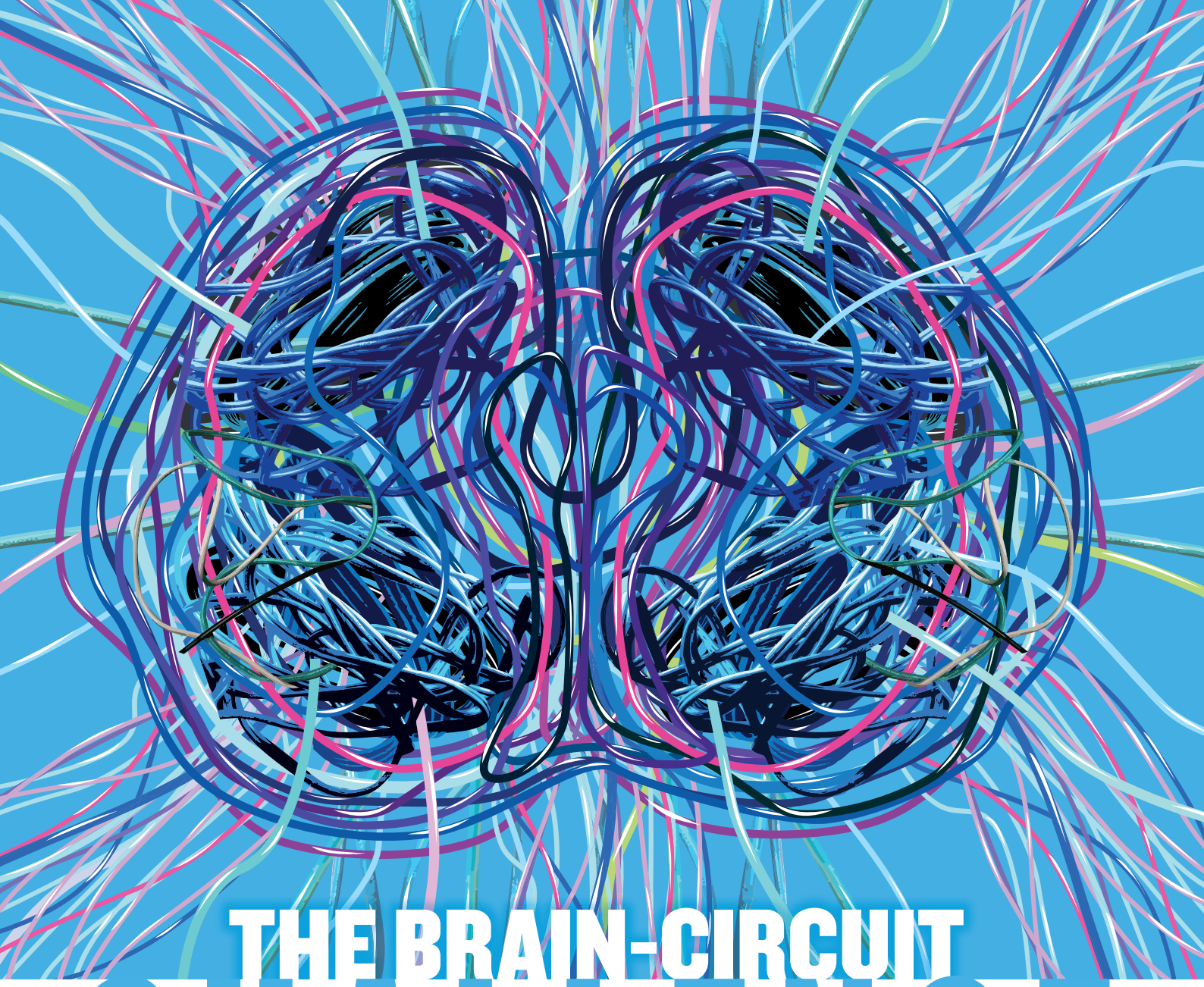


THE BRAIN-CIRCUIT CHALLENGE

EVEN THE SIMPLEST NETWORKS OF NEURONS DEFY UNDERSTANDING. SO HOW DO NEUROSCIENTISTS HOPE TO UNTANGLE BRAINS WITH BILLIONS OF CELLS?

BY KERRI SMITH

Marta Zlatic owns what could be the most tedious film collection ever. In her laboratory at the Janelia Research Campus in Ashburn, Virginia, the neuroscientist has stored more than 20,000 hours of black-and-white video featuring fruit-fly (*Drosophila*) larvae. The stars of these films are doing mundane maggoty things, such as wriggling and crawling about, but the footage is helping to answer one of the biggest questions in modern neuroscience: how the circuitry of the brain creates behaviour.

