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Teasing Apart Brain Function, Neuron by Neuron

With a new imaging tool developed by scientists at the Howard Hughes Medical Institute's Janelia Farm Research Campus, researchers can watch individual neurons in the brains of living animals light up as they work together to control the animal's behavior. The tool offers a more detailed perspective on neural circuits that is crucial to understanding the functional architecture of the brain.

Even simple actions – such as moving one's hand or recognizing a familiar object – require the coordinated activity of sets of neurons. Of the billions of neurons in the human brain, only a thousand or so might be coordinating each of these tasks at a given time. But when it comes to knowing exactly which neurons are used to initiate and carry out such behaviors, “we have a kindergarten-level understanding of the brain,” says Loren Looger, the Janelia Farm scientist whose research team developed the new imaging tool.

Looger's group has created a molecule that can be manufactured inside an animal's nerve cells so that those cells light up under a microscope whenever they fire. The protein works by signaling the presence of calcium ions, a sure-fire way of knowing that a neuron is active. The new genetically-encoded calcium indicator molecule is far more powerful than earlier-generation tools for imaging neural activity in worms, fruit flies, and mice.

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- Loren L. Looger

The team has already distributed the indicator, which they call GCaMP3, to about 100 labs around the world for use in their experiments. With it,

scientists can look into the brains of live animals as they are running, eating, or doing other activities and pinpoint exactly which neurons are active and when. The number of tools currently available to track activity in neural circuits is limited, and this new molecule, Looger says, “is a game-changer for the study of what neurons are doing in awake, behaving animals, and how and why brains get wired up the way they do.”

Looger and his colleagues, including Karel Svoboda, Vivek Jayaraman, and others at Janelia Farm, HHMI investigator Cornelia Bargmann at The Rockefeller University, and collaborators at the University of Puerto Rico, published their research on November 8, 2009, in the journal *Nature Methods*.

Researchers use a variety of approach to watch neural activity in living animals – but each of these has its limits. Magnetic resonance imaging (MRI) can show activity in large regions of the brain and is one of the few techniques amenable to routine use on humans, but is not detailed enough to reveal which neurons are firing. Electrodes can also be inserted into the brain to measure the electrical activity of nerve cells – but electrodes are imprecise and invasive.

More recent tools take advantage of the chemical changes that occur inside neurons as those cells switch between active and passive states. When a neuron receives a message from another neuron, its chemistry changes, triggering a brief period of positive charge before returning to its inactive, negatively charged state. This so-called action potential occurs within two milliseconds—at the limit of current imaging technology. But in the last 30 years, researchers have focused on developing dyes and other indicators that light up in the presence of calcium – an indirect measure of electrical neural activity. Those techniques allow researchers to measure the increase in calcium that occurs inside neurons after the action potential fires.

The earliest calcium indicators were dyes and proteins that had to be injected into the brain. This method is invasive and slightly toxic to cells. Such dyes typically produce strong signals in the presence of calcium, but have a short useful lifespan. “The Holy Grail of neural activity imaging,” Looger explains, “is to be able to look at the function of your 10,000 favorite neurons simultaneously, and then reproducibly over an extended period of time.”

In the late 1990s, researchers genetically modified animals so that their neurons contained genes capable of generating calcium indicators. A major advantage to these genetically encoded calcium indicators is that they can be targeted to specific groups of neurons, a feat impossible with synthetic dyes. When the calcium level inside a neuron rises due to neural signaling, these indicator molecules bind to the calcium and start glowing. Looger’s GCaMP3 is the latest in this family of molecules.

Looger likens the chatter between neurons in the brain to the hum of conversation at a party: With the constant conversation going on, it is hard to figure out who said what. “The ideal situation at a cocktail party is to have 1,000 microphones spread around the room and know who is wearing each one,” he says. That is essentially how genetically encoded calcium indicators work. Instead of microphones, Looger’s team has endowed each members of a specific set of neurons with GCaMP3, which acts as the cell’s personal “flashlight.” Once the scientists have engineered an animal to express GCaMP3, they can watch the cells in a defined neural circuit flick on their molecular flashlights in sequence -- like a relay in which a glowing baton is passed from player to player.

Looger says earlier genetically encoded calcium indicators “didn’t really produce enough useful fluorescence signal to observe modest neural activity above the noisy background of an awake and behaving animal.” But last year, when Looger and a team of colleagues determined the molecular structure of an indicator known as GCaMP2 (the predecessor of GCaMP3)– both alone and bound to calcium – it became clear to them that some chemical tinkering could make it more useful.

Looking at the structure of GCaMP2, Looger could see exactly how the molecule grabbed on to calcium and turned this into brighter fluorescence. It did not take long before he and his team roughed out ideas for making modifications to the molecule that would make it grab calcium more tightly. They also identified a second set of modifications would make the molecule glow more brightly. Looger’s team made those adjustments and wound up with GCaMP3, which he says is three times brighter, three times more sensitive to calcium, and binds the calcium 1.3 times more tightly than GCaMP2.

With the new indicator in hand, Looger collaborated with Janelia Farm fellow Vivek Jayaraman to test GCaMP3’s power to track neural activity in the olfactory system of the fruit fly brain. “The tiny size of the fruit fly brain makes recording from its neurons with electrodes exceedingly hard,” explains Jayaraman. “GCaMP3 provides a non-invasive way to measure the activity of specific neurons with a bright signal that is unprecedented for such sensors.” Jayaraman says his research group uses the tool for their studies of the neural circuitry that guides visual processing.

GCaMP3 also proved useful in revealing neural activity in another popular animal model, the flatworm *C. elegans*. Cornelia Bargmann’s lab at Rockefeller genetically engineered worms that expressed the new calcium indicator in a neuron known to detect odors, and watched that neuron light up as certain smells were presented and taken away. The increase in fluorescence when the neuron fired was far more dramatic than the team saw with earlier generation GCaMPs.

Looger also collaborated with Janelia group leader Karel Svoboda to use GCaMP3 for imaging brain activity in the mouse. They engineered mice that

produced GCaMP3 in a group of neurons that processes information from just one of the animal's whiskers. They were able to watch 13 neurons within that group light up in a particular sequence as the mouse walked and the whisker moved. "The new indicator for the first time allows us to image activity in large populations of neurons in the intact brain, and to track the same neurons over time," Svoboda says. "We hope to be able to relate the dynamics of large neuronal ensembles to neural computation and learning."

Looger considers these experiments proof of principle. "For instance, we don't know which cell does what precisely, or which ones responds when the whisker hit something or at what angle." The movies of the mouse brain do reveal that in this tiny section of the brain dedicated to moving a single whisker, neurons seem to be performing a wide array of tasks. The Svoboda lab hopes that GCaMP3 will now let them get to the business of deciphering exactly what information this brain region is receiving, and how it quickly processes this information into a decision about what to do next.

Looger believes that GCaMP3 will be useful in finding answers to fundamental questions about the neural circuits that guide behavior. "For the foreseeable future," he says, "we need to focus on simple-ish, but profoundly important questions." For example, he says, tracking neural activity with GCaMP3 in the brains of living mice will allow scientists to ask: "When the mouse looked left, which 1,000 neurons were used? Which ones came on first? Then which ones next? Was it repeatable, so when the mouse did it again, was it the *exact same* 1,000 neurons? If two animals are under the microscope, do they both use the same 1,000 neurons to do this?" Teasing out the contributions of individual neurons to a simple behavior, he says, is "step zero in cracking the functional wiring diagram of the brain."