NEUROSCIENCE

THE NEW CENTURY OF THE BRAIN

Big science lights the way to an understanding of how the world's most complex machine gives rise to our thoughts and emotions

By Rafael Yuste and George M. Church

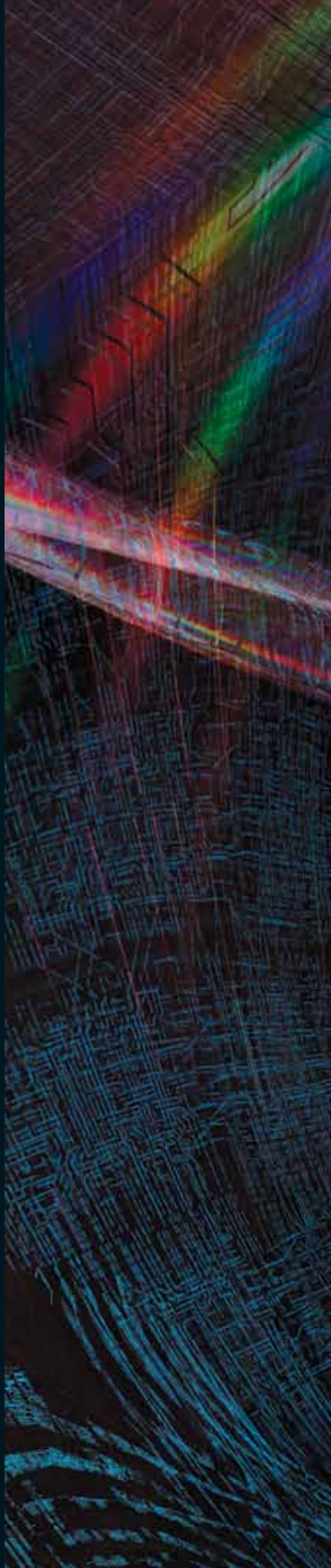
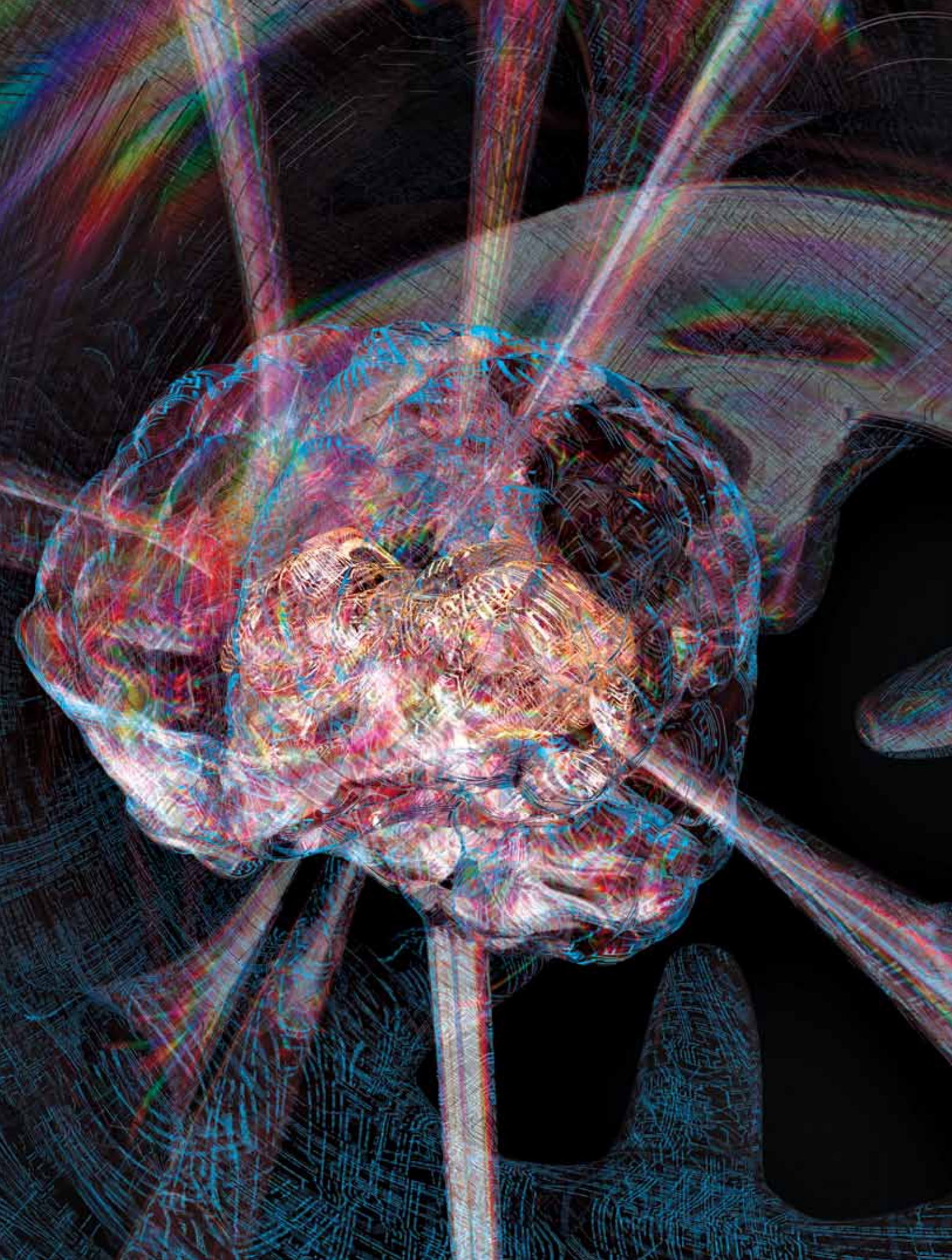


