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Optogenetics: Lightly Pressing More and More of the Cell's Buttons

The engineering of light sensitivity into specific parts of selected cells enables the dissection of signaling pathways.

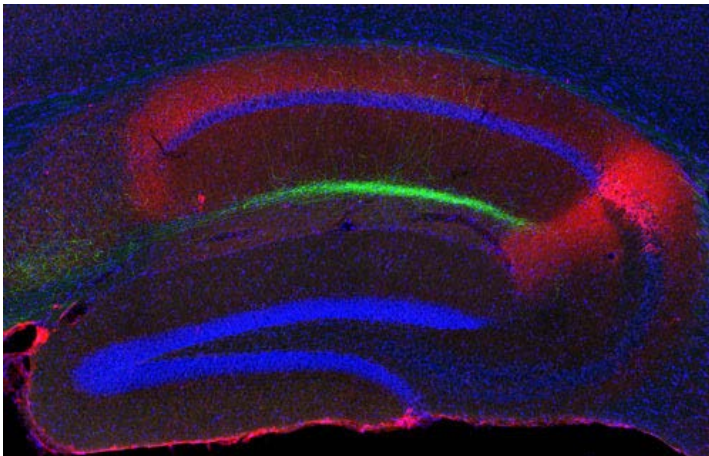
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MIT researchers using optogenetics to trace the neural connections involved in memory formation. Here, a cross-section of a mouse's hippocampus show s island cells (green) projecting to the CA1 region of the hippocampus. [Takashi Kitamura]

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"You can observe a lot just by watching," said the accidental sage, leaving us to wonder whether he

meant to celebrate the power or lament the futility of simply paying attention. Were he scientifically inclined, he might have added that mere observation is unsatisfying because many things can be learned only through experimentation—perturbing things, then seeing what happens next. So it is with cells: Once roused or stifled, cells may reveal much that would otherwise remain hidden.

The idea is to enact “what if” scenarios instead of simply watching and waiting. The trick, however, is to perturb cells without being too heavy-handed, always a risk when cells are electrically jolted or chemically lesioned. The lightest touch, it turns out, may be an actual “light touch”—the use of illumination to alter the behaviors of selected cells. This is the essence of [optogenetics](#).

Optogenetics relies on proteins that alter their functions when they are exposed to light. These proteins, which are expressed naturally by various organisms, may be introduced to targeted cells within living organisms by means of genetic engineering. Then, by supplying illumination of the appropriate wavelength to sensitized cells, the researcher may incite or suppress selected biological processes.

It is possible to perturb the cell's biological processes at several levels. To date, most optogenetic studies have deployed light-sensitive ion channels or pumps, mostly in [neurons](#). By switching specific populations of neurons on and off, scientists gain the ability to disentangle neural circuits and decode the brain's electrical signaling. Membrane-bound, light-sensitive proteins have also been used to explore the cell's internal signaling, the biochemical cascades triggered, for example, by G-protein coupled receptors.

With optogenetics, it is also possible to “push buttons” beyond the cell membrane. Instead of using optogenetics to open or close an ion channel, researchers have created an optogenetic system that controls customized transcription machinery. This system relies on a light-sensitive protein that has been fused to a DNA-binding protein, a transcription activator-like effector (TALE), which may be customized to bind to selected regions of the genome. When illuminated, the protein-TALE complex is activated, and transcription commences. Thus, the light-sensitive protein and the TALE, used together, determine not only where, but when, transcription occurs. A similar system, one that would use CRISPR instead of TALE technology, has been proposed.

Used at yet another level, optogenetics may activate or inactivate individual proteins that have been engineered to carry a bulky, artificial, light-sensitive amino acid. This amino acid, upon illumination, sheds a side chain to become cysteine, and the protein of which it is a part alters its shape and gains activity.

One such protein was incorporated into an ion channel. The protein, at the flick of a light switch, jettisoned its artificial amino acid's extra bulk, freeing the ion channel of an obstruction. Although this protein was used to optically open an ion channel, similar proteins could be devised to optically regulate protein modifications and protein-protein interactions.

Switching Neurons On and Off

Optogenetics got its start in the 2000s, when neuroscientists began realizing a plan that had been outlined decades earlier by Francis Crick. Noting that electrodes could not distinguish between different cell types, Crick proposed in 1979 that what neuroscientists needed was a method that could activate or deactivate “all neurons of just one type ... leaving the others more or less unaltered.” With such a method, neuroscientists could avoid the problem of activating conflicting neural pathways. Neuroscientists could finally dissect neural circuitry.

Subsequently, Crick even suggested that light could serve as a control tool. At the time, however, various light-sensitive proteins were just beginning to be characterized. In the early 1970s, researchers identified bacteriorhodopsin as a light-activated ion pump and the first of what was to become an expanding palette of so-called microbial opsins. Bacteriorhodopsin was soon joined by halorhodopsin and channelrhodopsin. In addition, engineered variants of these molecules eventually appeared.

