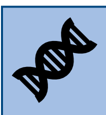

Discovering the Wiring Diagram of the Brain

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THE BRAIN, THE SEAT OF OUR COGNITIVE ABILITIES, is perhaps the most complex puzzle in all of biology. Every second in the human brain, billions of cortical nerve cells transmit billions of messages and perform extraordinarily complex computations. How the brain works—how its function follows from its structure—remains a mystery.

The brain's vast numbers of nerve cells are interconnected at synapses in circuits of unimaginable complexity. It is largely assumed that the specificity of these interconnections underlies our ability to perceive and classify objects, our behaviors both learned (such as playing the piano) and intrinsic (such as walking), and our memories—not to mention controlling lower-level functions such as maintaining posture and even breathing. At the highest level, our emotions, our sense of self, our very consciousness are entirely the result of activities in the nervous system.

At a macro level, human brains have been mapped into regions that can be roughly associated with specific types of activities. However, even this building-block approach is fraught with complexity because often many parts of the brain participate in completing a task. This complexity arises especially because most behaviors begin with sensory input and are followed by analysis, decision making, and finally a motor output or action.

At the microscopic level, the brain comprises billions of neu-

rons, each connected to other neurons by up to several thousand synaptic connections. Although the existence of these synaptic circuits has been appreciated for over a century, we have no detailed circuit diagrams of the brains of humans or any other mammals. Indeed, neural circuit mapping has been attempted only once, and that was two decades ago on a small worm with only 300 nerve cells. The central stumbling block is the enormous technical difficulty associated with such mapping. Recent technological breakthroughs in imaging, computer science, and molecular biology, however, allow a reconsideration of this problem. But even if we had a wiring diagram, we would need to know what messages the neurons in the circuit are passing—not unlike listening to the signals on a computer chip. This represents the second impediment to understanding: traditional physiological methods let us listen to only a tiny fraction of the nerves in the circuit.

To get a sense of the scale of the problem, consider the cerebral cortex of the human brain, which contains more than 160 trillion synaptic connections. These connections originate from billions of neurons. Each neuron receives synaptic connections from hundreds or even thousands of different neurons, and each sends information via synapses to a similar number of target neurons. This enormous fan-in and fan-out can occur because each neuron is geometrically complicated, possessing many receptive processes (dendrites) and one highly branched outflow process (an axon) that can extend over relatively long distances.

One might hope to be able to reverse engineer the circuits in the brain. In other words, if we could only tease apart the individual neurons and see which one is connected to which and with what strength, we might at least begin to have the tools to decode the functioning of a particular circuit. The staggering numbers and complex cellular shapes are not the only daunting aspects of the problem. The circuits that connect nerve cells are nanoscopic in scale. The density of synapses in the cerebral cortex is approximately 300 million per cubic millimeter.

Functional magnetic resonance imaging (fMRI) has provided glimpses into the macroscopic 3-D workings of the brain. However, the finest resolution of fMRI is approximately 1 cubic millimeter per voxel—the same cubic millimeter that can contain 300 million synapses. Thus there is a huge amount of circuitry in even the most finely resolved functional images of the human brain. Moreover, the size of these synapses falls below the diffraction-limited resolution of traditional optical imaging technologies.

Circuit mapping could potentially be amenable to analysis based on color coding of neuronal processes [1] and/or the use of techniques that break through the

diffraction limit [2]. Presently, the gold standard for analyzing synaptic connections is to use electron microscopy (EM), whose nanometer (nm) resolution is more than sufficient to ascertain the finest details of neural connections. But to map circuits, one must overcome a technical hurdle: EM typically images very thin sections (tens of nanometers in thickness), so reconstructing a volume requires a "serial reconstruction" whereby the image information from contiguous slices of the same volume is recomposed into a volumetric dataset. There are several ways to generate such volumetric data (see, for example, [3-5]), but all of these have the potential to generate astonishingly large digital image data libraries, as described next.

