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1. Abstract

Brain neural network is composed of densely packed, intricately wired neurons whose activity patterns ultimately give raise to every behavior, thought or emotion that we experience. Over the past decade, a novel neurotechnique, optogenetics that combines light and genetic methods to control or monitor neural activity patterns, has proven to be revolutionary in understanding the functional role of specific neural circuits. We here briefly describe recent advance in optogenetics, and compare optogenetics with deep brain stimulation technology that holds the promise for treating many neurological and psychiatric disorders.

2. Optogenetics

Optogenetics combines light and genetic methods to control or monitor cellular activities. For rhodopsin based optogenetic control techniques, light-sensitive rhodopsin molecules were genetically introduced into otherwise not-light sensitive neurons. Upon light illumination, genetically modified neurons that express rhodopsins can then be precisely controlled. Three major classes of rhodopsins, all microbial rhodopsins, have been developed as optogenetic molecular sensors, channelrhodopsins, halorhodopsins, and archaerhodopsins (Figure 1) (Han 2012). Because of their small sizes, these rhodopsins can be easily expressed in neurons, and thus optogenetics has been successfully applied in almost all experiment neural systems, from *Caenorhabditis elegans*, rodents, to nonhuman primates, as well as human retina. With the ability to rapidly and reversibly activate or silence genetically transduced cells, optogenetics has enabled the examination of the causal role of specific cells in neural computation, behavior and brain disorders. A number of recent reviews and books have summarized various aspects of the current state of this field (Bernstein and Boyden 2011; Miesenbock 2011; Yizhar, Fenno et al. 2011; Zhang, Vierock et al. 2011; Chow, Han et al. 2012; Han 2012; Knopfel and Boyden 2012). In parallel, a new generation of genetically encoded calcium/activity optogenetic sensors are being improved, with which neural activity patterns can now be monitored with high spatiotemporal resolution (i.e. (Chen, Wardill et al. 2013)). We here will focus our discussion on optogenetic control technologies that are rhodopsin based, and will not further discuss other calcium/activity sensors.

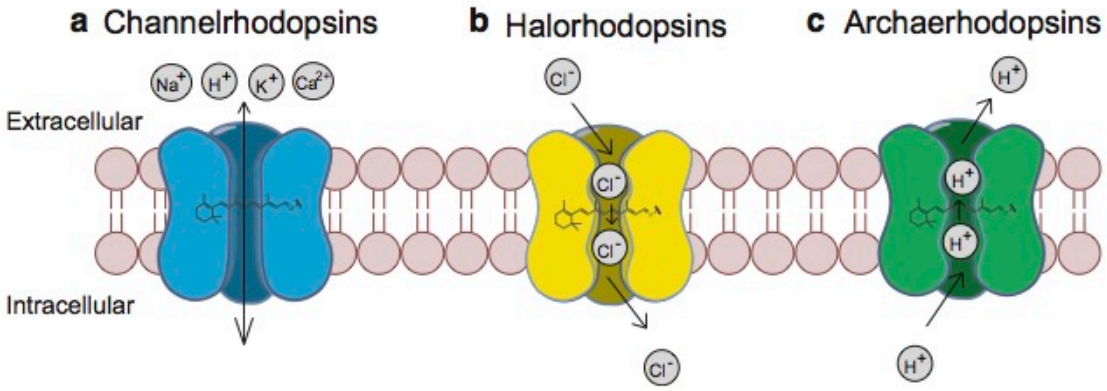

Fig. 1 Optogenetic molecular sensors. Upon light illumination, channelrhodopsins passively transport Na^+, K^+, H^+, Ca^{2+} down their electrochemical gradients to depolarize neurons (**a**); halorhodopsins actively pump Cl^- into the cell to hyperpolarize neurons (**b**); archaerhodopsins actively pump H^+ out of the cell to hyperpolarize neurons (**c**) (Han 2012a)

2.1 Rhodopsin based optogenetic sensors

Microbial rhodopsins are photoactive proteins with seven transmembrane domains. They are widely spread in archaea, bacteria, algae, and fungi, where they are critical for light-sensing or photosynthetic functions. Each rhodopsin molecule consists a protein domain, opsin that binds to the photoactive co-factor all-*trans*-retinal, and thus rhodopsin refers to the combination of the opsin protein and the bound retinal (Spudich, Yang et al. 2000; Spudich 2006). Light induced photoisomerization of all-*trans*-retinal to 13-*cis*-retinal leads to opsin protein conformational changes that result in direct ion conductance across the membrane. Rhodopsins have been studied since 1970s (Oesterhelt and Stoeckenius 1971; Oesterhelt and Stoeckenius 1973), but they were only recently adopted as optogenetic sensors.

