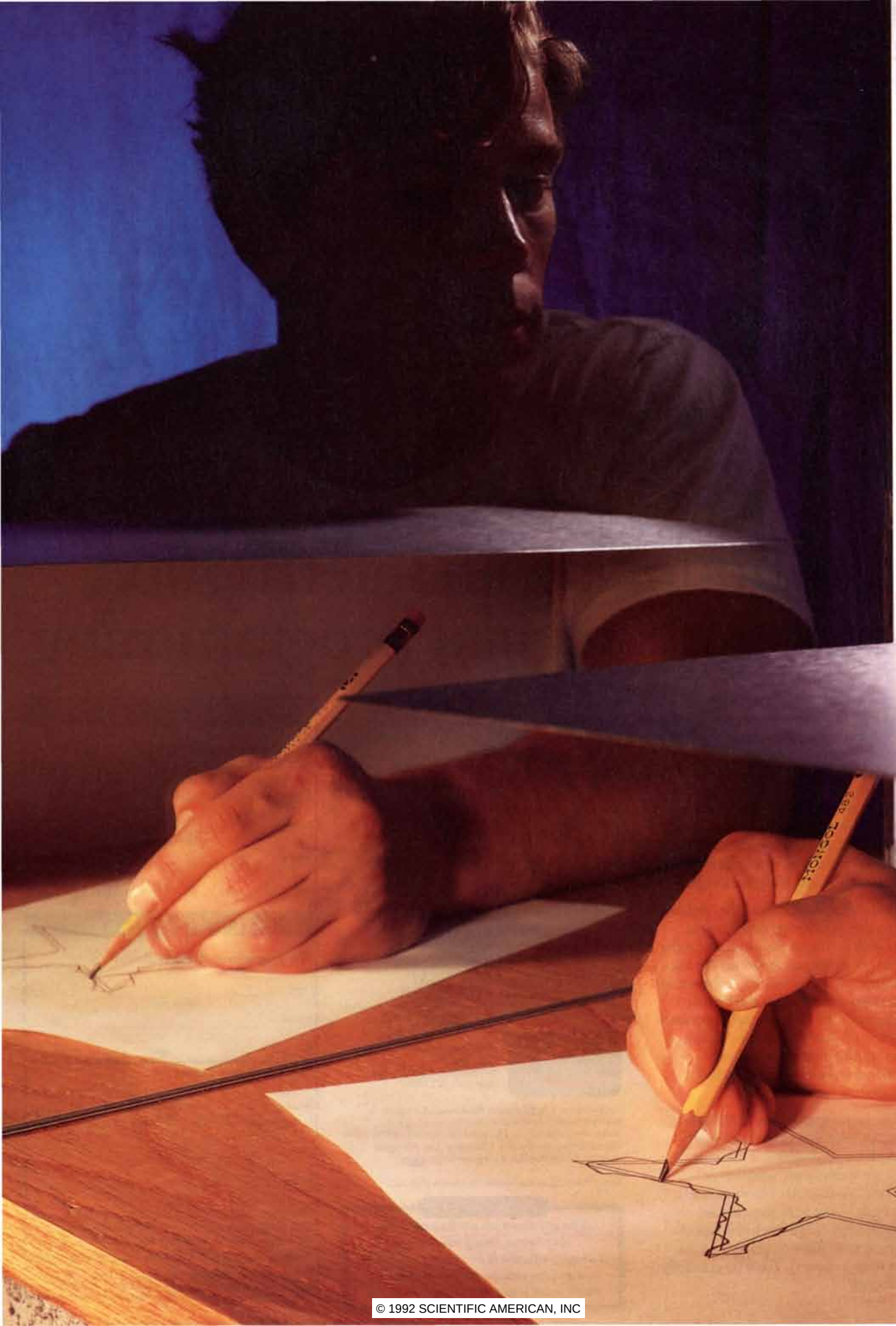
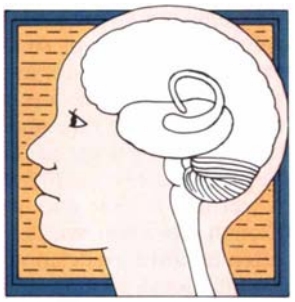


The Biological Basis of Learning and Individuality

Recent discoveries suggest that learning engages a simple set of rules that modify the strength of connections between neurons in the brain. These changes play an important role in making each individual unique

by Eric R. Kandel and Robert D. Hawkins



Over the past several decades, there has been a gradual merger of two originally separate fields of science: neurobiology, the science of the brain, and cognitive psychology, the science of the mind. Recently the pace of unification has quickened, with the result that a new intellectual framework has emerged for examining perception, language, memory and conscious awareness. This new framework is based on the ability to study the biological substrates of these mental functions. A particularly fascinating example can be seen in the study of learning. Elementary aspects of the neuronal mechanisms important for several different types of learning can now be studied on the cellular and even on the molecular level. The analysis of learning may therefore provide the first insights into the molecular mechanisms underlying a mental process and so begin to build a bridge between cognitive psychology and molecular biology.

Learning is the process by which we acquire new knowledge, and memory is the process by which we retain that knowledge over time. Most of what we know about the world and its civilizations we have learned. Thus, learning and memory are central to our sense of individuality. Indeed, learning goes beyond the individual to the transmission of culture from generation to generation. Learning is a major vehicle for behavioral adaptation and a powerful force for social progress. Conversely, loss of memory leads to loss of contact with one's immediate self, with one's life history and with other human beings.

Until the middle of the 20th century, most students of behavior did not believe that memory was a distinct mental function independent of movement, perception, attention and language. Long after those functions had been localized to different regions of the brain, researchers still doubted that memory could ever be assigned to a specific region. The first person to do so was Wilder G. Penfield, a neurosurgeon at the Montreal Neurological Institute.

In the 1940s Penfield began to use electrical stimulation to map motor, sensory and language functions in the cortex of patients undergoing neurosurgery for the relief of epilepsy. Because the brain itself does not have pain receptors, brain surgery can be carried out under local anesthesia in fully conscious patients, who can describe what they experience in response to electric stimuli applied to different cortical areas. Penfield explored the cortical surface in more than 1,000 patients. Occasionally he found that electrical stimulation produced an experiential response, or flashback, in which the patients described a coherent recollection of an earlier experience. These memorylike responses were invariably elicited from the temporal lobes.

