Janelia researchers are working their way up from simple to more complex organisms to measure brain activity.

by Helen Fields | photo illustration by Fredrik Broden

To Tim Harris, understanding the brain is like understanding a building—a really big building—from the vantage point of the sidewalk. "The brain is the Empire State Building, and it's opaque. You're standing there looking at the outside and wondering: is the hot water faucet on the third sink of the 65th-floor restroom on the left or the right?"

This is the situation neuroscientists find themselves in, Harris says. They can see your head, they can see you sensing your environment and doing things, but they have only the murkiest sense of your brain's inner workings. Harris, a physicist at HHMI's Janelia Farm Research Campus in Ashburn, Virginia, develops tools neuroscientists can use to measure the brain's activity, to give them a quantitative view inside the elaborate structure of the brain.

Harris spent the early part of his career at Bell Labs, where he developed optical methods for studying semiconductors. Later, at Helicos Biosciences and elsewhere, he became interested in biological measurements that generate huge amounts of data. He sees neuroscience as one big measurement problem. All science depends on good measurements. But the unbelievably complex brain makes measuring particularly challenging. The human brain has more than 80 billion neurons, and each neuron can have 10,000 connections to other neurons. There's no way to measure the whole thing at once.

Taking it apart, however, isn't the answer. The brain is a live, working system; cut out a piece and you're left with a blob of goo. Then there's the problem of the unyielding skull. Cutting a hole in it opens a window to the electrical signals that carry information but offers only a limited view: "If I punch a hole in a wall and look through the hole, I can see many things. I'm not sure what fraction of them are engaged in my problem and what fraction are not relevant to my problem," Harris says.

To study the brain, he adds, "the question is, where did the electricity go and when did it go? The essence of all neuroscience is summed up in that one thing." Since it's impossible to work out the entire human brain at once, Harris and the other instrument experts at Janelia help neuroscientists figure out what they can measure and how to do it. They're getting at the brain by studying simpler animals, like nematodes and fruit flies, with tools that can measure electricity either directly, with an electrode, or indirectly, with proteins that light up when an electrical pulse goes by.

Start Simple

One way to understand a behemoth like the Empire State Building, Harris says, is to first figure out the workings of a one-room, mud-brick hut. In neuroscience, that's the nematode *Caenorhabditis elegans*. The tiny, see-through worm has 302 neurons—much easier to study than a human brain. Rex Kerr, a fellow at Janelia Farm, is trying to understand how worms do what they do. And one of the tools he's using to measure the worm's brain was developed at Janelia by group leader Loren Looger's team: GCaMP3, a protein that lights up in the presence of calcium and is now used in labs throughout the world.

Neurons make their electrical impulses by moving ions around. One of the main ions is calcium. GCaMP3 is a kind of protein known as a genetically encoded calcium indicator, or GECI. The cell is engineered to express GCaMP, so when a blue light is shined on it, the GCaMP lights up—giving off green light—when it detects calcium. These proteins let neuroscientists see electricity in the brain, with the help of a microscope.

"The challenge here is that we have neurons in three-dimensional space," Kerr says. A worm's brain is tiny and clear, but it's still 3-D, with cells stacked on top of each other and intertwined. With instrument design experts at Janelia, Kerr developed a microscope that can image the whole brain. A laser sweeps through the brain over and over, lighting it in sheets from the side. As the laser beam touches each level, it hits the GCaMP3 proteins and they fluoresce, sending light to the waiting microscope to record which neurons are active.

Kerr can measure neuron activity in live worms while they are sensing the environment. An individual worm is placed under the microscope lens and herded into a wedge-shaped chute like a sheep waiting for a vaccination. A researcher uses a setup of syringes to squirt chemicals past it—and then watches to see how neurons that have been engineered to make GCaMP3 react to, for example, a scent that the nematode associates with food.

For now, the worm has to be stuck in a chute to line up its brain just so with the laser and microscope lens. But Kerr's dream is to be able to take a dish of free-swimming worms, "and tell the scope, 'Follow that worm! Tell me what it's thinking wherever it goes.' Or tell me what that small subset of neurons is doing wherever it goes." He's working on a system to do this—it involves putting the dish on a platform that tracks the worm's movement and moves the plate so the worm's head stays centered under the lens. He already has a system that can track worms as they squirm around under a microscope (see Web Extra, "Follow that Worm").

