Neuropeptides, Adenylyl Cyclase, and Memory Storage

Eric Kandel and Ted Abel

Genetically modified animals are providing us with a new approach to the molecular basis of behavior. In this issue of *Science*, Feany and Quinn (*1*) use *Drosophila* to revisit the idea that neuropeptides have a specific role in memory storage. Their cloning of the *Drosophila* memory gene *amnesiac* reveals that it encodes a novel neuropeptide homologous to mammalian pituitary adenylyl cyclase activating peptide (PACAP).

Peptide messengers have distinctive properties which suggest that they have special functions in the brain (*2*). Peptides usually are released from nerve terminals during high-frequency neuronal activity as cotransmitters along with a primary small molecule transmitter (such as glutamate or serotonin). Small molecule transmitters act rapidly because they can be quickly degraded and removed from the synaptic cleft. Peptides, in contrast, produce longer-lasting actions, in part because there is no uptake mechanism or degradative system for them in the synaptic cleft. Finally, small molecule transmitters are synthesized and enzymatically packaged in small vesicles at the presynaptic terminal, whereas peptide transmitters are synthesized on ribosomes in the cell body as part of inactive protein precursors, which are then cleaved into smaller, active forms and transported in large vesicles to the nerve terminals.

As cotransmitters, peptides typically enhance the primary transmitter's action but can also have distinct behavioral effects of their own, modulating homeostasis—particularly thirst (angiotensin) (*3*), feeding (neuropeptide Y and galanin) (*4*), and pain (enkephalin) (*5*). Do peptides also have roles in cognition? Vasopressin and adrenocorticotropic hormone can enhance memory storage, but this action on memory may be due to secondary effects on arousal, attention, or motivation (*6*).

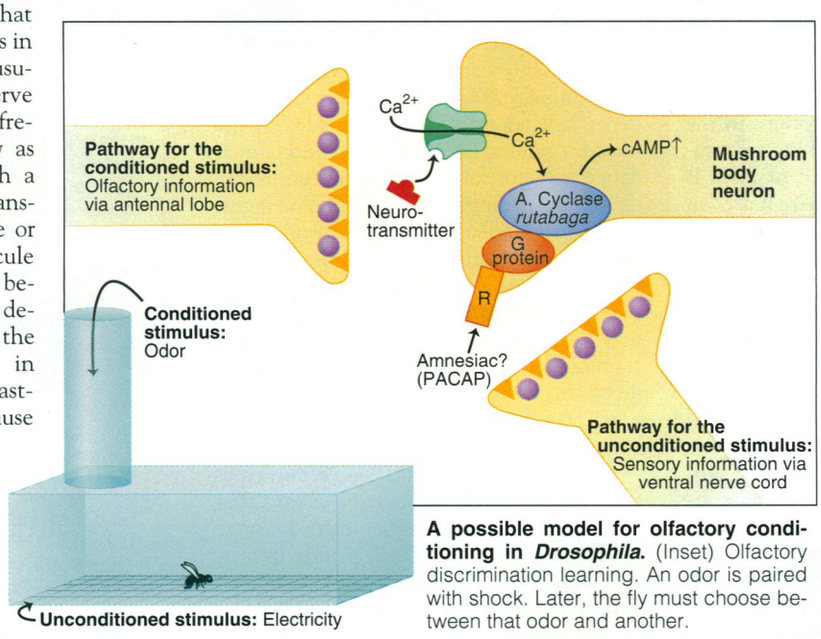
With the new results of Feany and Quinn (*1*), peptides and memory storage have again come to the fore, but now in a genetic context. Feany and Quinn examined *amnesiac*, a *Drosophila* mutant that learns normally but forgets rapidly, and found that it encodes a protein precursor related to mammalian PACAP. PACAP, a member of a family of polypeptide hormones that includes secretin, glucagon, and vasoactive intestinal polypeptide (VIP), is expressed in various peripheral tissues and in several regions of the brain, including the hypothalamus and hippocampus (*7*). PACAP appears to function as a neurotransmitter by interacting with two types of seven-transmembrane-domain receptors. One receptor, which is positively coupled to adenylyl cyclase, also recognizes VIP, the closest family member to PACAP. A second receptor, which recognizes only PACAP, is linked to phospholipase C and adenylyl cyclase (*8*). In neurons, PACAP increases intracellular cAMP (adenosine 3′,5′-monophosphate) and calcium and promotes neurite outgrowth (*9*).

