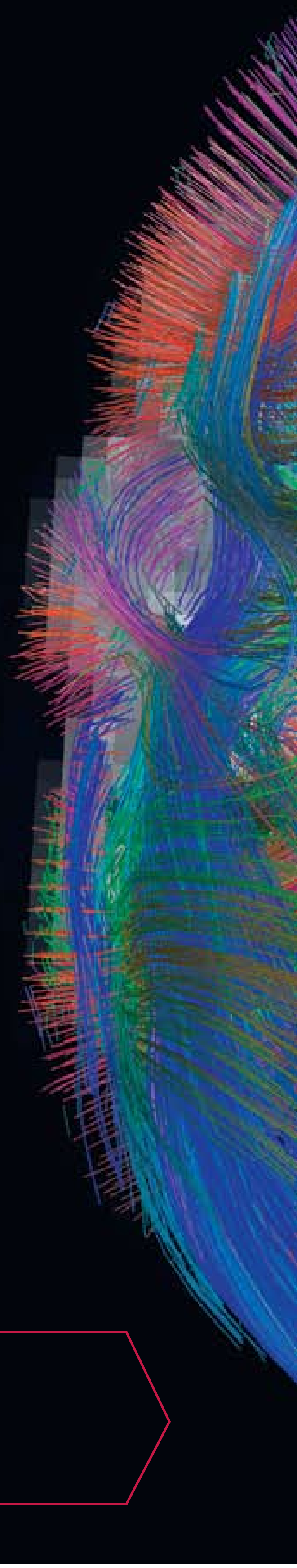
How Are We WIRED?

Cutting-edge imaging techniques are creating the first circuit diagram of the brain. With billions of neurons, and trillions of connections between them, it's going to take some of the most innovative minds in neuroscience to see it all



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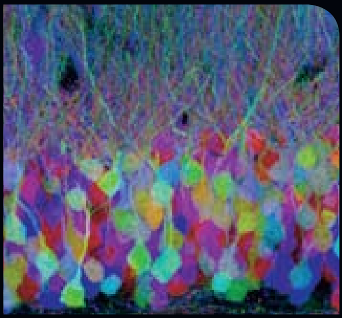

How do the brain's connections form?

Developmental neurobiologist Jeff Lichtman tracks how the brain's wiring changes during development.

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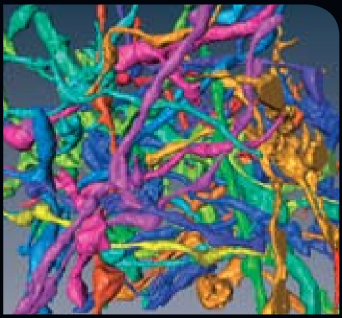

What do the brain's circuits look like?

Neurobiologist Winfried Denk maps the activity and structure of the brain's neuronal circuits.

