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## New Studies Illuminate the Computational Power of Neurons

Scientists have found that individual neurons have more computational power and contribute more to behavior than previously thought. The researchers used light to activate individual neurons in living mice and showed that even short bursts of activity in a few neurons can influence learning and decision making.

Karel Svoboda, Daniel Huber, and their colleagues at the Howard Hughes Medical Institute's Janelia Farm Research Campus and at Cold Spring Harbor Laboratory published their findings in two research articles in the journal *Nature*. In one paper, the researchers described how they trained genetically engineered mice to respond to activation of their neurons by light pulses. That paper was published December 19, 2007, in an advance online publication in *Nature*. In experiments described in a paper published in the December 20, 2007, issue of *Nature*, the researchers used light to study even more detailed aspects of how neurons function.

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When researchers want to learn how particular groups of neurons influence an animal's behavior, they activate those neurons and then study the effects of the stimulation. The most commonly used techniques allow researchers to target groups of neurons by their location, but do not permit them to understand the activity and output of these brain cells. In addition, different types of neurons are spatially intermingled. Huber, Svoboda and their colleagues wanted to examine the brain in more detail than traditional methods allow — to investigate how specific types of neurons affect learning and behavior - and so they turned to a new technique that relies on a light-sensing protein found in green algae called channelrhodopsin-2. Researchers have taken advantage of channelrhodopsin-2, a protein that enables algae to migrate toward light, to stimulate only neurons that express channelrhodopsin-2 with great precision.

In earlier studies, Svoboda and his colleagues genetically introduced channelrhodopsin-2 into mouse neurons. By experimenting on slices of brain tissue from the mice, the researchers showed that they could trigger nerve impulses by shining a laser on cells that contain channelrhodopsin-2. Only those neurons containing channelrhodopsin-2 fired when they were stimulated by light from the laser.

In their new experiments, Huber, Svoboda and their colleagues explored whether they could use the technique to influence the behavior of living mice. They began by implanting tiny glass slides - which literally served as windows into the brain — in the skulls of mice whose neurons contained channelrhodopsin-2. The researchers then mounted a light emitting diode (LED) light on the window.

The scientists trained the mice to respond to photostimulation of the channelrhodopsin-2-containing neurons. As part of the training exercise, the animals were placed in a chamber with two water ports. The animals learned to sip from one water port when they sensed photostimulation of their neurons, and to sip from the other port when they did not.

These animals learned the task remarkably quickly and very reliably, said Svoboda. So we knew we had a powerful method to ask how many stimuli are required for perception and in how many neurons. This was a very precise tool to not only stimulate just a particular cell type, but also control the fraction of cells that are stimulated.

The technique offered the researchers enough control to stimulate varying numbers of neurons by controlling light intensity. Their experiments revealed that relatively few neurons are needed to be activated for the mice to detect the photostimulation, said Svoboda. In the brain region that we targeted, the total number of cells that needed to be activated ranged from several tens of neurons to a couple of hundred, depending on how many stimuli there were, said Svoboda.

The sparseness of stimulation required for detection was surprising, because we know that there is considerable ongoing activity in the brain, he said. The activity produced by the light impulses is just a tiny fraction of the total activity. These findings tell us that there are mechanisms in the brain that can read out very sparse subsets of activated neurons. So, the take-home message from these experiments is quite powerful: that very few neurons need to be activated with very few action potentials to drive perceptions and behavior.

Svoboda said the broader lesson is that stimulating neurons optically is a powerful way to study brain circuitry. We can use these kinds of tools to figure out which neurons are connected to each other, he said. And we can also precisely manipulate particular neuronal populations and look at the effects on quantitative behaviors. That allows one to dissect how these circuits guide behavior.

This kind of neuronal targeting and stimulation might even have clinical applications, he said. Deep brain stimulation to treat Parkinson's disease and

other disorders activates brain tissue rather nondiscriminately, said Svoboda. One can imagine using the kind of genetic targeting and stimulation techniques we have used to target specific cell populations, reducing the side effects of deep brain stimulation.

In the second study, the researchers used light as a tool to study how the brain works on an even more intricate level.

Neurons propagate nerve signals by communicating with one another across junctions called synapses. These synapses are supported on tiny mushroom-shaped spines, a multitude of which sprout from dendrites that branch from neurons. Each spine acts as a receiving station for chemical signals—neurotransmitters—from neighboring neurons.

As synapses are repeatedly triggered to transmit an impulse, the strength of those connections can change through a process called long-term potentiation (LTP). Such modification enables the brain to modify circuits during learning.

Svoboda and his colleague Chris Harvey sought to understand whether LTP in one dendritic spine influences LTP in another. Such crosstalk would enable groups of synapses on the same dendritic branch to coordinate with each other to store more information.

Again, the scientists needed a way to stimulate neurons that was more precise than electrical stimulation. So they bathed slices of mouse brain tissue in a solution of the neurotransmitter glutamate in which the individual molecules had been trapped in light-sensitive molecular cages. The researchers then used precise laser pulses to unleash the caged glutamate at selected synapses, triggering the synapses to fire. At the same time, they used electrical pulses to induce LTP in the neurons.

When the researchers analyzed how stimulating an individual synapse affected its neighbors, they detected robust crosstalk. They found that once a synapse had undergone LTP, weaker stimuli now caused LTP at neighboring synapses.

Svoboda said that LTP at one synapse reduced the threshold for LTP at neighboring synapses for about 10 minutes. That could be very important from a learning perspective, he said, because it is on a time-scale in which learning takes place. In the learning process, animals usually have to associate one event with another event on a scale of minutes. In contrast, other neuronal mechanisms associated with synaptic plasticity have been on the order of seconds. So, we are exploring a new time scale for cellular plasticity, and I think people who model neuronal circuitry will be interested in seeing how these cell-level phenomena can explain learning and other behaviors, he said.