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CHAPTER 10

Synaptic remodeling, synaptic growth and the storage of long-term memory in *Aplysia*

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Abstract: Synaptic remodeling and synaptic growth accompany various forms of long-term memory. Storage of the long-term memory for sensitization of the gill-withdrawal reflex in *Aplysia* has been extensively studied in this respect and is associated with the growth of new synapses by the sensory neurons onto their postsynaptic target neurons. Recent time-lapse imaging studies of living sensory-to-motor neuron synapses in culture have monitored both functional and structural changes simultaneously so as to follow remodeling and growth at the same specific synaptic connections continuously over time and to examine the functional contribution of these learning-related structural changes to the different time-dependent phases of memory storage. Insights provided by these studies suggest the synaptic differentiation and growth induced by learning in the mature nervous system are highly dynamic and often rapid processes that can recruit both molecules and mechanisms used for de novo synapse formation during development.

Keywords: active zone; apCAM; *Aplysia*; cell adhesion molecules; learning and memory; long-term memory; long-term sensitization; nascent synapse; presynaptic facilitation; presynaptic terminal; silent synapse; structural changes; synapse formation; synaptic growth; synaptic plasticity; synaptic remodeling

Introduction

Studies of simple forms of implicit memory in higher invertebrates and more complex forms of explicit memory in mammals have found that the storage of long-term memory is represented at the cellular level by activity-dependent modulation of both the function and the structure of specific synaptic connections (Kandel, 2001). Although a number of molecular components that underlie the functional changes associated with memory storage have been characterized, little is known about how these are regulated by and coupled to the signaling pathways that give rise to the synaptic structural changes. This in turn raises two questions central to an understanding of the mechanisms that underlie long-term memory: (1) does the alteration in synaptic strength that accompanies memory storage result from a structural change in

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pre-existing connections — for example, from the conversion of nonfunctional (silent) synapses to functional synapses — from the addition of newly formed functional synapses, or from perhaps both? (2) how closely do the mechanisms and signaling interactions that regulate alterations in the structure of the synapse that are induced by learning resemble those that govern de novo synaptogenesis and the fine-tuning of synaptic connections during development?

In this chapter, we address these questions by focusing on molecular and structural studies of long-term memory storage in *Aplysia*. We begin by examining the remodeling and growth of identified sensory neuron synapses that accompany long-term sensitization — an elementary form of implicit memory. We then turn to recent in vitro studies of the sensory-to-motor neuron synapse reconstituted in dissociated cell culture and consider the cellular and molecular events that give rise to these learning-related structural changes and their functional contribution to the different temporal phases of long-term memory storage. Finally, we outline some of the insights that have been provided by these studies of synaptic remodeling and synaptic growth in *Aplysia* and discuss how molecules and mechanisms important for synapse formation during the development of the nervous system may be reutilized in the adult for the purposes of synaptic plasticity and memory storage.

Functional architecture of the synapse

The interaction between neurons is largely restricted to specialized cellular sites where one nerve cell comes into close apposition with its target cell. This junction is called the synapse, a term introduced by Charles Sherrington in 1897. Although the concept of the synapse was originally framed in physiological terms, it was also realized that there had to be a stable physical structure mediating the function of each synapse. Morphological support for this idea was first provided by Sherrington's contemporary, Santiago Ramon y Cajal, who demonstrated that all synapses had two conserved elements: a presynaptic terminal and a postsynaptic target site. Ramon y Cajal also inferred the existence of a third element, the synaptic cleft, the space between the presynaptic and postsynaptic elements. Collectively, these three components of the synapse underlie the ability of neurons to communicate with one another, the process of synaptic transmission.

Modern studies of the central nervous system (CNS), beginning with those of Palay and Palade, have revealed that chemical synapses are asymmetric sites of cell–cell contact designed for rapid and repetitive signaling between neurons. The presynaptic compartment contains a highly specialized and restricted region, known as the active zone, where synaptic vesicles preferentially dock and fuse with the presynaptic membrane. The fine structure of the active zone consists of an electron-dense meshwork of cytoskeletal filaments and associated proteins at the plasma membrane, embedded with clusters of synaptic vesicles, which is contiguous with the electron-dense postsynaptic density (PSD) of the target neuron. The cytoskeletal matrix associated with the active zone (CAZ) contains a large family of scaffolding and signaling molecules. These are thought to play a fundamental role in the formation and organization of sites along the presynaptic membrane where neurotransmitter is released, maintaining the presynaptic active zone in full alignment with the PSD, and regulating the mobilization of synaptic vesicles and the refilling of transmitter release sites in the presynaptic compartment.

Directly apposed to the active zone, and separated from it by a distance of typically 10–50 nm, is a postsynaptic membrane specialization — the PSD — that serves as a molecular apparatus for both the reception and transduction of the chemical information released by the presynaptic neuron. The PSD consists of a dense network of cytoskeletal filaments that extends across the synaptic cleft to the presynaptic active zone as well as into the cytoplasm of the postsynaptic compartment where it is also associated with a family of scaffolding and regulatory proteins. This postsynaptic matrix serves to anchor and cluster neurotransmitter receptors, ion channels regulated by the receptors and cell adhesion

molecules (CAMs) in the postsynaptic membrane and can recruit a variety of signaling cascades that link these structures with the cytoskeleton to coordinate electrical and more enduring cellular responses. The close apposition of the pre- and postsynaptic compartments as well as the precise structural alignment of the molecular components for transmitter release and transmitter reception across the synaptic cleft facilitates the efficacy of synaptic transmission.

Until approximately three decades ago chemical synapses were thought to convey information in only one direction — from the presynaptic to the postsynaptic neuron. It now is clear that synaptic transmission is a bidirectional and self-modifiable form of cell–cell communication (Jessell and Kandel, 1993). The bidirectional nature of signaling across synapses has been demonstrated in biophysical studies of synaptic transmission, and it also is evident in the assembly of synapses during development and during activity-dependent plasticity of synapses in the mature brain. The relative contributions of both the pre- and postsynaptic neuron and their reciprocal signaling interactions is consistent with the current view of intercellular communication that incorporates the biology of nerve cells and, specifically, signaling in the nervous system, into the broader field of cell biology. This emerging appreciation provides a conceptual framework for the interpretation of learning-related changes in the structure of the synapse.

Synaptic plasticity and memory storage

Modern behavioral and biological studies have revealed that memory is not a unitary faculty of the mind but consists of distinct families of mental processes that can be grouped into at least two general categories, each with its own rules (Polster et al., 1991; Squire and Zola-Morgan, 1991). Explicit or declarative memory is the conscious recall of knowledge about people, places and things, and is particularly well developed in the vertebrate brain. Implicit or nondeclarative memory is memory for motor and perceptual skills as well as other tasks and is expressed through performance, without conscious recall of past experience. Implicit memory includes simple associative forms of memory such as classical and operant conditioning, and nonassociative forms such as sensitization and habituation. Explicit and implicit memory have been localized to different neural systems within the brain (Milner, 1985; Polster et al., 1991). Explicit memory is critically dependent on structures in the medial temporal lobe of the cerebral cortex, including the hippocampal formation. Implicit memory is a family of different processes that are represented in a number of brain systems including the cerebellum, the striatum, the amygdala and in the simplest cases, the sensory and motor pathways recruited during the learning process for particular perceptual or motor skills. As a result, implicit memory can be studied in a variety of simple reflex systems, including those of higher invertebrates, whereas explicit memory can best (and perhaps only) be studied in mammals.

