

Memory Suppressor Genes: Enhancing the Relationship Between Synaptic Plasticity and Memory Storage

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Memory suppressor genes encode proteins that act as inhibitory constraints to impede memory storage. The study of memory suppressor genes is important not only for understanding the link between synaptic plasticity and learning but also for identifying potential targets for future pharmaceuticals to treat memory disorders. This article first reviews the evidence for proteins that impede memory storage from work in invertebrates and then explores recent evidence for the existence of memory suppressor genes in vertebrates in the context of hippocampus-dependent forms of memory. In *Aplysia*, memory suppressor gene products act at each step in long-term facilitation: in the cytoplasm to regulate kinase activity, in the nucleus to alter the activity of transcriptional regulatory proteins, and on the cell surface to modulate cell–cell interactions. Studies of genetically modified *Drosophila* have provided behavioral evidence for the existence of memory suppressor genes. One of the best candidates for a neuronal mechanism underlying learning is long-term potentiation (LTP), which has been extensively studied in the mammalian hippocampus. Recent work has identified a number of putative memory suppressor gene products that act in the hippocampus at the levels of LTP induction, regulation of intracellular signaling cascades, and transcriptional control. Using these gene products as tools to study enhancements rather than deficits in LTP and learning may generate more precise information about the relationship between synaptic plasticity and behavioral learning. The study of mammalian memory suppressor genes may provide insights into alleviating the learning and memory deficits that accompany both normal aging and a variety of human disorders. *J. Neurosci. Res.* 58:10–23, 1999. © 1999 Wiley-Liss, Inc.

Key words: synaptic plasticity; memory storage; LTP; *Aplysia*; genetically modified mice

INTRODUCTION

On a molecular level, one of the most important advances in our understanding of the basis of long-term

memory storage has been the idea that memory consolidation involves alterations in gene expression. A variety of molecular models for the processes by which synaptic events initiate the nuclear events leading to gene induction have been proposed (Abel and Kandel, 1998; Silva et al., 1998). These models have been guided by ideas arising from the study of signal transduction in the context of oncogenesis and development. The study of cell division and development has shown that the protein products of cellular oncogenes activate a variety of signal transduction pathways, spanning from the cell surface to the nucleus. Oncogenesis results when these pathways are constitutively activated by the increased or altered activity of these cellular oncoproteins.

Studies of the role of signal transduction mechanisms in memory storage have explored the molecules, such as calmodulin kinase II (CaMKII), protein kinase A (PKA), and cyclic AMP response element binding protein (CREB), necessary for synaptic plasticity, learning, and memory. One of the surprising findings to emerge from the studies of the molecular and cellular mechanisms of synaptic plasticity is that memory storage is mediated not only by positive but also by negative regulatory mechanisms, just as cell division is regulated by the proteins encoded by oncogenes and tumor suppressor genes. Tumor suppressor genes, whose products normally suppress cell division, were initially identified as loss-of-function mutations that result in increased cell proliferation in certain forms of cancer such as retinoblastoma. By homology, genes that encode proteins that act as inhibitory constraints to impede memory storage have been termed *memory suppressor genes* (Abel et al., 1998). Like tumor suppressor genes, memory suppressor genes may function at regulatory checkpoints during learning and memory consolidation to ensure that only salient features of an experience are learned.

The evidence for memory suppressor genes is based largely on studies of long-term facilitation in *Aplysia*.

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This article first reviews the evidence for proteins that impede memory storage from work in the *Aplysia* and *Drosophila* systems and then explores the recent evidence for the existence of memory suppressor genes in vertebrates in the context of hippocampus-dependent forms of memory. Candidate hippocampal memory suppressor genes will be identified, and experimental approaches to address their function will be described. This distinct way to view the molecular mechanisms underlying synaptic plasticity and memory storage may provide a different outlook on the relationship between the cellular mechanisms of synaptic plasticity and behavioral memory. Further, the identification and characterization of memory suppressor genes may be especially important in the development of pharmacological, molecular, or genetic methods to enhance learning and memory. It is intriguing to speculate that the products of memory suppressor genes may turn out to be attractive drug targets for the next generation of pharmaceuticals designed to alleviate the memory loss that accompanies Alzheimer's disease, mental retardation, and aging.

MEMORY SUPPRESSOR GENES IN *APLYSIA*

The study of the gill and siphon withdrawal reflex in the marine snail *Aplysia californica* has been important in outlining the molecular mechanisms that underlie changes in synaptic strength (Byrne and Kandel, 1996). During sensitization, a nonassociative form of learning, an animal learns to increase responses to a previously neutral stimulus, such as a weak stimulus to the siphon, following the presentation of an aversive stimulus, such as a shock to the tail. On a cellular level, sensitization involves an enhancement of synaptic transmission at the sensory-motor neuron synapse (Frost et al., 1985). This long-lasting increase in synaptic strength, termed *long-term facilitation*, is mediated by serotonin (5-hydroxytryptamine; 5-HT) released from a class of facilitatory interneurons activated by tail stimulation (Brunelli et al., 1976; Glanzman et al., 1989). In a coculture system consisting of sensory and motor neurons, long-term facilitation is produced by applying 5-HT. Long-term facilitation at the sensory-motor neuron synapse in culture differs with the number of 5-HT treatments (Montarolo et al., 1986), just as behavioral sensitization is a function of the number of stimuli applied to the tail (Frost et al., 1985). One pulse of 5-HT produces a synaptic enhancement that is transient and not affected by inhibitors of protein and RNA synthesis. In contrast, five pulses produces a long-term (24 hr) increase in synaptic strength that, like long-term behavioral sensitization, is blocked by inhibitors of protein and RNA synthesis (Montarolo et al., 1986).

On a molecular level, short-term facilitation is produced by the activation of PKA and protein kinase C

(PKC) in the presynaptic sensory neuron via 5-HT receptors (Byrne and Kandel, 1996). Both PKA and PKC enhance transmitter release in part by closing potassium channels, thereby increasing the calcium influx that is important for transmitter release. During long-term facilitation, both PKA and mitogen-activated protein (MAP) kinase translocate to the nucleus. One of their molecular targets may be CREB, a transcription factor thought to be critical for the induction of gene expression during this process (Bartsch et al., 1995, 1998; Dash et al., 1990). Indeed, treatment with five pulses of 5-HT leads to the phosphorylation of certain isoforms of CREB, and this phosphorylation is sufficient for long-term facilitation (Bartsch et al., 1998). A cytoplasmic form of CREB appears to modulate both short-term and long-term facilitation (Bartsch et al., 1998).

