How Does the Brain Learn? A Molecular View

by Mary B. Kennedy

THE BRAIN IS a remarkable organ; one of its most remarkable and least understood functions is its ability to learn and remember, to adapt to changes in the environment. Neuroscientists have been studying the brain for 100 years or more, but it is only within the last decade that we have begun to establish some basic facts about how it learns.

It is clear that learning must involve physical changes within the neuron — the cell that is the basic signaling and processing unit of the brain (Figure 1 and cover). Neurons have three main parts: the cell body, the dendrites, and the axon. The cell body forms the center of the neuron, the place where proteins and other structural components are synthesized. Radiating from the cell body are the dendrites — fine, dense, branching processes that receive information transmitted from other neurons. Each neuron also has an axon, a long, often branched cable that carries information to many other neurons. The axon branches end in tiny bulbs called synapses, which are the crucial points of contact with the dendrites of other neurons. They are also active information processing sites.

Billions of neurons, each one making contact with an average of 10,000 others, make up the brain. Although the brain is a soft, almost buttery tissue, the neurons are highly organized. Deep within the brain, neurons cluster in "nuclei." Some of the nuclei send axons to the surface of the brain, which is covered by the cerebral cortex, a highly folded and convoluted sheet of neurons. This cortex is important for most of the brain's higher functions.

Specific, highly organized areas within the brain perform many of its specialized functions. For instance, a part of the cerebral cortex called the motor cortex is contained in a narrow band over the top of the brain. Neurons there send axons directly to the spinal cord, where they signal spinal neurons that in turn activate our muscles. At the back of the brain lies the primary visual cortex, which receives signals starting in the retina. Cells in the visual cortex process information from the retina to produce our concept of the visual world. Figure 2 shows a cross section

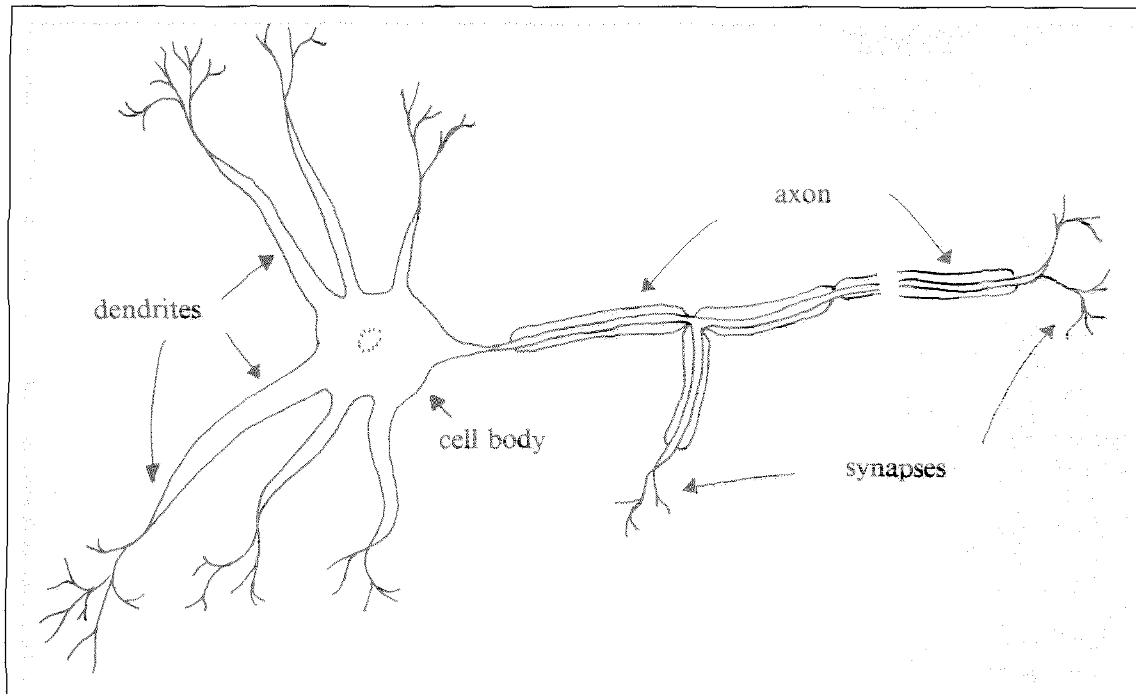

Figure 1. The typical neuron has three main parts — the cell body, the dendrites, and the axon.

through the visual cortex of a macaque monkey revealing its highly organized structure. The stripes indicate specialized layers of cells that interconnect in a regular way.

