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"Optogenetics" is the control of cellular biophysical properties by selective, genetically targeted expression of light-sensitive moieties. This strategy has rapidly become a widely pervasive tool in neuroscience, in large part because it arrived at a perfect historical moment to provide a logically essential but previously missing piece in controlling cellular activity, thereby testing how the *in vivo* brain works.

Evidence in science is based on the triangulation of three key modes of proof. The first is the widespread standard of correlative observations, in which two variables are argued to be interdependent because their co-occurrence is so suspiciously overlapping. Such evidence is the source of many of the most distinguished findings in science, with examples in neuroscience including such hallmarks as the observation and characterization of receptive fields in the neocortex.

The second key mode is negative causal evidence—the damage/removal/disruption of a specific process and the demonstration that negative consequences follow. Within neuroscience, examples of such evidence include lesioning of neural tissue that is followed by loss of behavioral function. The double and triple dissociation of the function of specific brain areas with specific and nonoverlapping behavioral capacities is a particularly powerful mode of such argument.

The third key mode, which is the least often employed but potentially the most important, is positive causal evidence. In such experiments, selective amplification or addition of a process leads to beneficial outcomes or a gain of function within a system. Examples of such evidence include the expression of behaviors during the microstimulation of specific neural systems. Such outcomes range from the instantaneous display of aggression after stimulation of specific hypothalamic regions to emergent rhythmic motion, including walking, trotting, and galloping, following the administration of increased levels of current in the mesencephalic locomotor region of decerebrated animals.

Historically, neuroscience has relied on all three modes of evidence. Prior to around 1960, seeking causal evidence of both types was a dominant paradigm. Lesion studies defined the seminal work of Florens and Lashley in animal models, and Henry Head and Penfield in human neuropsychology. Microstimulation was also widely employed with the hopes of obtaining both negative and positive causal proof. Remarkable demonstrations of the specificity of control of complex systems emerged. For example, animals could be made to suddenly display shame or directed rage through localized stimulation foci in the hypothalamus. At a more basic level, elemental motor actions could be demonstrated to emerge from highly localized stimulation of the mesencephalic locomotor region, the subthalamic locomotor region and the pontine peduncular nucleus, producing highly repeatable expression of complex rhythmic motor sequences involving diverse muscle groups and limbs, dissecting the differential contribution of several motor centers. One can appreciate the prominence of microstimulation *in vivo* as a paradigm prior to 1960 by reviewing entries in the expansive three-volume *Handbook of Neurophysiology* published at that time. "Neurophysiology" prior to 1960 was dominated by *in vivo* microstimulation in the pursuit of such data.

At around 1960, a relatively rapid and substantial shift away from microstimulation occurred. This shift occurred for a variety of reasons. First, and perhaps most importantly, Mountcastle and later Hubel and Wiesel demonstrated the power of correlative receptive field mapping in sensory neocortex, with studies approximately ten years later showing the remarkable discovery of highly specific place fields within the hippocampus. The new strategy of extracellular single-unit recording revealed that central neurons could be repeatedly driven by specific patterns of input, such as taps on a specific finger pad, and more macroscopic patterns, such as cortical columns, were discovered through the iterative application of this approach.

The radical specificity and power of this correlative experimental approach in anesthetized or paralyzed animals was in sharp contrast to the emerging realization that while microstimulation could causally evoke complex behaviors, their exact mechanistic origins—and therefore a more computationally satisfying account of the mechanisms of the mind—were not well elucidated by injecting current into an awake animal. The site of action of the current was hard to localize—did it drive local neurons, incoming afferent endings, axons passing nearby or all of the above? Further, even if one assumed that 'local neurons' were being driven, even the simplest brain structures include a diversity of cell types with a large variety of chemical receptors. This heterogeneity was assumed to play a role in neural processing, a role that could not be revealed by larger scale microstimulation.

Further, an alternative causal control paradigm gained ascendance in this period, the use of pharmacology. The

elucidation of the specific effects of dopamine and related precursors when administered peripherally led to not only landmark scientific findings that contributed to a Nobel Prize award but also to remarkable treatments for maladies such as Parkinson's disease. In the early days of pharmacology, single molecules were thought to drive single receptor types. The phenomenological zoo of receptor types and flavors of agonists and antagonists of receptors in neurons that now exist were not yet appreciated, making pharmacology appear to be a selective method. This trend was served by the refinement of methods for slice physiology, allowing the direct application of drugs to local circuits with intracellular recordings, particularly after development of the powerful control capabilities of whole cell configuration. The approach of dissective pharmacology in *in vitro* tissues appeared to provide the reductionistic causal specificity needed to fill the gap created by the loss of pharmacology.

