NEWS

Switching neurons on and off to probe autism circuits

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Much of modern biology is built on addition and subtraction.

To interrogate a gene's function, subtract it from the genome and watch what happens (often, death). To confirm a gene's role in some disease, replace a defective copy with an unmutated version and see if the addition renders a fix.

Such simple arithmetic is fundamental to the natural sciences, says **Karl Deisseroth**, professor of bioengineering and a psychiatrist at Stanford University in California. "It is the foundation of not just genetics, but biochemistry and developmental biology."

Past the level of a cell, however, that math gets exponentially more complex. The functions that interest neuroscientists depend on vast networks of neurons, calling for calculations across entire neural circuits.

Deisseroth and others developed the technique of optogenetics to do controlled experiments on neurons at this scale nearly 20 years ago. As its name suggests, the method uses pulses of light to activate **genetically modified neurons** that have channelrhodopsins, or opsins, dotted along their surface. These proteins shift their shape when struck by light, forming pores that usher sodium ions into the cell and trigger an action potential.

Neuroscientists have applied the tool to explore just about every behavior, "from social interaction to hunger, thirst, aggression, sleep, avoidance, anxiety and fear," Deisseroth says, but it lacks precision for some experiments. Any neuron with an opsin will fire an action potential when struck

by photons, which is good for studying larger brain regions but not for answering more nuanced questions about, as Deisseroth puts it, “how many neurons, of what kind, and with what relative action” underlie some condition.

A new technique called two-photon holographic optogenetics, developed only in the past few years, could change all that. Unlike traditional optogenetics, it stimulates individual neurons with much greater specificity. It can turn on or off hundreds of neurons at will, in almost any desired pattern. And it can mimic the natural rhythms of neuronal activity observed in mouse and, potentially, human brains, rendering previously unanswerable questions amenable to inquiry — including questions about autism.

Researchers have used standard optogenetics to characterize neural circuits that go awry in mouse models of Parkinson’s and Alzheimer’s disease, and to create interventions that can target and restore those circuits, potentially providing an alternative to pharmacological approaches. But studies of the neural circuits underlying autism — along with any possible interventions — have lagged behind.

> "We can interface with the brain, in the language that it naturally uses.” Ofer Yizhar

“The problem is that there’s very few people working on circuits right now in autism,” says **Carlos Portera-Cailliau**, professor of neurology and neurobiology at the University of California, Los Angeles. “There’s a huge emphasis on molecular studies and genetic studies,” sometimes to the neglect of more systems-level investigations, he says, where a full account of autism is likely to lie.

That neglect may not be unwarranted. The equipment used to study neural circuits — especially the lasers and optical systems — are expensive and require specialized knowledge to operate, says **Nicolas Pégard**, assistant professor of computational biophotonics at the University of North Carolina at Chapel Hill.

Despite these barriers, researchers could use two-photon holographic optogenetics to stress test key theories about the condition’s origins, including the idea that it results from **too much excitation** in the brain. Mice with excess neuronal excitation shy away from an unfamiliar mouse, for instance, which researchers take as a proxy for the altered social behaviors seen in autistic people. If researchers could selectively trigger those excitatory neurons in mice to find the cells responsible, and then fine-tune the number of neurons involved, they might unveil the tipping point at which the animals’ brains favor social aversion.

In other words, researchers could conceivably “come to a more tractable, material, physical understanding of the core problem with autism,” Deisseroth says, “that might lead to better understanding and treatment.”

Courtesy of Shai Berlin / Technion - Israel Institute of Technology in Haifa

A traditional optogenetics experiment goes something like this: Pack viruses with the gene that codes for an opsin and inject those viruses into mice. The viruses travel to the brain and dump their cargo into neurons. Genetic controllers ensure that the opsins are expressed in only a **specific neuronal subtype,** such as interneurons or GABAergic neurons.

After a few days, drill a hole into the skull of each mouse and plug in an LED light. Voilà! With the flip of a switch, researchers activate the engineered neurons.

Two-photon holographic optogenetics improves upon these basic steps in several ways.

First, the opsin protein is modified to **respond to infrared**, rather than visible, light. Whereas visible light illuminates only the surface of tissue, near-infrared light penetrates a millimeter or more, which gives researchers control over neurons deeper in the brain. These infrared-sensing opsins trigger an action potential only when struck by two photons in quick succession (hence the term ‘two-photon’), ensuring that nearby neurons are not switched on accidentally.

Second, the virus is also packed with the gene for a **calcium sensor protein**, which binds to calcium ions and emits fluorescent light. Calcium rushes into neurons during action potentials and is a proxy for neuronal activity. Using both an opsin and calcium sensor protein makes it possible to simultaneously read and write neuronal information using two separate lasers: Infrared light at one wavelength triggers neurons to fire, whereas infrared light at another wavelength monitors fluorescence and records neuronal activity like a camcorder. Modern lasers can emit pulses **80 million times per second,** fast enough to detect or trigger each action potential.

Third, the illumination technique allows for a depth dimension. Instead of wiring an LED into an animal’s brain, researchers swivel a microscope overhead and can zoom in on specific neurons, marking their location on a computer screen. From the neurons’ coordinates in 3D space, the system calculates a wave pattern that, as it propagates through the brain tissue, focuses energy at those locations.