Where in the brain are memories stored? The most correct answer to this difficult question is that memories seem to be stored in many different places, both in the cortex and in deeper structures. Different kinds of memories are apparently stored in different places — visual memories in the brain's visual areas, motor memories in motor areas, and so on. Complex memories may well be stored dispersed throughout areas of the brain called "association cortex." However, one brain structure, which lies just beneath the cortex, seems to play a particularly important role in the formation of memories. Its name, the hippocampus, comes from the Greek word for sea horse, which some early anatomists thought it resembled.

Several circumstantial lines of evidence suggest that the hippocampus plays a role in forming memories. The most dramatic evidence is clinical. It comes from a small set of

Figure 2. A cross section through the visual cortex of a Macaque monkey reveals its highly organized structure.

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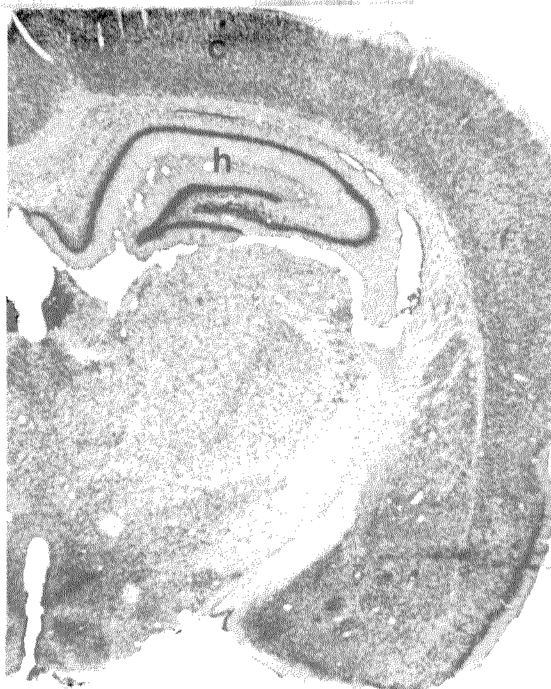
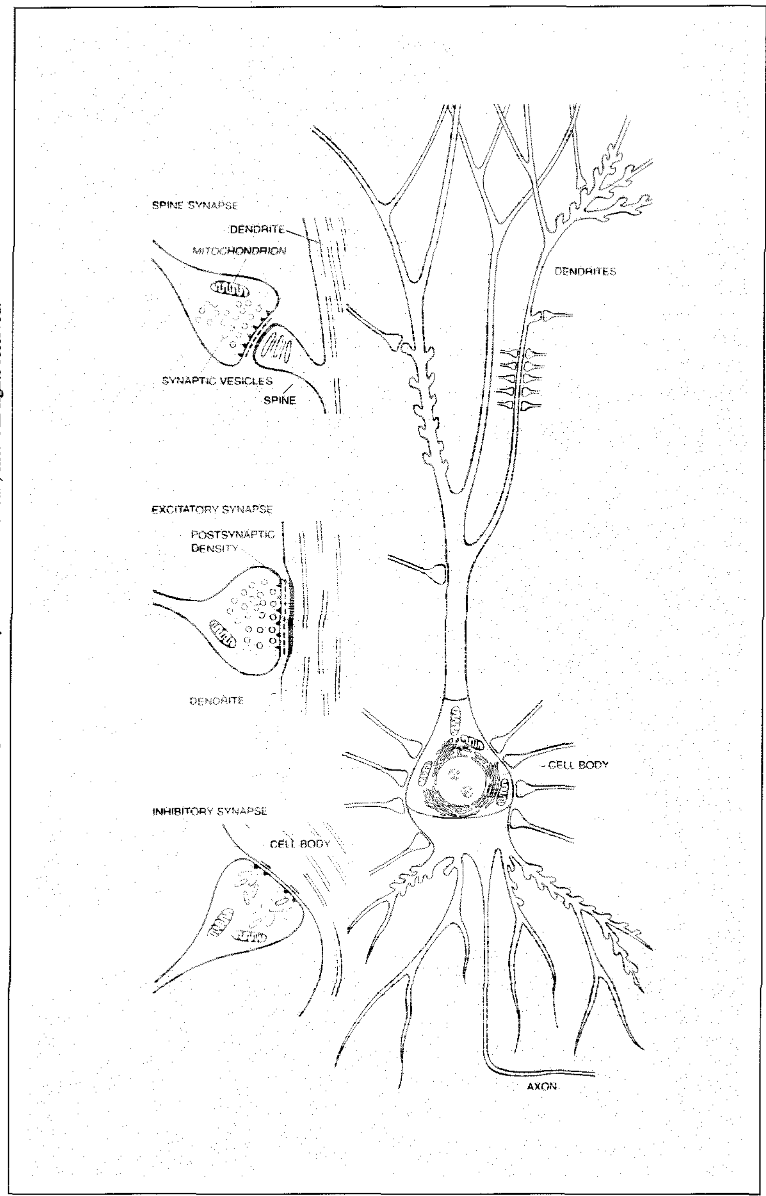


