LIGHTING OUR MEMORIES

Researchers are keenly interested in understanding brain function. Jeffrey Perkel takes a look at how optogenetics is providing new insights into learning and memory.

Credit: Dheeraj Roy.

As a boy, Dheeraj Roy spent summers in India with his paternal grandmother. It was a happy time, filled with camping, hiking, and delicious food—a time that his grandmother would also recall with fondness years later.

But by 2012, things had changed. Roy, newly installed as a graduate student at the Massachusetts Institute of Technology, went back to India for a visit with his parents and grandmother. He brought gifts—food and clothing from the United States—and was anxious to reminisce about the past. But when he saw his grandmother, he says, “she didn’t know who I was.”

Roy’s grandmother was suffering from Alzheimers, a disease that robs its victims first of their past and then of their dignity. There is no cure, nor even a reliable antemortem diagnostic for the disease. For Roy, it was a galvanizing moment, spurring him to design a graduate research project that he hopes might one day lead to advances that could spare others from his grandmother’s fate: “That experience is on my mind every day.”

Fortunately, Roy was in the right lab to make that happen. MIT professor Susumu Tonegawa, a 1987 Nobel laureate for his work on how B cells are able to generate a seemingly infinite variety of antibody designs, has been focusing on neuroscience, specifically memory and learning, since the 1990s. Roy was now a student in Tonegawa’s lab, and they were making headway in understanding the brain using a powerful neuroscience tool: optogenetics.

Lighting a revolution

Optogenetics exploded onto the neuroscience scene in 2005, allowing for spatial, temporal, and cell type-specific control of neural activity. In optogenetics, researchers express a light-sensitive ion channel or pump, such as channelrhodopsin or halorhodopsin, in

specific sets of neurons. By illuminating those cells with light of the appropriate wavelength, researchers can cause neurons to fire action potentials (or block them, depending on the specific actuator used) and thus, probe the cellular and molecular mechanisms of neural circuitry in real time.

According to Inbal Goshen, Assistant Professor at the Edmond and Lily Safra Center for Brain Sciences at the Hebrew University in Jerusalem, the technique offers two advantages in memory research: temporal precision and cellular specificity. Researchers have had the ability to manipulate neurons in the brain with electrode stimulation or pharmacologicals for years, but neither technique can be restricted to specific cellular populations, and the kinetics of small molecule compounds are simply too slow. “A minute is an eternity for a neuron,” Goshen explains.

Because the brain is awash in active neurons, the trick with optogenetics is keeping the area of interest as small as possible. This requires two elements. First, a light source, usually a thin fiber-optic fiber, is embedded in or above a specific part of the brain, such as the CA1 subregion of the hippocampus. At the same time, researchers try to restrict expression of the optogenetic protein to specific classes of neurons or to neurons that are physically connected to one another.

Tonegawa’s lab pioneered an alternative approach. As Steve Ramirez, a former graduate student in the lab who helped develop these methods explains, their system relies on two DNA constructs. The first places the doxycycline-activated transcription factor tTA under the control of a c-fos-dependent promoter; the second expresses a channelrhodopsin under the control of tTA, whose activity is regulated by the antibiotic doxycycline. Since c-fos activity is limited to neurons that happen to be active, tTA expression is confined to active neurons and, thus, so is channelrhodopsin. The net effect is

Example of an optogenetics experiment. Researchers identify and tag (with channelrhodopsin) cells involved in representing a blue box. Animals in a novel red box that they had never been in before are pulsed with light that reactivates the memory of the blue box, during which the animals receive a few mild foot shocks. The experiment tries to artificially create an association between the light-reactivated memory of the blue box and the footshocks themselves. When the animals are placed back in the blue box—the box in which nothing bad had ever happened to them—they now display fear behavior. Image courtesy of Collective Next.

to restrict optogenetic protein expression to neurons actually firing at a particular time. “We let the brain do the hard work for us,” Ramirez says.

How, though, to measure memory? One can’t simply ask rodents what they’re thinking. Researchers need to produce a memory that results in some measurable behavioral change, and most often, that memory is fear. Put a mouse in a box with a metal grid in the floor and apply a mild shock to its feet, and the animal will quickly learn to be afraid of that box, says Kiriana Cowansage, a fifth-year postdoc at the University of California, San Diego, who also uses the approach. The result is easily monitored, as the animals freeze upon re-entering the box. “It’s not a really strong shock, but it’s surprising, and the surprise of it is really what gets the mouse to remember,” says Cowansage.

