Shining a light on optogenetics

Advances in electronics and protein engineering advance the field of optogenetics to study the activity of neuronal networks

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Optogenetics is relatively new and has attracted much interest since the first publications from the field in the early 2000s. Both researchers and the media are fascinated by the notions it conjures of bionic implants. Notwithstanding potential applications, the technology has quickly become an important research tool in neuroscience with potential for developing a range of novel therapeutic approaches. Scientifically, optogenetics combines advances in optics, small-scale power generation, electronics, the manipulation of cellular activity and gene therapy, to enable the study of complete systems, including neural networks in animals and humans. Moreover, some recent studies have highlighted potential applications in plants as an alternative to genetic modification (GM) for targeted alteration of phenotypes.

Since the first experiments [1], optogenetics has consistently expanded in scope and definition. It can now be defined as the use of light to stimulate cells into which exogenous genes coding for light-sensitive proteins have been introduced, and to measure the response of these transformed cells to stimulation. Typically, the light is generated by an artificial source powered by a battery or external electric field. The definition also embraces treatments for some forms of blindness based on implanting light-sensitive proteins in the retina.

However, much of the fields' focus revolves around the optical components, their implantation in the body, and the interface with the target cells to stimulate gene expression with high spatial and temporal resolution. In practice, an implanted LED or other light source

illuminates a specific target area. Within this area, the target cells—genetically modified to express specific light-sensitive proteins—react to this light by changing their membrane potential, which in turn starts signal cascades that activate or shut down specific target genes. The key biological challenges of optogenetics are the development of appropriate light-sensitive proteins, mechanisms for delivering their genes to specific cells, and feedback systems for observing the resulting changes in behavior at the level of cells, tissues, organs, systems, or the whole organism. The engineering developments in turn have focused on reducing the size of the light sources and wireless power delivery, so the whole package can be implanted with the least possible disruption to the host, avoiding the need for external wires or headsets.

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Optogenetics mostly works with cells that are capable of generating electric currents in the form of ion flows in response to light. For this reason, it has been largely confined to the study of neurons, but has potential for similar excitable cell types, which include muscle cells or touch-sensitive cells. These cells can change their internal charge from negative to positive to generate transient electrical signals over the membrane, so-called spiking, which is the mechanism of

neuronal communication in the brain. Conversely, the reverse process of membrane hyperpolarization in which the cell's electrical potential across its membrane becomes more negative inhibits spiking. In neurons and muscle cells, the generation of an action potential involves first depolarization to trigger a spike and then hyperpolarization to inhibit it. Optogenetics manipulates this process by interacting with ion channel proteins in cell membranes that mediate electrical potential in the cytoplasm. Typically, these light-sensitive ion channels are opsins, which provide light sensitivity to a wide range of organisms from single-celled algae to humans, and which are also involved in molecular clocks and circadian rhythms.

While therapies derived from optogenetics are still some years away, the technology has already had a dramatic impact on fundamental research in neuroscience. A recent review of the subject argues that optogenetics is kick-starting progress and rescuing applied brain research from the doldrums it had slipped into toward the end of the last century [2]. According to the review's co-author, Thomas Knopfel, Chair of Optogenetics and Circuit Neurosciences at Imperial College in London, UK, brain drug discovery stalled after a few early successes in the mid-20th century because it was bogged down by the idea of “fixing brain chemistry” by targeting single molecules. One trouble with this approach, Knopfel explained, is that the human brain uses the same molecular building blocks for numerous functions and thereby gains much of its power through the combinatorial use of molecular mechanisms. A drug targeting a given molecule is

therefore unlikely to have a specific action against a single condition.

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An alternative approach is to target the whole phenotype relating to a condition without bothering with the underlying molecular mechanisms, but this requires suitable animal models. The problem is that the difference between humans and animals is most profound in the brain, which makes it hard to find suitable animal models that are predictive of human neurological diseases, especially psychotic conditions. For these reasons, neurological research has shifted its focus to the neuronal circuits and pathways that control particular components of behavior. The initial aim of this shift in focus has been to investigate neural mechanisms with molecular and cellular accuracy, but at the level of complete systems and behavior.

Optogenetics provides an ideal methodological platform for these circuit-centric approaches, Knopfel argued, because it is capable of both eliciting and recording neural activity in a relatively non-disruptive way. It can thus provide a conceptual and methodological bridge between molecular and cellular activities—which it can measure with high precision—and the higher system-level aspects of behavior and cognition. This is a bold statement, but one which Knopfel insisted is now being justified. “For sure there is already high impact on discovery and preclinical research and there will be much more”, he said.