Figure 3. The hippocampus (h) lies just beneath the cortex (c) in this cross section through one hemisphere of a rat brain. The dark lines in the hippocampus are densely packed cell bodies.

Figure 4. The pyramidal cell is the main type of output neuron in the cortex and hippocampus.



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patients who, for one reason or another, have suffered damage to the hippocampus and nearby areas. Sometimes the damage is due to disease or accident and sometimes to neurosurgical procedures. These patients often appear reasonably healthy except for one problem — they are unable to form new memories. They remember what has happened to them for just a few minutes, and then these memories disappear. In some cases, such a patient must be reintroduced to his doctors every day. But these people retain most of their memories from times before their hippocampal damage. Such evidence suggests to neuroscientists that the hippocampus may be involved in the formation, but not the permanent storage, of conscious memories. This has prompted several researchers to study synaptic connections within the hippocampus to see how they may be altered during learning.

The hippocampus can be seen in Figure 3, a cross section through one hemisphere of a rat's brain. For several reasons, biochemists like myself rarely study primate brains. Instead, we use the the rat brain. The rat cortex has about the same thickness as the cortex of a human brain, but it is much simpler and smaller. Just beneath the cortex lies the hippocampus. The neurons of the hippocampus receive information from many brain regions, both cortical and subcortical. They also interconnect with each other.

Neurobiologists have found that the strength of synapses within the hippocampus can be changed in a particular way as a result of their previous electrical activity ("previous experience"). To explain how this works, I must first explain more about the structure of synapses. Figure 4 depicts a major type of output neuron in the hippocampus and in the cortex, called a pyramidal neuron because of the triangular shape of its cell body. The diagram shows small protuberances along the dendrites, called dendritic spines. Although they are barely visible in the light microscope, they are the principal sites on the dendrites for synaptic contacts from other neurons.

Under the electron microscope, the detailed structure of the synapse becomes visible (Figure 5). The presynaptic terminal, the transmitting part of the synapse that comes from a distant neuron's axon, contains hundreds of small membranous vesicles filled with a particular chemical neurotransmitter (inset, Figure 4). The spine of the postsynaptic neuron contains a darkly staining fibrous

