronal subtypes within the electrical field generated by the current stimulus. Drug uncaging by light stimulation can overcome some of the limitations of electrical stimulation but lacks the ultrafast dynamics of optogenetics, which operates on a millisecond timescale[60].

Optogenetic tools offer new ways to analyze neuronal circuits. For example, the input axons can be selectively targeted by cell specific expression of the optogenetic tools. Because axons survive in a slice preparation, long-range connections remain viable and can be specifically activated by light, and the optical trigger does not interfere with the electrical recordings. Petreanu *et al* [61] mapped the synapses with pyramidal neurons in the mouse barrel cortex by expressed ChR-2 in the whisker motor cortex and local excitatory neurons. ChR-2 expression in layer-5 of the motor cortex of transgenic mice enabled the topographic mapping of motor cortex and muscle innervation [62]. Cardin *et al* [63] used this technology to analyze the neuronal origins of cortical gamma oscillations *in vivo*.

The artificial activation or inactivation of defined neuronal populations within a neuronal circuit can reveal which neurons are necessary for a speci c behavioral task. Optogenetic tools make it possible to determine which neurons are suf cient to elicit speci c behaviors. In *C. elegans*, when ChR-2 was expressed in mechanosensory neurons, light evoked withdrawal behaviors could be evoked that are normally elicited by mechanical stimulation [64]. Conversely illumination of NpHR in cholinergic motor neurons using the vesicular acetylcholine transporter promoter, strongly reduced or essentially stopped swimming behavior [33]. In ChR-2 transgenic Drosophila, stimulating a nociceptor with blue light elicited a 'pain' response. In freely behaving adult flies, rapid photoactivation of targeted gustatory sensory neurons, modulatory dopaminergic neurons, and motor neurons triggered a proboscis extension response, escape reflex, or changes in the pattern of locomotion, respectively, with precise temporal control [65]. By using complementary optogenetic activation and targeted silencing of sensory neurons it was found that Drosophila

larvae "roll" with a motor pattern that is completely distinct from the style of locomotion used for foraging. A single class of neuron (class IV multidendritic neuron) was found to be sufficient and necessary for triggering the unusual rolling behavior [66]. Similarly, an aversive locomotion response to CO_2 could be mimicked by light-activated sensory neurons specifically responsive to CO_2 [67]. In zebrafish, photoactivation of ChR-2 in genetically defined populations of somatosensory neurons triggered escape behaviors and even a single action potential in a single sensory neuron was at times sufficient to evoke escape behavior [68]. When ChR-2 was specifically expressed in dopaminergic neurons and activated by light stimulus paired with an odor stimulus this induced an aversive memory formation. In contrast, when tyraminergic and octopaminergic neurons were activated through ChR-2, a simultaneously perceived odor was learned to be more attractive [69]. Tsai *et al* [22] specifically targeted mouse dopaminergic neurons with ChR-2 and found that phasic activation of these neurons with an optic fiber was sufficient to drive behavioral conditioning and elicited dopamine transients with magnitudes not achieved by longer, lower-frequency spiking.

POSSIBLE CLINICAL APPLICATIONS

Optogenetics has the potential to advance clinical neuromodulation. Genetic control makes it possible to develop more precise therapies by restricting the excited or inhibited neurons to the disease-relevant population. Moreover, the ability to simultaneously record electrical activity during optical stimulation without electrical artefacts makes it possible to engage in responsive neuromodulation by dynamically adjusting the stimulation or inhibition intensity based on feedback from the activity state in the brain. Optogenetics opens new opportunities for more systematic delineation of disease mechanisms and better treatments.

Optogenetic is a potentially useful tool for restoring vision in patients with photoreceptor degeneration. The rod and cone cells of the mammalian retina are the principal photoreceptors for image-forming vision. They transmit information by means of a chain of intermediate cells to the retinal ganglion cells, which in turn send signals from the retina to the brain. The death of photoreceptor cells caused by retinal degenerative diseases often results in a complete loss of retinal responses to light. It has recently been shown that ectopic retinal expression of light sensitive protein, channelrhodopsin-2 [30,31,70] or melanopsin [16], can restore simple visual abilities in genetically blind mice and rats[71-73]. Coexpression of ChR2/HaloR in retinal ganglion cells can produce ON, OFF, and even ON-OFF responses, depending on the wavelength of the light stimulus [74]. These Experiments indicate that optogenetic tool can provide a viable and efficacious clinical therapy for photoreceptor disease-related blindness.