Two experimental model systems have been extensively studied as representative examples of these two forms of memory storage: sensitization in the marine snail *Aplysia californica* as an example of implicit memory, and spatial memory formation in the rodent hippocampus as an example of explicit memory. In both model systems, the elementary events that underlie synaptic plasticity, the ability of neurons to modulate the strength of their synapses in response to extra- or intracellular cues, are thought to be fundamental for both the fine-tuning of synaptic connections during development, as well as for behavioral learning and memory storage in the adult organism. Cell biological and molecular studies in both *Aplysia* and the hippocampus suggest that activity-dependent modulation of synaptic function and synaptic structure is an important mechanism by which information is encoded, processed and stored within the brain (Kandel, 2001; Bliss et al., 2003).

For both implicit and explicit memory, two general types of storage mechanisms have been described: short-term memory lasting minutes and long-term memory lasting days, weeks or longer. This temporal distinction in behavior is reflected in

specific forms of synaptic plasticity that underlie each form of behavioral memory as well as specific molecular requirements for each of these two forms of synaptic plasticity. The short-term forms involve the covalent modifications of pre-existing proteins by a variety of kinases (e.g., PKA and MAPK) and are expressed as alterations in the effectiveness of pre-existing connections. By contrast, in addition to the kinases recruited during the short-term forms, the long-term forms also require CREB-mediated gene expression, new mRNA and protein synthesis and are often associated with the growth of new synaptic connections (Bailey et al., 1996). For both implicit and explicit memory storage, the synaptic growth is thought to represent the final and self-sustaining cellular change that stabilizes the long-term process (Bailey and Kandel, 1993). Moreover, recent studies in *Aplysia* and mammals have provided evidence that activity-dependent remodeling of pre-existing synapses and the growth of new synapses might play an important role in the storage of information at both the level of individual synaptic connections as well as in more complex neuronal networks by modulating and perhaps reconfiguring the activity of the neural network in which this occurs (Bailey et al., 2004; Lamprecht and LeDoux, 2004).

Although each chemical synapse consists of two precisely aligned, tightly adherent, highly specialized and functionally coupled anatomical components, most studies of the structural changes that accompany long-lasting forms of synaptic plasticity have focused on either the presynaptic or postsynaptic compartment. For example, learning-related remodeling and growth of the presynaptic compartment have been most extensively studied in the sensory neurons of the gill-withdrawal reflex of *Aplysia* following long-term sensitization — a simple form of implicit memory storage. Conversely, activity-dependent structural changes in the postsynaptic compartment have been the major focus of studies on dendritic spines in the hippocampus during long-term potentiation (LTP) — a more complex form of explicit memory storage in mammals. Historically, this dichotomy reflects, at least in part, the unique experimental advantages of each model system as well as the underlying hypotheses regarding the mechanisms that give rise to these different forms of plasticity.

The significance of learning-related changes in either the structure of pre-existing synapses or changes in the number of synapses must ultimately be considered in a functional context, that is, the contribution of a specific structural modification to the change in effectiveness of that synaptic connection. When viewed in this light, it is readily apparent that studies of the structural changes associated with synaptic plasticity should consider each synaptic contact in its entirety and recognize that functionally relevant changes in the morphology of the presynaptic compartment are likely to be accompanied by reciprocal changes in the morphology of the postsynaptic compartment. Of the changes in pre-existing synapses, the most reliable and potentially best suited for correlation with changes in synaptic effectiveness are those that involve reorganization of the active zone and associated cytoskeletal matrix (CAZ) in the presynaptic compartment and the PSD in the postsynaptic compartment. Any change in the number, size, continuity or shape of one of these focal and highly specialized regions of the synapse should be reflected by a comparable remodeling in its cognate partner. Similarly, in order to modulate synaptic strength, an increase or decrease in the number of synapses must consist of parallel changes in both the presynaptic transmitter release machinery and the postsynaptic receptive apparatus. Indeed, alterations in the number and structure of both the presynaptic and postsynaptic compartments have been found to accompany long-term sensitization in *Aplysia* and LTP in mammals, and an increasing body of evidence now suggests that coordinated pre- and postsynaptic mechanisms may underlie each form of memory storage (Hawkins et al., 2006).

Growth of new sensory neuron synapses and the persistence of long-term sensitization

The CNS of *Aplysia* contains only approximately 20,000 large and frequently identifiable nerve cells, clustered into 9 major ganglia. The ability

to identify many of the individual neurons of this nervous system and record their activity has made it possible to define the major components of the neuronal circuits of specific behaviors, and to delineate the critical sites and underlying mechanisms used to store memory-related representations.

The mechanisms contributing to implicit memory storage have been most extensively studied for sensitization of the gill-withdrawal reflex in *Aplysia* (Kandel, 2001). Sensitization is an elementary type of nonassociative learning, a form of learned fear, by which an animal learns about the properties of a single noxious stimulus. When a light touch is applied to the siphon of the snail, the snail responds by withdrawing its gill and siphon. This response is enhanced when the animal is given a noxious, sensitizing stimulus. As with other forms of defensive behaviors, the memory for sensitization of the withdrawal reflex is graded, and repeated tail shocks lead to a longer-lasting memory: a single tail shock produces short-term sensitization that lasts for minutes, whereas repeated tail shocks given at spaced intervals produce long-term sensitization that lasts for up to several weeks (Frost et al., 1985).

The simplicity of the neuronal circuit underlying this behavioral modification — including direct monosynaptic connections between identified mechanoreceptor sensory neurons and their follower cells (Castellucci et al., 1970) — has allowed reduction of the analysis of the short- and long-term memory for sensitization to the cellular and molecular level. This monosynaptic sensory to motor neuron connection, which is thought to be glutamatergic, can be reconstituted in dissociated cell culture. Despite its simplification, this in vitro model system reproduces what is observed in the whole animal during behavioral training. In this simplified culture preparation tail shocks are replaced with brief applications of serotonin (5-HT), a modulatory transmitter normally released by sensitizing stimuli in the intact animal (Montarolo et al., 1986). A single brief application of 5-HT produces a short-term change in synaptic effectiveness (short-term facilitation) much as does a single tail shock, whereas repeated and spaced applications produce changes in synaptic strength that can last for more than a week (long-term facilitation or LTF). A component of the increase in synaptic strength observed during both the short- and long-term changes is due, in each case, to enhanced release of transmitter by the sensory neuron onto its follower cells, and is accompanied by an increase in the excitability of the sensory neuron attributable to the depression of specific sets of potassium channels (Klein and Kandel, 1980; Frost et al., 1985; Hochner et al., 1986; Montarolo et al., 1986; Dale et al., 1987; Scholz and Byrne, 1987).

Despite this superficial similarity, the short-term cellular changes differ fundamentally from the long-term changes in at least two important ways: (1) the long-term change, but not the short-term change, requires new protein synthesis (Schwartz et al., 1971; Montarolo et al., 1986; Castellucci et al., 1989) and (2) the long-term but not the short-term process involves a structural change (Bailey and Chen, 1983, 1988a, b, 1989). Long-term sensitization is associated with the growth of new synaptic connections by the sensory neurons onto their follower cells.