Additional evidence for the role of the temporal lobe in memory came in the 1950s from the study of a few patients who underwent bilateral removal of the hippocampus and neighboring regions in the temporal lobe as treatment for epilepsy. In the first and best-studied case, Brenda Milner of the Montreal Neurological Institute described a 27-year-old assembly-line worker, H.M., who had suffered from untreatable and debilitating temporal lobe seizures for more than 10 years. The surgeon William B. Scoville removed the medial portion of the temporal lobes on both sides of H.M.'s brain. The seizure disturbance was much improved. But immediately after the operation, H.M. experienced a devastating memory deficit: he had lost the capacity to form new long-term memories.

Despite his difficulty with the formation of new memories, H.M. still retained his previously acquired long-term memory store. He remembered his name, retained a perfectly good use of language and kept his normal vocabulary; his IQ remained in the range of bright-normal. He remembered well the

MIRROR DRAWING EXPERIMENT in patients with temporal lobe lesions gave the first hint, in 1960, that there are two distinct types of learning systems. One form, which is spared by the lesions, involves tasks that have an automatic quality such as the skilled movements illustrated in this experiment. The subject, who can see his hand only in the mirror, tries to trace the shape of a star. The second type of learning depends on conscious awareness and cognitive processes and is abolished by the lesions.

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events that preceded the surgery, such as the job he had held, and he remembered vividly the events of his childhood. Moreover, H.M. still had a completely intact short-term memory. What H.M. lacked, and lacked profoundly, was the ability to translate what he learned from short-term to long-term memory. For example, he could converse normally with the hospital staff, but he did not remember them even though he saw them every day.

The memory deficit following bilateral temporal lobe lesions was originally thought to apply equally to all forms of new learning. But Milner soon discovered that this is not the case. Even though patients with such lesions have profound deficits, they can accomplish certain types of learning tasks as well as normal subjects can and retain the memory of these tasks for long periods. Milner first demonstrated this residual memory capability in H.M. with the discovery that he could learn new motor skills normally [*see illustration on page 78*]. She, and subsequently Elizabeth K. Warrington of the National Hospital for Nervous Diseases in London and Lawrence Weiskrantz of the University of Oxford, found that patients such as H.M. can also acquire and retain memory for elementary kinds of learning that involve changing the strength of reflex responses, such as habituation, sensitization and classical conditioning.

It immediately became apparent to students of behavior that the difference between types of learning that emerged from studies of patients with temporal lobe lesions represented a fundamental psychological distinction—a division in the way all of us acquire knowledge. Although it is still not clear how many distinct memory systems there are, researchers agree that lesions of the temporal lobes severely impair forms of learning and memory that require a conscious record. In accordance with the suggestion of Neal J. Cohen of the University of Illinois and Larry R. Squire of the University of California at San Diego and of Daniel L. Schacter of the University of Toronto, these types of learning are commonly called declarative or explicit. Those forms of learning that do not utilize conscious participation remain surprisingly intact in patients with temporal lobe lesions; they are referred to as nondeclarative or implicit.

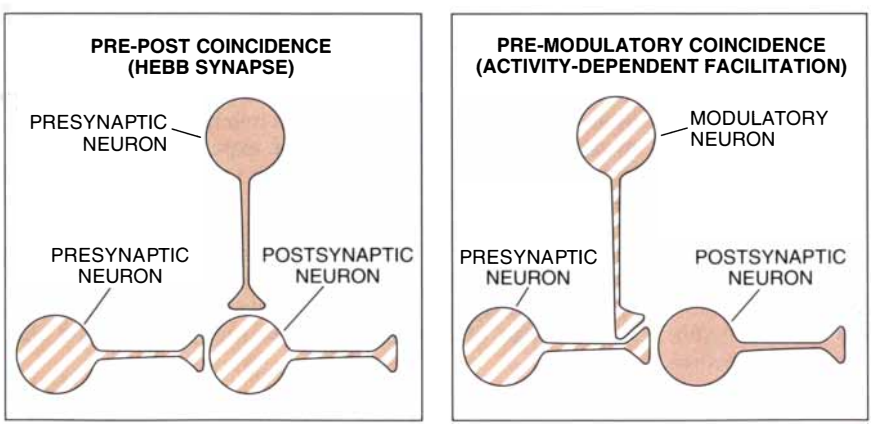
Explicit learning is fast and may take place after only one training trial. It often involves association of simultaneous stimuli and permits storage of information about a single event that happens in a particular time and place; it therefore affords a sense of familiarity about previous events. In contrast, implicit learning is slow and accumulates through repetition over many trials. It often involves association of sequential stimuli and permits storage of information about predictive relations between events. Implicit learning is expressed primarily by improved performance on certain tasks without the subject being able to describe just what has been learned, and it involves memory systems that do not draw on the contents of the general knowledge of the individual. When a subject such as H.M. is asked why he performs a given task better after five days of practice than on the first day, he may respond, "What are you talking about? I've never done this task before."

Whereas explicit memory requires structures in the temporal lobe of vertebrates, implicit memory is thought to be expressed through activation of the particular sensory and motor systems engaged by the learning task; it is acquired and retained by the plasticity inherent in these neuronal systems. As a result, implicit memory can be studied in various reflex systems in either vertebrates or invertebrates. Indeed, even simple invertebrate animals show excellent reflexive learning.

The existence of two distinct forms of learning has caused the reductionists among neurobiologists to ask whether there is a representation on the cellular level for each of these two types of learning process. Both the neural systems that mediate explicit memory and those that mediate implicit memory can store information about the association of stimuli. But does the same set of cellular learning rules guide the two memory systems as they store associations, or do separate sets of rules govern each system?

An assumption underlying early studies of the neural basis of memory systems was that the storage of associative memory, both implicit and explicit, required a fairly complex neural circuit. One of the first to challenge this view was the Canadian psychologist Donald O. Hebb, a teacher of Milner. Hebb boldly suggested that associative learning could be produced by a simple cellular mechanism. He proposed that associations could be formed by coincident neural activity: "When an axon of cell A…excite[s] cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficacy, as one of the cells firing B, is increased." According to Hebb's learning rule, coincident activity in the presynaptic and postsynaptic neurons is critical for strengthening the connection between them (a so-called pre-post associative mechanism) [*see illustration at left*].