In addition to positive regulatory mechanisms such as the activation of CREB by PKA, the switch from short-term to long-term facilitation in *Aplysia* also involves the inactivation of gene products that otherwise would inhibit memory consolidation. Three major inhibitory constraints have been identified thus far for the long-term process in *Aplysia*, each acting at a distinct step in the process of synaptic facilitation. These memory suppressor gene products include the regulatory subunit of PKA, inhibitory forms of CREB, and the cell adhesion molecule (CAM) apCAM (Fig. 1).

Long-term facilitation is characterized by the persistent activation of PKA even in the absence of 5-HT or elevated intracellular cyclic AMP (cAMP) levels. This persistent activation of PKA extends through the first 12 hr of long-term facilitation and is the result of the regulated proteolysis of the regulatory subunit of PKA (Chain et al., 1999; Hegde et al., 1997). Like long-term facilitation, this proteolysis requires protein synthesis. It appears that 5-HT induces the expression of *Aplysia* ubiquitin carboxyl-terminal hydrolase (Ap-uch), a rate-limiting component of the ubiquitin-dependent proteolytic pathway. As levels of Ap-uch increase, the regulatory subunit of PKA is degraded by the proteasome. Other proteins may also be degraded by this pathway, but their identity is unknown.

At the transcriptional level, the induction of new gene expression requires the inactivation of the transcriptional repressor ApCREB2 and an inhibitory isoform of ApCREB1 termed *ApCREB1b* (Bartsch et al., 1995, 1998). ApCREB2 appears to inhibit activating forms of ApCREB1 by forming heterodimers in which the activation domains on both proteins are masked. Phosphorylation of ApCREB2 by MAP kinase or PKC, both of which phosphorylate this protein in vitro, may inhibit the ability of ApCREB2 to repress transcription. After injection of ApCREB2 antiserum into sensory neurons, a single

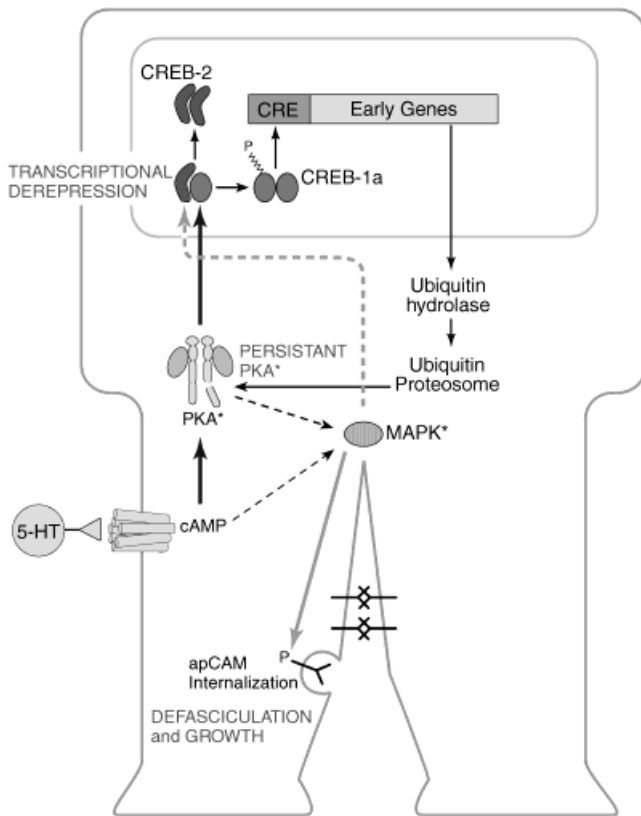


Fig. 1. Long-term synaptic facilitation in *Aplysia*. Three inhibitory constraints, or memory suppressor gene products, of long-term facilitation in *Aplysia* have been identified. These include the regulatory subunit of protein kinase A, inhibitory forms of CREB, and the cell adhesion molecule apCAM (modified from Abel et al., 1998).

treatment with 5-HT that normally produces only short-term facilitation now produces long-term facilitation (Bartsch et al., 1995). This long-term process produced by one pulse of 5-HT after the injection of ApCREB2 antiserum requires translation and transcription and occludes the effects of five pulses of 5-HT, suggesting that this synaptic facilitation is a phenocopy of long-term facilitation.

Cellular studies of memory storage in both *Aplysia* and mammals suggest that what sustains long-term memory is the growth of new synaptic connections (Bailey and Kandel, 1993). Long-term sensitization of the gill-withdrawal reflex involves structural changes at the sensory-motor neuron synapse, and the appearance of new synapses parallels synaptic facilitation. Like long-term facilitation and behavioral memory, these structural changes are blocked by inhibitors of RNA and protein synthesis (Bailey et al., 1992b). The first insight into the molecules that may contribute to these structural changes came from studies that used two-dimensional gel electro-

phoresis to identify a group of five proteins whose levels decrease within the first hour after 5-HT treatment (Barzilai et al., 1989). These downregulated proteins were later identified as apCAMs, *Aplysia* members of the immunoglobulin class of cell adhesion molecules that includes mammalian neural CAM and *Drosophila* fasciclin II (Mayford et al., 1992).

Although many studies have focused on the role of cell adhesion molecules during development, it is now clear from the work in *Aplysia* and *Drosophila* that this class of cell surface molecules can play a critical role in synapse formation and maintenance during synaptic plasticity. In particular, both apCAM and fasciclin II appear to inhibit the formation of new synaptic connections. At the *Drosophila* neuromuscular junction, the presynaptic downregulation of fasciclin II is both necessary and sufficient for activity-dependent synaptic sprouting (Goodman et al., 1997). The selective downregulation of the transmembrane form of the cell adhesion molecule apCAM, induced in part by the activation of the MAP kinase cascade, may remove an inhibition of synaptic growth (Bailey et al., 1992a, 1997) by leading to defasciculation. This defasciculation may act in a permissive way to allow neurites from the sensory neuron to interact with the motor neuron, thereby leading to the formation of new synaptic connections (Zhu et al., 1994).