SOME NUMBERS

If one were to reconstruct by EM all the synaptic circuitry in 1 cubic mm of brain (roughly what might fit on the head of a pin), one would need a set of serial images spanning a millimeter in depth. Unambiguously resolving all the axonal and dendritic branches would require sectioning at probably no more than 30 nm. Thus the 1 mm depth would require 33,000 images. Each image should have at least 10 nm lateral resolution to discern all the vesicles (the source of the neurotransmitters) and synapse types. A square-millimeter image at 5 nm resolution is an image that has ~4 $x10^{10}$ pixels, or 10 to 20 gigapixels. So the image data in 1 cubic mm will be in the range of 1 petabyte (2^{50} ~ 1,000,000,000,000,000 bytes). The human brain contains nearly 1 million cubic mm of neural tissue.

SOME SUCCESSES TO DATE

Given this daunting task, one is tempted to give up and find a simpler problem. However, new technologies and techniques provide glimmers of hope. We are pursuing these with the ultimate goal of creating a "connectome"—a complete circuit diagram of the brain. This goal will require intensive and large-scale collaborations among biologists, engineers, and computer scientists.

Three years ago, the Reid and Lichtman labs began working on ways to automate and accelerate large-scale serial-section EM. Focusing specifically on large cortical volumes at high resolution, the Reid group has concentrated on very high throughput as well as highly automated processes. So far, their work has been published only in abstract form [3], but they are confident about soon having the first 10 terabytes of volumetric data on fine-scale brain anatomy. Physiological experiments can now show the function of virtually every neuron in a 300 μm cube. The new EM data has the resolution to show virtually every axon, dendrite, and

synapse—the physical connections that underlie neuronal function.

The problem of separating and tracking the individual neurons within the volume remains. However, some successes have already been achieved using exotic means. Lichtman's lab found a way to express various combinations of red, green, and blue fluorescent proteins in genetically engineered mice. These random combinations presently provide about 90 colors or combinations of colors [1]. With this approach, it is possible to track individual neurons as they branch to their eventual synaptic connections to other neurons or to the end-organs in muscle. The multicolor labeled nerves (dubbed "brainbow"), shown in Figure 1, are reminiscent of the rainbow cables in computers and serve the same purpose: to disambiguate wires traveling over long distances.

Because these colored labels are present in the living mouse, it is possible to track synaptic wiring changes by observing the same sites multiple times over minutes, days, or even months.

Reid's lab has been able to stain neurons of rat and cat visual cortices such that they "light up" when activated. By stimulating the cat with lines of different orientations, they have literally been able to see which neurons are firing, depending on the specific visual stimulus. By comparing the organization of the rat's visual cortex to that of the cat, they have found that while a rat's neurons appear to be randomly organized based on the orientation of the visual stimulus, a cat's neurons exhibit remarkable structure. (See Figure 2.)

Achieving the finest resolution using EM requires imaging very thin slices of neural tissue. One method begins with a block of tissue; after each imaging pass, a

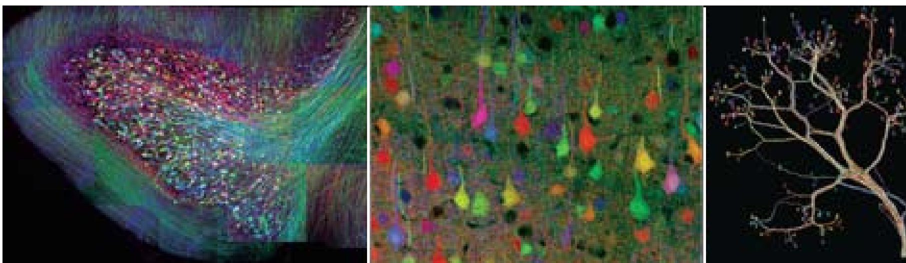

FIGURE 1.
Brainbow images showing individual neurons fluorescing in different colors. By tracking the neurons through stacks of slices, we can follow each neuron's complex branching structure to create the treelike structures in the image on the right.

