Using Light to Reinstate Respiratory Plasticity

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Arenkiel BR, Peca J. Using light to reinstate respiratory plasticity. J Neurophysiol 101: 1695–1698, 2009. First published February 18, 2009; doi:10.1152/jn.00009.2009. Restoring normal function to damaged or diseased nervous tissue remains a major goal of both basic and clinical neuroscience research. Advances in genetic technologies now allow targeted control of neuronal activity in the mammalian nervous system, providing novel therapeutic avenues to repair or bypass faulty circuits. Here we review recent work published in the Journal of Neuroscience by Alilain et al., demonstrating the use of Channelrhodopsin-2 to restore breathing in rodent models of spinal cord injury.

The mammalian nervous system is an astounding ensemble of cells that form exquisite patterns of connectivity and function in precise concert to enable complex physiological interactions with the surrounding environment. A multitude of different cell types contribute to the output of even the simplest neural circuits, making the identification, isolation, and manipulation of single-neuron activities indispensable toward our current understanding of brain function. Routine applications for targeted manipulation of neuronal activity include deciphering single-neuron firing properties (Bean 2005), dissecting complex circuit pathways (Shepherd 1998), and—arguably most relevant to the general population—repairing or bypassing damaged and diseased nervous tissue (DiMarco 2005; Videnovic and Metman 2008). To date, current methods for such manipulations use either electrical stimulation or pharmacological intervention. These approaches, although effective, fail to target neuronal subsets of given neurotransmitter classes and often show nonspecific, off-target effects by indiscriminately influencing the firing properties of cells in the general vicinity surrounding the target area. For example, deep brain stimulation (DBS) is a common treatment for the debilitating dyskinesias associated with Parkinson’s disease. In this procedure, small microelectrodes are surgically targeted to the subthalamic nucleus or globus pallidus and used to deliver focal trains of high-frequency stimulation. Patients undergoing DBS therapy often show marked improvement of involuntary movements and report a better quality of life. However, due to the fact that many critical brain functions are located in close proximity to each other, adverse side effects of DBS are not uncommon. In some patients the positive therapeutic effects of DBS are accompanied by seizures, speech impairment, mood changes, or even cognitive deficits (Videnovic and Metman 2008). Thus precise functional control of neuronal activity remains a major goal for both basic and clinical neuroscience research.

With the expansion of novel molecular and genetic tools, electrophysiology has naturally dovetailed with molecular neuroscience. This convergence has promoted a conscious effort to develop novel techniques that allow for targeted genetic control of neuronal activity (Luo et al. 2008). One of the most elegant approaches to surface has been the light-driven control of neurons through targeted expression of the algal protein Channelrhodopsin-2 (ChR2) (Arenkiel et al. 2007; Boyden et al. 2005; Nagel et al. 2003). ChR2 encodes a light-gated ion channel that when illuminated with blue light (~470 nm) drives a rapid influx of cations into expressing neurons, resulting in depolarization and action potential generation (Boyden et al. 2005). Unlike other genetic expression systems that allow for cell-specific control of neuronal output by activation of ligand-gated receptors, ChR2 offers many unique advantages: ChR2 is nontoxic to mammalian cells, encoded by a single subunit, readily trafficked to the plasma membrane, directly gated by photons, and shows very rapid on/off kinetics (Nagel et al. 2003; Zhang and Oertner 2007). Most important, however, ChR2 can be genetically targeted for restricted expression in individual neurons or even small neuronal subpopulations, thereby circumventing off-target effects. For tissue-specific patterns of channel expression, stereotaxic injection of viral vectors affords delivery to discrete regions of the nervous system, whereas the incorporation of cell-type–specific promoters allows further refinement of viral-mediated expression to neurons of given subtypes. All of these properties highlight ChR2 as an ideal molecular candidate for potential therapeutic applications in the mammalian nervous system. In a recent study published in the Journal of Neuroscience by Alilain and colleagues, titled “Light-Induced Rescue of Breathing After Spinal Cord Injury” (Alilain et al. 2008), the authors elegantly demonstrate that ChR2 expression and activation may indeed be a plausible approach to reinstate proper neuronal function to damaged or diseased neural circuits.