Illustration by Bryan Christie



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DESPITE A CENTURY OF SUSTAINED RESEARCH, BRAIN SCIENTISTS REMAIN IGNORANT of the workings of the three-pound organ that is the seat of all conscious human activity. Many have tried to attack this problem by examining the nervous systems of simpler organisms. In fact, almost 30 years have passed since investigators mapped the connections among each of the 302 nerve cells in the roundworm *Caenorhabditis elegans*. Yet the worm-wiring diagram did not yield an understanding of how these connections give rise to even rudimentary behaviors such as feeding and sex. What was missing were data relating the activity of neurons to specific behaviors.

The difficulty in establishing a link between biology and behavior in humans is still more acute. The media routinely report on scans showing that specific brain locations light up when we feel rejected or speak a foreign language. These news stories may give the impression that current technology provides fundamental insights into how the brain works, but that impression is deceiving.

A noteworthy example of the mismatch is a much publicized study identifying single brain cells that fired an electrical impulse in response to the face of actor Jennifer Aniston. Despite the hoopla, the discovery of a "Jennifer Aniston neuron" was something like a message from aliens, a sign of intelligent life in the universe but without any indication about the meaning of the transmission. We are still completely ignorant of how the pulsing electrical activity of that neuron influences our ability to recognize Aniston's face and then relate it to a clip from the television show *Friends*. For the brain to recognize the star, it probably has to activate a large ensemble of neurons, all communicating using a neural code that we have yet to decipher.

The Jennifer Aniston neuron also exemplifies the crossroads neuroscience has reached. We already have techniques to record the activity of single neurons in living humans. But to advance meaningfully, the field needs a new set of technologies that will enable investigators to monitor and also alter the electrical activity of thousands or even millions of neurons—techniques capable of deciphering what the pioneering Spanish neuroanatomist Santiago Ramón y Cajal called "the impenetrable jungles where many investigators have lost themselves."

Such breakthrough methods could, in principle, begin to bridge the gap between the firing of neurons and cognition: perception, emotion, decision making and, ultimately, consciousness itself. Deciphering the exact patterns of brain activity that underlie thinking and behavior will also provide critical insights into what happens when neural circuitry malfunctions in psychiatric and neurological disorders—schizophrenia, autism, Alzheimer's or Parkinson's.

Calls for a technological leap in studying the brain have started to be heard outside the laboratory. Indeed, the Obama administration announced last year that it was establishing a large-scale initiative: the Brain Research through Advancing Innovative Neurotechnologies Initiative, or simply the BRAIN Initiative, the most visible big science effort of the president's second term.

The BRAIN Initiative, with an initial funding level of more than $100 million in 2014, targets development of technologies to record signals from brain cells in much greater numbers and even from whole areas of the brain. BRAIN complements other large neuroscience projects outside the U.S. The Human Brain Project, funded by the European Union, is a 10-year, $1.6-billion effort to

IN BRIEF

The brain—and the way it gives rise to conscious thought—remains one of the great mysteries in all of science.

To better understand the brain, neuroscientists need new tools for analyzing the functioning of neural circuits.

Technologies that either record or control the activity of brain circuits may address these needs.

