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New Technique Visualizes the Function of Synaptic Channels

Using a new technique, Howard Hughes Medical Institute researchers have visualized calcium channels in their native environment, showing the number and activity of the voltage-sensitive molecules that allow calcium to flow into neurons.

In an article published in the November 30, 2000, issue of the journal *Nature*, HHMI investigator Karel Svoboda and colleague Bernardo Sabatini at Cold Spring Harbor Laboratory describe the use of optical fluctuation analysis to view the activity of calcium channels in intact synapses, the junctions between neurons. Synapses are located on tiny projections called spines that jut from dendrites on neurons.

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— Karel Svoboda

"These spine channels are functionally important because they serve to amplify synaptic currents, and they also couple synaptic activity to downstream biochemical events," said Svoboda. "These events include those that modify the receptors themselves, leading to changes in the efficacy of synapses, which are a basis for long-term plasticity in the brain."

A major technical hurdle in understanding the behavior of these channels, said Svoboda, is that the widely used electrical measurement technique, single-channel patch clamping, could not be applied to study the channels in intact synapses. In patch clamping, microscopic electrodes are used to measure the electrical properties of individual channels.

"The patch clamp technique can only be used in simplified preparations such as cell cultures," said Svoboda. "However, we are interested in synapses, which must be studied in intact nervous tissue because of the importance of their cellular environment. Synapses are far too small to be accessible to electrical measurements."

To overcome this hurdle, the scientists invented the optical fluctuation analysis technique, which uses a laser scanning microscope to visualize calcium channels by measuring the light emitted from a calcium-sensitive fluorescent dye injected into target neurons in slices of rat brain. The scientists use electrical action potentials to trigger open calcium channels repeatedly and then measure the fluctuations in the light emitted by the spines. These light fluctuations provide a measure of the calcium activity in the synapses caused by random fluctuations in the opening and closing of the channels. The scientists analyzed those fluctuations over many trials to deduce the number and properties of the calcium channels.

"We knew that the spines were very small, so there couldn't be that many calcium channels on them," said Svoboda. "So, we knew that we could gain useful information by analyzing these fluctuations. The process is like flipping coins. Let's say you have ten coins. On any given flip, you might get anywhere from zero through ten heads. On average, you'll get five heads, but the fluctuations in the number of heads from trial to trial can actually tell you how many coins there are and what the probability of heads is.

"We used that kind of logic to find out how many channels there are in the spines and what their properties are," said Svoboda. "For example, we could figure out the probability of a channel opening in response to an action potential." The scientists' studies revealed that the typical dendritic spine held only about six channels, ranging from one to 20 and that they opened with a high probability with each triggering.

"It was really surprising that an individual synapse only works with such a small number of channels," said Svoboda.

In additional studies, Sabatini and Svoboda discovered how to modulate the signals from the calcium channels. In those studies, the scientists used a chemical activator, or agonist, that switched on GABA_B receptors to reduce synaptic transmission. "When we added GABA_B receptor agonist, which presumably activates all GABA_B receptors along the dendrite, only the calcium channels in the spines were modulated by these activated receptors. So this is good evidence that these spines are actually separate signaling compartments and that the signaling environment in the spines is very different than in the dendrites."

According to Svoboda, additional studies should yield significant information about the nature and location of such key biochemical processes underlying neuronal function and plasticity. "In general, all of the evidence points towards these spines being isolated signaling compartments," he said. "We know that the synapse activates the spine, the calcium comes in and activates enzymes or receptors, and all of this biochemical action happens in the spine head."