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# A New Window to View How Experiences Rewire the Brain

Howard Hughes Medical Institute researchers have developed sophisticated microscopy techniques that permit them to watch how the brains of live mice are rewired as the mice learn to adapt to new experiences.

Their studies show that rewiring of the brain involves the formation and elimination of synapses, the connections between neurons. The technique offers a new way to examine how learning can spur changes in the organization of neuronal connections in the brain.

The researchers, postdoctoral fellow Josh Trachtenberg, graduate student Brian Chen and Karel Svoboda, a Howard Hughes Medical Institute investigator at Cold Spring Harbor Laboratory, published their findings in the December 19/26, 2002, issue of the journal *Nature.*

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> **- Karel Svoboda**

According to Svoboda, researchers had previously shown that the adult brain has a capacity to reorganize in response to new experience. However, it is not clear how this reorganization might occur. Svoboda and his colleagues wanted to see whether learning could induce restructuring of the neural circuitry in the brain that could not be picked up with conventional techniques.

To study those kinds of changes in a living animal, Svoboda and his colleagues started with transgenic mice that were engineered to produce green fluorescent protein within neurons in a portion of the brain that processes tactile sensory inputs from the whiskers. To observe changes in these neurons at high resolution, the scientists constructed a 2-photon laser scanning microscope. This microscope uses an infrared laser to excite green fluorescent protein in neurons, deep in the brain, through a tiny glass window installed in a portion of the mouse's skull.

"Since we had this great tool to look at the brain at unprecedented resolution we did not know what to expect and we began with no preconceived notions of what we might see in these animals," said Svoboda. "Our first observations of the large-scale structure of neurons, their axons and dendrites, revealed that they were remarkably stable over a month." Dendrites and axons are highly branched structures, where dendrites are the input side of neurons and axons the output side.

"However, when we zoomed in closer, we found that some spines on dendrites appeared and disappeared from day to day," said Svoboda. These spines stipple the surface of dendrites, like twigs from a branch, and form the receiving ends of synapses, which are the junctions between neurons where neurotransmitters are released.

This finding was quite unexpected, because the traditional view of neural development has been that when animals mature, the formation of synapses ceases, which is indicated by stable synaptic densities, said Svoboda. However, the flaw in this view has been that a stable density only indicates a balanced rate of birth and death of synapses. It doesn't imply the absence of the formation of new synapses, but it was often interpreted that way.

In their experiments, Svoboda and his colleagues observed that about twenty percent of spines disappeared from one day to the next, offset by the formation of new spines.

"While we were surprised at the rate of turnover of some spines, we were also surprised at the incredible stability of other spines," said Svoboda. The spines appeared to fall into different classes. And while there were those that turned over rapidly, other spines, typically the larger ones, persisted for months.

To test whether the new spines were actually forming synapses, the researchers used electron microscopy to analyze in brain slices the same regions that they had studied in the living animals. Those studies revealed that the sprouting spines had indeed formed synapses.

The researchers also explored whether sensory experiences could affect the turnover of spines. In this set of experiments, they trimmed individual whiskers from the mice, forcing them to experience their environment with a subset of whiskers. This manipulation expands the representation of the intact whiskers at the expense of trimmed whiskers. There was a dramatic effect on spine turn-over.

"We found in these animals that there was a pronounced increase in the rate of birth and death of these synapses, as evidenced by increased turnover of spines," said Svoboda. "This finding indicates that there's a pronounced rewiring of the synaptic circuitry, with the formation of new synapses and the elimination of other synapses," he said.

In an accompanying article published in *Nature,* researchers led by Wen-Biao Gan of the New York School of Medicine found almost no turnover of spines in a region of the visual cortex they studied in mice. Although the results of the experiments would appear to be contradictory, Svoboda said that is not necessarily the correct conclusion. Svoboda said that the visual cortex in adult animals might exhibit far less spine turnover than the tactile sensory region studied by his group. Also, he said, if the animals in the experiments by Gan and his colleagues lived in a visually impoverished environment, experience-dependent synaptic plasticity might not be as evident.

Svoboda said that his team's results suggest that a sample and hold model may operate to drive the plasticity of the adult brain. "We believe that the high turnover that we see might play an important role in neural plasticity, in that the sprouting spines reach out to probe different presynaptic partners on neighboring neurons," said Svoboda. "If a given connection is favorable -- that is, reflecting a desirable kind of brain rewiring -- then these synapses are stabilized and become more permanent. But most of these synapses are not going in the right direction, and they are retracted."

"The finding that structural plasticity in the adult brain is limited to synapses and spines could help explain the phenomenon of critical periods," said Svoboda. As an animal matures, there are certain critical periods early in development during which brain plasticity is highly active. By the time the animal reaches adulthood, plasticity is much reduced.

"It may be that in adulthood, since the large-scale structure of neurons does not change, the brain has become essentially an entangled mesh of neuronal processes. Axons and dendrites are stuck with each other as neighbors for life," said Svoboda. Each neuron thus may have a limited number of permanent neighbors, and any further rewiring with experience is limited to changes in the spines that connect those neurons.

In further studies, Svoboda and his colleagues plan to explore how brain circuitry changes on a larger-scale, by observing mice engineered to express different fluorescent proteins in different populations of neurons.