Molecular Tools and Approaches for Optogenetics

Yuan Mei and Feng Zhang

The mammalian brain poses a formidable challenge to the study and treatment of neuropsychiatric diseases, owing to the complex interaction of genetic, epigenetic, and circuit-level mechanisms underlying pathogenesis. Technologies that facilitate functional dissection of distinct brain circuits are necessary for systematic identification of disease origin and therapy. Recent developments in optogenetics technology have begun to address this challenge by enabling precise perturbation of distinct cell types based on molecular signatures, functional projections, and intracellular biochemical signaling pathways. With high temporal precision and reversible neuromodulation, optogenetics promises to improve existing disease models and advance our understanding of psychiatric conditions. In this review, we describe the current state of molecular optogenetic tools and future directions of development.

Key Words: Brain machine interface, channelrhodopsin, gene therapy, neuromodulation, neuropsychiatric disease, optogenetics

The brain is a particularly complicated organ to study because of its sheer diversity and heterogeneity of molecules, cells, and connections. Dysfunctions in specific cell types and circuits can lead to severe neuropsychiatric conditions (1). To dissect the mechanisms of psychiatric conditions, it is essential to investigate the pathogenic mechanisms at every level of brain function: from subcellular molecular signaling and cellular physiology to circuit-level functions. On the basis of clinical observations and insights to neuropsychiatric diseases, it is possible to generate cellular and animal models that recapitulate the multilevel symptoms of human patients (2). Systematic perturbation of the implicated circuit components in these disease models will help identify effective therapeutic targets and strategies. However, precise manipulation of the brain has been a difficult task to achieve. To fully recapitulate biological events, an ideal approach requires highly precise manipulations, including fast temporal control, cell-type specificity, and applicability within awake, behaving animals.

The development of optogenetic technologies enables such precise manipulations (3–6). It combines the temporal and spatial precision of light pulses with cellular specificity of genetic targeting; by coupling cellular behavior with light, it provides a fast, light-controlled approach to manipulate neural activities in intact brain circuits. The general strategy of optogenetics involves introducing a light-sensitive protein to a specific cell type, illuminating the targeted cells with defined parameters, and obtaining reliable readout of the cellular behavior. Compared with electrical microstimulation, optogenetic activation specifically targets the opsin-expressing neurons, whereas electrical stimulation may simultaneously affect diverse local, afferent, and passing axonal fibers (7,8) in addition to cellular somata near the electrode insertion site. Currently, the molecular toolbox of optogenetics (4,6) involves many different light-sensitive constructs capable of controlling a diverse range of neuronal functions, from electrical to biochemical signaling. Through a combination of molecular engineering and genomic discoveries of new light-sensitive proteins, the toolkit of neuron modulators is rapidly improving in specificity and versatility. At its present stage, optogenetics affords millisecond precision in controlling neuron activity, genetically defined cell type targeting, and the ability to modulate neural circuit function in awake, behaving animals. With these features, optogenetics can be used to causally probe circuitries underlying complex behavior, dissect signaling pathways, and construct models of psychiatric disease through loss- and gain-of-function experiments. This review describes the optogenetic tools available for investigating neural circuit function and future directions in the development of this technology.

From the Department of Brain and Cognitive Sciences (YM, FZ), Massachusetts Institute of Technology; and Broad Institute of MIT and Harvard (FZ) Cambridge, Massachusetts.

Address correspondence to Feng Zhang, Ph.D., Broad Institute of MIT and Harvard, 7 Cambridge Center, 5011, Cambridge, MA 02142; E-mail: zhang_f@mit.edu.

Received Nov 21, 2011; revised and accepted Feb 10, 2012.

Modulating Neural Activity Using Light

The heterogeneous nature of brain tissue presents major challenges for selectively controlling subsets of well-defined neuron types in intact circuits. Most current neuromodulation techniques (i.e., electrical stimulation or pharmacologic intervention) either simultaneously affect surrounding cells and processes in addition to the target population, or have slow kinetics and reversibility. The lack of modulation specificity frequently limits the strength of conclusions drawn from conventional neuromodulation experiments. To overcome the spatial and temporal limitations of electrical, pharmacologic, and genetic neuromodulation approaches, various microbial and engineered opsins (Figure 1A) have been developed recently to control electrical and biochemical activities of neurons with cell-type selectivity, high temporal precision, and rapid reversibility. Because most neurons in the brain are not naturally light-sensitive, selective expression of the opsin genes in targeted neural populations makes it possible to control their activity specifically. Additionally, the fast on–off kinetics of microbial opsins make it possible to evoke or inhibit neural activities within milliseconds, on a time scale relevant to physiologic brain functions.

