Optogenetics

Introduction

To understand the role of a given group of neurons in the behavior of a living organism, one could activate or deactivate the gene that governs their activity and observe the effects. Researchers have had the ability to suppress or enhance the expression of genes for many years. However, the process of initiating this change takes hours, days or even months with traditional genetic techniques, rendering it difficult to measure or even observe the resulting neurological change. Alternatively, electrical stimulation offers some degree of stimulation or suppression of neurons on much faster timescales and can be targeted to a given region. However, electrical stimulation is unable to specifically target individual types of cells.

Optogenetics combines optical and genetic techniques to allow direct control of electrical and biochemical events, such as the firing or suppression of neurons, triggered by direct light stimulation. This is achieved by introducing proteins with light-activated channels, called Opsins (Figure 1), into the cells of interest using targeted genetic techniques. Once in place, these channels allow manipulation of neurons on timescales of milliseconds rather than months. The result is a technique that can be used to both study the role of neurons and control the behavior of an organism, either *in vivo* or *in vitro*. *In vivo*, equipment to control neuron behavior and monitor neuron activity can be implanted into the brain of any freely-moving animal from a fruit fly to a primate. *In vitro*, optical stimulation can be combined with optical and electrophysiological techniques to observe the results on the cellular level.

The resulting techniques not only provide valuable insight into the normal functioning of brain tissues but also into neurological conditions such as Parkinson's disease, Alzheimer's disease and epilepsy. And in the future, optically-controllable cells may themselves be used to treat disorders.

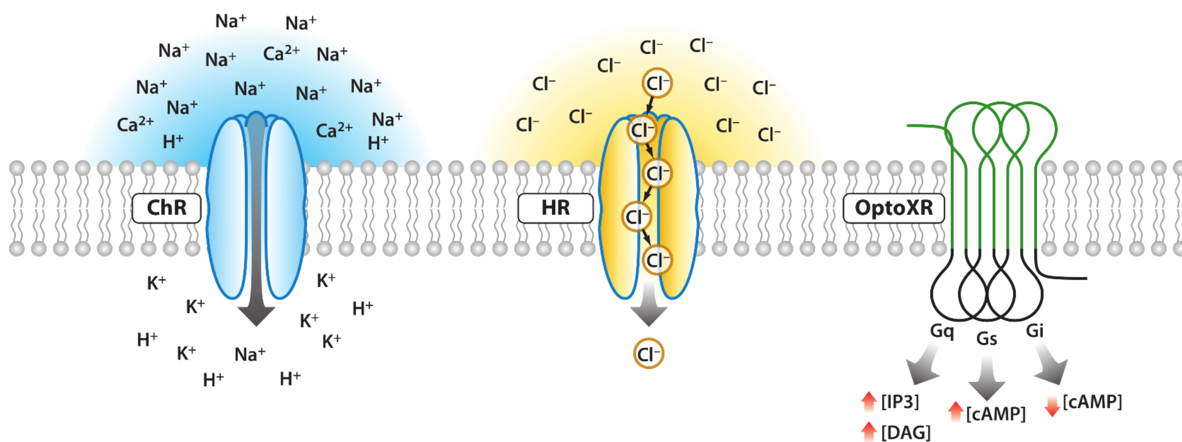

Figure 1: Opsins allow the optically-triggered flow of ions into or out of a cell. Source: (Fenno, Yizhar and Deisseroth, 2011)

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Optogenetic Control

There are three goals for optogenetic studies – stimulating the firing of a neuron, suppressing its response and biasing its response to make spontaneous firing more likely. Each of these goals requires a different class of opsins.

Opsins that cause the neuron to fire are the most common, and there are dozens of choices that can be activated with a broad range of wavelengths of light, also with a broad range of response times from milliseconds to tens of minutes. A summary of commonly used opsins can be found in Table 1.

Opsin tools	CHR2	NpHR	ReaChR	Chrimson	SFO*	VChR1	iC1C2
Action	Activator	Inhibitor	Activator	Activator	Activator	Activator	Inhibitor
Channel/pump	Cation channel	Chloride pump	Cation channel	Cation channel	Cation channel	Cation channel	Chloride channel
Sensitive wavelength spectrum (nm)	400–500	550–620	590–630	Near to 600 (peak)	450–590	500–550	450–500

*Two wavelengths are required for activation, wherein one initiates the current and the second terminates it.