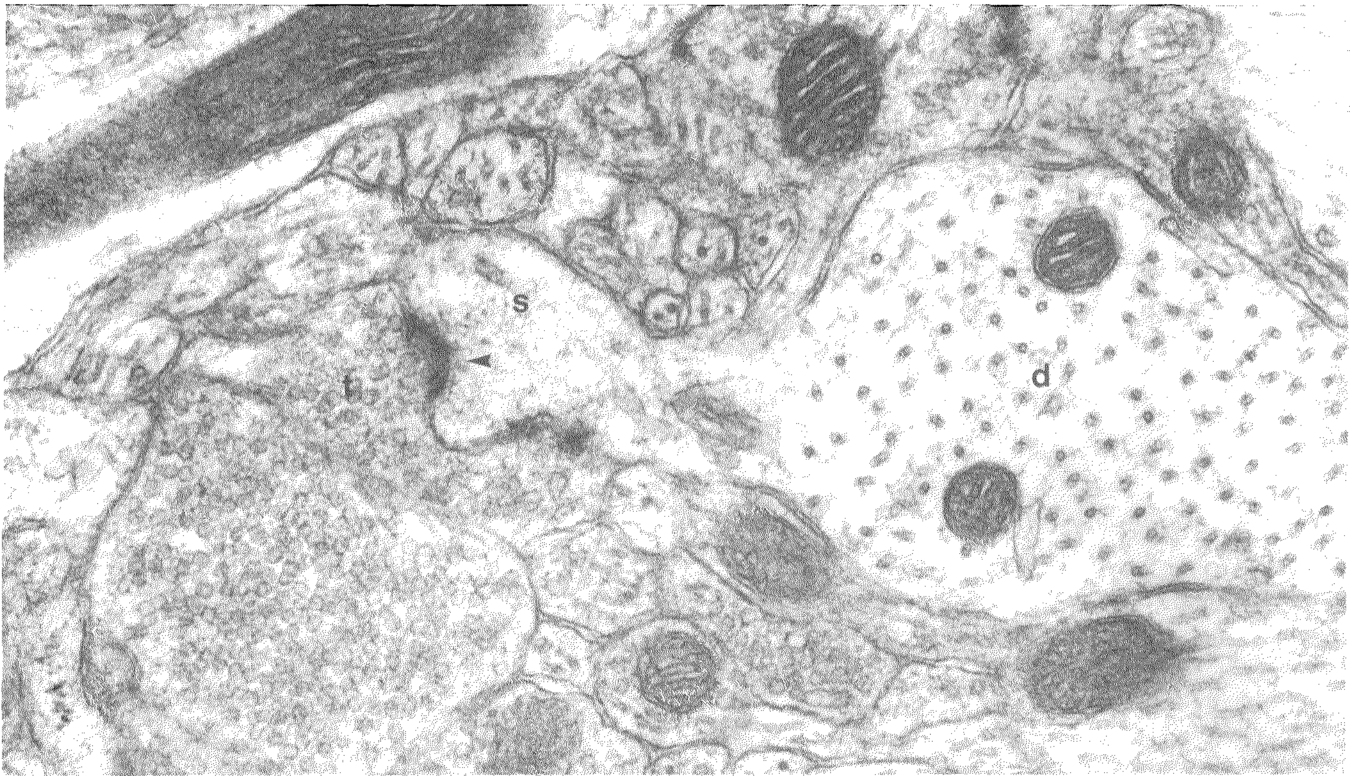

Figure 5. This electron micrograph of a hippocampal synapse shows a cross section of a dendrite (d), its spine (s), and the presynaptic terminal (t), which contains many synaptic vesicles. The arrow points to a postsynaptic density.

structure under its membrane, called by anatomists the postsynaptic density. Its function is not known, but, as you will see, we think that it may play a role in the storage of information by the synapse.

When the axon of a neuron is activated, calcium flows into the terminals at the ends of the axon, causing many of the synaptic vesicles to fuse with the outer membrane of the terminal and release their transmitter into the extracellular space. Protein receptors on the postsynaptic cell then bind this transmitter and initiate transmission of the signal.

The mechanism by which the presynaptic terminal excites the postsynaptic spine relies on its electrical properties. Neurons are tiny batteries. Neurophysiologists are able to poke microelectrodes into them and find that there is a difference in voltage between the inside of a neuron and the outside. At rest, the potential difference between the inside and the outside is between -80 and -50 millivolts (mV). But when a synapse ending on a neuron is activated, the postsynaptic receptors open little channels in the membrane, allowing sodium, potassium, and calcium ions to enter the postsynaptic cell. This flow of ions produces a small, brief depolarization (postsynaptic potential) in the neuron; that is, the resting potential may go from -80 mV to -75 mV or so for a few milliseconds. The neuron integrates information coming from its many

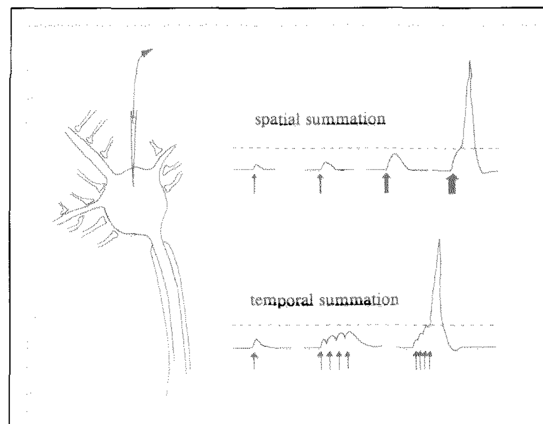

Figure 6. Subthreshold postsynaptic potentials can add together to produce an action potential through spatial or temporal summation. (After Aidley, 1971.)

synapses by summing the potential changes produced by each of them (Figure 6). If the summed depolarization becomes large enough, either because many synapses fire at once (spatial summation) or because one or two synapses fire many times very rapidly (temporal summation), a threshold will be reached. At the threshold, a large change in potential, called the action potential, is initiated. It is self-regenerating and it travels down the axon, ultimately activating presynaptic terminals, which in turn signal other neurons.

