Small Systems of Neurons

BY ERIC R. KANDEL

Such systems are the elementary units of mental function. Studies of simple animals such as the large snail Aplysia show that small systems of neurons are capable of forms of learning and memory

Many neurobiologists believe that the unique character of individual human beings, their disposition to feel, think, learn and remember, will ultimately be shown to reside in the precise patterns of synaptic interconnections between the neurons of the brain. Since it is difficult to examine patterns of interconnections in the human brain, a major concern of neurobiology has been to develop animal models that are useful for studying how interacting systems of neurons give rise to behavior. Networks of neurons that mediate complete behavioral acts allow one to explore a hierarchy of interrelated questions: To what degree do the properties of different neurons vary? What determines the patterns of interconnections between neurons? How do different patterns of interconnections generate different forms of behavior? Can the interconnected neurons that control a certain kind of behavior be modified by learning? If they can, what are the mechanisms whereby memory is stored?

Among the many functions that emerge from the interactions of neurons, the most interesting are the functions concerned with learning (the ability to modify behavior in response to experience) and with memory (the ability to store that modification over a period of time). Learning and memory are perhaps the most distinctive features of the mental processes of advanced animals, and these features reach their highest form in man. In fact, human beings are what they are in good measure because of what they have learned. It is therefore of theoretical importance, for the understanding of learning and for the study of behavioral evolution, to determine at what phylogenetic level of neuronal and behavioral organization one can begin to recognize aspects of the learning and memory processes that characterize human behavior. This determination is also of practical importance. The difficulty in studying the cellular mechanisms of memory in the brain of man or other mammals arises because such brains are immensely complex. For the human brain ethical issues also preclude this kind of study. It would therefore be congenial scientifically to be able to examine these processes effectively in simple systems.

It could be argued that the study of memory and learning as it relates to man cannot be pursued effectively in simple neuronal systems. The organization of the human brain seems so complex that trying to study human learning in a reduced form in simple neuronal systems is bound to fail. Man has intellectual abilities, a highly developed language and an ability for abstract thinking, which are not found in simpler animals and may require qualitatively different types of neuronal organization. Although such arguments have value, the critical question is not whether there is something special about the human brain. There clearly is. The question is rather what the human brain and human behavior have in common with the brain and the behavior of simpler animals. Where there are points of similarity they may involve common principles of brain organization that could profitably be studied in simple neural systems.

The answer to the question of similarity is clear. Ethologists such as Konrad Lorenz, Nikolaas Tinbergen and Karl von Frisch have shown that human beings share many common behavioral patterns with simpler animals, including elementary perception and motor coordination. The capacity to learn, in particular, is widespread; it has evolved in many invertebrate animals and in all vertebrates. The similarity of some of the learning processes suggests that the neuronal mechanisms for a given learning process may have features in common across phylogeny. For example, there appear to be no fundamental differences in structure, chemistry or function between the neurons and synapses in man and those of a squid, a snail or a leech. Consequently a complete and rigorous analysis of learning in such an invertebrate is likely to reveal mechanisms of general significance.

Simple invertebrates are attractive for such investigation because their nervous systems consist of between 10,000 and 100,000 cells, compared with the many billions in more complex animals. The cells are collected into the discrete groups called ganglia, and each ganglion usually consists of between 500 and 1,500 neurons. This numerical simplification has made it possible to relate the function of individual cells directly to behavior. The result is a number of important findings that lead to a new way of looking at the relation between the brain and behavior.

The first major question that students of simple systems of neurons might examine is whether the various neurons of a region of the nervous system differ from one another. This question, which is central to an understanding of how behavior is mediated by the nervous system, was in dispute until recently. Some neurobiologists argued that the neurons of a brain are sufficiently similar in their properties to be regarded as identical units having interconnections of roughly equal value.

These arguments have now been strongly challenged, particularly by studies of invertebrates showing that many neurons can be individually identified and are invariant in every member of the species. The concept that neurons are unique was proposed as early as 1912 by the German biologist Richard Goldschmidt on the basis of his study of the nervous system of a primitive worm, the intestinal parasite *Ascaris*. The brain

of this worm consists of several ganglia. When Goldschmidt examined the ganglia, he found they contained exactly 162 cells. The number never varied from animal to animal, and each cell always occupied a characteristic position. In spite of this clear-cut result Goldschmidt's work went largely unheeded.

More than 50 years later two groups at the Harvard Medical School returned to the problem independently. Masanori Otsuka, Edward A. Kravitz and David D. Potter, working with the lobster, and Wesley T. Frazier, Irving Kupfermann, Rafiq M. Waziri, Richard E. Coggeshall and I, working with the large marine snail *Aplysia*, found a similar but less complete invariance in the more complex nervous systems of these higher invertebrates. A comparable invariance was soon found in a variety of invertebrates, including the leech, the crayfish, the locust, the cricket and a number of snails. Here I shall limit myself to considering studies of *Aplysia*, particularly studies of a single ganglion: the abdominal ganglion. Similar findings have also emerged from the studies of other invertebrates.

In the abdominal ganglion of *Aplysia* neurons vary in size, position, shape, pigmentation, firing patterns and the chemical substances by which they transmit information to other cells. On the basis of such differences it is possible to recognize and name specific cells (R1, L1, R15 and so on). The firing patterns illustrate some of the differences. Certain cells are normally "silent" and others are spontaneously active. Among the active ones some fire regular action potentials, or nerve impulses, and others fire in recurrent brief bursts or trains. The different firing patterns have now

been shown to result from differences in the types of ionic currents generated by the membrane of the cell body of the neurons. The cell-body membrane is quite different from the membrane of the axon, the long fiber of the neuron. When the membrane of the axon is active, it typically produces only an inflow of sodium ions and a delayed outflow of potassium ions, whereas the membrane of the cell body can produce six or seven distinct ionic currents that can flow in various combinations.

Whether or not most cells in the mammalian nervous system are also unique individuals is not yet known. The studies in the sensory systems of mammals reviewed by David Hubel and Torsten Wiesel in this book, however, have revealed fascinating and important differences between neighboring neurons [see "Brain Mechanisms of Vision," by David H. Hubel and Torsten N. Wiesel, page 84]. Studies of the development of the vertebrate brain reviewed by Maxwell Cowan lead to a similar conclusion [see "The Development of the Brain," by W. Maxwell Cowan, page 56].

The finding that neurons are invariant leads to further questions. Are the synaptic connections between cells also invariant? Does a given identified cell always connect to exactly the same follower cell and not to others? A number of investigators have examined these questions in invertebrate animals and have found that cells indeed always make the same kinds of connections to other cells. The invariance applies not only to the connections but also to the "sign," or functional expression, of the connections, that is, whether they are excitatory or inhibitory.

Therefore Frazier, James E. Blan-

kenship, Howard Wachtel and I next worked with identified cells to examine the rules that determine the functional expression of connections between cells. A single neuron has many branches and makes many connections. We asked: Are all the connections of a neuron specialized for inhibition or excitation, or can the firing of a neuron produce different actions at different branches? What determines whether a connection is excitatory or inhibitory? Is the sign of the synaptic action determined by the chemical structure of the transmitter substance released by the presynaptic neuron, or is the nature of the postsynaptic receptor the determining factor? Does the neuron release the same transmitter from all its terminals?

One way to explore these questions is to look at the different connections made by a cell. The first cell we examined gave a clear answer: it mediated different actions through its various connections. The cell excited some follower cells, inhibited others and (perhaps most unexpectedly) made a dual connection, which was both excitatory and inhibitory, to a third kind of cell. Moreover, it always excited precisely the same cells, always inhibited another specific group of cells and always made a dual connection with a third group. Its synaptic action could be accounted for by one transmitter substance: acetylcholine. The reaction of this substance with different types of receptors on the various follower cells determined whether the synaptic action would be excitatory or inhibitory.