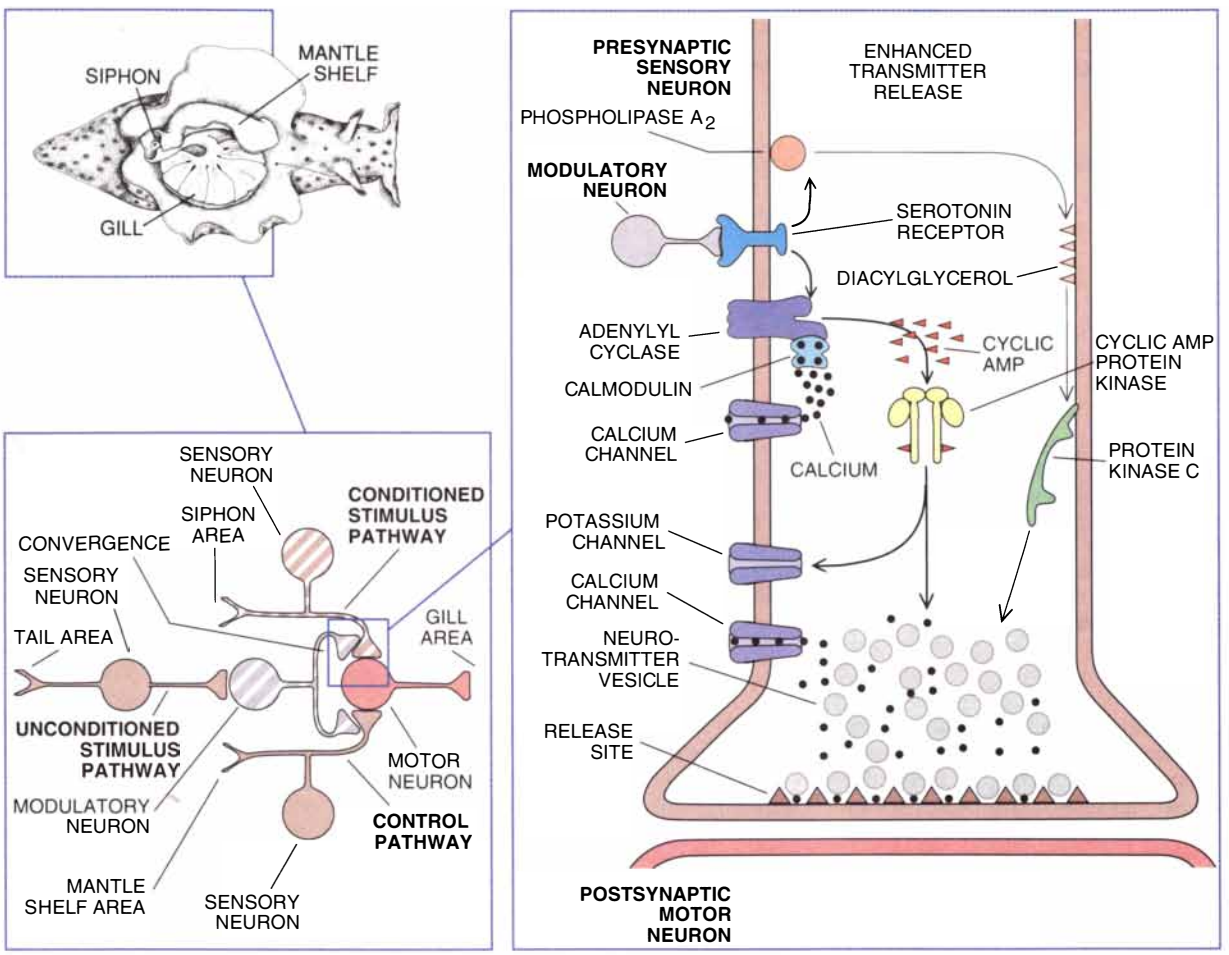
Ladislav Tauc and one of us (Kandel) proposed a second associative learning rule in 1963 while working at the Institute Marey in Paris on the nervous system of the marine snail *Aplysia*. They found that the synaptic connection between two neurons could be strengthened without activity of the postsynaptic cell when a third neuron acts on the presynaptic neuron. The third neuron, called a modulatory neuron, enhances



TWO CELLULAR MECHANISMS are hypothesized for associative changes in synaptic strength during learning. The pre-post coincidence mechanism, proposed by Donald O. Hebb in 1949, posits that coincident activity in the presynaptic and postsynaptic neurons is critical for strengthening the connections between them. The pre-modulatory coincidence mechanism proposed in 1963, based on studies in *Aplysia*, holds that the connection can be strengthened without activity of the postsynaptic cell when a third neuron, the modulatory neuron, is active at the same time as the presynaptic neuron. Stripes denote neurons in which coincident activity must occur to produce the associative change.

Classical Conditioning in *Aplysia*

The marine snail *Aplysia* (*top left*) is used in studies of the biological basis of learning because its simple nervous system consists of only 20,000 relatively large neurons. The diagram (*bottom left*) traces one of the pathways involved in classical conditioning of the gill-withdrawal reflex in *Aplysia*. An increase in the release of neurotransmitter due to activity-dependent facilitation is a mechanism that contributes to conditioning. The molecular steps in activity-dependent facilitation are shown in the enlargement at the right. Serotonin released from the modulatory neuron by the unconditioned stimulus activates adenylyl cyclase in the sensory neuron. When the sensory neuron is active, levels of calcium are elevated within the cell. The calcium binds to calmodulin, which in turn binds to adenylyl cyclase, enhancing its ability to synthesize cyclic AMP. The cyclic AMP activates protein kinase, which leads to the release of a substantially greater amount of transmitter than would occur normally.