It's a major goal across the field: to work out how neurons wire up, how signals move through the networks and how these signals work together to pilot an animal around, to make decisions or — in humans — to express emotions and create consciousness.

Even under the most humdrum conditions — "normal lighting; no sensory cues; they're not hungry", says Zlatic — her fly larvae can be made to perform 30 different actions, including retracting or turning their heads, or rolling. The actions are generated by a brain comprising just 15,000 neurons. That is nothing compared with the 86 billion in a human brain, which is one of the reasons Zlatic and

ILLUSTRATION BY DANIEL HERTZBERG

her teammates like the maggots so much.

"At the moment, really, the *Drosophila* larva is the sweet spot," says Albert Cardona, Zlatic's collaborator and husband, who is also at Janelia. "If you can get the wiring diagram, you have an excellent starting point for seeing how the central nervous system works."

Zlatic and Cardona lead two of the dozens of groups around the world that are generating detailed wiring diagrams for brains of model organisms. New tools and techniques for slicing up brains and tracing their connections have hastened progress over the past few years. And the resulting neural-network diagrams are yielding surprises — showing, for example, that a brain can use one network in multiple ways to create the same behaviours.

But understanding even the simplest of circuits — orders of magnitude smaller than those in Zlatic's maggots — presents a host of challenges. Circuits vary in layout and function from animal to animal. The systems have redundancy that makes it difficult to pin one function to one circuit. Plus, wiring alone doesn't fully explain how circuits generate behaviours; other factors, such as neurochemicals, have to be considered. "I try to avoid using the word 'understand'," says Florian Engert, who is putting together an atlas of the zebrafish brain at Harvard University in Cambridge, Massachusetts. "What do you even mean when you say you understand how something works? If you map it out, you haven't really understood anything."

Still, scientists are beginning to detect patterns in simple circuits that may operate in more complex brains. "This is what we hope," says Willie Tobin, a neuroscientist at Harvard Medical School in Boston, Massachusetts: "that we can come across general principles that can help us understand larger systems."

> "I DON'T EVEN KNOW IF THERE IS A WORD TO DESCRIBE THIS. IT'S BEYOND PETABYTES."

CIRCUIT TRAINING

The simplest brain for which scientists have the full wiring diagram is that of the nematode worm *Caenorhabditis elegans*, which has just over 300 neurons. Its connectome — a map of every single neural connection — was completed in the 1980s[1]. But getting a close look at those connections in action has been difficult. And some neuroscientists are sceptical that the worm brain works in the same way as larger brains.

That's why many, like Zlatic, have relied on another invertebrate bastion of the biology lab, the fruit fly. *Drosophila* larvae are complex enough to display some interesting behaviours, but have few enough neurons to make a circuit-mapping project feasible. Plus, Zlatic and her colleagues have a suite of techniques such as optogenetics, in which light-sensitive proteins are used to control or monitor neuronal activity as the flies go about their business.

Zlatic and Cardona are developing methods for collecting high-resolution cross-sectional images of the larval fly brain, and for automating the laborious process of tracing all the connections from section to section. Then, by matching up behaviours and activity patterns with their maps, the teams can find out which circuits contribute to which behaviours.

One puzzle, for example, is how brains choose between two competing actions. Last year, Cardona, Zlatic and their teams traced the circuitry that allows maggots, when faced with an annoying puff of air, to choose between scrunching their heads in or bending them away[2] (the same animal, puffed with air twice, might choose to bend the first time and scrunch the next). The teams identified which neurons they thought were responding to the air-puff, and used optogenetics to activate them in turn. They could watch the circuit for scrunching become inhibited while the one for bending strengthened, all in the space of a few milliseconds. Then they built a computational model that predicts the response when larvae are stimulated in a particular way.

Plenty of labs are studying the adult fruit-fly connectome, too. The whole brain, at 135,000 neurons, is too big to reconstruct in its entirety, so instead scientists are looking at smaller pieces of the nervous system, where they can study the wiring and the activity together.

Tobin, for example, works with a speck of the fruit-fly brain that helps to process odours — a circuit called an olfactory glomerulus. The fly brain has 50 such glomeruli, each hosting a few dozen neurons in a region measuring no more than 20 micrometres across, and each split in half to receive signals from the fly's left and right antennae. In Tobin's latest study[3], published in May, he and his team took one glomerulus, sliced it finely and used electron microscopy to reconstruct the layout of all 50 neurons of a particular type, including which others they connect to and how strongly. Comparing the two halves revealed some noticeable differences in the number of cells and the wiring, even though the function of the circuit was unchanged.