The Obama administration has a large-scale initiative under way to promote development of these technologies.

develop a computer simulation of the entire brain. Ambitious neuroscience research projects have also been launched in China, Japan and Israel. The global consensus that is now propelling investment in brain science recalls other postwar science and technology initiatives focused on pressing national priorities: nuclear power, atomic weaponry, space exploration, computers, alternative energy and genome sequencing. The Century of the Brain is now upon us.

THE TV SCREEN PROBLEM

TRACKING HOW BRAIN CELLS compute the concept of Jennifer Aniston—or anything comparable that we encounter through subjective experience or perceptions of the outside world—is currently an insurmountable obstacle. It requires moving from measuring one neuron to gaining an understanding of how a collection of these cells can engage in complex interactions that give rise to a larger integral whole—what scientists call an emergent property. The temperature or solidity of any material or the magnetic state of a metal, for instance, emerges only from the interactions of a multitude of molecules or atoms. Consider carbon atoms. The same atoms can bond to create either a diamond's durability or the softness of graphite, which exfoliates so easily it forms words on paper. Whether hard or soft, these emergent properties depend not on the individual atoms but on the set of interactions among them.

The brain, too, probably exhibits emergent properties that are wholly unintelligible from inspection of single neurons or even from a coarse, low-resolution picture of the activity of large groups of neurons. The perception of a flower or the retrieval of a childhood memory may be discerned only by observing the activity of brain circuits that pass electrical signals along intricate chains of hundreds or thousands of neurons. Although neuroscientists have long been familiar with these challenges, they still lack the tools to record the activity of the individual circuits that underlie a perception or a memory or that give rise to complex behaviors and cognitive functions.

What it takes to perceive a flower may only be discerned by observing the activity of brain circuits that pass electrical signals along chains of thousands of neurons.

One attempt to overcome this bottleneck involves assembling a map of the anatomical connections, or synapses, among neurons—an endeavor called connectomics. The recently launched Human Connectome Project in the U.S. will provide a structural wiring diagram of the brain. But, as with the roundworm, that map is only a starting point. By itself, it will be unable to document the constantly varying electrical signals that produce specific cognitive processes.

To make such a recording, we need wholly new methods of measuring electrical activity that go beyond existing technologies—which provide either a precise picture of the activity of relatively small groups of neurons or else sweeping imagery of large brain areas but without the resolution required to identify specific brain circuits switching on or off. Fine-scale recordings are made currently by inserting needlelike electrodes into the brains of laboratory animals to record the firing of a single neuron, the electrical impulse triggered after the cell receives chemical signals from other neurons. When a neuron is properly stimulated, the voltage across the cell's outer membrane reverses. This voltage shift induces membrane channels to usher in sodium or other positively charged ions. The inflow, in turn, produces an electrical "spike" that travels down the cell's long projection—the axon—spurring it to send a chemical signal of its own to other neurons and thus continue to propagate the signal. Recording from just one neuron is analogous to trying to follow the plot of a high-definition movie while viewing only a single pixel, making viewing all but impossible. It is also an invasive technique that can cause tissue damage when electrodes penetrate brain tissue.

At the other end of the spectrum, methods that track the collective activity of neurons across the whole brain are also inadequate. In the familiar electroencephalograph (EEG), invented by Hans Berger in the 1920s, electrodes sit on the skull and measure the combined electrical activity of more than 100,000 nerve cells underneath—the EEG records the oscillating "waves" of rising and falling amplitude over a few milliseconds, although it cannot resolve whether any individual neuron is active. Functional magnetic resonance imaging (fMRI)—producing the splotches of color illuminating active brain areas—records activity throughout the brain noninvasively but only slowly and with poor spatial resolution. Each image element, or voxel (a three-dimensional pixel), is a composite of about 80,000 neurons. Moreover, fMRI does not track neuronal activity directly but records only secondary changes in blood flow within voxels.

To gain a picture of emergent patterns of brain activity, investigators need new sensing devices that can record from assemblages of thousands of neurons. Nanotechnology, with novel materials that sometimes measure less than the dimensions of individual molecules, may assist in making large-scale recordings. Prototype arrays have been built that incorporate more than 100,000 electrodes on a silicon base; such devices could record the electrical activity of tens of thousands of neurons in the retina. Further engineering of this technology will allow stacking of these arrays into three-dimensional structures, shrinking the electrodes to avoid damage to tissue and lengthening shafts to penetrate deep within the cerebral cortex, the brain's outermost layer. These developments could make it possible to record tens of thousands of neurons in a human patient while discerning the electrical properties of each cell.