To demonstrate the feasibility of optogenetic therapy the authors use a rodent system that models acute spinal cord injury (SCI) by cervical lesion (Goshgarian 2003), followed by direct motor neuron activation using ChR2. Patients with cervical SCI often suffer from lesions to descending fibers that regulate the phrenic motor neurons that innervate the diaphragm. This is the most common form of SCI and often requires mechanical ventilation due to the inability to regulate normal respiratory patterns. Inputs to the phrenic motor nuclei come from descending ipsilateral bulboSpinal tracts, which receive contributions from the pre-Bötzinger complex, reticulum formation, and the solitary tract nucleus (Grant and Robertson 2004). These command centers are thought to establish the primary respiratory rhythms and pacemaker functions via direct readout from the carotid body, which is a cluster of...
chemosensory cells associated with the carotid artery in the throat. The carotid body functions to monitor changes in the levels of $O_2$, $CO_2$, and pH in the blood and signals to regulate breathing patterns via circuitry connecting to the cardiorespiratory centers in the hindbrain. When this circuit is interrupted by trauma, disease, or lesion, respiratory control fails. However, despite the loss of input control, the output functions innate to the phrenic motor neurons remain intact. Remarkably, the authors were able to restore near-normal breathing patterns in rodent SCI models by acute photostimulation of ChR2-expressing phrenic motor neurons while bypassing premotor inputs from the hindbrain. This discovery is notable since many human conditions that interfere with proper breathing are severely incapacitating or fatal. Circuit architecture linking central command centers to motor output is common throughout the nervous system, suggesting that direct and controlled stimulation of motor neuron pools, while bypassing higher brain regions, may be a feasible approach to restore muscle function following SCI, a concept clearly validated in the study reported by Alilain et al.

By infecting neurons that constitute the phrenic motor nucleus with a sindbis virus that expresses both ChR2 and green fluorescent protein (GFP), the authors were able to both visualize and photostimulate the target cells that directly innervate the diaphragm muscles. Following ipsilateral hemisection of the spinal cord (Fig. 1A), the authors demonstrate restoration of respiratory function on the lesioned side of the body by illuminating motor neurons that express ChR2. Due to the relatively superficial location of the phrenic motor nuclei within the spinal column, the authors targeted motor neuron activation by simply illuminating the surface of the spinal cord using a small fiber-optic implant placed alongside the motor column, thereby avoiding invasive damage to the spinal cord itself. Photostimulation not only restored output function to the denervated motor pool, but also indirectly influenced the firing properties of the contralateral phrenic motor neurons. By imaging GFP expression they observed that the virally transduced cells in the phrenic motor nucleus included both motor neurons and interneurons, each class of which send projections to the contralateral side of the spinal cord (Fig. 1A). Interestingly, only repetitive, intermittent pulses of light were able to evoke sustained and rhythmic diaphragmatic responses, whereas prolonged periods of illumination typically produced arrhythmic patterns of breathing that completely ceased with light termination. The most striking feature of light-evoked breathing recovery in SCI animals was the observation of a long-term and dynamic period of respiratory plasticity following photostimulation. This plasticity exhibited a unique contralateral volley of activity between the two phrenic motor nuclei (Fig. 1A); patterns of high respiratory activity on one side of the animal were often initially coupled with lower activity on the opposite side. However, within several minutes following photostimulation, the nonsynchronized unilateral diaphragm contractions oscillated until the phase offsets between the two sides coincided. A regular breathing pattern on both sides often remained for hours following activation. Moreover, light-evoked restoration of breathing was repeatable over multiple trials. The significance of this observation is that it reveals an interesting and previously unknown circuit interaction between the opposing phrenic motor nuclei that is unaffected by the anterior afferent lesion, suggesting a direct synaptic pathway between the cells comprising the two nuclei (Fig. 1A).

Interestingly, the novel approach of directly targeting the phrenic motor neurons for photostimulation, via expression of the genetically encoded activator ChR2, has revealed completely new information regarding the cellular connectivity of the respiratory network. These results not only provide greater insight into the overall respiratory circuit function, but also highlight the potential for experiments of this nature to unveil both interesting and unexpected findings.