In the early 1980s, studies in *Aplysia* first began to explore the morphological basis of the synaptic plasticity that might underlie the transition from short-term to long-term memory. By combining selective intracellular labeling techniques with the analysis of serial thin sections and transmission electron microscopy, complete reconstructions of unequivocally identified sensory neuron synapses were quantitatively analyzed from both control and behaviorally modified animals. The storage of long-term memory for sensitization (lasting several weeks) was accompanied by a family of distinct structural changes at identified sensory neuron synapses. These changes reflected a learning-induced remodeling of the functional architecture of presynaptic sensory neuron varicosities at two different levels of synaptic organization: (1) alterations in focal regions of membrane specialization of the synapse that mediate transmitter release — the number, size and vesicle complement of sensory neuron active zones were larger in sensitized animals than in controls (Bailey and Chen, 1983, 1988b) and (2) a growth process that appeared similar to synaptogenesis during

development and led to a pronounced increase in the total number of presynaptic varicosities per sensory neuron (Bailey and Chen, 1988a). Thus, sensory neurons from long-term sensitized animals exhibited a twofold increase in the number of synaptic varicosities, as well as an enlargement in the linear extent of each neuron's axonal arbor when compared to sensory neurons from untrained animals (Fig. 1).

To determine which class of structural changes at sensory neuron synapses might contribute to the retention of long-term sensitization, Bailey and Chen (1989) compared the time course for each morphological change with the behavioral duration of the memory. They found that not all of the structural changes persisted as long as the memory. The increase in the size and synaptic vesicle complement of sensory neuron active zones present 24 h following the completion of behavioral training returned to control levels when tested 1 week later. These data indicated that, insofar as this relatively transient modulation of active zone size and associated synaptic vesicles is one of the structural mechanisms underlying long-term sensitization, it is associated with the initiation and early expression of the long-term process and not with its persistence. By contrast, the duration of changes in varicosity and active zone number, which persisted unchanged for at least 1 week and were partially reversed at the end of 3 weeks, paralleled the behavioral time course of memory storage indicating that only the increase in the number of sensory neuron synapses contributes to the stable maintenance of long-term sensitization. These results directly linked a change in synaptic structure to a long-lasting behavioral memory and suggested that the morphological alterations could represent an anatomical substrate for memory consolidation. In addition, the finding that some components of the learning-induced changes in synaptic architecture were transient whereas others endured suggested that not all of these modifications were regulated synchronously. At the structural level, the sensory neuron appears to have multiple mechanisms and parameters of plasticity available to it. Thus, during the later phases of long-term memory storage for sensitization, although there are more synapses, each individual synapse may recruit all of the mechanisms of plasticity that were present before training.

Unlike the extensive anatomical changes observed at sensory neuron synapses following long-term training, the structural correlates of short-term memory in *Aplysia* (lasting minutes to hours rather than days to weeks) are far less pronounced (Bailey and Chen, 1988c). For example, the decrease in the strength of the sensory to motor neuron connection that accompanies short-term habituation is not associated with any detectable alterations in either the number of sensory neuron presynaptic varicosities or the number of active zones within the presynaptic varicosities. Nor does it alter the size of active zones or the total number of synaptic vesicles within the presynaptic varicosity. Rather, there is a reduction in the number of vesicles that are docked at the active zones and thus there are fewer packets of transmitter ready to be released.

Taken together, these initial morphological studies of short- and long-term memory in *Aplysia* began to suggest a clear difference in the nature, extent and time course of changes in the functional architecture of the synapse that may underlie memories of differing durations. The transient durations of short-term memories involving covalent modifications of pre-existing proteins (proteins that turn over slowly) are accompanied only by modest structural rearrangements that appear to be restricted to shifts in the proximity of synaptic vesicle populations contiguous to the release site. By contrast, the prolonged durations of long-term memories depend on altered gene expression and the synthesis of new proteins and are associated with more substantial and potentially more enduring structural alterations that are reflected by frank changes in both the number of synaptic contacts and their active zone morphology.

These studies also demonstrated, for the first time, that learning-induced structural changes could be detected at the level of specific, identified synaptic connections known to be critically involved in the behavioral modification and

