

Crystal clear. CLARITY allows researchers to image specific neurons and molecules within an intact mouse brain (*left*) and hippocampus (*right*) without making a single cut.

NEUROSCIENCE

Tissue Imaging Method Makes Everything Clear

Karl Deisseroth is on a roll. In March, the neuroscientist-psychiatrist-engineer of Stanford University in Palo Alto, California, was awarded a share of the world's largest neuroscience prize for his work developing optogenetics, a tool which allows researchers to manipulate neuronal activity with light. Last week, he was appointed to advise President Barack Obama's new \$100 million brain research initiative. Now, this week in *Nature*, Deisseroth presents a new way of imaging the brain, which many researchers say will fundamentally change the way labs study the intricate organ.

"This is one of those singular events in the history of neuroscience," says Terrence Sejnowski, a neuroscientist at the Salk Institute for Biological Studies in San Diego, California, referring to CLARITY, Deisseroth's new method of rendering tissue transparent. Sejnowski compares the technique to the Golgi stain, which more than a century ago allowed scientists to trace specific neurons and their projections. "Until you actually see it, you don't quite appreciate how spectacular [CLARITY] is," he says. "You can see not just very detailed images of single neurons but whole populations of neurons, their projections, the inner structure of the brain. It's very revealing and has almost infinite potential to image anything that could be labeled."

The technique can be used to make any organ transparent, but it was the challenges of imaging the brain that motivated Deisseroth, who hopes to dissect psychiatric disorders such as schizophrenia and depression. Creating a 3D image of a brain today requires carving its tissue into hundreds or thousands of hair-thin slices, scanning an image of each slice in microscopic detail into a computer, and then painstakingly realigning the sections. Digitally stitching axons

back together—each roughly a hundredth the diameter of a human hair—not only takes a lot of computational time, but is also prone to significant error, says neuroscientist Arthur Toga of the University of California (UC), Los Angeles. After spending decades refining techniques for slicing and imaging brains, Toga says CLARITY may soon make that work irrelevant: "I'm gonna go up there and talk to these guys," he says, impressed.

The CLARITY technique, which Deisseroth and colleagues have been working on for about 6 years, skips over slicing entirely and instead tackles the biggest obstacle to tra-

Deisseroth says. "Everything would just slosh around." Instead, CLARITY first replaces the lipids with a water-based gel by infusing a brain, or any other tissue, with single molecules of acrylamide—the same clear, jelly-like substance used to separate and analyze DNA molecules through electrophoresis. The fatty molecules are then flushed out with a combination of electrical current and detergent. For a roughly 4-mm-diameter mouse brain, the process takes about 9 days, Deisseroth says.

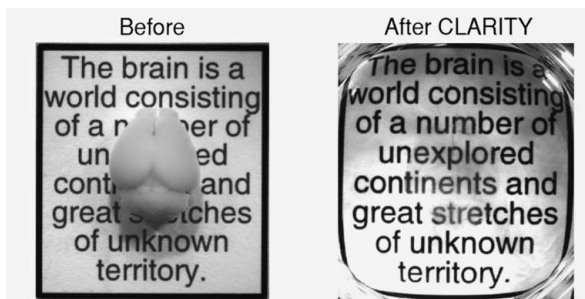
"This is a significant improvement" over previous methods of removing lipids from

brain tissue, notes neurophysicist David Kleinfeld of UC San Diego. Although other labs have attempted to render the brain clear, he says, previous attempts have left the tissue far too fragile to work with. Kleinfeld says that he plans to use CLARITY to study the human brainstem, a region that is difficult to image because of all the lipid-packed myelin that surrounds neurons in that area. "We're

pretty psyched."

One of the most exciting things about the technique is that it allows researchers to infiltrate the brain with labels for specific cell types, neurotransmitters, or proteins; wash them out; and image the brain again with different labels, Sejnowski says. "In one shot, you can look at every molecule in the brain that has that label." Digitizing the process will speed up by a hundredfold tasks such as counting all the neurons in a given brain region, he adds.

In the *Nature* paper, Deisseroth and colleagues describe using the technique to image



Disappearing act. CLARITY-treated mouse brain (*right*) reveals quote from famous neuroanatomist Santiago Ramón y Cajal.

ditional brain imaging: the fatty molecules, called lipids, that form the cellular membranes that keep neurons and other brain cells and organelles intact. Much as oil on sunlit water produces a rainbow sheen, these molecules scatter light in all directions, making it impossible to use light-based microscopy to see more than a few hundred microns deep into the brain. Lipids also repel many substances, such as antibodies, making it difficult to label specific types of cells without cutting the brain into sections.

Because lipids maintain the brain's structure, just getting rid of them isn't an option,

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not only the mouse brain, but also small blocks of postmortem brain tissue from a person with autism. The experiment confirmed that the method works with brains preserved in formalin, a common fixative.

