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The Persistence of Long-Term Memory: A Molecular Approach to Self-Sustaining Changes in Learning-Induced Synaptic Growth

Review

Craig H. Bailey,[2,3,4] Eric R. Kandel,[1,2,3,4,*] and Kausik Si[2,3]
[1]Howard Hughes Medical Institute
[2]Center for Neurobiology and Behavior
[3]Kavli Institute for Brain Sciences
College of Physicians and Surgeons
Columbia University
[4]New York State Psychiatric Institute
1051 Riverside Drive
New York, NY 10032

Recent cellular and molecular studies of both implicit and explicit memory storage suggest that experience-dependent modulation of synaptic strength and structure is a fundamental mechanism by which these diverse forms of memory are encoded and stored. For both forms of memory storage, some type of synaptic growth is thought to represent the stable cellular change that maintains the long-term process. In this review, we discuss recent findings on the molecular events that underlie learning-related synaptic growth in *Aplysia* and discuss the possibility that an active, prion-based mechanism is important for the maintenance of the structural change and for the persistence of long-term memory.

Although altered gene expression, the synthesis of new proteins, and synaptic growth have been found to accompany various forms of long-term memory, surprisingly little is known about the cell biological and molecular mechanisms that initiate and maintain the structural changes (Bailey and Kandel, 1993; Bliss et al., 2003). This in turn raises two questions central to an understanding of the molecular biology of memory storage: (1) Do the alterations in synaptic strength that underlie long-term memory storage result from a structural change in preexisting connections—for example, by the conversion of nonfunctional (silent) synapses to functional synapses—or from the addition of newly formed synapses or both? (2) Is the maintenance of long-term memory achieved, at least in part, because of the relative stability of synaptic structure? If so, the stability of the synapse would seem to require some mechanisms that can survive molecular turnover and thereby serve to stabilize learning-induced changes in synapse number and structure.

In this review, we address these questions by focusing on recent molecular studies of long-term structural changes in *Aplysia*. We begin by examining the structural 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 that have provided some initial molecular insights into the signaling pathways and mechanisms that underlie the initiation of these learning-related structural changes and their functional contribution to the different temporal phases of long-term facilitation. Finally, we consider the role of local protein synthesis and specifically a novel molecular mechanism for the self-perpetuating activation of a translational regulator in the stabilization of learning-related synaptic growth and the maintenance of long-term memory.

*Correspondence: erk5@columbia.edu

Growth of New Sensory Neuron Synapses and the Persistence of Long-Term Sensitization

The gill-withdrawal reflex of the marine mollusk *Aplysia californica* undergoes several forms of both nonassociative and associative behavioral modification (Kandel, 2001). The molecular mechanisms contributing to implicit memory storage have been most extensively studied for sensitization—an elementary type of nonassociative learning exhibited by this reflex. Sensitization is a form of learned fear in which the animal learns about the properties of an aversive stimulus applied to another site, such as the neck or tail. As with defensive behaviors in other species, the memory for sensitization of the gill-withdrawal reflex is graded, and retention is proportional to the number of training trials. A single tail shock produces short-term sensitization that lasts for minutes. Repeated tail shocks given at spaced intervals produce long-term sensitization that lasts for days or even weeks (Castellucci et al., 1986).

An important component of the memory for both the short- and long-term forms of sensitization is represented on an elementary level by the monosynaptic connections between identified mechanoreceptor sensory neurons and their follower cells (Castellucci et al., 1970). The simplicity of this synaptic component of the behavioral modification has allowed the reduction of the analysis of the short- and long-term memory of sensitization to the cellular and molecular level. For example, this monosynaptic pathway can be reconstituted in dissociated cell culture (Montarolo et al., 1986), where serotonin (5-HT), a modulatory neurotransmitter normally released by sensitizing stimuli, can substitute for the shock to the neck or tail used during behavioral training in the intact animal (Glanzman et al., 1989). A single application of 5-HT produces short-term changes in synaptic effectiveness, whereas five spaced applications given over a period of 1.5 hr produce long-term changes lasting several days.

Studies of this monosynaptic connection between sensory and motor neurons both in the intact animal and in culture indicate that, phenotypically, the long-term changes are surprisingly similar to the short-term changes, consistent with the idea that long-term memory is a direct extension of short-term memory. 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, accompanied by a broadening of the action potential and an increase in excitability attributable to the depression of specific sets of potassium channels

(Byrne and Kandel, 1996; Castellucci et al., 1986; Dale et al., 1987; Hochner et al., 1986; Klein and Kandel, 1980; Montarolo et al., 1986; Scholz and Byrne, 1987).