The advantage of microbial opsins is that they are single-component systems—they combine light

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sensitivity and effector function in a single protein. Although the protein requires a cofactor, retinal, this cofactor naturally occurs in animal tissues. In fact, in all animal tissues tested to date, retinal is present in such quantities that no retinal supplementation is needed. And so, a microbial opsin optogenetic system need deploy only a microbial opsin gene and a suitable promoter. (Multicomponent systems, in contrast, deploy not microbial opsin genes but rather combinations of different genes that are sometimes accompanied by custom-synthesized chemicals.)

A lucky break, to be sure. But luck has had little to do with the development of optogenetics. Without its enabling technologies, optogenetics would have remained little more than an intriguing notion. These technologies encompass microbial opsins; gene constructs (including promoters, viral vectors, and transgene lines); and light-delivery and signal-detection tools. Thanks to diligent effort, microbial opsins have been engineered to provide desirable tradeoffs with respect to light sensitivity and expression level as well as the timing and magnitude of physiological effect. Gene constructs are available that permit the targeting of specific neuronal cell types. And optrodes, implantable devices that are capable of both optical neuromodulation (via fiber optics and laser diodes) and electrical recording (via electrodes), have been developed that hardly impede the self-directed movements of live animals.

Dissecting Neural Circuits

Optogenetics may be used for the temporally and spatially precise activation or deactivation of specific kinds of neurons. But that's hardly the point. What's really interesting is seeing how neuronal signaling propagates from neuron to neuron, or through clusters of neurons. Such signaling is optogenetically initiated when an ion channel or pump, stimulated by light, shuttles ions across a membrane, setting up an action potential, an electrical signal that triggers the release of neurotransmitters. It is also possible to target neuromodulatory neurons, which release dopamine or serotonin.

Signals that propagate through a neural circuit may be traced by means of electrode recording, calcium imaging, and other neural activity readout methodologies. Neural activity may also be correlated with functional magnetic resonance imaging. Of particular interest, to judge by the volume of news coverage, are the connections between specific kinds of neural stimulation and changes in the behaviors of lab animals.

Here is a sampling of recently announced optogenetic behavioral studies in animals:

- Researchers at the University of North Carolina School of Medicine asserted that they had pinpointed the precise cellular connections responsible for overeating. By optogenetically stimulating gaba neurons in the bed nucleus of the stria terminalis (BNST), the researchers caused well-fed mice to eat voraciously. Shutting down the BNST pathway caused the mice to show little interest in eating, even if they had been deprived food.
- A team of scientists based at the Douglas Mental Health University Institute reported that they had identified a precise causal link between neuronal activity in the lateral hypothalamus and the state of REM sleep.
- Rather than focus on principal neurons, which are excitatory, researchers at Cold Spring Harbor Laboratory widened their view to take in interneurons, which are inhibitory. This approach helped the researchers discover that certain inhibitory neurons—that is, neurons expressing vasoactive intestinal polypeptide (VIP)—specialize in inhibiting other inhibitory neurons in multiple regions of cortex, and does so under specific behavioral conditions. For example, in mice engaged in a learning exercise, discriminating between sounds of different pitches, VIP neurons in the auditory cortex were activated by rewards and punishments. In fact, these neurons appeared to mediate the impact of reinforcements, “turning up the lights” on principle cells, to use a dimmer-switch analogy.
- INSERM-based scientists were able to demonstrate that fear expression is related to the inhibition of highly specific interneurons—the parvalbumin-expressing prefrontal interneurons. More specifically, inhibition of their activity disinhibits the activity of the prefrontal projection neurons, and synchronizes their action.
- At Wake Forest Baptist Medical Center, researchers selectively controlled a specific population of

dopamine cells in the ventral tegmental area that serves as part of the brain-reward system. Using this technique, the researchers discovered distinct pattern of dopamine cell activation that seemed to be able to disrupt alcohol-drinking behavior.

- Scientists at the California Institute of Technology found a neural circuit that connects the lateral septum (LS) with other brain structures in a manner that directly influences anxiety. Even brief, transient activation of neurons in the LS could produce a state of anxiety lasting for at least half an hour. That activating LS neurons, which are inhibitory neurons, should heighten anxiety, not diminish it, puzzled the researchers, who hypothesized that a double-inhibitory mechanism might be involved. Further investigation revealed that LS neurons were inhibiting neurons in the hypothalamus. Importantly, most of those hypothalamic neurons were themselves inhibitory. Moreover, those hypothalamic inhibitory neurons, in turn, connected with a third brain structure called the paraventricular nucleus, which is well known to control the release of hormones like cortisol in response to stress and has been implicated in anxiety.
- At the Okinawa Institute of Science and Technology, scientists found that inactivating neurons in the nucleus accumbens shell enhanced behavioral flexibility. Specifically, lab rats in which the neurons were turned off were able to adapt more quickly to a shift in reward or error feedback during a lever-pressing exercise. This finding, the scientists suggested, shows that the nucleus accumbens shell neurons somehow integrate a history of reward, which may be consulted during decision making.
- A team of investigators at Stanford University reported that stimulating a particular circuit in the ventral tegmental area enhances the drive to engage in social behavior. Inhibiting the circuit reduces it. Such manipulations, however, did not seem to affect the exploration of novel objects or even the propensity to move around.

