Optogenetics and the future of neuroscience

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Over the last 10 years, optogenetics has become widespread in neuroscience for the study of how specific cell types contribute to brain functions and brain disorder states. The full impact of optogenetics will emerge only when other toolsets mature, including neural connectivity and cell phenotyping tools and neural recording and imaging tools. The latter tools are rapidly improving, in part because optogenetics has helped galvanize broad interest in neurotechnology development.

It was precisely 10 years ago that this journal published a study[1] describing how a microbial opsin, a natural light-sensitive ion-transporting membrane protein, could be expressed in neurons to make their electrical activity controllable by light. This special issue of *Nature Neuroscience* reflects on how this field, now known as optogenetics, has developed over the ensuing decade. The journal has asked scientists for their personal thoughts on the past, present and future of optogenetics in neuroscience, in a Q&A[2] and in a Commentary by Deisseroth[3]. Here I reflect on the impact optogenetics has had on neuroscience, not just in the answering of specific scientific questions but on the direction of the field as a whole.

The study, which originated in ideas and experiments generated by Karl Deisseroth and myself, collaborating with Georg Nagel and Ernst Bamberg and later with the assistance of Feng Zhang, was not immediately a smash hit. Rejected by *Science*, then *Nature*, the discovery perhaps seemed too good to be true. Could you really just express a single natural algal gene, channelrhodopsin-2 (ChR2)[4], in neurons to make them light-activatable? Our paper showed that the gene product was safe in neurons, and had appropriate kinetics and current amplitude to cause neurons to fire action potentials upon delivery of millisecond-timescale pulses of light, without the need for chemical supplementation. This of course was serendipitous: there was no a priori reason for an algal protein to successfully express and operate in mammalian neurons. This serendipity was the result of the intersection of decades of basic curiosity about how microbial proteins use light for energy storage and sensation, and modern neuroscience-question-driven technology need.

Another reason for skepticism may have been that many technologies for neuroscience had been published in the years before, some of which were not robust nor easy to use, and skepticism about technology seemed, to me anyway, to abound in neuroscience. "Don't develop tools, just learn to answer scientific questions," one person advised me when I first arrived at Stanford in 1999. Thus, there was some question about the actual utility of optogenetics. In addition, neuroengineering was not the fast-growing field that it is now. There was no BRAIN initiative (http://www.whitehouse.gov/share/brain-initiative), and one could argue that neurotechnology development was not then a fully respectable profession. Indeed, after our first optogenetics paper appeared, my faculty job search was hit-or-miss. The MIT Media Lab, which had decided that one of its mandates was to hire eccentric misfits, thankfully took a chance on me.

I've discussed the early history of the optogenetics toolset previously[5]. In the years following our first paper, the toolset rapidly expanded, and adoption of the tools began to spread—first slowly, then faster and faster. In 2007, neural silencing was achieved with the use of light-sensitive chloride pumps to mediate neural silencing with light[6,7]. Over the last few years, the optogenetics toolset has expanded to include molecules that mediate neural activation with green and even red light[8–10], neural silencing with red light[11], neural silencing via light-driven chloride conductances[12–14], and high speed and light-sensitive neural activation[10], among other advances. Arguably, the optogenetic tools are maturing, and in some cases may even be near their physical limits. Usage of optogenetic tools spread throughout the genetic model organisms of neuroscience—*Caenorhabditis elegans*, *Drosophila melanogaster*, zebrafish, mice—and later nongenetic model organism such as nonhuman primates.

What have we learned from optogenetics? As far as direct scientific impact goes, one could argue that no major paradigm shift in neuroscience has resulted from the use of optogenetic tools in their first 10 years—in comparison with, say, the discovery of neurons in the first place, or of synaptic release. What optogenetics has done so far is make the study of circuits more tractable, especially when it comes to causally probing what a neuron means to a circuit, since neural activation and silencing enable the testing of the sufficiency and necessity of specific cell populations in the generation of behaviors and pathologies. Optogenetics has also greatly helped with the linkage of cellular types and mechanisms to circuit- and systems-level emergent phenomena. But we still cannot fully explain any sensation, behavior, emotion, movement or cognitive process. Some of the studies done in the first

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10 years of optogenetics tested old hypotheses, tying up loose ends. As the toolset gained adoption, work exploring new pathways and sets of cells and functions has become more prominent. Of course, 10 years is not a long time in science terms, and we are just at the beginning.