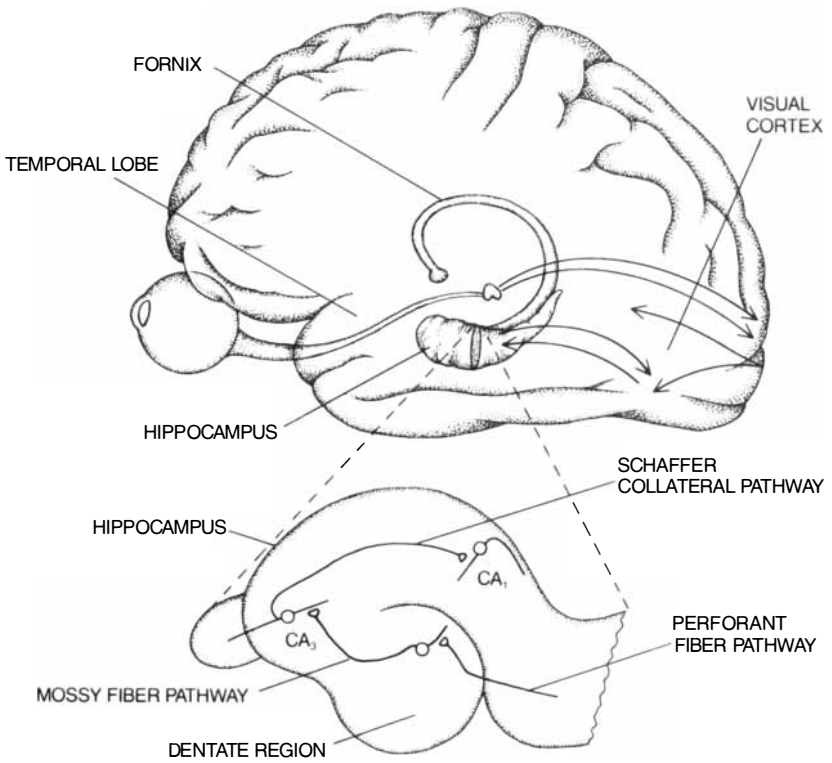


transmitter release from the terminals of the presynaptic neuron. They suggested that this mechanism could take on associative properties if the electrical impulses known as action potentials in the presynaptic cell were coincident with action potentials in the modulatory neuron (a pre-modulatory associative mechanism).

Subsequently, we and our colleagues Thomas J. Carew and Thomas W. Abrams of Columbia University and Edgar T. Walters and John H. Byrne of the University of Texas Health Science Center found experimental confirmation. We observed the pre-modulatory associative mechanism in *Aplysia,* where it contributes to classical conditioning, an implicit form of learning. Then, in 1986, Holger J. A. Wigström and Bengt E. W. Gustafsson, working at the University of Göteborg, found that the pre-post associative mechanism occurs in the hippocampus, where it is utilized in types of synaptic change that are important for spatial learning, an explicit form of learning.

The finding of two distinct cellular learning rules, each with associative properties, suggested that the associative mechanisms for implicit and explicit learning need not require complex neural networks. Rather the ability to detect associations may simply reflect the intrinsic capability of certain cellular interactions. Moreover, these findings raised an intriguing question: Are these apparently different mechanisms in any way related? Before considering their possible interrelation, we shall first describe the two learning mechanisms, beginning with the pre-modulatory mechanism contributing to classical conditioning in *Aplysia*.

Classical conditioning was first described at the turn of the century by the Russian physiologist Ivan Pavlov, who immediately appreciated that con-



HIPPOCAMPUS stores long-term memory for weeks and gradually transfers it to specific regions of the cerebral cortex. The diagram illustrates an example of this process involving a visual image. Neural input travels to the visual cortex and then to the hippocampus, where it is stored for several weeks before it is transferred back to the cortex for long-term memory. The hippocampus (*enlargement*) has three major synaptic pathways, each capable of long-term potentiation (LTP), which is thought to play a role in the storage process. LTP has different properties in the CA_1 and CA_3 regions of the hippocampus.

ditioning represents the simplest example of learning to associate two events. In classical conditioning, an ineffective stimulus called the conditioned stimulus (or more correctly, the to-be-conditioned stimulus) is repeatedly paired with a highly effective stimulus called the unconditioned stimulus. The conditioned stimulus initially produces only a small response or no response at all; the unconditioned stimulus elicits a powerful response without requiring prior conditioning.

As a result of conditioning (or learning), the conditioned stimulus becomes capable of producing either a larger response or a completely new response. For example, the sound of a bell (the conditioned stimulus) becomes effective in eliciting a behavioral response such as lifting a leg only after that sound has been paired with a shock to the leg (the unconditioned stimulus) that invariably produces a leg-lifting response. For conditioning to occur, the conditioned stimulus generally must be correlated with the unconditioned stimulus and precede it by a certain critical period. The animal is therefore thought to learn predictive relations between the two stimuli.

Because *Aplysia* has a nervous system containing only about 20,000 central nerve cells, aspects of classical conditioning can be examined at the cellular level. *Aplysia* has a number of simple reflexes, of which the gill-withdrawal reflex has been particularly well studied. The animal normally withdraws the gill, its respiratory organ, when a stimulus is applied to another part of its body such as the mantle shelf or the fleshy extension called the siphon. Both the mantle shelf and the siphon are innervated by their own populations of sensory neurons. Each of these populations makes direct contact with motor neurons for the gill as well as with various classes of excitatory and inhibitory interneurons that synapse on the motor neurons. We and our colleagues Carew and Walters found that even this simple reflex can be conditioned.

A weak tactile stimulus to one pathway, for example, the siphon, can be paired with an unconditioned stimulus (a strong shock) to the tail. The other pathway, the mantle shelf, can then be used as a control pathway. The control pathway is stimulated the same number of times, but the stimulus is not paired (associated) with the tail shock. After five pairing trials, the response to stimulation of the siphon (the paired pathway) is greater than that of the mantle (the unpaired pathway). If the procedure is reversed and the mantle shelf is paired rather than the siphon, the response to the mantle shelf will be greater than that to the siphon. This differential conditioning is remarkably similar in several respects to that seen in vertebrates.

To discover how this conditioning works, we focused on one component: the connections between the sensory neurons and their target cells, the interneurons and motor neurons. Stimulating the sensory neurons from either the siphon or the mantle shelf generates excitatory synaptic potentials in the interneurons and motor cells. These synaptic potentials cause the motor cells to discharge, leading to a brisk reflex withdrawal of the gill. The unconditioned reinforcing stimulus to the tail activates many cell groups, some of which also cause movement of the gill. Among them are at least three groups of modulatory neurons, in one of which the chemical serotonin is the transmitter. (Neurotransmitters such as serotonin that carry messages between cells are called first messengers; other chemicals known as second messengers relay information within the cell.)