Will scientists ever be able to use CLARITY to look at an entire human brain? Although the notion is “not outrageous,” it would require building a much, much bigger microscope lens, Kleinfeld says. “We haven’t pushed that limit yet,” Deisseroth says. Now, existing microscopes can image up to only 6 to 8 mm in



On a roll. Karl Deisseroth wants to revolutionize neuroscience.

diameter of clarified brain tissue at a time—about the size of a whole adult mouse brain, he says. That’s convenient, Deisseroth says, because it’s about the size of what many researchers believe to be the core computational unit of the human cortex.

Although he agrees that CLARITY will lead to a “major advance” in tissue morphology, the technique may be dangerous and expensive for other labs to adopt, warns neuroimaging expert Hans Ulrich Dodt of the Vienna University of Technology, who has been explor-

ing other methods to render tissues transparent. For one thing, acrylamide is highly toxic and carcinogenic, “so it’s going to be a mess, and severe safety precautions have to be taken.” Deisseroth acknowledges the need for caution, but he expects that other labs will find the new technique easy to adopt. He has already run several workshops to share the method.

Nora Volkow, director of the National Institute on Drug Abuse in Bethesda, Maryland, and one of the reviewers of the National Institutes of Health Director’s Transformative Research Award that funded the experiment, describes CLARITY as “magnificent.” Deisseroth is an “extraordinary individual and extremely creative,” she says.

—EMILY UNDERWOOD

PALEOANTHROPOLOGY

A Human Smile and Funny Walk for *Australopithecus sediba*

If you happened to be in South Africa about 2 million years ago, you might have seen an odd sight: an older female hominin sashaying down a wooded slope, perhaps in search of water. She walked upright, but she wasn’t human, and she moved with what to our eyes would have looked like a distinctly strange gait. She was a member of *Australopithecus sediba*, and according to new analyses of fossils published online in *Science* this week (see p. 163), she may have twisted from side to side, rolling her feet inward with each step.

“*Sediba*’s got swag,” says paleoanthropologist Lee Berger of the University of the Witwatersrand in Johannesburg, South Africa. At least, she would have had swag until she fell into a death pit—a deep cave—at the site of Malapa, where she was buried with a youth and at least one other of her kind for nearly 2 million years before Berger and his son found them in 2008 (*Science*, 9 April 2010, p. 154).

The six new papers analyze two partial skeletons of *Au. sediba*, plus a shinbone of a third individual. They offer the most detailed picture yet of how this species moved and its relationship with other hominins, including our own ancestors. The remarkably complete skeletons reveal a strange creature with a small brain and primitive walk—but some surprisingly human teeth. “When you look at *Au. sediba* from head to toe, you get a package that’s very different from anything we’ve ever seen or predicted,” Berger

says. The analyses detail many traits shared with our genus *Homo*, in the spine, vertebral column, knee, teeth, and jaws, and Berger argues that *Au. sediba* could be the long-sought species that gave rise to *Homo*.

Few paleoanthropologists agree. But they say that at the very least, the new papers



Jaw dropper. *Au. sediba*’s skull shows a surprising mix of primitive features, such as a small brain, coupled with more human features in the jaw and teeth.

offer a detailed view of a novel way to be a member of the extended human family. They also show how evolution remodels the same body plan in diverse ways in different habitats. “If you look at the discoveries of hominins 2 to 3 million years ago, each one

seems to shuffle the deck [of traits] in different ways,” says paleoanthropologist William Kimbel of Arizona State University, Tempe. “It adds another subtle layer of information about the diversity of African hominins.”

The outlines of *Au. sediba* have been presented before, but the latest work brings this strange hominin into ever sharper focus. For example, ever since Berger’s team discussed a chimpanzee-like heel from one of the skeletons at a meeting last year (*Science*, 4 May 2012, p. 538), researchers have wondered how the species could walk upright. The bottom of the heel, preserved in the older female, is “exceptionally small,” twisted, and pointed as in a chimp, compared with the broad, flat base of a human heel bone, the authors write in *Science* (see image, p. 133). The narrow heel offers far less surface area on which to distribute weight when the foot strikes the ground, explains biological anthropologist Jeremy DeSilva of Boston University, first author of the foot and leg paper. (Chimps knuckle-walk, using their arms for added stability to support their skinny heels.)

In their analysis of bones from the female skeleton’s leg, DeSilva and his colleagues propose that *Au. sediba* had an arched foot that allowed it to put weight simultaneously on its heel and the outside of its midfoot. As it took each step, it must have pronated the foot, rolling it markedly inwards.

If modern humans try such a maneuver, they’ll quickly find that the hyperpronation puts torque on the foot, and also causes the lower leg and thigh to rotate excessively,

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