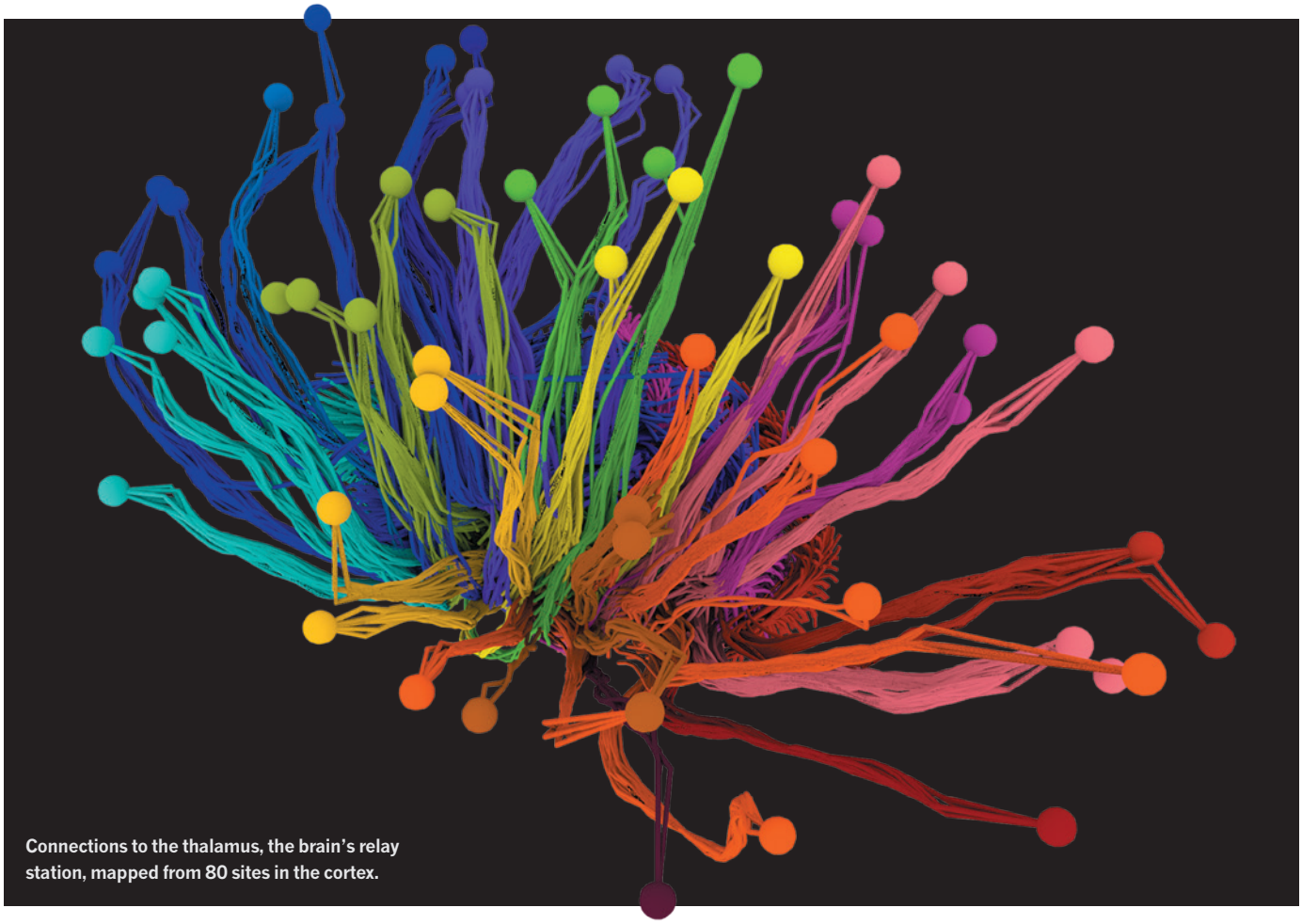
TECHNOLOGY FEATURE

CONNECTOMES MAKE THE MAP

Working at a variety of scales and with disparate organisms and technologies, researchers are mapping how parts of the brain connect.

ALLEN INSTITUTE FOR BRAIN SCIENCE



Connections to the thalamus, the brain's relay station, mapped from 80 sites in the cortex.

BY AMBER DANCE

A newborn baby, well fed and sleepy, is swaddled in a blanket and lying on what looks like a tea tray with a helmet attached to one end. Once the infant falls asleep, researchers pull special tabs on the blanket to ease the baby into the helmet. It is a customized receiver coil used for magnetic resonance imaging (MRI), a common method for visualizing brains in living people. The researchers slide the baby-holding contraption along a special trolley into the MRI tube and start collecting images.