By using optogenetic neuromodulation, several brain diseases had be treated or studied in animal models. Experiment showed that light activation of ChR-2-expressing cells was sufficient to bring about recovery of respiratory diaphragmatic motor activity. Alilain *et al*[75] induced spinal neurons in and around the phrenic motor pool to express ChR-2 and subsequent photostimulation restored respiratory motor function in adult animals with cervical spinal cord injuries. Optical deep brain stimulation may be a refined substitute for electrical stimulation to alleviate symptoms for different pathological conditions such as Parkinson's disease [76], depression [77], obsessive-compulsive disorder (OCD) [78], chronic pain [79,80] and Epilepsy[81].

In conclusion, optogenetic is still a relatively new technology but it has already been proven to be a highly effective tool for dissecting normal brain function and disease mechanisms. Although the therapeutic potentials for its human clinical application are still to be proven, several encouraging studies, together with the constant development of more advanced tools for optogenetics, have outlined great hope. The limiting factor here is the safe delivery of transgenes into somatic cells of humans. If this hurdle can be overcome in the future, optogenetics might provide a useful strategy for many medical applications.

REFERENCES

1 Wilson BS, Dorman MF. Cochlear implants: a remarkable past and a brilliant future. *Hear Res* 2008;242(1-2):3-21

2 Benabid AL, Chabardes S, Mitrofanis J, Pollak P. Deep brain stimulation of the subthalamic nucleus for the treatment of Parkinson's disease. *Lancet Neurol* 2009;8(1):67-81

3 Hales CM, Rolston JD, Potter SM. How to culture, record and stimulate neuronal networks on micro-electrode arrays (MEAs). *J Vis Exp* 2010;30 (39):2056

4 Tufail Y, Matyushov A, Baldwin N, Tauchmann ML, Georges J, Yoshihiro A, Tillery SI, Tyler WJ. Transcranial pulsed ultrasound stimulates intact brain circuits. *Neuron* 2010;66(5):681-694

5 Tyler WJ, Tufail Y, Finsterwald M, Tauchmann ML, Olson EJ, Majestic C. Remote excitation of neuronal circuits using low-intensity, low-frequency ultrasound. *Plos One* 2008;3(10): e3511

6 Gorostiza P, Isacoff EY. Optical switches for remote and noninvasive control of cell signaling. *Science* 2008; 322(5900):395-399

7 Deisseroth K, Feng G, Majewska AK, Miesenbock G, Ting A, Schnitzer MJ. Next-generation optical technologies for illuminating genetically targeted brain circuits. *J Neurosci* 2006;26(41):10380-10386

8 Nagel G, Szellas T, Huhn W, Kateriya S, Adeishvili N, Berthold P, Ollig D, Hegemann P, Bamberg E. Channelrhodopsin-2, a directly light-gated cation-selective membrane channel. *Proc Natl Acad Sci U S A* 2003;100 (24): 13940-13945

9 Boyden ES, Zhang F, Bamberg E, Nagel G, Deisseroth K. Millisecond-timescale, genetically targeted optical control of neural activity. *Nat Neurosci* 2005;8(9):1263-1268

10 Deisseroth K, Feng GP, Majewska AK, Miesenbock G, Ting A, Schnitzer MJ. Next-generation optical technologies for illuminating