These modulatory neurons act on the sensory neurons from both the siphon and the mantle shelf, where they produce presynaptic facilitation, that is, they enhance transmitter release from the terminals of the sensory neurons. Presynaptic facilitation contributes to a nonassociative form of learning called sensitization, in which an animal learns to enhance a variety of defensive reflex responses after receiving a noxious stimulus [see "Small Systems of Neurons," by Eric R. Kandel; SCIENTIFIC AMERICAN, September 1979]. This type of learning is referred to as nonassociative because it does not depend on pairing between stimuli.

The finding that modulatory neurons act on both sets of sensory neurons—those from the siphon as well as those from the mantle—posed an interesting question: How is the specific associative strengthening of classical conditioning achieved? Timing turned out to be an important element here. For classical conditioning to occur, the conditioned stimulus generally must precede the unconditioned stimulus by a critical and often narrow interval. For condi-

tioning gill withdrawal by tail shock, the interval is approximately 0.5 second. If the separation is lengthened, shortened or reversed, conditioning is drastically reduced or does not occur.

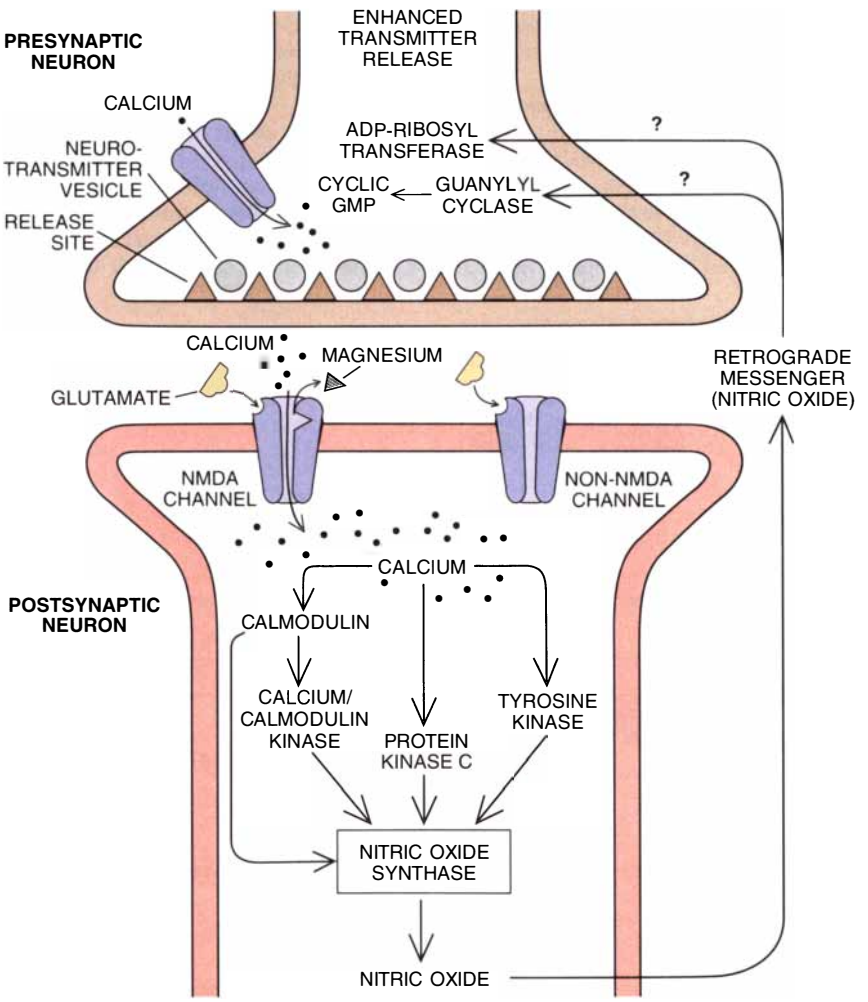
In the gill-withdrawal reflex, the specificity in timing results in part from a convergence of the conditioned and unconditioned stimuli within individual sensory neurons. The unconditioned stimulus is represented in the sensory neurons by the action of the modulatory neurons, in particular the cells in which serotonin is the transmitter. The conditioned stimulus is represented by activity within the sensory neurons themselves. We found that the modulatory neurons activated by the unconditioned stimulus to the tail produce greater presynaptic facilitation of the sensory neurons if the sensory neurons had just fired action potentials in response to the conditioned stimulus. Action potentials in the sensory neurons that occur just after the tail shock have no effect.

This novel property of presynaptic facilitation is called activity dependence. Activity-dependent facilitation requires the same timing on the cellular level as does conditioning on the behavioral level and may account for such conditioning. These results suggest that a cellular mechanism of classical conditioning of the withdrawal reflex is an elaboration of presynaptic facilitation, a mechanism used for sensitization of the reflex. These experiments provided an initial suggestion that there might be a cellular alphabet for learning whereby the mechanisms of more complex types of learning may be elaborations or combinations of the mechanisms of simpler types of learning.

The next piece in the puzzle of how classical conditioning occurs was to discover why the firing of action potentials in the sensory neurons just before the unconditioned tail stimulus would enhance presynaptic facilitation. We had previously found that when serotonin is released by the modulatory neurons in response to tail shock, it initiates a series of biochemical changes in the sensory neurons [*see illustration on page 81*]. Serotonin binds to a receptor that activates an enzyme called adenylyl cyclase. This enzyme in turn converts ATP, one of the molecules that provides the energy needed to power the various activities of the cell, into cyclic AMP. Cyclic AMP then acts as a second messenger (serotonin is the first messenger) inside the cell to activate another enzyme, a protein kinase. Kinases are proteins that phosphorylate (add a phosphate group to) other proteins, thereby increasing the activity of some and decreasing the activity of others.

The activation of the protein kinase in sensory neurons has several important short-term consequences. The protein kinase phosphorylates potassium channel proteins. Phosphorylation of these channels (or of proteins that act on these channels) reduces a component of the potassium current that normally repolarizes the action potential. Reduction of potassium current prolongs the action potential and thereby allows calcium channels to be activated for longer periods, permitting more calcium to enter the presynaptic terminal. Calcium has several actions within the cell, one of which is the release of transmitter vesicles from the terminal. When, as a result of an increase in the duration of the action potentials, more calcium enters the terminal, more transmitter is released. Second, as a result of protein kinase activity, serotonin acts to mobilize transmitter vesicles from a storage pool to the release sites at the membrane; this facilitates the release of transmitter independent of an increase in calcium influx. In this action, cyclic AMP acts in parallel with another second messenger, protein kinase C, which is also activated by serotonin.

