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Nerve Cells Possess a Previously Unknown Form of Plasticity

Researchers have discovered a new form of synaptic plasticity, the changes to nerve cells in the brain that underlie learning and memory. The phenomenon, the scientists say, may help govern how a single neuron integrates and processes multiple stimuli.

The researchers, led by HHMI investigators Lily Jan and Yuh Nung Jan at the University of California San Francisco, published their findings in the October 7, 2005, issue of the journal *Cell*. Coauthors on the paper include the Jans' colleagues at UCSF and Robert B. Darnell, an HHMI investigator at The Rockefeller University.

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— Lily Y. Jan

Scientists have long known that long-term potentiation (LTP) can strengthen the connections between neurons, so that a nerve cell more readily responds to a signal from its neighbor. This heightened sensitivity can persist for several hours.

LTP is best studied in excitatory synapses, where a neurotransmitter molecule is released by one cell and tends to trigger an electrical impulse in the receiving cell. Lily Jan compares the phenomenon to Pavlov's famous experiment in which ringing a bell while feeding a dog causes the dog to associate the two stimuli. LTP, she says, "reminds you of that. If you have two different excitatory inputs and they happen at the same time, it can cause a very long-lasting change."

But not all the signals that neurons send are excitatory. Some neurotransmitters act as inhibitory signals, which reduce the likelihood of generating electrical impulses in the receiving cell. Inhibitory signals come in two types, Jan explained, fast and slow. Fast inhibition occurs through a mechanism very similar to fast excitation of a nerve cell, by activating transmitter receptors that are ion channels, and takes place on essentially the same time scale - tens of milliseconds. Some inhibitory neurotransmitter

receptors, however, exert their effects through a more complicated signaling pathway, and so take longer to act - more like hundreds of milliseconds.

Work on fast inhibition by other labs, Jan said, has shown that stimulations causing LTP of fast excitation could induce long-term depression of fast inhibition - an opposite effect to long-term potentiation that makes the synapse less responsive to incoming signals. But there was not yet any evidence to indicate whether slow inhibition could be affected by similar neuronal activity.

Jan said the researchers' curiosity was piqued by earlier work that showed that two molecules involved in slow synaptic inhibition, known as GABA_B receptors and GIRK channels, could be found in parts of the neuron known as the spines. These tiny mushroom-shaped protrusions line the dendrite - the portion of a nerve cell that receives neurotransmitter signals - and are home to the cell's excitatory synapses. Inhibitory synapses, on the other hand, reside on the main body of the dendrite - the shaft - and so it was a surprise to find GABA_B receptors and GIRK channels there. All the machinery for synaptic plasticity, Jan says, is also in the spine. "And so the question was," Jan said, "what would happen to the slow inhibition that's happening right there on the spine."

To find out, the scientists studied the electrical signals in neurons from the hippocampus, the region of the brain involved in memory. Since LTP requires signaling from multiple synapses, the researchers simulated a signal from a neighboring neuron by depolarizing the cell, thereby reducing its charge. They then gave an electric stimulus at the same time as the depolarization and measured the resulting current with a glass electrode applied directly to the cell - and found that the slow inhibition did indeed undergo LTP.

The researchers went on to explore the pathway controlling this response. They found that by blocking the receptor responsible for long-term potentiation of excitation, the NMDA receptor, they could prevent LTP of the inhibition, as well. Similarly, both calcium and an enzyme known as calcium/calmodulin kinase II (CaMKII), essential for LTP of excitatory synapses, were also necessary for inhibitory LTP. In fact, they found that by activating CaMKII, they could directly induce inhibitory LTP. From this evidence, they were able to conclude the LTP of slow inhibition occurs through the same pathway as LTP of excitatory synapses.

Once they knew that the same pathway could generate LTP of both excitatory and inhibitory synapses, the scientists wondered whether this plasticity might be controlled by a "master" regulatory protein. A good candidate, they thought, was a protein found in the brain called Nova-2.

Nova, the target of autoimmune attack in an uncommon neurological disorder known as paraneoplastic opsoclonus myoclonus ataxia, is the focus of study in Bob Darnell's lab. When Jan learned years ago during a lecture by Darnell at UCSF that GABA_B receptors, GIRK channels, and other molecules thought to be involved in synaptic plasticity were among those under

Nova-2's control, the labs began to collaborate.

Darnell recently found that Nova-2 controls a coordinated network of synaptic proteins, and one-third of these are molecules involved in inhibitory synaptic transmission - so it was reasonable to think it might play a role in the newly discovered inhibitory LTP. Using mice engineered by the Darnell lab to lack Nova-2, Jan and colleagues found that the protein is indeed required for LTP of slow inhibition. It is not, however, necessary for LTP of excitation. "It was totally surprising to us, but it's really intriguing that there could be controls at that level," Jan said.

Jan and her colleagues are proceeding with additional experiments to understand the how and why of this new phenomenon. They are investigating the finer details of how the molecules involved in slow inhibition might be altered during potentiation. "The other that's fun to consider," Jan says, "is why do we do this? Why do we potentiate slow inhibition at the same time as we potentiate fast excitation?"

At this point, the scientists can only speculate, but they have some ideas. Returning to Pavlov's experiment, Jan points out that in order for the dog to associate a bell with food, the two stimuli must come at the same time. "But when we say they happen at the same time, what do we really mean? Is it within the same millisecond? Within the same second? It's not within the same millisecond; the neuron cannot be that precise."

So a possible role of LTP of slow inhibition, she says, is to shorten the window during which two excitatory signals are perceived as coming "at the same time." Since slow inhibition counteracts the impulse generated by excitatory signals, signals that arrive after a few hundred milliseconds will be "put at a disadvantage" and no longer considered synchronous with the original signal, she explains.