The receptors determined the sign of the synaptic action by controlling different ionic channels in the membrane: primarily sodium for excitation and chloride for inhibition. The cells that received the dual connection had two types of receptor for the same transmitter, one receptor that controlled a sodium channel and another that controlled a chloride channel. The functional expression of chemical synaptic transmission is therefore determined by the types of receptor the follower cell has at a given postsynaptic site. (Similar results have been obtained by JacSue Kehoe of the École Normale in Paris, who has gone on to analyze in detail the properties of the various species of receptors to acetylcholine.) Thus, as was first suggested by Ladislav Tauc and Hersch Gerschenfeld of the Institute Marey in Paris, the chemical transmitter is only permissive; the instructive component of synaptic transmission is the nature of the receptor and the ionic channels it interacts with. This principle has proved to be fairly general. It applies to the neurons of vertebrates and invertebrates and to neurons utilizing various transmitters: acetylcholine, gamma-aminobutyric acid (GABA), serotonin, dopamine and histamine. (The principle
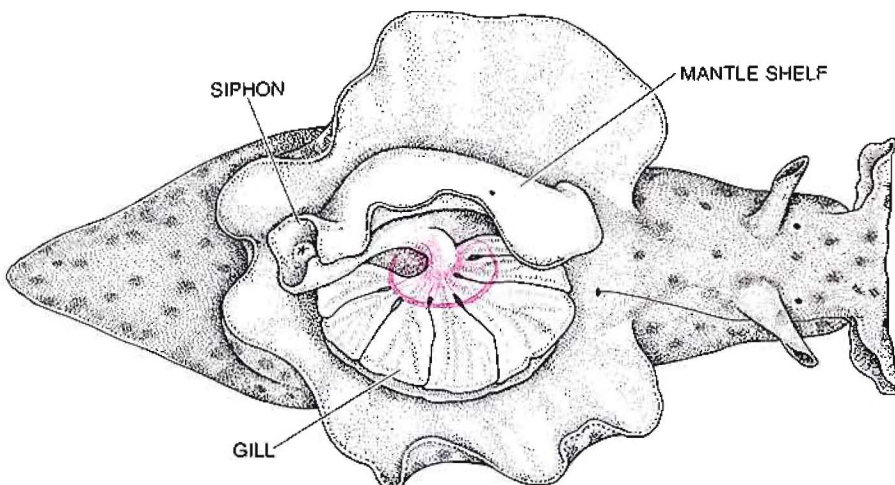
GILL-WITHDRAWAL REFLEX of *Aplysia* results when the siphon or the mantle shelf is somehow stimulated. The animal then retracts the gill to the position that is indicated in color.

also applies to the actions of certain peptide hormones on neurons, a subject to which I shall return.)

The discovery in invertebrate ganglia of identifiable cells that make precise connections with one another has led to the working out of the "wiring diagram" of various behavioral circuits and has therefore made possible an exact study of the causal relation of specific neurons to behavior. The term behavior refers to the observable actions of an organism. These range from complex acts such as talking or walking to simple acts such as the movement of a body part or a change in heart rate. Types of behavior that have been at least partly worked out in leeches, crayfishes and snails include feeding, various locomotor patterns and a variety of escape and defensive reactions.

The first finding to emerge from these studies is that individual cells exert a control over behavior that is specific and sometimes surprisingly powerful. The point can be illustrated by comparing the neural control of the heart in *Aplysia* with that in human beings.

The human heart beats spontaneously. Its intrinsic rhythm is neuronally modulated by the inhibitory action of cholinergic neurons (acetylcholine is the transmitter substance) with their axons in the vagus nerve and the excitatory action of noradrenergic neurons with their axons in the accelerator nerve. The modulation involves several thousand neurons. In *Aplysia* the heart also beats spontaneously; it is neuronally modulated by the inhibitory action of cholinergic neurons and the excitatory action of serotonergic neurons, but the modulation is accomplished by only four cells! Two cells excite the heart (only the "major excitor" cell is really important) and two inhibit it. Three other cells give rise to a constriction of the blood vessels and thereby control the animal's blood pressure.

Since individual cells connect invariably to the same follower cells and can mediate actions that have a different sign, certain cells at a critical point in the nervous system are in a position to control an entire behavioral sequence. As early as 1938 C. A. G. Wiersma, working with the crayfish at the California Institute of Technology, had appreciated the importance of single cells in behavior and had called them "command cells." Such cells have now been found in a variety of animals. A few of them have proved to be dual-action neurons. Hence John Koester, Earl M. Mayeri and I, working with *Aplysia* at the New York University School of Medicine, found that the dual-action neuron described above is a command cell for the neural circuit controlling the circulation. This one cell increases the rate and output of the heart by exciting the
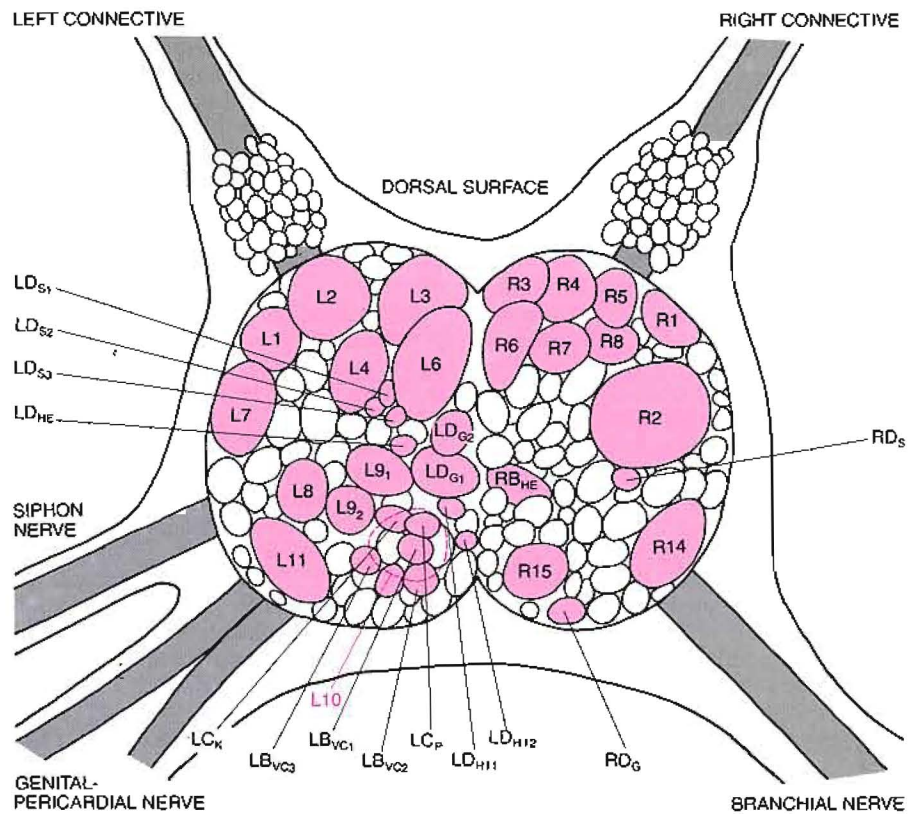
MAP OF ABDOMINAL GANGLION in *Aplysia californica* shows the location of the identified neurons, which have been labeled L or R (for left or right hemiganglion) and assigned a number. Neurons that are members of a cluster, consisting of cells with similar properties, are further identified by a cluster letter (LD) and a subscript representing the behavioral function of the neuron, such as HE for heart excitor and G1 and G2 for two gill motor neurons.

major cell that excites the heart while inhibiting the cells that inhibit the heart and the cells that constrict the major blood vessels. As a result of the increased activity of this one cell the heart beats faster and pumps more blood.

This is only a simple example of the behavioral functions of a command cell. In the crayfish and even in a more complex animal, the goldfish, a single impulse in a single command neuron causes the animal to flee from threatened danger. Recently Vernon Mountcastle of the Johns Hopkins University School of Medicine has suggested in this context that small groups of cells may serve similar command functions in the primate brain to control purposeful voluntary movements.

Hence a functional purpose of dual-action cells is to bring about a constellation of different physiological effects. A similar constellation can be achieved by the action of neuroendocrine cells, neurons that release hormones (the chemical substances that are usually carried in the bloodstream to act at distant sites). The abdominal ganglion of *Aplysia* contains two clusters of neuroendocrine cells, which are called bag cells because each cluster is bag-shaped. Kupfermann, working in our division at the Columbia University College of Physicians and Surgeons, has shown, as have

Stephen Arch of Reed College and Felix Strumwasser and his colleagues at Cal Tech, that the bag cells release a polypeptide hormone that controls egg laying. Mayeri has found that this hormone has long-lasting actions on various cells in the abdominal ganglion, exciting some and inhibiting others.

One of the cells excited by this hormone is the dual-action command cell that controls the heart rate. As a result the heart speeds up to provide the extra flow of blood to the tissues that the animal requires during egg laying. Thus superimposed on a precise pattern of connections that provide short-range interaction of neurons is an equally precise pattern of long-range interactions achieved by the hormones released by neuroendocrine cells. The precise effect of each hormone seems to be determined, as synaptic effects are, by the nature of the receptors on the target cells.

