Lessons from Snails and Other Models

Not that we learn like snails, but some cellular changes are probably quite similar

"LEARNING is a heterogeneous phenomenon and is unlikely to have a simple solution. But we may be able to find some underlying order." The order, referred to by Eric Kandel of the Howard Hughes Medical Institute at Columbia University, is now emerging in the form of similar cellular changes that may be important for learning in very different organisms.

Researchers also have new evidence that long-term learning and memory may depend on changes in protein synthesis, possibly because of changes in gene expression. This information, based largely on four model systems, came to light at a recent Dahlem conference in West Berlin.*

In large pyramidal neurons in the mammalian hippocampus and in sensory neurons of the marine invertebrates, *Hermissenda* and *Aplysia*, activity in one set of nerve cells induces changes in others. Cell membranes become less permeable to certain ions, often potassium, and more permeable to others, especially calcium. Furthermore, changes in neuronal activity trigger critical changes inside cells that are mediated by substances called second messengers. In chick skeletal muscle, changes in neuronal activity regulate protein synthesis and gene expression.

Learning is broadly defined as a change in behavior due to experience, but no one can measure very intricate changes associated with learning in a tissue as complex as the human brain. So researchers develop models for learning using only parts of the mammalian brain, or simple animals, or even tissue culture systems to study basic molecular and cellular changes.

Often they focus on how a given stimulus produces an altered response (usually larger) after training and they measure changes in nerve cells thought to mediate the response. There are many model systems for learning, but four, in particular, are providing some of the most exciting new information. They include the mammalian hippocampus, two marine invertebrates, *Hermissenda* and *Aplysia*, and chick skeletal muscle.

Within the past few years, Richard Thompson of Stanford University, Theodore Berger, now of the University of Pittsburgh, and their colleagues demonstrated a relationship between classical conditioning in rabbits and altered responses in the hippocampus, a part of the mammalian brain involved in learning. Many neuroscientists, including Thompson and Berger, believe that a phenomenon in the hippocampus called long-term potentiation (LTP) underlies certain forms of learning. Essentially, LTP is a long-lasting increase in the communication of neurons across a synapse, and researchers argue whether the critical changes are presynaptic or postsynaptic.

Speaking at the recent Dahlem conference, Timothy Bliss of the National Institute for Medical Research in London said, "The mechanisms for the induction of long-term potentiation may be different than those for its maintenance." Bliss proposed that neurons on both sides of synapses (presynaptic and postsynaptic) must be activated to a threshold level to initiate LTP. Presynaptic changes probably result in more transmitter release, and postsynaptic changes may involve increased sensitivity to transmitter or changes in cell excitability. To maintain LTP, Bliss thinks, depends largely on changes in presynaptic neurons.

Roger Nicoll of the University of California in San Francisco thinks that "there is evidence that second messenger systems play a role in LTP induction." However, as Michel Baudry of the University of California in Irvine points out, "There is no hard evidence that second messengers are involved in LTP. The only evidence is pharmacological."

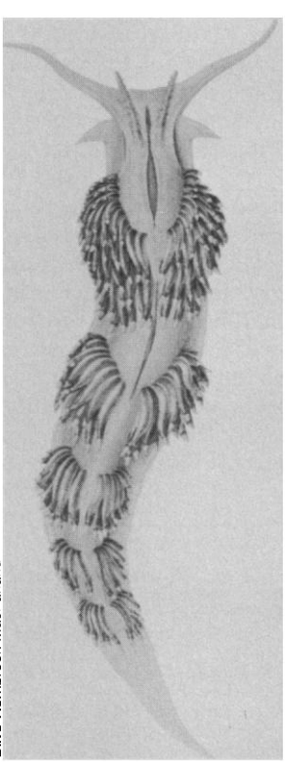
But the pharmacological evidence is tantalizing. Recently, Nicoll, Daniel Madison, and Alison Cole, also of UCSF, applied norepinephrine and acetylcholine (ACh), neurotransmitters normally released in the hippocampus that stimulate second messengers, to slices of hippocampus and saw increased synaptic transmission.