Applying the technology

Ramirez and Tonegawa used their activity-based system in three key memory studies between 2012 and 2015. First, they, with late postdoc Xu Liu, demonstrated optogenetically that activating the neurons firing as mice are taught to fear an aversive foot

shock is sufficient to make the animals recall—or “jump-start,” in Ramirez’s words—that memory, even in an environment not otherwise associated with it. That this would work wasn’t necessarily obvious at the start, Ramirez notes. After all, it’s not clear precisely how or where memories are stored, nor how they are activated. “We could’ve needed to activate perhaps more brain cells, or perhaps we need to activate them in a very particular temporal pattern, which we don’t do.”

A year later, Ramirez and Liu used the same strategy to implant a false memory—essentially to make a mouse believe it had been shocked in a cage in which that had never happened—by activating a “neutral” memory while delivering a shock. Memory, Ramirez explains, “is not a carbon copy of the past or a tape recorder of the past; when a memory is actively recalled, it’s actually very susceptible to modification.”

The final paper on this work, published in 2015, addressed depression. Ramirez and Tonegawa showed that repeatedly activating a positive memory—in this case, the memory of encountering a female mouse—in a mouse model of depression was sufficient to provide long-term resto-

ration of pleasure-seeking behavior, which is normally dampened in depressive mice.

According to Ramirez, that study suggests the possibility of treating depression in humans using compounds that can strengthen synaptic connections. That’s a long way off, he stresses. “We’re not showing how memory works per se, we’re showing how memory can work. What we’re doing is we’re hot-wiring the car and we’re saying: ‘Here’s one way to jump-start this car.’”

Roy applied the same approach to study neural deficits in Alzheimers disease. The conventional wisdom has held that the reason patients with early Alzheimers cannot remember recent events—where they placed their keys, for instance—is because memories cannot form. Roy decided to test this idea directly.

Earlier, Tonegawa’s lab used a mouse model to demonstrate that amnesia is a breakdown in memory recall. In that work, a mouse exposed to a foot shock didn’t seem to remember the experience when treated with the protein-synthesis inhibitor anisomycin. (Protein synthesis seems to be required for memory formation.) But optogenetic excitation of the cells that

had been activated during the learning process—a collection of cells called an “engram”—could force recall of the “lost” memory, meaning it wasn’t so much lost as misplaced. The effect was only temporary though—when the light was switched off, amnesia returned.

Roy wanted to know if a similar mechanism was at work in Alzheimer’s patients. Using a mouse model of early Alzheimer’s disease, he introduced DNA vectors required to tag active neurons with optogenetic reporters, focusing on a region of the hippocampus called the dentate gyrus and its connectivity with a higher brain region called the entorhinal cortex. He then trained animals to optogenetically tag the neurons active during learning.

A published photograph (reproduced on this page) beautifully illustrates the surgical precision of this approach. The image is of the dentate gyrus. A bright green tongue of cells enters the frame from the left, surrounded by a thick padding of blue cells. The blue cells, Roy says, represent the “major excitatory neuron cell layer,” and it is sparsely dotted with green neurons—the cells that were active when the memory was formed.

As predicted, when early Alzheimer’s model mice were asked to remember the fear-inducing memory—that is, to recall that they had been shocked in a par-

As a graduate student at MIT, Dheeraj Roy developed a research project to understand memory formation and loss. Credit: W. Yu.

The hippocampus, the site of memory formation. Credit: S. Ramirez courtesy of *Science* magazine.

ticular box—they couldn’t. But if the engram was activated optogenetically, they froze, implying that the fear memory was formed and contradicting conventional wisdom. “We restored memories that were assumed to not be stored in the first place,” Roy says.

More importantly, Roy and his team demonstrated that by repeatedly stimulating the engram cells with a protocol intended to strengthen synaptic connections (called long-term potentiation), they could restore recall of the memory even in the absence of light.

Such a strategy cannot be applied to humans, of course, as it requires genetic manipulation of the brain. But for Roy, the findings suggest a possible therapeutic strategy: using drugs to strengthen neural connections. “That’s, to be honest, my dream experiment,” he says—to identify drugs that strengthen synaptic contacts between the entorhinal cortex and the hippocampus and boost memory recall in a mouse model of early Alzheimer’s. “For me, that’s the home run,” he says.

Timing is everything

When memories of places and events—so-called “context memories”—are recorded, their first stop is the hippocampus. But some time later, those memories, or at least “the gist” of them, appear to move into higher brain regions in the

cortex, like archiving data from a hard drive onto the cloud. Cowansage, a post-doctoral fellow in Mark Mayford’s laboratory at UCSD, was interested in learning just when this happens.