Channelrhodopsin-2 (ChR-2), cloned from green algae *Chlamydomonas reinhardtii*, is the first optogenetic sensor adapted to activate neurons (Boyden, Zhang et al. 2005). Light-induced photoisomerization of all-*trans*-retinal results in protein conformational changes that lead to a passive conductance to both monovalent and divalent cations such as Na^+, K^+, H^+ and Ca^{2+}. The duration of ion flow is determined by the subsequent ChR2 conformation changes that led to channel closure (Nagel, Szellas et al. 2003). Because ion flow is independent of photon absorption, engineered ChR2 mutants that alter the process of light induced protein conformational changes have led to a number of variants that operate on varying time scales from milliseconds to minutes, and present varying permeability to different ions (Bamann, Gueta et al. 2010; Gunaydin, Yizhar et al. 2010; Berndt, Schoenenberger et al. 2011; Berndt, Lee et al. 2014; Wietek, Wiegert et al. 2014).

Two classes of light activated ion pumps have been used to silence neurons, halorhodopsins and Archaerhodopsins. Halorhodopsins, such as that from *Natronomonas pharaonis* (Halo, NpHR), are light-activated inward chloride pumps (Han and Boyden 2007; Zhang, Wang et al. 2007). Archaerhodopsin, such as that from *Halorubrum sodomense* (Arch), are light-activated outward proton pumps (Chow, Han et al. 2010; Han, Chow et al. 2011). These light-activated pumps lead to a net outward current flow in

neurons, thereby silencing neural activities. For Halo and Arch, photonic energy is directly coupled to ion transport, and thus photo current depends upon continuous light illumination.

Much effort has been directed to enhance the efficiency, temporal precision and spectrum properties of these rhodopsin molecules. While it remains to be established whether these optogenetic molecules from archeabacteria or algea produce any side effects in neurons, these molecules are widely used and have so far proven safe and effective in most neural systems.

2.2. Target rhodopsin expression though genetic modification

A major advantage of optogenetic technologies over other brain stimulation technologies is the ability to control specific cells with distinct genetic markers. Such specificity is achieved by expressing rhodopsins in desired cell populations through genetic modification. Because of the intrinsic difficulty in transducing neurons, genetic modification of neurons is mainly limited to whole animal transgenic approaches and viral based gene delivery approaches (Han 2012).

Transgenic mice represent a versatile and powerful platform to target a variety of distinct cells of interest, in particular in conjunction with the phage derived Cre-LoxP recombination technology (Sauer and Henderson 1988; Tsien, Chen et al. 1996). Cre recombinase selectively catalyzes the recombination between a pair of LoxP recognition DNA sequences. Through strategic placement of LoxP sequences, a specific gene, such as rhodopsins can be expressed only in the presence of Cre enzymes. A large number of Cre transgenic mice are available with targeted Cre expression in specific cells. Upon injection of a virus that mediates Cre-dependent expression of rhodopsin molecules, one can selectively express rhodopsins only in cells that also express Cre. Alternatively, Cre transgenic mice can be crossed with transgenic mice with Cre-dependent rhodopsin expressions (Madisen, Mao et al. 2012).

In genetically intractable species, viruses remain the most effective methods to transduce brain cells. Over the years, viral based gene delivery methods have been well established and are widely used in basic research and in human gene therapy clinical trials (Waehler, Russell et al. 2007; Han 2012). The most commonly used viral vectors, lentivirus and adeno-associated virus (AAV), have been engineered to exhibit little or no toxicity, with excellent transduction efficiency. However, two major limitations remain for viral vectors. First, the packaging ability of a virus is limited, which cannot be easily overcome due to the intrinsic stability of viral particles. Second, different viruses display distinct tropism, likely because specific membrane receptors are required for viral entry into target cells. As a result, it remains difficult to target specific cells with virus, which has presented a major challenge in realizing the full potential of optogenetics in genetically intractable species.

A number of non-viral methods have been developed for gene delivery, i.e. using cationic lipids, cationic polymers, nanoparticles, carbon nanotubes, gene guns, or calcium phosphate *(Luo and Saltzman 2000)*. Although these methods exhibit excellent

transduction efficiency in a number of cells, they have largely failed to transduce neurons effectively. A potential advantage of non-viral based gene delivery method is the ability to introduce large pieces of DNAs into cells, and thus may enable improved targeting to specific cells. However, further optimization of non-viral gene delivery methods is necessary for the use of these methods in the brain.

2.3 Light illumination of cells expressing rhodopsins

Optogenetic control of neural activities critically relies on the amount of light reaching a neuron, the number of rhodopsin molecules present on the plasma membrane, and the light sensitivity of the rhodopsins. The control efficacy may also be influenced by the intrinsic neuronal membrane biophysical properties and the surrounding neural network environment, and both factors cannot be controlled by experimenters. Having discussed the genetic modification methods that control the expression level of rhodopsin on the plasma membrane, and the molecular properties that dictate a rhodopsin's light sensitivity, we here describe the consideration of light illumination.