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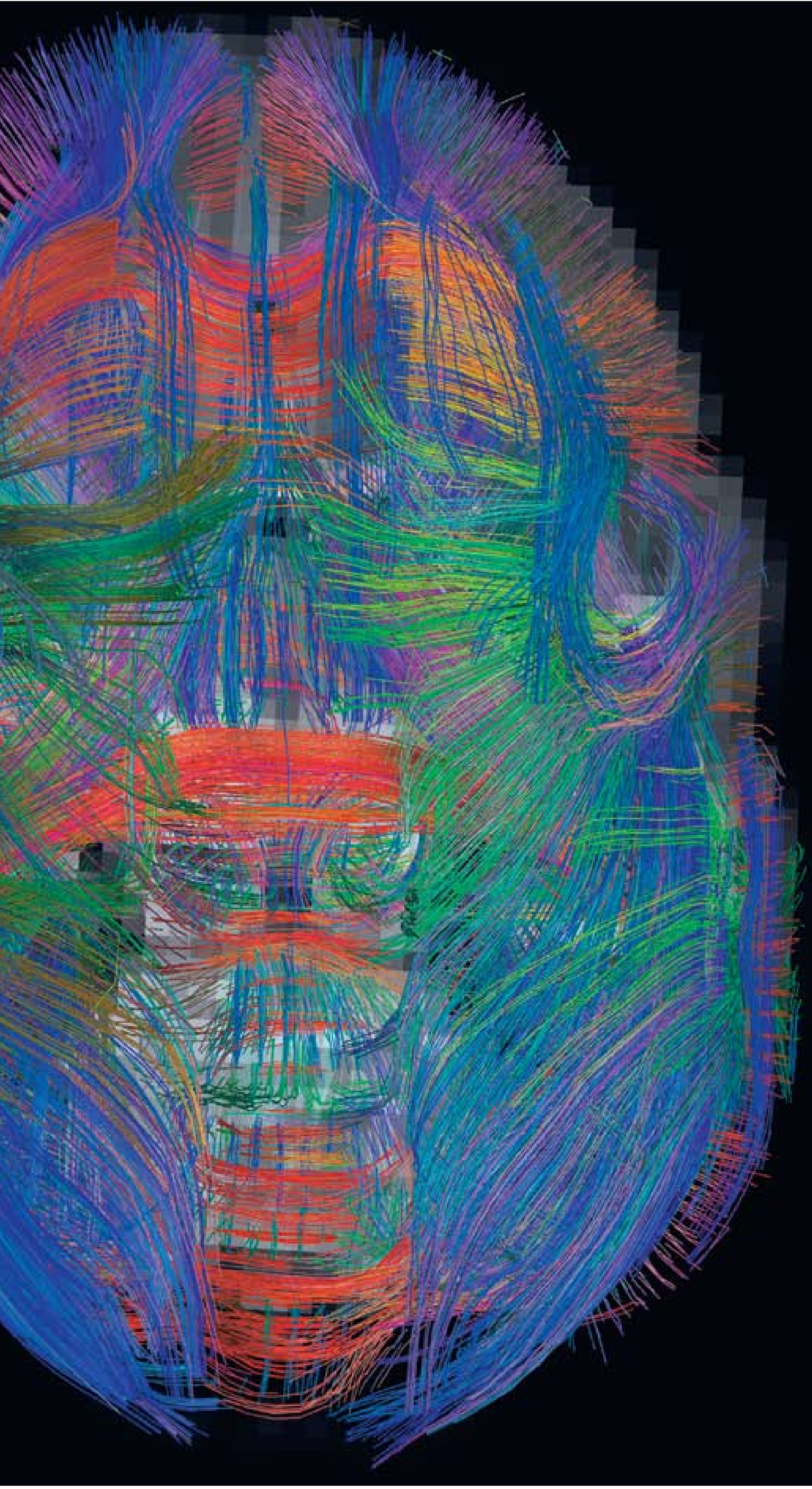
How are the different regions of the brain networked?

Imaging scientist Van Wedeen tracks the connections between different areas of the brain.



