Published in final edited form as:

Science. 2014 April 04; 344(6179): 44–45. doi:10.1126/science.1253088.

Optogenetic Regeneration

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Abstract

Applying tools from optogenetics with ideas from regenerative medicine may herald a new era of translational optogenetics.

The first decade of optogenetics has seen many efforts to improve our understanding of normal and pathological neural circuitry (1). The great impact of these efforts has stemmed from an alliance between systems neuroscientists and protein engineers—the first group identifying neural circuits amenable to causal dissection, the second developing tools that enable unprecedented degrees of control over neural activity. The next decade of optogenetics is likely to see the development of a new alliance that may have similarly important implications—one between optogenetics and translational medicine. On page 94 of this issue, Bryson *et al.* describe one model for how such an alliance may proceed, applying tools from optogenetics in concert with ideas in regenerative medicine to restore muscle function in a mouse model of peripheral nerve injury (2).

The threshold question that all optogenetic experiments face is that of achieving stable expression of opsins in the desired cell population (1). Bryson *et al.* adopted an approach to solve this problem that has some precedent in the field (3). The authors genetically engineered mouse embryonic stem cells to stably express channelrhodopsin-2 (ChR2), a cation channel sensitive to blue light, and then differentiated these cells in vitro to obtain optogenetically activatable ChR2 motoneurons. Thus, shining blue light on these ChR2 motoneurons could robustly drive neuronal firing. They then grafted aggregates of stem cells (embryoid bodies) containing these ChR2 motoneurons into a mouse model of muscle denervation in which the sciatic nerve was ligated. The engrafted ChR2 motoneurons survived, matured, and grew to innervate the denervated muscles of the lower limb, allowing Bryson *et al.* to restore muscle function through illumination of the graft site in anesthetized mice with blue light.

For reasons that remain somewhat unclear but are the subject of active study through computational modeling (4), optogenetic stimulation of ChR2 motoneurons results in motor unit recruitment patterns that closely track physiological motor unit recruitment order (5), unlike electrical stimulation, which produces a reverse or random recruitment order (6). Bryson *et al.* were therefore able to use their engrafted ChR2 motoneurons to achieve “orderly recruitment” of reinnervated muscles. These results confirm previous reports indicating that optogenetic stimulation activates muscles in a way that induces less fatigue

than electrical stimulation, thus enabling optogenetically induced force to be sustained for long durations through preferential recruitment of fatigue-resistant motor units (5).

The capabilities that Bryson *et al.* demonstrate are likely to spur many subsequent studies. One critical question is whether the restoration of muscle function achieved can be extended to mice that are not under anesthesia. This will likely require the use of chronically implantable light-emitting nerve cuffs, which allow for optogenetic activation of peripheral nerves in freely moving animals. (7). Also of great interest will be the quality and persistence of the enabled control. Bryson *et al.* describe ChR2 motoneuron endplates (innervated regions of muscle) that are malformed, and hypothesize that this is due to initial in vivo inactivity of the transplanted ChR2 motoneurons. Chronic cuff implantation would allow for optogenetic activation of these neurons immediately after engraftment, which may help prevent such malformation. The long-term survival of engrafted ChR2 motoneurons is another major challenge that must be overcome.

The cell transplantation framework used by Bryson *et al.* may also have applicability in the treatment of other forms of nervous pathology. Stem cell grafts and electrochemical neuroprostheses may have potential use in the treatment of spinal cord injury (8, 9). Combining these strategies with the stimulation specificity provided by the optogenetic approach of Bryson *et al.* may be a productive direction for future research efforts.

By demonstrating how results from regenerative medicine may be integrated with new techniques in muscle physiology to restore function, Bryson *et al.* exemplify the type of interdisciplinary synthesis that will be essential for developing translational optogenetics. Like Bryson *et al.*, others have identified neurons outside the brain as the likely first target for optogenetic translation (10). In addition to control over peripheral motoneurons (2, 5, 7), foundational work has been done in this area to demonstrate that optogenetics may be used to control retinal cells (11) and pain circuits (12, 13).

However, several challenges remain to be overcome before the first successful optogenetic therapy is realized. Among these is the extension of optogenetic techniques beyond murine models to nonhuman primates (14), particularly in neural circuits outside the brain. Equivalently important is improved assessment of the long-term safety of opsin expression across a variety of delivery strategies, including both viral vectors (such as adeno-associated viruses), and cell transplants such as those used by Bryson *et al.* The development of robust light-emitting devices that are well tolerated upon implantation is also critical; these may potentially be wirelessly powered. And opsins will need to be developed that exhibit improved light sensitivity (particularly to red light) and a wide range of different temporal characteristics. These challenges notwithstanding, this study by Bryson *et al.* provides an elegant step along the path to optogenetic translation (see the figure).

Acknowledgments

Supported by National Institute of Neurological Disorders and Stroke grant R01-NS080954 and the Stanford Bio-X NeuroVentures program. S.M.I. is supported by a Howard Hughes Medical Institute International Student Research Fellowship. We thank K. Montgomery for assistance with the illustration concept.

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Figure. The dawn of translational optogenetics

Bryson *et al.* restored muscle function in a mouse model of peripheral nerve injury in an anesthetized animal. In the future, neural circuits in live animals might be controlled with robust light-emitting devices to restore physiological functions.