According to Nicoll, "the net effect of both norepinephrine and acetylcholine is to interrupt a braking action of potassium channels that normally limits activity in these pyramidal cells." With potassium efflux inhibited, cells are more excitable and have bigger excitatory responses to synaptic stimulation.

Nicoll and his co-workers find that the potassium current blocked by norepinephrine and acetylcholine depends on calcium ions flowing into the cell. Apparently, one way norepinephrine works is by stimulating a second messenger system involving cyclic AMP and protein phosphorylation.

Whether or not these changes are directly involved in long-term potentiation is an open question. However, Daniel Johnston and William Hopkins, of the Baylor College of Medicine in Houston, recently showed that norepinephrine increases " . . . the magnitude, duration, and probability of induction of long-term synaptic potentiation . . ."†, in an area of hippocampus receiving noradrenergic input in vitro. With these pieces of evidence about norepinephrine in mind, its ability to increase synaptic communication and also to enhance LTP induction, Dahlem participants suggested that this neurotransmitter may play a role in learning.

Now, Nicoll and his colleagues can identify another possible second messenger system that seems to regulate two kinds of ion channels, the potassium channels affected by norepinephrine and cyclic AMP and a separate population of chloride channels. It requires the breakdown of a kind of phospholipid (phosphatidylinositol-4,5-bisphos-



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The marine snail, Hermissenda.

*"Neural and molecular bases of learning," 8–13 December 1985, West Berlin. Proceedings of the meeting can be obtained from Dahlem Konferenzen, Wallostrasse 19, D-1000 Berlin 33, Federal Republic of Germany.

phate) located in the plasma membranes of many cells, including neurons. Normally, when certain transmitters bind to membrane receptors, they stimulate the breakdown of these phospholipids to produce two second messenger substances. One raises intracellular levels of calcium, and the other activates protein kinase C, an enzyme that phosphorylates proteins.

Nicoll, Robert Malenka, and Rodrigo Andrade use a drug, phorbol ester, to stimulate protein kinase C directly. Somehow, by activating this enzyme, phorbol esters decrease both potassium and chloride ion flow. The chloride channels affected by C kinase are regulated by electrical potentials across neuronal membranes, rather than neurotransmitters. They are open when cells are at rest (not firing spikes), allowing chloride ions to flow into neurons. Protein kinase C activation reduces this chloride influx and makes neurons more excitable.

The UCSF group hypothesizes that these chloride channels are located primarily in pyramidal cell dendrites. A stimulus (as yet unknown) activating C kinase might close the channels, thus changing the electrical relationship between remote dendrites and cell bodies of the neurons. Then, any excitatory input at distant synapses in the dendrites would lead to a larger excitatory potential in the cell body. Such a sustained increase in postsynaptic response is the ma-

Are There Common Cellular Mechanisms in Learning?

Aplysia. During short-term sensitization and classical conditioning of the gill-withdrawal reflex in the marine snail, *Aplysia*, interneurons release serotonin or a peptide, setting in motion an entire cascade of specific changes in sensory neurons. Transmitter binding to receptors stimulates production of second messenger molecules of cyclic AMP which in turn activate a calcium-independent protein kinase. This somehow leads to the closure of potassium channels ($\downarrow gK^+_S$), increased spike duration, and more calcium influx ($\uparrow [Ca^{2+}]_i$). Although these events are measured in sensory neuron cell bodies, they may also occur in presynaptic terminals, resulting in more neurotransmitter release and an increased response from postsynaptic motor neurons controlling gill withdrawal.

Hermissenda. When the marine snail, *Hermissenda*, undergoes short-term or long-term classical conditioning, light (conditioned stimulus) and rotation of the animal (unconditioned stimulus) induce changes in type B photoreceptors and decrease the animal's response to move toward light. Light and stimulation from excitatory (E) and inhibitory (I) neurons cause transient membrane depolarization and calcium influx ($\uparrow [Ca^{2+}]_i$) into B cells. This activates two different protein kinases, calcium/calmodulin-dependent kinase type II and phospholipid-dependent protein kinase C, and leads to long-term reduced potassium fluxes through two kinds of ion channels ($\downarrow gK^+_A$ and $\downarrow gK^+_{Ca^{2+}}$). The identity of the neurotransmitter mediating these events is in question, but norepinephrine, serotonin, and acetylcholine are implicated.

