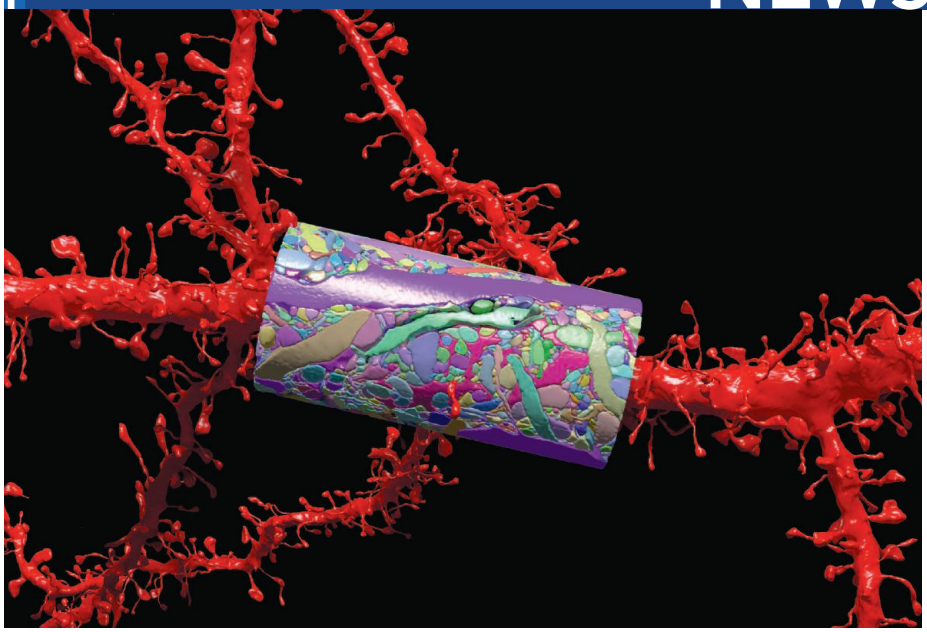

CREDIT: BOBBY KASTHURI/DANIEL BERGER

Fine structure. A composite of artificially colored EM images reveals details within a cylinder of mouse brain tissue, smaller than a grain of sand, that contains 680 nerve fibers and 774 synapses.

NEUROSCIENCE

New Tools Light Up the Intricacies of the Brain

SAN DIEGO, CALIFORNIA—Last week, nearly 5000 of the roughly 30,000 researchers at the Society for Neuroscience conference here crammed into an auditorium to see neuroscientist Jeff Lichtman of Harvard University present a few salt grain–sized chunks of mouse brain. When magnified to fill several large projection screens, a small part of one of these revealed a cylinder of tissue in unprecedented detail: 680 nerve fibers, 79 branched projections called dendrites, and 774 synapses—the junctions where chemical signals jump from neuron to neuron.

Such a precise inventory of the contents of one tiny speck of cortex—just one-billionth of a mouse brain—was unthinkable only a few years ago. But recent advances in high-throughput, automated electron microscopy, from Lichtman's group and others, are "opening a new era" in which investigators study brain circuitry at the level of individual synapses, says Yuan Liu, a program director at the National Institute of Neurological Disorders and Stroke in Bethesda, Maryland, which funds Lichtman's work.

For Liu, Lichtman's talk was a highlight of the meeting because it shows that new technologies and techniques could one day fulfill the promise of President Barack Obama's controversial BRAIN Initiative (*Science*, 1 March, p. 1022), which aims to speed up the mapping of the human brain's structure and activity. "We cannot afford to give all our money to tech development," she says, but the value of such investment "really shows" in work such as Lichtman's.

To others, the dizzying complexity of Lichtman's data, assembled from many thousands of 30-nanometer-thin sections over the course of months, was worrisome. Despite producing 100 terabytes of data and some beautiful images, it provided scant new insight into how the brain's complexity is organized, contends neuroscientist Partha Mitra of Cold Spring Harbor Laboratory in New York. Simply describing neural circuitry at this minute level "is far from adequate for anyone wanting to make sense of the brain, and we are not even down to the atomic or molecular scale," he says. "If you take apart any object around you atom by atom, you will get a mass of complicated detail, but that is not how we have gained insight into the chemical and physical properties of the world."

Lichtman has heard such skepticism for years as one of the early pioneers in creating detailed wiring diagrams of neural circuits. In 2007, he and colleagues released Brainbow, a method of distinguishing among nerve cells by breeding animals to express random combinations of red, green, and blue fluorescent protein in their neurons. The technique has aided the mapping of connections in the peripheral system, but has fallen short in distinguishing the densely packed and overlapping neurons of the central nervous system (CNS). New versions of Brainbow that allow more colors, highlight cell membranes rather than cell bodies, and employ fluorescent proteins from different species that allow better antibody labeling have been released this year. Lichtman predicts they will finally start to unravel the CNS connectome, but for now electron microscopy remains the most effective method for tracing its cells, he says.

Mapping the brain at the synaptic level—a resolution 1 trillion times finer than that of a functional magnetic resonance imaging scan—strikes many people as crazy, admits Bobby Kasthuri, one of Lichtman's postdoctoral students. But the detail in the new electron micrographs can be revelatory, Lichtman says. For example, one clear finding from the cortex images is that "you can't just infer synapses" based on where axons and dendrites intersect, he says. "Really seeing what's there gives you a way of framing your ideas better than imagining it," adds neuroscientist Eve Marder of Brandeis University in Waltham, Massachusetts.