From about 1,000 such scans, and another 500 of developing fetuses, UK scientists in the Developing Human Connectome Project plan to map how regions of the brain communicate with each other during development. They then hope to work out why preterm babies are at risk for conditions such as autism spectrum disorder or attention deficit hyperactivity disorder, and perhaps to do similar scans to check whether methods to prevent such disorders are working.

The project is one of many to unravel the 'connectome', the links between the brain's hundreds of areas and millions of neurons. "The days of just looking at one part of the brain are waning," says Arthur Toga, director of the Laboratory of Neuro Imaging at the University of Southern California (USC) in Los Angeles. He and other scientists are already starting to compare the connections in healthy brains with those of people who have connectopathies, diseases caused by aberrant connections, such as schizophrenia, or disrupted connections, like Alzheimer's disease.

The subjects studied by connectome researchers range from living people to the preserved brains of tiny animals such as worms ▶

and flies. The investigative technologies range from MRI scanners to light microscopes and electron microscopes. Irrespective of the specifics, scientists — with the aid of computers — painstakingly chart connections to build an atlas. The map-makers hope that revealing the connectome's structure will help neuroscientists to navigate as they work out how different parts of the brain function together.

Like traditional cartography, brain mapping is a matter of scale (see 'Maps across magnitudes'). Researchers such as Toga who study the brains of living people are limited to a global view. "It's basically a fly-over at 39,000 feet," Toga says. This approach, called macroscale by some, shows how bundles of axon fibres connect large regions together. With millimetre resolution, it is like a country map that marks major highways. Scientists studying animal brains slice by slice get more detail. At this mesoscale, researchers see how smaller regions of the brain communicate along single axons at micrometre or submicrometre resolution. It is like adding in the lanes of highways and local streets. Finally, microscale images reveal individual neurons and synapses at resolutions of a few nanometres — akin to a map that shows even footpaths and stepping stones.

FLY-OVER

In a major effort to visualize the brain's superhighways, 100 researchers across 10 institutions are close to wrapping up the 5-year, US$30-million Human Connectome Project (HCP), funded by the US National Institutes of Health[1]. By early 2016, they expect to complete MRI scans on 1,200 healthy young adults. They recruited twins — both identical and fraternal — and their non-twin siblings to investigate how patterns in brain connectivity might be inherited; they also collected data such as IQ scores and smoking habits to look for correlations with the connectome. By the end of the project, they will have amassed a petabyte's (10^{15}) worth of pictures.

HCP researchers image the basic structure of the brain and bundles of axon fibres. They measure blood oxygen levels across the brain as an indicator of activity, looking for areas that fire as people perform tasks or just zone out. Brain areas that are active at the same time are likely to work together.

To get the most information out of each subject, HCP collaborators worked with Siemens Healthcare in Erlangen, Germany, to soup up a standard MRI scanner. It generates a 3-tesla magnetic field — comparable to that in standard machines — but can control the field more precisely. MRI scanners use gradients of magnetic fields to aim at parts of the brain, and the stronger gradient of the HCP machine offers faster imaging and better resolution. That creates more-detailed images of axon bundles. A version of their machine is now available commercially, known as the MAGNETOM Prisma.

Many standard MRI machines collect images through the brain one slice at a time, but others, including the HCP one, collect eight cross-sectional images of the brain at once, helping researchers see which brain regions are working at the same time. HCP collaborators have also scanned some subjects with a 7-tesla machine, getting even higher-quality data.

In a separate arm of the HCP project, Toga and another group of collaborators are pushing MRI technology in another way. They are improving how machines visualize axon bundles by making use of the restricted movement of water molecules within them. Such diffusion imaging can typically detect water moving in no more than 64 directions. With the HCP's diffusion spectrum imaging software, MRI machines detect hundreds of directions, and thus reveal smaller axon bundles than regular MRI can.

But high-quality images are not enough. To compare images between subjects, researchers use academic-written software such as FSL and FreeSurfer to stretch and squeeze each brain image into a standard shape. The programs must also track how each image was warped, because therein lie key data on what differentiates one human brain from another.

Wide-scale comparisons are planned. The 1,200 young adults, aged 22–35, who were scanned for the HCP are just the beginning. The NIH will now fund projects that look at children and older adults. Combining those connectomes with results from the Developing Human Connectome Project, scientists will have brain maps of the whole human lifespan. The NIH also plans to sponsor work focusing on people with particular diseases or genetic profiles.

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Despite the advances, David Van Essen, a neurobiologist at Washington University in St. Louis, Missouri, and co-leader of the HCP, cautions that MRI images can only approximate the wiring of the brain. Scientists working at the mesoscale level get more detail by using light microscopes to look at brain slices.

At this scale, scientists work to pick out groups of neurons and their outgoing axons. Mesoconnectome cartographers therefore inject tracers to label a specific brain region and its conversational partners. Most of the work is in mice, but some researchers are getting started with marmosets, primates the size of kittens.