The hippocampus. In a giant leap up the evolutionary ladder, mammalian hippocampal pyramidal neurons in vitro show reduced spike afterhyperpolarizations (AHP) and increase cell excitability to norepinephrine and acetylcholine. Both transmitters act by inhibiting a calcium-dependent potassium conductance ($\downarrow gK^+_{Ca^{2+}}$), and NE works through two second messenger systems. It stimulates a cyclic AMP–dependent kinase as well as the phospholipid-dependent protein kinase C stimulated by ACh. Additionally, drugs called phorbol esters activate kinase C directly and block both the potassium channels and voltage-dependent chloride ion channels ($\downarrow gCl^-_V$) thought to be located in dendrites. Although neither NE nor ACh has been associated directly with learning, both are endogenous transmitters in hippocampus and may enhance long-term potentiation in vivo.

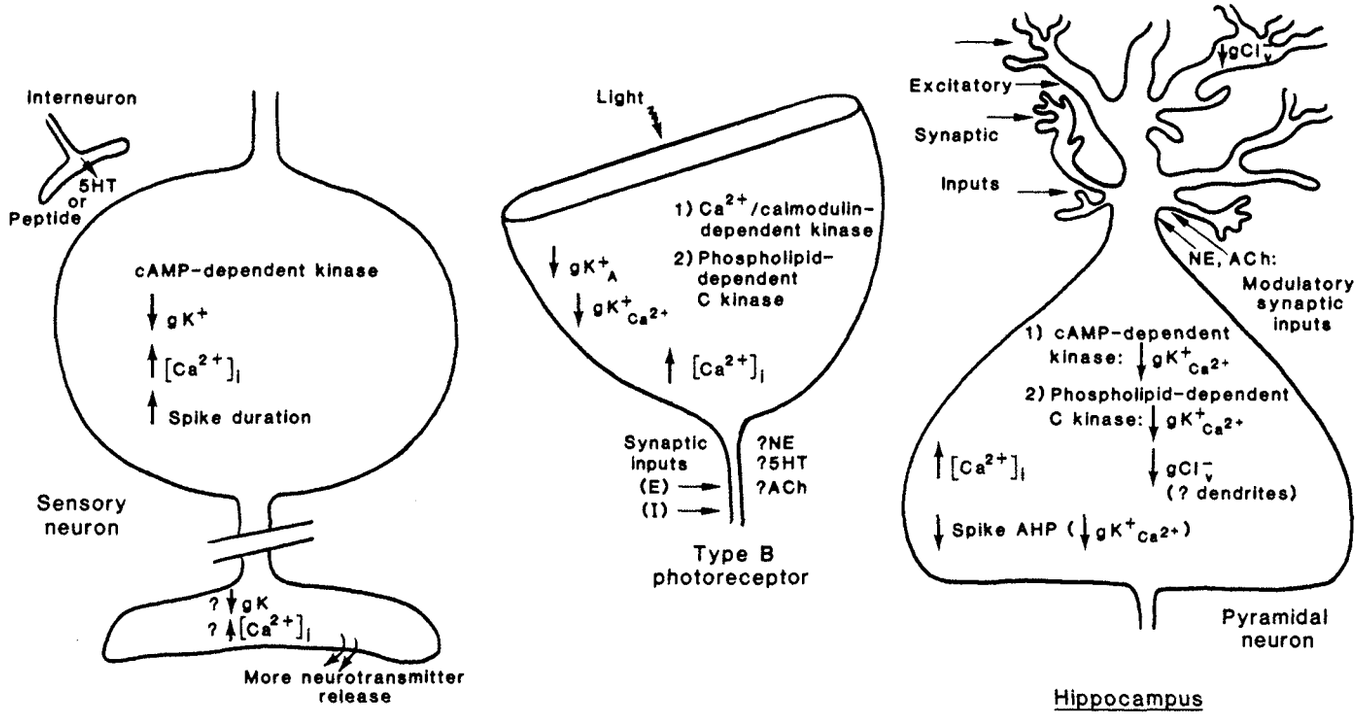


Diagram: Deborah Barnes. Execution: Holly Bishop

jor physiological hallmark of long-term potentiation in the hippocampus.