genetically targeted brain circuits. *J Neurosci* 2006;26(41):10380-10386
11 Zhang YP, Oertner TG. Optical induction of synaptic plasticity using a light-sensitive channel. *Nat Methods* 2007;4(2):139-141
12 Zhao SL, Cunha C, Zhang F, Liu Q, Gloss B, Deisseroth K, Augustine GJ, Feng GP. Improved expression of halorhodopsin for light-induced silencing of neuronal activity. *Brain Cell Biol* 2008;36(1-4):141-154
13 Airan RD, Thompson KR, Fenno LE, Bernstein H, Deisseroth K. Temporally precise *in vivo* control of intracellular signalling. *Nature* 2009; 458(7241):1025-1029
14 Berndt A, Prigge M, Gradmann D, Hegemann P. Two open states with progressive proton selectivities in the branched channelrhodopsin-2 photocycle. *Biophys J* 2010;98(5):753-761
15 Berndt A, Tsunoda S, Hegemann P. Mutations in channelrhodopsin 2 (ChR2) affect changes in photocycle, ion specificity and absorption maximum. *Biophys J* 2007;149a-149a
16 Lin B, Koizumi A, Tanaka N, Panda S, Masland RH. Restoration of visual function in retinal degeneration mice by ectopic expression of melanopsin. *Proc Natl Acad Sci USA* 2008;105(41):16009-16014
17 Bamann C, Gueta R, Kleinlogel S, Nagel G, Bamberg E. Structural guidance of the photocycle of channelrhodopsin-2 by an interhelical hydrogen bond. *Biochemistry* 2010;49(2):267-278
18 Gunaydin LA, Yizhar O, Berndt A, Sohal VS, Deisseroth K, Hegemann P. Ultrafast optogenetic control. *Nat Neurosci* 2010;13(3):387-392
19 Kleinlogel S, Feldbauer K, Dempski RE, Fotis H, Wood PG, Bamann C, Bamberg E. Ultra light-sensitive and fast neuronal activation with the Ca^{2+}-permeable channelrhodopsin CatCh. *Nat Neurosci* 2011;14 (4): 513-518
20 Schultheis C, Liewald JF, Bamberg E, Nagel G, Gottschalk A. Optogenetic long-term manipulation of behavior and animal development. *Plos One* 2011;6(4):e18766
21 Zhang F, Gradinaru V, Adamantidis AR, Durand R, Airan RD, de Lecea L, Deisseroth K. Optogenetic interrogation of neural circuits: technology for probing mammalian brain structures. *Nature Protocols* 2010;5(3):439-456
22 Tsai HC, Zhang F, Adamantidis A, Stuber GD, Bonci A, de Lecea L, Deisseroth K. Phasic firing in dopaminergic neurons is sufficient for behavioral conditioning. *Science* 2009;324(5930):1080-1084
23 Zhang F, Aravanis AM, Adamantidis A, de Lecea L, Deisseroth K. Circuit-breakers: optical technologies for probing neural signals and systems. *Nat Rev Neurosci* 2007;8(8):577-581
24 Berndt A, Schoenenberger P, Mattis J, Tye KM, Deisseroth K, Hegemann P, Oertner TG. High-efficiency channelrhodopsins for fast neuronal stimulation at low light levels. *Proc Natl Acad Sci U S A* 2011; 108(18):7595-7600
25 Aravanis AM, Wang LP, Zhang F, Meltzer LA, Mogri MZ, Schneider MB, Deisseroth K. An optical neural interface: *in vivo* control of rodent motor cortex with integrated fiberoptic and optogenetic technology. *J Neural Eng* 2007;4(3):S143-156
26 Adamantidis AR, Zhang F, Aravanis AM, Deisseroth K, De Lecea L. Neural substrates of awakening probed with optogenetic control of hypocretin neurons. *Nature* 2007;450(7168):420-429
27 Carter ME, Adamantidis A, Ohtsu H, Deisseroth K, de Lecea L. Sleep homeostasis modulates hypocretin-mediated sleep-to-wake transitions. *J Neurosci* 2009;29(35):10939-10949
28 Bruegmann T, Malan D, Hesse M, Beiert T, Fuegemann CJ, Fleischmann BK, Sasse P. Optogenetic control of heart muscle *in vitro* and *in viva Nat Methods* 2010;7(11):897-900
29 Stroh A, Tsai HC, Wang LP, Zhang F, Kressel J, Aravanis A, Santhanam N, Deisseroth K, Konnerth A, Schneider MB. Tracking stem cell differentiation in the setting of automated optogenetic stimulation. *Stem Cells* 2011;29(1):78-88