One objective is to identify and engineer new opsins with desirable characteristics in order to fine-tune the response of the target cells to light, according to Ed Boyden, Leader of the Synthetic Neurobiology Group at MIT in the USA, and one of the pioneers of optogenetics. “Recently, a number of groups have mutated key amino acids within channel-rhodopsin-2 [ChR2], creating versions that

stay open for long periods of time after a brief pulse of light, or that close quickly after the light pulse ends”, he said. “Other versions have been created that do not inactivate as quickly, or that are more light sensitive when expressed in neurons”. Channelrhodopsins belong to the rhodopsin protein family of light-driven ion channels and sensory photoreceptors in organisms such as unicellular green algae. As such, they are ideal candidates for protein engineering to tailor their responses to light. One key goal is to engineer proteins that can either trigger or suppress activity in target cells in response to red light. It has the lowest frequency of the visible colors and is therefore both least damaging to tissue and penetrates deeper into it.

The other major focus is on the light-generating electronics. Even though experiments are currently confined to animals, it is still important to minimize the electronics package to allow the animals as much freedom of movement as possible to study behavior under relatively natural conditions. In the early days, light was applied to brain circuits mostly through optical fibers inserted directly into the head through implanted tubes. This restricted the freedom of movement of the animals, usually mice or rats, owing to the need for external cables. MIT’s Polina Anikeeva, who specializes in the development of hybrid materials and devices that act as interpreters between electronics and neural circuits, explained that this limitation was reduced through the use of internal LEDs powered by implanted batteries or wirelessly via head-mounted radiofrequency (RF) power receivers. Nevertheless, the size and weight of the hardware prohibited many experiments that would, for instance, require mice to enter confined spaces. In addition, the need for head-mounted components largely restricts the application to the brain and rules out studies of the spinal cord, peripheral nerves, and muscles. These tissues demand greater flexibility of the implanted optical interfaces, and the RF field has to penetrate deeper into the tissue to deliver uniform power.

Anikeeva pointed to two recent studies that made substantial progress using wireless power generation for the implanted LED. One, involving researchers from the USA and South Korea, combined thin and flexible neural interfaces with a fully implantable and stretchable wireless power and control system to modulate the spinal cord

and peripheral nervous system in target animals [3]. The other team, at Stanford University in the USA, developed small implantable coils about 2 mm across and weighing 20 mg, which the authors claimed was two orders of magnitude smaller than any previously reported wireless optogenetic system [4].

These advances should allow an entire device to be implanted subcutaneously anywhere in the nervous system, including brain and spinal cord. So far, the Stanford technology has been used in the brain of mice to stimulate the motor cortex by expressing ChR2 via blue light in excitatory neurons. The team also stimulated peripheral nerves by implanting the device under the skin of hind limbs, triggering unconditioned aversion through activation of unmyelinated nociceptors in the sciatic nerve virally transfected with ChR2, which shows the potential of optogenetics for manipulating the pain response.

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According to Anikeeva, the Stanford work has yielded a device for which the fabrication of the implantable receiver-LED assemblies is straightforward, but for which the RF field to generate the power must be applied directly from close proximity to the animal, thus restricting freedom of movement. In contrast, the USA/South Korea device made use of RF transmitters driven by off-the-shelf power supplies that can be located up to 20 cm away, allowing the animal more space and enabling it to perform a wider variety of tasks. In principle, this method could be extended to allow complete freedom of movement, but at the cost of requiring highly specialized fabrication methods to produce the flexible antenna in the implant.

Anikeeva concluded that both groups had made substantial technical progress, but that more work is needed. She added that the foreign body response remains a major challenge for implantable neuromodulators. All biological tissue exerts adverse responses to

most materials identified by the immune system as non-self, depending on their size and reactivity. This is known as the foreign body response and can be minimized by making the implants as small and similar to surrounding tissue as possible. One team attempted to address this issue through extreme miniaturization and the other by matching the mechanical properties of the device to the target tissue.

Another study conducted primarily at the ETH Zurich in Switzerland made further progress in reducing the foreign body effect by encasing the whole implant in inert plastic [5]. “The embedded electronic module simplifies the sterilization, as no electrode gets in contact with the patient”, said Marc Folcher, lead author of the study. This study was more notable, though, for demonstrating a further step in optogenetics by involving the human brain to control light signals and the expression of transgenes in mice. In this clever experiment, human volunteers wore headsets monitoring their EEG (electroencephalography) activity, which was correlated with different brain states such as relaxation, concentration, and biofeedback. This information was translated via a computer to electrical fields, which powered up an implanted LED in the mice. It emitted near-infrared light, causing transgenic human stem cells in the mice to express the protein secreted alkaline phosphatase (SEAP), a so-called reporter protein used to study gene expression. By altering their brain state, the human volunteers could vary the expression of SEAP in the mice.

