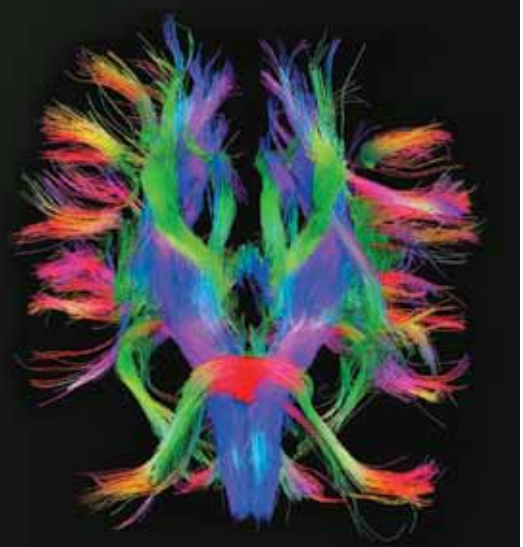
This is Your Brain: Mapping the Connectome



It's been 20 years since Francis Crick and Edward Jones, in the midst of the so-called Decade of the Brain, lamented science's lack of even a basic understanding of human neuroanatomy. "Clearly what is needed for a modern human brain anatomy is the introduction of some radically new techniques," the pair wrote in 1993. Clearly, researchers were listening. Today, they are using novel technologies and automation to map neural circuitry with unparalleled resolution and completeness. The NIH has dedicated nearly $40 million to chart the wiring of the human brain, and the Allen Brain Institute has poured in millions more to map the mouse brain. The data will take years to compile, and even longer to understand. But the results may reveal nothing less than the nature of human individuality. As MIT neuroscientist Sebastian Seung writes, "You are more than your genes. You are your connectome."

By Jeffrey M. Perkel

When Seung says in *Connectome: How the Brain's Wiring Makes Us Who We Are*, "You are your connectome," what he means is that neural connectivity is like a fingerprint. Each person has their own unique blend of genetics, environmental influences, and life experience. Those factors influence the detailed circuitry of the brain, such that even identical twins likely differ at the level of neural connectivity.

By mapping those connections, researchers hope to understand the normal variability of human connectomes and how they change and rewrite themselves as humans learn, mature, and age. They can begin to probe how connectomes become dysfunctional in traumatic brain injury or neurodegenerative disorders, or in patients with, say, schizophrenia or autism—conditions that Seung terms "connectopathies."

Yet the very scale of the problem is daunting. Only one connectome has been mapped to completion, and that was the roundworm, *Caenorhabditis elegans*. *C. elegans* contains just 300 neurons joined by 7,000 connections, yet charting its neural connectivity took more than a decade to complete. "Your connectome is 100 billion times larger [than *C. elegans*], with a million times more connections than your genome has letters," Seung writes. "Genomes are child's play compared with connectomes."

Nevertheless, researchers are making a stab at the problem. From the so-called macroscale of magnetic resonance imaging, to the microscale of electron microscopy, the connectome is slowly coming into focus, one synapse at a time.

The Human Connectome Project

When thinking about the connectome, says Hongkui Zeng, senior director of research science at the **Allen Institute for Brain Science,** think Google Maps. Neuroscientists would like to navigate the brain in virtual space as modern travelers do on the Internet: by zooming in and out and panning at will, from entire brain regions down to individual cells and synapses. In this metaphor, says Zeng, macroscopic MRI efforts reveal only neural superhighways. Still, she says, that can be useful, providing "an overview of the global sense of how regions are connected to each other, and how the world is organized."

That goal lies at the heart of the **Human Connectome Project** (HCP), a $40 million NIH effort launched in September 2010 to map the wiring of the live human brain. Two research consortia were funded under the HCP, with $30 million going to Washington University in St. Louis and the University of Minnesota, and $8.5 million to Massachusetts General Hospital (MGH) and the University of California, Los Angeles (UCLA).

While both teams are pursuing technology development, the WashU/Minnesota team also focuses on production, pushing 1,200

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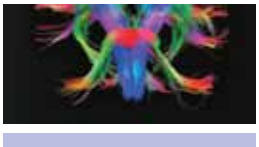

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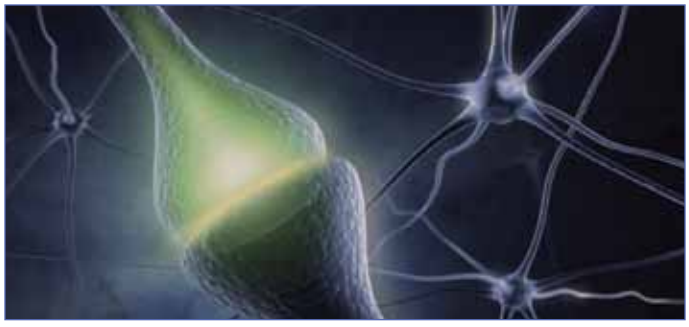

normal adults—400 sets of twins and their non-twin siblings—through a series of behavioral, genetic, and imaging scans to produce a reference against which other connectomes may be compared.

