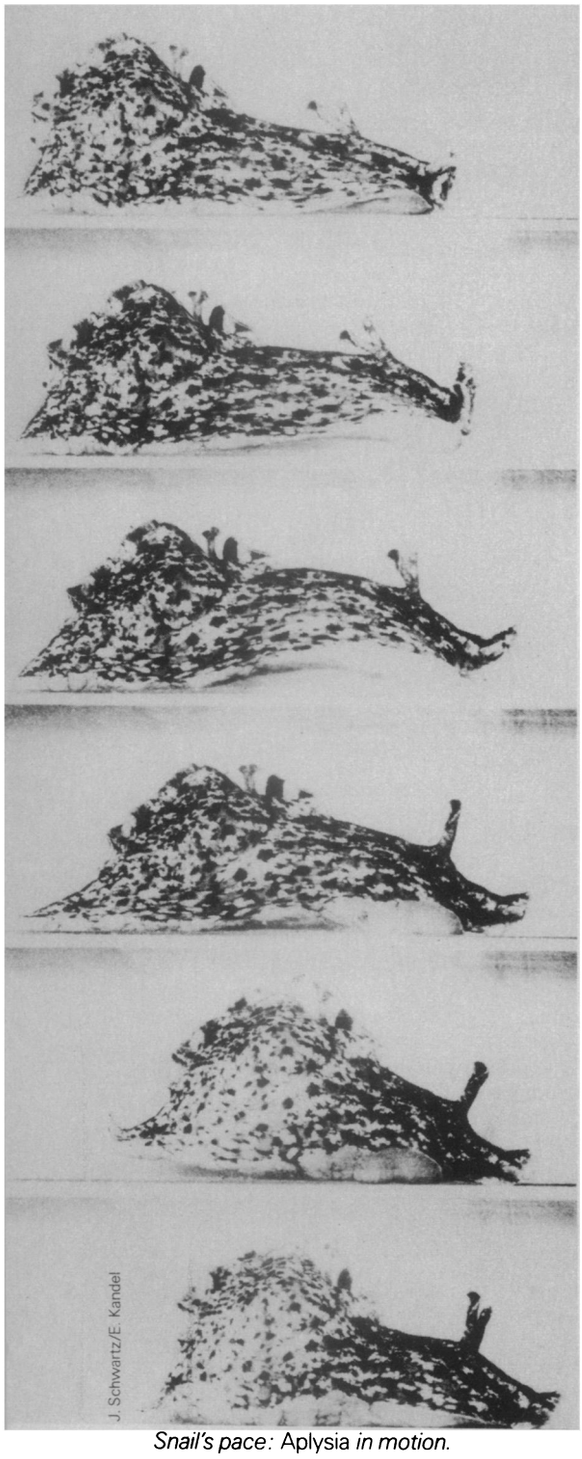
Soft-Core Learning

Neurophysiologists are beginning to unlock some of the brain's best-kept secrets — with the help of undeniably simple-minded creatures



Snail's pace: Aplysia *in motion.*

By ROBERT POLLIE

First of two articles

In its native Pacific coastal waters its primary activities are, in the words of one zoologist, "eating and copulating." Removed from the water, it resembles nothing so much as a quart of failed Jell-O. No one has ever accused the squirrel-sized shell-less snail *Aplysia californica* of oversophistication. The same applies to *Aplysia*'s spineless comrades the crayfish, the many-hued sea slug *Hermissenda* and the giant garden slug, *Limax maximus:* simple beasts all. But it is precisely because of their simplicity that these invertebrates have become leading figures in an assault on one of biology's most complex problems.

How does the brain — essentially a living electrical system — change with experience, acquiring new abilities and information? Put simply, how do animals learn and remember? There has been no shortage of speculation over the years, "but the hard evidence," as one neuroscientist laments, "has been depressingly thin." Yet that assessment is changing, thanks largely to invertebrates. Researchers studying these creatures have lately gotten their first glimpses of learning's microscopic foundations — the physiological changes that take place in nerve cells as organisms learn. After decades of theorizing, the neurosciences have a new empirical footing. "We're finally in a position to make some really important statements about the cellular mechanisms of learning," says neurophysiologist Terry Crow of the University of Pittsburgh.

With their modest behavioral repertoires and uncomplicated nervous systems, it is no wonder that *Aplysia* and company have become some neurophysiologists' best friends. The simplicity of these invertebrates has allowed researchers to dissect some elementary behaviors down to the neural circuitry. The scientists can then outline the behaviors in "wiring diagrams" of specific nerve cells, much as an electrician might depict the workings of a household appliance.

