Optogenetics
Illuminating the brain

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ABSTRACT
The nature of the brain presents many challenges to its study, from the intricacy of its structure to the minute timescale at which it functions. Traditional research techniques, such as electrophysiological manipulation and pharmacologic intervention, are limited by their inability to operate with both high temporal and spatial resolution. Optogenetics is a novel technology that provides unparalleled specificity in this regard. It allows for control of neural activity with high temporal-spatial resolution in a manner that does not disrupt the normal physiology of the system. It is an elegant research tool that uses light to control the electrical activity of genetically defined neuron populations with millisecond precision in systems as complex as freely moving live animals. First demonstrated in 2005, it was identified by Nature as the Scientific Method of the Year in 2010 and is currently used by thousands of labs across the world. It has already yielded new discoveries in a variety of neuroscience subfields and will undoubtedly continue to do so. The technology currently exists in a basic science capacity, but has potential for therapeutic application. It is not without its own limitations, but has advantages over more crude alternatives and has proven to be a powerful tool in the hand of the neuroscientist.

INTRODUCTION
The human brain is the most intricate organ in the body and has been referred to as “the most complex object in the known universe.”1 A recent estimate put the number of neurons in the human brain at 86 billion2 with each neuron forming hundreds, if not thousands, of individual connections through which they communicate on the millisecond scale. The complexity of its structure and function is astounding. For neuroscientists, this makes the brain both irresistibly fascinating and prohibitively difficult to study. In order to overcome the challenges that result from the inherent complexity of the brain, investigators must be creative and innovative in their approach to its examination. The recent development of a research tool known as optogenetics provides an ideal example of such innovation.

BACKGROUND
Traditional methods of study, such as with electrophysiological devices or pharmacologic intervention, have provided countless insights into how the brain operates. However, each technique has limitations. Direct electrical stimulation of tissue has high temporal resolution, but indiscriminately affects all cells around the microelectrode and thus has poor spatial resolution.3,4 Given that specifically targeted neurons are often sparsely embedded within tissue, this is a significant drawback. Pharmacologic intervention on the other hand has high spatial resolution, as drugs can be designed to act on only certain neuron populations, but because the drug can stay in the system for anywhere from minutes to hours such an approach lacks temporal resolution.4,5 Given the minute timescale at which the brain operates, this is also a significant hindrance.

The idea of using light as a medium to control the activity of neurons is not novel. It was speculated on by Francis Crick as far back as 1979;6 however it was only first demonstrated in 2005.7 Since then, the lab of Karl Deisseroth at Stanford University has published extensively on the topic8–17 and optogenetics has spread to thousands of labs across the world.18 It is an elegant technique that uses light to control the activity of genetically engineered neurons and has significant advantages over its more crude alternatives. Its significance has been recognized throughout the scientific world, notably with Nature naming it Scientific Method of the Year in 2010 and its early developers being honored as recipients of The Brain Prize by the Grete Lundbeck European Brain Research Foundation in 2013.

DETAILS
Optogenetics, quite literally, refers to the convergence of optics and genetics. When applied to neuroscience, it is a technology that uses light to control the electrical activity of neurons with both high temporal and spatial resolution in a manner that minimally disrupts the normal physiology of the system. Thus, it overcomes some of the limitations associated with traditional research techniques as described above.

In order to achieve this, single-component, microbial-derived, light-activated ion channel proteins called opsins are artificially expressed in the membranes of neurons. This is predominantly accomplished through the use of viral vectors carrying specially designed genetic constructs, but can also be achieved using transgenic animals.18 Regional specificity when using viral vectors can be ensured via the use of specific promoters, by localized viral injection, and by restriction of opsin activity via targeted light delivery.19 The family of proteins in play, opsins, was first discovered as a component of bacterial cell membranes over 40 years ago.19 Since then, further research has enlarged the family and proteins have even been altered in the laboratory to be more suitable for optogenetics research purposes.20 There currently exist dozens of opsin types with unique characteristics, thus allowing for a variety of experimental configurations.21

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The genetic engineering techniques employed ensure that the opsins are expressed exclusively in genetically defined target population(s) of neurons and thus provide spatial resolution to the cell-type level. Light is delivered via surgically implanted fiber optics24 or other means. Upon exposure, the opsins alter their conformation such that the flow of ions in or out of the neuron of which they are a part is altered.22 Depending on both the type of opsin involved and the wavelength of light used, the target cell(s) are either depolarized (excited) or hyperpolarized (inhibited).22 This use of light as the metaphorical on/off switch for neuron activity provides the high temporal resolution. The light source can be pulsed with enough speed and precision so as to reliably elicit single spikes in neuron electrical activity. Furthermore, light is an ideal medium to use for this purpose as it does not affect unaltered neurons (neurons without opsins) and thus minimally disrupts normal brain physiology.

