Mammalian Learning and Memory Studied by Gene Targeting

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This is a unique and interesting conference, and I wish to extend my congratulations to Jim Watson, Frances Crick, and Maurice Wilkins on the 40th anniversary of the discovery of the double helix. In this brief presentation I would like to focus on recent studies in which we employed a genetic approach to study mammalian learning and memory.

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The gene-targeting technology to study learning and memory in mammals was pioneered by Mario Capecchi and others and is revolutionizing many subfields of mammalian biology. Neurobiology or neuroscience should be no exception. In this paper, I will focus on our recent attempt to apply this technique to dissect the cellular and molecular mechanisms underlying mammalian learning and memory. The key phenomenon is the long-term potentiation, or LTP, which is an electrophysiological manifestation of a long-lasting increase in the strength of a neuronal synapse that has been stimulated in an appropriate fashion. The initial phase of LTP induction is relatively clear; LTP is induced by the binding of a neurotransmitter, glutamate, combined with depolarization of the postsynaptic membrane, which leads to an influx of calcium ion. What follows the rise of the calcium concentration is less clear at this time, but various studies suggest that the increased calcium concentration triggers a biochemical cascade that involves calcium/calmodulin–dependent kinase-II, calcium-dependent protein kinase, protein kinase C, and phosphotases, etc. Eventually, the signal is thought to be transmitted to the nucleus of the postsynaptic cell to establish the LTP; that is, new protein synthesis may be required. A role of so-called retrograde messenger which would increase the neurotransmitter release from the presynaptic terminal has also been suggested. Although LTP has been widely studied as a candidate information-storage mechanism, at least for some forms of learning and memory, the actual evidence supporting this notion is not very extensive. The main support for LTP as a memory mechanism is the observation that the pharmacological

agents such as AP5 known to block hippocampal NMDA receptors prevent the induction of LTP as well as spatial learning in rodents. However, the problem with this evidence is that blocking NMDA receptors disrupts the synaptic function and potentially interferes with the *in vivo* computational ability of hippocampal circuits. Perhaps the impairment of learning that was observed results not from the deficit in LTP *per se*, but simply from incorrect operation of hippocampal circuits that lack NMDA receptor function.

In order to re-examine the relation between LTP and learning, we focused on kinases that are presumably in the downstream of NMDA receptors in the biochemical cascade triggered by the calcium ion influx. We produced a strain of mouse mutants by knocking out the corresponding gene by the gene-targeting procedure. The first mutant mouse of this program in my laboratory was made by Alcino Silva, who is now at the Cold Spring Harbor Laboratory. In this strain of mutant mouse, the α subtype of CaM kinase II is deleted, and I will mention only the summary of the data obtained with the α CaMKII mutant mice because it is now more than a year since these data were published. The first important conclusion drawn was that LTP in the CA1 region of the hippocampus of the mutant mouse was severely impaired, but no impairment occurred in the ordinary postsynaptic mechanisms such as the NMDA receptor function. The second important message we got was that the capability of hippocampus-dependent spatial learning was severely impaired. So these results supported the notion which had been held by some neuroscientists on the basis of earlier pharmacological studies, namely, that LTP is the cellular basis for some types of learning.

We have continued our program of investigating the molecular mechanism of LTP and LTP's relation to learning and memory by producing a second mutant strain—mice lacking the γ isoform of the calcium and phospholipid–dependent protein kinase C (PKC). PKC γ is one of the nine known PKC isoforms and is expressed specifically in the brain and spinal cord, being particularly abundant in the hippocampus. As far as ordinary synaptic transmission is concerned, we could not distinguish the slices from the hippocampus of the PKC γ mutants and those from the wild-type littermates. However, when we looked at the LTP in the CA1 region of the hippocampus, we saw a greatly diminished LTP in the mutant compared to the wild-type littermates.