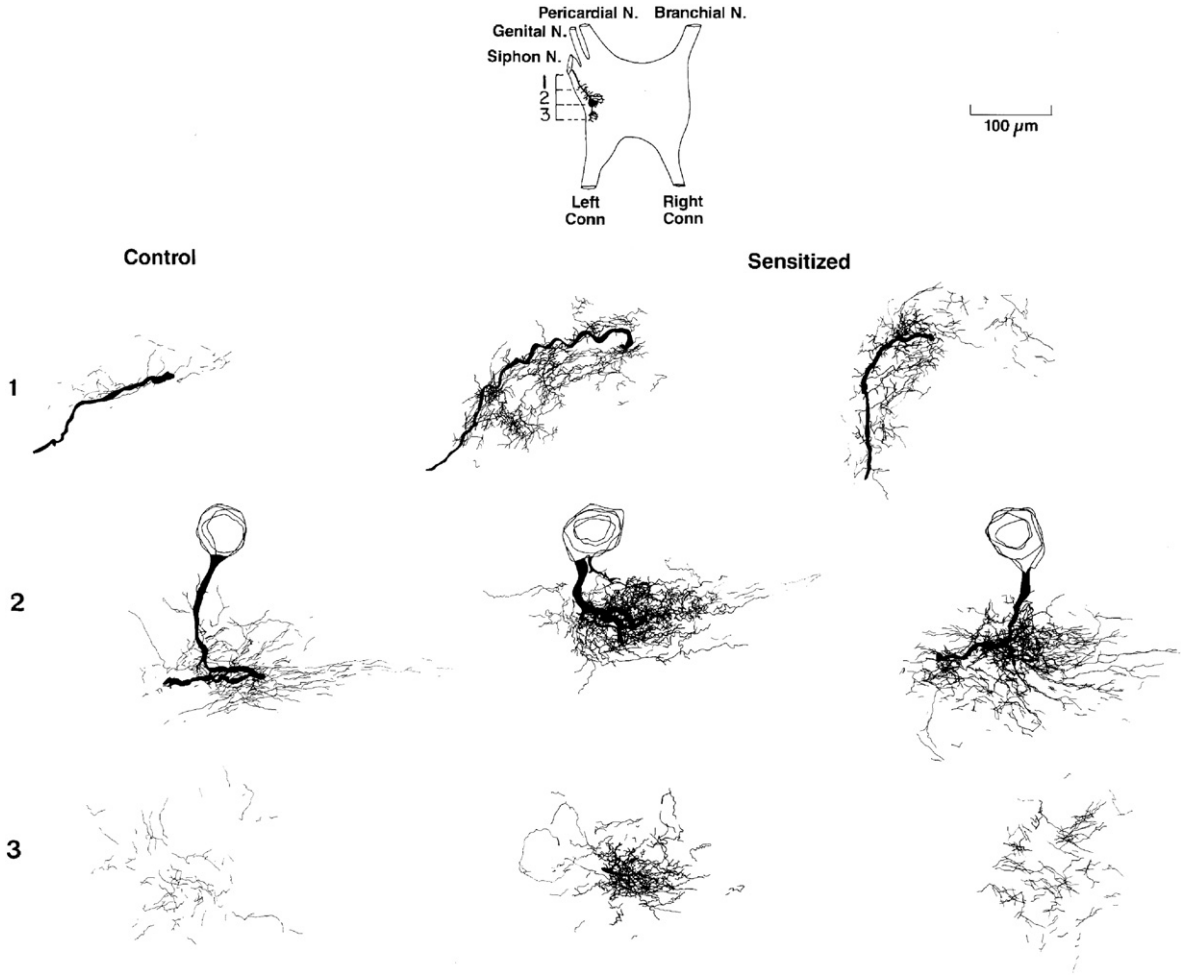

Fig. 1. Growth of sensory neurons induced by long-term sensitization in *Aplysia*. Serial reconstruction of identified sensory neurons labeled with horseradish peroxidase (HRP, Bailey et al., 1979) from long-term sensitized and control animals. Total extent of the neuropil arbors of sensory neurons from one control (untrained) and two long-term-sensitized animals are shown. In each case, the rostral (row 3) to caudal (row 1) extent of the arbor is divided roughly into thirds. Each panel was produced by the superimposition of camera lucida tracings of all HRP-labeled processes present in 17 consecutive slab-thick Epon sections and represents a linear segment through the ganglion of roughly 340 μm. For each composite, ventral is up, dorsal is down, lateral is to the left, and medial is to the right. By examining images across each row (rows 1, 2, and 3), the viewer is comparing similar regions of each sensory neuron. In all cases, the axonal arbor of long-term-sensitized cells is markedly increased compared to cells from control (untrained) animals and parallels the concomitant increase in the number of sensory neuron presynaptic varicosities. (From Bailey and Chen, 1988a.)

provided evidence for an intriguing notion — that active zones are plastic rather than immutable components of the synapse. Even elementary forms of learning can remodel the basic anatomical scaffolding of the neuron, in this case by altering the number and organization of transmitter release sites in the presynaptic compartment, to modulate the functional expression of synaptic connections. In addition, complete serial reconstructions of identified sensory neuron varicosities in untrained (naïve) animals revealed that approximately 60% of these presynaptic terminals lacked a structurally detectable active zone suggesting the possibility of nascent synapses in the adult

brain. The extent to which learning and memory can convert these immature, and presynaptically silent synapses into mature and functionally competent synaptic connections is discussed below. Finally, these initial studies in *Aplysia* suggested that the growth of new sensory neuron synapses may represent the final and perhaps most stable phase of long-term memory storage, and raised the possibility that the stability of the long-term process might be achieved, at least in part, because of the relative stability of synaptic structure.

Subsequent studies by Wainwright et al. (2002) have examined the effects of different sensitization training protocols on the structure of sensory neurons in the pleural ganglion mediating the tail-siphon withdrawal reflex in *Aplysia*. A 4-day training period produced a robust and localized outgrowth of both neuritic processes and presynaptic varicosities in these sensory neurons observed 24 h after the end of training. These structural changes were consistent with the previous results of Bailey and Chen (1988a) in siphon sensory neurons. By contrast, 1 day of sensitization training, which can induce both long-term behavioral sensitization and synaptic facilitation (Frost et al., 1985; Cleary et al., 1998), was not associated with a comparable outgrowth of tail sensory neurons indicating that storage of the memory for sensitization induced by the 1 day of training and the spaced 4 day protocols are likely to recruit different mechanisms. These investigators have also reported a dissociation of morphological and physiological changes associated with long-term sensitization of the tail-siphon withdrawal reflex in *Aplysia* (Wainwright et al., 2004). The behavioral effects of long-term sensitization training were restricted to the trained side of the animal as were changes in the strength of sensory to motor connections. By contrast, long-term training produced varicosity formation on both sides of the animal. Interestingly, on the trained side, this outgrowth was reflected by an increase in the number of putative contacts with follower neurons as well as with an increase in synaptic strength and behavioral enhancement suggesting a causal relationship between these changes.

The long-lasting growth of new synaptic connections between sensory neurons and their follower cells during long-term sensitization can be reconstituted in sensory–motor neuron co-cultures by five repeated applications of 5-HT (Glanzman et al., 1990; Bailey et al., 1992b) as well as induced in the intact ganglion by the intracellular injection of cAMP, a second messenger activated by 5-HT (Nazif et al., 1991). In culture, the synaptic growth can be correlated with the long-term (24–72 h) enhancement in synaptic effectiveness and depends on the presence of an appropriate target cell similar to the synapse formation that occurs during development.

Time-lapse imaging reveals LTF is associated with presynaptic activation of silent synapses and growth of new functional synapses

In most model learning systems, the functional contribution of the structural changes that accompany long-lasting forms of synaptic plasticity remains largely unknown. In particular one would like to know if changes in the number or structure of synaptic connections induced by learning are functionally effective and capable of contributing to the storage of long-term memory. Both technical and experimental limitations prevented the earlier studies in *Aplysia* discussed above from examining whether the increase in synaptic strength during long-term sensitization resulted from the conversion of pre-existing but nonfunctional (silent) synapses to active synapses, or from the addition of newly formed functional synapses, or perhaps both. To address these issues directly, more recent in vitro studies of the sensory-to-motor neuron synapse in *Aplysia* culture have monitored both functional and structural changes simultaneously so as to follow remodeling and growth at the same specific synaptic varicosities continuously over time and to examine the functional contribution of these presynaptic structural changes to the different time-dependent phases of LTF.

Toward that end, Kim et al. (2003) combined time-lapse confocal imaging of individual presynaptic varicosities of sensory neurons labeled with

three different fluorescent markers: the whole cell marker *Alexa-594*, and two presynaptic marker proteins: *synaptophysin-eGFP* which monitors changes in the distribution of synaptic vesicles within individual varicosities and *synapto-PHluorin* (synPH), a monitor of active transmitter release sites (Miesenbock et al., 1998). They found that repeated pulses of 5-HT induce two temporally, morphologically and molecularly distinct classes of presynaptic changes: (1) the rapid activation of silent presynaptic terminals through the filling of pre-existing empty varicosities with synaptic vesicles, which requires translation but not transcription and (2) the generation of new synaptic varicosities which occurs more slowly and requires both transcription and translation. The enrichment of pre-existing but empty varicosities with synaptophysin is completed within 3–6 h, parallels intermediate-term facilitation and accounts for approximately 32% of the newly activated synapses evident at 24 h. By contrast, the new sensory neuron varicosities, which account for 68% of the newly activated synapses at 24 h, do not form until 12–18 h after exposure to 5 pulses of 5-HT. The rapid activation of silent presynaptic terminals suggests that in addition to its role in LTF, this modification of pre-existing synapses may also contribute to the intermediate phase of synaptic plasticity and memory storage (Ghirardi et al., 1995; Mauelshagen et al., 1996; Sutton et al., 2001) (Fig. 2).

This temporal analysis of the synaptic remodeling and growth that underlie the development of LTF served to bring the structural changes into register with the physiological correlates of the different phases of long-term synaptic plasticity. For example, the different time windows for the two classes of presynaptic structural changes — a rapid enrichment of pre-existing empty varicosities with synaptic vesicle proteins and a slower generation of new varicosities — appear to be consistent with the onset and duration of intermediate-term and LTF, respectively. Since intermediate-term facilitation lasts for about 3–6 h whereas LTF first appears at 10–15 h and lasts for days (Ghirardi et al., 1995; Mauelshagen et al., 1996), these two classes of presynaptic changes may represent distinct structural correlates of the two phases of memory storage (Sutton et al., 2001). This idea is further supported by the finding that the 5-HT-induced clustering of synaptic vesicles into pre-existing empty varicosities is blocked by the inhibition of de novo protein synthesis, which has previously been shown to attenuate both intermediate-term facilitation and the corresponding intermediate phase of memory (Ghirardi et al., 1995; Sutton et al., 2001).

