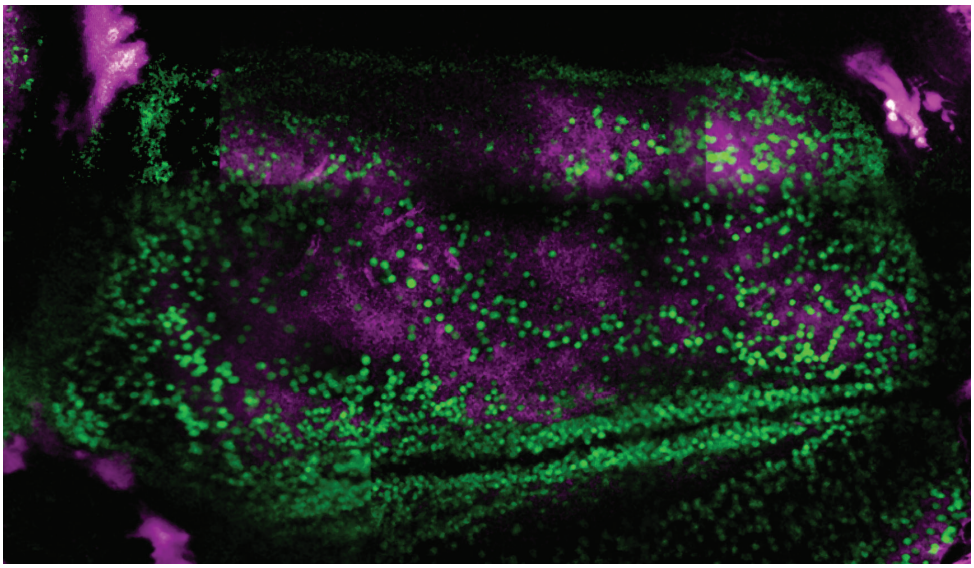
Imaging Brains of Mice On The Go



Scientists can now follow the activity of multiple neurons as a mouse sees, responds and behaves, thanks to advances in multiphoton microscopy.

It's one of neuroscience's biggest questions, and researchers have pursued it for decades: How do the firing patterns in specific neural circuits determine an animal's behavior? Early studies on excised brain slices only hinted at an answer, suggesting how firing patterns of individual neurons might change during activity.

To better answer this question, Bernd Kuhn set out to visualize neural firing patterns in an animal's brain as it reacts to stimuli or performs various behaviors. This required Kuhn, a neuroimaging expert at Okinawa Institute of Science and Technology Graduate University in Japan, to expand the capabilities of an imaging method called multiphoton microscopy.

Since its early days in the 1990s, multiphoton microscopy has helped reveal cellular changes during brain development or neurodegenerative disease in animal models. As it has improved, it has offered a window into the workings of the cerebral cortex — the brain's outermost layer — which is responsible for a variety of essential and higher functions.

To help neuroscientists discover what goes wrong in mouse models of Alzheimer's, Parkinson's, and other brain disorders, Kuhn set himself a challenging task: to build a foundation of knowledge about how the brain integrates sensory, motor, and cognitive information as mice perform a variety of actions. This meant observing a deep, previously inaccessible layer of the visual cortex that communicates with an even deeper layer of the brain called the thalamus, and doing so for more than an hour as each mouse ran, slept or woke.

Recently, Kuhn succeeded. As a mouse ran on a moving ball trying to reach a reward of water, with its head fixed in place, a behavioral camera observed its eyes to gauge alertness, while also measuring how often it licked its whiskers and other parameters. Kuhn then matched these behaviours with neural activity across more than two hours of observations — long enough for the mouse to tire and fall asleep, he and his colleagues reported in Current Biology in October.1 The experiment must last that long to observe the different behavioural states, Kuhn says.

"It's a beginning for us to find a connection between behavior and neuronal activity," Kuhn says. "You need this as a base for understanding what is going wrong if you have any neuronal disease."

Penetrating vision

Multiphoton microscopy addressed a longstanding problem in biological microscopy. Scientists often add fluorescent markers to cells to help spotlight particular structures. But in confocal microscopy, then the state-of-the-art method, the visible light used to excite these fluorophores can damage brain tissue. In the early 1990s, Winfried Denk and the late Watt Webb at Cornell University figured out how to avoid that damage by exciting the fluorophores with infrared light instead of visible light. Infrared light is less energetic, so it takes two photons rather than one to excite the fluorophore. But because it is less energetic, it can penetrate deeper into the brain without damaging tissue. This let neuroscientists label specific types of neurons with a fluorescent protein or other fluorescent marker, then illuminate buried neural circuits by shining infrared light through a transparent window placed in the animal's skull.