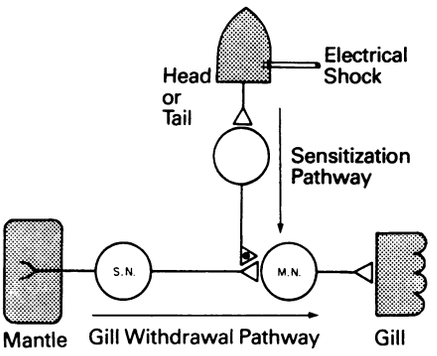
Take *Aplysia*'s gill withdrawal reflex, studied extensively by several groups of researchers. Normally the gill lies flat on the animal's back, flanked by a membrane called the mantle shelf. But if the membrane is disturbed by a touch, the gill retreats into a protective "mantle cavity." Eric Kandel and his colleagues at Columbia University have managed to map many of the nerve cells involved in the response, and the diagram that emerges is essentially a simple one. Touching the mantle shelf sets off nerve impulses in sensory neurons. The impulses are passed, both directly and through intermediary neurons, to a set of motor neurons. The gill is pulled in as motor neurons activate the withdrawal muscles.

But gill withdrawal is not merely a preprogramed reflex; it also can be modified by experience, an ability known to neuroscientists as "plasticity." For example, the gill's response to stimulation, though normally energetic enough, becomes downright vigorous if *Aplysia* is first "sensitized" by an electrical shock to the head or tail. "The noxious stimulus arouses the animal and makes it more alert to incoming sensations," explains James Schwartz of the Columbia group. *Aplysia*'s heightened sensitivity to touch lasts for an hour or so following moderate shocks; after a heavy shock treatment, the arousal can persist for weeks. This is learning of an unambitious sort, but learning nonetheless.

Sensitization, along with many other types of learning, has been studied and characterized by psychologists for some time. Researchers understood decades ago many of the behavioral principles involved when laboratory animals learn to run mazes or push levers dispensing food. But the *physiological* processes underlying this behavioral plasticity were anybody's guess.

And guess they did. Some scientists conjectured that learning occurred when the sprouting branches of brain cells formed new neural circuits; memories might be stored in the networks so formed. An alternative view held that it was only the electrical activity of the brain, and not its cellular architecture, that changed during learning. Nerve cells were turned on or off, up or down, and memories were encoded in the myriad impulses that caromed nonstop through the gray matter.

But evidence was hard to come by. In



How Aplysia *learns: The gill is retracted when a touch activates mantle sensory neurons (SN) and motor neurons (MN). The connection between these nerve cells is strengthened by a shock, through the process of sensitization.*

one of neurobiology's most celebrated efforts, the eminent physiological psychologist Karl Lashley set out in 1917 to locate the brain's "memory trace": the hypothetical structure or region of activity where memory reposed. Lashley probed the brains of rats trained in simple tasks, seeking to determine whether the destruction of selected brain areas would also eliminate an animal's newly learned skills. But after nearly 20 years of searching, Lashley hadn't a clue. No sites corresponding to any specific memories could be found. Summing up, Lashley later wrote, "I sometimes feel in reviewing the evidence on the location of the memory trace, that the necessary conclusion is learning is just not possible. It is difficult to conceive of a mechanism that can satisfy the conditions set for it." Lashley's work had a considerable impact on the neurosciences, discouraging the hope that experimental, physiological approaches could ever solve the abstruse riddles of mental activity.

Nevertheless, neurophysiology was eventually to come into its own. Through gradual refinements in experimental techniques it became possible by the late 1960s to examine learning on the level of individual nerve cells. And one of the first problems to come under experimental attack was the sensitization of *Aplysia*'s gill reflex.

In 1976, Kandel and co-worker Vincent Castelucci succeeded in pinpointing the exact site of sensitization: the synaptic terminals of the mantle neurons. Synapses are the junctions where impulses are relayed from one neuron to the next, via chemical messengers called neurotransmitters. Kandel and Castelucci found that after *Aplysia* is sensitized the mantle sensory neurons release larger amounts of neurotransmitter (the particular chemical is still unidentified) in response to a touch. This evokes a stronger impulse in the motor neurons and causes a more vigorous retraction of the gill. Thus, Kandel says, "Learning is achieved through changes in the strength of connections between nerve cells."