The predicted *amnesiac* protein potentially comprises three peptides, two of which are homologous to PACAP. Although the final proof that this PACAP-related gene is indeed *amnesiac* will require germline rescue experiments, the homology to PACAP suggests that *amnesiac* increases cAMP by activating adenylyl cyclase. Genetic evidence also supports this idea: In fact, the *amnesiac* gene was isolated by transposon (P-element) mutagenesis designed to identify mutations that suppress female sterility caused by elevated cAMP levels in *dunce* mutant flies.

These findings about *amnesiac* rekindle interest in the role of peptides in memory and further enliven the study of *Drosophila* learning. These studies date to 1974, when Seymour Benzer and his colleagues, having successfully isolated a variety of important behavioral mutants (in circadian rhythms, courting, visual function, and neural development), turned their genetic screens to the study of learning by testing how flies remember to avoid an odor that has been paired with a shock (*10*). Using classical chemical mutagenesis of the X chromosome, Dudai *et al.* next identified *dunce*, the first *Drosophila* mutant in learning and memory (*11*). Additional mutants were then identified quite rapidly, including *amnesiac*, *rutabaga*, *cabbage*, *turnip*, and *radish* (*12*).

Soon thereafter, biochemical experiments found that two of the memory mutants were components of the adenylyl cyclase pathway: *dunce* encodes a cAMP-dependent phosphodiesterase, and *rutabaga* encodes a Ca^{2+}-dependent adenylyl cyclase (*13*). However, the identification of other mutations in these screens proved difficult because behavioral testing is time-consuming and the chemically mutated genes were not tagged, making cloning tedious. These obstacles slowed progress in the study of *Drosophila* learning over the next decade and almost brought it to a halt.

Now, several technical advances have converged to overcome these impediments, moving the study of *Drosophila* learning to a new level (*12*, *14*). First, Tully *et al.* greatly improved the behavioral assays and developed techniques for studying both short- and long-term memory (*14*). Second, Davis (*12*) carried out enhancer-trap screens for genes expressed in the mushroom body, a portion of the fly's brain thought to be critical for olfactory learning. Third, Quinn and his colleagues (*15*) used a heat-shock pro-



A possible model for olfactory conditioning in *Drosophila*. (Inset) Olfactory discrimination learning. An odor is paired with shock. Later, the fly must choose between that odor and another.

The authors are at the Howard Hughes Medical Institute and Center for Neurobiology and Behavior, College of Physicians and Surgeons of Columbia University, 722 West 168 Street, New York, NY 10032, USA.

moter to study the effects of transient expression of a transgene on memory in the adult fly, thereby avoiding potential developmental defects. Their demonstration that the acute expression of a peptide inhibitor of protein kinase A (PKA) blocked learning provided direct evidence for the importance of the cAMP pathway in the adult fly. Finally, P-element mutagenesis, the method of tagging the mutated gene that was used by Feany and Quinn (*1*), was introduced to the study of memory in *Drosophila* by Tully and his colleagues (*16*).