The finding that behavior is mediated by invariant cells interconnecting in precise and invariant ways might suggest that simple animals differ from more complex ones in having stereotyped and fixed repertories of activity. It is not so. Studies in different invertebrates have shown that behavior in simple animals is quite capable of being modified by learning.

We have explored this subject most

fully in one of *Aplysia*'s simplest kinds of behavior: a defensive reflex action in which the gill is withdrawn after a stimulus. The gill is in a respiratory chamber called the mantle cavity. The chamber is covered by a protective sheet, the mantle shelf, that terminates in a fleshy spout, the siphon. When a weak or a moderately intense stimulus is applied to the siphon, the gill contracts and withdraws into the mantle cavity. This reflex is analogous to the withdrawal responses found in almost all higher animals, such as the one in which a human being jerks a hand away from a hot object. *Aplysia* and the other animals exhibit two forms of learning with such reflexes: habituation and sensitization.

Habituation is a decrease in the strength of a behavioral response that occurs when an initially novel stimulus is presented repeatedly. When an animal is presented with a novel stimulus, it at first responds with a combination of orienting and defensive reflexes. With repeated stimulation the animal readily learns to recognize the stimulus. If the stimulus proves to be unrewarding or innocuous, the animal will reduce and ultimately suppress its responses to it. Although habituation is remarkably simple, it is probably the most widespread of all forms of learning. Through

habituation animals, including human beings, learn to ignore stimuli that have lost novelty or meaning; habituation frees them to attend to stimuli that are rewarding or significant for survival. Habituation is thought to be the first learning process to emerge in human infants and is commonly utilized to study the development of intellectual processes such as attention, perception and memory.

An interesting aspect of habituation in vertebrates is that it gives rise to both short- and long-term memory and has therefore been employed to explore the relation between the two. Thomas J. Carew, Harold M. Pinsker and I found that a similar relation holds for *Aplysia*. After a single training session of from 10 to 15 tactile stimuli to the siphon the withdrawal reflex habituates. The memory for the stimulus is short-lived; partial recovery can be detected within an hour and almost complete recovery generally occurs within a day. Recovery in this type of learning is equivalent to forgetting. As with the repetition of more complex learning tasks, however, four repeated training sessions of only 10 stimuli each produce profound habituation and a memory for the stimulus that lasts for weeks.

The first question that Vincent Castellucci, Kupfermann, Pinsker and I asked

was: What are the loci and mechanisms of short-term habituation? The neural circuit controlling gill withdrawal is quite simple. A stimulus to the skin of the siphon activates the 24 sensory neurons there; they make direct connections to six motor cells in the gill, and the motor cells connect directly to the muscle. The sensory neurons also excite several interneurons, which are interposed neurons.

By examining these cells during habituation we found that short-term habituation involved a change in the strength of the connection made by the sensory neurons on their central target cells: the interneurons and the motor neurons. This localization was most fortunate, because now we could examine what happened during habituation simply by analyzing the changes in two cells, the presynaptic sensory neuron and the postsynaptic motor neuron, and in the single set of connections between them.

The strength of a connection can be studied by recording the synaptic action produced in the motor cells by an individual sensory neuron. It is possible to simulate the habituation training session of from 10 to 15 stimuli by stimulating a sensory neuron following the exact time sequence used for the intact animal. The stimulus can be adjusted so that it generates a single action potential. The first time the neuron is caused to fire an action potential it produces a highly effective synaptic action, which is manifested as a large excitatory postsynaptic potential in the motor cell. The subsequent action potentials initiated in the sensory neuron during a training session give rise to progressively smaller excitatory postsynaptic potentials. This depression in the effectiveness of the connection parallels and accounts for the behavioral habituation. As with the behavior, the synaptic depression resulting from a single training session persists for more than an hour. Following a second training session there is a more pronounced depression of the synaptic potential, and further training sessions can depress the synaptic potential completely.

What causes the changes in the strength of the synaptic connection? Do they involve a change in the presynaptic sensory neuron, reflecting a decrease in the release of the transmitter substance, or a change in the postsynaptic cell, reflecting a decrease in the sensitivity of the receptors to the chemical transmitter? The questions can be answered by analyzing changes in the amplitude of the synaptic potential in terms of its quantal components.

As was first shown by José del Castillo and Bernhard Katz at University College London, transmitter is released not as single molecules but as "quanta," or multimolecular packets. Each packet contains roughly the same amount

R2

|←— 10 SECONDS —→|

R3

|←— 10 SECONDS —→|

R15

|←— 10 SECONDS —→|

L10

|←— 50 SECONDS —→|
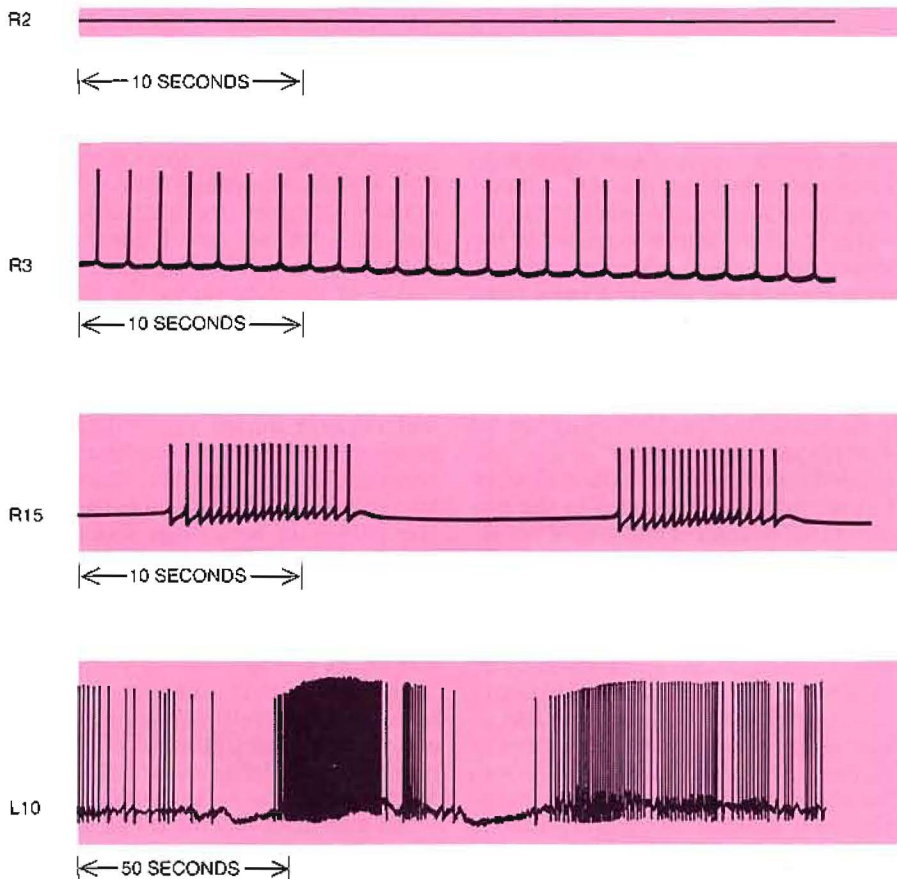
FIRING PATTERNS of identified neurons in *Aplysia*'s abdominal ganglion are portrayed. R2 is normally silent, R3 has a regular beating rhythm, R15 a regular bursting rhythm and L10 an irregular bursting rhythm. L10 is a command cell that controls other cells in the system.
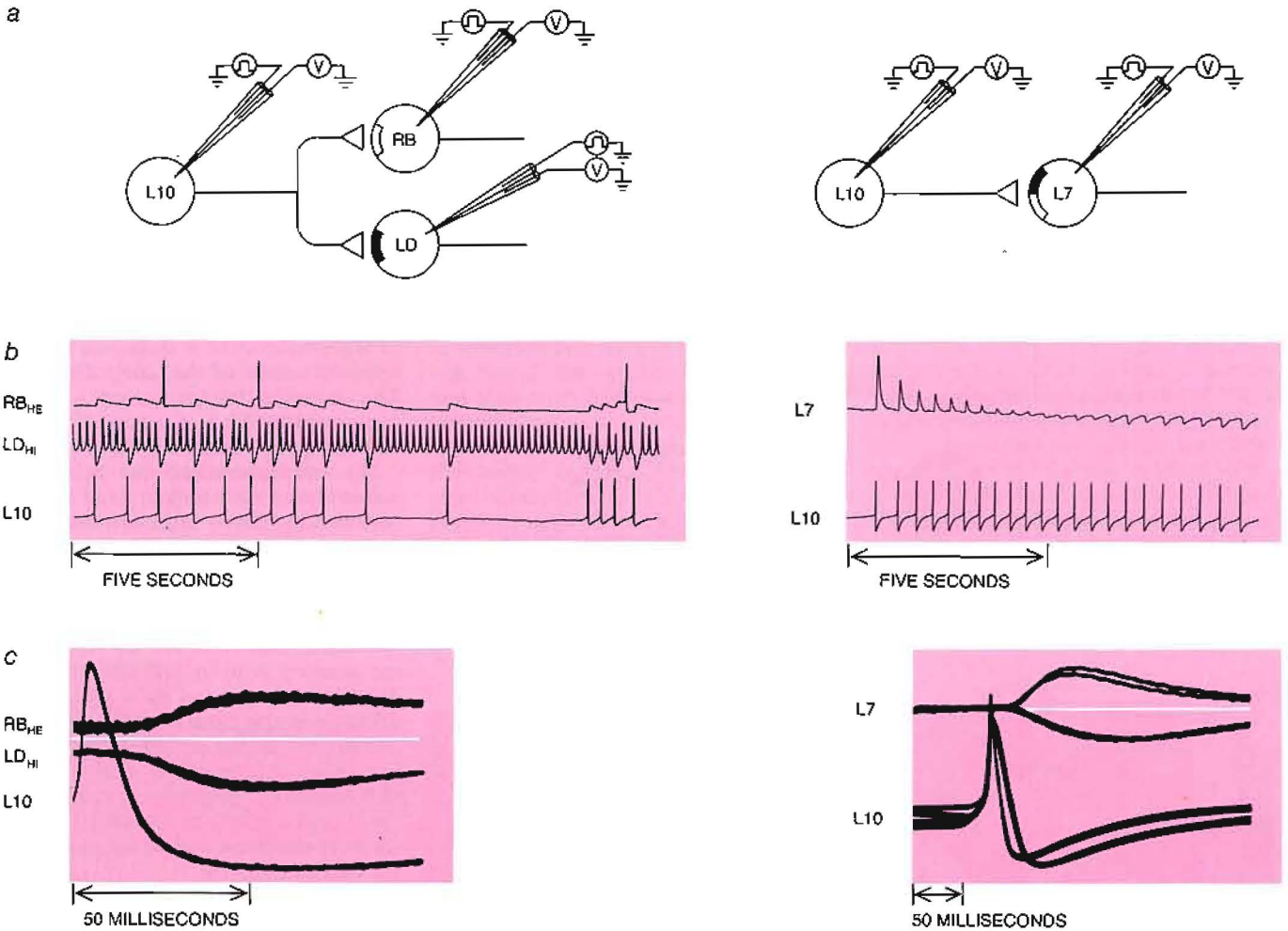
INVARIANCE OF CONNECTIONS between cell L10 and some of its follower cells was ascertained (a) by an arrangement in which double-barrel microelectrodes for recording and passing current were inserted in L10, which is a presynaptic neuron, and three of its follower cells. L10 produces excitation (*white*) in RB, inhibition (*black*) in LD and both excitation and inhibition in L7. The respective firing patterns are shown at b. Several superposed sweeps (*at the left in* c) illustrate the brief but constant latency between an impulse in the presynaptic neuron and the response of two follower cells. Superposed traces from L10 and L7 (*at the right in* c) show that effect is excitatory when L10 fires initially, as indicated by tall and narrow impulses, and inhibitory when it fires repeatedly, as shown by short and broad impulses.