Thus, memory suppressor gene products act at each step in long-term facilitation: in the cytoplasm to regulate kinase activity, in the nucleus to alter the activity of transcriptional regulatory proteins, and on the cell surface to modulate cell-cell interactions (Fig. 1). It is interesting to note that MAP kinase appears to be required for long-term facilitation (Martin et al., 1997). For at least two of these memory suppressor gene products, ApCREB2 and the transmembrane isoform of apCAM, MAP kinase may be critically involved in removing these inhibitory constraints on synaptic facilitation.

ENHANCING LEARNING AND MEMORY IN *DROSOPHILA*

The power of *Drosophila* genetic approaches has been instrumental in providing behavioral evidence for the existence of memory suppressor genes. Classic genetic studies of olfactory conditioning, initially performed by Seymour Benzer and his colleagues, have identified a number of mutants impaired in this conditioning paradigm (Dubnau and Tully, 1998). Two of these, the mutants *dunce* and *rutabaga*, have been particularly important in defining the role of the cAMP/PKA signaling pathway in learning and memory. Initial biochemical studies, followed by molecular cloning, have shown that the *dunce* locus encodes a cAMP-specific phosphodiesterase and the *rutabaga* locus encodes a calcium-sensitive

form of adenylyl cyclase. They have distinct alterations in the cAMP/PKA pathway, with *dunce* mutants showing increased cAMP levels and *rutabaga* mutants exhibiting decreased cAMP levels. Both of these mutants exhibit deficits in learning and short-term memory for olfactory conditioning, suggesting that cAMP levels must be modulated within a narrow window for normal learning to occur.

This idea has been supported and strengthened by the work of Tully and others who observed learning deficits in transgenic flies expressing a constitutively active form of the G protein, $G_{\alpha s}$, selectively in neurons within the mushroom bodies, a portion of the fly brain required for learning (Connolly et al., 1996). Further experimental support comes from the observation that the heat shock promoter-induced expression of an inhibitor of PKA blocks learning (Drain et al., 1991). Beyond an optimum range, therefore, the constitutive alteration of levels of either a positive or a negative regulator of a signaling pathway may be deleterious.

Just as CREB plays a central role in long-term facilitation in *Aplysia*, this transcription factor also plays a critical role in long-term memory in the conditioned odor avoidance response in *Drosophila* (Dubnau and Tully, 1998; Silva et al., 1998). Spaced training in olfactory conditioning results in long-term memory that is blocked by protein synthesis inhibitors (Tully et al., 1994). This long-term memory is disrupted by the induced expression of an inhibitory form of CREB, whereas the shorter-lasting memory is unaffected (Yin et al., 1994). Further, just as inhibiting the action of a CREB repressor, ApCREB2, enhanced long-term facilitation in *Aplysia*, transgenic flies expressing an activator isoform of CREB exhibit enhanced long-term memory (Yin et al., 1995). In an important experiment, it was observed that transgenic flies expressing a mutant activator isoform in which the consensus PKA phosphorylation site is disabled did not exhibit enhanced memory, demonstrating that the phosphorylation of CREB is critical for this enhancement. This observation that increasing the levels of an activating form of CREB enhances memory is intriguing because most models for the role of CREB have proposed that CREB is constitutively present, with the activity of CREB regulated by kinases that modify the activity of CREB rather than protein levels (Silva et al., 1998).

Thus reverse genetic experiments using inducible and cell-type-specific expression systems have been instrumental in demonstrating that the learning impairments in *dunce* and *rutabaga* mutant flies are not solely the result of developmental deficits. These experiments have also delineated the neuronal circuits within the *Drosophila* brain necessary for classical conditioning. Further, they have demonstrated that both long-term

facilitation in *Aplysia* and long-term memory for olfactory conditioning in *Drosophila* require CREB- and cAMP-induced gene expression. Strikingly, enhancing the activity of CREB by increasing levels of an activating form or decreasing levels of an inhibitory form results in single trial learning in each of these systems.

The difficulty in these invertebrate systems, however, has been to study synaptic plasticity and behavioral memory in the same system. In *Drosophila*, this relationship has been investigated by analyzing synaptic transmission at the larval neuromuscular junction in mutant flies impaired in learning and memory (Zhong and Wu, 1991). The mutants *dunce* and *rutabaga* exhibit impaired synaptic facilitation and posttetanic potentiation. Interestingly, *dunce*, but not *rutabaga*, mutants exhibit enhanced basal synaptic transmission. However, these physiological studies have been performed at the larval neuromuscular junction rather than in adult mushroom body neurons, although some studies have begun to analyze mushroom body neurons in culture (Wright and Zhong, 1995). To directly examine synaptic plasticity and behavior in the same system, researchers have turned to the rodent hippocampus.

MAMMALIAN HIPPOCAMPUS: ENHANCING SYNAPTIC PLASTICITY AND SPATIAL MEMORY

One of the best candidates for a neuronal mechanism underlying learning is a form of synaptic plasticity called long-term potentiation (LTP). LTP is an increase in synaptic strength resulting from repeated stimulation of the afferents to that synapse (Fig. 2). It has been extensively studied in the mammalian hippocampus, a brain region critical for certain types of learning and memory. There are several identified forms of LTP, including *N*-methyl-D-aspartate (NMDA) receptor-dependent LTP in the Schaffer collateral pathway and NMDA receptor-independent LTP in the mossy fiber pathway (Fig. 3; Bliss and Collingridge, 1993). In rodents, interruption of LTP by pharmacological, genetic, or electrophysiological means causes an impairment of the ability of animals to learn hippocampus-based tasks such as the Morris water maze and contextual fear conditioning. Thus, blockade of LTP by treatment with NMDA receptor antagonists was found to impair spatial memory under certain circumstances (Bannerman et al., 1995; Morris, 1989; Saucier and Cain, 1995), and the saturation of LTP in the perforant path of unilateral hippocampallectomized rats was found to impair performance on the hidden platform version of the Morris water maze (Moser et al., 1998). In genetically modified mice, null mutations in the genes encoding a variety of protein kinases or other signal