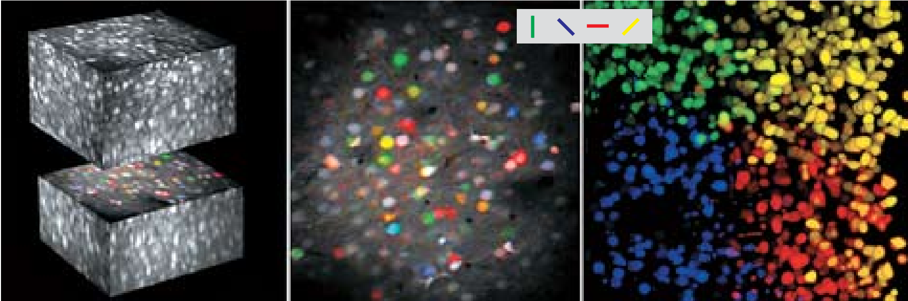

FIGURE 2.
Neurons in a visual cortex stained in vivo *with a calcium-sensitive dye. Left: A 3-D reconstruction of thousands of neurons in a rat visual cortex, obtained from a stack of images (300 µm on a side). The neurons are color coded according to the orientation of the visual stimulus that most excited them. Center: A 2-D image of the plane of section from the left panel. Neurons that responded to different stimulus orientations (different colors) are arranged seemingly randomly in the cortex. Inset: Color coding of stimulus orientations. Right: By comparison, the cat visual cortex is extremely ordered. Neurons that responded preferentially to different stimulus orientations are segregated with extraordinary precision. This image represents a complete 3-D functional map of over 1,000 neurons in a 300x300x200 µm volume in the visual cortex [6, 7].*

thin slice is removed (and destroyed) from the block, and then the process is repeated. Researchers in the Lichtman group at Harvard have developed a new device—a sort of high-tech lathe that they are calling an Automatic Tape-Collecting Lathe Ultramicrotome (ATLUM)—that can allow efficient nanoscale imaging over large tissue volumes. (See Figure 3 on the next page.)

The ATLUM [3] automatically sections an embedded block of brain tissue into thousands of ultrathin sections and collects these on a long carbon-coated tape for later staining and imaging in a scanning electron microscope (SEM). Because the process is fully automated, volumes as large as tens of cubic millimeters—large enough to span entire multi-region neuronal circuits—can be quickly and reliably reduced to a tape of ultrathin sections. SEM images of these ATLUM-collected sections can attain lateral resolutions of 5 nm or better—sufficient to image individual synaptic vesicles and to identify and trace all circuit connectivity.

The thin slices are images of one small region at a time. Once a series of individual images is obtained, these images must be stitched together into very large images

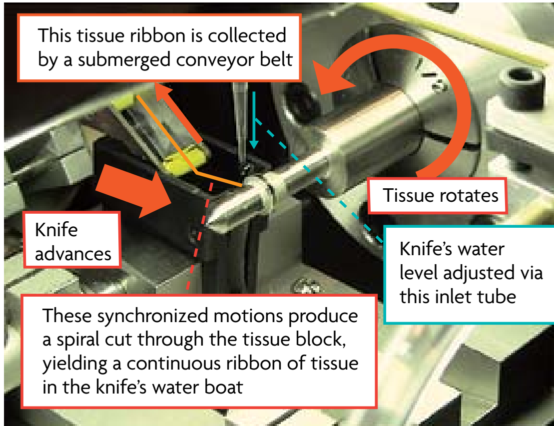

FIGURE 3.
The Automatic Tape-Collecting Lathe Ultramicrotome (ATLUM), which can allow efficient nanoscale imaging over large tissue volumes.

and possibly stacked into volumes. At Microsoft Research, work has proceeded to stitch together and then interactively view images containing billions of pixels.[1] Once these gigapixel-size images are organized into a hierarchical pyramid, the HD View application can stream requested imagery over the Web for viewing.[2] This allows exploration of both large-scale and very fine-scale features. Figure 4 shows a walkthrough of the result.

Once the images are captured and stitched, multiple slices of a sample must be stacked to assemble them into a coherent volume. Perhaps the most difficult task at that point is extracting the individual strands of neurons. Work is under way at Harvard to provide interactive tools to aid in outlining individual "processes" and then tracking them slice to slice to pull out each dendritic and axonal fiber [8, 9]. (See Figure 5.) Synaptic interfaces are perhaps even harder to find automatically; however, advances in both user interfaces and computer vision give hope that the whole process can be made tractable.