of transmitter (several thousand molecules). The quanta are thought to be stored in subcellular organelles called synaptic vesicles that are seen in abundance at synaptic endings examined with the electron microscope. Since the number of transmitter molecules in each quantum does not ordinarily change, the number of quanta released by each action potential is a fairly reliable index of the total amount of transmitter released. Each quantum in turn produces a miniature excitatory postsynaptic potential of characteristic size in the postsynaptic cell. The size is an indication of how sensitive the postsynaptic receptors are to the several thousand molecules of transmitter released by each packet.
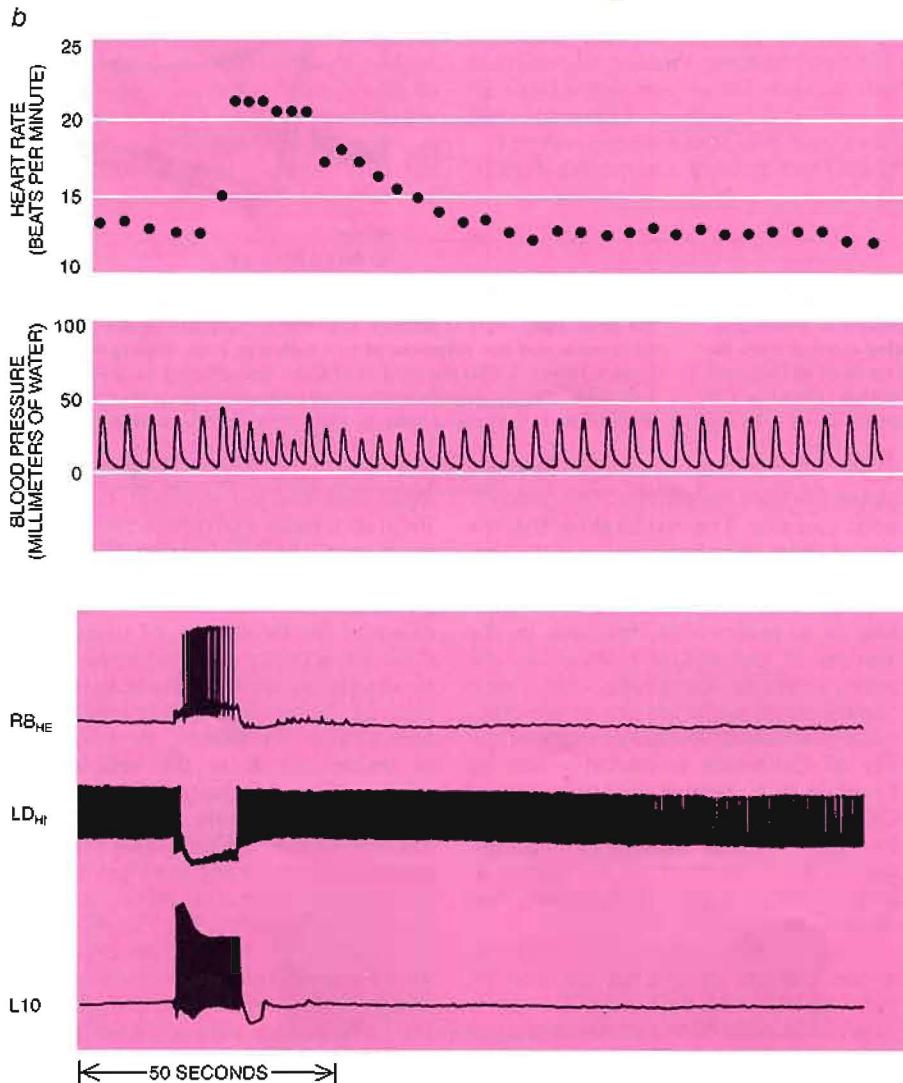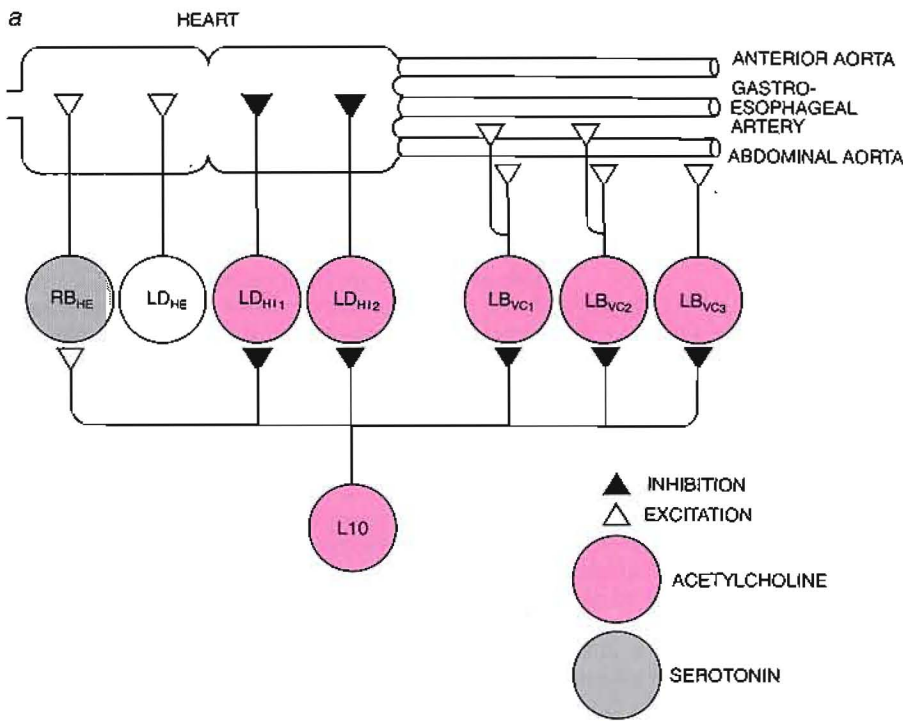
Castellucci and I, working with *Aplysia*, found that the decrease in the amplitude of the synaptic action potential with habituation was paralleled by a decrease in the number of chemical quanta released. In contrast, the size of the miniature postsynaptic potential did not change, indicating that there was no change in the sensitivity of the postsynaptic receptor. The results show that the site of short-term habituation is the presynaptic terminals of the sensory neurons and that the mechanism of habituation is a progressive decrease in the amount of transmitter released by the sensory-neuron terminals onto their central target cells. Studies in the crayfish by Robert S. Zucker of the University of California at Berkeley and by Franklin B. Krasne of the University of California at Los Angeles and in the cat by Paul B. Farel and Richard F. Thompson of the University of California at Irvine indicate that this mechanism may be quite general.