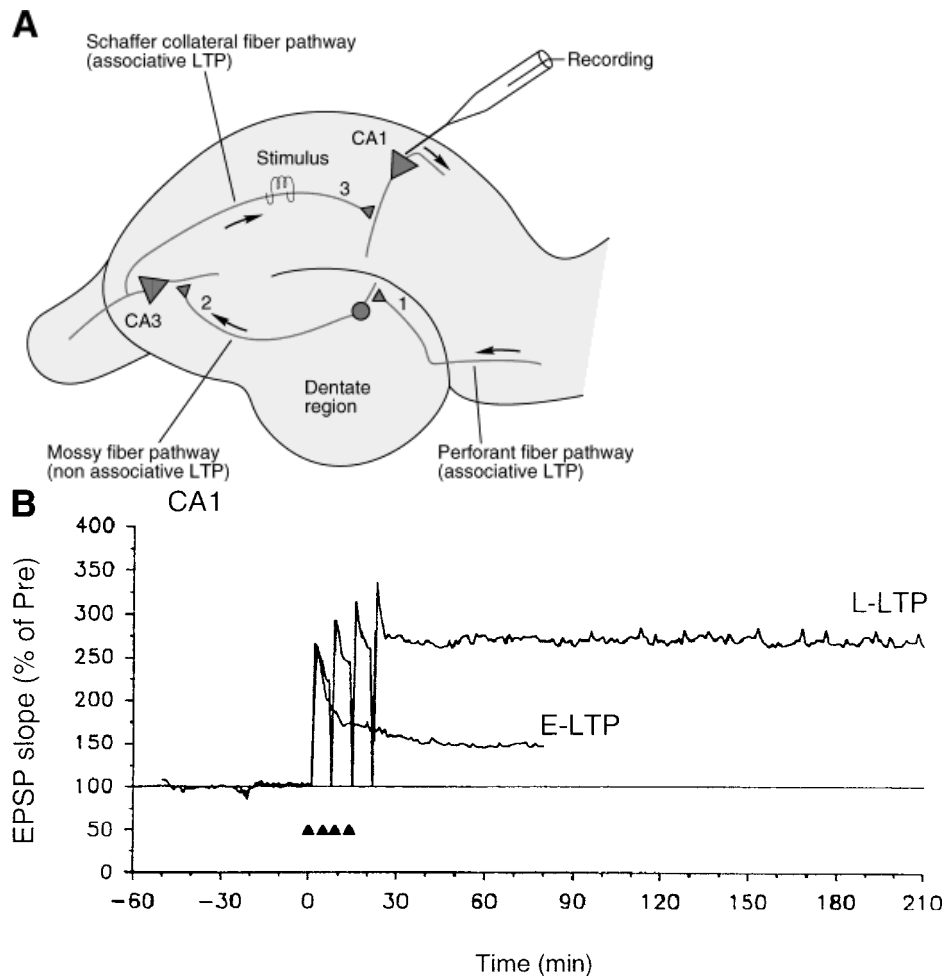


Fig. 2. **A:** The hippocampus contains a trisynaptic circuit: from the perforant pathway to the dentate gyrus, from the dentate gyrus to CA3, and from CA3 to CA1. NMDA receptor-dependent long-term potentiation (LTP) has been found at Schaffer collateral and perforant path synapses, whereas NMDA receptor-independent LTP has been found at mossy fiber synapses. **B:** Early LTP (E-LTP), which is independent of protein kinase A (PKA), can be induced in CA1 by one train of tetanic stimulation to the Schaffer collateral pathway at 100 Hz. In contrast, late LTP (L-LTP), which is PKA and protein synthesis dependent, requires three to four trains of stimulation to be induced. Whereas L-LTP lasts for many hours *in vitro*, E-LTP lasts only 1–2 hr (modified from Abel and Kandel, 1998).

transduction molecules involved in LTP were found to alter synaptic plasticity and spatial memory (Chen, 1997).

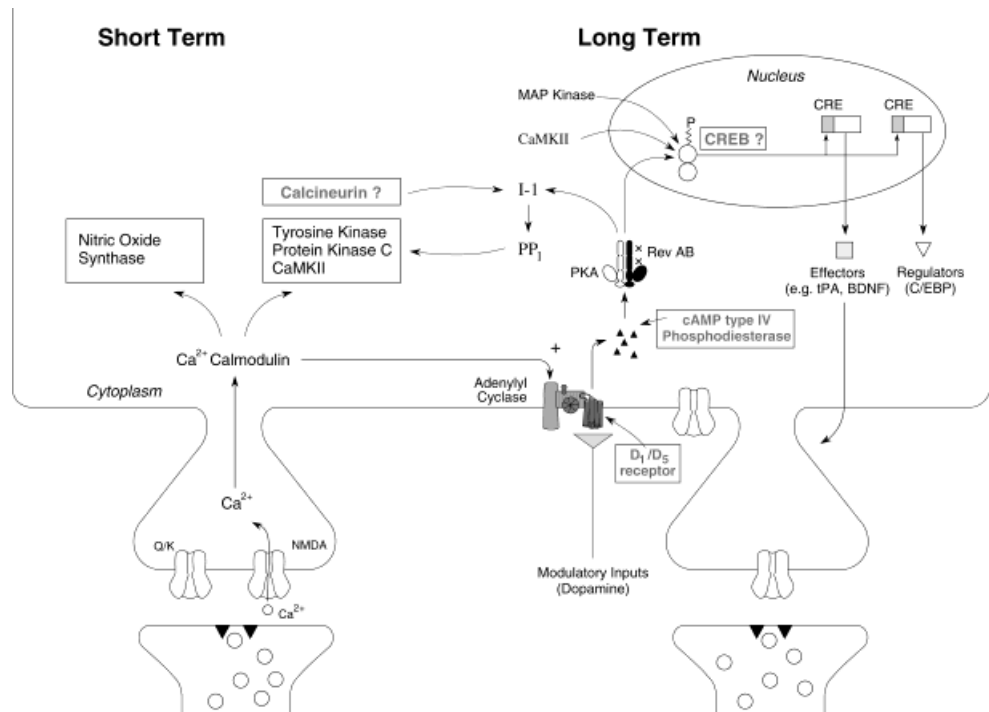
The general view that memory may be stored as alterations in synaptic strengths in appropriate neural circuits has been supported by many experiments. One criticism of the field, however, is that it is dominated by the LTP, or synaptic plasticity hypothesis, largely because of the lack of alternatives. Place cells, neurons within the hippocampus that fire at high rates only when the animal is in a particular spatial location (Muller, 1996), were originally postulated to be the cellular basis for the role of the hippocampus in spatial memory (O'Keefe and Nadel, 1978). The recent analysis of hippocampal place cells in genetically modified mice (Cho et al., 1998; McHugh et al., 1996; Rotenberg et al., 1996) and after pharmacological blockade of NMDA receptors (Kentros et al., 1998) is promising because it represents a distinct hypothesis that can be experimentally tested.