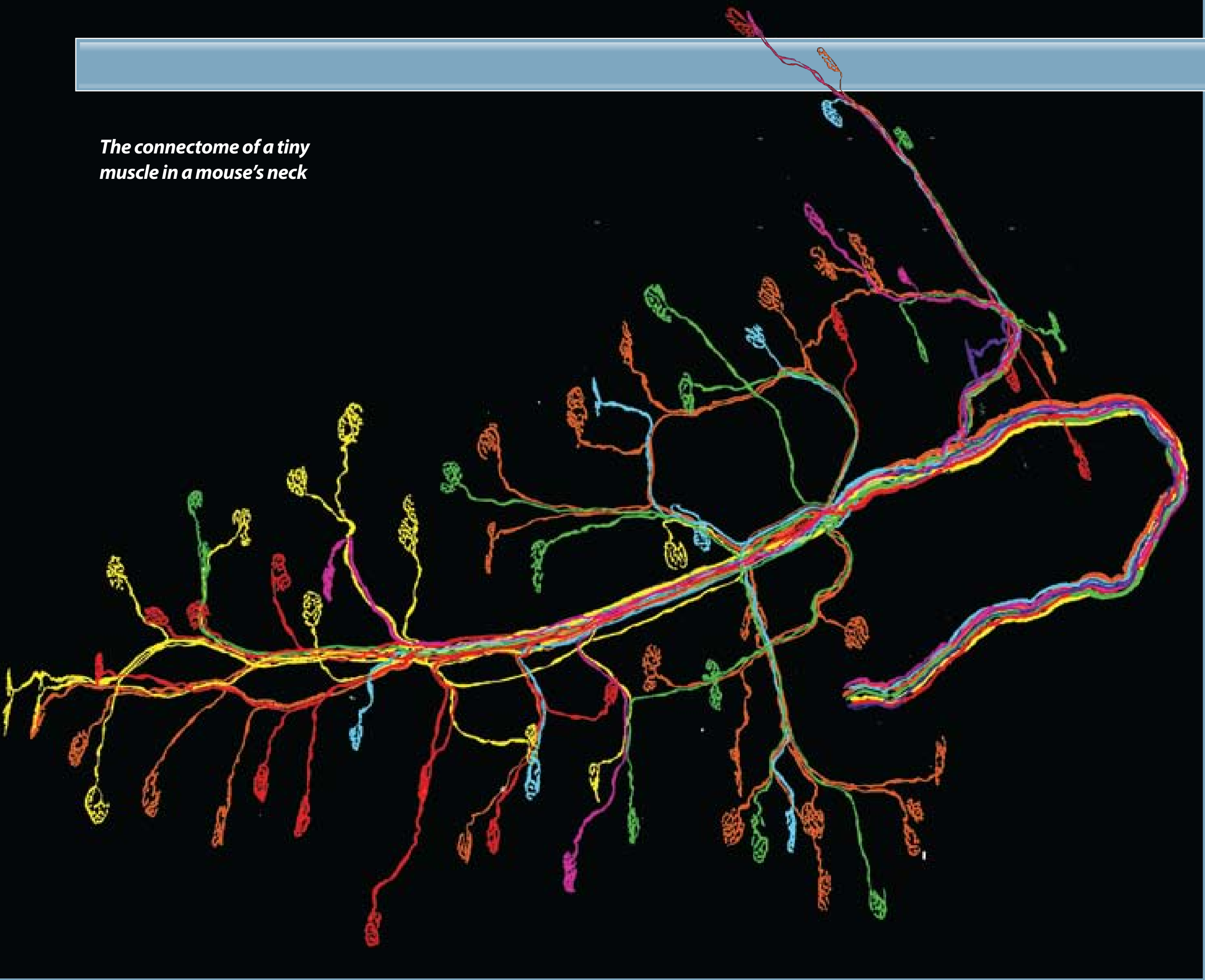
A composite of neural pathways in the brain of an owl monkey

Ever since individual nerve cells were first imaged in the late 1800s, biologists have tried to understand how these cells give the brain its incredible abilities. We now know quite a bit about nerve cells, or neurons, and how they generate and transmit signals. We can also pinpoint the roles played by many regions of the brain. But the more we learn, the clearer it becomes that the brain is more than just the sum of its parts.

Researchers are now investigating the bigger picture—how the connections between individual neurons and between different brain centers allow our brain to function the way it does. For example, although we know where visual stimuli are processed, we have less understanding of the physical path visual information takes from the eyes to the visual centers, or the connections between these centers and other regions of the brain that allow us to interpret what we see and react to it. As a key step toward grasping how the brain functions, scientists are using a variety of new techniques to map how it is wired.

The Connectome Takes Shape

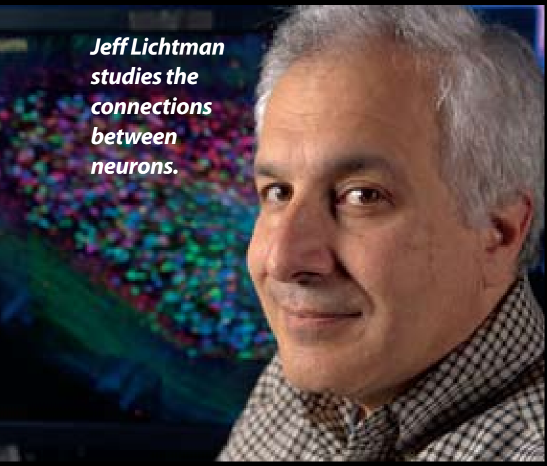
Several of the world's leading biologists are interested in mapping the brain's connections. But the challenge is colossal. The human brain is a tremendously complicated



The connectome of a tiny muscle in a mouse's neck

1 How do the brain's connections form?

Jeff Lichtman, a developmental neurobiologist at Harvard University, studies how the brain's wiring changes as we learn. He hopes that studying the structural changes in the brain will eventually show him how information is encoded in our wiring. "Where is your memory of your grandmother—how is that arranged, what does it look like?" Lichtman asks.