To test this hypothesis, Kim et al. (2003) developed a reduced 5-HT protocol to induce selectively facilitation in the intermediate-term time domain without inducing LTF (see also Ghirardi et al., 1995). They found that the isolated intermediate-term facilitation is also accompanied by the redistribution and clustering of synaptophysin-eGFP into empty varicosities at 0.5 and 3 h similar to what occurs when intermediate- and LTF are recruited together. However, the presynaptic structural changes induced by the reduced 5-HT protocol differed from those induced by the long-term protocol in at least two ways. First, there was no growth of new sensory neuron varicosities in the isolated intermediate phase. Second, unlike the filling of pre-existing empty varicosities during the intermediate-term phase induced by the long-term protocol, the newly filled varicosities did not persist for 24 h and were unaffected by inhibitors of protein synthesis suggesting that the structural remodeling activated by the reduced 5-HT protocol involved only a simple rearrangement of pre-existing synaptic components. This may reflect a fundamental difference in the molecular mechanisms that underlie the two 5-HT protocols. Although both protocols induce intermediate-term facilitation, the long-term protocol may recruit additional molecular events (including the machinery for translational activation) required to set up the long-term phase, perhaps by stabilizing the intermediate phase.

To quantitatively assess the contribution of these two distinct classes of presynaptic structural changes to LTF, Kim et al. (2003) monitored the functional state of individual sensory neuron varicosities in living cells before and 24 h after 5 pulses of 5-HT using the activity-sensitive fusion protein synPH. With synPH, release of labeled synaptic vesicles yields an increase in fluorescence

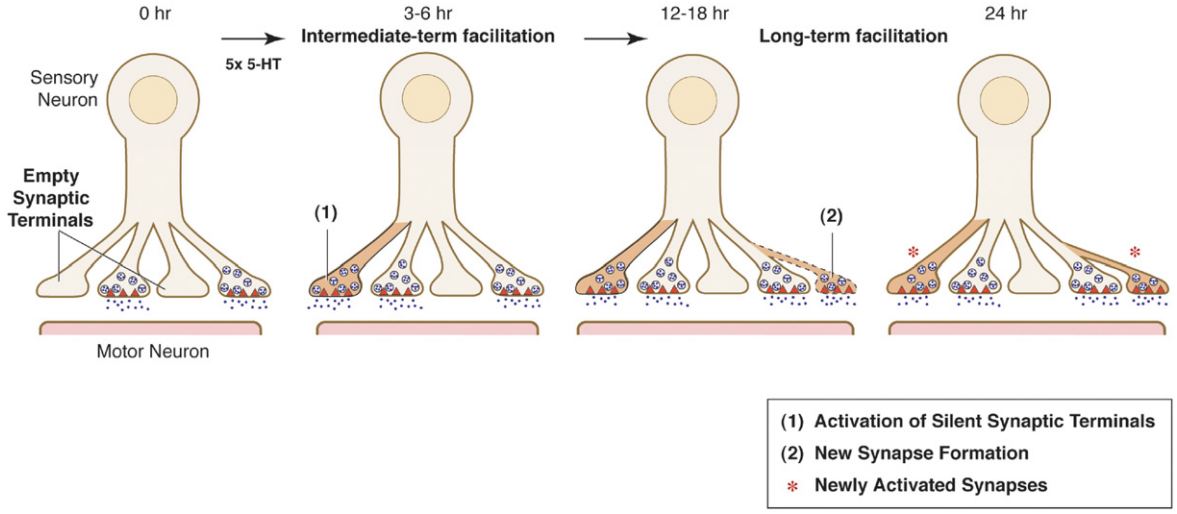

Fig. 2. Time course and functional contribution of two distinct presynaptic structural changes associated with intermediate- and long-term facilitation in *Aplysia*. Repeated pulses of 5-HT in sensory to motor neuron co-cultures trigger two distinct classes of presynaptic structural changes: (1) the rapid clustering of synaptic vesicles to pre-existing silent sensory neuron varicosities (3–6 h) and (2) the slower generation of new sensory neuron synaptic varicosities (12–18 h). The resultant newly filled and newly formed varicosities are functionally competent (capable of evoked transmitter release) and contribute to the synaptic enhancement that underlies LTF. The rapid filling and activation of silent presynaptic terminals at 3 h suggests that, in addition to its role in LTF, this modification of pre-existing varicosities may also contribute to the intermediate phase of synaptic plasticity. Red triangles represent transmitter release sites (active zones). (Modified from Kim et al., 2003.) (See Color Plate 10.2 in color plate section.)

due to the externalization of pHluorin to a more basic exterior medium, which returns to basal levels by the re-acidification of synaptic vesicles following endocytosis in a Ca^{2+}-dependent fashion (Sankaranarayanan and Ryan, 2001). When expressed in *Aplysia* sensory neurons, depolarization by bath application of KCl leads to the evoked exocytotic release of synaptic vesicles within individual varicosities as indicated by an increase in the fluorescence signal of synPH as has been previously reported in cultured hippocampal neurons (Miesenbock et al., 1998).

In this fashion, Kim et al. (2003) were able to monitor continuously over time individual 5-HT-induced structural changes at the same sensory neuron varicosities and to examine directly the functional contribution of two distinct classes of presynaptic structural changes to the different temporal phases of LTF. They found that 24 h after repeated pulses of 5-HT there was a 59% increase in the total number of synPH-active sensory neuron varicosities. These real time experiments on living synapses in culture were remarkably consistent with the earlier electron microscopic studies of Bailey and Chen (1983) which had demonstrated that long-term sensitization in the intact animal is accompanied by an increase of 65% in the number of sensory neuron varicosities that contained fully differentiated presynaptic active zones (transmitter release sites) when compared to varicosities from untrained (naïve) animals. The enrichment of pre-existing but empty varicosities accounted for approximately 32% of these newly activated synapses at 24 h, whereas newly formed varicosities accounted for approximately 68%. These results suggested that both classes of structural changes — the presynaptic activation of pre-existing silent synapses and the growth of new functional synapses — appear to contribute to the synaptic enhancement that characterizes LTF at 24 h.

Previous studies have shown that specific 5-HT protocols (Sun and Schacher, 1998; Casadio et al., 1999) or experimental manipulation in *Aplysia*

(Hatada et al., 2000; Udo et al., 2005) can induce LTF at 24 h without the formation of new varicosities. How might such an increase in synaptic strength persist for 24 h in the absence of synaptic growth? The results of Kim and associates suggest that additional modifications of pre-existing connections including the activation of previously silent synapses, may play a role in the initial phases of synaptic maintenance and highlight the fact that there are likely to be multiple types of structural mechanisms that can contribute to LTF at 24 h (Schacher et al., 1997; Bailey et al., 2000; Sutton and Carew, 2000).

Of the two classes of presynaptic structural plasticity induced by 5-HT in culture, synaptic growth appears to contribute more to the synaptic enhancement present at 24 h than does the activation of pre-existing silent synapses. It will be of interest to see if the functional contribution by newly formed synapses increases with time when the growth process is more fully developed and memory storage is likely to be more stable. This would be consistent with the earlier studies in the intact animal outlined above, which have shown that only the increases in the number of sensory neuron varicosities and active zones persist for several weeks in parallel with the behavioral duration of the memory, as well as more recent work in culture which has demonstrated that synaptic growth plays a more prominent role in the expression of the later phases of LTF (Martin et al., 1997b; Casadio et al., 1999).