Electrodes are only one way to track the activity of neurons. Methods that move beyond electrical sensors are making their way into the lab. Biologists, borrowing from technologies developed by physicists, chemists and geneticists, are beginning

to visualize living neurons in awake animals going about their daily paces.

A hint of what might be in store came last year, when Misha Ahrens of the Howard Hughes Medical Institute's Janelia Farm Research Campus in Ashburn, Va., used a larval zebra fish to perform microscopic whole-brain imaging. The zebra fish is one of neurobiologists' favorite organisms because the species is transparent in its larval state, allowing for easy inspection of the fish's innards, including the brain. In the experiment, the neurons of the zebra fish were genetically engineered to fluoresce when calcium ions entered the cell after it fired. A novel type of microscope illuminated the zebra fish brain by projecting a sheet of light over the entire organ while a camera took second-by-second snapshots of the neurons lighting up.

The technique used, called calcium imaging, which was pioneered by one of us (Yuste) to record the electrical activity of neural circuits, enabled the recording of 80 percent of the zebra fish's 100,000 neurons. It turns out that when the fish was at rest, many regions of the nervous system of the larval zebra fish switched on and off in mysterious patterns. Ever since Berger introduced the EEG, researchers have known that the nervous system is essentially always active. The zebra fish experiment gives hope that newer imaging technologies could help tackle a major challenge in neuroscience—the understanding of the persistent, spontaneous firing of large groups of neurons.

The zebra fish experiment is just the beginning because neuroscientists require still better technologies to discover how brain activity gives rise to behavior. New types of microscopes need to be designed to image simultaneously neuronal activity in three dimensions. In addition, calcium imaging operates too slowly to track the rapid firing of neurons and is also unable to measure the inhibitory signals that tamp down electrical activity in the cell.

Neurophysiologists, working side by side with geneticists, physicists and chemists, are trying to improve optical techniques that—instead of sensing calcium—record neuronal activity directly by detecting changes in membrane voltage. Dyes that alter their optical properties as voltage fluctuates—either deposited on the neuron or integrated through genetic engineering into the cell membrane itself—could improve on calcium imaging. This alternative technique, known as voltage imaging, may ultimately enable researchers to record the electrical activity of every neuron in an entire neural circuit.

Voltage imaging is still in its infancy, however. Chemists need to enhance the ability of the dyes to change color or other characteristics as a neuron fires. The dyes must also be designed to ensure that the chemicals do not damage the neuron. Already, though, molecular biologists are building genetically encoded voltage sensors; these cells read a genetic sequence to produce a fluorescent protein that is delivered to the cells' outer membrane. Once there such proteins can change the degree to which they fluoresce in response to alterations in a neuron's voltage.

As with electrodes, advanced nonbiological materials borrowed from nanotechnology may help. In place of organic dyes or genetic indicators, a new type of voltage sensor can be made of quantum dots—small semiconductor particles that exhibit quantum-mechanical effects and can be precisely tailored in their optical properties, such as the color or intensity of the light emitted. Nanodiamonds, another novel material imported from quantum optics, are highly sensitive to changes in electrical fields that occur as a cell's electrical activity fluctuates. Nanoparticles could also be combined with conventional organic or genetically engineered dyes to produce hybrid molecules in which a nanoparticle could serve as an "antenna" to amplify low-intensity signals produced by fluorescent dyes when a neuron is activated.

GOING DEEP

ANOTHER IMPOSING TECHNICAL CHALLENGE to visualizing neuronal activity is the difficulty of delivering light to, and collecting it from, neural circuits deep below the surface of the brain. To solve this problem, neurotechnology developers are beginning to undertake collaborations with researchers in computational optics, materials engineering and medicine who also need to see through solid objects noninvasively, whether skin, skull or the inside of a computer chip. Scientists have long known that some of the light that hits a solid object gets scattered and that the scattered photons may, in principle, reveal details of the object from which it is reflected.

For example, the light from a flashlight on one side of a hand shines through, exiting as a diffuse glow yet without giving any clue about the location of the bones or vasculature underneath the skin. But information about the path the light takes through the hand has not been lost entirely. The disordered waves of light scatter and then interfere with one another. This light pattern can be imaged with a camera, and new computational methods can then reconstruct an image of what lies within—a technique used last year by Rafael Piestun and his colleagues at the University of Colorado Boulder to see through an opaque material. These methods might be combined with other optical techniques, including those used by astronomers to correct image distortions caused by the atmosphere's effects on starlight. So-called computational optics may help visualize the fluorescent glow from dyes that light up when subsurface neurons fire.