Daniel Alkon of the National Institute of Neurological and Communicative Disorders and Stroke and the Marine Biological Laboratory in Woods Hole, Massachusetts, and his colleagues study learning in a second model system, the marine snail *Hermissenda*. In many experiments Alkon and Izja Lederhendler train *Hermissenda* according to a Pavlovian model of classical conditioning. As a result, the animals learn to decrease their usual response (phototaxis) to move toward light.

Hermissenda learning depends on "a highly integrated system," according to Alkon. Multiple stimuli, including light and input from other neurons, activate sensory neurons called type B photoreceptors. In turn, B cells activate neurons controlling movement.

In recent work, Alkon and his co-workers show that very specific changes in photoreceptor neurons are associated with both short-term and long-term learning in *Hermissenda*. They measure a decreased outflow of potassium ions through two different kinds of channels, changes that occur quickly and last for days. Alkon now thinks that "calcium can reduce both of these currents. It both activates and inactivates the same potassium currents in a time-dependent way."

Alkon and his colleagues demonstrated that one kind of potassium channel opens and closes quickly. The other type, recently described by Alkon, Bruce Hay, and Manabu Sakakibara, now at Nagoya University in Japan, and Joseph Farley, now at Princeton University, operates more slowly.

Alkon's group found that two different kinases modulate the activity of both channel types in *Hermissenda*. One is calcium/calmodulin type II kinase and the other is protein kinase C. "With the two kinases activated together there is a larger effect than with either alone," says Alkon. Further, "the physiological trigger for activating both kinases is cell depolarization."

Research teams, led independently by Alkon and Farley, demonstrated that protein kinase C decreases both currents by providing *Hermissenda* photoreceptors with second messenger substances or drugs to activate the enzyme directly or indirectly. By stimulating the enzyme artificially, they produced the same cellular changes that occur during learning.

Alkon believes the transmitter responsible for mediating these effects in vivo is norepinephrine, because it reduces both potassium currents in vitro and some *Hermissenda* neurons appear to stain for norepinephrine. Other neuroscientists at the conference dispute this conclusion and point out that no known invertebrate nervous system uses norepinephrine as a transmitter. Moreover, Farley was the first to report norepinephrine's effects, but has since " . . . discarded the idea because no norepinephrine was detectable in the system." Farley and Sidney Auerbach of Rutgers University have just reported that serotonin, not norepinephrine, may modulate the two potassium conductances.

Kandel also takes " . . . a reductionist approach to the study of memory and learning, in which behavior can be related to nerve cells in a specific and causal way." Kandel, Vincent Castellucci, James Schwartz, and other Columbia colleagues study various forms of learning in a third model system, *Aplysia*.

Perhaps the best understood behavior is sensitization, or an increase, of the gill withdrawal reflex. In collaboration with Samuel Schacher and Philip Goelet, also of Columbia, the group has examined both short- and long-term sensitization of gill withdrawal in *Aplysia*. They find that synapses between sensory neurons and motor neurons are critical sites for change, but different mechanisms may underlie short-term and long-term learning.