Future optogenetics experiments, especially performed in conjunction with other new technologies, may realize the dream of fully understanding neural circuits with single-cell precision. For example, many optogenetic studies have activated or silenced neurons as populations, engaging them synchronously as an ensemble. Of course, in the brain even adjacent neurons of the same kind can have very divergent activity patterns, raising the question of whether one could instead dial in a truly arbitrary, naturalistic activity pattern. Recently, tools such as spatial light modulators have begun to enter more common use in neuroscience and are beginning to enable optogenetics with single-cell-targeting precision[15,16]. This technology improvement raises hope for individual control of multiple cells throughout neural networks.

Many optogenetic studies have focused on classically defined cell types, such as somatostatin-positive neurons or norepinephrine-producing neurons, raising the question of whether it would be possible to control any desired kind of cell in the brain. Of course, complete cell taxonomies for almost all species and brain circuits do not yet exist, much less good genetic handles that enable specific targeting of those cell types for gene expression. Recently, many groups have begun to develop strategies for enumerating, describing and achieving molecular handles on cell types, ranging from new ways of assessing cell genetic programs and transcriptomes[17] to new ways of describing neural morphologies and molecular locations[18,19]. If complete descriptions of cell types are achievable, and in particular if selective genetic handles that allow specific cells to be targeted for gene expression are found, it might be possible to optogenetically activate or silence any specific kind of neuron that is hypothesized to be involved in a behavior or a disease. If connectivity maps of neural circuits can be derived, that may help provide new hypotheses of neural circuit functions that can then be causally tested with optogenetics.

Many optogenetic studies have been focused on open-loop control of neurons, activating or silencing cells or sets of cells without involving neural recording in conjunction with real-time data analysis to sculpt the stimulation paradigm. The ability to record activity and, in real time, to responsively compute specific patterns of illumination to be delivered to neurons in the brain would enable detailed studies of neural dynamics that could be more powerful than open-loop control. We are now starting to see the creation of strategies for simultaneous recording and perturbation of neural dynamics—for example, in the context of silencing seizures in mouse models of epilepsy[20] or for precisely causing cells to fire in specific patterns regardless of the inputs being received by those neurons[21]. As optical three-dimensional neural activity imaging methods become faster in their volumetric acquisition speeds[22,23] and as fluorescent reporters of neural activity also increase in speed, signal-to-noise ratio and fidelity[24], one appealing goal is the ability to image and control neural circuits continuously in real time, enabling all-optical interfaces to neural circuits that can tease apart how individual cells work together to generate dynamics in real time.

The aforementioned examples of technologies that are enhancing the power of optogenetic tools hint at a broader impact of optogenetics: because optogenetics is so easy to use and has spread so fast—in part because the groups involved in optogenetic tool development disseminated molecules freely to the neuroscience community—optogenetics has, through its utility to experimentalists, catalyzed a desire for more kinds of powerful tool. Indeed, neuroscience studies performed in the first decade of optogenetics have often helped to raise awareness of, and sometimes even accelerate development of, other toolsets to meet complementary needs in neuroscience. This cultural shift in neuroscience toward celebrating tool development has resulted in efforts like the US BRAIN initiative, which aims to catalyze new neurotechnologies, and the creation of many centers and institutes for neuroengineering throughout the world. This indirect impact of optogenetics, making the neuroscience world safe, as it were, for tool development has helped create a sense of optimism and confidence that new technologies can assist in making the big mysteries of the brain more tractable.

The impact of optogenetics in basic neuroscience has been significant, and it will probably only grow as the aforementioned synergistic tools yield, in the years to come, potentially near-complete powers for mapping, recording the dynamics of, and controlling the dynamics of neural circuits. An open question remains as to how optogenetic tools might be used in the clinic. As we have only rudimentary lists of cell types of the human brain and their functions, solid rationale from basic science experiments is required to define the precise neural substrates that could serve as clinical targets in humans. Perhaps not surprisingly, the detailed knowledge of the cell types and wiring of the retina has helped make photoreceptor-loss disorders such as retinitis pigmentosa into early candidates for optogenetics treatment[25]. Of course, without human trials it is impossible to know whether microbial gene products will be well tolerated in the human body, especially over long timescales, and thus fundamental risks remain to be resolved. But it is clear that the results emerging from the use of optogenetics in basic neuroscience, and from neurotechnology as a whole, will provide in the years to come a variety of insights into new molecular targets for drug development, new circuit sites for electrical brain stimulation, new protocols of regenerative medicine, and other strategies for helping repair the brain.

COMPETING FINANCIAL INTERESTS
The authors declare competing financial interests: details are available in the online version of this paper.

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Erratum: Optogenetics and the future of neuroscience

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In the version of this article initially published, page ranges were missing for refs. 2 and 3 and the journal abbreviation was missing for ref. 21. They are, respectively, pp 1202–1212, pp 1213–1225 and *Elife*. The error has been corrected in the HTML and PDF versions of the article.