A particularly striking example of the use of genetically modified mice to study the relationship between LTP and memory comes from the studies of Tsien, Tonegawa, and others who used a site-specific recombi-

nase to selectively ablate the gene encoding the R1 subunit of the NMDA receptor only in neurons within hippocampal area CA1 (Tsien et al., 1996a,b). This regionally restricted knockout resulted in impairments in NMDA receptor-dependent forms of synaptic plasticity in the Schaffer collateral pathway within the hippocampus, including deficits in LTP and long-term depression (LTD; Tsien et al., 1996b). These mice also exhibited impairments in spatial memory and alterations in the properties of place cells in CA1 (McHugh et al., 1996; Tsien et al., 1996b). The behavioral deficits observed in these mice with a regionally restricted deletion of the NMDA receptor R1 subunit provide evidence supporting a selectively important role for hippocampal area CA1 in learning and memory, as suggested by the study of the patient R.B. by Rempel-Clower et al. (1996).

Nonetheless, it has been difficult to demonstrate definitively that LTP is the cellular basis of learning for several reasons. First, LTP can be evoked in each of the three projection pathways in the hippocampal circuit and these pathways may contribute differently to hippocampal function (Huang et al., 1995, 1996b; Nosten-

Fig. 3. Molecular model for short-term and long-term plasticity and hippocampus-based memory. Early long-term potentiation (E-LTP) is produced by Ca^{2+} influx through NMDA receptors, which activates a variety of kinases. In contrast, late LTP (L-LTP) is produced by Ca^{2+} or dopamine receptor activation of adenylyl cyclase, which leads to increased levels of cyclic AMP. Cyclic AMP activates protein kinase A, which phosphorylates a number of targets, including ion channels and CREB (modified from Abel et al., 1997).



Bertrand et al., 1996). Several forms of LTP have been identified, each with a distinct duration and involvement of underlying signal transduction pathways (Huang et al., 1996b). Further, an implication of the hypothesis that LTP mediates memory storage is that enhancing LTP should lead to increased learning, but few studies have found this to be the case. In some instances, enhancing LTP has resulted in behavioral impairments (Migaud et al., 1998). Although the magnitude of hippocampal LTP may be important in determining learning, there may be a limited range in which LTP is effective. It is also possible that the threshold and frequency response characteristics of each synapse within the hippocampal circuit are especially important. In addition, many studies have focused on aversive tasks, such as the Morris water maze and contextual fear conditioning, which measure spatial and contextual aspects of hippocampal function rather than nonaversive tasks, such as social transmission of food preference and olfactory paired associate learning, which may measure other hippocampal functions. The results described above suggest that LTP may be the underlying cellular mechanism of hippocampus-based learning. However, because blocking LTP may potentially involve changing other basic cellular mechanisms, it is important to determine whether enhancing LTP results in improvements in behavioral learning.

Glutamate Receptors, LTP, Learning, and Memory

Examining enhancements, rather than deficits, in LTP and learning may generate more precise information

about the relationship between synaptic plasticity and behavioral learning. One way to study enhancements in LTP and learning is to identify negative regulatory constraints on learning, such as memory suppressor genes, and use them as tools in examining the relationship between synaptic plasticity and learning. Genetic manipulations of NMDA and other glutamate receptors have suggested several inhibitory mechanisms that regulate the expression of hippocampal LTP. One of these is postsynaptic density protein 95 (PSD-95), which contains several PDZ domains and which is thought to function in localizing NMDA receptors to synapses. PSD-95 modulates NMDA channel activity, possibly by promoting phosphorylation of the NR2A subunit (Tezuka et al., 1999; Yamada et al., 1999). To investigate the possible role of PSD-95 in hippocampal synaptic plasticity, Migaud et al. (1998) generated mice with a targeted mutation of PSD-95 that excised one PDZ domain, a Src homology 3 domain, and a guanylate kinase homology domain and left the two amino terminal PDZ domains intact. NMDA receptor localization, synaptic plasticity, and learning were examined in hippocampal neurons in these mutant mice.

Hippocampal slices from the PSD-95 mutant mice showed normal NMDA receptor localization and function and enhanced paired-pulse facilitation. The mutant slices demonstrated an increased magnitude of LTP in CA1 as compared with controls in response to a single 100-Hz train of tetanization. Whereas control slices required three to four trains of stimulation for LTP saturation, slices

from PSD-95 mutants demonstrated saturated LTP only after five to six trains. In addition, the PSD-95 mutant slices exhibited a greater magnitude of LTP than did controls in response to 5-Hz stimulation and of LTP rather than LTD in response to 1-Hz stimulation. This suggests that the loss of functional PSD-95 increases the magnitude of LTP, alters LTP saturation, and modifies the frequency-response function of the Schaffer collateral pathway. These results suggest that PSD-95 may normally inhibit the expression of LTP at CA3–CA1 synapses. This increased LTP resulting from PSD-95 knockout, however, appears to lead to deficits in spatial learning. In the Morris water maze, the mutants had significantly longer swim paths in the hidden platform task than did controls and did not show a quadrant preference during the probe trial. They also showed some evidence of thigmotaxis during the probe trial. The mutants had longer latencies to find the platform than did the controls early in the visible platform version of the water maze.

Because the function of PSD-95 has not yet been well characterized, it is difficult to draw detailed conclusions about the relationship between this protein and mechanisms of synaptic plasticity. One particular concern is the role of the first two PDZ domains of PSD-95, which were not removed in the mutant mice. The increased paired-pulse facilitation observed in the PSD-95 mutants may be the result of a modified interaction of the remaining PDZ domains with retrograde signaling mechanisms or with K^+ channels or other proteins (Burke et al., 1999; Xia et al., 1999). It is possible that the behavioral impairments observed in the PSD-95 mutant mice are due to the altered frequency response of CA3–CA1 synapses. Such altered frequency responses have been observed previously to result in behavioral impairments in spatial tasks (Bach et al., 1995; Mayford et al., 1995).