She optogenetically tagged cells that were activated during learning in the retrosplenial cortex, a cortical region connected to the hippocampus. The mice were taught to fear a box that had been furnished with a variety of memorable sensory cues, including an odor such as peppermint, a checkerboard pattern on the walls, and a metal gridded floor, through which the mice receive a mild shock. Some time later, the mice were placed in a second neutral box, to which they are not normally afraid, and the light in the retrosplenial cortex was flipped on. The animals exhibited sudden fear—they froze.

“We don’t, of course, know exactly what the mouse is thinking, but it gives us some idea that the fear-inducing experience they had the day before in the real box is now being triggered somehow artificially by stimulating those retrosplenial cells with the LED,” Cowansage explains.

The results surprised her. Neuroscientists traditionally have argued that memories migrate to the cortex only slowly, perhaps within weeks of initial creation. “We’re showing that actually fairly early, pretty much on the same time frame that we normally think of hippocampal

memory, we're also seeing the involvement of cortex."

Researchers are even using optogenetics to probe the role of cells other than neurons in memory formation. Goshen, for instance, focuses on the cells that constitute the majority of the brain, glial cells, including astrocytes. Glia were often thought of as performing housekeeping functions in the brain, but recent work indicates these cells perform more interesting functions as well.

Getting at those "interesting functions" requires a different kind of optogenetic actuator, since unlike neurons, glial cells do not fire action potentials. But they do respond to extracellular stimuli through G-protein-coupled receptors. In 2009, Raag Airan, in the lab of optogenetics pioneer Karl Deisseroth, described a collection of light-activated signaling receptors, called optoXR_s, which allow researchers to simulate ligand binding with light. Goshen and her team are now using these constructs to determine whether glial cells "have a computational role in neuronal activity and behavior."

Strong memories

How closely the ability of animals to recall fear models the higher learning capabilities of humans is, of course, debatable. But it's clear, says Mayford, that not all memory is the same. Learning to play the piano is different, for instance, from recalling a particular event.

Sadegh Nabavi, a group leader at the Danish Research Institute of Translational Neuroscience (DANDRITE), is interested in what differentiates strong memories from weak ones. Most Americans can easily recall where they were, what they were doing, and who else was with them when they first heard of the terrorist attacks on Sept. 11, 2001, Nabavi says.

The difference could be protein synthesis. Memory formation seems to require protein synthesis, and it may be that protein synthesis associated with a "strong" memory can strengthen an otherwise weak memory that is created at around the same time. "The weak memory can use the protein resources of the strong memory too and become strong," Nabavi suggests.

Nabavi is currently testing this hypothesis using optogenetics. He plans to induce, in a single population of neurons, two memories that can selectively be made weak or strong using a dual-color light-activation system. While a "strong" memory persists long-term, the "weak" memory fades within hours. If Nabavi strengthens one with light, he wonders, will the second be strengthened as well?

University of California, Los Angeles Distinguished Professor Alcino Silva studies mechanisms by which the brain "time-stamps" memories. Temporal relationships matter in the brain, Silva says, because events linked in time are also often related in content. "The closer the time between events, the more likely it is that they have something to do with one another." How, though, does the brain make such connections? That is, how are memories "linked across time?"

To answer his question, Silva and his colleagues at UCLA determined how time can affect the storage of two memories. He used a transcription factor called CREB, which activates neurons, to trap memories in a specific location by selectively infecting those cells with a virus carrying CREB. "You can imagine having a group of people to which you give a flag," Silva explains, "and then you give them instructions that at a specific time, they raise their flag. Essentially that's what

Steve Ramirez helped to pioneer the optogenetic approach used by Susumu Tonegawa's laboratory at MIT for studying memory formation. Credit: Joshua Sarinana.

we do with those neurons." That virus also encodes an optogenetic actuator, such as channelrhodopsin.

Silva's team used mice whose activated neurons fluoresce to investigate the overlap in neural circuits that are written when mice experience two different environments within hours of one another, or within days. The results suggest a high degree of overlap in linked memories, he says. "There is a lot of the same cells reacting to cage A and cage B when they are 5 hours apart, but very different populations ... when they are 7 days apart."

For all they have learned using optogenetics, researchers have only begun to scratch the surface of how memories are created, retrieved, and lost; it isn't even clear if an engram actually represents the entirety of a memory or if it is simply a marker that points to a location elsewhere.

Tool development to answer such questions continues apace. Yet according to Cowansage, that's only the first step. "After a technology has been validated and tested, determining the best way to apply it to clearly answer a complex experimental question is often equally difficult," she says. For a structure as complex as the brain, that's hardly surprising.

Written by Jeffrey Perkel, Ph.D.

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