30 Bi A, Cui J, Ma YP, Olshevskaya E, Pu M, Dizhoor AM, Pan ZH. Ectopic expression of a microbial-type rhodopsin restores visual responses in mice with photoreceptor degeneration. *Neuron* 2006;50(1):23-33
31 Lagali PS, Balya D, Awatramani GB, Munch TA, Kim DS, Busskamp V, Cepko CL, Roska B. Light-activated channels targeted to ON bipolar cells restore visual function in retinal degeneration. *Nat Neurosci* 2008;11 (6):667-675
32 Weick JP, Johnson MA, Skroch SP, Williams JC, Deisseroth K, Zhang SC. Functional control of transplantable human ESC-derived neurons via optogenetic targeting. *Stem Cells* 2010;28(11):2008-2016
33 Zhang F, Wang LP, Brauner M, Liewald JF, Kay K, Watzke N, Wood PG, Bamberg E, Nagel G, Gottschalk A, Deisseroth K. Multimodal fast optical interrogation of neural circuitry. *Nature* 2007;446(7136):633-634
34 Han X, Boyden ES. Multiple-color optical activation, silencing, and desynchronization of neural activity, with single-spike temporal resolution. *Plos One* 2007;2(3):e299
35 Gradinaru V, Thompson KR, Deisseroth K. eNpHR: a Natronomonas halorhodopsin enhanced for optogenetic applications. *Brain Cell Biol* 2008;36(1-4):129-139
36 Gradinaru V, Zhang F, Ramakrishnan C, Mattis J, Prakash R, Diester I, Goshen I, Thompson KR, Deisseroth K. Molecular and cellular approaches for diversifying and extending optogenetics. *Cell* 2010;141(1):154-165
37 Lin JY, Lin MZ, Steinbach P, Tsien RY. Characterization of engineered channelrhodopsin variants with improved properties and kinetics. *Biophys J* 2009;96(5):1803-1814
38 Chow BY, Han X, Dobry AS, Qian X, Chuong AS, Li M, Henninger MA, Belfort GM, Lin Y, Monahan PE, Boyden ES. High-performance genetically targetable optical neural silencing by light-driven proton pumps. *Nature* 2010;463(7277):98-102
39 Zhang F, Prigge M, Beyriere F, Tsunoda SP, Mattis J, Yizhar O, Hegemann P, Deisseroth K. Red-shifted optogenetic excitation: a tool for fast neural control derived from Volvox carteri. *Nat Neurosci* 2008;11(6): 631-633
40 Lin JY. A user's guide to channelrhodopsin variants: features, limitations and future developments. *Exp Physiol* 2011;96(1):19-25
41 Pastrana E. Perfecting ChR2. *Nat Methods* 2011;8(6):447
42 Dittgen T, Nimmerjahn A, Komai S, Licznerski P, Waters J, Margrie TW, Helmchen F, Denk W, Brecht M, Osten P. Lentivirus-based genetic manipulations of cortical neurons and their optical and electrophysiological monitoring *in viva Proc Natl Acad Sci U S A* 2004;101(52):18206-18211
43 Lewis TL, Jr., Mao T, Svoboda K, Arnold DB. Myosin-dependent targeting of transmembrane proteins to neuronal dendrites. *Nat Neurosci* 2009;12(5):568-576
44 Lemaillet G, Walker B, Lambert S. Identification of a conserved ankyrin-binding motif in the family of sodium channel alpha subunits. *J Biol Chem* 2003;278(30):27333-27339
45 Garrido JJ, Giraud P, Carlier E, Fernandes F, Moussif A, Fache MP, Debanne D, Dargent B. A targeting motif involved in sodium channel clustering at the axonal initial segment. *Science* 2003;300 (5628): 2091-2094
46 Grubb MS, Burrone J. Activity-dependent relocation of the axon initial segment fine-tunes neuronal excitability. *Nature* 2010;465 (7301): 1070-1074
47 Kuhlman SJ, Huang ZJ. High-resolution labeling and functional manipulation of specific neuron types in mouse brain by Cre-activated viral gene expression. *Plos One* 2008;3(4):e2005
48 Atasoy D, Aponte Y, Su HH, Sternson SM. A FLEX switch targets