But how does a shock boost the output of the mantle neurons? Over the past few years, the Columbia researchers have been piecing together many details of this process, known as "presynaptic facilitation" (SN: 8/1/81, p. 71). According to a model recently developed by the group, a sensitizing shock is received by nerves in *Aplysia*'s head and tail, and certain nerve cells respond by squirting the chemical serotonin on the synaptic terminals of the mantle neurons. The serotonin sets in motion a cascade of chemical events within these cells. First, it stimulates the cells' production of an important regulatory molecule, cyclic adenosine monophosphate (cyclic AMP). This leads to changes in the electrical properties of the cell membranes. The outflow of potassium ions — which normally limits the duration of nerve impulses and cuts off the release of neurotransmitter — is reduced. The next time the impulse comes traveling down the neuron, its later stages will last longer, and more transmitter will be released. The result: a stronger nerve cell connection and a sensitized snail.

Many phases of this scheme are still hypothetical, having been observed only under artificial laboratory conditions and not during the actual process of sensitization. Serotonin, for instance, *can* shut down membrane potassium channels when it is applied directly to the upper parts of mantle neurons. No one, however, has ever yet witnessed these events taking place in the mantle synaptic terminals themselves, which are very small and hard to study.

In any case, the sensitization model is generally consistent with a number of experimental findings. In studies of crayfish learning, for example, Frank Krasne of the University of California at Los Angeles has recently discovered a sensitization process that "so far appears similar to the one proposed by Kandel's group, although it's more widely distributed in the nervous system," he says. Krasne has found that a large shock can sensitize the crayfish's "tail-flip escape reflex," apparently by strengthening synaptic connections. Unlike sensitization in *Aplysia*, however, this presynaptic facilitation takes place in both sensory neurons and interneurons, and it seems to be induced by the neurotransmitter octopamine, rather than serotonin.

The Columbia team's model also offers a convenient explanation of the short- and long-term forms of memory seen in the sensitization of *Aplysia*. The short-term, hours-long arousal resulting from moderate shocks might be caused by a temporary jump in cyclic AMP levels. Schwartz and others have observed just such a jump in sensory neurons exposed to serotonin.

The mechanism of long-term memory storage is less clear, but Kandel and Schwartz have a few "tentative" speculations. Repeated shocks might cause sustained cyclic AMP increases that actually alter the activity of genes in the neurons. Newly switched-on genes could produce proteins that cause lengthy shut-downs of potassium flow and lead to prolonged facilitation.

A second line of conjecture follows from some striking findings made recently by Kandel's Columbia colleague Craig Bailey, who studied the cellular anatomy of neurons in *Aplysia*. He discovered that in long-term sensitized animals the sensory neurons had unusually large numbers of "active zones" — the sites where neurotransmitters are launched across the synapse. This sort of learning-related structural change, never observed before, suggests another way nerve cell connections might be strengthened.

Kandel and Schwartz believe that a proliferation of active zones could be brought about by changes in genetic activity (prompted, once again, by elevated cyclic AMP). That genes are somehow involved in long-term memory has been suspected by scientists for some time. In a number of experiments, researchers have noted that long-term memory retention in a variety of organisms seems to depend on the synthesis of proteins and nucleic acids — signs of genetic activity. Kandel has even made the suggestion, on a somewhat whimsical note, that psychotherapists may practice a bit of unwitting genetic manipulation when they counsel patients. "Insofar as psychotherapy works, it produces long-lasting changes in the behavior of people. It is unlikely, it seems to me, to produce those changes without inducing changes in gene expression," he says.

While research on *Aplysia* has yielded remarkably detailed insights into the mechanism of sensitization (as well as another rudimentary learning process called habituation), many neuroscientists have been quick to point out the limited applicability of such invertebrate studies. Both habituation and sensitization are forms of "nonassociative" learning, in which an organism learns about only one event, such as an electrical shock. But, as University of Pennsylvania learning theorist Robert Rescorla explains, "learning in higher organisms has been mainly equated with associative learning — cases where animals discover relationships *between* things in their environment." Skeptics have doubted whether invertebrate research could explain such "higher-order" forms of learning.

"Initially, there was a tremendous prejudice against the intellectual abilities of invertebrates," says neurophysiologist Terry Walters of the University of Texas Medical School at Houston. "Most people did not expect them to show anything as sophisticated as associative learning." It was thus considered a major advance when researchers recently began reporting associative capabilities in a number of invertebrates. □

Next: From invertebrates to vertebrates