Table 1: The common optogenetic proteins for neural activity modulation, from (Mahmoudi, Veladi and Pakdel, 2017)

Proteins capable of inhibiting neuronal activity with light are rarer, however Wietek *et al.* and Berndt *et al*. have each engineered channelrhodopsins that are capable of inhibiting action potentials (Wietek *et al*. 2014; Berndt *et al*. 2014).

A more subtle approach is to avoid directly causing the neuron to fire with a given trigger, and instead to put the neuron into an 'excited state' where firing becomes more likely. This can be achieved using a 'step-function opsin'. These further have the advantage that the neuron remains in the excited state long after the light is deactivated, meaning the optical fibre to supply the pulse can then be disconnected, allowing the animal free movement. Developments in step-function opsins have led to neurons that can exhibit up to 30 minutes of biased activity after a single 10ms blue light pulse, which can also be deactivated with a pulse of yellow light (Yizhar *et al.* 2011).

Sample Preparation

The proteins for optogenetic control are typically introduced to neuron cells via a virus vector. Viruses provide both fast and versatile insertion, with a high proportion of target cells being infected, providing robust expression. There are a number of ways of achieving cell specificity, including using specific promoters, spatial targeting of the injection of the virus, and precisely targeting the light delivery. However, most viral vectors are restricted in the amount of genetic information they can carry.

An alternative way to introduce the desired proteins is to use genetic 'knock-in' techniques to rear animals that express the proteins as part of their own genetic code, which avoids the limited carrying ability of viruses. However, introducing opsins in this way requires more time, effort and cost (Fenno, Yizhar and Deisseroth, 2011).

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Control and Observation

While *in vivo* measurements can study the macroscopic behavior of an organism under optogenetic influence, *in vitro* experiments can provide control and observation with single-cell resolution. For *in vitro* experiments, neuronal cells are typically prepared in culture. Cells are simply illuminated with either filtered light from mercury arc lamps or LEDs within a microscope setup, though more advanced lighting setups to very specifically target subregions of the sample are available.

Illumination *in vivo* is a little more complicated as typically the animal still needs to be able to move and behave as normal, but typically involves the implantation of a flexible optical fibre into the brain, with laser or high-power LED light used for illumination. However, this approach is fairly invasive and still restricts movement. In 2013, Bruchas and Rogers developed a wireless, ultrathin needle-based system for control and observation that can be injected deep into soft tissues allowing a greater degree of freedom for the animal (T. -i. Kim *et al.* 2013). This device uses 8.5 µm thick micro-LEDs, a microelectrode, an integrated photodetector and a temperature sensor to both optically stimulate and monitor the brain of a moving animal.

Both *in vivo* and *in vitro* studies must occasionally image deep into thick tissues. One method to achieve this is two-photon microscopy with a pulsed laser. This technique allows simultaneous control and observation, with very good spatial and temporal resolution (Andrasfalvy *et al.* 2010). *In vivo,* 2-photon calcium imaging can allow deeper penetration into living tissues to observe the influence of optogenetic stimulation in real time. However, these techniques require the animal to be stationary, such as on a fixed treadmill (C. K. Kim *et al.* 2017).

Optogenetics Discoveries

To observe the results of optogenetic control on a microscopic level as well as the macroscopic 'behavioral' level, electrophysiological techniques are frequently used in parallel, often with control and observation hardware built into a single unit. For *in vitro* studies, microscopy techniques are also possible, such as studying the beating of heart muscle cells that are under optogenetic control (Bruegmann *et al*. 2010). This allowed the researchers to stimulate, study and control heart muscles without the toxic effects of electrical stimulation, providing a mechanism for investigating pacemaking. Fluorescence imaging shown in Figure 2a confirms the cell specificity of the light-sensitive proteins, and parts 2b and 2c confirm the electrical response of the heart tissues to light.

A recent *in vivo* study in mice used optogenetics to discover pathways of the brain responsible for 'reward-seeking' behavior (C. K. Kim *et al.* 2017). Through optogenetic enhancement of these pathways, the researchers caused a mouse to be less likely to press a lever that had a likelihood of delivering either a morsel of food or a mild shock. This may pave the way for treatments of psychiatric illnesses that heighten reward-seeking such as substance abuse, or suppress it, such as depression. By confining the mouse to a treadmill, researchers were able to mount apparatus to its cranium in order to perform two-photon calcium imaging during behavior, confirming the microscopic response.