Rhodopsins typically operate at visible wavelength light (450-650 nm) that are also highly absorbed by blood hemoglobin. Monte Carlo simulations (Mobley and Vo-Dinh 2003), along with experimental evidence, demonstrated that tissue penetration by visible light drops sharply to less than 10% within the first few hundred microns (Bernstein, Han et al. 2008; Chow, Han et al. 2010). To circumvent this, rhodopsins with red- and far-red light sensitivity (> 650 nm) have been developed to allow for more efficient illumination of brain tissue, thereby improving stimulation volume and reducing possible risk of heat-induced tissue damage associated with high intensity light illumination (Zhao, Cunha et al. 2008; Chuong, Miri et al. 2014).

A variety of light sources with decent light power are well suited for illuminating neurons expressing rhodopsins. For example, lasers and LEDs that are low cost and easy to handle can provide excellent light illumination for in vivo optogenetic experiments. When coupled with fiberoptics, they allow the delivery of light with a narrow wavelength and high spatiotemporal resolution. The use of thin fibers or fiber arrays is advantageous in reducing mechanical tissue damage (Bernstein, Garrity et al. 2012). Light induced tissue heating may alter tissue integrity, cell metabolism and neuronal excitability (Wells, Kao et al. 2005), and thus the amount of light delivered into brain tissue needs to be properly evaluated and controlled during an optogenetic experiment. Another consideration when using optogenetics in conjunction with metal recording electrode is laser-induced electrical artifact due to photoelectric effects (Han, Qian et al. 2009; Han, Chow et al. 2011). Development of novel electrode materials may overcome some of these photoelectric problems (Zorzos, Dietrich et al. 2009).

2.4 Application of optogenetics

Optogenetics have been used in experimental organisms from *C. elegans,* zebrafish to mice, primates to analyze neural circuits relevant for many behaviors, from motor behavior (Cavanaugh, Monosov et al. 2012) to learning and memory (Liu, Ramirez et al. 2012). With the proof of principle demonstration that optogenetics can be safely performed in non-human primates (Han, Qian et al. 2009), optogenetics has been

explored for its translational potential in treating blindness (Doroudchi, Greenberg et al. 2011).

3. Deep Brain Stimulation (DBS)

DBS represents a revolutionary brain-region specific neuromodulation therapy that first gained FDA approval in 1997 for treating essential tremor and Parkinson's disease (PD) tremor, and later in 2002 for PD and in 2003 for dystonia. Though invasive, DBS has been proven to be highly effective, and is now actively explored as therapies for a number of brain disorders. Because of the greater understanding of DBS, we will focus the following discussion on DBS, and compare DBS to optogenetics.

A set of other noninvasive electrical brain stimulation technologies have been historically applied to treat neurological and psychiatric disorders, such as transcranial magnetic stimulation (TMS), transcranial direct current stimulation (tDCS), and transcranial alternating current stimulation (tACS). These noninvasive technologies stimulate a large and often distributed neural network with little spatial resolution, and thus it has been difficult to pinpoint their action mechanisms. However, the noninvasive nature of these technologies attracts much enthusiasm on their therapeutic potentials, and there is much effort on improving the spatial resolution of these tools. Recently, TMS gained FDA approval in the United States for treating migraine in 2013.

3.1 Discovery of DBS therapy

The use of electrical stimulation can be traced back to Fritsch and Hitzip in the 1870s, and later in the 1940s, Penfield systematically stimulated different parts of the human brain and established the map of human motor and sensory cortices that remain instrumental in our understanding of the functional organization of the brain. With the advance of stereotaxic surgeries and the establishment of Parkinson's disease animal models, much research and clinical effort have finally led the FDA approval of DBS for treating PD, in 1997 for stimulating thalamus and in 2002 for stimulating STN and GPi.

Current DBS electrode designs consist of four contacts that are 0.5mm or 1.5mm apart. Electrical currents are controlled via an integrated pulse generator that can stimulate using different electrode pairs, at certain polarity, amplitude, pulse width, and frequencies. A specific set of stimulation parameters is determined through trial and error for each patient to achieve optimal clinical efficacy with minimal side effects (Volkmann, Moro et al. 2006). While DBS has been remarkable in treating several key motor symptoms presented in PD patients, such as bradykinesia, akinesia and tremor (Anderson, Burchiel et al. 2005; Rodriguez-Oroz, Obeso et al. 2005), it is often associated with other side effects, such as mood disorders, depression and impulsivity (Uc and Follett 2007).