Although the role of NMDA receptors in synaptic plasticity has received a great deal of attention, the possible role of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors, which mediate most of the fast excitatory synaptic transmission in the hippocampus, is less well characterized. Recent ideas about silent synapses have begun to focus attention on the AMPA class of glutamate receptors (Malenka and Nicoll, 1997). Four glutamate receptor (GluR) genes encode the heteromeric glutamate AMPA receptors (Hollmann and Heinemann, 1994). GluR2, which has a much lower Ca^{2+} permeability than the other AMPA receptor subunits, may play a dominant role in inhibiting Ca^{2+} influx through AMPA receptors (Brusa et al., 1995; Hollmann et al., 1991; Koh et al., 1995). Thus, GluR2 may have an inhibitory effect on the Ca^{2+} permeability of hippocampal neurons. Because Ca^{2+} influx is an important step in the induction of LTP, it is possible that GluR2 is the product

of a memory suppressor gene that negatively regulates synaptic plasticity.

To assess the role of GluR2 in hippocampal synaptic plasticity, Jia et al. (1996) generated knockout mice lacking the GluR2 gene. CA1 pyramidal neurons from the GluR2 mutants demonstrated higher Ca^{2+} permeability than did those from controls. Mutant hippocampal slices also exhibited significantly increased LTP magnitude in CA1 for over 2 hr in response to five trains of tetanic stimulation as compared with controls. In control slices, application of 2-amino-5-phosphovaleric acid (APV), an NMDA receptor antagonist, blocked induction of LTP by five trains of tetanic stimulation. In contrast, mutant slices demonstrated some residual LTP in response to the same protocol in the presence of APV. This residual LTP was blocked by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), suggesting that it was due to AMPA receptors. In addition, whereas LTP in the control slices saturated after three to four stimulation trains, LTP in the GluR2 mutant slices was unsaturable even after five trains. Although these results suggest that the loss of functional GluR2 leads to increased LTP magnitude following five trains of stimulation, it is important to test one- and two-train LTP, which may have different underlying molecular mechanisms, in the GluR2 mutant mice to compare these knockouts with data available from other mutant mice.

Behavioral analysis of the GluR2 knockout mice by Gerlai et al. (1998) suggests that the GluR2 knockout may cause both motor and learning deficits. The mutants were impaired in open-field, locomotor, and sensory perception tests and exhibited significant impairments in learning the visible and hidden platform versions of the Morris water maze task. The GluR2 mutant mice showed no preference for the training quadrant during the probe trial and spent most of their time in the starting quadrant. Further, the mutants were observed to float for long periods of time in the water maze. These results suggest that the GluR2 mutant mice may be impaired in spatial learning, but because the observed sensory and motor impairment may have affected performance in the water maze, it is difficult to interpret these results with respect to the relationship between synaptic plasticity and learning. It is possible that testing these mice in other hippocampus-based learning tasks, which do not have as stringent motor requirements, such as social transmission of food preference (Bunsey and Eichenbaum, 1995; Galef et al., 1988; Galef and Wigmore, 1983; Kogan et al., 1997) or object recognition (Mansuy et al., 1998), may shed more light on the nature of the behavioral effects of the GluR2 knockout. It would be especially interesting to investigate whether a hippocampus-specific GluR2 knockout, which might avoid confounding sensory and motor deficits, would result in enhancements in hippocampus-dependent tasks. In addition, a temporally regulated

hippocampal GluR2 knockout under the control of the tetracycline system might allow the study of transient increases in LTP and learning.

In contrast to the results from genetic manipulations of specific AMPA receptor subunits, pharmacological evidence suggests that facilitation of hippocampal AMPA receptors may reduce the threshold for LTP induction without changing the properties of LTP itself. Systemic administration of AMPA receptor-facilitating drugs enhances both spatial and nonspatial hippocampus-dependent learning in young and aged rats (Granger et al., 1996; Hampson et al., 1998; Larson et al., 1995; Staubli et al., 1994). In addition, facilitation of AMPA receptors in hippocampal slices significantly increases the magnitude of LTP produced by tetanic stimulation, thereby reducing the number of tetanic stimulations necessary to produce maximal LTP (Arai et al., 1996). These results suggest that facilitating hippocampal AMPA receptors enhances neuronal depolarization, leading to increased NMDA receptor currents, increased Ca^{2+} influx, and increased LTP.

L-LTP and Long-Term Memory Storage

Although our discussion has focused thus far on the early, transient phase of LTP (E-LTP) that lasts 1–2 hr, hippocampal LTP, like behavioral memory, has distinct temporal phases (Figs. 2, 3). The late phase of LTP (L-LTP) lasts for up to 8 hr in hippocampal slices (Frey et al., 1993) and for days in the intact animal (Abraham et al., 1993). L-LTP can be induced by repeated bursts of tetanic stimulation in each of the three major pathways in the hippocampal trisynaptic circuit (Huang et al., 1996b), by treatments with activators of PKA (Frey et al., 1993; Nguyen et al., 1994), or with dopamine receptor D1/D5 agonists (Huang and Kandel, 1995). L-LTP in each of the hippocampal pathways shares with long-term memory a requirement for translation and transcription (Davis and Squire, 1984; Frey et al., 1988, 1993; Huang and Kandel, 1994; Nguyen et al., 1994).

Based on pharmacological experiments suggesting that PKA plays a critical role in L-LTP (Frey et al., 1993; Huang and Kandel, 1994), Abel et al. (1997) created a line of transgenic mice with reduced hippocampal PKA activity to examine the behavioral role of different temporal stages of LTP (Abel et al., 1997). These mice express R(AB), a dominant negative inhibitor of PKA, under the control of the $\text{CaMKII}\alpha$ promoter. The R(AB) transgenic mice do not differ significantly from wild-type animals in basal synaptic transmission or short-term plasticity but have selective deficits in L-LTP in hippocampal area CA1 and corresponding deficits in long-term, but not short-term, contextual memory (Abel et al., 1997). Further, pharmacological inhibition of PKA or protein synthesis also selectively impairs long-term contextual memory (Abel et al., 1997; Bourtoouladze et al., 1998).