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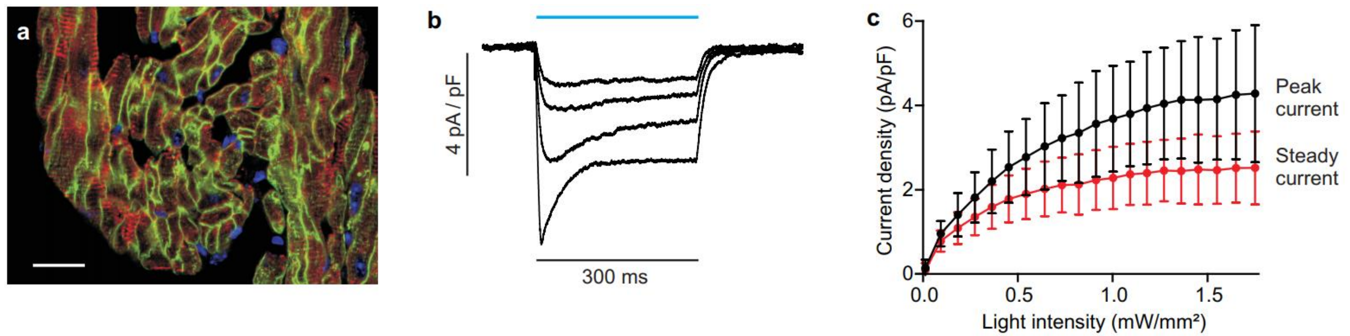

Figure 2: ChR2 expression and light-induced currents in atrial cardiomyocytes from CAG-ChR2 mice. (a) Membrane-bound ChR2-EYFP fluorescence (green) in -actinin (red) positive cardiomyocytes of the atrium. Nuclei shown in blue, bar: 20 µm. (b) Inward currents evoked at a holding potential of -40 mV by light stimulation at 0.09, 0.18, 0.45 and 1.75 mW/mm (from top to bottom). (c) Relationship between light intensity and peak (black) or steady state (red) currents (n = 7, holding potential -40 mV).
From (Bruegmann *et al*. 2010), Supplementary Information.

Challenges in Optogenetics

One major consideration for any behaviorological study is to ensure that the experimental apparatus, along with any genetic changes to the organism, don't influence behavior. For optogenetics, this often requires running parallel experiments with and without cranially-implanted apparatuses, and with and without genetic modification. This is on top of any control groups necessary to isolate the effect of the optogenetic stimulation. Additionally, to prepare a sample for optogenetic control or observation with cell-type specificity often requires difficult and advanced techniques, and requires careful validation, especially check avoidance non-targeted populations.

Optogenetics Imaging

The demands for camera imaging in optogenetics are very broad, from the macroscopic observation of organisms as large as primates to the high-speed, high-magnification microscopic observation of individual cells. In vivo studies typically observe animal behavior as a key experimental outcome. Video or time-lapse imaging of the animal freely moving within its environment is often used.

On the microscopic scale, fluorescence imaging of the targeted cells can be a vital tool in verifying the accuracy of cell-type targeting methods. For in vitro experiments, the tools of optogenetics often go hand-in-hand with those of electrophysiology in general such as patch-clamping and calcium imaging, which are covered in greater detail in the application notes on electrophysiology and calcium imaging, also available in this section of the Photometrics website.

Conclusion

For microscopists with powerful LED and laser illumination on hand and the ability to use electrophysiological techniques in parallel, the ability to control neuronal cells with millisecond time resolution and cell-type specificity allows an unprecedented degree of insight into the cellular-level behavior of the brain.

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Optogenetics is an excellent tool in the arsenal of neuroscientists, and the wealth of research using these techniques in everything from small-scale in vitro experiments to human clinical trials of medical procedures is a testament to its potential.

Further Reading

An historical perspective review by K. Deisseroth provides a wealth of information on the development of optogenetics, the breadth of techniques that utilize it, and an overview of the huge range of discoveries optogenetic techniques have led to (Deisseroth, 2015).

A more recent review by P. Mahmoudi, H. Veladi and F. Pakdel shows the latest developments in the field (Mahmoudi, Veladi, and Pakdel, 2017).

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