Decoding the complete connectome of the human brain is one of the great challenges of the 21st century. Advances at both the biological level and technical level are certain to lead to new successes and discoveries, and they will hopefully help answer fundamental questions about how our brain performs the miracle of thought.

[1] http://research.microsoft.com/en-us/um/redmond/groups/ivm/ICE
[2] http://research.microsoft.com/en-us/um/redmond/groups/ivm/HDView

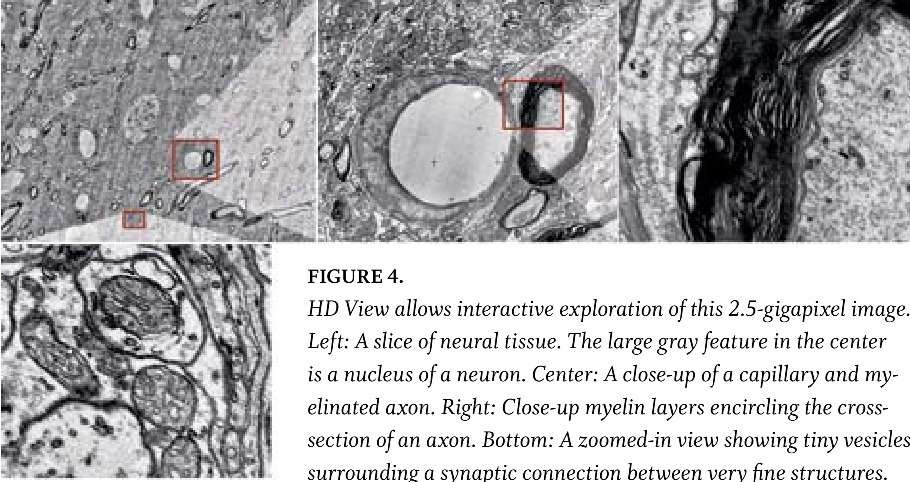

FIGURE 4.
HD View allows interactive exploration of this 2.5-gigapixel image. Left: A slice of neural tissue. The large gray feature in the center is a nucleus of a neuron. Center: A close-up of a capillary and myelinated axon. Right: Close-up myelin layers encircling the cross-section of an axon. Bottom: A zoomed-in view showing tiny vesicles surrounding a synaptic connection between very fine structures.

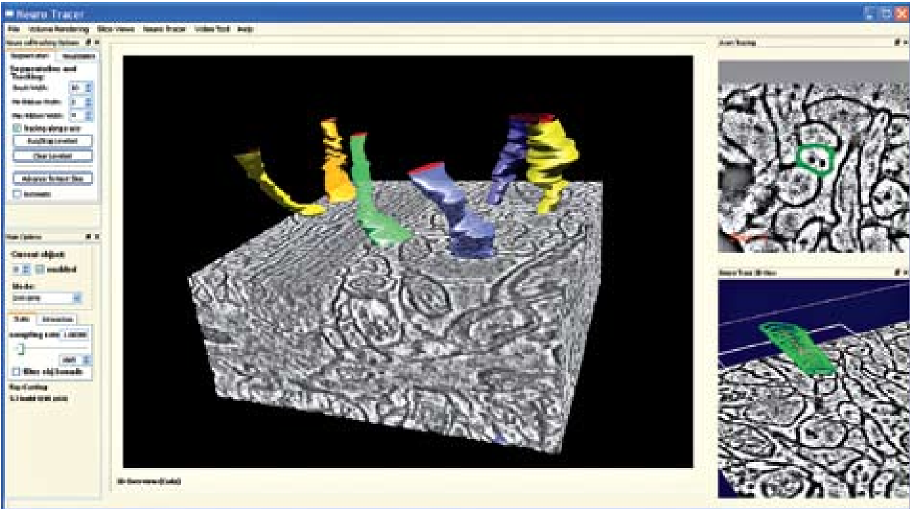

FIGURE 5.
NeuroTrace allows neuroscientists to interactively explore and segment neural processes in high-resolution EM data.

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