While pharmacology flourished in many ways (and is of course the basis of modern biomedicine), there was increasing appreciation of the incredible diversity of impact similar molecules have at differing targets. Further, there was appreciation that the strong reductionistic assumptions from *in vitro* models did not always hold—such data could often be unreliable in predicting *in vivo* outcomes, even when all of the players in a circuit were supposedly understood. Such progress led to increasing skepticism that the specific computational role of select neuron types in brain function could be elucidated in this way. Further, pharmacological approaches were "slow," acting on the time course of minutes to hours as they diffuse over a slice or through a body. Put simply, while there was appreciation that *in vivo* causal studies were essential, no clear consensus existed on how they could be conducted with the precision needed to provide a computationally satisfying quality of data. An approach was needed that had cell-type specificity and millisecond level precision (at least in some of its applications) and could be robustly applied *in vivo*.

Into this context, Francis Crick offered a prescient *Scientific American* article in 1979. In it he argued that causal methods needed to be reasserted within neuroscience and that revolutions in the exact methodology were therefore needed:

> A major first step, then, is to identify the many different types of neuron existing in the cerebral cortex and other parts of the brain. One of the next requirements (as discussed above) is to be able to turn the firing of one or more types of neuron on and off in the alert animal in a rapid manner. **The ideal signal would be light**, probably at an infrared wavelength to allow the light to penetrate far enough. This seems rather far-fetched but it is conceivable that molecular biologists could engineer a particular cell type to be sensitive to light in this way.[1]

This call-to-optical-arms was taken up, knowingly or through parallel intellectual evolution, by a variety of new methods in the early 2000s. In one key approach, light pulses were used to uncage molecules such as glutamate that then acted locally to impact their targets. The local action of these molecules—for example at different points along the dendritic tree of a neuron—produced substantial advances in our understanding of biophysical principles of neural function. This approach was, however, limited largely to *in vitro* systems where the molecules could be introduced.

An alternative approach was offered by Miesenbock and colleagues. Using visual rhodopsins incorporated into cultured vertebrate neurons, they showed robust modulation of neural activity with light pulses.[2] This strategy was subsequently used to effectively regulate behavior in flies *in vivo*.[3]

At approximately the same time, Nagel and colleagues[4,5] showed that light-activated cation channels were present in bacterial algae. In 2003, they showed that robust currents could be generated through activation of a molecule they dubbed channelrhodopsin-2, including significant influx of calcium. They observed in that paper that this molecule could readily meet the conditions sought in the charge offered by Crick:

> **ChR2 May Be Used to Depolarize Animal Cells.** We reasoned that ChR2 should be able to significantly depolarize a Chlamydomonas cell and, second, that heterologous expression of ChR2 **should become a useful tool to manipulate intracellular Ca2+ concentration (Fig. 1g) or membrane potential, especially in mammalian cells**. Current-clamp experiments with ChR2-expressing oocytes (Fig. 4d) or HEK293 cells of similar diameter as a Chlamydomonas cell (Fig. 4e) to test this promise clearly demonstrated a fast light-induced depolarization by tens of mV when the cells were illuminated with blue light.

While all of these precedents set the stage for optogenetics as it is currently practiced, the intersecting requirements of robust proof-of-concept in mammalian cells, genetic targeting of specific cell types, and elaboration of the toolkit to include a broad array of options, had not yet been met. Deisseroth, Boyden, and Zhang collaborated with Nagel and Bamberg in 2005[6] to begin a series of publications that have provided exactly these advances. In later papers, they and others further discovered or invented the wide variety of highly useful variants of light-activated pumps and channels, offering a large array of options for neural control. Further, and as importantly, while the logical need for this new kind of tool was apparent to some in the field, these authors have done a remarkable job of making the power of these tools widely appreciated (including such advances as coining the descriptive name "optogenetics" itself). This effort included not only many publications reviewing the potential of these tools for a wide variety of applications, these authors also generously shared the new advances they were making immediately and developed a variety of practical viral delivery strategies for realizing the genetic specificity that is central to the method's efficacy.

With optogenetics, a wide variety of *in vivo* studies have now been conducted, bringing in a new era of precise causal study of the mechanism's underlying behavior. Combination of this approach with other techniques has also proven particularly powerful, including electrophysiology, optical imaging using wide-field and multi-photon microscopy, and fMRI. Critically, optogenetic drive has proven highly amenable to study of behaving animals, thereby demonstrating the result of neural control on behavior in addition to its effect on the neural substrates.