Why should the firing of action potentials in the sensory neurons just before the unconditioned stimulus enhance the action of serotonin? Action potentials produce a number of changes in the sensory neurons. They allow sodium and calcium to move in and potassium to move out, and they change the membrane potential. Abrams and



IN LONG-TERM POTENTIATION the postsynaptic membrane is depolarized by the actions of the non-NMDA receptor channels. The depolarization relieves the magnesium blockade of the NMDA channel, allowing calcium to flow through the channel. The calcium triggers calcium-dependent kinases that lead to the induction of LTP. The postsynaptic cell is thought to release a retrograde messenger capable of penetrating the membrane of the presynaptic cell. This messenger, which may be nitric oxide, is believed to act in the presynaptic terminal to enhance transmitter (glutamate) release, perhaps by activating guanylyl cyclase or ADP-ribosyl transferase.

Kandel found that the critical function of the action potential for activity dependence was the movement of calcium into the sensory neurons. Once in the cell, calcium binds to a protein called calmodulin, which amplifies the activation of the enzyme adenylyl cyclase by serotonin. When calcium/calmodulin binds to the adenylyl cyclase, the enzyme generates more cyclic AMP. This capacity makes adenylyl cyclase an important convergence site for the conditioned and the unconditioned stimuli.

Thus, the conditioned and the unconditioned stimuli are represented within the cell by the convergence of two different signals (calcium and serotonin) on the same enzyme. The 0.5-second interval between the two stimuli essential for learning in the gill-withdrawal reflex may correspond to the time during which calcium is elevated in the presynaptic terminal and binds to calmodulin so as to prime the adenylyl cyclase to produce more cyclic AMP in response to serotonin.

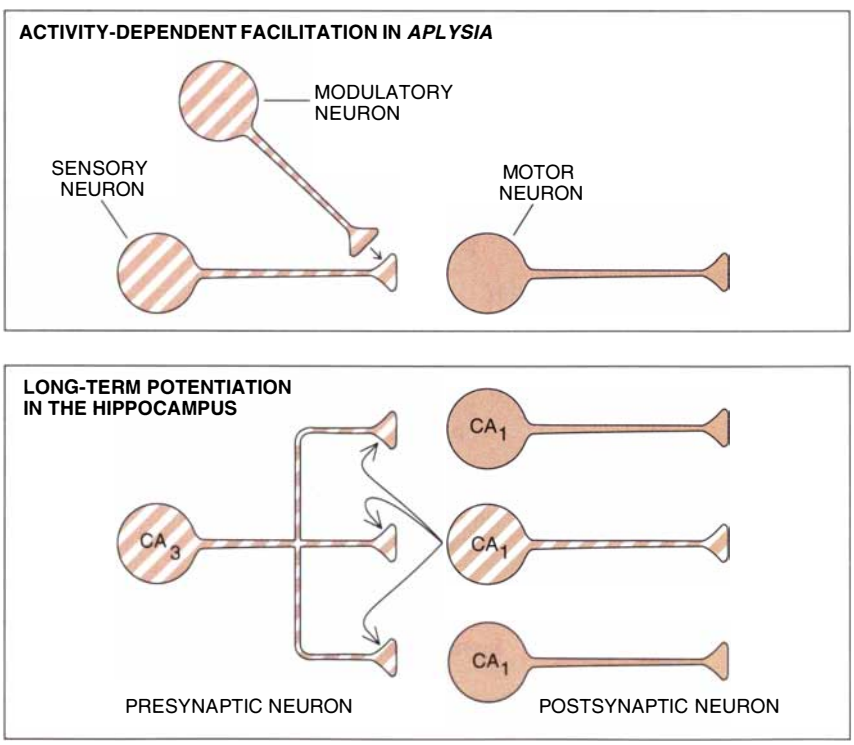
Activity-dependent amplification of the cyclic AMP pathway is not unique to the gill- or tail-withdrawal reflexes of *Aplysia*. Genetic studies in the fruit fly *Drosophila* have implicated a similar molecular mechanism for conditioning. *Drosophila* can be conditioned, and single-gene mutants have been discovered that are deficient in learning. One such mutant, called *rutabaga,* has been studied by William G. Quinn of the Massachusetts Institute of Technology and Margaret Livingstone of Harvard University and by Yadin Dudai of the Weizmann Institute in Israel. The gene encoding the defective protein in this mutant has now been shown to be a calcium/calmodulin-dependent adenylyl cyclase. As a result of the mutation in *rutabaga,* the cyclase has lost its ability to be stimulated by calcium/calmodulin. Moreover, Ronald L. Davis and his colleagues at Cold Spring Harbor Laboratory have found that this form of the adenylyl cyclase is enriched in the mushroom bodies, a part of the fly brain critical for several types of associative learning. Thus, both cell biological studies in *Aplysia* and genetic studies in *Drosophila* point to the significance of the cyclic AMP second-messenger system in certain elementary types of implicit learning and memory storage.

What about explicit forms of learning? Do these more complex types of associative learning also have cellular representations for associativity? If so, they must differ from the mechanisms for implicit learning, because, unlike classical conditioning, explicit learning is often most successful when the two events that are associated occur simultaneously. For example, we recognize the face of an acquaintance most easily when we see that acquaintance in a specific context. The stimuli of the face and of the setting act simultaneously to help us recognize the person.

As we have seen, explicit learning in humans requires the temporal lobe. Yet it was unclear at first how extensive the bilateral lesion in the temporal lobe had to be to interfere with memory storage. Subsequent studies in humans and in experimental animals by Mortimer Mishkin of the National Institutes of Health and by Squire, David G. Amaral and Stuart Zola-Morgan of the University of California at San Diego help to answer the question. They suggest that one structure within the temporal lobe particularly critical for memory storage is the hippocampus. And yet lesions of the hippocampus interfere only with the storage of new memories: patients like H.M. still have a reasonably good memory of earlier events. The hippocampus appears to be only a temporary depository for long-term memory. The hippocampus processes the newly learned information for a period of weeks to months and then transfers the information to relevant areas of the cerebral cortex for more permanent storage [see "Brain and Language," by Antonio R. Damasio and Hanna Damasio, page 88]. As discussed by Patricia S. Goldman-Rakic, the memory stored at these different cortical sites is then expressed through the working memory of the prefrontal cortex [see "Working Memory and the Mind," page 110].