Jeff Lichtman studies the connections between neurons.

One imaging technique he uses, called thin-section-scanning electron microscopy, uses a diamond knife to shave slices of tissue from a rotating mouse brain. The slices are fixed onto a plastic strip prior to microscopy. Afterward, the path of each individual nerve cell must be traced. Initially researchers did this by manually identifying the same cells in different slices, but Lichtman and his colleagues at Harvard and the Massachusetts Institute of Technology are working on computer algorithms to speed up the process.

A Mouse Neuron Tree

In February 2009, Jeff Lichtman's lab imaged the entire network of neurons that control the movements of a mouse muscle, chosen for its relatively simple connections. The researchers genetically modified mice to express yellow fluorescent protein in nerves that control muscles. A computer created 3-D stacks of images just 0.2 micron thick. Nerve connections from about 150 stacks were identified and manually aligned to form the final model. Each muscle neuron was then given a distinct color, ranging from red, signifying the largest number of muscle-fiber connections, to blue, representing the smallest number.

FROM TOP: JU LU/HARVARD UNIVERSITY; VOLKER STEGER; PRECEDING PAGES, FROM LEFT: JEFF LICHTMAN/JEAN LIVET; WINFRIED DENK; VAN J. WEDEEN

organ, containing billions of neurons that form trillions of connections with one another. Many scientists are starting their work on smaller brains, like those of mice and rabbits, before they tackle human ones. Still, the task demands that researchers automate existing analytic techniques to run like an assembly line. They will also need to create technologies to attack the problem in new and more effective ways.

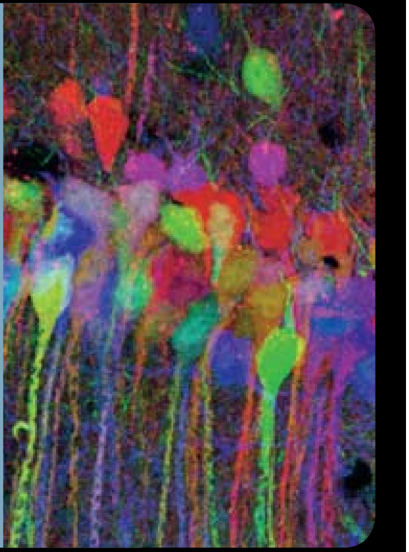
Currently scientists have two basic approaches to choose from—one uses microscopy and the other magnetic resonance imaging. Microscopy provides detailed insights into individual neurons' connections to one another. The downside is that it can be used to study only a tiny part of the brain at a time, so it provides little insight into connections between various parts of the brain. Using a variant of the classic MRI scanner, however, researchers can study large parts of the brain, or even the entire brain at once, but without much detail. Although individual neurons cannot be distinguished from one another with this method, scientists can study them as bundles of nerve cells. Each method has its own advantages and limitations, and it is only by using both and combining results that researchers will be able to get a complete picture of the brain's structure.

Researchers have already begun to draw a map of the brain's neural pathways, dubbed the connectome. Interest in this field is increasing rapidly thanks to the promise it holds for understanding many conditions that may have their origins in abnormal brain connections, such as schizophrenia, autism and learning disorders. If scientists can find the underlying causes of these disorders, they may be able to develop new and more effective ways of diagnosing, treating, or even preventing them.