To further investigate the nature of this long-term respiratory plasticity, the authors pharmacologically probed the synaptic components that might be mediating this effect by application of the noncompetitive $N$-methyl-$d$-aspartate receptor (NMDAR) blocker (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate (MK-801). Direct MK-801 treatment largely abolished all light-evoked responses, with the exception of minimal activation to the intact respiratory circuit contralateral to the lesioned side. The authors reasoned that ChR2-mediated neuronal stimulation leads to a form of long-term potentiation (LTP; or facilitation) in phrenic motor neurons, and this is dependent on classic NMDAR signaling to modulate cellular and respiratory plasticity (Fig. 1B).

The discovery that ChR2-mediated stimulation is capable of inducing a classic LTP-like mechanism in targeted neurons in vivo is significant. For years, and in many experimental systems both in vitro and in vivo, LTP induction has been difficult to spatially restrict to desired circuit pathways. A major drawback to the predominant LTP induction methods is the reliance on either general bath application of pharmacological stimulants or the injection of frequency-dependent, cell-indiscriminant fields of electrical current. Genetically targeting subsets of neurons for photostimulation provides an elegant alternative to current LTP induction methods (Zhang and Oertner 2007). The extremely fast kinetics of ChR2 lends itself to the stimulus frequency variations required for driving LTP responses in nearly every type of known synaptic circuit. This approach will undoubtedly be generally applicable to future experimental paradigms that require LTP induction.

Since the original description of ChR2 as a tool for manipulating neuronal activity (Boyden et al. 2005), there has been a paucity of studies that demonstrate potential translational applications. Only a handful of experiments have implemented optogenetic approaches toward this goal, all of which focus on arguably less-tractable therapies than that being reviewed, the first of which described the restoration of light-encoded signals to the visual cortex in mice lacking photoreceptor cells by expressing and activating ChR2 in subsets of inner retinal neurons (Bi et al. 2006). This was a pioneering study that set the stage for similar manipulations in other cell types of the retina (Lagali et al. 2008; Lin et al. 2008). However, our lack of full understanding as to how retinal maps translate to visual perception precludes the immediate use of this technology for repairing damaged vision. Another study that demonstrated the use of ChR2 to probe brain circuit function was conducted by Adamantidis and colleagues (2007), where the authors showed that manipulating the activity of orexin-producing neurons in the hypothalamus influences arousal and sleep/wake cycles of behaving mice, thus serving as a foray into treating sleep disorders such as narcolepsy. A limitation of this particular application is the need for deep-brain fiber-optic implants, which require invasive brain surgery. This raises the point that
the experiments conducted by Alilain et al. may be unique in that restoration of breathing in their model can be achieved by a relatively straightforward circuit manipulation that does not require deep-brain manipulation. Strong and direct photostimulation of ChR2-expressing phrenic motor neurons results in the concomitant contraction of diaphragm muscles. Most neural circuits are not this simple. Often physiological outputs are controlled by multiple modulatory synapses from different brain regions, posing a significant challenge to accurately manipulate complex neural circuit function with a stimulus such as light. Nonetheless, genetically targeting pools of output neurons (when possible) for synthetic stimulation provides a convenient and promising avenue for neural therapy.

A common caveat to all of the studies to date that have investigated ChR2 as a tool for therapeutic applications is that neuronal introduction of ChR2 cDNA has been largely restricted to viral transduction. Current recombinant viral vectors are still not acceptable for therapeutic use in humans and have
the potential for unpredictable cytotoxicity and/or tumorogenicity, limiting their current therapeutic prospects and placing importance on continued viral vector design. Alternative approaches for ChR2 delivery into nervous tissue might include the use of local electroporation or some form of differentiated stem cell therapy, both of which represent nascent technologies that clearly await further development and testing. However, the future prospects of repairing or restoring proper neuronal function to faulty brain circuits through genetically encoded actuators places emphasis toward progress in these fields of research.

The report by Alilain et al. represents a body of work rightfully deserving of accolades. The authors convincingly demonstrate the potential for using heterologous expression of the algal-derived ChR2 protein as a means to directly activate phrenic motor neurons that drive breathing in SCI models lacking proper presynaptic control. This represents one of the first studies that implements ChR2 as a feasible means for genetically targeted neuronal therapy. Moreover, observations describing the ability of ChR2 to effectively modulate neuronal plasticity at both cellular and circuit levels further highlight the general utility of this tool for basic neuroscience research.

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REFERENCES