We then subjected the PKC γ mutant mice to several different learning tasks. The one I will focus on here is called the Morris water maze, which consists of a circular pool 1.2 meters in diameter and tests the animal's ability to acquire spatial learning. The pool is filled with opaque water and a small platform is submerged just below the surface of the water. The platform location is kept constant throughout the training, but a mouse to be trained is allowed to start swimming from a random location in multiple training sessions. Mice are born swimmers, but they don't like to be in water so they try to find a way to escape from it. However, since the water is opaque they cannot see

the platform, so the mouse will initially swim more or less randomly trying to find an exit and run into the platform only accidentally. However, as the training is repeated, a wild-type mouse learns the location of the platform fairly precisely and eventually swims directly to it regardless of the starting site. It has previously been shown that the strategy employed by the mouse is to map the location of the hidden platform using the multiple objects surrounding the pool as landmarks. This learning is termed spatial learning and has been shown to be hippocampus-dependent. If the mouse receives a lesion in the hippocampus it cannot learn this task well. So, what one does is to repeat the trials, record the time required for the mouse to find the platform, and plot it against the number of trials. The PKC γ mutant mice improved their performance as well as the wild-type mice did.

In order to confirm that the PKC γ mutant mice indeed use the spatial strategy to improve the performance we must examine the trained mice by appropriate tests. One test is called the probe trial. In this test we first attempt to train a mouse for a particular location of the platform so that the mouse has a memory of a specific location of the platform that could easily last for at least a few weeks. We then put the mouse back in the cage and remove the platform from the pool. We return the mouse to the pool and let it swim for a fixed period of time, like 60 seconds, and then record the fraction of the 60 seconds spent in each of the four equivalent imaginary quadrants (namely, the training, left, right, and opposite quadrants).

As expected, wild-type mice spend more time in the training quadrant compared to any of the other three quadrants, indicating that they have the spatial memory. The mutant mice also have the spatial memory; they showed clear selectivity to the training platform.

We then subjected the trained mice to a second test, called a platform crossing test. In this test trained mice are again tested in a pool without a platform, but in this case one counts the number of times a trained mouse crosses the site at which the platform was situated during the training session. We then compared this number with the number of times the mouse crossed the equivalent site in each of the three non-training quadrants. Again, the wild-type mice showed clear site-selectivity. Mutant mice also showed site-selectivity, but when we analyzed it statistically, we noticed that there was a slight deficiency in the site-selectivity with mutants. Nevertheless, mutant mice clearly indicated the acquisition of spatial learning.

So we are left to conclude that the PKC γ mutant mice can acquire spatial learning despite the severe deficit in the LTP in the hippocampal CA1 region. This is an apparent contradiction of the findings made with α CaMKII mutant mice that I summarized above. So we then asked what hippocampal synaptic plasticity could be the basis for the spatial learning in the PKC γ mutants. We went back to electrophysiology and found that the second major form of synaptic plasticity, called long-term depression (LTD), is intact in the CA1 re-

TABLE 1. Summary of Data from Mutant and AP5-Treated Mice

Analysis	(−/−)	(−/−)	AP5-Treated
LTP (conventionally induced)	−	−	−
LTP (primed)	+	−	Not done
LTD	+	−	−
Learning (spatial/contextual)	+[a]	−	−

[a] Partial impairment.

NOTE: Data are from the following sources: LTP and LTD in PKCγ-mutant mice are from Abeliovich *et al.*[3,4] LTP in αCaMKII-mutant mice is from Silva *et al.*[1]; LTD in αCaMKII mice is from Stevens *et al.*[6] Learning in αCaMKII mice is from Silva *et al.*[2] AP5 LTP is from Collingridge and Singer[7]; AP5 LTD is from Dudek and Bear[8]; and AP5 learning is from Morris *et al.*[9]

gion of the PKC γ mutant. LTD is similar to LTP except that it is a long-lasting reduction of synaptic efficacy. Like LTP, LTD depends on NMDA receptors and calcium ion influx and can potentially serve as a synaptic mechanism for learning and memory. However, because of the delay in the discovery, LTD has not been studied as a memory mechanism as much as LTP. In any case, our data indicated that the hippocampal LTD could be the basis for the observed spatial learning by PKC γ mutant mice. However, the matter was more complex.