Both consortia employ magnetic resonance imaging of one form or another. At WashU, subjects are scanned for anatomic features and functional connectivity (i.e., regions linked by common purpose). To map physical connections, the consortia use diffusion MRI, a form of imaging that tracks the motion of water molecules as a marker of axonal fiber orientation. "Water molecules move more rapidly parallel to fibers than perpendicular to fibers," explains Van Wedeen, who heads the MGH team.

Wedeen invented and uses one form of diffusion MRI, called diffusion spectrum imaging (DSI); the WashU and Minnesota teams use HARDI, or high-angular resolution diffusion imaging. In both cases, the idea is to divide the brain into thousands of volumetric pixels, or "voxels," each about one cubic millimeter in size, and calculate for each one the different directions in which water diffuses. Then, in a process called "tractography," or track tracing, those vectors are connected to produce brilliant multicolor images of cables, or "fiber tracks," snaking their way through the brain's white matter.

The result is a map not of individual axons but rather thousands of axons in aggregate. "These are just numerical integrals of differential equations," says Wedeen. "These are not microscopic images of fibers." Nevertheless, collecting even those relatively low-resolution data requires some souped-up hardware. A standard clinical MRI, Wedeen says, has a magnetic field strength of 3 Tesla (T) and a gradient strength of 40 mT/m. The WashU/Minnesota group is using a specially made **Siemens** 3T scanner with a gradient strength of 100 mT/m, while the MGH/UCLA team's "Connectome Scanner" sports a 300 mT/m gradient.

That increased gradient strength offers two benefits for connectivity mapping, Wedeen says. "You get both more signal and better signal," he says, just as a telescope with a larger mirror can peer deeper into space.

Kamil Ugurbil, director of the **Center for Magnetic Resonance Research at the University of Minnesota** and co-PI of the WashU/Minnesota consortium, says his team has seen "significant technological gains" with their new scanner—resolution has been increased two- to three-fold and some 30 subjects have already been scanned, each over a two-day period.

But Ugurbil is no longer working with the 100 mT/m 3T scanner, which was shipped to WashU for the project's "production" mode. He has taken possession of a new 7T scanner, also from Siemens, which should provide even sharper images, and is awaiting shipment of an even larger $10 million, 10.5T instrument. At the moment, though, that latter magnet is sitting untested on the floor of a factory, he says, thanks to a "worldwide shortage of liquid helium."

"To cool this huge magnet we need something like 40,000 L of liquid helium, and we can't get it."

Mapping Mesoscale Connections

The Allen Institute is mapping the mouse connectome at what Zeng calls the "mesoscopic" scale—a mapping strategy first articulated by **Cold Spring Harbor Laboratory** neuroscientist Partha Mitra and colleagues in 2009. To build that map, Zeng's team uses "serial two-photon tomography."

Mice are injected in discrete brain regions with a recombinant adeno-associated virus (AAV, supplied by **University of Pennsylvania Vector Core**) that expresses a fluorescent protein. The mice are subsequently sacrificed and their brains fixed and embedded in agarose. That block is then mounted inside a two-photon fluorescent microscope tricked out with an ultrafine cutting apparatus, or vibratome—a system that has been commercialized by **TissueVision.**

"To really show that there's a connection, I'd have to show you there is a synapse and there are neurotransmitters crossing that synapse."

In this configuration, the top face of the block is fluorescently imaged at 0.35 μm lateral resolution, revealing the neuronal "arbors" traced out by the cells in whatever region was injected. Then the vibratome slices off the top 100 μm to reveal the next surface, and the process repeats.

"You image, cut, image, cut, image, cut," Zeng says. The entire process is automated, she explains, producing about 750 gigabytes of raw image data in about 18 hours—*per brain*. A complete dataset comprises approximately 500 injection points, and thus at least 500 brains, all of which must then be integrated and registered onto a three-dimensional template for comparison and navigation and to generate a detailed, brain-wide connectivity matrix.

Technically, says Zeng, the Allen Institute is not collecting a "connectome." Their virus is nonreplicative, meaning it can only infect cells once. It also cannot cross neural synapses. Therefore, she says, what her project is really imaging is a "projectome."

According to Zeng, data for most brain regions has already been collected, and some has been publicly released. (These data are freely navigable using the Institute's Brain Explorer software and freely downloadable via the Allen Connectivity Atlas data portal, www.brain-map.org.) Now she is going back and repeating the process with viruses that are specific to individual neural subtypes, to understand, for instance, how projectomes of excitatory and inhibitory neurons differ.

At the Cold Spring Harbor Laboratory, Mitra is pursuing a similar strategy. He injects each of 262 grid points on each mouse brain, but does so using four tracers—two "anterograde" and two "retrograde." That's about 1,000 mouse brains per dataset.