Though this approach is a long way from clinical application, Folcher said that the study represents a significant step forward in proof-of-concept terms and technical progress. “It laid a proof of concept that optogenetics can play a key role as an interface to control a cell-based drug delivery device”, he said.

Yet, as Boyden noted, even if the implanted package is inert, it still leaves the fundamental need for opsins from other organisms for light-controlled gene expression. “The biggest problem is that these genes must be delivered by gene therapy”, he said. “Most of the genes come from non-human organisms, meaning that their gene products must be tolerated well by the immune system and the body, to be successful as a therapy”.

This issue has not prevented the clinical application of optogenetics to treat some categories of blindness. Boyden pointed to a gene therapy called RST-001, developed by US biotech firm RetroSense Therapeutics, which received FDA clearance in August 2015 for Phase I/II testing to treat patients with retinitis pigmentosa (RP), a genetic condition resulting in progressive degeneration of both rod and cone photoreceptors. An injection into the eye delivers a gene encoding the ChR2 protein to cells in the retina. Because it is a photoswitch, ChR2 can be depolarized by light, which in this case is admitted by the pupil, rather than an implanted LED. The signal generated in response is then transmitted to the brain. This restores some light sensitivity to the eye and, according to RetroSense, could restore some degree of vision for patients for whom there is no effective treatment at present.

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This is only one application, however, and one for which there are no real alternatives at present. But there are many more potential therapeutic applications of optogenetics in which it will compete with existing or other emerging technologies. Targeted drug delivery is one example, where optogenetics will compete with conventional drug delivery by injection or mouth. “Moving a therapeutic innovation to the clinic can be pushed only if the device brings a real patient health or lifestyle benefit. At this point, the priority is to secure the necessary funding to complete the development. We could then move forward and show that for certain disease areas, implants offer a valuable alternative to regular injections of biopharmaceutical drugs”, Folcher said.

Folcher added that fundamental research is still needed to connect feedback from sensors with controlling expression, which would usher in a host of potential novel applications. “We are currently designing new communication paths between cells and machines, but we are building the bridge from the biology

side”, he said. “The most challenging step that is still ahead of us is to develop feedback signals emitted by the cell that could be integrated with a telemetric function by the implanted electronic circuit. As an example, wirelessly powered light might activate transcription and secretion of the desired therapeutic protein in the implanted cells and then, once activated, the cell would send a signal back that ‘the job is done’”.

While such biological/electric feedback mechanisms are still some way off, controlled drug delivery could be closer to clinical applications. Significant progress has been made in miniaturizing microfluidic devices to deliver drugs internally in response to light signals. Until now, metal cannulas similar in dimension to optical fibers have typically been used for targeted drug delivery from supplies outside the body. But a study at the University of Illinois at Urbana-Champaign in the USA demonstrated a much smaller optofluidic device one-fifth the thickness of a human hair—about 20 μm —that can be connected to LED arrays and store the drug internally without tethering to external supplies [6]. The authors demonstrated the use of these devices to modify gene expression and manipulate reward-related behavior in animals.

Although much focus has been on neurons, there has been some work on other cell groups, such as cardiac myocytes. A study at Stony Brook University in the USA delivered light-sensitive ChR2 proteins to myocyte cells via viral vectors and demonstrated optical actuation of the cells by inducing activity in the ChR2 proteins and measuring the response [7]. This has some potential for treating irregularities in heart rhythm if optogenetic techniques could demonstrate advantages over conventional pacemakers for example.

Optogenetics also has potential in plants for controlling cellular functions with light, though there is some gap between speculated applications and the actual work done thus far. As Ove Nilsson, Director of the Umea Plant Science Centre at the Swedish University of Agricultural Sciences (SLU), pointed out, there is great excitement about potential applications like turning plants into batteries or fuel cells and developing interfaces between electronics and living cells for electronically

controlled pumps and sensors for small molecules. “These could be coupled to small transmitting devices connected to the Internet, so that your window plants for example might send you a text message when they need water or are attacked by aphids”. However, as Nilsson noted, the real work at present is more at the research stage for sensing and manipulating small molecules in plants via optogenetically generated signals. One avenue would be to develop alternatives to GM plants for applications such as improving yield or drought tolerance. “This is rather speculative and I cannot think of any obvious direct applications at the moment”, Nilsson said. “The line of reasoning goes that this type of ‘electronic modification’ of plant properties would be more acceptable since it does not interfere with the genetic material and will not be transmitted to the offspring”.

At present, most optogenetics research remains focused on animal models, both for studying the link between neural networks and behaviors and for evaluating potential therapies and targeted drug delivery. As a new research tool though, optogenetics is well established and has vast potential to yield new insights, particularly in neurobiology.

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