Researchers also hope the connectome can reveal how the brain's network changes as we develop from birth. The plasticity of the human brain's wiring is one of its greatest strengths, and scientists hope to learn more about how we learn and adapt as we grow. Ultimately, brain researchers want to

Plastic Brains

Researchers cut thin slices from mouse brains and affix them to plastic strips before imaging them with an electron microscope. Neurobiologist Bobby Kasthuri displays a strip of the plastic.



Brainbow of Neurons

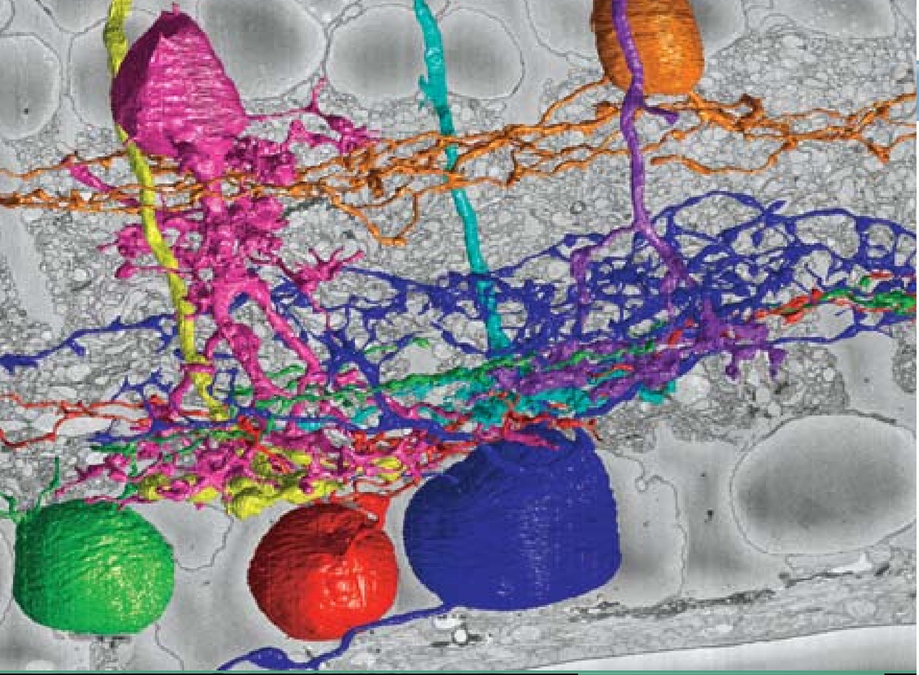
In the Brainbow technique, mice are genetically engineered to produce yellow, blue and red fluorescent proteins in neurons. The colors combine to mark individual neurons in about 100 hues in images like this one, from the hippocampus region of a mouse brain. The method allows researchers to quickly distinguish and follow the path made by each neuron.

FROM TOP: VOLKER STEGER; JEFF LICHTMAN/JEAN LIVET

Lichtman also relies on optical microscopy, which he has used to map the nerve fibers controlling a muscle in an adult mouse ear. Axons—fibers that carry signals away from nerve cells—had to be manually traced between different three-dimensional stacks of images, a task Lichtman calls "sobering." The first tracing of ear axons took six months.

Advancements to an imaging technique first developed by Lichtman's team in 2007 have recently helped simplify the task of tracking individual nerve fibers. In this method, dubbed Brainbow, a snippet of DNA with genes that code for random amounts of yellow, blue and red fluorescent proteins in nerve cells are inserted into mice. By manipulating DNA, researchers can give individual neurons different colors. The colors allow them to determine which synapses—the junctions where axons transmit their signals to other cells—correspond to which axons.

The real advantage to understanding the brain's connections, Lichtman believes, may come from connectomics, or the ability to find patterns in the wiring. Connectomics offers a way to investigate thought disorders, such as mental illnesses and learning disabilities, that may be caused by wiring errors. In his latest research, Lichtman tracked the nerve connections in an ear muscle of a juvenile mouse using Brainbow. When he looked at only a few axons, "it was just a massive tangle of wires," he says. But after imaging all the axons, connectomics revealed a strict arrangement, with each axon usually connecting to only one other axon.



Reconstructing the Retina

In a rabbit retina, bipolar cells [here, yellow, aqua and violet] transmit electrical signals to ganglion cells [dark blue], which make up the optic nerve that carries visual information to the brain. Amacrine cells [pink, orange, green and red] send signals between ganglia.