Despite this phenotypic similarity, the short-term cellular changes for sensitization differ fundamentally from the long-term changes in at least two important ways. First, the long-term change requires new protein synthesis (Castellucci et al., 1989; Montarolo et al., 1986; Schwartz et al., 1971). Second, the long-term process involves a structural change (Bailey and Chen, 1983, 1988a, 1988b). Long-term sensitization is associated with the growth of new synaptic connections by the sensory neurons onto their follower cells. The persistence of this structural change parallels the behavioral duration of the memory (Bailey and Chen, 1989). This synaptic growth also can be reconstituted in sensory-motor neuron cocultures by repeated presentations of 5-HT (Bailey et al., 1992a; Glanzman et al., 1990). Whereas these learning-related anatomical changes are considerably regulated and involve both pre- and postsynaptic changes, we will limit ourselves in this review to the presynaptic changes.

Functional Contribution of Two Distinct Presynaptic Structural Changes to Long-Term Facilitation: Rapid Activation of Silent Synapses and Slower Generation of Newly Formed Synapses

These earlier studies did not examine the functional contribution of presynaptic structural changes to the different time-dependent phases of long-term facilitation and, thus, could not determine to what degree the increase in synaptic strength resulted from the conversion of preexisting but nonfunctional (silent) synapses to active synapses or from the addition of newly formed functional synapses or both. Recently, Kim et al. (2003) have addressed these issues by monitoring functional and presynaptic structural changes continuously during long-term facilitation using time-lapse confocal imaging of sensory neurons labeled with the red fluorescent dye Alexa Fluor-594 hydrazide (Alexa-594). To determine the functional contribution of the structural changes in any given varicosity, they used, in addition, two other fluorescent protein markers specific to different presynaptic functions: synaptophysin tagged with the enhanced form of the green fluorescent protein (synaptophysin-eGFP) to monitor changes in the distribution of synaptic vesicles within each sensory neuron varicosity and an activity-sensitive fluorescent protein, ecliptic synapto-PHluorin (synPH), to monitor active transmitter release sites (Miesenbock et al., 1998).

With these markers, Kim et al. (2003) found that in a typical sensory neuron cocultured with the postsynaptic motor neuron L7, approximately 12% of the total presynaptic varicosities lack markers for synaptic vesicles (synaptophysin-eGFP) and for transmitter release (synPH). These varicosities therefore are not competent to release transmitter. Within 3–6 hr following five pulses of 5-HT, synaptic vesicle proteins accumulate in approximately 60% of these preexisting "empty" varicosities. These varicosities now contain functional release sites, suggesting that the clustering of synaptic vesicle proteins may represent a critical step leading to the activation of presynaptic silent synapses. The newly filled varicosities persist and account for one-third of the 5-HT-induced newly activated synapses (capable of evoked transmitter release) at 24 hr. In addition, 12–18 hr after five pulses of 5-HT, completely new sensory neuron synapses form. These account for about two-thirds of the newly activated synapses at 24 hr.

Thus, 5-HT induces two temporally and morphogically distinct presynaptic structural changes: (1) a rapid activation of preexisting silent synapses and (2) a slower growth of new functional synapses (Figure 1). The rapid activation of silent presynaptic terminals suggests that, in addition to its role in long-term facilitation, this modification of preexisting 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).

One clue to the underlying molecular mechanisms responsible for these two discrete learning-related structural changes comes from a study by Ahmari et al. (2000), who demonstrated that in hippocampal neurons puncta labeled by VAMP-GFP are transported only at those synapses defined by FM4-64. Moreover, these puncta contained not only synaptic vesicles but also other molecular components of the presynaptic active zone. Thus, the 5-HT-induced clustering of synaptic vesicle proteins to sensory neuron varicosities might represent a recruitment of not only synaptic vesicles but also the molecular precursors for active zone assembly. This redistribution of synaptic vesicle proteins and active zone components in both preexisting and newly formed sensory neuron synapses is also likely to involve cytoskeleton rearrangement (Benfenati et al., 1999). For example, structural remodeling of synapses in response to physiological activity requires reorganization of the actin network (Colicos et al., 2001; Huntley et al., 2002), and the inhibition of actin function blocks synapse formation and interferes with long-term synaptic plasticity (Hatada et al., 1999; Krucker et al., 2000; Zhang and Benson, 2001). Furthermore, several synaptic proteins, such as synapsin, can bind to the actin cytoskeleton and participate in synaptic vesicle trafficking (Humeau et al., 2001).

