Optogenetics: Molecular and Optical Tools for Controlling Life with Light

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Abstract: Optogenetic tools are genetically-encoded reagents that, when expressed in specific neurons in the brain, enable their electrical activity to be precisely controlled in response to millisecond timescale pulses of light.
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Over the last several years we and our colleagues have developed a toolbox of fully genetically encoded molecules that, when expressed in neurons, enable the electrical potentials of the neurons to be controlled in a temporally precise fashion by brief pulses of light. Some of the molecules enable the neurons to be electrically activated, and others enable the neurons to be electrically silenced. Because the tools are genetically encoded, and optically driven, they have come to be known as "optogenetic." These molecules are microbial (type I) opsins, seven-transmembrane proteins found in organisms throughout the tree of life, where they mediate light-sensing or photosynthetic functions, capturing light energy and using the energy to convey ions across cell membranes. These molecules had been studied since the 1970s for the biophysical and biological insights they yielded. We found that these molecules express well in neurons (perhaps surprisingly, given that they function natively in organisms such as fungi and algae) and can mediate light-driven depolarizations and hyperpolarizations [1]. Furthermore, although these molecules require the vitamin A derivative all-trans-retinal to function (as the light capturing chromophore component), enough all-trans-retinal is present in mammalian neurons in culture or in vivo to sustain the function of these molecules (and, for organisms such as C. elegans, Drosophila, and other non-mammalian species, the all-trans-retinal is easily enough supplemented in the food supply). The illumination power required to activate these molecules is in typically in the range of 0.1-10 mW/mm2, easily achieved in vivo (but far higher than seen in the ambient lab environment, thus reducing worries about background side effects).

Three classes of such molecules are in widespread use in neuroscience. Channelrhodopsins are light-driven inward cation channels from green algae. When expressed in neurons, they localize to the cell membrane, and when illuminated, they open up a channel that lets in positive charge (chiefly sodium ions and protons, but also potassium and calcium), thus depolarizing the cell [2]. The first one to be used in neurons was channelrhodopsin-2 (ChR2), from the green alga *C. reinhardtii* [3]; when expressed in neurons, it reacts rapidly to brief pulses of blue light, with large enough depolarizing photocurrents to mediate action potentials at rates of tens of hertz. Due to the utility of ChR2 to mediate the driving of specific cells or pathways in vivo, many variants of channelrhodopsins have been found or engineered, including channelrhodopsins that exhibit higher amplitude currents or currents slower to run down than the original, channelrhodopsins that are faster or slower to turn off after illumination, and color shifted channelrhodopsins, with new variants arising at a rapid pace [4]. Ongoing work is leading to multi-color activation of independent neural populations with multiple channelrhodopsins.

Halorhodopsins are light-driven inward chloride pumps from archaeal species that live in very high-salinity environments. When expressed in neurons, and illuminated, they pump chloride ions into the cells, thus hyperpolarizing them. The first halorhodopsin to be used in neurons was the halorhodopsin from the archaea *N. pharaonis* (Halo/NpHR) [5, 6]. When expressed in neurons, and illuminated with yellow light, halorhodopsins mediate hyperpolarizations, enabling the quieting of neural activity. This halorhodopsin is capable of supporting the perturbation of specific neurons to study their role in the brain functions of behaving mice [7]. Many other organisms bear halorhodopsins that express and can mediate hyperpolarization in neurons [8]. Halorhodopsins in general, however, have poorer expression in neurons than channelrhodopsins, and require the addition of trafficking sequences (e.g., from potassium channels) to express well, at very high levels, in mammalian cells [9-12]. They also recover slowly after extended illumination, taking tens of minutes to recover full photocurrent amplitude after use [8, 13, 14]. A third class of light-activated protein, the light-driven outward proton pumps, express well and also recover rapidly after use. One subclass of these light-driven proton pumps, the archaerhodopsins, as exemplified by the molecule archaerhodopsin-3 (Arch) from *H. sodomense*, are also found in archaeal species. When expressed in neurons, and illuminated with yellow or green light, they pump positive charge out of the cells, hyperpolarizing them. Arch enables 100% neural silencing of neurons in the cortex of awake behaving mice [8]. Recently, the molecule ArchT, 3.5x more light-sensitive than Arch, has become available [15], enabling silencing of large brain regions (such as might be of use in the brain of non-human primates). Light-driven proton pumps also exist in many different color variants; for example, the light-driven outward proton pump Mac (from the fungus *L. maculans*) supports blue-light driven neural silencing [8], thus enabling, alongside the earlier molecule Halo (which is drivable by yellow, and to some extent red, light), two-color neural silencing of two separate neural populations expressing Mac and Halo [8]. Ongoing work is leading to very powerful neural silencers.

The usage of these opsins spread rapidly throughout neuroscience in the months and years following the publication of the initial tool papers, with the molecules being expressed in many different kinds of targeted neuron, enabling them to be activated and silenced with light, to assess their contribution to behavior. These molecules are encoded for by small genes (<1000 DNA bases long), and thus can be delivered into organisms for expression in targeted neurons via practically any commonly used method of gene delivery,

including, for the case of the mammalian brain, viral delivery via lentiviruses, adeno-associated viruses (AAV), and other viruses [3, 16, 17], in utero electroporation [18], and transgenic mouse generation [19-22]. Viral delivery has been utilized to enable optical control of neurons in a wide variety of species, including non-human primates [23-25].

Light delivery into the brain has been supported by the wide availability of fiber-coupled lasers that can be inserted into the brain via cannulas [26], or coupled to fibers that are chronically implanted into the brain. As optics is a rapidly changing field, we are maintaining a web page with current part numbers and best-practices method for assembling, calibrating, and utilizing these fiber-coupled lasers and accessory parts [27]. Wireless light delivery devices [28] and multisite microfabricated 3-D illumination devices [29, 30] are enabling more convenient, and more patterned, delivery of light into the brains of awake behaving mammals. These devices are serving not only as tools for systematic neural circuit analysis, but may in the future support new kinds of clinical precision biological control prosthetic, for treatment of intractable disorders [31]. Such devices can also be used in conjunction with new methods for automated, scalable, neural activity measurement [32, 33].

Note: the text above is adapted heavily from the previously published works [34] and [31].

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