2 What do the brain's circuits look like?

Winfried Denk, a physicist and neurobiologist at the Max Planck Institute for Medical Research in Heidelberg, Germany, used to study the movement and activity of nerve cells. But he became frustrated by the lack of a more fundamental understanding of the brain's connections.

Working toward this understanding, Denk now uses a technique called serial block-face scanning electron microscopy, in which a diamond knife cuts thin slices off the top of a block of tissue. By imaging the block with an electron microscope while going through the tissue section by section, Denk is able to reconstruct the entire block in 3-D. He stains the tissues with heavy-metal atoms to identify nerve fibers within each block, but the difficulty comes in tracking them across blocks. Denk's latest efforts have focused on using computers to take over this arduous task. A major strength of the technique is that Denk can first image nerve-cell activity with one type of microscopy and then figure out the circuitry responsible for it by imaging the same sample with serial block-face scanning.

Winfried Denk uses his scanning electron microscope to examine brain slices.

understand how the organ stores information and how its wiring influences our personality and behavior.

Mission Impossible?

At first glance, it might seem impossible to map the brain's incredibly complex network. But by joining forces, research teams could achieve results far more quickly than expected. To this end, the National Institutes of Health launched the Human Connectome Project last July. This $30-million effort aims to combine a variety of imaging technologies to systematically map the brain's wiring. Researchers will collect information on the connections of a healthy adult human brain, and the data will be freely available to the research community, forming a basis for future studies. The project also aims to develop a combination of noninvasive tools that might allow healthy brains to one day be compared with those of people with neurological disorders, to look for clues about the source of these abnormalities.

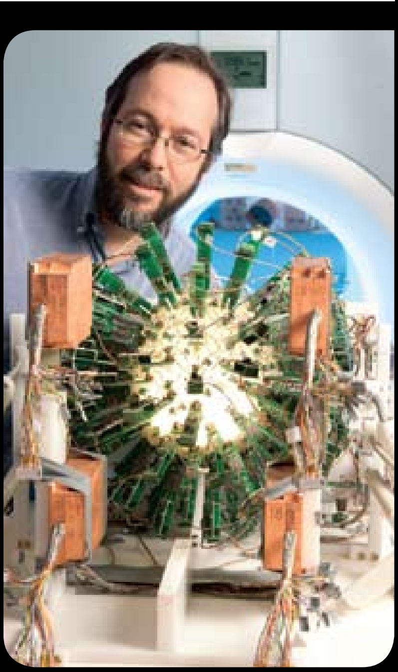
The mapping, which is one of three NIH neuroscience challenges, will also provide information about higher-level organization and patterns in the brain. That could help us understand how the connections between billions of neurons give rise to a single conscious mind. ■

FROM TOP: WINFRIED DENK; VOLKER STEGER

3

How are the different regions of the brain networked?