How does an extracellular signal such as 5-HT lead to a reorganization of the actin cytoskeleton? The balance between actin polymerization and depolymerization is tightly regulated by extracellular signaling molecules, many of which act through the Rho family of GTPases. These small GTPases are thought to participate at different stages during the development of the central nervous system, for example, in the establishment of polarity, axon guidance, dendritic growth, and maintenance of dendritic spines (Bradke and Dotti, 1999; Nakayama et al., 2000; Sin et al., 2002; Threadgill et al., 1997; Yuan et al., 2003). Their participation, in turn, can be regulated by neuronal activity in vivo (Li et al., 2002). In *Aplysia*, the application of toxin B, a general inhibitor of the Rho family, blocks 5-HT-induced long-term facilitation, as well as growth of new synapses in sensory-motor neuron cocultures (Udo et al., submitted). Udo et al. (submitted) have recently found that, in *Aplysia*, repeated pulses of 5-HT selectively activate the small GTPase Cdc42, leading to rearrangement of the presynaptic actin network and the assembly, insertion, and functional matu-

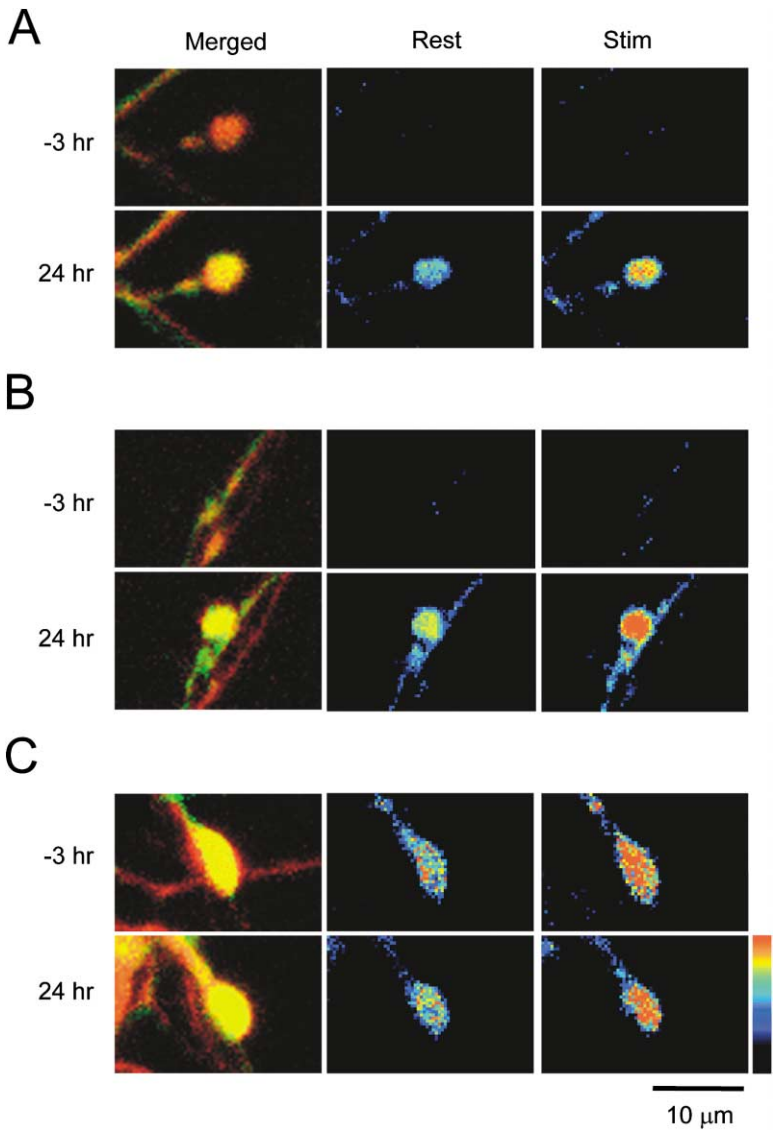

Figure 1. 5-HT-Induced Activation of Silent Presynaptic Varicosities and the Growth of New Functional Synaptic Varicosities

The functional state of individual sensory neuron varicosities as determined before and 24 hr after 5 × 10 μM 5-HT. (A) The merged images (red, Alexa-594; green, synPH) reveal that a preexisting empty varicosity lacking synPH (red) at −3 hr becomes enriched (yellow) at 24 hr after 5-HT treatment. The pseudocolor images before (rest) and after (stim) depolarization of the sensory neuron indicate that there is no significant change in fluorescence intensity at −3 hr (presynaptically silent and not competent for evoked transmitter release) but illustrate a significant increase in fluorescence intensity (presynaptically active and competent for evoked transmitter release) 24 hr after 5-HT treatment. (B) Only sensory neuron neurites are present at −3 hr, but a new varicosity is formed and enriched in synPH (yellow) at 24 hr after 5-HT treatment. The pseudocolor images show an increase in fluorescence intensity, indicating that the newly formed presynaptic varicosity is functional. (C) A preexisting and synPH-enriched varicosity is competent both before and after 5-HT treatment. There is no substantial change in varicosity structure or synPH distribution. The pseudocolor images also indicate that the varicosity is functional at both −3 hr and 24 hr following 5-HT treatment. The pseudocolor scale shows fluorescence intensity of synPH (in arbitrary fluorescence units) for rest/stim panels of (A)–(C). (From Kim et al., 2003.)

ration of active transmitter release sites at sensory neuron varicosities. 5-HT activation of Cdc42 is dependent on signaling through the P13K and PLC pathways, and in turn Cdc42 activates the downstream effectors PAK and N-WASP, leading to the growth of new sensory neuron varicosities associated with long-term facilitation.