Channelrhodopsin-2 to multiple cell types for imaging and long-range circuit mapping. *J Neurosci* 2008;28(28):7025-7030

49 Gradinaru V, Thompson KR, Zhang F, Mogri M, Kay K, Schneider MB, Deisseroth K. Targeting and readout strategies for fast optical neural control *in vitro* and *in viva*. *J Neurosci* 2007;27(52):14231-14238

50 Navarro-Quiroga I, Chittajallu R, Gallo V, Haydar TF. Long-term, selective gene expression in developing and adult hippocampal pyramidal neurons using focal in utero electroporation. *J Neurosci* 2007;27 (19): 5007-5011

51 Borrell V, Yoshimura Y, Callaway EM. Targeted gene delivery to telencephalic inhibitory neurons by directional in utero electroporation. *J Neurosci Methods* 2005;143(2):151-158

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

53 Wang H, Peca J, Matsuzaki M, Matsuzaki K, Noguchi J, Qiu L, Wang D, Zhangn F, Boyden E, Deisserothl K, Kasai H, Hall WC, Feng G, Augustine GJ. High-speed mapping of synaptic connectivity using photostimulation in Channel rhodopsin-2 transgenic mice. *Proc Natl Acad Sci USA* 2007;104(19):8143-8148

54 Kokaia M, Sorensen AT. The treatment of neurological diseases under a new light: the importance of optogenetics. *Drugs Today (Barc)* 2011;47(1): 53-62

55 Tominaga T, Tominaga Y, Ichikawa M. Optical imaging of long-lasting depolarization on burst stimulation in area CA1 of rat hippocampal slices. *J Neurophysiol* 2002;88(3):1523-1532

56 Muschol M, Kosterin P, Ichikawa M, Salzberg BM. Activity-dependent depression of excitability and calcium transients in the neurohypophysis suggests a model of "stuttering conduction". *J Neurosci* 2003;23 (36): 11352-11362

57 Grinvald A, Hildesheim R. VSDI: a new era in functional imaging of cortical dynamics. *Nat Rev Neurosci* 2004;5(11):874-885

58 Airan RD, Meltzer LA, Roy M, Gong Y, Chen H, Deisseroth K. High-speed imaging reveals neurophysiological links to behavior in an animal model of depression. *Science* 2007;317(5839):819-823

59 Bamann C, Nagel G, Bamberg E. Microbial rhodopsins in the spotlight. *Curr Opin Neurobiol* 2010;20(5):610-616

60 Goard M, Aakalu G, Fedoryak OD, Quinonez C, St Julien J, Poteet SJ, Schuman EM, Dore TM. Light-mediated inhibition of protein synthesis. *Chem Biol* 2005;12(6):685-693

61 Petreanu L, Mao T, Sternson SM, Svoboda K. The subcellular organization of neocortical excitatory connections. *Nature* 2009;457(7233): 1142-1145

62 Ayling OGS, Harrison TC, Boyd JD, Goroshkov A, Murphy TH. Automated light-based mapping of motor cortex by photoactivation of channelrhodopsin-2 transgenic mice. *Nature Methods* 2009;6(3):219-224

63 Cardin JA, Carlen M, Meletis K, Knoblich U, Zhang F, Deisseroth K, Tsai LH, Moore CI. Driving fast-spiking cells induces gamma rhythm and controls sensory responses. *Nature* 2009;459(7247):663-667

64 Nagel G, Brauner M, Liewald JF, Adeishvili N, Bamberg E, Gottschalk A. Light activation of channelrhodopsin-2 in excitable cells of Caenorhabditis elegans triggers rapid behavioral responses. *Curr Biol* 2005;15(24):2279-2284

65 Zhang W, Ge WP, Wang ZR. A toolbox for light control of Drosophila behaviors through Channelrhodopsin 2-mediated photoactivation of targeted neurons. *European J Neurosci* 2007;26(9):2405-2416

66 Hwang RY, Zhong L, Xu Y, Johnson T, Zhang F, Deisseroth K, Tracey WD. Nociceptive neurons protect Drosophila larvae from parasitoid wasps. *Curr Biol* 2007;17(24):2105-2116