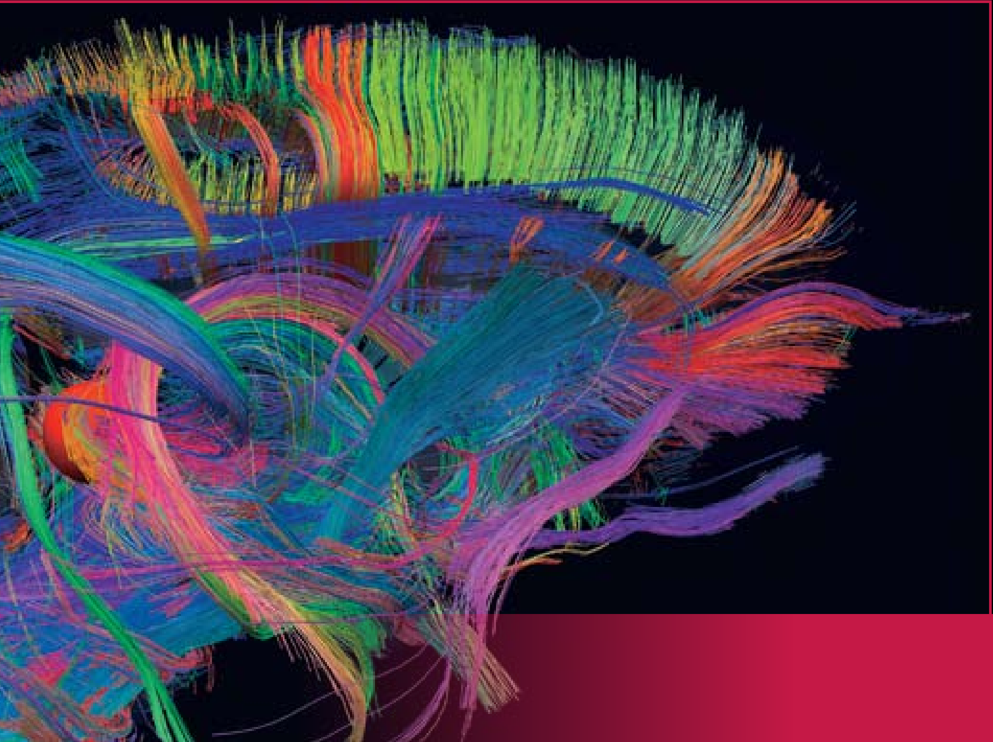
To study the brains of live subjects, scientists use diffusion-sensitive MRI, a scanning technique that allows them to analyze the movement of water molecules within brain tissues. Water moves along nerve fibers, so a computer can use this information to reconstruct their path. Van Wedeen, an imaging scientist at Massachusetts General Hospital in Boston, has used a version of this technique, called high-angular-resolution diffusion MRI, to get a 3-D map of the networks between regions in human and monkey brains. These images, taken from live subjects during 20- to 30-minute scanning sessions, have a spatial resolution of a tenth of an inch. This resolution is good enough to find differences between brains, but to identify previously unknown anatomy, Wedeen uses brains from human or animal cadavers, which he leaves in the scanner for days at a time. These images have a resolution of a hundredth of an inch. According to Wedeen, diffusion-sensitive MRI will have a central role in the NIH's Human Connectome Project. He would like to image hundreds of healthy human subjects to fulfill the project's goal of developing a circuit diagram of the brain's wiring. This, he hopes, would eventually allow him to scan for neurological problems, including psychiatric disorders and autism, that currently can't be detected by MRI.



Experimental MRI equipment allows Van Wedeen to scan the brain faster.

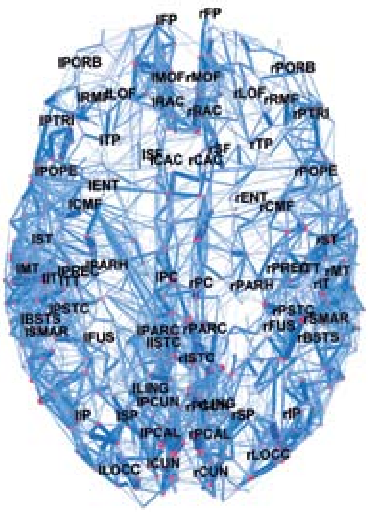
Vision's Long Path through the Brain

Using high-angular-resolution diffusion MRI scans like this one, Van Wedeen can use the movement of water molecules in nerve fibers to track the path of the fibers. This image shows nerve fibers crossing a virtual slice of an owl monkey brain. The colors represent the direction of each fiber, with the x, y and z axes in red, green and blue, respectively, and all other colors representing diagonals. The blue arc toward the top left relays visual information from the optic nerves [hidden behind the green-orange bundle, bottom right] to the visual cortex toward the back of the brain. The green treelike structures are the pathways of the cerebellum, which coordinates rapid muscle responses to sensory input.



Highways and Byways

MRI scans show that the brain's network connections are organized around three or four hubs consisting of many interconnected subregions. A map of the network of nerve connections among different anatomical subsections is shown here in a human brain.



CLOCKWISE FROM TOP: VOLKER STEGER; OLAF SPORNS/PATRIC HAGMANN/PLOS BIOLOGY; VAN J. WEDEEN