What is responsible for the decrease in the number of quanta released by each action potential? The number is largely determined by the concentration of free calcium in the presynaptic terminal. Calcium is one of three kinds of ion involved in the generation of each action potential in the terminal. The depolarizing upstroke of the action potential is produced mainly by the inflow of so-dium ions into the terminal, but it also involves a lesser and delayed flow of calcium ions. The repolarizing downstroke is largely produced by the outflow of potassium ions. The inflow of calcium is essential for the release of transmitter. Calcium is thought to enable the synaptic vesicles to bind to release sites in the presynaptic terminals. This binding is a critical step preliminary to the release of transmitter from the vesicles (the process termed exocytosis). It therefore seems possible that the amount of calcium coming into the terminals with each action potential is not fixed but is variable and that the amount might be modulated by habituation.

The best way to examine changes in the flow of calcium into terminals would be to record from the terminals directly. We have been unable to do so because the terminals are very small. Because the properties of the calcium channels of the cell body resemble those of the terminals, however, one of our graduate students, Marc Klein, set about examining the change in the calcium current of

a

HEART

ANTERIOR AORTA
GASTRO-ESOPHAGEAL ARTERY
ABDOMINAL AORTA

RB$_{HE}$ LD$_{HE}$ LD$_{HI1}$ LD$_{HI2}$ LB$_{VC1}$ LB$_{VC2}$ LB$_{VC3}$

L10

▲ INHIBITION
△ EXCITATION

● ACETYLCHOLINE

● SEROTONIN

b

HEART RATE (BEATS PER MINUTE)
25
20
15
10

BLOOD PRESSURE (MILLIMETERS OF WATER)
100
50
0

RB$_{HE}$

LD$_{HI}$

L10

|←——50 SECONDS——→|

BEHAVIORAL CONTROL exerted by the single neuron L10 is shown by its effect on cardiovascular motor neurons of *Aplysia*. L10 is known to make synaptic connections (*a*) with six of the cells (LD$_{HE}$ has not yet been examined for this synaptic connection); the color of each cell indicates what chemical transmitter it utilizes. It can be seen (*b*) that activity in L10 increases the animal's heart rate and blood pressure by exciting RB$_{HE}$ and inhibiting LD$_{HI}$.

the cell body that accompanies the synaptic depression.

The calcium current turns on slowly during the action potential and so is normally overlapped by the potassium current. To unmask the calcium current we exposed the ganglion to tetraethylammonium (TEA), an agent that selectively blocks some of the delayed potassium current. By blocking the repolarizing action of the potassium current the agent produces a significant increase in the duration of the action potential. Much of this prolongation is due to the unopposed action of the calcium current. The duration of the action potential prolonged by TEA is a good assay for changes in calcium current.

We next examined the release of transmitter by the terminals of the sensory neurons, as measured by the size of the synaptic potential in the motor cell, and the changes recorded simultaneously in the calcium current, as measured by the duration of the action potential. We found that repeated stimulation of the sensory neuron at rates that produce habituation led to a progressive decrease in the duration of the calcium component of the action potential that paralleled the decrease in the release of transmitter. Spontaneous recovery of the synaptic potential and of the behavior were accompanied by an increase in the calcium current.

What we have learned so far about the mechanisms of short-term habituation indicates that this type of learning involves a modulation in the strength of a previously existing synaptic connection. The strength of the connection is determined by the amount of transmitter released, which is in turn controlled by the degree to which an action potential in the presynaptic terminal can activate the calcium current. The storage of the memory for short-term habituation therefore resides in the persistence, over minutes and hours, of the depression in the calcium current in the presynaptic terminal.

What are the limits of this change? How much can the effectiveness of a given synapse change as a result of learning, and how long can such changes endure? I have mentioned that repeated training sessions can completely depress the synaptic connections between the sensory and the motor cells. Can this condition be maintained? Can long-term habituation give rise to a complete and prolonged inactivation of a previously functioning synapse?

These questions bear on the longstanding debate among students of learning about the relation of short- and long-term memory. The commonly accepted idea is that the two kinds of memory involve different memory processes. This idea is based, however, on rather indirect evidence.

Castellucci, Carew and I set out to

examine the hypothesis more directly by comparing the effectiveness of the connections made by the population of sensory neurons on an identified gill motor cell, L7, in four groups of *Aplysia:* untrained animals that served as controls, and groups examined respectively one day, one week and three weeks after long-term habituation training. We found that in the control animals about 90 percent of the sensory neurons made extremely effective connections to L7, whereas in the animals examined one day and one week after long-term habituation the figure was 30 percent. Even in the three-week group only about 60 percent of the cells made detectable connections to L7. Here, then, are previously effective synaptic connections that become inactive and remain that way for more than a week as a result of a simple learning experience.

Hence whereas short-term habituation involves a transient decrease in synaptic efficacy, long-term habituation produces a more prolonged and profound change, leading to a functional disruption of most of the previously effective connections. The data are interesting for three reasons: (1) they provide direct evidence that a specific instance of long-term memory can be explained by a long-term change in synaptic effectiveness; (2) they show that surprisingly little training is needed to produce a profound change in synaptic transmission at synapses critically involved in learning, and (3) they make clear that short- and long-term habituation can share a common neuronal locus, namely the synapses the sensory neurons make on the motor neurons. Short- and long-term habituation also involve aspects of the same cellular mechanism: a depression of excitatory transmission. One now needs to determine whether the

long-term synaptic depression is presynaptic and whether it involves an inactivation of the calcium current. If it does, it would support on a more fundamental level the notion that short- and long-term memory can involve a single memory trace.