Some of these new optical techniques have already been used successfully to image the inner reaches of animal or human brains with a piece of the skull removed, enabling scientists to see more than a millimeter into the cortex. And with further refinement, these techniques might potentially offer a way to look through the thickness of the skull. But see-through optical imaging will not penetrate far enough to detect structures deep within the brain. Yet another recent invention may help address this problem. In a technique called microendoscopy, neuroradiologists currently insert a narrow but flexible tube into the femoral artery and then maneuver it to many parts of the body, including the brain, allowing microscopic light guides inserted in the tube to do their work. In 2010 a team at the Karolinska Institute in Stockholm demonstrated an "extroducer"—a device that allows the artery or vessel through which the endoscope is threaded to be safely perforated, which makes any part of the brain, not just the vasculature, accessible for inspection by various imaging or electrical recording technologies.

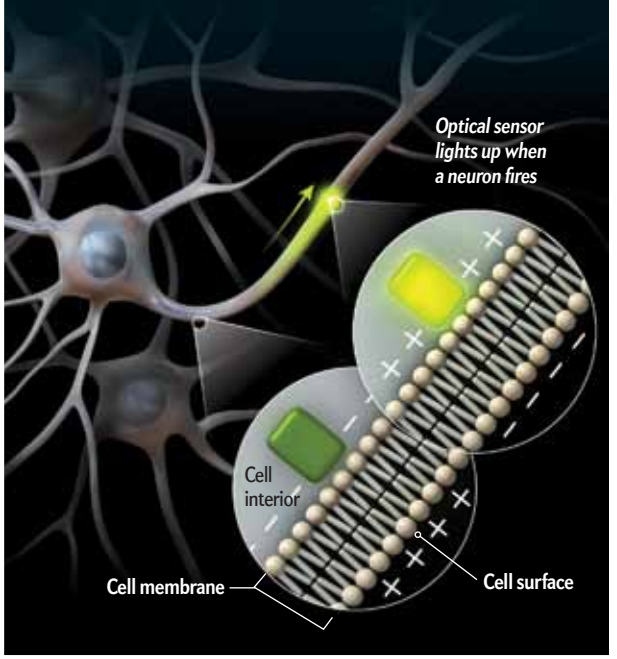
Electrons and photons are the most obvious candidates for recording brain activity, though not the only ones. DNA technology could also play a critical role in a still distant future for monitoring neuronal activity. One of us (Church) has gained inspiration from the field of synthetic biology, which tinkers with biological materials as if they were machine parts. As research goes forward, lab animals could be genetically engineered to synthesize a "molecular ticker tape"—a molecule that changes in spe-

Listening in on Millions of Neurons

Neuroscientists need more efficient and less intrusive ways to observe brain circuits, in which electrical signals pass from one neuron to the next. A range of technologies—some in use, others just a glint in a researcher's eye—may enable scientists to record from thousands, even millions, of neurons. They will replace slow and imprecise methods that often require invasive electrical probes.

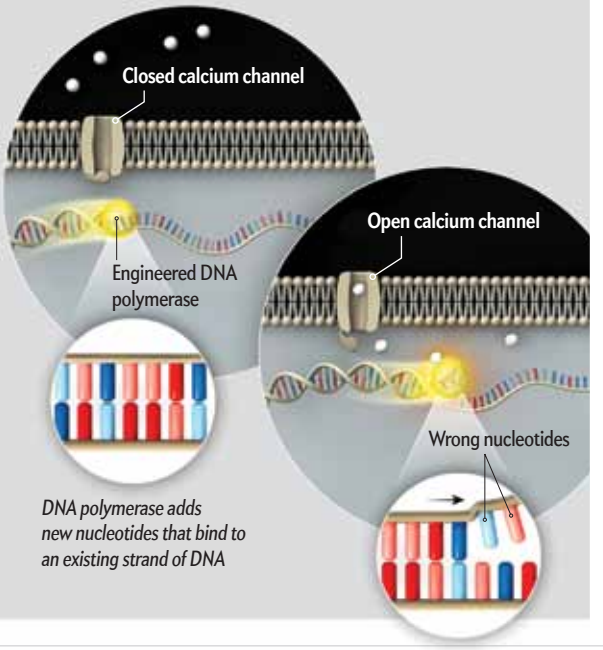
Voltage Imaging

This technique implants a dye into a neuron to determine if the cell is active. This sensor fluoresces when the electrical field across the cell membrane flips its charge as an electrical signal passes by. A detector (*not shown*) registers the event and may also monitor the activity of many other neurons, labeled with the same dye.