The dependence of L-LTP on cAMP and PKA suggests that increasing cAMP signaling at active synapses without changing basal levels of cAMP or altering basal synaptic transmission might enhance L-LTP and thus increase long-term memory. Barad et al. (1998) used low levels of rolipram, a type IV phosphodiesterase inhibitor, to selectively enhance cAMP levels at active synapses while leaving basal levels of cAMP unchanged. The effects of this manipulation were tested on synaptic plasticity and learning. In control slices, one train of stimulation to the Schaffer collateral pathway in murine hippocampal slices produced E-LTP, which decayed to baseline levels after several hours. In contrast, in the presence of a low dose of rolipram that did not alter basal cAMP levels, one train of stimulation to the Schaffer collateral pathway produced L-LTP that persisted for several hours. These effects were seen only when rolipram was present at the time of tetanus.

To examine the effects of rolipram on learning, Barad et al. (1998) treated mice with a low dose of rolipram and tested them on the contextual fear conditioning task. Mice treated with a low dose of rolipram prior to training showed no differences from controls 1 hr after training but exhibited increased freezing to context 24 hr after training. The results from low-dose rolipram treatment fit well with those from the R(AB) mice, which exhibit normal freezing to context 1 hr after training but decreased freezing as compared with controls 24 hr after training (Abel et al., 1997). One potential confounding variable is that rolipram treatment caused a significant impairment in performance on the hippocampus-independent visible platform version of the Morris water maze. These results suggest that rolipram enhances contextual learning, but without testing the effects of rolipram on other hippocampus-independent control tasks, such as cued fear conditioning, and other hippocampus-based tasks, such as the hidden platform version of the Morris water maze, it is difficult to determine whether rolipram specifically augments hippocampus-based learning.

The results of Barad et al. (1998) suggest that type IV phosphodiesterase is a memory suppressor gene product that negatively regulates L-LTP and long-term memory. Increasing cAMP at active hippocampal synapses without affecting basal levels appears to increase L-LTP and thereby enhance contextual learning. In contrast, the *Drosophila dunce* mutant, which also shows increased cAMP levels as a result of phosphodiesterase manipulation, demonstrates decreased synaptic plasticity, increased basal synaptic transmission, and learning deficits. Alterations in basal cAMP levels in *dunce* may be responsible for the observed behavioral impairments. Alternatively, the genetic manipulation may result in

developmental abnormalities or compensations not seen after transient pharmacological treatment.

With advanced age, the ability of rodents to learn and remember spatial tasks declines (Gallagher and Rapp, 1997). Aged rodents appear to have a deficit in the consolidation of short-term into long-term memory. To examine the relationship between L-LTP and age-related memory deficits, Bach et al. (1999) investigated learning and hippocampal L-LTP in aged and young mice.

The Barnes circular maze task is sensitive to manipulation of hippocampal LTP, and aged animals exhibit learning deficits in this task (Bach et al., 1995; Barnes et al., 1994, 1996; Geinisman et al., 1995). Bach et al. found that, although aged and young mice performed equally well in the cued portion of the Barnes task, 18-month-old mice performed significantly worse than younger mice (3–12 months old) on the spatial version of the task. In addition, hippocampal L-LTP was significantly decreased in the aged mice. The number of errors made in the Barnes task was significantly and negatively correlated with the magnitude of hippocampal L-LTP across all ages.

Previous evidence in young rodents has suggested that D1/D5 receptors may be critically involved in L-LTP (Fig. 3; Huang and Kandel, 1995). To determine whether D1/D5 agonists could attenuate age-related deficits in L-LTP, the researchers applied the full D1/D5 agonist 6-bromo-ApB and the partial D1/D5 agonist SKF 38393 to hippocampal slices from aged mice (Bach et al., 1999). Although 6-bromo-ApB has been shown to increase basal hippocampal synaptic transmission in young mice (Huang et al., 1996a), neither compound altered baseline synaptic responses in hippocampal slices from aged mice. Both 6-bromo-ApB and SKF 38393 significantly attenuated the age-related deficit in L-LTP when applied prior to tetanus. The agonist 6-bromo-ApB did not enhance L-LTP in hippocampal slices from young mice. When SKF 38393 was administered to aged mice, a significant dose-dependent improvement was seen in both the percentage of animals that learned the Barnes task and the number of errors made during the last block of trials. These results suggest that one of the changes in aged mice responsible for impairments in synaptic plasticity and learning may be altered signaling through D1/D5 receptors. This alteration could be at the level of the D1/D5 receptor itself or at the level of the signal transduction pathway coupled to the receptor. This hypothesis could be tested directly by comparing the effects of D1/D5 agonists, forskolin, and Sp-cAMPs on L-LTP in hippocampal slices from aged mice.

To test further whether modulation of cAMP signaling facilitates memory in aged mice, Bach et al. (1999) administered a low dose of rolipram to aged mice prior to each day of training on the Barnes task. The dose

administered to the subjects was chosen to avoid affecting basal levels of cAMP, based on the studies of Barad et al. (1998). A significant increase in the number of animals that learned the task and a significant reduction in errors during the last block of trials were observed in the rolipram-treated aged mice. It will be interesting to see if future genetic or pharmacological experiments will directly demonstrate that similar alterations specifically limited to the hippocampus result in similar enhancements in learning and memory.

New information on negative regulatory mechanisms of LTP and learning was generated from a series of studies on a member of the opioid family, nociceptin/orphanin FQ, and its receptor, ORL-1/KOR-3 (hereafter referred to as nociceptin and ORL-1). ORL-1, a G-protein coupled receptor similar to other known opioid receptors, is expressed in projection neurons in all areas of the hippocampus (Bunzow et al., 1994; Meunier et al., 1995; Mollereau et al., 1994; Reinscheid et al., 1995). ORL-1 activates G-protein coupled K^+ (GIRK) channels and Ca^{2+} -activated K^+ channels, inhibits voltage-gated Ca^{2+} channels (VGCCs) and L- and N-type Ca^{2+} channels, inhibits adenylyl cyclase, and activates the PKC pathway (Bevan et al., 1998; Connor et al., 1996; Fukuda et al., 1998; Hawes et al., 1998; Knoflach et al., 1996).

There is evidence that nociceptin and ORL-1 are involved in modulating hippocampus-based learning. Rats injected intrahippocampally with nociceptin before training show little learning in the hidden platform version of the Morris water maze task (Sandin et al., 1997). Mice lacking the ORL-1 gene learn the hidden platform version of the Morris water maze faster than controls, although visible platform performance and long-term retention do not appear to differ (Manabe et al., 1998). Whereas application of nociceptin to normal hippocampal slices attenuates the induction of L-LTP by four trains of stimulation (Yu et al., 1997; Yu and Xie, 1998), hippocampal slices from the ORL-1 mutant mice demonstrate increased E-LTP magnitude in response to one train of stimulation as compared with slices from control mice (Manabe et al., 1998).

