Optogenetic Regeneration

Shrivats M. Iyer and Scott L. Delp

Science **344**, 44 (2014);
DOI: 10.1126/science.1253088

NEUROSCIENCE

Optogenetic Regeneration

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

Shrivats M. Iyer[1] and Scott L. Delp[1,2]

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*).

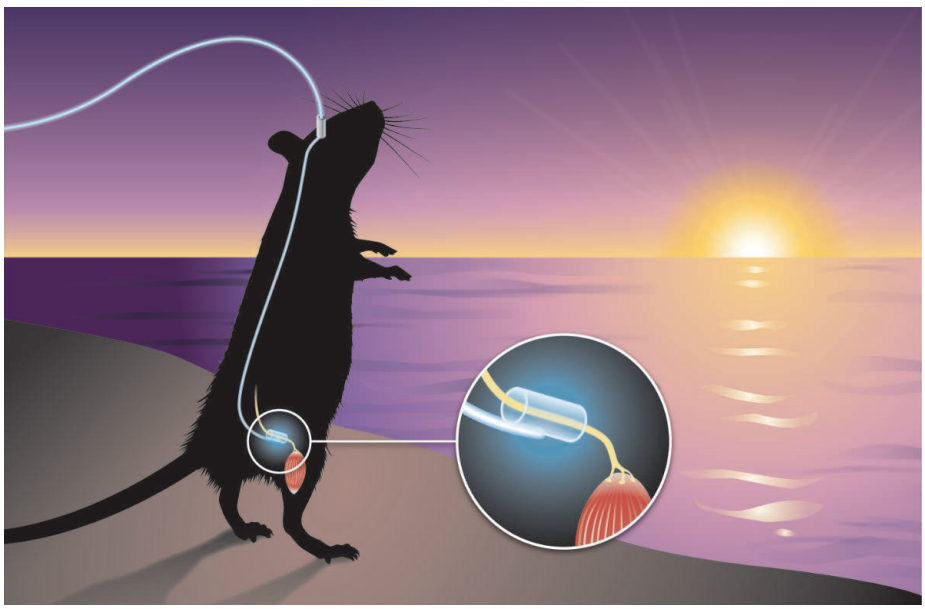
The threshold question that all optogenetic experiments face is that of achieving stable expression of opsins in the desired cell population (*1*). Bryson *et al*. adopted an approach to solve this problem that has some precedent in the field (*3*). The authors genetically engineered mouse embryonic 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 motorneurons. Thus, shining blue light on these ChR2 motorneurons could robustly drive neuronal firing. They then grafted aggregates of stem cells (embryoid bodies) containing these ChR2 motorneurons into a mouse model of muscle denervation in which the sciatic nerve was ligated. The engrafted ChR2 motorneurons 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 motorneurons 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 motorneurons 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 motorneuron endplates (innervated regions of muscle) that are malformed, and hypothesize that this is due to initial in vivo inactivity of the transplanted ChR2 motorneurons. 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 motorneurons 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 motorneurons (*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*).



CREDIT: H. MCDONALD/*SCIENCE*

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.

[1]Department of Bioengineering, Stanford University, Stanford, CA 94305, USA. [2]Department of Mechanical Engineering, Stanford University, Stanford, CA 94305, USA. E-mail: delp@stanford.edu

Published by AAAS

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).

References and Notes

1. K. M. Tye, K. Deisseroth, *Nat. Rev. Neurosci.* **13**, 251 (2012).
2. J. B. Bryson *et al*., *Science* **344**, 94 (2014).
3. J. P. Weick *et al*., *Stem Cells* **28**, 2008 (2010).
4. R. L. Arlow, T. J. Foutz, C. C. McIntyre, *Neuroscience* **248**, 541 (2013).
5. M. E. Llewellyn, K. R. Thompson, K. Deisseroth, S. L. Delp, *Nat. Med.* **16**, 1161 (2010).
6. C. S. Bickel, C. M. Gregory, J. C. Dean, *Eur. J. Appl. Physiol.* **111**, 2399 (2011).
7. C. Towne, K. L. Montgomery, S. M. Iyer, K. Deisseroth, S. L. Delp, *PLOS ONE* **8**, e72691 (2013).
8. P. Lu *et al*., *Cell* **150**, 1264 (2012).
9. R. van den Brand *et al*., *Science* **336**, 1182 (2012).
10. J. C. Williams, T. Denison, *Sci. Transl. Med.* **5**, 177ps6 (2013).
11. V. Busskamp, B. Roska, *Curr. Opin. Neurobiol.* **21**, 942 (2011).
12. S. M. Iyer *et al*., *Nat. Biotechnol.* **32**, 274 (2014).
13. I. Daou *et al*., *J. Neurosci.* **33**, 18631 (2013).
14. I. Diester *et al*., *Nat. Neurosci.* **14**, 387 (2011).

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.