DNA Ticker Tape

A radically new approach—a molecular ticker tape—would, in one scenario, place a single strand of DNA with a known sequence of letters, or nucleotides, inside a cell but near its surface. An enzyme, DNA polymerase, would then add new nucleotides that bind to form a double-stranded molecule (*left*). When a neuron fires, an influx of calcium ions coming through a newly opened membrane channel would cause the enzyme to add the wrong nucleotides (*right*), an error that could be detected when the DNA strand is later sequenced.



cific, detectable ways when a neuron becomes active. In one scenario, the ticker tape would be made by an enzyme called a DNA polymerase that starts off by continuously building a long strand of DNA that binds to another strand consisting of a preestablished sequence of nucleotides (the "letters" that are the building blocks of DNA). An influx of calcium ions, generated after the neuron fires, would then cause the polymerase to produce a different sequence of letters—in short, causing "errors" in the expected placement of nucleotides. The resulting double strand of nucleotides could be sequenced later from each neuron of the brain of an experimental animal. An innovative technique called fluorescent in situ sequencing would yield a record of different patterns of changes, the errors from the original ticker tape, corresponding to either the intensity or the timing of each of many neurons in a given volume of tissue. In 2012 the Church lab reported on the feasibility of this idea using a DNA ticker tape altered by magnesium, manganese and calcium ions.

Down the road, synthetic biology envisages the prospect of artificial cells acting as biological sentinels that patrol the human body. A genetically engineered cell could serve as a biological electrode, much smaller than a hair's width in diameter, that could be placed near a neuron to detect its firing. This pattern of firing could be recorded by a nanosize integrated circuit inside the synthetic cell—"electronic dust," which could transmit the collected data by a wireless link to a nearby computer. These nanosize devices, a hybrid concoction of electronic and biological parts, might be powered by an external ultrasound transmitter or even from within the cell using glucose, adenosine triphosphate or another molecule.

TOGGLING ON OR OFF SWITCHES

TO UNDERSTAND WHAT IS HAPPENING in the brain's vast web of neural circuitry, researchers need to do more than just snap photographs. They must switch selected groups of neurons on or off at will to test what the cells are doing. Optogenetics, a technique widely adopted by neuroscientists in recent years, involves using animals that have been genetically engineered so that their

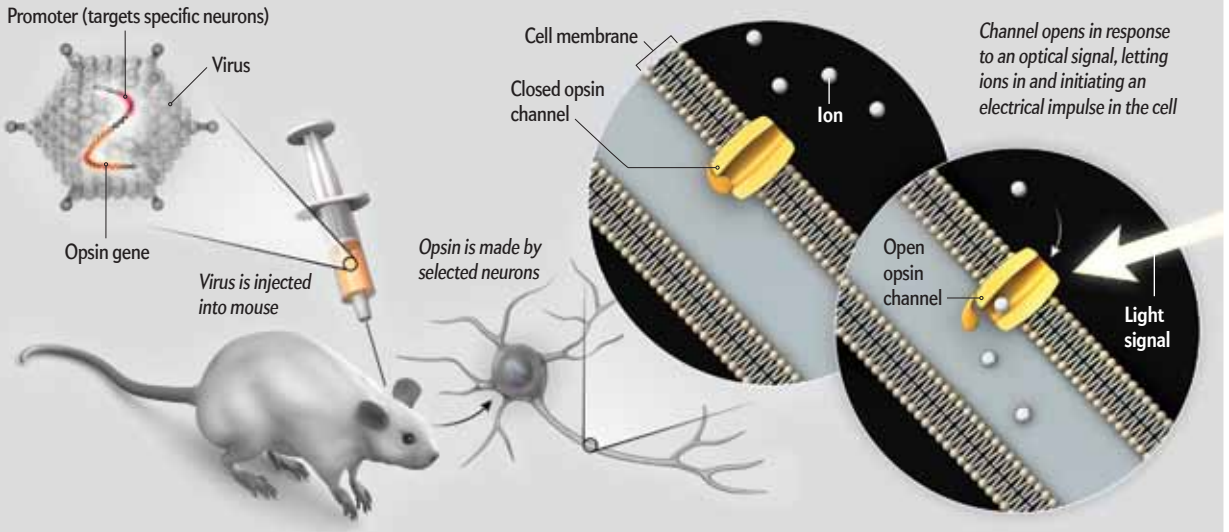
Illustrations by Emily Cooper

Installing a Neural Light Switch

Beyond observing electric currents flowing through circuits, neuroscientists increasingly want to turn individual circuits on and off at will so they can learn how to control specific forms of brain activity. One day these nascent technologies, two of which rely on optical signals (*below*), may quell epileptic seizures or parkinsonian tremors.