At the Allen Institute for Brain Science in Seattle, Washington, Hongkui Zeng and her colleagues put together a mesoconnectome by injecting the brains of living mice with viruses carrying the gene for the glowing marker green fluorescence protein (GFP). The neurons at each injection site accumulate GFP along their axons, and so point to the other neurons that they communicate with.

To image the brain, microscopists typically slice it as thinly as possible, but that can mangle the tissue and reduce the quality of the image. Zeng therefore uses a technique called serial two-photon tomography[2] in a system called the TissueCyte 1000 that she helped microscope company TissueVission in Cambridge, Massachusetts, to design. In conventional microscopy, GFP requires only one photon to fluoresce, but Zeng's set-up requires the marker to take a double hit. Any wayward photons that flow above or below her plane of focus make no difference to the image, because it is unlikely that two off-target photons will hit the same GFP molecule.

The next trick is that sections are scanned before they are sliced. Researchers embed the mouse brain in a stabilizing matrix of agarose, then image just below the top surface. A cutter integrated into the microscope then shaves off a 100-micrometre-thick section, and the microscope images beneath the new top surface, repeatedly, all through the brain. "It's never damaged before we do the imaging," says Tim Ragan, president of TissueVision. The system can scan a whole mouse brain overnight, Zeng says.

But imaging is only part of the battle. The microscope yields 2D sections of 3D axons that are tangled together. Scientists — or rather, their computer algorithms — must examine each section, tracking the axons through more than 100 images. That can take more time than the scanning.

USC neuroscientist Houri Hintiryan, who is working to generate a mouse mesoconnectome using multiple coloured tracers[3], says that the gold-standard tool for this analysis is the human eye. She spends a lot of time lining up structures in sequential images. "That is very exhausting," she says. "However, that is probably the most reliable way to do it up to this point."

Eventually, however, the process will need to be automated, says neuroscientist Partha Mitra of Cold Spring Harbor Laboratory in New York. "One has to build a virtual neuroanatomist," he says. "I want a machine to look at the slides and make sense of them." He and other researchers are working toward this goal. At the Allen Institute, Zeng's team already uses in-house software that quantifies the GFP signal in blocks measuring 25 micrometres per side, about the size of a single cortical neuron, rather than tracing individual cells directly. The team has already processed images from more than 2,100 mice, and made the data available online. It expects to add connection information from hundreds more mice over the next couple of years.

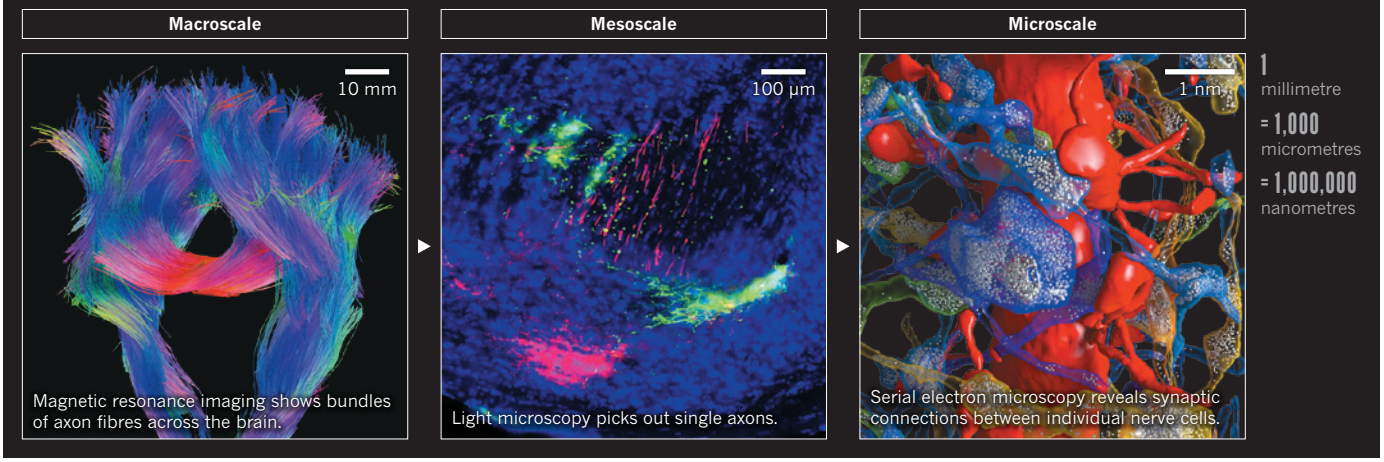
ZOOMING IN

Even the mesoscale connectome offers only part of the brain's story. Microscale neural map-makers want to see connections between neurons — individual synapses where an outstretched axon meets a spiny dendrite. Every neuron talks to thousands of others, so each might have thousands of synapses.