Initiation of Long-Term Facilitation: PKA and MAP Kinase Activate CREB-Related Transcription Factors

5-HT, released in vivo during sensitization or applied directly to cultured neurons, binds to a cell surface receptor on the sensory neurons that activate the enzyme adenylyl cyclase, which converts ATP to the diffusible second-messenger cAMP, thereby activating the cAMP-dependent protein kinase (PKA). PKA recruits MAP kinase and both translocate to the nucleus. PKA activates gene expression by phosphorylating the transcription factors that bind to the cAMP-responsive element (CRE), the CRE binding protein (CREB1). Microinjection of CRE containing oligonucleotides into sensory neurons inhibits the function of CREB1 and blocks long-term facilitation but has no effect on the short-term process (Dash et al., 1990). Injection of recombinant CREB1a phosphorylated in vivo by PKA leads to an increase in EPSP amplitude at 24 hr in the absence of any 5-HT stimulation (Bartsch et al., 2000). Not only is the CREB1 activator necessary for long-term facilitation, it is also sufficient to induce long-term facilitation, albeit in reduced form and in a form that is not maintained beyond 24 hr.

The transcriptional switch in long-term facilitation is not only composed of the CREB1 regulatory unit but also of the CREB2 repressor. Injection of anti-ApCREB2 antibodies into *Aplysia* sensory neurons causes a single pulse of 5-HT, which normally induces only short-term facilitation lasting minutes, to evoke facilitation that lasts more than 1 day. This response requires both transcription and translation and is accompanied by the growth of new synaptic connections (Bartsch et al., 1995). ApCREB2 has both protein kinase C and MAP kinase phosphorylation sites, and MAP kinase is activated by 5-HT

in *Aplysia* neurons. Like PKA, MAP kinase translocates to the nucleus with prolonged 5-HT treatment so as to activate the activators (CREB1) and relieve the repressors (CREB2) (Martin et al., 1997b).

The balance between CREB activator and repressor isoforms is also critically important in long-term behavioral memory, as first shown in *Drosophila*. Expression of an inhibitory form of CREB (dCREB-2b) blocks long-term olfactory memory but does not alter short-term memory (Yin et al., 1994). Overexpression of an activator form of CREB (dCREB-2a) increases the efficacy of massed training in long-term memory formation (Yin et al., 1995).

The CREB-mediated response to extracellular stimuli can be modulated by a number of kinases (PKA, CaMKII, CaMKIV, RAK2, MAPK, and PKC) and phosphatases (PP1 and calcineurin). The CREB regulatory unit may therefore serve to integrate signals from various signal transduction pathways. This ability to integrate signaling as well as mediate activation or repression may explain why CREB is so central to memory storage in different contexts (Martin and Kandel, 1996).

This question has been studied by Guan et al. (2002), who examined the role of CREB-mediated responses in long-term synaptic integration by studying the long-term interactions of two opposing modulatory transmitters important for behavioral sensitization in *Aplysia*. Toward that end, they utilized a single bifurcated sensory neuron that contacts two spatially separated postsynaptic neurons (Martin et al., 1997a). They found that when a neuron receives 5-HT, and at the same time receives input from the inhibitory transmitter FMRFamide at another set of synapses, the synapse-specific long-term depression produced by FMRFamide dominates. These opposing inputs are integrated in the neuron's nucleus and are evident in the repression of the CCAAT-box-enhanced binding-protein (C/EPB), a transcription regulator downstream from CREB that is critical for long-term facilitation. Whereas 5-HT induces C/EPB by activating CREB1 and recruiting the CREB binding protein, a histone acetylase, to acetylate histones, FMRFamide displaces CREB1 with CREB2, which recruits a histone deacetylase to deacetylate histones. When 5-HT and FMRFamide are given together, FMRFamide overrides 5-HT by recruiting CREB2 and the deacetylase to displace CREB1 and CBP, thereby inducing histone deacetylation and repression of C/EBP. Thus, both the facilitatory and inhibitory modulatory transmitters that are important for long-term memory in *Aplysia* activate signal transduction pathways that alter nucleosome structure bidirectionally through acetylation and deacetylation of chromatin (Figure 2).