Even as some neuroscientists debated the merits of Lichtman's images, other emerging technologies beckoned in San Diego. A crowd of graduate students buzzed around Stanford University neuroscientist Karl Deisseroth, the inventor of optogenetics, a widely adapted technique for making neurons responsive to light, and CLARITY, a technology that makes brain tissue transparent. Next to him, ghostly fields of firing neurons flickered on a video screen, demonstrating Deisseroth's latest, yet-to-be published brainchild.

Called SWIFT-volume imaging, the technique captures firing of extensive neural circuits in animals whose neurons have been genetically engineered to glow when triggered. To watch the neurons in action, researchers drill a hole into a rodent's skull and employ a microscope that, thanks to new optics and computational techniques, can image and analyze the firing patterns of large, 3D groups of neurons—more than 1000 cells at once, according to Logan Grosenick,

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a graduate student in the Deisseroth lab who spearheaded the technique. The neurons can be recorded while the animal runs in place on a ball or learns different tasks in a virtual reality environment.

Similarly, in zebrafish, whose nerve cells are visible through largely transparent flesh, the technique allows the activity of individual neurons to be monitored as a fish swims and learns to avoid certain areas or go to others containing rewards. The technique promises to track real-time activity in major brain structures known to influence behavior, says Mehmet Fatih Yanik, a neuroscientist and engineer at the Massachusetts Institute of Technology in Cambridge. "I think it will be adopted very rapidly by other labs, including my own."

Warranted or not, enthusiasm for the new techniques is irrepressible. "I won't ever stop doing this," says Kasthuri of his electron microscopy work. "I'm going to try to convince every person I ever meet that this is the way to do this." The young researchers clustered around Deisseroth's posters clearly felt similar excitement: At 5 p.m. on Wednesday night, at the tail end of a weeklong conference, security had to shoo them out of the building.

–EMILY UNDERWOOD

CLIMATE CHANGE

Humans Fueled Global Warming Millennia Ago

People were already pumping greenhouse gases into the atmosphere 5000 years before the Industrial Revolution, air bubbles in Antarctic ice suggest. The new evidence—in part from an exceptional record of atmospheric methane freshly cored from the West Antarctic Ice Sheet—supports a paleoclimatologist's provocative idea that humanity began warming the world early, as methane bubbled out of early rice farmers' paddies.

Closer look. An exceptionally detailed Antarctic ice-core record shows that expanding rice cultivation, plus natural forces, kicked off global warming 5000 years ago.

Paleoclimatologist William Ruddiman, now a professor emeritus at the University of Virginia in Charlottesville, proposed his early-warming hypothesis 10 years ago after studying ice core records available at the time (*Science*, 16 January 2004, p. 306). Bubbles of ancient air, trapped when fallen snow turned to ice, showed that methane levels declined as the last ice age was ending 10,000 years ago—just as they had done at the end of previous glacial periods. But then, about 5000 years ago, methane began to rise in a way that no one could explain.

Delving into the archaeological literature, Ruddiman noted that the methane increase occurred at about the same time as people started cultivating rice in what were essentially manmade, methane-producing wetlands. The coincidence suggested humans were behind the rise. And 8000 years ago, another greenhouse gas, carbon dioxide, also began rising suspiciously—perhaps because early farmers were clearing forests for agriculture. Together, Ruddiman calculated, the added greenhouse gases could have warmed the world by 0.8°C, about as much as humans have warmed the world over the past century or two.

Skepticism was widespread in the paleoclimate community. Methane molecules don't come labeled as human-generated or purely natural, so lone ice cores couldn't give Ruddiman's hypothesis a rigorous test. Then geochemist Logan Mitchell, now at the University of Utah in Salt Lake City, and colleagues decided to compare the methane trapped over time in two ice cores: one retrieved from Greenland in the 1990s and the other from the West Antarctic Ice Sheet in late 2011—the best record so far from the Southern Hemisphere. Because air circulation tends to be confined within one hemisphere or the other, atmospheric methane produced in one is slow to spread to the other. So methane concentrations can differ between the north and south in ways that reflect the relative sizes and locations of sources.

The results, reported on page 964, show that from 2800 to 600 years ago—the period most easily tested—atmospheric methane rose 17%. Levels started out higher in the Northern Hemisphere, as they are today, but they rose in tandem so that the north-south difference remained unchanged. Natural wetlands could have driven the global rise in the Southern Hemisphere, but Mitchell and his colleagues found a need for a human source—presumably Ruddiman's expanding rice cultivation—to drive the rise in the Northern Hemisphere.

"Fully one or the other [source] wouldn't fit the data," says ice-core paleoclimatologist Eric Wolff of the University of Cambridge in the United Kingdom. "They end up with a bit of both, which is probably okay. It's reasonably persuasive."

Ruddiman himself is encouraged. The community's attitude "is changing slowly," he says, adding that "it's in the right direction." But he notes that modelers have failed to show how natural processes on their own could have boosted methane, much less carbon dioxide, thousands of years ago. Even in the early days of global warming, he thinks, humans must have been the major force.

–RICHARD A. KERR

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