Although these results suggest that nociceptin and ORL-1 are memory suppressor gene products that play inhibitory roles in regulating hippocampus-based synaptic plasticity and learning, additional experiments are necessary. It would be interesting to investigate whether the ORL-1 knockout mice also exhibit increased hippocampal L-LTP and whether the ORL-1 knockout alters the hippocampal frequency response function. It would also be interesting to explore learning and memory in mice lacking ORL-1 only in the hippocampus. Mice with a hippocampus-specific knockout of ORL-1 would offer an opportunity to investigate in detail the role of hippocampal ORL-1 in various forms of LTP. In addition,

testing ORL-1 knockouts on other hippocampus-based learning tasks, such as contextual fear conditioning, might generate more information on the nature of the observed learning enhancement.

As described above, transcription and translation are necessary for both L-LTP and long-term memory. C/EBP δ is a member of the CCAAT/enhancer binding protein family of transcriptional regulators that has been implicated in long-term facilitation in *Aplysia*. In *Aplysia*, C/EBP is induced in response to cAMP signals, perhaps by the transcription factor CREB, and blocking C/EBP inhibits long-term facilitation (Alberini et al., 1994). In mice, C/EBP δ , an isoform of C/EBP, is highly expressed in each region of the hippocampus. Murine C/EBP δ expression is induced by cAMP and Ca²⁺ (Yukawa et al., 1998). To explore further the role of this regulator protein in learning and memory, Sterneck et al. (1998) generated mice with a targeted deletion of the C/EBP δ gene. The C/EBP δ mutant mice were tested in both the context conditioning and Morris water maze tasks. The mutants showed no differences from controls when tested immediately after context conditioning but exhibited enhanced freezing as compared with controls 24 hr after training, suggesting selectively enhanced long-term memory. Mutants and controls performed similarly in the cued conditioned fear task. In the hidden platform version of the water maze, escape latencies were similar between controls and mutants, as was time spent in the target quadrant during the probe trial. During reversal learning, the mutants had significantly shorter escape latencies than did controls during the first block of trials. Performance on the probe trial was the same between the two groups.

These results suggest that C/EBP δ may be selectively important for long-term memory retention of contextual fear conditioning, a task sensitive to hippocampal lesions (Kim et al., 1993; Phillips and LeDoux, 1992). Because CREB and C/EBP can compete for binding to cyclic AMP response elements (CREs), it is possible that C/EBP δ normally heterodimerizes with an inhibitory CREB isoform, thereby preventing activation by CREB (Vallejo et al., 1993). In the C/EBP δ knockout mice, this inhibitory constraint on CREB is absent. In this sense, the knockout of C/EBP δ may be similar to the inactivation of ApCREB2 which leads to enhanced synaptic facilitation in *Aplysia*. Because genetic manipulation of C/EBP δ enhanced only contextual learning, it would be interesting to discover whether this transcriptional regulator is activated only by fear or contextual learning and not by spatial learning. In addition, it would be intriguing to investigate the effects of C/EBP δ knockout on the various forms of hippocampal LTP. Because C/EBP δ knockout affected only long-term memory for contextual conditioning, the C/EBP δ knockout mice may have a corresponding selective increase in hippocampal L-LTP.

CONCLUSION

The initial evidence for memory suppressor genes came from electrophysiological and molecular biological studies in *Aplysia* and from behavioral genetic studies in *Drosophila*, but recent work in rodents has begun to address this issue at both the electrophysiological and behavioral levels. Although this work is at an early stage and not all of the described experiments have examined effects on both synaptic plasticity and behavior, the experiments described above have identified putative mammalian memory suppressor genes whose products function at three distinct points in the cascade of molecular events underlying synaptic plasticity. GluR2 and PSD-95 affect LTP induction by regulating the response to glutamate, whereas ORL-1 may modulate membrane potential. Rolipram inhibits type IV phosphodiesterase, which functions at an intermediate point in the cascade of signaling pathways activated during L-LTP. ORL-1 may also act at this intermediate level by inhibiting adenylyl cyclase or altering PKC activity. In contrast, C/EBP δ may function as a memory suppressor by regulating transcription. Thus, memory suppressor genes, like tumor suppressor genes, may play roles at multiple regulatory checkpoints during hippocampal synaptic plasticity and learning.

One of the best tools for investigating the relationship between synaptic plasticity and learning may be to study enhancements of learning. Some of the memory suppressor gene products described in the present article, when inhibited, led to some form of increased learning, although others led to learning impairments. However, the precise nature of the changes in LTP which lead to enhanced learning are not clear. One possibility is that changes in LTP magnitude, LTP threshold, or the hippocampal frequency response function may be responsible for the observed increases in learning. However, it is also possible that only a narrow range of such manipulations of LTP will lead to enhanced learning. Thus, future experiments should include manipulations that selectively enhance LTP magnitude without altering threshold or frequency response. An important element of these studies should be to look at the effects of memory suppressor gene products on all three measurements, as in Migaud et al. (1998), and on the various forms of LTP. In addition, regional and temporal control of genetic manipulations of memory suppressor genes may also shed some light on these issues.

The important role of the hippocampus in a variety of behaviors provides the opportunity to examine the role of memory suppressor gene products more closely. This can be accomplished by using nonspatial and nonstressful hippocampus-dependent tasks and all the versions of the Morris water maze and contextual and cued fear conditioning. Precise behavioral information from these tasks will

generate a comprehensive picture of the selective effects of memory suppressors on various types of memory.

The study of memory suppressor genes and their products is important not only for understanding the link between synaptic plasticity and learning but also for identifying potential targets for future pharmaceuticals. Recent evidence has shown that it is possible to bring the results of animal research to bear on human problems of learning and memory. In particular, treatment with AMPA receptor facilitating drugs attenuates some of the age-related learning and memory deficits observed in aged humans (Lynch et al., 1997). Similarly, further study of mammalian memory suppressor genes may provide new insights into alleviating the learning and memory deficits that accompany both normal aging and a variety of human disorders.

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