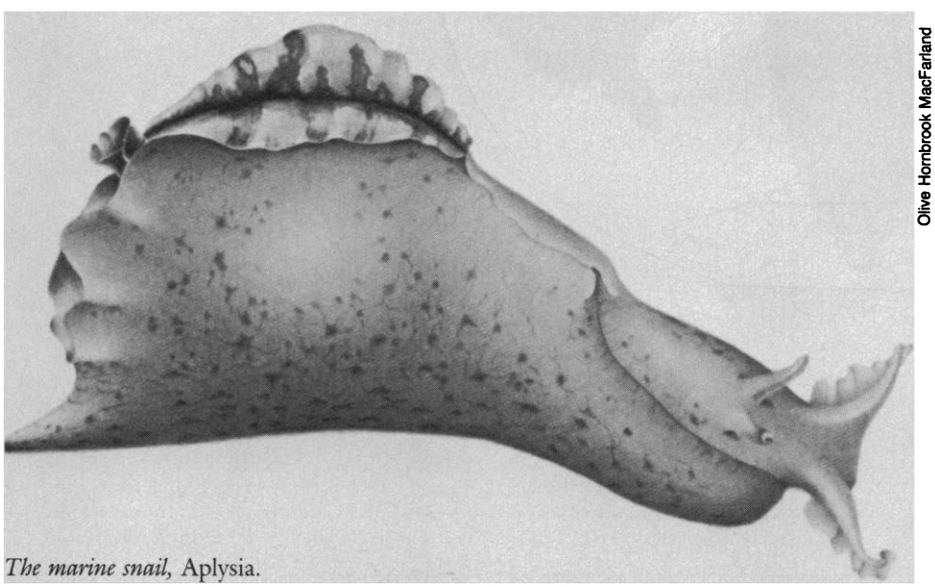
The Columbia group has discovered that short-term increases in *Aplysia*'s gill withdrawal reflex are due to an increased amount of neurotransmitter released from sensory neuron terminals. This causes stronger responses in motor neurons controlling the gill and an increased withdrawal reflex.

In recent years, Kandel, Schwartz, Marc Klein, and their co-workers have shown that key events are the production of cyclic AMP as a second messenger and protein phosphorylation. According to Kandel, this " . . . leads directly, or through a regulatory protein, to the closure of a specific group of potassium channels called serotonin-sensitive potassium channels." As a result, action potentials last longer, intracellular calcium ion concentration increases, and sensory neurons release more transmitter to motor neurons. Kandel and his colleagues measure all of these events in the cell bodies of sensory neurons, and propose that similar phenomena occur in presynaptic terminals.

Now, Schacher, Piergiorgio Montarolo, Kandel, Castellucci, and Goelet have preliminary evidence that the cellular bases for short-term and long-term learning in *Aplysia* are different. Long-term changes seem to require protein synthesis, but short-term changes do not. They draw this conclusion from studies of *Aplysia* neurons in culture and in a semi-intact preparation.

Schacher and Montarolo culture the sensory and motor neurons from *Aplysia* responsible for mediating its gill-withdrawal reflex. The researchers add serotonin to the culture in brief, repetitive doses to simulate what happens in the intact animal during training. "Twenty-four hours later," according to Schacher, they "re-examine the connections between sensory and motor neurons and find an increase in synaptic strength," similar to the change during long-term sensitization in vivo.

But when the researchers add either protein synthesis inhibitors or messenger RNA synthesis inhibitors to the culture, long-term increases in the motor neuron response are blocked. In contrast, "short-term changes are not affected by blockers, indicating that we have dissociated short-term mechanisms from long-term ones," says Schacher.



Olive Hornbrook MacFarland

The marine snail, Aplysia.

Working with the same set of neurons in a semi-intact preparation of *Aplysia* in which behavior can be studied, Hal Blumenfeld, Goelet, and Schwartz, of Columbia University, also blocked long-term increases in synaptic efficacy by adding protein synthesis inhibitors. Again, the changes associated with short-term learning were not affected.

There may be a problem with all of this, according to several Dahlem participants. For instance, as Alkon notes, "protein synthesis effects are seen, but these may not be unique to learning and the storage of information. General protein synthesis inhibitors may interfere with the synthesis of other essential proteins needed for normal cell function."

If new proteins are needed for learning, their roles are still hypothetical, but may include structural changes. Moreover, Kandel and his colleagues do not know if the requirement for protein synthesis associated with long-term learning in *Aplysia* is due to changes in gene expression. It is possible that new proteins are not produced, but that proteins continually synthesized by sensory neurons are modified. Nevertheless, the notion that changes in neurons during long-term learning and memory are stable because they involve changes in gene expression is an intriguing one.