Anterograde tracers, like AAV and biotinylated dextran (obtained from **Life Technologies**), penetrate the cell body and then "piggyback on anterograde transport mechanisms that carry molecules away from the [cell body] along the axon to the [synaptic] terminals," Mitra explains. Retrograde tracers like cholera toxin (obtained from continued>

Featured Participants

Allen Institute for Brain Science
www.alleninstitute.org

Carl Zeiss International
www.zeiss.com

Center for Magnetic Resonance Research, UMinn
www.cmrr.umn.edu/index.shtml

Cold Spring Harbor Laboratory
www.cshl.edu

FEI
www.fei.com

Gatan
www.gatan.com

Hamamatsu
www.hamamatsu.com

Human Connectome Project
www.humanconnectomeproject.org

Human Connectome Project (WashU/UMinn)
humanconnectome.org

JEOL
www.jeol.com

Life Technologies
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List Biological Laboratories
www.listlabs.com

Siemens
www.medical.siemens.com

TissueVision
www.tissuevision.com

University of Pennsylvania Vector Core
www.med.upenn.edu/gtp/vectorcore

List Biological Laboratories) and rabies viruses (Duke University Viral Vector Core), enter cells via synapses, travel up axonal arbors to the cell body, and do actually provide some long-range connectivity information, Mitra says.

Mitra images each mouse brain (manually cryosectioned into 20 µm sections spaced 40 µm apart) on a **Hamamatsu** Nanozoomer 2.0 automated slide scanning fluorescence microscope. When reconstructed, the resulting dataset contains a trillion voxels measuring half a micron on a side. Those are just one-billionth the size of a diffusion MRI voxel. At one terabyte per injection site, he says, his lab has collected nearly a petabyte of information, some of which was released in June (www.brainarchitecture.org).

To The Microscale

Dense as mesoscale information is, it doesn't actually reveal synaptic connections. "If one is going to be a purist about this, we are not mapping connections per se," says Mitra. "To really show that there's a connection, I'd have to show you there is a synapse and there are neurotransmitters crossing that synapse."

Such information certainly isn't available on the mesoscale. But it is at least partially observable on the microscale. In Zeng's Google Maps analogy, this is like viewing the driveways and walkways leading into individual houses. The tool for seeing those details is electron microscopy.

At Harvard University, for instance, neuroscientist Jeff Lichtman embeds pieces of thalamus measuring just 400 x 400 x 250 µm in plastic ("That's not even one fMRI voxel," he notes), and sections them into 9,000 ultrathin slices on a home-built instrument, basically a deli-slicer, called an automatic tape-collecting ultramicrotome. Each slice is attached to a moving strip of tape as it emerges from the blockface, producing something like an old movie film reel of brain slices. That tape is then fed into a scanning electron microscope (Lichtman has instruments from Zeiss, **FEI,** and **JEOL**), which images each section one by one like a movie projector.

According to Lichtman, sections are imaged at 4 nm resolution in 16 tiles of 25,000 x 25,000 pixels each, collected at 20 megapixels per second. The process generates a terabyte of image data per day, 24/7, for 100 days, Lichtman says.

The goal, he says, is to map the organization of retinal ganglion cells in the thalamus. "We will get a good sense of the way that first stage of central processing of retinal information is organized from this dataset."

Lichtman recently acquired a new EM that collects data at twice the current speed, 40 megapixels per second. Yet even at that rate it is wholly impractical to map an entire human brain at this nanoscale resolution, both for reasons of data management—a single cubic millimeter is about 1,000 terabytes—and of time; even at 40 megapixels per second, it would still take years to image just a cubic millimeter.

A next generation instrument, though, could help. **Zeiss** is developing a new automated EM, Lichtman says, that will image sections with 61 electron beams at once (current machines use only one), speeding data acquisition up some 60-fold; he hopes to receive a prototype of this new device within a few years.

But collecting the data is only half the battle, says Moritz Helmstaedter of the **Max-Planck Institute of Neurobiology** in Martinsried, Germany; data analysis is the other.

As a postdoc, Helmstaedter worked with Winfried Denk at the Max-Planck Institute in Heidelberg. There Helmstaedter, with postdoc Kevin Briggman, used serial blockface electron microscopy (SBEM)—in which a piece of plastic-embedded brain is imaged and cut, imaged and cut, much as the Allen Institute does but on a nanometer scale—to image a piece of retinal tissue comprising about 1,000 neurons.

According to Helmstaedter, the SBEM-enabled scanning EM ran continuously for some eight weeks straight, collecting 13,000 images, each 2.5 gigapixels in size. (Both Zeiss and FEI EMs were used with a custom microtome; a complete system called 3View is now available from **Gatan**.) But it took more than two years to reconstruct the resulting neuronal circuits.

Helmstaedter's solution to that problem borrows from the crowd-sourced protein-folding game, FoldIt. His team trains computers to assemble the images to trace neurites. But to ensure accuracy, they have hired some 200 undergraduates, at $10/hour, to sit in front of a computer and navigate through the computed neurite forest by essentially "flying through the data" as if with a flight simulator. These students helped validate much of a 900-neuron retinal connectome, Helmstaedter says.

Now Helmstaedter is upping the ante with a piece of neocortex 500 µm on a side, containing some 10,000 neurons. For that, they'll need an even wider hive-mind, which they hope to tap using an in-development game version of their application for use on mobile devices.

In connectomics, says Helmstaedter, the bottleneck is network reconstruction. "We have to take these extreme measures to get it done."

Radical new techniques, indeed.

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