In 1973 Timothy Bliss and Terje Lømo, working in Per Andersen's laboratory in Oslo, Norway, first demonstrated that neurons in the hippocampus have remarkable plastic capabilities of the kind that would be required for learning. They found that a brief high-frequency train of action potentials in one of the neural pathways within the hippocampus produces an increase in synaptic strength in that pathway. The increase can be shown to last for hours in an anesthetized animal and for days and even weeks in an alert, freely moving animal.

Bliss and Lømo called this strengthening long-term potentiation (LTP). Later studies showed that LTP has different properties in different types of synapses within the hippocampus. We will focus here on an associative type of potentiation that has two interrelated characteristics. First, the associativity is of the Hebbian pre-post form: for facilitation to occur, the contributing presyn-



ASSOCIATIVE PROCESSES believed to contribute to learning in *Aplysia* and in the hippocampus of mammals may share similar mechanisms. Both may involve a modulatory substance that produces activity-dependent enhancement of transmitter release from the presynaptic neuron. Stripes denote neurons in which coincident activity must occur to produce the associative change.

aptic and postsynaptic neurons need to be active simultaneously. Second, and as a result, the long-term potentiation shows specificity: it is restricted in its action to the pathway that is stimulated.

Why is simultaneous firing of the presynaptic and postsynaptic cells necessary for long-term potentiation? The major neural pathways in the hippocampus use the amino acid glutamate as their transmitter. Glutamate produces LTP by binding to glutamate receptors on its target cells. It turns out that there are two relevant kinds of glutamate receptors: the NMDA receptors (named after the chemical *N*-methyl D-aspartate, which also binds to these receptors) and the non-NMDA receptors. Non-NMDA receptors dominate most synaptic transmission because the ion channel associated with the NMDA receptor is usually blocked by magnesium. It becomes unblocked only when the postsynaptic cell is depolarized. Moreover, optimal activation of the NMDA receptor channel requires that the two signals—glutamate binding to the receptor and depolarization of the postsynaptic cell—take place simultaneously. Thus, the NMDA receptor has associative or coincidence-detecting properties much as does the adenylyl cyclase. But its temporal characteristics, a requirement for simultaneous activation, are better suited for explicit rather than implicit forms of learning.

Calcium influx into the postsynaptic cell through the unblocked NMDA receptor channel is critical for long-term potentiation, as was first shown by Gary Lynch of the University of California at Irvine and by Roger A. Nicoll and Robert S. Zucker and colleagues at the University of California at San Francisco. Calcium initiates LTP by activating at least three different types of protein kinases.

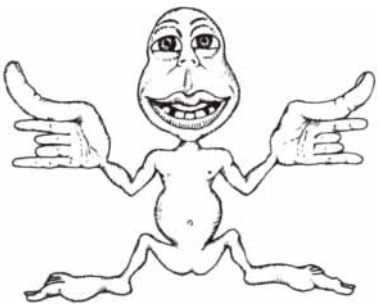
The *induction* of LTP appears to depend on postsynaptic depolarization, leading to the influx of calcium and the subsequent activation of second-messenger kinases. For the *maintenance* of LTP, on the other hand, several groups of researchers have found that enhancement of transmitter from the presynaptic terminal is involved. These workers include Bliss and his colleagues, John Bekkers and Charles Stevens of the Salk Institute and Roberto Malinow and Richard Tsien of Stanford University.

If the induction of LTP requires a postsynaptic event (calcium influx through the NMDA receptor channels) and maintenance of LTP involves a presynaptic event (increase in transmitter release), then, as first proposed by Bliss, some message must be sent from the postsynaptic to the presynaptic neurons—and that poses a problem for neuroscientists. Ever since the great Spanish anatomist Santiago Ramón y Cajal first enunciated the principle of dynamic polarization, every chemical synapse studied has proved to be unidirectional. Information flows only from the presynaptic to the postsynaptic cell. In long-term potentiation, a new principle of nerve cell communication seems to be emerging. The calcium-activated second-messenger pathways, or perhaps calcium acting directly, seem to cause release of a retrograde plasticity factor from the active postsynaptic cell. This retrograde factor then diffuses to the presynaptic terminals to activate one or more second messengers that enhance transmitter release and thereby maintain LTP [*see illustration on page 83*].

Unlike the presynaptic terminals, which store transmitter in vesicles and release it at specialized release sites, the postsynaptic terminals lack any special release machinery. It therefore seemed attractive to posit that the retrograde messenger may be a substance that rapidly diffuses out of the postsynaptic cell across the synaptic cleft and into the presynaptic terminal. By 1991 four groups of researchers had obtained evidence that nitric oxide may be such a retrograde messenger: Thomas J. O'Dell and Ottavio Arancio in our laboratory, Erin M. Schuman and Daniel Madison of Stanford University, Paul F. Chapman and his colleagues at the University of Minnesota School of Medicine and Georg Böhme and his colleagues in France. Inhibiting the synthesis of nitric oxide in the postsynaptic neuron or absorbing nitric oxide in the extracellular space blocks the induction of LTP, whereas applying nitric oxide enhances transmitter release from presynaptic neurons.

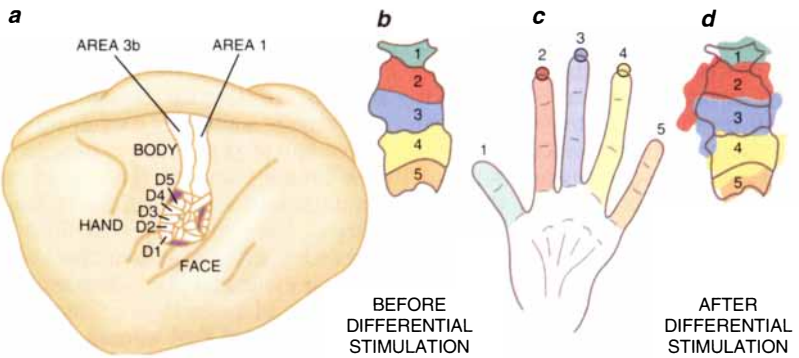
In the course of studying the effects of applying nitric oxide in slices of hippocampus, we and Scott A. Small and Min Zhuo made a surprising finding: we discovered that nitric oxide produces LTP only if it is paired with activity in the presynaptic neurons, much as is the case in activity-dependent presynaptic facilitation in *Aplysia*. Presynaptic activity, and perhaps calcium influx, appears to be critical for nitric oxide to produce potentiation. These experiments suggest that long-term potentiation uses a combination of two independent, associative, synaptic learning mechanisms: a Hebbian NMDA receptor mechanism and a

Representation of the Surface of the Body in the Cortex



The homunculus ("little man") is a traditional way of illustrating how the surface of the body is represented in the somatosensory cortex. Larger areas of the cortex are devoted to parts of the body that have greater sensitivity, such as the fingers and lips.