We also found that if a low-frequency stimulation is given prior to a tetanus, LTP can be induced in PKC γ mutant mice. So this type of LTP, which we call primed LTP, can also serve as the mechanism for spatial learning.

We also went back to the α CaMKII mutant mice in light of these new findings with the PKC γ mutant mice. We found that LTP cannot be induced with α CaMKII mutant slices even when a low-frequency stimulation was given prior to the high-frequency stimulation (i.e., no primed LTP). We also found that LTD is impaired in the α CaMKII mutant slices. TABLE 1 lists three different types of mice (AP5-treated α CaMKII mutant, PKC γ mutant, and normal mice) as well as absence or presence of the three different types of synaptic plasticity (LTP, LTD, and primed LTP) and spatial learning capability. This table shows that LTD and primed LTP correlate better with the spatial learning capability than does the conventional LTP, which is not essential for spatial learning. However, the moderate learning deficit observed in PKC γ mutant mice suggests that conventional LTP contributes to learning. Contextual learning is a broader term which includes spatial learning. We have carried out another set of behavioral experiments in which contextual learning capability was tested using a different paradigm (fear conditioning). The results were very similar to those of the Morris water maze.

We are encouraged by the progress made to date with this new genetic approach. Our goal is to uncover the relationship between a function of a specific gene, that for synaptic plasticity, and learning. We are aware of some shortcomings of this approach, however. For instance, one would like to be able

to improve the gene-targeting technology such that a disruption of a specific gene can be induced in an adult animal in response to an appropriate agent (inducible knockout). Another desired improvement would be to restrict the knockout to a particular region of the brain (tissue-specific knockout). These technical improvements are currently actively pursued not only in my laboratory, but also in several other laboratories throughout the world. Considering the number of highly qualified applicants I receive for postdoctoral and graduate studies in this field, I would predict that this new genetic strategy for the analysis of mammalian behaviors will really flourish in the coming years.

REFERENCES

1. SILVA, A., R. PAYLOR, J. M. WEHNER & S. TONEGAWA. 1992. Impaired spatial-learning in α calcium/calmodulin kinase II mutant mice. Science 257: 206–211.
2. SILVA, A., C. STEVENS, J. WEHNER, S. TONEGAWA & Y. WANG. 1992. Deficient hippocampal long term potentiation in α calcium/calmodulin kinase II mutant mice. Science 257: 201–206.
3. ABELIOVICH, A., C. CHEN, Y. GODA, A. J. SILVA, C. F. STEVENS & S. TONEGAWA. 1993. Modified hippocampal long term potentiation in PKCγ mutant mice. Cell 75: 1253–1262.
4. ABELIOVICH, A., R. PAYLOR, C. CHEN, J. J. KIM, J. M. WEHNER & S. TONEGAWA. 1993. PKCγ mutant mice exhibit moderate deficits in contextual learning. Cell 75: 1263–1271.
5. GRANT, S. G., T. J. O'DELL, K. A. KARL, P. L. STEIN, P. SORIANO & E. R. KANDEL. 1992. Impaired long-term potentiation, spatial learning, and hippocampal development in *fyn* mutant mice. Science 258: 1903–1910.
6. STEVENS, C. F., S. TONEGAWA & Y. WANG. 1994. The role of calcium-calmodulin kinase II in three forms of synaptic plasticity. Curr. Biol. 4(8): 687–693.
7. COLLINGRIDGE, G. L. & W. SINGER. 1990. Excitatory amino acids and synaptic plasticity. Trends Pharmacol. Sci. 11: 290–296.
8. DUDEK, S. M. & M. F. BEAR. 1992. Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade. Proc. Natl. Acad. Sci. USA 89: 4363–4367.
9. MORRIS, R. G. M., E. ANDERSON, G. S. LYNCH & M. BAUDRY. 1986. Selective impairment of learning and blockade of long-term potentiation by an *N*-methyl-D-aspartate receptor antagonist AP5. Nature 319: 774–776.