Kerr thinks it might be possible to learn how a worm does what it does in the next decade or so. And those lessons could be applied to understanding more complicated animals.

Moving on Up

It's still just a worm, but Tim Harris says that's a good start. "Learning how to build a one-story, mud building is a pretty good idea," he says. "Then people think, 'ok, so, mud is never going to get us to the Empire State Building. We've got to learn how to build using bricks and do plumbing and all that jazz.' So that's now another measurement problem that's even harder."

A fruit fly brain is a lot easier to study and less complex than a human brain, but more complicated than a worm brain. When dealing with a lot more neurons, you want more measurements. It's possible to buy a probe from a supply company with many tiny wires on the end. Ease it into the brain and the tip of each wire records the electrical impulses around it. The probe can record data for many neurons at once, Harris says. "But, you're still poking a stick into a brain. You've probably caused some damage. We'd rather have a magic microscope that could see through the brain and measure the electricity, but we don't know how to make that."

Instead, he's making better probes. Along with fruit fly researcher Vivek Jayaraman, Harris and Mladen Barbic in his group have developed smaller, skinnier probes for fly brains. Because they're 10 times narrower than commercial probes, they destroy less tissue on the way in, and the tips of the wires are tiny, suited to flies' small neurons.

Like Kerr, Jayaraman wants to measure neuron activity in flies living in a sort of virtual reality arena. An individual fruit fly is glued by its head to a bracket and then allowed to fly or to walk on a ball, like a treadmill. Meanwhile, the researchers display moving patterns on a U-shaped bank of light-emitting diodes designed by Janelia group leader Michael Reiser. The fly sees and reacts to those patterns, trying to walk or fly toward a fixed line or fly straight when it seems the world is moving to the left.


REX KERR (LEFT) CAN MEASURE NEURON ACTIVITY IN WORMS AS THEY SENSE CHEMICALS IN THEIR ENVIRONMENT. TIM HARRIS SAYS WORMS AND FRUIT FLIES ARE A GOOD START FOR EVENTUALLY ANSWERING QUESTIONS ABOUT THE BRAINS OF LARGER ANIMALS.

Kerr: James Kegley Harris: Paul Fetters

Crucially, the top of the fly's head is open and bathed in saline under a microscope; a researcher removes a smidgen of the fly's cuticle, and nudges a probe into the working brain. Harris's improved probes should help Jayaraman get better measurements from neurons and understand more about how the brain makes decisions.

Illuminating Windows

The next step on the way up to the Empire State Building, Harris says, is the mouse. "The mouse brain is even bigger, with even more neurons. So you have to study smaller parts of it to understand what's going on."

Karel Svoboda, a group leader at Janelia Farm, studies mouse brains. His team builds a tiny glass window into each animal's head. This doesn't seem to bother the mice, and the researchers can follow one mouse for months as its brain changes to accommodate its new knowledge.

He uses GCaMP3 and other tools to measure electrical activity in mouse brains. But he says the tools available to do neuroscience today still aren't good enough. "In brain research, we make up a lot of stories based on incomplete information," Svoboda says. "We're still looking at large populations of neurons, but we have only probed a small part of the brain. In many ways we're still very much limited by measurements."

As part of the GECI project at Janelia, Svoboda, Jayaraman, and Kerr are working with protein engineer Looger to develop improved versions of GCaMP3. The new proteins should be better at binding calcium, so they will respond when there's less calcium. They hope newer versions will also light up sooner after calcium rushes into the cell. And while the current version can impair cells when it builds up, the next proteins may do less damage.

"The major discoveries of neuroscience in the modern era correlate directly with advances in measurement technology," Svoboda says. Around the turn of the 20th century, Spanish physiologist Santiago Ramón y Cajal perfected a technique for looking at slices of brains and determined that brains were made of cells. Neuroscientists figured out some basics about how the visual cortex works because they invented a technique for recording electrical signals from cells.

This work continues at Janelia Farm, as its neuroscientists keep working to understand the brain. Harris thinks neuroscientists won't understand the human brain for a thousand years, at least; but with new tools, they can keep chipping away at the problem—and make a little bit more sense of what goes on inside our heads. ■

WEB EXTRA: To learn how scientists quantify worm behavior and study salamanders at dinner, visit www.hhmi.org/bulletin/aug2011.