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Researchers Find Enzyme Crucial to Preservation of Memories

Using a technique to eliminate the function of one enzyme in a restricted memory-related region in the brains of mice, researchers have shown that the enzyme is important in consolidating long-term memories.

According to the researchers, their experiments — which showed that defects in a key biochemical signaling pathway were responsible for the animals' inability to improve their long-term memory in a series of maze tests — constitute a powerful approach to understanding molecules involved in learning and memory.

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— Susumu Tonegawa

In an article published in the September 21, 2001, issue of *Cell*, Howard Hughes Medical Institute investigator Susumu Tonegawa and colleagues at the Massachusetts Institute of Technology and the Vollum Institute reported that elimination of the enzyme, calcium-calmodulin dependent kinase (CaMKIV), in the forebrains of mice had profound effects on signaling pathways in the brain and learning behavior.

The scientists began their studies to clarify the enzyme's role in late long-term potentiation (L-LTP), the process by which enduring memories are established through a mechanism of activating genes that trigger protein synthesis. This protein synthesis, in turn, alters the synapses — connections between neurons — and "etches" permanent memory pathways.

"CaMKIV had been implicated in long-term memory pathways in the past, but previous studies had involved global knockout of the enzyme in the entire animal," said Tonegawa. "Such knockouts gave inconsistent results because

they affected the whole brain throughout development. We decided to use a technique to inhibit the protein only in the forebrain, which is more involved in higher brain function.”

Tonegawa said that other research groups had attempted to knock out a protein called CREB, which is involved in turning on gene transcription in L-LTP, and which was believed to be activated by CaMKIV. The results of these studies were inconclusive, Tonegawa said, because there appeared to be multiple forms of CREB that could compensate for any knockout.

Tonegawa and his colleagues used a genetic technique that allowed them to replace the normal CaMKIV with a “dominant negative” mutant enzyme that would be produced only in the forebrains of the mice. Dominant negative enzymes have all of the characteristics of the functioning enzyme — such as an ability to bind normally to other molecules — but they lack the ability to carry out an appropriate enzymatic reaction.

The scientists first studied the molecular details of the lack of CaMKIV activity in brain slices from the transgenic mice. They discovered that the base level of CaMKIV activity in the mouse brains was normal, but when chemicals were added that mimicked the conditions of neuronal activity, as in memory formation, the enzyme function was significantly lower. The brain slice studies also revealed that CREB activation by phosphorylation in the transgenic mice was suppressed, strongly implicating a role for the CaMKIV in normal CREB activation as a result of neuronal activity.

Experiments with brain slices also revealed that the transmission of nerve impulses in the transgenic mice was normal, except under conditions mimicking protein-synthesis-dependent L-LTP.

“These results pinpointed for us the role of CaMKIV in the protein-synthesis-dependent type of LTP,” emphasized Tonegawa. “This is very important, because in the past people have published studies implicating another enzyme, called protein kinase A, in LTP. However, that enzyme was not specific to the protein-synthesis-dependent type of LTP.”

With clear physiological evidence that they had specifically disrupted the CaMKIV pathway, the researchers next tested how well the transgenic mice could consolidate memories of a water maze. The mice were placed in a pool of water made opaque by floating beads, and required to find a platform submerged just beneath the surface. The transgenic mice initially learned the task as well as normal mice, but as training continued, they became significantly less able to find the platform.

“Thus, while these mice have a normal ability to acquire memories, they have problems converting those memories into a long-term form,” said Tonegawa. However, he noted, maze experiments still present problems in interpretation. “This training takes place over a two-week period, so the memory acquisition and consolidation processes are superimposed,” said Tonegawa. “Thus, it is difficult to know whether the deficit is primarily in the acquisition phase or the consolidation phase.”

In an additional set of experiments, the scientists compared normal and transgenic animals' ability to acquire and consolidate the memory that involves associating a mild shock to the footpads to the specific context of the chamber in which the shock is administered. In these experiments, memory acquisition could be more clearly separated from memory consolidation, said Tonegawa. These experiments demonstrated that the CaMKIV-deficient mice could learn to associate the shocks to the chamber context, but they had difficulties in converting such memories to long lasting memories, said Tonegawa.

“Our conclusion from these tests was that the CaMKIV pathway was primarily involved in memory consolidation and retention,” he said.

Tonegawa noted that memory consolidation in the transgenic animals was not completely extinguished, suggesting that there may be parallel signaling pathways involved in consolidation, or that there may have been incomplete knockout of CaMKIV activity.

“However, we believe that further studies using this technique will allow us to dissect in greater detail the differential roles and interactions of these signal transduction pathways, and how they contribute to this very complex mechanism of memory consolidation,” he said. “Also, we want to know which genes are activated in this process and how these gene products help establish these long-term changes in synaptic strengths.”