To follow further the sequence of steps whereby CREB leads to the stable, self-perpetuating long-term process, Alberini and colleagues (Alberini et al., 1994) focused on the CCAAT-box enhanced binding protein (C/EBP) transcription factors which they found were induced by exposure to 5-HT. Inhibition of ApC/EBP activity blocked long-term facilitation but had no effect on short-term facilitation. Thus, the induction of ApC/EBP seems to serve as an intermediate component of a molecular switch activated during the consolidation period.

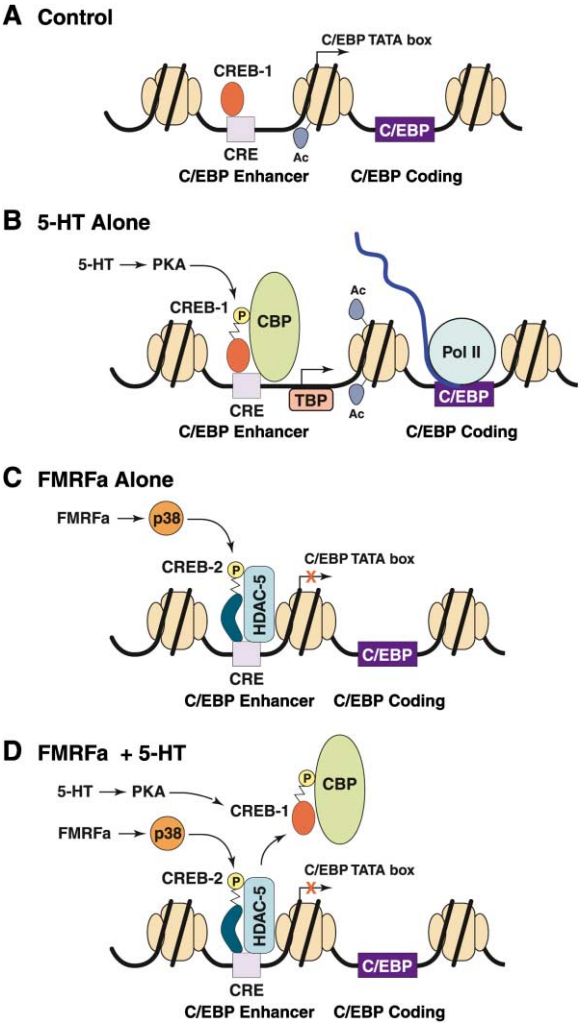

Figure 2. 5-HT and FMRFa Bidirectionally Regulate Histone Acetylation

(A) At the basal level, CREB1a resides on the C/EBP promoter and some lysine residues of histones are acetylated.

(B) 5-HT, through PKA, phosphorylates CREB1 that binds to the C/EBP promoter. Phosphorylated CREB1 then forms a complex with CBP at the promoter. CBP then acetylates lysine residues of the histones (e.g., K8 of H4). Acetylation modulates chromatin structure, enabling the transcription machinery to bind and induce gene expression.

(C) FMRFa activates CREB2, which displaces CREB1 from the C/EBP promoter. HDAC5 is then recruited to deacetylate histones. As a result, the gene is repressed.

(D) If the neuron is exposed to both FMRFa and 5-HT, CREB1 is replaced by CREB2 at the promoter, even though it might still be phosphorylated through the 5-HT-PKA pathway, and HDAC5 is then recruited to deacetylate histones, blocking gene induction. (From Guan et al., 2002.)

Initiation of Synaptic Growth: Learning-Induced Internalization of apCAM

How does this sequential gene activation lead to the growth of new sensory neuron synapses? 5-HT-induced synaptic growth in sensory-motor neuron cocultures is associated with downregulation of the neuronal cell adhesion molecule (NCAM)-related apCAMs on the surface membrane of the sensory neuron (Mayford et al., 1992).

Downregulation is particularly prominent at sites at which the processes of the sensory neurons contact one another and is achieved by the protein synthesis-dependent activation of a coordinated program of clathrin-mediated endocytosis leading to the internalization and apparent degradation of apCAM (Bailey et al., 1992b). *Aplysia* neurons express two isoforms of apCAM—a transmembrane form and a GPI-linked form. Only the transmembrane isoform is internalized following exposure to 5-HT (Bailey et al., 1997). The internalization was blocked by overexpression of transmembrane 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 apCAMs and may represent one of the initial and perhaps permissive stages of learning-related synaptic growth in *Aplysia*. Furthermore, the combined actions of MAPK both in the cytoplasm and in the nucleus suggest that MAPK plays multiple roles in long-lasting synaptic plasticity and appears to regulate each of the two distinctive processes that characterize the long-term process: activation of transcription and growth of new synaptic connections.