You can see that if the postsynaptic potential produced by a particular synapse were suddenly to become larger, it would have a greater influence on the total depolarization of the postsynaptic cell. Changes in

synaptic strength probably occur frequently in the brain. They can be produced either by local chemical changes in the brain or by previous electrical activity in the synapse itself. Some of these changes last for only short periods of time, but a certain class of changes appears to last for a very long time indeed — several hours in the laboratory. In the animal these changes may last as long as several weeks and perhaps for an animal's lifetime if they are sufficiently reinforced. Neuroscientists think that these very long-lasting changes in synaptic efficacy, collectively called long-term potentiation (LTP), may underlie part of the process of memory formation.

The hippocampus provides a particularly good place to study long-term potentiation, although it is by no means the only place in the brain that displays the phenomenon. To produce LTP in the laboratory, a neurophysiologist stimulates a group of presynaptic terminals at a high frequency for a short time — say 100 times per second for a few seconds. After this rapid burst of stimulation, whenever one of the synapses is again activated at a more usual low frequency, it will produce a larger postsynaptic potential than it had previously. The larger potential can be observed for hours after the burst. This long-lasting change in the synapse produced by a brief strong stimulus is what one might expect of a memory mechanism. Furthermore, long-term potentiation contains a possible mechanism for the association of one stimulus with another. It is produced more easily (that is, by lower frequency stimulation) in one set of synapses in the hippocampus if another set ending on the same neurons is stimulated simultaneously.

In my laboratory, we have been studying the biochemical structure of synapses in an effort to understand how they are regulated. As I described earlier, synapses transmit signals from the presynaptic side to the postsynaptic side by releasing molecules of neurotransmitter. When an action potential reaches the presynaptic terminal, it causes a rapid influx of calcium ions. The calcium triggers a series of events that causes many of the synaptic vesicles to fuse with the presynaptic membrane and dump their content of neurotransmitter into the synaptic cleft. When the molecules of neurotransmitter bind to their postsynaptic receptors, they initiate a flow of ions across the membrane, producing the brief postsynaptic potential change.

Within this sequence of events there are several ways that the strength of synapses could be modified in order to store information. For example, it is possible to increase the strength of a synapse by increasing the amount of neurotransmitter released from the presynaptic terminal during each impulse. Such an increase actually happens in long-term potentiation, but it does not seem to account for all of its properties. It is also possible to increase the strength of a synapse by increasing either the number or the sensitivity of the transmitter receptor molecules. This would lead to an increased flow of ions and a greater potential change. Another way of changing synaptic strength might be to change the shape of the postsynaptic spines. Many spines have very narrow necks, and if the necks were widened, the potential change produced in the spine might travel out into the rest of the cell more effectively.

This is where biochemistry merges with neurophysiology, because the biochemist would like to determine the chemical mechanisms that cause physiological changes. We have determined that one particularly interesting molecule found in synapses is regulated in a way that suggests that it may be a crucial link in some of the chains of events involved in alterations of synaptic efficacy. This molecule is an enzyme called a calcium-dependent protein kinase.

Most of the proteins that make up cells are enzymes — catalysts that facilitate biochemical reactions important to the life of the cell. A protein is a long chain of amino acids that folds up into a characteristic three-dimensional shape, which is important to the protein's function. Protein kinases regulate the activity of other proteins by altering their shapes. To be specific, protein kinases catalyze the transfer of a phosphate group from ATP (adenosine triphosphate, the cell's energy currency) to specific sites on other proteins (Figure 7) — a process known as phos-

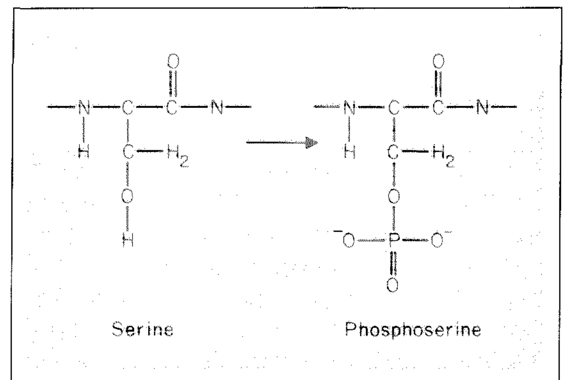

Figure 7. Within a protein, the amino acid serine can be modified by phosphorylation to form phosphoserine.

phorylation. When a big, negatively charged, phosphate group is added to a protein, it often forces the protein to assume a different shape and thus alters its function.

