Turkish Journal of Molecular Biology & Biotechnology
(2017) 2: 82-85

ORIGINAL ARTICLE

Turkish JMBB

Optogenetics: Controlling Neurons with Light

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Keywords

Optogenetics, channelrhodopsin, halorhodopsin, archeorhodopsin

ABSTRACT • There are many obstacles for uncovering the physiological and pathophysiological mechanism of nervous system. One of most difficult challenges is the heterogeneity of cell types which come with billions of synaptic connections. Neurons are among the most challenging cells to figure out their working principles which will help our understanding of the brain. During the past decades, specific light-sensitive proteins and molecules helped scientists to develop an important technical approach called "Optogenetics," with which precise inhibition or activation of neural pathways in nervous system can be achieved temporally and spatially using light. Interestingly, these light sensitive proteins come from mostly unicellular organisms such as algae and bacteria. Combination with genetically engineered tools like adeno-associated viruses and ion-gated channels like channelrhodopsins, halorhodopsins and archaerhodopsins, the activity of neurons can be manipulated by excitation and silencing. In this article, I reviewed basic principles of optogenetics to provide the reader with current updates.

INTRODUCTION

Brain is one of the most complex organs in our body and difficult to understand how nervous system works properly under normal conditions. Up to decade ago, neuronal circuits have been mainly probed by traditional electric and magnetic stimulations that are impossible to investigate selectively specific subtypes of neurons under physiological and pathological conditions. Other techniques, such as lesion studies that do not offer any chance to neuronal selectivity, or microinjection of neurotransmitters like dopamine, glutamate, serotonin etc., are limited to spatially and temporally constrained applications in neuroscience studies until the appearance of optogenetics techniques which give researchers important details regarding not only for specific neuronal activities but also for neuronal receptors (1,2). Optogenetics was initially used within the context of neuroscience to describe the approach of using light to drive or silence neuronal activity in the intact, living brain in wild type or transgenic animals, for instance, mice or rats (3). Optogenetics composed of two important research fields that are optics (light) which are used to activate or inhibit neurons, thanks to specific light-sensitive rhodopsins such as channelorhodopsin-2 (ChR2), halorohodosin (NpHR), archaerhodopsin (Arch), and genetic modifications which are used to synthesis of various kinds of

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rhodopsins by using viral approaches such as adeno-associated virus (AAV). The success of optogenetics in neuroscience has taken attention of many neuroscientists and engineers in other fields, and now the definition of optogenetics has expanded to including the general field of biotechnology (2,4-6). In this review, I will briefly explain the most fundamentals of optogenetics.

1) LIGHT SENSITIVE PROTEINS

a) Channelrhodopsins (ChRs)

Channelrhodopsins (ChRs) are light-gated ion channels found in a unicellular alga *(Chlamydomonas reinhardtii)* (7-9). The use of microbial opsin to control the activity of neurons utilize channelrhodopsin-2 (ChR2), one of two channelrhodopsins have by this alga (10). The most obvious and important feature of ChR2 is a light-gated nonspecific cation channel which, when illuminated with blue light, opens and permits the passage of cations (positively charged sodium and calcium ions) and the subsequent depolarization of the cell (8,9). In 2005, ChR2 was introduced into hippocampal neurons in petri dish, and control neuronal spiking activity with fine temporal precision (10). Very brief (millisecond level) pulses of blue light may be used to induce single action potentials in ChR2-expressing neurons, and neuronal spiking activity driven by the activation of this opsin can be controlled with high precision. This preliminary experiments of the usefulness of ChR2 for the control of neural activity was immediately followed by a number of reports and scientific papers confirming its function in neurons (11,12) and usefulness for investigate basic questions in neuroscience (13-15). ChR2 has subsequently been transferred from in vitro to in vivo experiments, to optimize expression and photocurrent in mammalian systems (13,16). After these pionnering reports, the optogenetic toolbox has greatly become indispensible for neuroscientists, and many different opsins with a variety of spectral, temporal, and conductive features have been discovered or engineered (17-19).

b) Halorhodopsin (NpHR)

Like activation of neurons, inhibition of neuronal activity is critical for understanding the mechanism of neural networks, and might complement excitatory tools by allowing researchers to investigate the individual circuit components. One of the most efficient and widely used inhibitory opsins, NpHR, is a halorhodopsin from the archaeon *Natronomonas pharaonis* (20,21). NpHR pumps chloride ions into the cell upon light activation, resulting in hyperpolarization. With an excitation maximum at 590 nm, eNpHR3.0 can be stimulated by green, yellow, or red light.

c) Archaerhodopsins (Arch)

Proton pumps might also be used to inhibit neurons through hyperpolarization, by pumping protons like (H+ ions) out of the cell, and have some features that make them another option to chloride pumps, which include fast recovery from inactivation and high light-driven currents. Arch (archaerhodopsin-3 from *Halorubrum sodomense*), is proton pumps that provide strong efficiency in inhibition of neurons (22-24).

2) OPSIN EXPRESSION

To control specific neural circuit with optogenetics, one of the most crucial approaches to take consideration is the targeting specific neurons in brain. There are so many ways to target subpopulations of neurons such cell body, axonal terminations (25). Genetically modified experimental animal models (mostly mice and rats) that express the enzyme Cre recombinase (Cre) under the transcriptional control of a specific gene are typically used to target neuronal subpopulations. For instance, vesicular gamma aminobutyric acid transporter (VGAT)-Cre mice express Cre only in inhibitory neurons that express VGAT. Many different transgenic rodent

lines with stable and heritable expression of Cre are commercially available through Jackson Laboratory (www.jax.org), Charles River Laboratories (www.criver.com) and other breeding facilities, provide to researchers to target and manipulate a variety of different neuronal subpopulations (26).

In order to provide anatomically local specificity of opsin expression, it is necessary to make stereotaxic injections of viral vectors encoding these proteins in the brain regions of interest. Cre is an important enzyme that catalyzes site-specific recombination between two LoxP sites, and modern Cre-driven viral vectors are constructed with "double-floxed" genes encoding the various types of opsin, causing targetted gene expression only in transfected cells that have Cre. A fluorescent tag is also encoded in the viral construct such as green fluorescent protein (GFP), allowing for postmortem histological confirmation of gene expression in the targeted cell type and brain region. Cre-inducible adeno-associated viruses (AAVs) are commercially available from Addgene (wwww.addgene.org), North Carolina University-Vector Core (https://www.med.unc.edu). These viruses are genetically engineered, therefore, replication deficient and it is not known to cause disease in humans. The numerous types of AAV strains (e.g., AAV 2, 5) have unique transfection features in brain; hence, it is important to control efficiency of the viral vectors for proper expression in the targeted brain region. After virus is injected to targeted brain area, at least 3 weeks are recommended prior to beginning experiments, in order to allow enough time for opsin expression in neurons (27).

CONCLUSION

Optogenetics has changed the way of neuroscience to new horizons, and has produced a new generation of experiments that dissect the causal roles of specific neural network components in physiological and pathological conditions. It has been used to increase our understanding of the neural circuits underlying psychiatric and neurological disorders (28), addiction (29), Parkinson's disease (30), obsessive compulsive disorder (31), social behavior (32) and reward (33), and many others (3). There is still an explosion in the development of new generation optogenetic tools, both through discovery in nature and engineering in laboratories. The coming years should see exciting progress in the development and application of these tools to deconstruct the neural networks underlying normal behavior and their dysfunction in psychiatric and neurological diseases.

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