Activating Neurons Using Light

The discovery and introduction of algal light-sensitive cation conducting channelrhodopsins into neurons has enabled fast optical control of genetically defined neuronal populations in intact circuits, both in vitro and in vivo (9). So far, channelrhodopsin genes from two algal species (*Chlamydmonas reinhardtii* and *Volvox carteri*) have been cloned and characterized in neurons (Figure 1A). Most notably, channelrhodopsin-2 from *Chlamydmonas reinhardtii* (ChR2) (10–12) and channelrhodopsin-1 from *Volvox carteri* (VChR1) (13) have proven to be a powerful pair of ChRs for controlling intact neural circuits using light. ChR2 is maximally activated by blue light around 480 nm, whereas VChR1 remains significantly light sensitive even at 589 nm, a wavelength at which ChR2 is no longer responsive. Therefore, ChR2 and VChR1 can be integrated in combinatorial neural control experiments both in vitro and in vivo to test the necessity and sufficiency of a variety of neural circuit functions. Both ChR2 and VChR1 have fast onset kinetics with time constants on the order of milliseconds and are therefore able to transduce high frequencies of millisecond-lasting light flashes into reliably evoked action potentials. However, because wildtype VChR1 has lower levels of photocurrent compared with ChR2,

0006-3223/$36.00
doi:10.1016/j.biopsych.2012.02.019

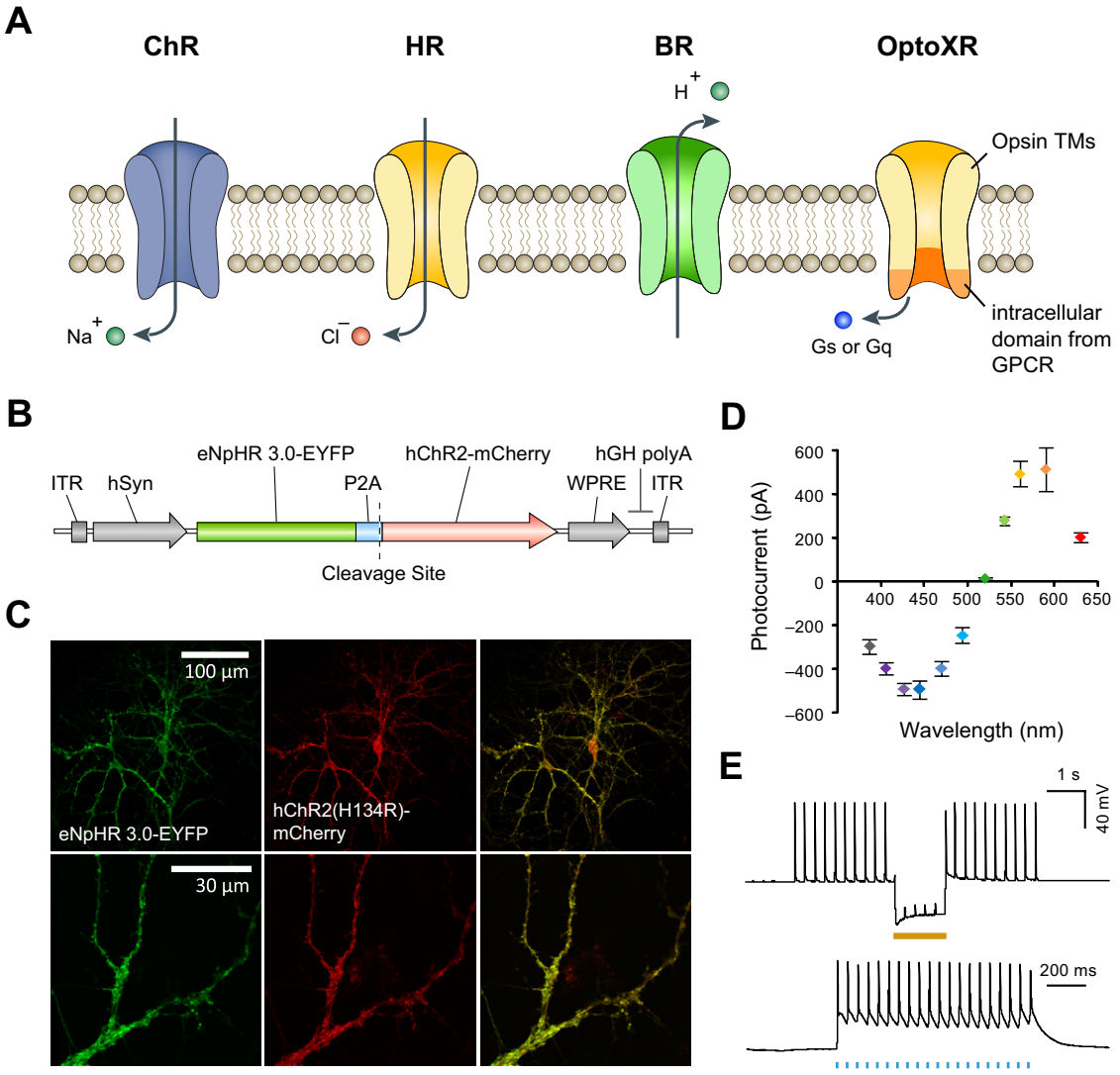

Figure 1. Microbial opsins as optogenetic tools. **(A)** A variety of natural and engineered opsins can be used to control the membrane potential and intracellular biochemical signaling of neurons. Channelrhodopsins (ChR) are light-activatable cation-conducting channels capable of activating neurons by depolarizing the membrane potential. Halorhodopsins (HR) and bacteriorhodopsins (BR) are light-activatable chloride and proton pumps capable of inhibiting neurons by hyperpolarizing the membrane potential. OptoXRs are engineered rhodopsin-G protein-coupled receptor (GPCR) chimeras useful for controlling intracellular G-protein-coupled signaling cascades. OptoXRs are engineered by replacing the intracellular domain of vertebrate rhodopsin with the intracellular loops from GPCRs. **(B)** A bi-cistronic vector used to express channelrhodopsin-2 (ChR2) and halorhodopsin (eNpHR3.0) in the same neuron. The two opsin genes