Recently, Han et al. (2004) have found that overexpression of the transmembrane isoform, but not the GPI-linked isoform of apCAM, blocked both long-term facilitation and 5-HT-induced synaptic growth. Long-term facilitation was also blocked by overexpression of the cytoplasmic tail portion of apCAM fused with GFP, designed to bind proteins such as MAP kinase p42. These studies confirm that the extracellular domain of transmembrane apCAM has an inhibitory function that needs to be neutralized by internalization to induce long-term facilitation and synaptic growth and that the cytoplasmic tail provides an interactive platform for both signal transduction and the internalization machinery.

A Molecular Model for the Stabilization of Synaptic Growth and Maintenance of Long-Term Facilitation: Self-Perpetuating Activation of Translational Regulators

As outlined above, the stability of long-term facilitation seems to result from the activation of a nuclear program and the persistence of the growth of new sensory neuron synapses, the decay of which parallels the decay of the behavioral memory. This raises two fundamental questions in the cell biology of memory storage. First, the activation of nuclear program suggests that long-term memory could potentially be cell-wide. On the other hand, there might be a cellular mechanism to utilize a cell-wide process in a synapse-specific way. Second, if a change in synaptic strength and structure is indeed the underlying mechanism of long-term memory storage, then the experience-dependent molecular changes at the synapse must somehow also be maintained for the duration of the memory. Since biological molecules have a relatively short half-life (hours to days) compared to the duration of memory (years), how is the altered molecular composition of a synapse maintained for such a long time?

To begin to address this question, Martin et al. (1997a) focused on the role of local protein synthesis in the maintenance of synapse-specific, long-term plasticity. Toward that end, they developed a culture system in *Aplysia* in which a single bifurcated sensory neuron of the gill-withdrawal reflex was plated in contact with two spatially separated gill motor neurons. In this culture system, repeated application of 5-HT to one synapse produces a CREB-mediated, synapse-specific long-term facilitation that is accompanied by the growth of new synaptic connections and persists for at least 72 hr. This long-term facilitation, as well as the long-lasting synaptic growth, can be captured by a single pulse of 5-HT applied at the opposite sensory-to-motor neuron synapse. In contrast to the synapse-specific forms, cell-wide long-term facilitation generated by repeated pulses of 5-HT at the cell body is not associated with growth and does not persist beyond 48 hr. However, this cell-wide facilitation also can be captured and growth can be induced in a synapse-specific manner by a single pulse of 5-HT applied to one of the peripheral synapses (Casadio et al., 1999).

Thus, CREB-mediated transcription appears to be necessary for the establishment of all four forms of long-term facilitation and for the initial maintenance of the synaptic plasticity at 24 hr. However, CREB-mediated transcription is not sufficient to maintain the changes beyond this time. To obtain persistent facilitation, and specifically to obtain the growth of new synaptic connections, one needs, in addition to CREB-mediated transcription, a marking signal produced by a single pulse of 5-HT applied to the synapse. This single pulse of 5-HT has at least two marking functions. First, it produces a PKA-mediated covalent modification that marks the captured synapse for growth. Second, it stimulates rapamycin-sensitive local protein synthesis, which is required for the long-term maintenance of the plasticity and stabilization of the growth beyond 24 hr.

The finding of two distinct components for the marking signal first suggested that there is a mechanistic distinction between the initiation of long-term facilitation and of synaptic growth (which require central transcription and central translation but do not require local protein synthesis) and the stable maintenance of the long-term functional and structural changes that require, in addition, local protein synthesis. Thus, the structural change once initiated is not sufficient as a maintenance mechanism for long-term memory. The structural change itself must be actively maintained. How might this local protein synthesis at the synapse, necessary for stabilizing and maintaining synaptic growth, be regulated? Since mRNAs are made in the cell body, the need for the local translation of some mRNAs suggests that these mRNAs may be dormant before they reach the activated synapse. If that were true, one way of activating protein synthesis at the synapse would be to recruit a regulator of translation that is capable of activating translationally dormant mRNAs.