To create this pattern, the system uses a spatial light modulator that consists of a liquid crystal, like those in a digital watch or old-school calculator. The crystals modify the wave phase of light impinging upon them. When the researchers shine a laser beam on the spatial light modulator, out comes the desired wave pattern. The laser beam is split, and each beamlet is redirected to a different neuron, like spotlights on a Broadway stage.

“Basically, it’s a fancy and expensive projector,” says **Shai Berlin**, assistant professor of neuroscience at Technion - Israel Institute of Technology in Haifa. “But instead of using small

mirrors, we use very, very complicated physics and filters to modulate the phase of the light."

In short, the approach generates an electronic hologram not unlike the 3D images in science-fiction movies. But instead of an image of, say, a rebel princess, this hologram is a 3D constellation of points of light that target specific locations in brain tissue. Two-photon holographic optogenetics, studies show, can **monitor 3,000 neurons at once** in 3D and activate **a few hundred** more. **Rafael Yuste**, professor of biology at Columbia University, equates this to "playing neurons like a piano."

The technology is superior to conventional optogenetics because two neurons that lie next to each other in the brain may be genetically identical yet encode entirely distinct functions; it can tease those cells apart.

"With historical optogenetics, you would flood the brain with light and activate all the neurons that are genetically identical," Pégard says, "which is kind of equivalent to hitting the person with a phone book on the head. You will get a reaction, but the level of control you have is pretty much all or nothing."

https://www.youtube.com/watch?v=KE4v8iipwXM

A researcher showcases his two-photon holographic microscope set up.

The technique has already shed actual light on complex behaviors.

In 2019, for example, Deisseroth and Yuste's teams independently applied it to mice that had learned to recognize horizontal or vertical stripes. They **trained the animals to drink water** only when they saw the vertical pattern and used the calcium sensors to **record which neurons** in the primary visual cortex fired in response to the visual cue.

"They actually could change the perception of a mouse, by just playing with a very small number of neurons," says **Ofer Yizhar**, professor of neurobiology at the Weizmann Institute of Science in Rehovot, Israel. In other words, "we can interface with the brain, in the language that it naturally uses."

Today, Deisseroth says his group is interested in even more fundamental questions that no other technique can crack: "How many neurons can bias a decision? Which layer of cortex are they in? What type of neuron might they be? Which neurons and how many of them can give rise to a

perception?”

> “”[This technique could help scientists] come to a more tractable, material, physical understanding of the core problem with autism that might lead to better understanding and treatment.” Karl Deisseroth

As part of those efforts, the team has designs on applying two-photon holographic optogenetics to autism. In 2017, they **used standard optogenetics** in a mouse model of autism — namely, mice that avoid strangers. Switching on inhibitory neurons in the medial prefrontal cortex — a brain region involved in social interactions — or switching off excitatory neurons made the animals more apt to approach and interact with unfamiliar mice, the researchers found. In other words, they made the mice more outgoing with the flip of a switch.

To extend that work, Deisseroth aims to establish the threshold at which this excitation-inhibition imbalance begins to alter social interactivity. It may be possible that there’s a threshold at which the brain undergoes a sort of phase transition, he says, switching from a state in which it can quickly process and engage in social interactions to one in which it cannot.

“And we can really only prove that sort of phase transition if we can smoothly and precisely modulate the number of cells that we’re recruiting of each type,” Deisseroth says. Two-photon holographic optogenetics can do that by activating dozens or hundreds of neurons of a specific cell type, with pinpoint precision.

For all its power, two-photon holographic optogenetics has not been widely adopted. The lasers alone cost more than $100,000. Learning about digital holography and the requisite animal surgery is time intensive.

Portera-Cailliau suggests that researchers aiming to map autism-centric neural circuits begin by recording neuronal activity from more brain regions in mouse models of the condition. A tiny electrical probe called **NeuroPixels**, for example, can capture neuronal activity at more than 6,000 sites in a mouse’s brain, enabling researchers to examine differences in brain activity that may underlie autism.

“It’s sort of a cost-benefit thing,” says **Audrey Brumback**, assistant professor of neurology and pediatrics at the University of Texas at Austin. These new technologies are sure to reveal interesting insights, she says, but “at this point, I still don't know enough about the circuits I’m studying. There’s things we need to figure out before we can jump into the micro-specifics.”

Once researchers map more neural circuits linked to autism across these mouse models, however, two-photon holographic optogenetics could help pinpoint the specific cell types and firing patterns that drive different traits, Portera-Cailliau adds.

Studies in autism mouse models consistently implicate neural circuits in the **amygdala**, **prefrontal cortex** and **cerebellum** in the **social problems** and repetitive behaviors commonly seen in those animals.

Single-cell optogenetics could help researchers probe those circuits more deeply and “distinguish whether there are certain cell populations that are more vulnerable to autism mutations or play a role in a certain behavior but not in other behaviors,” says **Simon Chen**, assistant professor of cellular and molecular medicine at the University of Ottawa, Canada.

In the distant future, Portera-Cailliau says, perhaps a highly targeted form of optogenetics, acting as a kind of neural pacemaker, could even be used to alleviate specific traits in people with autism.

It’s an idea that motivates Yuste, who says there are two reasons to make optogenetics a **viable tool** to manipulate the human brain: “One is to understand the human mind, and the other one is to help patients with mental and neurological conditions.”

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