are linked using a 2A self-cleavage linker. **(C)** Images of neurons coexpressing ChR2 and eNpHR3.0. **(D)** Activation spectra of neurons coexpressing ChR2 and eNpHR3.0. **(E)** Bidirectional control of neurons coexpressing ChR2 and eNpHR3.0. Blue light (470 nm) flashes induced action potential firing and yellow light (583 nm) facilitated spike inhibition. Panel A adapted with permission from Zhang *et al.* (4), and panels B through E are reprinted with permission from Gradinaru *et al.* (22), with permission from Elsevier, copyright 2010. Cl, chloride; Gq, G-protein Gq; Gs, G-protein Gs; hSYN, human synapsin I promoter; ITR, inverted terminal repeat; Na, sodium; TMs, transmembrane domains.

a chimera between *Chlamydamonas* ChR1 and *Volvox* ChR1 has been generated called C1V1. In conjunction with ChR2, C1V1 forms a powerful pair of opsins to control the activity of distinct groups of neurons in vivo simultaneously (14).

Mutagenesis studies could expand the function of channelrhodopsins by altering the spectral properties, conductance, or kinetics of the channels. Indeed, one mutant ChR2(H134R) has been shown to exhibit enhanced photocurrent (15), and three point-mutants of humanized ChR2 convert a brief pulse of light into a period of stable current influx that can last for many minutes (16). These latter mutant channels were generated by substitution of the C128 position in the original ChR2 sequence. All three mutants are activated by blue light (470 nm). Photocurrents generated by the opening of ChR2(C128A) and ChR2(C128S) can be effectively terminated by a pulse of green light (542 nm). Other slow mutants were generated by mutating residue D156; this mutation has been shown to result in similar stabilization of the conducting state (14,17).

Substituting ChR2 residue E123 with T or A was found to accelerate channel closure kinetics and significantly increase the fidelity of fast optogenetic control (18). These E123 mutations can be combined with other gain-of-function modifications such as the H134R and T159C mutations (18,19) or membrane trafficking signals (14,20–22). The E123 mutations appear unique thus far because they eliminate the sensitivity of channel kinetics to membrane potential (19). Opsins of this class (E123 mutations alone or in combination with other modifications (18) are termed ChETAs (*Ch*R