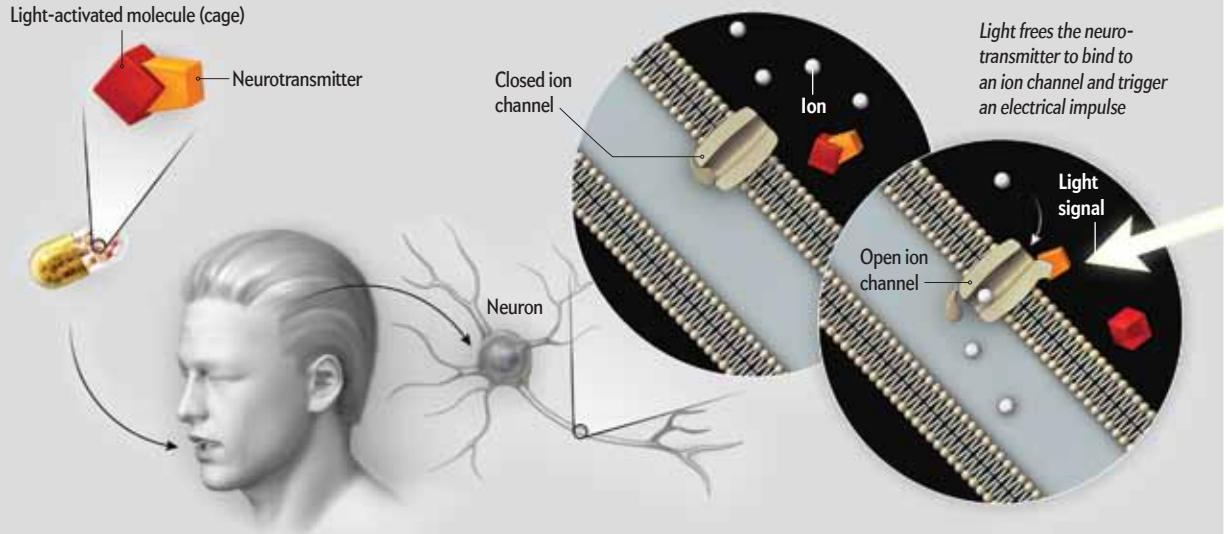
How Optogenetics Works

As the name implies, optical signaling and genetic engineering combine to activate a brain circuit in a living animal. First, a gene for a light-sensitive protein, an opsin, is placed inside a virus that, after injection into an animal, delivers the gene into neurons. Promoter DNA in the inserted genetic material ensures that only certain neurons make the opsin, an ion channel, and insert it in their surface membranes. A signal from an optical fiber inside a mouse skull opens the channel, allowing charged ions to enter the neuron and triggering a current through the cell.



How Optochemistry Works

An alternative technique known as optochemistry avoids the need for cumbersome genetic engineering. A patient would first swallow a pill that contains a light-activated molecule—a cage—that attaches to a neurotransmitter, which regulates a neuron's activity. After the pill's content reached the brain, a pulse of light from an endoscope, or one delivered from outside the skull, would detach the neurotransmitter, which would go on to bind to and open a channel on the cell membrane that lets ions enter. The ions would then trigger the firing of the neuron, sending an electrical impulse traveling into the cell.



neurons produce light-sensitive proteins derived from bacteria or algae. When exposed to light of a particular wavelength, piped in through an optical fiber, these proteins cause neurons to either switch on or shut down. Researchers have applied the technique to activate neural circuits involved in pleasure and other reward responses and in the impaired movements characteristic of Parkinson's. They have even used optogenetics to "implant" false memories into mice.