As with the development of all new methods that inspire widespread adoption, there has been increasing realization

of the limitations of these approaches as well, particularly in how inferences are made from strong and homogenous recruitment of a large population of cells at a single point in the complex and interconnected brain.

There are several forefronts on the cutting edge of optogenetics. These include but are not limited to the application of optogenetic methods to non-neural excitable and nonexcitable cell types throughout the body. Adoption to non-neural cells has been steady since the first demonstration of the methods but will likely show increased adopting as other fields of study outside neuroscience increasingly appreciate the powerful features of this approach. Another key area that has seen progress but that will likely show rapid innovation is in optogenetic use in nonhuman primates.

Another key cutting edge is in the advancement of new methods for light delivery. A particularly important area of effort that is now seeing significant progress is the use of 2-photon excitation to provide a high degree of specificity in the targeting of specific cells in three dimensions. Such targeting will allow, for example, the testing of the importance for behavior of discrete ensembles of cells, such as those shown by correlative means to be implicated in specific forms of perceptual processing. Other cutting-edge advances include new ways of embedding light delivery in probes, such as laminar arrays of light delivery systems. A particularly unique approach in this regard is the use of bioluminescence to drive optogenetics. This method, termed "BL-OG" (for BioLuminescent OptoGenetics) allows the selective production of light in cells expressing bioluminescent enzymes, letting cells become their own light source for controlling activity. This advance transforms optogenetics into a chemical genetic method, in some ways akin to designer receptors exclusively activated by designer drugs (DREADDs) but exceeding DREADDs in key ways, including by providing access to the entire optogenetic toolkit for such applications.

In the current special section, a collection of original research, methods, and review papers are presented that add to many of the forefronts discussed above. The papers reflect the rapid maturation of the optogenetic approach, the development of novel methods to concurrently stimulate and record or image activity, application in non-neural excitable cells, and the use of optogenetics in nonhuman primates, all necessary steps in advancing the methods and towards leveraging optogenetics to the treatment of human disease. Lu et al. characterize terminal dopaminergic dynamics by precise optogenetic modulation of neurotransmitter release. Nussinovich and Gepstein apply optogenetic control to suppress cardiac electrical activity in human and murine cardiomyocyte cultures, opening the path to the application of optogenetic control to treat cardiac failure.

The development of methods to stimulate and record activity were an essential building block to the broad adoption of optogenetics, and novel developments allowing miniaturization, energy efficiency, and compatibility with technologies such as MRI will ease its adoption in humans. Paluch-Siegler et al. present all-optical bidirectional control using a hybrid of multiphoton holographic stimulation and imaging. Shen, Lai, and Campbell report on red fluorescent protein based biosensors for imaging applications. The available approaches for combined optical drive and electrophysiological recordings are reviewed by Dufour and De Koninck and the history and state-of-the-art in optogenetic microdevices by Kale et al.

Beyond the development of novel methods, a prerequisite in the adoption of optogenetics in humans, and the utility of these methods for a major area of neuroscience, is testing it in nonhuman primates. Providing the necessary building blocks to translate optogenetics to use in human disease, Dai et al. present an improved toolbox for the use of optogenetics in nonhuman primates, and Gerits et al. report on the efficiency of adeno-associated viral vector transduction in monkey neocortex.

Collectively, this special issue reflects the fact that optogenetics has become a commonly used tool in neuroscience, providing the means to identify the neural substrates driving specific functions and complementing the parallel tremendous achievements made over the past two decades in recording the activity of large-scale neural populations.

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Christopher Moore is an associate professor of neuroscience and a member of the Brown Institute for Brain Sciences. His work focuses on understanding neural dynamics and how they contribute to in vivo brain function. He has worked extensively with optogenetic tools, including advancing them through contributions such as development opto-fMRI (with Itamar Kahn), optimal implanted drives for combined optical stimulation and electrophysiology (the flexDrive), and more recent work on bioluminescent optogenetics (BL-OG).

Itamar Kahn is an assistant professor of neuroscience in the Rappaport Faculty of Medicine at Technion—Israel Institute of Technology. His research focuses on understanding the basic principles of communication between distal brain systems. He has worked on methods for awake mouse imaging, including the combination optogenetic control with fMRI in mice (with Christopher Moore) and more recent work on fMRI of awake behaving mice in intact animals and genetic models of developmental disorders.