The activation of silent synapses also seems to play a major role in LTP in the hippocampus. Although in mammals the term refers to a very specific molecular configuration found in synapses in different regions of the CNS of vertebrates (Malinow et al. 2000; Malinow and Malenka, 2002). In this case, the term *silent synapse* refers to excitatory glutamatergic synapses whose postsynaptic membrane contains NMDARs but no AMPARs. The lack of AMPAR-mediated signaling renders these synapses inactive, or "silent", under normal conditions. Synaptic stimulation activates these silent synapses through the insertion of AMPARs into the postsynaptic membrane, a phenomenon some times referred to as *AMPA-fication*. Calcium/calmodulin-dependent protein kinase II (CaMKII) plays a critical role in this process. Once this kinase is activated by high frequency stimulation, it phosphorylates AMPARs or associated proteins, triggering their insertion into the postsynaptic membrane. The synapse is then no longer silent and postsynaptic responses are, by consequence, enhanced.

Remodeling of the presynaptic actin network for learning-related synaptic growth requires activation of Cdc42-mediated signaling pathways

Actin is enriched in both the pre- and postsynaptic compartment of neurons (Matus, 2000). Although the activity-dependent modulation of actin dynamics at postsynaptic spines has been well documented, the extent and role of actin regulation in presynaptic terminals is less clear (Colicos et al., 2001; Antonova et al., 2001). During development, reorganization of actin in growth cones has been shown to play an important role in axonal path finding (Yuan et al., 2003). However, in mature neurons, it has been suggested that the presynaptic actin network may function more as an intracellular scaffold rather than providing a propulsive force that could contribute directly to the type of rapid structural remodeling reported for postsynaptic dendritic spines (Sankaranarayanan et al., 2003).

In *Aplysia*, repeated applications of 5-HT that lead to LTF induce a slower and more persistent alteration in the dynamics of the presynaptic actin network leading to the remodeling and growth of sensory neuron synapses. Both the 5-HT-induced enrichment of synaptic proteins in pre-existing varicosities and the formation of new and functionally competent sensory neuron varicosities during LTF involve an activity-dependent rearrangement of the presynaptic actin cytoskeleton (Udo et al., 2005). These findings in *Aplysia* are consistent with previous reports that structural remodeling of synapses in response to physiological activity requires the reorganization of actin (Colicos et al., 2001; Huntley et al., 2002) and that the inhibition of actin function blocks synapse formation and interferes with long-term synaptic

plasticity (Hatada et al., 2000; Krucker et al., 2000; Zhang and Benson, 2001). Furthermore, several synaptic proteins such as synapsin can bind to the presynaptic actin cytoskeleton and participate in synaptic vesicle trafficking (Humeau et al., 2001) that may contribute to the 5-HT-induced enrichment of pre-existing varicosities observed during LTF.

One attractive molecular candidate for the 5-HT-induced reorganization of the cytoskeleton at sensory neuron presynaptic varicosities is the Rho-family of small GTPases, which can modulate actin polymerization in response to extracellular signals and can be regulated by neuronal activity in vivo (Luo et al., 1996; Hall, 1998). In *Aplysia*, Udo et al. (2005) found that the application of toxin B, a general inhibitor of the Rho family, blocks 5-HT-induced LTF, as well as growth of new synapses in sensory–motor neuron co-cultures. Moreover, repeated pulses of 5-HT selectively induce the spatial and temporal regulation of the activity of only one of the small GTPases-Cdc42-at a subset of sensory neuron presynaptic varicosities. The activation of ApCdc42 induced by 5-HT is dependent on both the P13K and PLC pathways and, in turn, recruits the downstream effectors PAK (p21-Cdc42/Rac-activated kinase) and N-WASP (neuronal Wiskott–Aldrich syndrome protein) to regulate the presynaptic actin network. This initial molecular cascade leads to the outgrowth of filopodia, some of which represent morphological precursors for the growth of new sensory neuron varicosities associated with the storage of LTF.

5-HT-induced internalization of apCAM: a preliminary and permissive step for initiation of learning-related synaptic growth

Cell adhesion molecules (CAMs) are cell surface glycoproteins that mediate cell-to-cell and cell-to-extracellular matrix adhesions. In the CNS, CAMs are involved in cell migration, neurite outgrowth and more recently have been shown to participate in synapse formation during development (Scheiffele, 2003; Washbourne et al., 2004) as well as various forms of learning-related synaptic plasticity in the adult brain (Martin and Kandel, 1996; Fields and Itoh, 1996; Benson et al., 2000). Some of the first evidence for a role of CAMs during learning and memory came from studies of an immunoglobulin-related CAM in *Aplysia*, designated apCAM, which is homologous to NCAM in vertebrates and Fasciclin II in *Drosophila* (Mayford et al., 1992). Following the application of 5-HT or cAMP, there is a decrease in the expression of apCAM and this occurs in a transcriptionally dependent manner. Furthermore, imaging of fluorescently labeled MAbs to apCAM indicates that not only is there a decrease in the level of expression but that even pre-existing protein is lost from the surface membrane of the sensory neurons within 1 h after the addition of 5-HT. This transient modulation by 5-HT of CAMs, therefore, may represent one of the early molecular steps required for initiating learning-related growth of synaptic connections. Indeed, blocking the expression of the antigen by MAb causes defasciculation, a step that appears to precede synapse formation during development in *Aplysia* (Keller and Schacher, 1990).

To examine the mechanisms that underlie the 5-HT-induced down-regulation of apCAM and, in particular, how these relate to the initiation of synaptic growth, Bailey et al. (1992a) combined thin-section electron microscopy with immunolabeling using a gold-conjugated MAb specific to apCAM. They found that a 1 h application of 5-HT led to a 50% decrease in the density of gold-labeled apCAM complexes at the surface membrane of the sensory neuron. This down-regulation was particularly prominent at adherent processes of the sensory neurons and was achieved by a heterologous, protein synthesis-dependent activation of the endosomal pathway, leading to internalization and apparent degradation of apCAM. As is the case for the down-regulation at the level of expression, the 5-HT-induced internalization of apCAM can be simulated by cAMP. Concomitant with the down-regulation of apCAM, Hu et al. (1993) further demonstrated

that, as part of this coordinated program for endocytosis, 5-HT and cAMP also induce an increase in the number of coated pits and coated vesicles in the sensory neurons and an increase in the expression of the light chain of clathrin (apClathrin). Since the apClathrin light chain contains the important functional domains of both LCa and LCb of mammalian clathrin thought to be essential for the coated pit assembly and disassembly, the increase in clathrin may be an important component in the activation of the endocytic cycle required for the internalization of apCAM.

The learning-induced internalization of apCAM is thought to have at least two major structural consequences: (1) disassembly of homophilically associated fascicles of the sensory neurons (defasciculation), a process that may destabilize adhesive contacts normally inhibiting growth, and (2) endocytic activation that may lead to a redistribution of membrane components to sites where new synapses form. Thus, aspects of the initial steps in the learning-related growth of synaptic connections that is a hallmark of the long-term process may eventually be understood in the context of a novel and targeted form of receptor-mediated endocytosis.

These initial effects of 5-HT on the remodeling of the surface and internal membrane systems of sensory neurons in *Aplysia* bear a striking similarity to the morphological changes induced in non-neuronal systems by growth factors, which suggests that modulatory transmitters important for learning, such as 5-HT, may serve a double function. In addition to producing a transient regulation of the excitability of neurons, with repeated or prolonged exposure they may also produce an action comparable to that of a growth factor, which results in a more persistent regulation of the architecture of the neuron.