10.1126/science.1253088

CHEMISTRY

A CO_2 Cloak for the Cyanide Dagger

The fleeting stability of the cyanoformate ion formed from CO_2 and cyanide has implications for plant enzymology and CO_2 sequestration.

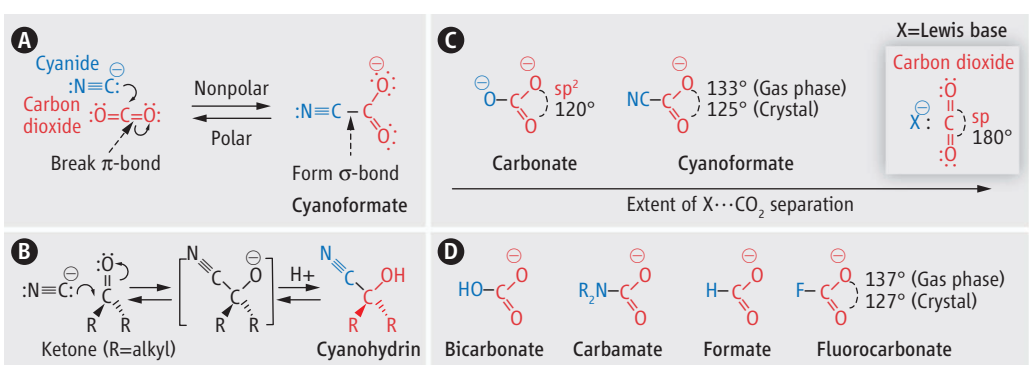
Igor Alabugin and Rana K. Mohamed

In the ever-expanding universe of compounds prepared to date, it is remarkable that a two-carbon ion with an apparently simple electronic structure could have eluded structural characterization until now. It is especially notable because this ion is formed from carbon dioxide (CO_2) and cyanide (CN^-), each with a rich chemical history. On page 75 of this issue, Murphy *et al*. (*1*) report trapping the elusive cyanoformate ion as a crystalline salt with a bulky and unreactive cation. Their crystallographic and spectroscopic analysis along with quantum-mechanical calculations reveal a seemingly ordinary carbon-carbon (C–C) bond with the length of ~1.5 Å, yet cyanoformate balances on the brink of fragmentation in nonpolar environments and its C–C bond breaks in more polar solvents.

On first glance, bonding of CO_2 and CN^- could be expected because the carbon atom in CN^- is nucleophilic (electron rich), whereas the carbon in CO_2 is electrophilic (electron poor) (see the figure, panel A). Every discussion on the chemistry of ketones, esters, and similar functional groups in an undergraduate organic chemistry class includes attack of the lone pair of electrons of a nucleophile at the carbonyl (the C=O group). In this process, a π bond is sacrificed to give a σ bond. Usually, σ bonds are stronger than π bonds, so the overall transformation is thermodynamically favorable. For example, interaction of cyanide with ketones and aldehydes (formation of cyano alcohols, also known as cyanohydrins) dates back to the classic 1850 work of Strecker (see the figure, panel B) (*2*).

Formation and decomposition of cyanonohydrins is commonly used by bacteria, plants, fungi, and a few animals (e.g., millipedes) as a way to store cyanide and release it on demand (cyanogenesis) (*3*). Rumor has it that this reaction offered protection from cyanide to Grigori Rasputin, a controversial figure of Russian history (*4*). One attempt to poison Rasputin failed because of his apparent immunity to cyanide. The theory is that his killers put the cyanides in sweet pastries, and the carbonyl functionality on the sugar molecules served as an antidote by reacting with the cyanide and forming cyanohydrins.

However, CO_2 is a much tougher nut to crack than a typical ketone, and its reaction with cyanide is considerably less favorable. Not only is the energy cost for distorting the linear CO_2 molecule into the bent sp^2 geometry (~75 kcal/mol) (*5*) comparable to the C–C bond energy, but π bonds at sp-hybridized carbons are stronger than π bonds at sp^2-hybridized carbons (*6*, *7*). The relatively small enthalpic preference for cyanoformate formation is wiped out by unfavorable entropy. The 133° O–C–O angle in cyanoformate suggests that CO_2 is poised, like a spring, for the escape to its relaxed linear geometry. The ephemeral nature of cyanoformate illustrates that C–C bonds can, under



Traps for CO_2 and CN^-. (**A**) Traps for cyanide can be transient, such as capture in cyanoformate, where, as shown by Murphy *et al*., stability depends on solvent polarity. (**B**) Long-term capture of cyanide in cyanohydrins. (**C**) CO_2 trapping can bend this linear molecule into the sp^2 geometry of carbonate ions or the intermediate structure of cyanoformate, depending on the degree of interaction. (**D**) Lewis bases can act as traps for CO_2.

Department of Chemistry and Biochemistry, Florida State University, Tallahassee, FL 32306, USA. E-mail: alabugin@chem.fsu.edu