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Lighting the Way to More Detailed Brain Mapping

Researchers have found that genetically installing light-sensitive proteins in the brains of mice enables them to map functional brain wiring over long distances at unprecedented levels of detail. The technique overcomes significant obstacles that have until now limited neuronal circuit mapping, the researchers said.

Karel Svoboda, a group leader at Howard Hughes Medical Institute's Janelia Farm Research Campus, led the research group that reported its results on April 15, 2007 in an advance online publication of *Nature Neuroscience*.

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Scientists working to understand how the brain processes information would like to reconstruct the circuit diagram of the brain, by mapping exactly which neurons communicate with one another. To do this, they often study preparations of brain slices from laboratory animals. This approach allows researchers to isolate specific regions of the brain and analyze connections formed by individual neurons within each brain slice. However, slicing disrupts three-dimensional brain circuitry. The vast majority of connections—some of which stretch across great distances—are inevitably severed.

Neuroscientists have devised methods of probing pairs of neurons for connections. However, these techniques are limited to studying the small subset of neurons and connections that remain intact in the brain slice.

Using a light-sensing protein normally produced by green algae, Svoboda and his colleagues found a way around this problem. In nature, the protein, which is known as channelrhodopsin-2, enables the algae to migrate toward light. Other researchers had already found that the protein could help them

manipulate neurons in the lab. They showed that neurons that contain channelrhodopsin-2 can be triggered to generate nerve impulses simply by exposure to light.

To explore how this protein might help in mapping neural circuits, Svoboda's team genetically engineered mouse embryos so that their neurons produced channelrhodopsin-2. To target the channelrhodopsin-2 to specific neurons, the researchers introduced its gene at a specific time during embryonic development when that subset of neurons was forming. They used a technique called electroporation, in which high-voltage current renders the cell membranes permeable, to allow infusion of genetic material.

They then showed that directing laser pulses at the engineered neurons reliably triggered nerve impulses. They measured these impulses using electrodes inserted into the engineered neurons. The researchers next explored whether they could use channelrhodopsin-2 to trace neuronal connections in slices of brain tissue. They found that by directing their laser at specific areas of a brain slice, they could trigger nerve impulses—even in axons that were severed from their parent cell bodies. This observation indicated that channelrhodopsin-2 protein was present throughout the neuron, including the axon. They then recorded synaptic transmission in neurons receiving input from the engineered neurons. The researchers called their technique channelrhodopsin-2 assisted circuit mapping (CRACM). This technique allows circuit mapping between presynaptic neurons, defined by channelrhodopsin-2 expression, and postsynaptic neurons, defined by electrophysiological recordings.

CRACM overcomes two major drawbacks of functional circuit mapping, compared to other methods, said Svoboda. One is that with other methods you don't really have the ability to genetically target specified neuronal populations. You just grab neurons and ask, based on their position and shape, which one connects to which. And the other drawback is that brain slices contain only a small fraction of the brain, and the vast majority of axons are separated from their cell bodies. But with CRACM, we can target neuronal subpopulations, and we can map connections, even if only the isolated axons remain in their target area.

In a related study, HHMI investigator Michael Ehlers used a different genetic technique to introduce the channelrhodopsin-2 gene into mice. In that paper, published in the April 19, 2007 issue of the journal *Neuron*, Ehlers and his colleagues reported mapping neuronal circuitry in the brains of living mice.

Svoboda and his colleagues used CRACM to take on a mapping project that has been particularly daunting for neuroscientists. Neural connections between the two hemispheres of the brain—known as corpus callosum—are critical to integrating the distinct functions of the brain's hemispheres. However, because the connections project over long distances, researchers have been unable to determine precisely how the hemispheres connect.

Using CRACM, the researchers could perform such long-distance mapping in brain slices from mouse cortex very precisely. The results were quite

surprising, said Svoboda. We have preliminary evidence for what I would call a simplifying principle of connectivity. This is important, because we in the field worry that we won't be able to make sense of the complexity of cortical circuits—that axons connecting one brain region to another could choose many different synaptic partners, perhaps a different subset in every case. If that were true, connections between different brain areas would demand new and detailed investigation.

Svoboda and his colleagues found instead that axons originating in particular layers of the brain sought out exactly the same types of neurons as in different brain regions. Similarly, they also appeared to avoid the same types of neurons. Svoboda said he believes this finding demonstrates the great value of channelrhodopsin-2-assisted mapping to understanding neural circuitry in a way never before possible.

CRACM allows exploration of functional anatomy across all length scales of the brain, said Svoboda. It further promises to provide the kind of mechanistic and biophysical insights that other techniques have only hinted at. Beyond mapping connectivity, we can explore the properties of the synaptic connections that these neurons make, he said. Analyzing the dynamics of synaptic connections is ultimately critical to get a hint of the computations that these circuits perform.

Svoboda and his colleagues plan to improve CRACM by developing ways to genetically target more specific neuronal subpopulations. They also plan to improve the electrical properties of channelrhodopsin-2, to enable lower levels of the protein to still trigger robust nerve impulses.