Tobin suggests that the circuit's wiring is compensating for vagaries of development that led to the two halves looking slightly different. This robustness, he says, is likely to be a general characteristic of all brains, and could be lost in some disorders. "Disease is a failure of robustness that the system has been unable to compensate for," he says.

Engert is focusing his efforts on the brain of the larval zebrafish (*Danio rerio*), which has about 100,000 neurons. In May this year, his team published[4] a reconstruction of a whole larval zebrafish brain, and used it to look at the paths that similar neurons take as they extend and connect during development. They expected some degree of randomness in the journey from the brain to the spinal cord, because in mammals such projections often become tangled and haphazard. But the zebrafish neurons they surveyed stayed together in bundles, and took mirror-image routes on each side of the animal. What seems to be important to guiding these cells, Engert says, is their genetic programs. These wiring cues "are much more dogmatic than we thought previously", he says.

Some teams are building up circuit diagrams for regions of the mouse brain. In 2014, for instance, a group led by Sebastian Seung, now at Princeton University in New Jersey, published a map of neurons and their connections in the mouse retina[5]. By looking at the shapes of neurons and the connections they made — star-shaped neurons have more synapses than have neurons with fewer branches, for example — the team could speculate about how the cells were passing signals along. Some of the newly mapped cells were known to send signals to others with a time delay, which might explain how the eye transmits information about an object in motion.

TRAFFIC JAM

If neural circuits can teach one lesson, it is that no network is too small to yield surprises — or to frustrate attempts at comprehension. For 30 years, neuroscientist Eve Marder of Brandeis University in Waltham, Massachusetts, has been working on a simple circuit of 30 neurons in the crab gastric system. Its role is simple and the wiring diagram has been in hand for decades. Still, the circuit has mysteries to offer. Marder has shown, for instance, that although the circuits of individual animals may look the same and produce the same output, they vary widely in the strength of their signals and the conductance at their synapses[6]. Today, she is preoccupied with how circuits maintain their identity over time, as things such as ion channels and receptors are replaced. "What rules do you use to replace all the components while maintaining a circuit?" she asks, adding that all these challenges will also apply to larger networks. "We are so far from knowing how to confront the kinds of information you get from an animal behaving and doing a complex task."

Scientists are preparing for that confrontation. The effort has required

several new ways of collecting and analysing data, and these have come into their own in the past five years or so. Zlatic's group worked with others at Janelia to fine-tune its optogenetic tools. And to analyse the maggot videos, Zlatic enlisted statisticians and computer scientists who specialize in machine learning to devise ways of classifying the larvae's movements.

Then, in Cardona's lab, scientists worked through mapping the larval brains, compiling thousands of images of brain slices taken with electron microscopes and painstakingly tracing the connections between neurons. This map forms the starting point for the rest of their efforts — map the circuit, manipulate the circuit, watch the behaviour (see 'Connecting the dots'). On page 175, the team uses this protocol to reveal how a circuit in the *Drosophila* brain called the mushroom body controls learning and memory, by linking feelings of reward or punishment with sensory information[7]. But the mapping process is a big hold-up in the field right now, Cardona says. Reconstructing a 160-neuron portion of the fly smell-detection circuit for another paper[8] took Cardona's team more than 1,100 hours. One estimate[9], extrapolating from previous fruit-fly work, suggests that a map of the full adult fly brain would take hundreds of person-years to complete. Automating the process would help, but algorithms can add bogus connections or miss some entirely.

Those working on larger circuits often break the problem down — assembling a list of cell types first. The Mouse Brain Connectivity Atlas at the Allen Institute for Brain Science in Seattle, Washington, is taking this approach. In work published in 2014, the team identified[10] 49 types of cell in the mouse visual cortex alone; the cells vary in size and shape, how fast they fire and what genes they express. The team expects orders of magnitude more cell types across the whole brain. "Up to 10,000 neuronal types would be my guess," says Hongkui Zeng, who works on the atlas at the Allen Institute.

When asked to estimate the amount of data required to map the whole mouse brain, Zeng first laughs. Then she says: "It's going to be astronomical numbers. I don't even know if there is a word to describe this. It's beyond petabytes. Petabytes of petabytes."

That quantity of data would be generated by just one animal's connectome, but many scientists would like to get to a point where they could produce several and compare them. Tobin thinks that different animals' wiring maps are likely to show important — and perhaps functionally interesting — differences. So far, "it's been a land of $n = 1$", he says.