Jean-Pierre Changeux and his colleagues at the Pasteur Institute in Paris study how activity regulates gene expression in a fourth model system, cultured chick muscle cells. In muscle, electrical activity affects protein synthesis in a quantitative way. Changeux's group has shown that decreased electrical activity in muscle cells induces an increased synthesis of acetylcholine receptor protein.

This response is opposite to that which occurs during development, when the onset of neuronal activity to muscle represses receptor synthesis. But taken together, the two phenomena indicate that gene expression and acetylcholine receptor synthesis in muscle cells can be either increased or decreased by the level of activity.

Motor neurons normally innervate skeletal muscle and release acetylcholine as a neurotransmitter. The muscle cell receptors to which ACh binds can change in both transient and more permanent ways. Short-term fluctuations in activity simply induce shifts in the conformation of existing receptor proteins, rather than altering protein synthesis.

To induce more lasting changes, Changeux and his co-workers disrupt electrical input to muscle chemically by blocking sodium entry with tetrodotoxin. Then, muscle cells increase their synthesis of several proteins, one of which is the acetylcholine receptor. Functionally, this increases the sensitivity of muscle to ACh, a phenomenon called "denervation supersensitivity."

In their most recent work, Andre Klarsfield and Changeux showed that tetrodotoxin treatment of cultured muscle induces a 14-fold increase in the content of mRNA for the alpha subunit of the acteylcholine receptor. Under the same conditions, the number of mature receptor molecules increases by only a factor of 2. Therefore, "the effect at the gene level is much larger than the effect on the protein itself," according to Changeux.

"If genes have been triggered for long-term storage then they must be switched off to lose the memory. It is a way of forgetting," Jean-Pierre Changeux.

Changeux does not know the precise mechanism responsible for translating a change in muscle activity to a long-term change in protein synthesis. The level of intracellular calcium seems to be critical, and second messenger systems such as cyclic AMP and inositol phospholipid turnover may also be involved. Nevertheless, the regulation of acetylcholine receptor synthesis is a convincing example of how activity influences gene expression.

Changeux relates changes in protein synthesis and gene regulation in muscle to processes that may determine how long a memory is stored or how quickly it is forgotten. "If a memory is due simply to a changed conformation of postsynaptic receptors because of a certain signal, then when the signal is removed, the receptors change back. But if the receptors are covalently modified (by adding phosphate groups for example), then the protein may need to be replaced to lose the memory. And if genes have been triggered for long-term storage, then they must be switched off to lose the memory. Therefore selection goes with elimination. It is a way of forgetting."

Changeux continues. "The acquisition of memory is associated with both stabilization and elimination of synapses, like the processes that occur with morphogenesis during development. During development there may be an exuberant and redundant innervation, in muscle and in the brain. The synapses finally retract and stabilize to a fixed number."

Thomas Carew of Yale University summarized four kinds of cellular changes that seem to share mechanisms during short-term learning.

First, changes occur at preexisting synapses. Rather than form new connections or eliminate old ones, existing synapses become stronger or weaker. Second, the changes depend on activation of second messenger systems and increased levels of intracellular calcium ions. Third, phosphorylation of existing proteins, including ion channels and enzymes, occurs. And fourth, modulation of ion channel conductances, potassium channels in particular, takes place. These changes have been documented best in *Aplysia* and *Hermissenda* sensory neurons during learning and may also be involved in the induction of long-term potentiation in hippocampus and regulation of gene expression in muscle and nerve tissue.

This is not to say that there are identical mechanisms for cellular and molecular changes during learning throughout the animal kingdom. In fact, plasticity may be a general property of many kinds of synapses rather than a unique feature of neurons involved in learning. There are obvious differences among mechanisms for learning in the model systems discussed here, not the least of which are the neurotransmitter and neuromodulator substances that initiate many of these processes. An important next step will be to identify which changes are essential or causal for learning and to distinguish them from the changes that are nonessential or the result of learning. ■

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ADDITIONAL READING

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