To further define the mechanisms whereby 5-HT leads to apCAM down-regulation, Bailey et al. (1997) used epitope tags to examine the fate of the two apCAM isoforms (transmembrane and GPI-linked) and found that only the transmembrane form (TM-apCAM) is internalized (Fig. 3). This internalization was blocked by overexpression of TM-apCAM with a point mutation in the two MAPK phosphorylation consensus sites, as well as by injection of a specific MAPK antagonist into sensory neurons. These data suggest that activation of the MAPK pathway is important for the internalization of TM-apCAM and may represent one of the initial and perhaps permissive stages of learning-related synaptic growth in *Aplysia*. In addition, the differential down-regulation of the GPI-linked and transmembrane forms of apCAM raised the interesting possibility that learning-related synaptic growth in the adult is initiated by an activity-dependent recruitment of specific isoforms of CAMs, similar to the modulation of cell-surface receptors during the fine-tuning of synaptic connections in the developing nervous system. One consequence of isoform recruitment is that it would allow neuronal activity to regulate the surface expression of each isoform, a process that might take on additional functional significance if these surface molecules were distributed differentially along the three-dimensional extent of the neuron and, thus may provide a regulatory unit capable of acting sequentially at multiple cytoplasmic and plasma membrane sites during the early inductive phases of the long-term process.

Han et al. (2004) have examined more closely the relationship between the 5-HT-induced down-regulation of TM-apCAM and synaptic growth by overexpressing various HA-epitope tagged recombinant apCAMs in *Aplysia* sensory neurons. They found that overexpression of TM-apCAM, but not the GPI-linked isoform of apCAM, blocked both LTF as well as the associated increase in the number of sensory neuron varicosities. By interrupting the adhesive function of apCAM with an anti-HA antibody, this inhibition of LTF induced by the overexpression of TM-apCAM was restored. Moreover, LTF could be completely blocked by overexpression of the cytoplasmic tail portion of apCAM alone. These studies indicated that the extracellular domain of TM-apCAM has an inhibitory function that is neutralized by internalization to induce LTF and suggested that the cytoplasmic domain provides an interactive platform for both signal transduction and the internalization machinery.

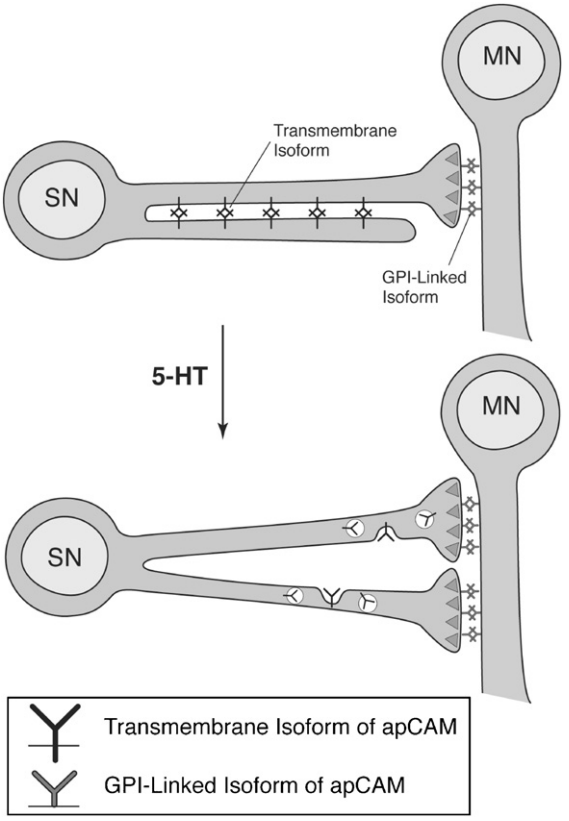

Fig. 3. Regional specific down-regulation of the transmembrane isoform of apCAM. This model is based on the assumption that the relative concentration of the GPI-linked versus transmembrane isoforms of apCAM is highest at points of synaptic contact between the sensory neuron and motor neuron and reflects the results of studies done in dissociated cell culture. Thus, previously established connections might remain intact following exposure to 5-HT since they would be held in place by the adhesive, homophilic interactions of the GPI-linked isoforms and the process of outgrowth from sensory neuron axons would be initiated by down-regulation of the transmembrane form at extrasynaptic sites of membrane apposition. In the intact ganglion, the axons of sensory neurons are likely to fasciculate not only with other sensory neurons but also with the processes of other neurons and perhaps even glia. One of the attractive features of this model is that the mechanism for down-regulation is intrinsic to the sensory neurons. Thus, even if some of the sensory neuron axonal contacts in the intact ganglion were heterophilic in nature, i.e., with other neurons or glia, we would still expect the selective internalization of apCAM at the sensory neuron surface membrane at these sites of heterophilic apposition to destabilize adhesive contacts and to facilitate disassembly. (From Bailey et al., 1997.) (See Color Plate 10.3 in color plate section.)

Nuclear translocation of apCAM-associated protein (CAMAP) activates presynaptic gene transcription for induction of LTF

Lee et al. (2007) have examined the 5-HT-induced signaling interactions mediated by the cytoplasmic domain of TM-apCAM and found an additional, and novel role for this CAM in synapse-specific forms of long-lasting plasticity. LTF at the sensory to motor neuron synapse requires the activation of CREB1 in the nucleus of the sensory neuron (Bartsch et al., 1998). Activated CREB1 induces the transcription factor ApC/EBP that in turn acts on downstream genes encoding proteins important for synaptic growth and the stable maintenance of LTF (Alberini et al., 1994). As discussed above, an initial step, thought to be permissive, for the initiation of learning-related growth is the clathrin-mediated internalization and consequent down-regulation of TM-apCAM.

To examine directly how the internalization of TM-apCAM is related to the initiation of transcription, Lee et al. (2007) first looked for molecules that could bind to the cytoplasmic tail of TM-apCAM and cloned an CAMAP by yeast two-hybrid screening. They found that 5-HT signaling at the synapse activates PKA which in turn phosphorylates CAMAP to induce the dissociation of CAMAP from apCAM and that this dissociation is a prerequisite for the internalization of apCAM. The 5-HT-induced dissociated CAMAP is subsequently translocated to the nucleus of the sensory neurons. In the nucleus, CAMAP acts as a transcriptional co-activator for CREB1 that is essential for the activation of ApC/EBP required for the initiation of LTF. Combined, these data suggest that CAMAP is one of the retrograde signals from the synapse to the nucleus where it acts as a co-regulator of the presynaptic gene expression associated with the induction of LTF in *Aplysia*. In addition, these findings demonstrate the importance, for learning-related synaptic plasticity, of signal propagation into the nucleus from the surface membrane of activated synaptic sites mediated by a molecule directly interacting with a cell surface adhesion molecule and suggest a novel presynaptic molecular mechanism to turn

on the gene transcription required for long-term memory.

An overall view

Perhaps the most striking finding in the biology of memory storage is that long-term memory involves transcription in the nucleus and structural changes at the synapse. The structural changes associated with the storage of long-term memory can be grouped into two general categories: remodeling of pre-existing synapses and growth of new synapses (Greenough and Bailey, 1988; Bailey and Kandel, 1993; Yuste and Bonhoeffer, 2001; Bailey et al., 2004; Lamprecht and LeDoux, 2004). Despite an increasing body of evidence for changes in the number or structure of synaptic connections and long-term memory, it has so far proven difficult to follow individual structural changes at the same synapse over time and to relate directly this remodeling to physiological function and memory storage.