The finding of Feany and Quinn (*1*) that *amnesiac* encodes a PACAP-like peptide emphasizes once again the general importance of the cAMP system in learning and memory. This role for cAMP extends beyond *Drosophila* to *Aplysia* and mice. In *Aplysia*, cAMP is required for a learning-induced short-term facilitation in the synaptic connections between sensory and motor neurons of the gill- and tail-withdrawal reflexes (*17*). During short-term facilitation, PKA enhances transmitter release by modifying the activity of various proteins in the presynaptic terminal. During long-term facilitation, the catalytic subunit of PKA is translocated to the nucleus, where it acts on transcription factors such as the cAMP response element binding protein (CREB) to produce the growth of new synaptic connections. Thus, CREB is essential for the switch from a covalently modified short-term process to a transcriptionally dependent long-term process (*18*). CREB is similarly important for long-term behavioral memory in *Drosophila* (*19*). Induction of an inhibitor of CREB under a heat shock promoter blocks long-term memory, and conversely, induction of a CREB activator enhances the formation of long-term memory. CREB and PKA are also important for some forms of spatial learning in mammals and for the transcriptionally dependent late phase of long-term potentiation (LTP) in the hippocampus (*20*).

The central role of cAMP in memory storage in *Drosophila*, *Aplysia*, and rodents suggests that insights into learning in one context may be useful for analysis of the others. In *Aplysia*, reinforcing (unconditioned) stimuli applied to the tail initiate both short- and long-term facilitation by causing the release of the monoamine serotonin (5-HT). Serotonin activates adenylyl cyclase (*21*), which strengthens synaptic connections in the reflex pathway of the conditioned stimulus. A peptide modulatory transmitter (small cardioactive peptide) seems to act in parallel with 5-HT to enhance the short-term process (*22*). By analogy, it is tempting to suggest that *amnesiac* neuropeptides may be one type of modulatory transmitter released by the unconditioned stimulus (electric shock) pathway in *Drosophila*, and that these peptides engage receptors that activate adenylyl cyclase in neurons of the conditioned stimulus (olfactory) pathway (see figure). If the analogy holds, it would suggest the interesting possibility that reinforcing (unconditioned) stimuli may activate monoaminergic or peptidergic modulatory systems and that these may produce functional changes in the pathway of the conditioned stimulus by activating the cAMP cascade. Dopamine, acting through adenylyl cyclase–coupled D1/D5 receptors in the prefrontal cortex of mammals, seems to function in this way to modulate working memory (*23*). Dopamine similarly modifies LTP in the Schaffer collateral pathway of the rat hippocampus by acting through these receptors (*24*).

Common to all of these learning-related pathways is the activation of adenylyl cyclase isoforms that are Ca^{2+}-dependent and therefore well suited to associate two stimuli: They can be stimulated by both Ca^{2+} influx and $G_{\alpha s}$ (*25*). Thus, the Ca^{2+}-dependent adenylyl cyclase encoded by the *rutabaga* gene in *Drosophila* may be a coincidence detector for classical conditioning in olfactory learning in *Drosophila*. The *amnesiac* peptides released by the unconditioned stimulus may activate the cyclase through $G_{\alpha s}$, whereas Ca^{2+} influx, produced by activity in the conditioned stimulus pathway, may activate cyclase through Ca^{2+}-calmodulin. That PACAP-mediated transmission at the *Drosophila* neuromuscular junction is reduced in *rutabaga* mutant larvae supports this idea (*26*).

This emphasis on cAMP in certain types of memory storage does not mean that other second messengers cannot have equally important roles in learning. In fact, although cAMP is present in bacteria and may be the most ancient second-messenger system, it typically acts in combination with other second-messenger cascades in eukaryotic cells.

In a broad sense, the role of adenylyl cyclase, PKA, and CREB in memory storage clearly shows that common peptides and second-messenger cascades can be used for uncommon purposes. To understand how specific memories can be stored in the brain by modulation of kinases that are shared with other cells of the body, we should recall François Jacob's admonition that evolution is a tinkerer—it does not design from scratch. By means of their highly precise connections, the nerve cells of the brain can endow even common molecules with the specific actions necessary to achieve uncommon ends. This is reassuring for the molecular biology of memory storage, as it suggests that even mental functions have not escaped the conservative forces of evolution.

References and Notes

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