3.2 Therapeutic Mechanisms of DBS for PD

The therapeutic mechanisms of DBS for PD remain largely unclear. There are several promising hypothesis. DBS may achieve its therapeutic effects through inhibiting the brain structures being stimulated. This hypothesis is largely based on the understanding that the brain regions targeted by DBS, i.e. STN, thalamus and GPi, when surgically lesioned, are equally effective in treating PD. This hypothesis is further supported by the observation that the firing rates of STN neurons decreased drastically upon local STN

DBS (Dostrovsky, Levy et al. 2000; Welter, Houeto et al. 2004; Foffani, Ardolino et al. 2006). However, electrical stimulation also stimulates the fibers of passage, as well as neurons that project to the site of stimulation antidromically. Thus, while DBS may inhibit local brain structures under stimulation, its effects likely extend to other brain regions that connect to the site of stimulation or have fibers bypassing the stimulation sites.

A second hypothesis is that DBS may reduce pathological oscillations. Implantation of DBS electrodes provides a unique opportunity for recording neural activities from PD brains. Much evidence has suggested the presence of exaggerated oscillations in the cortical-basal ganglion circuit at beta frequencies (oscillations around 20Hz). Exaggerated beta oscillations closely parallel the key PD motor deficits, bradykinesia, rigidty, akinesia, and tremor (Levy, Hutchison et al. 2000; Brown, Oliviero et al. 2001; Levy, Hutchison et al. 2002; Boraud, Brown et al. 2005; Weinberger, Hutchison et al. 2009; Weinberger, Hutchison et al. 2009), and are largely suppressed by effective dopamine replacement treatment (Brown, Oliviero et al. 2001; Levy, Ashby et al. 2002; Williams, Tijssen et al. 2002; Priori, Foffani et al. 2004; Silberstein, Pogosyan et al. 2005) and DBS (Wingeier, Tcheng et al. 2006; Kuhn, Kempf et al. 2008; Kuhn, Tsui et al. 2009; Lehmkuhle, Bhangoo et al. 2009). It has thus been suggested that DBS therapeutic effect is through reducing beta oscillations. However, it remains unknown whether the exaggerated beta oscillation is a cause or a correlate of motor deficits, and where and how beta oscillations arise in PD.

Finally, it has also been hypothesized that replacement of DBS electrodes could recruit glia related neurotransmission to inhibit neuron activities at the target brain structures (Bekar, Libionka et al. 2008). While the surgical placement of DBS electrodes presents serious risks intrinsic to any surgery, interestingly electrode implantation within STN may be neuroprotective and could slow down dopamine neuron degeneration in SNpc, as demonstrated in MPTP monkey PD models (Doroudchi, Greenberg et al. 2011). With the development of optogenetics, researchers are now able to start to investigate the specific neural circuit mechanisms underlying DBS therapeutic actions.

4. Optogenetics and DBS

The amazing efficacy of DBS in treating PD has motivated much effort in developing DBS based therapy for many neurological and psychiatric disorders beyond motor deficits, such as major depression, obsessive and compulsive disorders, and Alzheimer's disease. Because of the non-selective nature of electrical stimulation, DBS may not be able to stimulate any specific cell type, or avoid the stimulation of fiber of passages. However, with the simplicity of electrode placement, and the superb resolution of the spatiotemporal specificity, DBS represents a new generation of site-specific neuromodulation therapies, and could revolutionize the treatment of neurological and psychiatric diseases.

Optogenetics however requires both gene therapy to express light activated rhodopsin proteins in neurons and the delivery of light illumination to target neurons. While much progress has been made in improving the efficacy of optogenetics, its clinical translation

may be limited by the requirement of gene therapy, and any potential damage from light illumination. In addition, optogenetics controls neuron activities through altering the biophysical properties of a neuron by adding an exogenerous light-sensitive ion conductance, and thus the precision may not be as superb as that achieved with DBS. For example, DBS can stimulate at very high frequencies, i.e. >120Hz, to achieve therapeutic effects for PD, and indeed DBS often needs to be delivered at >120Hz to be effective. However, it is difficult for optogenetics to stimulate neurons at such high frequency. But as a research tool, optogenetics holds the promise to provide mechanistic understanding of neural circuits underlying behavioral and therapies, and for certain systems, such as the retinal, optogenetics may be proven effective as a therapy.

Figure Legend

Figure 1, Optogenetic molecular sensors. Upon light illumination, channelrhodopsins passively transport Na^+, K^+, H^+, Ca^{2+} down their electrochemical gradients to depolarize neurons (A); halorhodopsins actively pump Cl^- into the cell to hyperpolarize neurons (B); archaerhodopsins actively pump H^+ out of the cell to hyperpolarize neurons (C) (Han 2012).

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