Si et al. (2003a) searched for such a molecule by focusing on the *Aplysia* homolog of cytoplasmic polyadenylation element binding protein (CPEB), a protein capable of activating dormant mRNAs through the elongation of their polyA tail. CPEB was first identified in oocytes and subsequently in hippocampal neurons (Hake and Richter, 1994; Wu et al., 1998). In *Aplysia*, a novel, neuron-specific isoform of CPEB is present in the processes

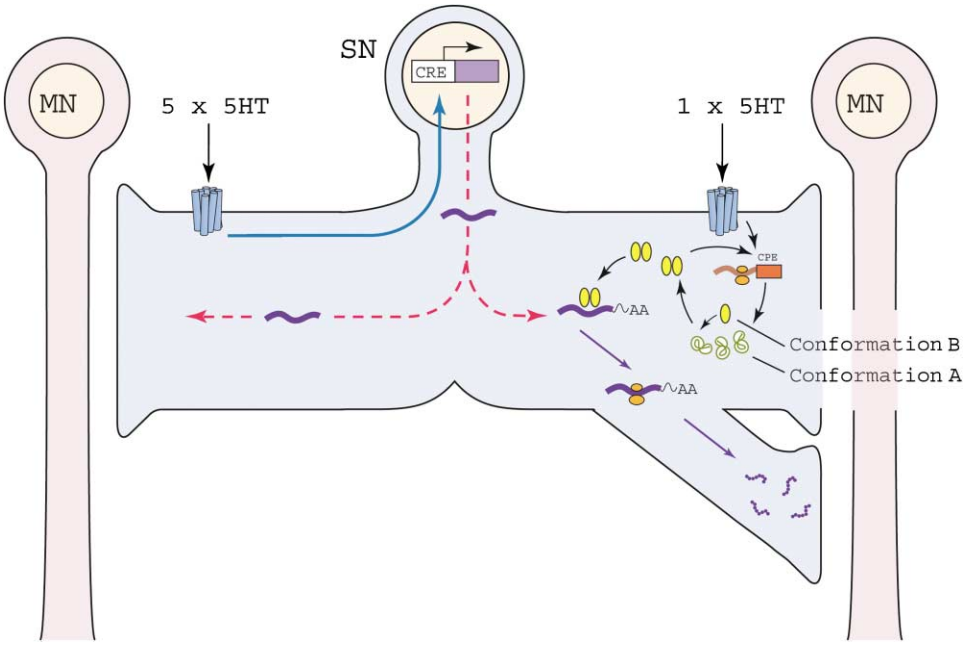

Figure 3. A Prion-Based Model for Self-Perpetuating Synaptic Change

Repeated pulses of 5-HT (5X5-HT) to one branch send a retrograde signal to the cell body activating transcription. The newly synthesized mRNAs, some of which are translationally inactive, are distributed to all synapses. One pulse of 5-HT applied to the other branch is sufficient to increase the level of CPEB protein. The newly synthesized CPEBs (conformation A) are the inactive conformational state of the protein. Some of the protein in conformation A, either spontaneously or in a regulated way, converts into the dominant, self-perpetuating active conformation B. A few molecules in conformation B are sufficient to convert all of conformation A to that of conformation B. The protein in conformation B can activate the translationally inactive mRNAs by elongating their polyA tail. The CPEB mRNA itself has a putative CPE element. Thus, once activated, the conformation B proteins can potentially regulate the availability of the proteins in conformation A. This can lead to a self-sustaining, autoregulatory feedback loop that could contribute to the stabilization of learning-related synaptic growth and the persistence of memory storage. (Based on Si et al., 2003b.)

of sensory neurons (Liu and Schwartz, 2003; Si et al., 2003a), and stimulation with 5-HT increases the amount of CPEB protein at the synapse. The induction of CPEB is independent of transcription but requires new protein synthesis and is sensitive to rapamycin and to inhibitors of P13 kinase. Moreover, the induction of CPEB coincides with the polyadenylation of neuronal actin, and blocking CPEB locally at the activated synapse blocks the long-term maintenance of synaptic facilitation but not its early expression at 24 hr. Thus, CPEB has the properties required of the local protein synthesis-dependent component of marking and supports the idea that there are separate mechanisms for initiation of the long-term process and its stabilization. Moreover, these data suggest that the maintenance but not the initiation of long-term synaptic plasticity requires a new set of molecules in the synapse and some of these new molecules are made by CPEB-dependent translational activation. A similar neuronal isoform of CPEB, CPEB-3, has been found in mouse hippocampal neurons, and CPEB-3 is induced by the neurotransmitter dopamine (Theis et al., 2003). Interestingly, Frey and Sajikumar (Sajikumar and Frey, 2004) recently reported that activation of the dopaminergic pathway is critical for the synaptic marking during mouse hippocampal LTP. This raises the possibility that dopamine-dependent regulation of mouse CPEB-3 might be similar to the serotonin-mediated regulation of *Aplysia* neuronal CPEB and that CPEB-3 might perhaps act as a positive synaptic mark at mammalian synapses.