Sensitization is a slightly more complex form of learning that can be seen in the gill-withdrawal reflex. It is the prolonged enhancement of an animal's preexisting response to a stimulus as a result of the presentation of a second stimulus that is noxious. Whereas habituation requires an animal to learn to ignore a particular stimulus because its consequences are trivial, sensitization requires the animal to learn to attend to a stimulus because it is accompanied by potentially painful or dangerous consequences. Therefore when an *Aplysia* is presented with a noxious stimulus
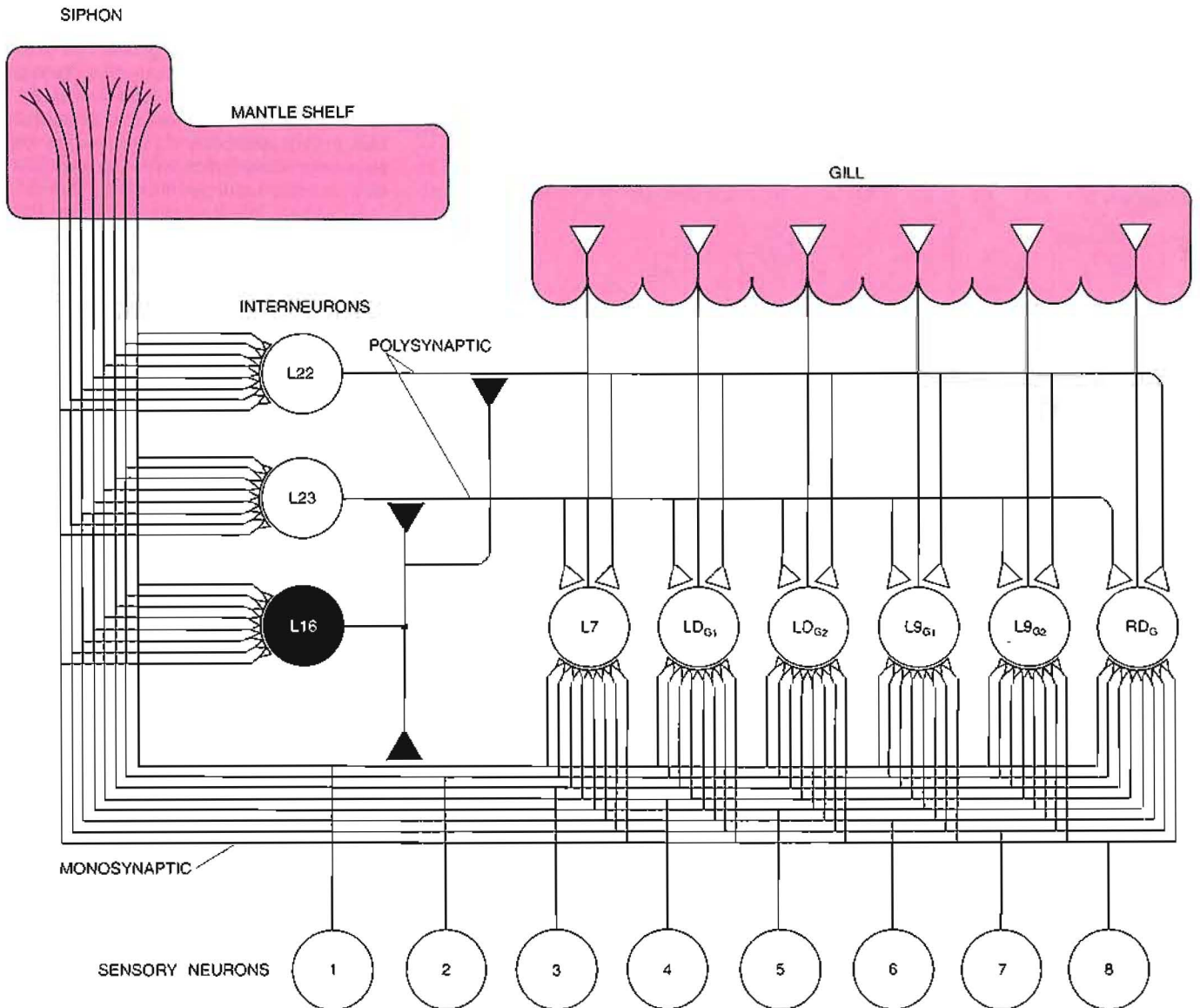
NEURAL CIRCUITRY of a behavioral reflex of *Aplysia*, the gill-withdrawal reflex, is depicted schematically. In the reflex action the animal withdraws its gill when a fleshy spout (the siphon) on a protective sheet (the mantle shelf) is stimulated in some way. The skin of the siphon is innervated by about 24 sensory neurons; the diagram has been simplified to focus on only eight of them. The sensory neurons make monosynaptic, or direct, connections to six identified gill motor neurons, which are shown in the row beginning with L7, and to at least one inhibitory cell (L16) and two interposed excitatory interneurons (L22 and L23), which make synapses with motor neurons.

to the head, the gill-withdrawal reflex response to a repeated stimulus to the siphon is greatly enhanced. As with habituation, sensitization can last from minutes to days and weeks, depending on the amount of training. Here I shall focus only on the short-term form.

Castellucci and I found that sensitization entails an alteration of synaptic transmission at the same locus that is involved in habituation: the synapses made by the sensory neurons on their central target cells. Our physiological studies and subsequent morphological studies by Craig Bailey, Mary C. Chen and Robert Hawkins indicate that the neurons mediating sensitization end near the synaptic terminals of the sensory neurons and enhance the release of transmitter by increasing the number of quanta turned loose by each action potential in the sensory neuron. The process is therefore called presynaptic facilitation. It is interesting because it illustrates (as does the earlier finding of presynaptic inhibition in another system by Joseph Dudel and Stephen Kuffler of the Harvard Medical School) that neurons have receptors to transmitters at two quite different sites. Receptors on the cell body and on the dendrites determine whether a cell should fire an action potential, and receptors on the synaptic terminals determine how much transmitter each action potential will release.

The same locus—the presynaptic terminals of the sensory neurons—can therefore be regulated in opposite ways by opposing forms of learning. It can be depressed as a result of the intrinsic activity within the neuron that occurs with habituation, and it can be facilitated by sensitization as a result of the activity of other neurons that synapse on the terminals. These findings at the level of the single cell support the observation at the behavioral level that habituation and sensitization are independent and opposing forms of learning.

This finding raises an interesting question. Sensitization can enhance a normal reflex response, but can it counteract the profound depression in the reflex produced by long-term habituation? If it can, does it restore the completely inactivated synaptic connections produced by long-term habituation? Carew, Castellucci and I examined this question and found that sensitization reversed the depressed behavior. Moreover, the synapses that were functionally inactivated (and would have remained so for weeks) were restored within an hour by a sensitizing stimulus to the head.

Hence there are synaptic pathways in the brain that are determined by developmental processes but that, being predisposed to learning, can be functionally inactivated and reactivated by experience! In fact, at these modifiable synapses a rather modest amount of training or experience is necessary to produce profound changes. If the finding were applicable to the human brain, it would imply that even during simple social experiences, as when two people speak with each other, the action of the neuronal machinery in one person's brain is capable of having a direct and perhaps long-lasting effect on the modifiable synaptic connections in the brain of the other.

Short-term sensitization is particularly attractive from an experimental point of view because it promises to be amenable to biochemical analysis. As a first step Hawkins, Castellucci and I have identified specific cells in the abdominal ganglion of *Aplysia* that produce presynaptic facilitation. By injecting an electron-dense marker substance to fill the cell and label its synaptic endings we found that the endings contain vesicles resembling those found in *Aplysia* by Ludmiela Shkolnik and James H. Schwartz in a neuron whose transmitter had previously been established to be serotonin. Consistent with the possible serotonergic nature of this cell, Marcel-

a

b

L7

S.N.

STIMULUS 1   2   5   10   15

L7

S.N.