To monitor neural circuits while simultaneously tracking behaviour, Kuhn took advantage of an imaging platform he had spent five years co-developing with Sutter Instrument, a Novato, California-based scientific instrument company. It includes Sutter's movable objective microscope (MOM) — a two-photon instrument that enables spatial scanning by moving an objective through space and using lasers to spotlight one small area at a time. Meanwhile, the platform records behaviours with a camera for up to three hours, and uses the company's proprietary MScan software to control the experiment and integrate the data.

This builds up a 3-D picture of as many as 10,000 neurons over a wide area of tissue — an ability that helped Daniel Dombeck, a neuroscientist at Northwestern University in Evanston, Illinois, and his team make a surprise discovery.To pinpoint circuits that might enable a mouse to monitor its own activity, the researchers placed each mouse on a rolling ball under the microscope with its head fixed in place, and projected a virtual-reality maze around it. As the mouse ran through the virtual maze, they recorded its neural activity to see which brain circuits were actually in use.

Sure enough, the study revealed a circuit that lit up when a mouse was active. But it also revealed nearby neural circuits that lit up after the animal stopped moving. Those circuits, they showed, kept rough track of how long the mouse had rested, he and his team reported in Neuron.2 The newly discovered circuits sit in the part of the brain where Alzheimer's starts. Since healthy people estimate time better than those with early-stage Alzheimer's, observing those circuits could lead to a simple test for early stages of the disease, Dombeck suggests.

Visualizing the deeper brain

To peer even deeper into the brain, researchers are now beginning to excite buried fluorophores with three photons that have even longer wavelengths and lower energy. Two-photon microscopy uses 800-1100 nm photons that can penetrate 0.5-1.0 mm into the brain. But Chris Xu, a physicist at Cornell University, and his colleagues use three photons with wavelengths of 1,700 nm that can penetrate up to 1.5 mm into brain tissue without scattering. The method also focuses the excitation light more sharply, leaving less stray light to blur the image.

"That additional depth is actually quite important," says Jack Waters, a researcher at the Allen Institute for Brain Science. "We're able to access much of cortex with a two-photon microscope. But certainly there are circumstances where you really can't get to the deeper layers of that network. Then you may need to go to the three-photon." Three-photon microscopy — which currently relies on custom-built or customized setups— can even see through a skull and image about 0.5 mm into the brain. Xu and neurobiologist Joseph Fetcho, who studies autism, recently used a three-photon microscope to image the brain of a live adult zebrafish.3 Zebrafish are popular in brain-development studies because their skulls are translucent when young, but they grow opaque with age. Peering through the skull could help better compare an adult with a youngster.

Three-photon microscopy can also explore the brains of larger animals. Waters has colleagues aiming two-photon microscopes at a macaque's visual cortex, which resembles that of a human. But this method only penetrates to layer two of the six-layer cortex. Much of the interesting activity takes place in layers four and five. With the three-photon technique, he says, "we're expecting to be able to image most of the way, perhaps all the way, through the cortex."

The lasers used in multiphoton microscopy are continuing to advance, as are the fluorescent labels and the methods of delivering them, and the technology can now visualize neurons and synapses across larger brain volumes in multiple areas of the brain. As the technology improves, and the industry provides more turnkey systems, it will help answer even more questions in brain science, Kuhn says. "There are many people doing this," he says. "It's just too exciting for only a few people to do this."

To learn more about multiphoton microscopy, click here.

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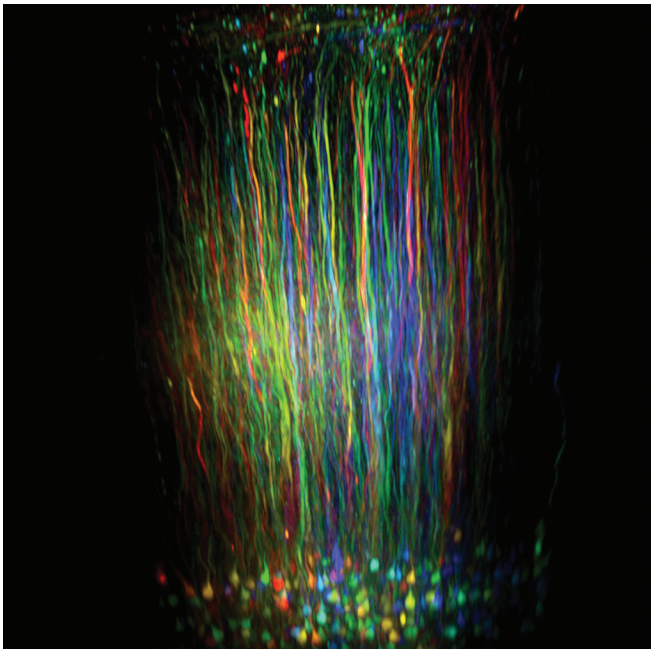

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