How might CPEB stabilize the late phase of long-term facilitation? The 5-HT-induced structural changes at the synapses between sensory and motor neurons include the remodeling of preexisting facilitated synapses, as well as the growth and establishment of new synaptic connections. The reorganization and growth of new synapses have two broad requirements: (1) structural (changes in shape, size, and number) and (2) regulatory (where and when to grow). The genes involved in both of these aspects of synaptic growth might be potential targets of apCPEB. The structural aspects of the synapses are dynamically controlled by reorganization of the cytoskeleton, which can be achieved either by redistribution of preexisting cytoskeletal components or by their local synthesis. The observation that N-actin and Tα1 tubulin (Moccia et al., 2003) are present in the peripheral population of mRNAs at the synapse and can be polyadenylated in response to 5-HT suggests that at least some of the structural components for synaptic growth can be controlled through apCPEB-mediated local synthesis (Kim and Lisman, 1999). In addition, recently, CPEB has been found to be involved in the regulation of local synthesis of EphA2 (Brittis et al., 2002), a member of the family of receptor tyrosine kinases, which have been implicated in axonal pathfinding and the formation of excitatory synapses in the mammalian brain.

Thus, CPEB might contribute to the stabilization of learning-related synaptic growth by controlling the synthesis of both the structural molecules such as tubulin and N-actin and the regulatory molecules such as CAMKII and members of the Ephrin family.

These findings in turn raise further questions: Is there a continuous need for the local synthesis of a set of molecules to maintain the learning-related synaptic changes over long periods of time? If so, how can these enduring changes be achieved by a translational regulator such as CPEB in the face of a continuous turnover of the protein? One possible answer to how a population of unstable molecules can produce a stable change in synaptic form and function comes from the subsequent finding by Si et al. (2003b) that the neuronal isoform of CPEB shares properties with prion-like proteins. Prions are proteins that can assume at least two stable conformational states (Prusiner, 1998; Uptain and Lindquist, 2002; Wickner et al., 1999). Usually, one of these conformational states is active, while the other is inactive. Furthermore, one of the conformational states, the prion state, is self-perpetuating, promoting the conformational conversion of other proteins of the same type. Work on yeast suggests that the *Aplysia* neuronal CPEB exists in two stable, physical states that are functionally distinct. As with other prions, one of these states has the ability to self-perpetuate in a dominant epigenetic fashion. However, unlike the known prion proteins where the dominant state is the inactive form of the protein, surprisingly, in the case of *Aplysia* CPEB, the dominant form is the active form of the protein capable of activating translationally dormant mRNAs.

An Overall View

One of the persistent questions in the molecular study of the storage mechanisms of memory is the nature of the enduring change that underlies long-term memory. Since proteins have a relatively short half-life compared to the duration of memory, structural changes at the synaptic level were postulated to confer stability to the memory, and it was implicitly assumed that the requirement of activity-dependent molecular changes was transient. However, it is now clear that the maintenance of learning-related structural alterations requires ongoing macromolecular synthesis. Crick (1984) first addressed the possibility of a sustained molecular alteration as the basis of long-term memory storage using protein phosphorylation as a candidate mechanism. John Lisman (Lisman et al., 1997) developed a model based on the autocatalytic properties of CamKII. According to Lisman's model, synaptic stimulation activates CamKII, which can then convert inactive CamKII molecules to their active form in the absence of any further synaptic input.

Based on the properties of *Aplysia* neuronal CPEB, it now appears that a prion-like switch could serve as another mechanism to maintain a self-sustained activated molecular state (Figure 3). In this model, CPEB in the sensory neuron has at least two conformational states: one is inactive, or acts as a repressor, while the other form is active. In a naive synapse, the basal level of CPEB is low, and unlike conventional prions, the protein in this state is in its inactive, or repressive, state. An increase in the amount of neuronal CPEB induced by 5-HT, either by itself or in conjunction with other signals, results in the conversion to the prion-like state, which might be more active or be devoid of the inhibitory function of the basal state. Once the prion state is established at an activated synapse, dormant mRNAs, made in the cell body and distributed globally to all synapses, can be activated only locally through the activated CPEB. Because the activated CPEB can be self-perpetuating, it could contribute to a self-sustaining synapse-specific long-term molecular change and provide a mechanism for the stabilization of learning-related synaptic growth and the persistence of memory storage.

According to this model, this variant form of prion mechanism evident in CPEB requires the action of a neurotransmitter for switching the protein to its active self-perpetuating state. This may be equivalent to a posttranslational modification induced by a physiological signal, a regulatory mechanism commonly found in the nervous system. A prion-like mechanism, however, introduces an additional feature into signal transduction; once the protein achieves its prion state it is self-perpetuating and no longer requires for maintenance continued signaling either by kinases or phosphatases. Moreover, its activity state is less easily reversed. This argument would imply that memory storage is much more dynamically regulated than one would predict from the alterations in synaptic structure alone. Moreover, since these presynaptic structural changes share their postsynaptic counterpart, there must be transynaptic signals both orthograde and retrograde to coordinate and regulate the structural remodeling in an ongoing manner.

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