Typically, studies that identify the neuronal circuits behind animal behaviors speculate about human conditions. For example, the studies listed above suggested that dysfunctional neuronal circuits in humans could help explain overeating, sleep disorders, anxiety or post-traumatic stress disorder, and alcoholism.

Mapping the Brain

The litany of circuit/behavior pairings may put you in mind of gene/disease pairings—the propensity of news organizations to run “gene for this, gene for that” stories. If so, you may also wonder if optogenetics is still accumulating disparate findings, or whether it is already trying to integrate these findings into a larger framework. After all, isolated genetic findings can be related to a grander, genomic vision. Could something of the like be accomplished for neuronal circuits?

As a matter of fact, projects roughly analogous to the Human Genome Project are underway. Instead of mapping the genome, however, these projects are mapping the connectome. Some projects are surveying neural connections; others, spatiotemporal patterns of gene expression. Examples of such projects in the United States include the Allen Institute’s brain connectivity atlases, the Human Connectome Project, and Eye Wire.

Still in its formative stages is the BRAIN Initiative (Brain Research through Advancing Innovative Neurotechnologies), or Brain Activity Map Project, which has announced that it intends to map the activity of every neuron in the human brain. Research supported by the BRAIN initiative includes the development of CLARITY, a technique that essentially renders the brain transparent, allowing investigators to stain and image different molecules and cell types that would otherwise remain hidden.

CLARITY, introduced by optogenetics pioneer Karl Deisseroth of Stanford, removes the fatty outer covering of the brain’s nerve cells while keeping the rest of the brain intact. “Using CLARITY with techniques such as optogenetics, or using it to analyze brains after behavioral studies, will be a powerful way to extract information about how brain networks function,” Eve Marder, a neuroscientist at Brandeis University in Waltham, MA, told Nature.

Developing Better Optogenetic Tools

To shift back to the fine-grained scale individual neural circuitry, it should be pointed out that several research groups continue to refine their optogenetic techniques. For example, Karl Deisseroth and colleagues recently published an article in *Science* announcing that they had addressed a persistent shortcoming: Microbial opsins are potent at switching cells on, but less effective at turning them off. The researchers, however, indicated that they had re-engineered light-sensitive proteins to switch off more efficiently.

"This latest discovery by the Deisseroth team is the kind of neurotechnology envisioned by President Obama when he launched the BRAIN Initiative," said Thomas Insel, M.D., director of the National Institute of Mental Health, which funded the study. "It creates a powerful tool that allows neuroscientist to apply a brake in any specific circuit with millisecond precision, beyond the power of any existing technology."

Even more recently, at MIT, researchers led by another optogenetics pioneer, Ed Boyden, reported that they had developed the first light-sensitive molecule that enables neurons to be silenced noninvasively, using a light source outside the skull. This makes it possible to do long-term studies without an implanted light source. The protein, known as Jaws, also allows a larger volume of tissue to be influenced at once—a capability that could enhance the study of larger animals.

Jaws, which is sensitive to penetrating red light, points to noninvasive optogenetic interventions. That is, optogenetics could conceivably move from research to clinical use. For example, optogenetic treatments could be devised for diseases such as epilepsy, should it prove practical to shut off the misfiring neurons that cause seizures.

Beyond the Neuron

Whereas the various microbial rhodopsins are useful in neurological research, another class of optogenetically sensitized membrane channel called optoXR has potential for cancer research. The microbial rhodopsins, or type I opsins, can shuttle charged ions into and out of cells, setting up action potentials. OptoXRs, however, may serve as G-protein coupled receptors (GPCRs).

OptoXRs, which are derived from vertebrate (or type II) opsins, are chimeric receptors that may be fashioned by replacing the cytoplasmic loops of a vertebrate rhodopsin with those of specific adrenergic receptors, taking advantage of common structure-function relationships amongst GPCRs. Then, light activation may be used to modulate intracellular signaling with precision in both space and time.

Such modulation, in the hands of scientists at IST Austria and Vienna Medical University, was recently used to remotely control cancer cells. According to these scientists, light activation of the receptor tyrosine kinases (RTKs) that they had engineered led to receptor dimerization, which in turn activated signaling within the cell. Light-activated dimerization in cancer cells caused changes in cell morphology, proliferation, and gene expression characteristic of increased malignancy. In blood cells, activation led to cell sprouting typical of the formation of new blood vessels.

The researchers assert that theirs is the first application of optogenetics in cancer research. They speculate that optogenetics could be used to identify new drugs or even maintain control over [cell signaling](#), rescuing cell function in degenerative disease.

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