Protein kinases are not turned on all the time but are themselves regulated in a variety of ways by signals external to the cell. One of the signals that can switch on a certain group of protein kinases is an increase in calcium concentration. As I mentioned, calcium levels rise when an action potential invades an axon terminal. My laboratory group is investigating a particular calcium-activated protein kinase that occurs in very high concentration in the brain. We call it brain type II calcium/calmodulin-dependent protein kinase (type II CaM kinase, for short), a name that indicates that it is activated by calcium ions in combination with another small protein called calmodulin. Ngozi Erondu and I have found that this kinase constitutes fully two percent of all the protein in the hippocampus, an extraordinarily high level for a regulatory molecule. It accounts for one percent of the protein in the cortex, and its concentration becomes progressively smaller in lower brain regions. This suggests that it is synthesized at high levels within the cortex and hippocampus in order to be used for some specialized function in those regions.

Mark Bennett, Venise Jennings, and I have also demonstrated that within the neuron this kinase appears to be concentrated in the synapse. Although it is present throughout the neuron — in the cell body and at both pre- and postsynaptic sites — it is especially abundant in the postsynaptic density underneath the postsynaptic membrane where the transmitter receptors are located. It makes up about 20 percent of the total protein in the postsynaptic density; thus the density can be thought of as a cluster of kinase molecules sitting under the synaptic membrane and waiting for a calcium signal.

An important property of type II CaM kinase is its ability to add phosphate groups to itself — a process called autophosphorylation. Steve Miller and I showed the significance of autophosphorylation in an experiment in which we measured the ability of the kinase to phosphorylate other proteins. In the absence of calcium, the kinase has virtually no ability to phosphorylate itself or other proteins; when we add calcium, the kinase begins to phosphorylate both. But if we first allow the enzyme to autophosphorylate for a few seconds before mixing it with the other proteins, the dependence on calcium looks very different. After autophosphorylation, the enzyme has a high catalytic rate in the *absence* of calcium, and adding calcium stimulates this rate only about two-fold. We say that the kinase has been switched by autophosphorylation from State 1, in which its activity depends completely on calcium, to State 2, in which it has a significant calcium-independent activity (Figure 8). In solution, the type II CaM kinase is a cluster of 12 individual catalytic subunits. Autophosphorylation of only a few of these subunits changes the calcium requirement of all of them. So it seems that these few phosphate groups can have a dramatic effect on the shape and function of the whole kinase molecule.

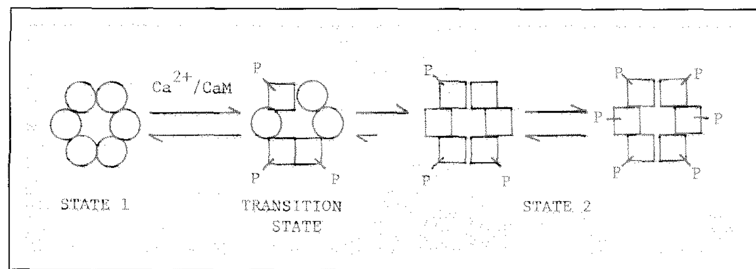

Figure 8. This schematic diagram shows the changes produced by autophosphorylation in the individual subunits of type II CaM kinase.

What does this mean? It means that, given a calcium signal lasting a few seconds, the CaM kinase can modify itself so that the effects of calcium persist even after the calcium levels have fallen. If nothing happens to remove the phosphate from the kinase, the enzyme's increased activity can last for a very long time. This kind of molecular switch is intriguing from the point of view of synaptic regulation. It is a mechanism one might imagine for an enzyme whose job is to produce a long-lasting signal from a very brief change in the chemical environment of the synapse.

At the moment, this model of a molecular switch within the synapse is really only that — a model. We are just beginning to test whether the autophosphorylation of the kinase actually occurs in the hippocampus during the generation of long-term potentiation. It may well be that this property of the molecule serves some other purpose entirely. Another question we must address is the nature and function of the other synaptic proteins that the kinase phosphorylates in addition to itself. But we cannot help being intrigued by the interesting properties of this molecule that appears to be positioned at a strategic location within neurons in areas of the brain thought to control learning and memory. □