Recent studies have found that activity-dependent remodeling of pre-existing synapses and the growth of new synaptic connections occurs in the mammalian CNS (Buchs and Muller, 1996; Engert and Bonhoeffer, 1999; Maletic-Savatic et al., 1999; Toni et al., 1999; Colicos et al., 2001; De Paola et al., 2003). However, in the mammalian brain these structural changes are difficult to study because the effects are often modest. Moreover, the specific role of this structural plasticity remains unclear because the functional contribution of individual synapses to memory processes in these more complex neuronal networks is not yet well defined (Lamprecht and LeDoux, 2004; Hayashi and Majewska, 2005; Segal, 2005). For example, although the generation and enlargement of dendritic spines has been associated with the production of LTP and synaptic activity in organotypic hippocampal slices (Matsuzaki et al., 2004; Nagerl et al., 2004) and acute slices of neonatal animals (Zhou et al., 2004), these structural changes are much more subtle in the adult brain (Lang et al., 2004). In adults there is only a modest production of new spines (Zuo et al., 2005), and learning-related plasticity seems to rely more on subcellular changes than on anatomical changes. Thus, neuronal activity regulates the transport of polysomes from dendritic shafts to active spines (Ostroff et al., 2002), as well as the trafficking of neurotransmitter receptors (Malinow and Malenka, 2002).

By contrast, in *Aplysia* the learning-induced structural changes that accompany long-term sensitization in vivo and LTF in vitro are robust, highly reproducible and easy to study and can be shown to be both functionally effective and capable of contributing to memory storage. Time-lapse imaging studies of the sensory to motor neuron synapse in culture have revealed that LTF is accompanied by two temporally and morphologically distinct classes of presynaptic structural change: the rapid activation of silent pre-existing varicosities by filling with synaptic vesicles and the slower growth of new functional varicosities. These findings, the first to be made on individually identified presynaptic varicosities, suggest that the duration of the changes in synaptic effectiveness that accompany memory storage may be reflected by the differential regulation of two fundamentally disparate forms of presynaptic compartment: (1) nascent (silent) varicosities that can be rapidly and reversibly remodeled into active transmitter release sites and (2) mature, more stable and functionally competent varicosities that following long-term training may undergo a process of fission to form new stable synaptic contacts.

Recent live imaging studies in the mammalian CNS have reported a comparable remodeling and differentiation of the presynaptic compartment in developing synapses (for review, see Ziv and Garner, 2004). For example, the establishment of functional transmitter release sites in cultured hippocampal neurons can occur in less than 1 h (Ahmari et al., 2000; Friedman et al., 2000). This short delay is similar to the 5-HT-induced enrichment and subsequent activation of pre-existing silent varicosities in *Aplysia*, which also occurs very rapidly — initial changes in the recruitment of synaptic vesicle proteins to empty presynaptic varicosities can be detected as early as 30 min after the completion of 5-HT training.

How is this rapid differentiation of nascent presynaptic compartments achieved? One model of active zone assembly suggests proteins that comprise the cytoskeletal matrix of the active zone (CAZ) are packaged into transport vesicles for delivery and fusion with the plasma membrane at nascent synaptic contacts (Zhai et al., 2001; Shapira et al., 2003). These transport vesicles contain multiple molecular components including Piccolo and Bassoon — important for assembly and structural maintenance of the active zone — as well as other CAZ scaffolding molecules implicated in synaptic vesicle exocytosis but typically not synaptic vesicle proteins. The rapid remodeling and presynaptic activation of nascent sensory neuron varicosities induced by 5-HT in *Aplysia* culture is consistent with this molecular model for active zone assembly in developing synapses of the mammalian CNS. Moreover, the apparent heterogeneity in the content of these mobile preassembled packets — some of which contain synaptic vesicle proteins and the others which contain components required for assembly of the active zone (Friedman et al., 2000; Zhai et al., 2001) could explain why more than half of the sensory neuron varicosities enriched in only synaptophysin following 5-HT-induced LTF were not functional.

The second general class of learning-related presynaptic structural change associated with LTF in *Aplysia* involves a slower generation of new and functionally effective sensory neuron varicosities. How are these new varicosities formed? Time-lapse imaging of individual sensory neuron varicosities indicates that the 5-HT-induced recruitment of synaptic proteins to a pre-existing varicosity leads directly to both an enrichment of these presynaptic constituents as well as to an overall increase in the size of the varicosity. The presynaptic remodeling and growth is followed by the apparent division or splitting of a subset of these pre-existing varicosities (Hatada et al., 2000; Kim et al., 2003; Udo et al., 2005). This physical process may lead to the budding off of components of the active zone and associated synaptic vesicle cluster from each pre-existing presynaptic compartment that could then serve as a nucleation site to seed the formation of a new varicosity. Aspects of these structural transformations of the presynaptic compartment that precede learning-related synaptic growth in *Aplysia* have also been reported at developing synapses in mammals. For example, imaging studies of the early stages of synapse formation have shown that presynaptic sites formed immediately after initial contact of axonal and dendritic processes are highly unstable. Moreover, even apparent mature presynaptic sites are relatively unstable as occasionally ''orphan release sites'' break off from fully formed boutons and either migrate to adjacent presynaptic sites or participate in the formation of completely new ones (Friedman et al.; 2000; Hopf et al., 2002; Krueger et al., 2003).

Taken together, these time-lapse observations of living synapses indicate that differentiation and growth of the presynaptic compartment, either induced by learning in the mature nervous system or as mechanistic steps during development, are highly dynamic and rapid processes that can recruit both pre-existing proteins as well as preassembled synaptic components. The instability of nascent presynaptic compartments during the early stages of development is characterized by the dispersion of mobile packets of synaptic components and a renewal of their migration once the transient pre- and postsynaptic contacts breakup. As the neurons mature, an increasing proportion of these initial contacts develop into more stable and fully functional presynaptic terminals (Ziv and Garner, 2004). Results in the *Aplysia* sensory to motor neuron culture preparation further indicate that even some of these mature presynaptic contacts can be selectively destabilized during learning and memory leading to the generation of new and functionally competent synaptic varicosities by processes that appear similar to those that govern developmental synaptogenesis.

Conclusions

Over the past two decades, it has become clear that synaptic growth and the formation of new synapses are hallmarks of long-term, learning-related synaptic plasticity. The morphological corres-

pondence between the studies of long-term sensitization in *Aplysia* and LTP in the mammalian hippocampus indicates that learning resembles a process of growth and neuronal differentiation across a broad segment of the animal kingdom and suggests that learning-related synapse formation may be a highly conserved feature for the storage of both implicit and explicit forms of long-term memory. Such changes are likely to reflect the recruitment by environmental stimuli of developmental processes that are latent or inhibited in the fully differentiated neuron. Recent studies of the sensory to motor neuron connection in *Aplysia* have begun to characterize the cellular and molecular events that underlie the structural changes induced by learning at the level of individual identified synapses. This in turn has suggested the synaptic remodeling and synaptic growth that accompany learning and memory storage in the adult brain may reutilize mechanisms important for de novo synapse formation and the fine-tuning of synaptic connections during development of the nervous system.

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