Another priority on many neuroscientists' wish lists is recording from lots of neurons simultaneously. In that way, researchers could stimulate one neuron and see which others are activated, then build up a dynamic picture of the chain of command that leads to behaviour. That will be "the next huge challenge for the more complex brains", says Zeng.

Even in the 30-cell circuit favoured by Marder, this is still hypothetical. Marder can stick electrodes into a handful of cells at once. Others studying small circuits use various techniques to provide a proxy for which cell is firing and when. For example, researchers can measure calcium released from neurons after they fire, or look at fluorescence in response to a change in voltage across a cell's membrane. But this is like measuring the speed of a car by the strength of the breeze it creates: the proxies aren't as fast as the firing rate itself. "Right now you can record from all neurons but a bit slowly, twice a second," says Zlatic. "Things happened in between that you missed."

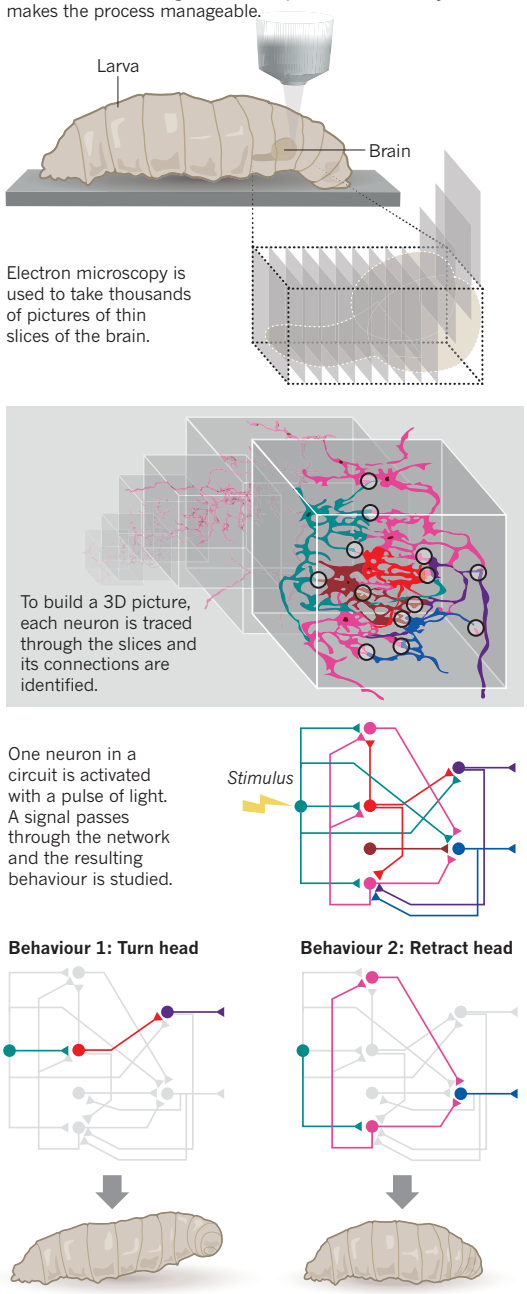
Grasping the dynamics of circuits with more precision could help inform medical questions. Marder has spent 25 years teaching students about brain networks, including those drawn up by specialists in Parkinson's disease. "The more I stare at their circuit diagrams, the more the paths connect." She admits that the details of the circuit don't really matter if the treatment works, but they might help get to the bottom of why drugs are effective in some people and not others, or what correlates with success. Clinical evidence suggests that different people with Parkinson's disease have different underlying abnormalities in certain brain regions and circuits[11].

But some researchers find it short-sighted to insist on clinical relevance, arguing that the circuit-mapping quest is worthwhile in its own right. "It's difficult for me to develop a research plan that will end at the bedside," says Engert of his work on zebrafish.

For now, at least, many researchers are content to embrace the dizzying complexity of the task at hand. Zlatic takes some comfort in the fact that she is starting to see repeating patterns in how neurons in her fly larvae arrange themselves and how they create feedback loops. This modular arrangement, she says, could make the going easier once the team has a finished map. "When you have partial information it looks like a big mess," she says. "Maybe the most surprising thing is that once you start seeing a relatively complete system, how much sense it makes." ■

CONNECTING THE DOTS

To find out how the brain creates behaviour, researchers follow a multistep process, combining a neural wiring diagram with data on behaviour. Working with the simple brains of fruit-fly larvae makes the process manageable.



NIK SPENCER/*NATURE*

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