The need for genetic engineering means that optogenetics may require lengthy approval protocols before it can be tested, or used as a therapy, in humans. A more practical alternative for some applications has been demonstrated by attaching neurotransmitters, the chemicals that regulate the activity of neurons, to a light-sensitive chemical called a "cage." Once exposed to light, the cage breaks apart, and the chemical escapes and becomes active. In a 2012 study, Steven Rothman of the University of Minnesota, in collaboration with the Yuste lab, placed ruthenium cages joined to GABA, a neurotransmitter that ratchets down neural activity, on the exposed cerebral cortex of rats that were chemically induced to produce epileptic seizures. Shining a pulse of blue light on the brain released the GABA and caused the seizures to abate. Similar "optochemical" approaches are currently used to probe the function of selected neural circuits. If further developed, they might serve as therapies for some neurological or mental disorders.

A long path still stretches from basic research to clinical applications. Each new idea for the large-scale measurement and manipulation of neural activity will have to be tested in fruit flies, roundworms and rodents before moving on to humans. An intensive effort could allow researchers to image and optically control a large number of the 100,000 neurons in a fruit fly brain within perhaps five years. Instruments to capture and modulate the neural activity of the brain of an awake mouse might not be possible for up to 10 years. Some technologies, such as thin electrodes to correct malfunctions in neural circuits in depressed or epileptic patients, could find their way into medical practice in the next few years, whereas some will take a decade or more.

As neurotechnologies grow in sophistication, investigators will need improved ways to manage and share enormous compilations of data. Imaging the activity of all the neurons in a mouse cortex could generate 300 terabytes of compressed data in an hour. But this is by no means an insurmountable task. Elaborate research facilities, akin to astronomical observatories, genome centers and particle accelerators, could acquire, integrate and distribute this type of digital data flood. Just as the Human Genome Project spawned the field of bioinformatics to cope with sequencing data, the academic discipline of computational neuroscience could decode the workings of entire nervous systems.

The ability to analyze petabytes of data will do more than bring order to floods of new information; it could lay the groundwork for new theories about how the cacophony of nerve firings translates into perception, learning and memory. The mega data analysis may also help confirm or dispel theories that could not be tested before. One intriguing theory postulates that the many neurons involved in the activity of a circuit develop particular sequences of firing known as attractors that may represent emergent brain states—a thought, a memory or a decision. In one recent study, a mouse had to make decisions about whether to traverse one section or another of a virtual maze projected on a screen. That action switched on dozens of neurons that exhibited dynamic changes in activity that resembled that of an attractor.

A better understanding of neural circuits could improve diagnosis of brain diseases from Alzheimer's to autism and give a deeper understanding of their causes. Instead of diagnosing and treating these conditions based on symptoms alone, doctors could look for specific alterations in the activity of particular neural circuits found to underlie each disorder and administer therapies to correct those abnormalities. By extension, knowledge about the roots of disease will likely translate into economic benefits for medicine and biotechnologies. As with the genome project, ethical and legal issues will need to be dealt with, particularly if this research leads to ways of discerning or altering mental states—outcomes that would necessitate careful safeguards for patient consent and privacy.

For the various brain initiatives to succeed, however, scientists and their backers must stay closely focused on the goal of imaging and controlling neural circuitry. The idea for the BRAIN Initiative grew from an article in the journal *Neuron* in June 2012. In it, we and our colleagues suggested a long-term collaboration among physicists, chemists, nanoscientists, molecular biologists and neuroscientists to develop a "brain activity map" derived by applying new technologies to measure and control the electrical activity of entire brain circuits.

We would urge that as the ambitious BRAIN project evolves, our original emphasis on tool building be retained. The scope of brain research is vast, and the BRAIN Initiative could easily devolve into a composite wish list that attempts to satisfy the broad-ranging interests of neuroscience's many subdisciplines. It could thus become nothing more than a supplement to already existing projects pursued by many individual labs working independently.

If this occurs, progress will be haphazard, and major technical challenges may never be met. We need collaboration among academic disciplines. Building instruments to image voltage in millions of neurons simultaneously throughout entire brain regions may be achieved only by a sustained effort of a large interdisciplinary team of researchers. The technology could then be made available at a large-scale, observatorylike facility shared by the neuroscience community. We are passionate about retaining a focus on new technology to record, control and decode the pattern of electrical spikes that are the language of the brain. We believe that without these new tools, neuroscience will remain bottlenecked and fail to detect the brain's emergent properties that underlie a virtually infinite range of behaviors. Enhancing the ability to understand and use the language of spikes and neurons is the most productive way to derive a grand theory of how nature's most complex machine functions. SA

MORE TO EXPLORE

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FROM OUR ARCHIVES

A Push to Map All the Brain's Neurons. *Scientific American Mind;* May/June 2013.