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Mouse brain made transparent: Method enables 3-D analysis of brain's fine structure and connections (w/ video)

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Combining neuroscience and chemical engineering, researchers at Stanford University have developed a process that renders a mouse brain transparent. The postmortem brain remains whole—not sliced or sectioned in any way—with its three-dimensional complexity of fine wiring and molecular structures completely intact and able to be measured and probed at will with visible light and chemicals.

The process, called CLARITY, ushers in an entirely new era of whole-organ imaging that stands to fundamentally change our scientific understanding of the most-important-but-least-understood of organs, the brain, and potentially other organs, as well.

The process is described in a paper to be published online April 10 in *Nature* by bioengineer and psychiatrist Karl Deisseroth, MD, PhD, leading a multidisciplinary team, including postdoctoral scholar Kwanghun Chung, PhD.

"Studying intact systems with this sort of molecular resolution and global scope—to be able to see the fine detail and the big picture at the same time—has been a major unmet goal in biology, and a goal that CLARITY begins to address," Deisseroth said.

"This feat of chemical engineering promises to transform the way we study the brain's anatomy and how disease changes it," said Thomas

Insel, MD, director of the National Institute of Mental Health. "No longer will the in-depth study of our most important three-dimensional organ be constrained by two-dimensional methods."

The research in this study was performed primarily on a mouse brain, but the researchers have used CLARITY on zebrafish and on preserved human brain samples with similar results, establishing a path for future studies of human samples and other organisms.

"CLARITY promises to revolutionize our understanding of how local and global changes in brain structure and activity translate into behavior," said Paul Frankland, PhD, a senior scientist in neurosciences and mental health at the Hospital for Sick Children Research Institute in Toronto, who was not involved in the research. Frankland's colleague, senior scientist Sheena Josselyn, PhD, added that the process could turn the brain from "a mysterious black box" into something essentially transparent.

An inscrutable place

The mound of convoluted grey matter and wiring that is the brain is a complex and inscrutable place. Neuroscientists have struggled to fully understand its circuitry in their quest to comprehend how the brain works, and why, sometimes, it doesn't.

CLARITY is the result of a research effort in Deisseroth's lab to extract the opaque elements—in particular the lipids—from a brain and yet keep the important features fully intact. Lipids are fatty molecules found throughout the brain and body. In the brain, especially, they help form cell membranes and give the brain much of its structure. Lipids pose a double challenge for biological study, however, because they make the brain largely impermeable both to chemicals and to light.

Neuroscientists would have liked to extract the lipids to reveal the brain's fine structure without slicing or sectioning, but for one major hitch: removing these structurally important molecules causes the remaining tissue to fall apart.

Prior investigations have focused instead on automating the slicing/sectioning approach, or in treating the brain with organic molecules that facilitate the penetration of light only, but not macromolecular probes. With CLARITY, Deisseroth's team has taken a fundamentally different approach.

"We drew upon chemical engineering to transform biological tissue into a new state that is intact but optically transparent and permeable to macromolecules," said Chung, the paper's first author.

This new form is created by replacing the brain's lipids with a hydrogel. The hydrogel is built from within the brain itself in a process conceptually similar to petrification, using what is initially a watery suspension of short, individual molecules known as hydrogel monomers. The intact, postmortem brain is immersed in the hydrogel solution and the monomers infuse the tissue. Then, when "thermally triggered," or heated slightly to about body temperature, the monomers begin to congeal into long molecular chains known as polymers, forming a mesh throughout the brain. This mesh holds everything together, but, importantly, it does not bind to the lipids.

With the tissue shored up in this way, the team is able to vigorously and rapidly extract lipids through a process called electrophoresis. What remains is a 3-D, transparent brain with all of its important structures—neurons, axons, dendrites, synapses, proteins, nucleic acids and so forth—intact and in place.

Going things one better

CLARITY then goes one better. In preserving the full continuity of neuronal structures, CLARITY not only allows tracing of individual neural connections over long distances through the brain, but also provides a way to gather rich, molecular information describing a cell's function is that is not possible with other methods.

"We thought that if we could remove the lipids nondestructively, we might be able to get both light and macromolecules to penetrate deep into tissue, allowing not only 3-D imaging, but also 3-D molecular analysis of the intact brain," said Deisseroth, who holds the D.H. Chen Professorship.

Using fluorescent antibodies that are known to seek out and attach themselves only to specific proteins, Deisseroth's team showed that it can target specific structures within the CLARITY-modified—or "clarified"—mouse brain and make those structures and only those structures light up under illumination. The researchers can trace neural circuits through the entire brain or explore deeply into the nuances of local circuit wiring. They can see the relationships between cells and investigate subcellular structures. They can even look at chemical relationships of protein complexes, nucleic acids and neurotransmitters.

"Being able to determine the molecular structure of various cells and their contacts through antibody staining is a core capability of CLARITY, separate from the optical transparency, which enables us to visualize relationships among brain components in fundamentally new ways," said Deisseroth, who is one of 15 experts on the "dream team" that will map out goals for the $100 million brain research initiative announced April 2 by President Obama.

And in yet another significant capability from a research standpoint, researchers are now able to destain the clarified brain, flushing out the fluorescent antibodies and repeating the staining process anew using

different antibodies to explore different molecular targets in the same brain. This staining/destaining process can be repeated multiple times, the authors showed, and the different data sets aligned with one another.

Opening the door

CLARITY has accordingly made it possible to perform highly detailed, fine-structural analysis on intact brains—even human tissues that have been preserved for many years, the team showed. Transforming human brains into transparent-but-stable specimens with accessible wiring and molecular detail may yield improved understanding of the structural underpinnings of brain function and disease.

Beyond the immediate and apparent benefit to neuroscience, Deisseroth cautioned that CLARITY has leapfrogged our ability to deal with the data. "Turning massive amounts of data into useful insight poses immense computational challenges that will have to be addressed. We will have to develop improved computational approaches to image segmentation, 3-D image registration, automated tracing and image acquisition," he said.

Indeed, such pressures will increase as CLARITY could begin to support a deeper understanding of large-scale intact biological systems and organs, perhaps even entire organisms.

"Of particular interest for future study are intrasystem relationships, not only in the mammalian brain but also in other tissues or diseases for which full understanding is only possible when thorough analysis of single, intact systems can be conducted," Deisseroth said. "CLARITY may be applicable to any biological system, and it will be interesting to see how other branches of biology may put it to use."

More information: dx.doi.org/10.1038/nature12107