STIMULUS 1   2   5   10   15

|← →|
200 MILLISECONDS
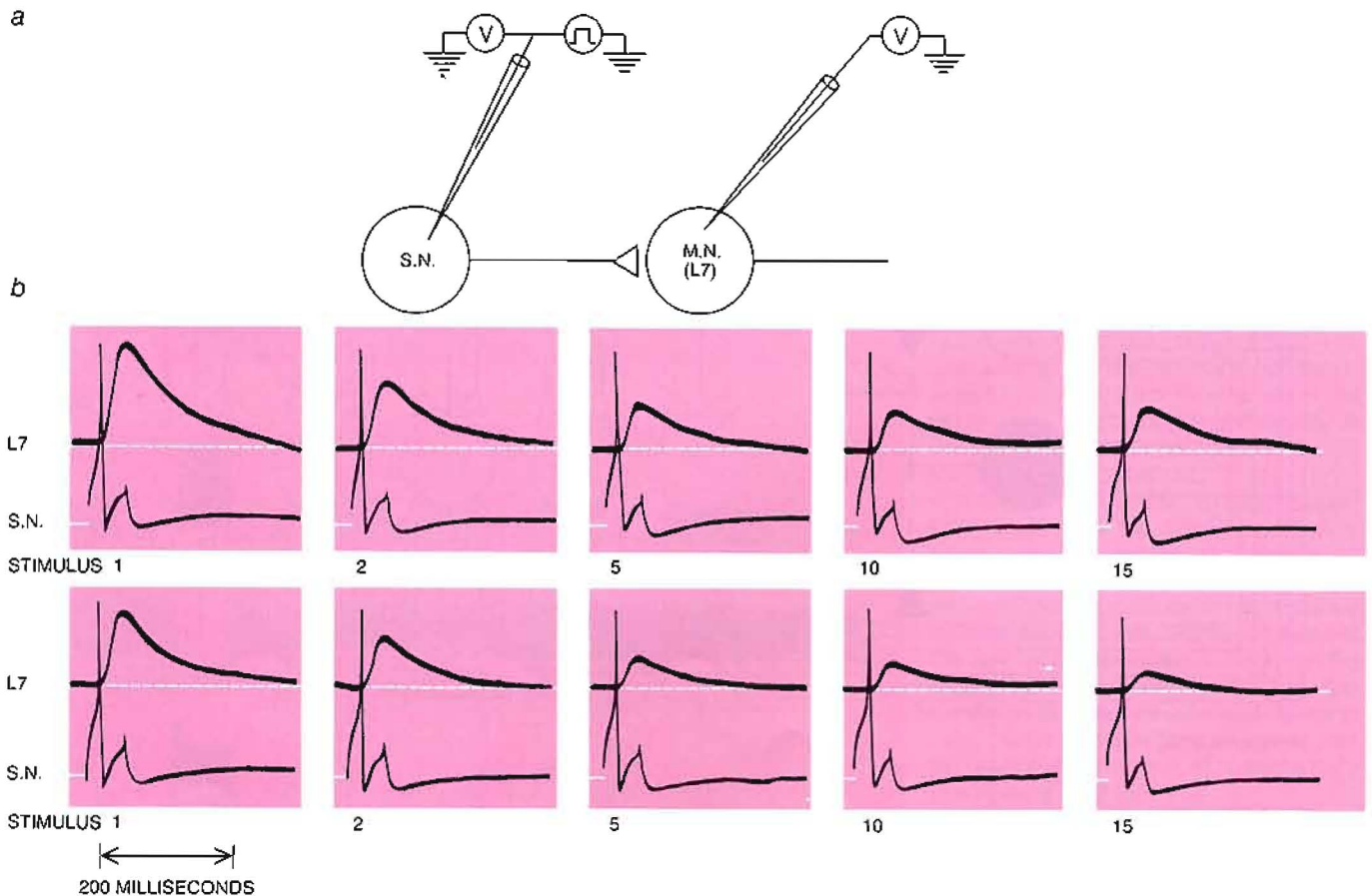
HABITUATION PROCESS, in which an animal's response to a stimulus gradually declines if the stimulus proves to be unimportant, is an elementary form of learning and memory that can be seen at the level of the single motor neuron. Here a sensory neuron (*S.N.*) from *Aplysia* that synapses on motor neuron L7 has been set up (*a*) so that the sensory neuron can be stimulated every 10 seconds. Selected records from two consecutive training sessions of 15 stimuli, separated by 15 minutes, show that the response of L7 declines and vanishes.

lo Brunelli, Castellucci, Tom Tomosky-Sykes and I found that serotonin enhanced the monosynaptic connection between the sensory neuron and the motor cell L7, whereas other likely transmitters did not.

We next uncovered an interesting link between serotonin and the intracellular messenger cyclic adenosine monophosphate (cyclic AMP). It has been known since the classic work of Earl W. Sutherland, Jr., and his colleagues at Vanderbilt University that most peptide hormones do not enter the target cell but instead act on a receptor on the cell surface to stimulate an enzyme called adenylate cyclase that catalyzes the conversion in the cell of adenosine triphosphate (ATP) into cyclic AMP, which then acts as a "second messenger" (the hormone is the first messenger) at several points inside the cell to initiate a set of appropriate changes in function.

Howard Cedar, Schwartz and I found that strong and prolonged stimulation of the pathway from the head that mediates sensitization in *Aplysia* gave rise to a synaptically mediated increase in cyclic AMP in the entire ganglion. Cedar and Schwartz and Irwin Levitan and Samuel Barondes also found that they could generate a prolonged increase in cyclic AMP by incubating the ganglion with serotonin. To explore the relation between serotonin and cyclic AMP, Brunelli, Castellucci and I injected cyclic AMP intracellularly into the cell body of the sensory neuron and found that it also produced presynaptic facilitation, whereas injection of 5'-AMP (the breakdown product of cyclic AMP) or still another second messenger, cyclic GMP, did not.

Since habituation involves a decrease in calcium current, it was attractive to think that cyclic AMP might exert its facilitating actions by increasing the calcium current. As I have mentioned, the calcium current is normally masked by the potassium current. Klein and I therefore examined action potentials in the sensory neurons with the potassium current reduced by TEA. Stimulating the pathway from the head that mediates sensitization or a single facilitating neuron enhanced the calcium current, as was evident in the increased duration of the action potential in TEA, and the enhancement persisted for 15 minutes or longer. The increase in calcium current paralleled the enhanced transmitter release, and both synaptic changes in turn paralleled the increase in the reflex response to a sensitizing stimulus.

The enhancement of the calcium current, as it is seen in the prolongation of the calcium component of the action potential after stimulation of the sensitizing pathway, could be produced by extracellular application of either serotonin or two substances that increase the intracellular level of cyclic AMP by in-

L7

S.N.

$\xleftarrow{\hspace{2cm}}$
100 MILLISECONDS
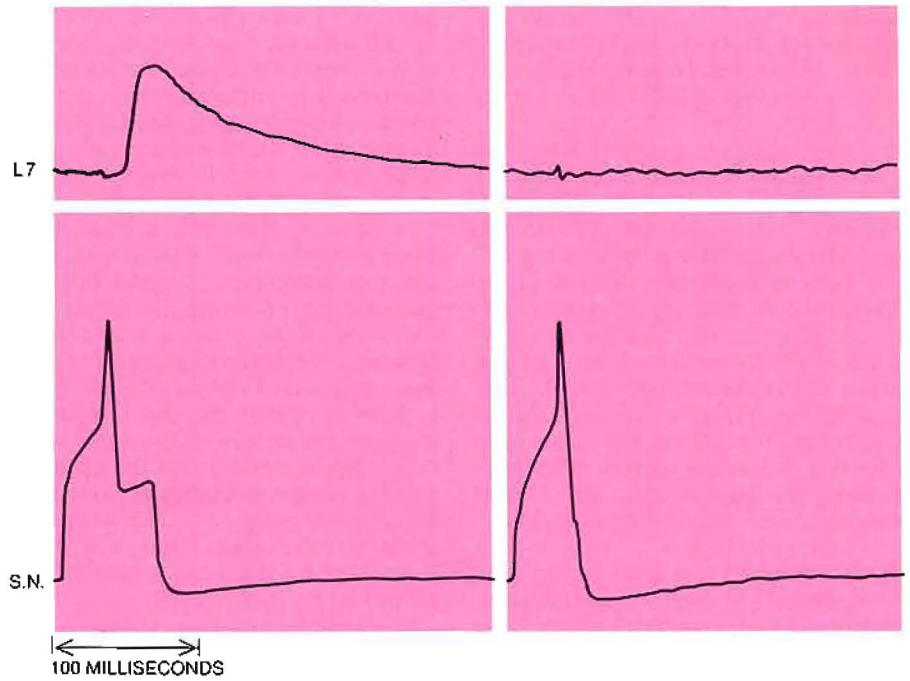
LONG-TERM HABITUATION is revealed in a comparison of synaptic connections between a sensory neuron (*S.N.*) and the motor neuron L7 in untrained *Aplysia* (*left*), which served as controls, and in *Aplysia* that had received long-term habituation training (*right*). In the control animals an impulse in the sensory neuron is followed by a large excitatory synaptic response from the motor neuron. In the trained animals the synaptic connection is almost undetectable.
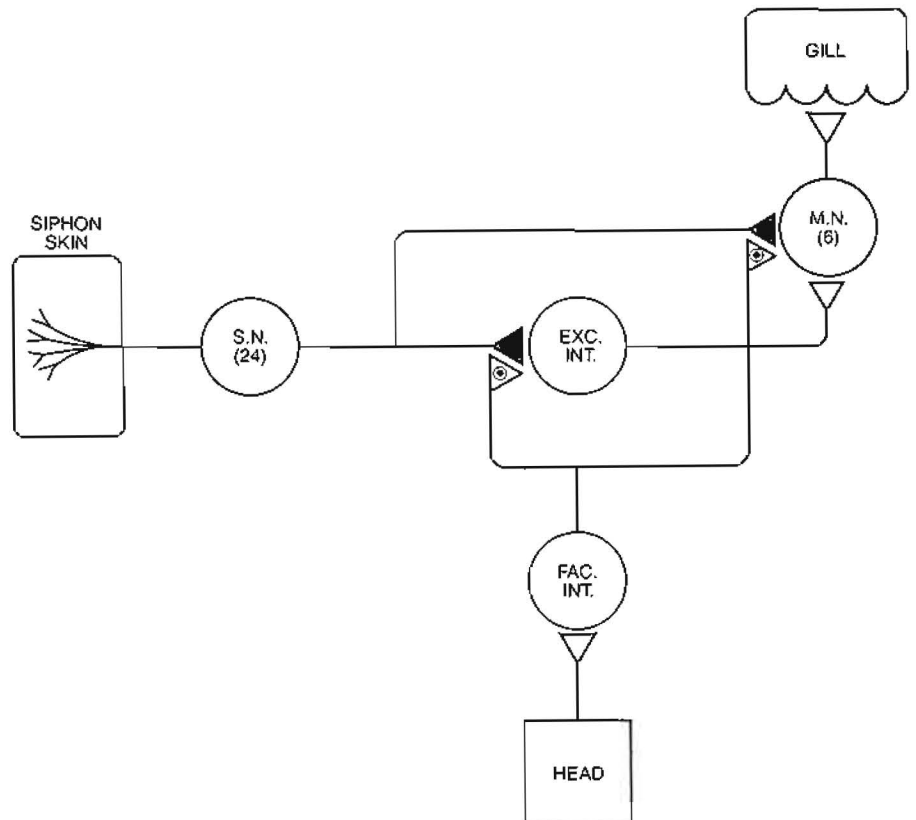
SENSITIZATION is a form of learning and memory in which the response to a stimulus is enhanced because of another and more noxious stimulus. Here gill-withdrawal reflex of *Aplysia* is intensified because of a noxious stimulus to the head. This stimulus activates neurons that excite facilitating interneurons, which end on the synaptic terminals of the sensory neurons. Those neurons are plastic, that is, capable of changing the effectiveness of their synapse. The transmitter of the facilitating interneurons, thought to be serotonin (*circled dots*), modulates the release of sensory-neuron transmitter to the excitatory interneurons and motor neurons.