Recently the effects of sensitivity training have been shown in the owl monkey. The monkey's digits are represented in areas 3b and 1 of the somatosensory cortex (*a*). The diagrams (*b* and *d*) outline the regions that map the surface of the digits of an adult monkey (*c*) before and after training. During training the monkey rotated a disk for one hour a day, using only digits 2, 3 and occasionally 4. After three months of this activity, the area representing the stimulated fingers in the brain had increased substantially.



non-Hebbian, activity-dependent, presynaptic facilitating mechanism. According to this hypothesis, the activation of NMDA receptors in the postsynaptic cells produces a retrograde signal (nitric oxide). The signal then initiates an activity-dependent presynaptic mechanism, which facilitates the release of transmitter from the presynaptic terminals.

What might be the functional advantage of combining two associative cellular mechanisms, the postsynaptic NMDA receptor and the activity-dependent presynaptic facilitation, in this way? If presynaptic facilitation is produced by a diffusible substance, that substance could, in theory, find its way into neighboring pathways. In fact, studies by Tobias Bonhoeffer and his colleagues at the Max Planck Institute for Brain Research in Frankfurt indicate that LTP initiated in one postsynaptic cell spreads to neighboring postsynaptic cells. Activity dependence of presynaptic facilitation could be a way of ensuring that only specific presynaptic pathways—those that are active—are potentiated. Any inactive presynaptic terminals would not be affected [*see illustration on page 84*].

The changes in synapses that are thought to contribute to these instances of implicit and explicit learning raise a surprising reductionist possibility. The fact that associative synaptic changes do not require complex neural networks suggests there may be a direct correspondence between these associative forms of learning and basic cellular properties. In the cases that we have reviewed, the cellular properties seem to derive in turn from the properties of specific proteins—the adenylyl cyclase and the NMDA receptor—that are capable of responding to two independent signals, such as those from the conditioned stimulus and the unconditioned stimulus. Of course, these molecular associative mechanisms do not act in isolation. They are embedded in cells that have rich molecular machinery for elaborating the associative process. And the cells, in turn, are embedded in complex neural networks with considerable redundancy, parallelism and computational power, adding substantial complexity to these elementary mechanisms.

The finding that LTP occurs in the hippocampus, a region known to be significant in memory storage, made researchers wonder whether LTP is involved in the process of storing memories in this area of the brain. Evidence that it is has been provided by Richard Morris and his colleagues at the University of Edinburgh Medical School by means of a spatial memory task. When NMDA receptors in the hippocampus are blocked, the experimental animals fail to learn the task. These experiments suggest that NMDA receptor mechanisms in the hippocampus, and perhaps LTP, are involved in spatial learning.

Having now considered the mechanisms through which learning can produce changes in nerve cells, we are faced with a final set of questions. What are the mechanisms whereby the synaptic changes produced by explicit and implicit learning endure? How is memory maintained in the long term?

Experiments in both *Aplysia* and mammals indicate that explicit and implicit memory storage proceed in stages. Storage of the initial information, a type of short-term memory, lasts minutes to hours and involves changes in the strength of existing synaptic connections (by means of second-messenger-mediated modifications of the kind we have discussed). The long-term changes (those that persist for weeks and months) are stored at the same site, but they require something entirely new: the activation of genes, the expression of new proteins and the growth of new connections. In *Aplysia,* Craig H. Bailey, Mary C. Chen and Samuel M. Schacher and their colleagues at Columbia University and Byrne and his colleagues at the University of Texas Health Science Center have found that stimuli that produce long-term memory for sensitization and classical conditioning lead to an increase in the number of presynaptic terminals. Similar anatomic changes occur in the hippocampus after LTP.

If long-term memory leads to anatomic changes, does that imply that our brains are constantly changing anatomically as we learn and as we forget? Will we experience changes in our brain's anatomy as a result of reading and remembering this issue of *Scientific American?*

This question has been addressed by many investigators, perhaps most dramatically by Michael Merzenich of the University of California at San Francisco. Merzenich examined the representation of the hand in the sensory area of the cerebral cortex. Until recently, neuroscientists believed this representation was stable throughout life. But Merzenich and his colleagues have now demonstrated that cortical maps are subject to constant modification based on use of the sensory pathways. Since all of us are brought up in somewhat different environments, are exposed to different combinations of stimuli and are likely to exercise our sensory and motor skills in different ways, the architecture of each of our brains will be modified in slightly different ways. This distinctive modification of brain architecture, along with a unique genetic makeup, contributes to the biological basis for the expression of individuality.

This view is best demonstrated in a study by Merzenich, in which he encouraged a monkey to touch a rotating disk with only the three middle fingers of its hand. After several thousand disk rotations, the area in the cortex devoted to the three middle fingers was expanded at the expense of that devoted to the other fingers [*see illustration on preceding page*]. Practice, therefore, can lead to changes in the cortical representation of the most active fingers. What mechanisms underlie the changes? Recent evidence indicates that the cortical connections in the somatosensory system are constantly being modified and updated on the basis of correlated activity, using a mechanism that appears similar to that which generates LTP.

Indeed, as we have learned from Carla J. Shatz [see "The Developing Brain," page 60], early results from cell biological studies of development suggest that the mechanisms of learning may carry with them an additional bonus. There is now reason to believe that the fine-tuning of connections during late stages of development may require an activity-dependent associative synaptic mechanism perhaps similar to LTP. If that is also true on the molecular level—if learning shares common molecular mechanisms with aspects of development and growth—the study of learning may help connect cognitive psychology to the molecular biology of the organism more generally. This broad biological unification would accelerate the demystification of mental processes and position their study squarely within the evolutionary framework of biology.

FURTHER READING

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