And scientists still want to look at wide sections of the brain. "This is an attempt to

MAPS ACROSS MAGNITUDES

Some connectome researchers track large-scale connections in living brains; others drill down into finer details using thinly sliced brain tissue.



MACROSCALE: TOGA, USC; MESOSCALE: MOUSE CONNECTOME PROJECT, USC

BERGER, KASTHURI, LICHTMAN HARVARD UNIV.

step back, but keep the detail," says Moritz Helmstaedter, director of the Max Planck Institute for Brain Research in Frankfurt, Germany. "This is why it's an enormous endeavour."

For this, researchers rely on electron microscopy. In the Fly EM project at the Howard Hughes Medical Institute's Janelia Research Campus in Ashburn, Virginia, collaborators use focused ion beam scanning electron microscopy (FIB-SEM) for a serial approach analogous to what Zeng does with light microscopy. They scan the top of a fruit-fly brain, then use the ion beam to sandblast just 8 nanometres off the top before scanning again, then repeat all through the brain for a total of about 500,000 slices.

It takes two or three years to section and image just one fly brain with FIB-SEM, although the researchers can reduce that time by splitting the task between a few microscopes. Fast machines are essential for larger mouse brains, and so Carl Zeiss Microscopy collaborated with connectome scientists to develop the Zeiss MultiSEM microscope. The device uses not one electron beam, but 61 or even 91, so it can do the work of dozens of electron microscopes at once. Imaging one square millimetre of tissue, in a single plane, takes just eight minutes, says Stephan Nickell, a product manager at Zeiss in Oberkochen, Germany. By tiling images together, users can get a picture representing a slice of brain that is several millimetres, and sometimes even centimetres, across, but can still zoom in for nanometre-scale details[4].

Again, the hard part is the data processing, and humans still do it best, says neurobiologist Jeff Lichtman of Harvard University in Cambridge. He and his colleagues are working on an algorithm to take over. "It's about 95% accurate, which is terrible," he says; he thinks that they can improve on that. Janelia scientists do not fully trust the computer yet, either; they let it make the first pass at identifying cells and synapses, but then use human proofreaders.

Others crowdsource the challenge. For example, Helmstaedter developed a game, called Brainflight, in which players 'fly' through the brain's nerves and software captures those movements to the define the borders of the axons. "Even lay people can do it within minutes," he says.

Helmstaedter and Winfried Denk — director of the Max Planck Institute for Neurobiology in Martinsried — have published the largest microconnectome reported so far: a cube of mouse retina measuring 100 micrometres to a side and encompassing about 1,000 neurons and 250,000 synapses[5]. That was about two-millionths of the mouse brain. Helmstaedter's next goal is a cubic millimetre of cortex, which is roughly 1,000 times bigger. Denk's ambition is a full mouse brain.

CONNECTOME IN ACTION

The number and extent of connectomes that are ready for mining will grow quickly over the next decade. Meanwhile, scientists are making headway with the bits and pieces. Thousands have accessed the partial HCP data set, Van Essen says, and Zeng says that thousands visit the Allen connectome database every month.

Neuroscientist Ian Meinertzhagen of Dalhousie University in Halifax, Canada, offers a straightforward example of how connectomics contributed to his work with the *Drosophila* vision system. Fruit flies are attracted to ultraviolet light, and certain photoreceptor cells are known to detect this wavelength. Armed with his electron-microscopy maps, Meinertzhagen predicted that certain neurons in the optic lobe would receive input from those photoreceptors. Sure enough, when his collaborators deactivated those connections, the flies no longer preferred ultraviolet light[6].

These connectomes will provide fundamental information for many neuroscientists, says Mark Mattson, chief of the Laboratory of Neurosciences at the US National Institute on Aging in Baltimore, Maryland. "It's important to know what neurons connect with other neurons in the brain; it's important to know how much variability there is between individuals."

But there is still debate about what information is needed, and the level of detail that will be most useful. Tony Movshon of New York University holds that the mesoconnectome hits the sweet spot to understand neural circuits — the level of brain function that neuroscientists most want to understand. For example, scientists interested in how the brain processes sound or touch could follow the mesoconnectome pathways to identify possible members of the relevant circuits. The microconnectome, to his mind, provides too much detail to ask those kinds of questions. At that level, scientists are "doomed to be lost in the forest by looking at all the individual branches", he says. And the macroconnectome fails to pick up many connections, so scientists will miss important components of the circuit.

But others say that all scales are essential to the next phase of neuroscience, even if it is still too early to predict precisely how. Advances such as the light microscope, and the electron microscope after it, revealed a cellular universe unimagined by those lacking such equipment, Lichtman points out. The connectome will do the same, he says, even at the microscale. "For that reason alone, looking at brains at this level is likely to be interesting." Eventually, such information will be a resource that scientists depend on, predicts Denk. "It's like the genome. It'll be something that nobody will want to do without." ■

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