hibiting phosphodiesterase, the enzyme that breaks down cyclic AMP. Similar effects were observed after direct intracellular injection of cyclic AMP, but not of 5'-AMP.

On the basis of these results Klein and I have proposed that stimulation of the facilitating neurons of the sensitizing pathway leads to the release of serotonin, which activates a serotonin-sensitive enzyme (adenylate cyclase) in the membrane of the sensory-neuron terminal. The resulting increase in cyclic AMP in the terminal leads to a greater activation of the calcium current either directly by activation of the calcium channel or indirectly by a decrease in an opposing potassium current. With each action potential the influx of calcium rises and more transmitter is released.

The availability of large cells whose electrical properties and interconnections can be thoroughly studied was the major initial attraction for using *Aplysia* to study behavior. The size of these cells might now prove to be an even greater advantage for exploring the subcellular and biochemical mechanisms of learning on the one hand and possible changes in membrane structure on the other. For example, it will be interesting to see more precisely how the increase in the level of cyclic AMP during sensitization is linked to the activation of a calcium current, because the linkage could provide the first step toward a molecular understanding of this simple form of short-term learning.

A number of mechanisms come to mind. The channels through which ions traverse the neuronal membranes are thought to consist of protein molecules. An obvious possibility is therefore that

cyclic AMP activates one or more protein kinases, enzymes that Paul Greengard of the Yale University School of Medicine has suggested may provide a common molecular mechanism for mediating the various actions of cyclic AMP within the cell. Protein kinases are enzymes that phosphorylate proteins, that is, they link a phosphoryl group to a side chain of the amino acids serine or threonine in the protein molecule, thereby changing the charge and configuration of proteins and altering their function, activating some and inactivating others. Phosphorylation could serve as an effective mechanism for the regulation of memory. One way sensitization might work is that the calcium-channel protein becomes activated (or the opposing potassium-channel protein becomes inactivated) when it is phosphorylated by a protein kinase that is dependent on cyclic AMP.

Sensitization holds an interesting position in the hierarchy of learning. It is frequently considered to be a precursor form of classical conditioning. In both sensitization and classical conditioning a reflex response to a stimulus is enhanced as a result of the activation of another pathway. Sensitization differs from conditioning in being nonassociative; the sensitizing stimulus is effective in enhancing reflex responsiveness whether or not it is paired in time with the reflex stimulus. Several types of associative learning have now been demonstrated in mollusks by Alan Gelperin of Princeton University, by George Mpitsos and Stephen Collins of Case Western Reserve University and by Terry Crow and Daniel L. Alkon of the National Institutes of Health. Recently Terry Walters, Carew and I have

obtained evidence for associative conditioning in *Aplysia*. We may therefore soon be in a position to analyze precisely how the mechanisms of sensitization relate to those of associative learning.

Another direction that research can now take is to examine the relation between the initial development of the neural circuit in the embryo and its later modification by learning. Both development and learning involve functional changes in the nervous system: changes in the effectiveness of synapses and in other properties of neurons. How are such changes related? Are the mechanisms of learning based on those of developmental plasticity, or do completely new processes specialized for learning emerge later?

Whatever the answers to these intriguing questions may be, the surprising and heartening thing that has emerged from the study of invertebrate animals is that one can now pinpoint and observe at the cellular level, and perhaps ultimately at the molecular level, simple aspects of memory and learning. Although certain higher mental activities are characteristic of the complex brains of higher animals, it is now clear that elementary aspects of what are regarded as mental processes can be found in the activity of just a very few neurons. It will therefore be interesting both philosophically and technically to see to what degree complex forms of mentation can be explained in terms of simpler components and mechanisms. To the extent that such reductionist explanations are possible it will also be important to determine how the units of this elementary alphabet of mentation are combined to yield the language of much more complex mental processes.
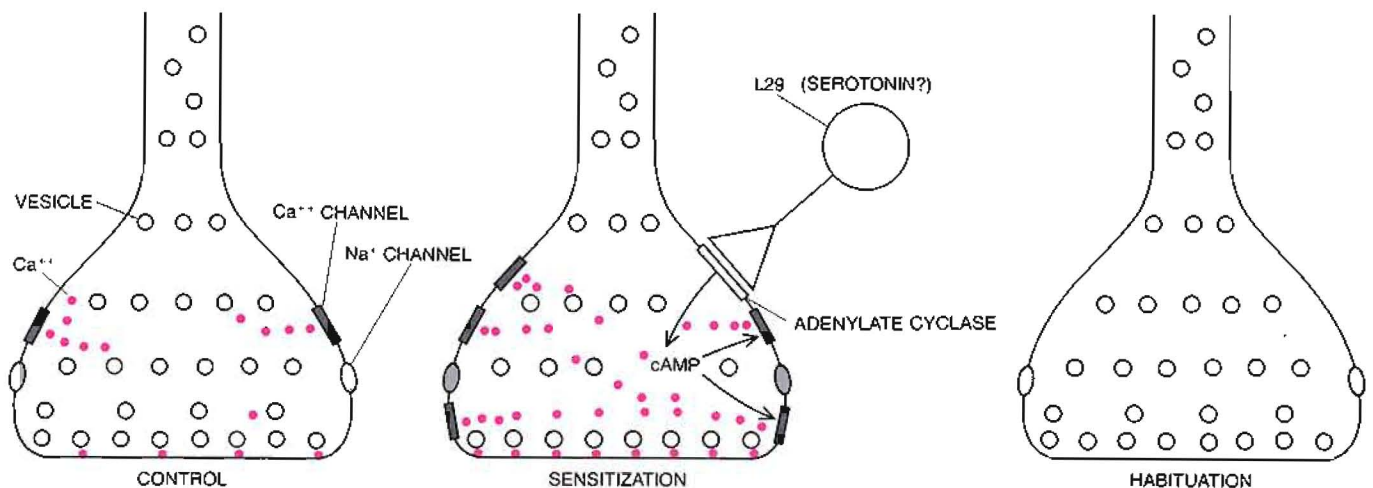
SHORT-TERM SENSITIZATION AND HABITUATION at the level of the single sensory neuron are modeled, beginning with what happens in a control situation (*left*) in which a cell fires before either sensitization or habituation has set in. A nerve impulse in the terminal membrane of the neuron opens up a number of channels for calcium ions (Ca^{++}) in parallel with the sodium channels (Na^{+}). Sensitization is produced by cell group L29 (perhaps more) that are believed to release the transmitter serotonin. It acts on an adenylate cyclase, an enzyme that catalyzes the synthesis of cyclic adenosine monophosphate (cyclic AMP) in the neuron terminals. The cyclic AMP increases the influx of calcium ions, perhaps by making more calcium channels available. The calcium causes a greater binding of transmitter-bearing vesicles to release sites, increasing the probability that the neuron will release transmitter. In habituation repeated impulses in the terminals could decrease the number of open calcium channels, depressing the calcium influx and inactivating the synapse.