67 Suh GS, Ben-Tabou de Leon S, Tanimoto H, Fiala A, Benzer S, Anderson DJ. Light activation of an innate olfactory avoidance response in Drosophila. *Curr Biol* 2007;17(10):905-908

68 Douglass AD, Kraves S, Deisseroth K, Schier AF, Engert F. Escape behavior elicited by single, channelrhodopsin-2-evoked spikes in zebrafish somatosensory neurons. *Curr Biol* 2008;18(15):1133-1137

69 Schroll C, Riemensperger T, Bucher D, Ehmer J, Voller T, Erbguth K, Gerber B, Hendel T, Nagel G, Buchner E, Fiala A. Light-induced activation of distinct modulatory neurons triggers appetitive or aversive learning in Drosophila larvae. *Curr Biol* 2006;16(17):1741-1747

70 Doroudchi MM, Greenberg KP, Liu J, Silka KA, Boyden ES, Lockridge JA, Arman AC, Janani R, Boye SE, Boye SL, Gordon GM, Matteo BC, Sampath AP, Hauswirth WW, Horsager A. Virally delivered channelrhodopsin-2 safely and effectively restores visual function in multiple mouse models of blindness. *Mol Ther* 2011;19(7):1220-1229

71 Tomita H, Sugano E, Isago H, Hiroi T, Wang Z, Ohta E, Tamai M. Channelrhodopsin-2 gene transduced into retinal ganglion cells restores functional vision in genetically blind rats. *Exp Eye Res* 2010;90 (3): 429-436

72 Tomita H, Sugano E, Fukazawa Y, Isago H, Sugiyama Y, Hiroi T, Ishizuka T, Mushiake H, Kato M, Hirabayashi M, Shigemoto R, Yawo H, Tamai M. Visual properties of transgenic rats harboring the channelrhodopsin-2 gene regulated by the Thy-1.2 promoter. *Plos One* 2009;4(11): e7679

73 Sugano E, Isago H, Wang Z, Murayama N, Tamai M, Tomita H. Immune responses to adeno-associated virus type 2 encoding channelrhodopsin-2 in a genetically blind rat model for gene therapy. *Gene Ther* 2011;18(3):266-274

74 Zhang Y, Ivanova E, Bi AD, Pan ZH. Ectopic expression of multiple microbial rhodopsins restores ON and OFF light responses in retinas with photoreceptor degeneration. *J Neurosci* 2009;29(29):9186-9196

75 Alilain WJ, Li X, Horn KP, Dhingra R, Dick TE, Herlitze S, Silver J. Light-induced rescue of breathing after spinal cord injury. *J Neurosci* 2008;28(46):11862-11870

76 Gradinaru V, Mogri M, Thompson KR, Henderson JM, Deisseroth K. Optical Deconstruction of Parkinsonian Neural Circuitry. *Science* 2009; 324(5925):354-359

77 Schlaepfer TE, Cohen MX, Frick C, Kosel M, Brodesser D, Axmacher N, Joe AY, Kreft M, Lenartz D, Sturm V. Deep brain stimulation to reward circuitry alleviates anhedonia in refractory major depression. *Neuropsychopharmacology* 2008;33(2):368-377

78 Rauch SL, Dougherty DD, Malone D, Rezai A, Friehs G, Fischman AJ, Alpert NM, Haber SN, Stypulkowski PH, Rise MT, Rasmussen SA, Greenberg BD. A functional neuroimaging investigation of deep brain stimulation in patients with obsessive-compulsive disorder. *J Neurosurg* 2006;104(4):558-565

79 Machado A, Ogrin M, Rosenow JM, Henderson JM. A 12-month prospective study of gasserian ganglion stimulation for trigeminal neuropathic pain. *Stereotact Funct Neurosurg* 2007;85(5):216-224

80 Rasche D, Ruppolt M, Stippich C, Unterberg A, Tronnier VM. Motor cortex stimulation for long-term relief of chronic neuropathic pain: a 10 year experience. *Pain* 2006;121(1-2):43-52

81 Tonnesen J, Sorensen AT, Deisseroth K, Lundberg C, Kokaia M. Optogenetic control of epileptiform activity. *Proc Natl Acad Sci U S A* 2009;106(29):12162-12167