RECENT RESEARCH
Before discussing relevant current research, it is important to note that optogenetics currently plays a role in basic science research exclusively. That is, its goal is not directly therapeutic, but to give neuroscientists the ability to more closely examine the brain and thus better understand how it functions. It is used in models of both health and disease with the hope that the knowledge gained will lead to the development of future therapies.

Optogenetics has been applied to nearly every area of neuroscience research18 and in models ranging from ex vivo cell lines to nonhuman primates. A sample of notable work using murine models includes controlling behavior in freely moving animals, increasing functional recovery after stroke, mapping of Parkinsonian neural circuitry, inhibiting symptoms in a model of Parkinson’s, eliminating cocaine seeking behavior after addiction, and inducing REM sleep. Last year researchers used optogenetics to control in vivo transcription of endogenous genes in the brain, as opposed to electrical neural activity. These represent some of the most dramatic recent advances in optogenetics, but there are countless examples of labs applying this technology to less complex in vitro systems. In vitro application of optogenetics, such as in vitro slice electrophysiology, benefits from the same principles that allow for exciting research in in vivo models, but is significantly less expensive (a few thousand dollars will provide for a good start).

FUTURE DIRECTIONS
Although optogenetics has come a long way since its debut a decade ago, it remains in its infancy. There is enormous ongoing research into its refinement and further development with exciting papers being published almost monthly. One intriguing topic is the development of optical systems capable of the simultaneous control and recording of neural activity. This is not possible to perform with electrophysiological devices and is just beginning to be performed using optogenetic systems. It would provide unprecedented information about the organization and function of intact neural networks.

The use of optogenetics as therapy for human disease is a natural direction to look in when considering the future of this technology. This, for reasons discussed below, is a distant, but not impossible, prospect. Based on current research, it could be applied in various forms to assist in treating disease states including Parkinson’s, addiction, stroke recovery, epilepsy and a variety of neuropsychiatric diseases.

LIMITATIONS
As with any new technology, the limitations of optogenetics are slowly appearing. Relating to basic science use, the pattern of ion concentration changes induced by opsins does not perfectly replicate the actions potentials observed in normal physiology. The conductance tends to be smaller and the magnitude of the ion concentration changes risks exceeding physiologic ranges. Exposure of multiple opsin-containing neurons to light means their resulting activity is synchronized and, given the precise timing of neural networks, potentially results in nonphysiologic patterns of activity. Furthermore, although light has desirable properties the heat created by its use poses a significant risk of damaging delicate tissue and is something researchers must be cognizant of. These limitations, among many others, threaten the validity of conclusions drawn solely from the interpretation of optogenetic results.

Besides the onerous task of compiling the knowledge of neural function and dysfunction required to even hypothesize about the use of optogenetics in the treatment of human disease, a major limitation to this application is the dependence on genetic manipulation. Proving the long-term safety of gene therapy in humans to the satisfaction of regulatory bodies is an immense undertaking. Combined with additional objectives, such as refining the methods of light delivery in intact living tissue and demonstrating therapeutic effects beyond the scope of other methods, an enormous deal of work needs to be completed before therapeutic application of optogenetics can be considered.

SUMMARY
Optogenetics is a novel and sophisticated research tool. Through the convergence of genetics, optics, and neurobiology it allows for the control of neural activity with unparalleled temporal resolution in systems as complex as freely moving live animals. It is an elegant technique with significant advantages over more crude alternatives and has provided us with new insights into how the brain functions. With continued application and refinement it will continue to do so. It is currently applied only in terms of basic science research, but has potential for a therapeutic role in a variety of diseases.

Given its intriguing core principle (controlling the brain with light), optogenetics is a topic prone to sensationalization and one needs to keep in mind that significant limitations exist at both the basic science and potential therapeutic levels.

In closing, I paraphrase the cautious champion of optogenetics, Karl Deisseroth, who said that optogenetics, although exciting, should be viewed as only one tool among the many available to a neuroscientist. Nevertheless, considering the task at hand, it is one that I would want in my kit.
REFERENCES


