

Molecular and Cellular Cognitive Studies of the Role of Synaptic Plasticity in Memory

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ABSTRACT: Synaptic plasticity has a central role in nearly all models of learning and memory. Besides experiments documenting changes in synaptic function during learning, most of the evidence supporting a role for synaptic plasticity in memory comes from manipulations that either enhance or lesion synaptic processes. In the last decade, mouse transgenetics (knock outs and transgenics) have provided compelling evidence that the

molecular mechanisms responsible for the induction and stability of synaptic changes have a critical role in the acquisition and storage of information. Here, I will review this literature, with a special focus on studies of hippocampal-dependent learning and memory. © 2003

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INTRODUCTION

Genetic approaches have had a major impact in nearly all fields of biology, including neuroscience. A decade ago, my colleagues and I showed that the genetic deletion of the α -calcium calmodulin-dependent kinase II (α CaMKII) impaired hippocampal long-term potentiation (Silva et al., 1992b) and disrupted spatial learning (Silva et al., 1992a) in a hippocampal-dependent task. A few months later, Grant and colleagues reported that deleting the tyrosine kinase Fyn also resulted in impairments in hippocampal long-term potentiation (LTP) and spatial learning deficits (Grant et al., 1992). These studies marked the beginning of a new field (molecular and cellular cognition) that has grown and expanded considerably in the last 10 years.

MOLECULAR AND CELLULAR COGNITION

Fields in science are defined by a subject matter and by a set of approaches and assumptions. The field of molecular and cellular cognition is no exception. The subject matter of this field is the study of mechanisms

that allow an organism to modify and fine-tune its behavioral responses to stimuli in the environment. For example, memories about past encounters allow mice to avoid certain territories of rival males where confrontation and injury is likely.

Molecular and cellular cognition is built around the idea that to understand brain processes such as memory, it will be essential to understand the basic macromolecular interactions as well as the intra- and intercellular mechanisms that allow circuits to hone in, process, store, retrieve, and edit behaviorally relevant information. Although it is extremely challenging to causally connect molecular, cellular, network, and behavioral processes, it is increasingly common to read papers that attempt to do just that. These reports are normally the result of collaborations between closely knit groups of scientists from one or more laboratories. Results from one level of analysis often become intertwined with results from the others, each level both constraining and adding to the evolving explanation of the behavior studied.

There are natural constraints in the activity and scope of any field, such as the number of technologies that members are expected to master, the information they are familiar with, the duration of training, the size and scope of manuscripts, etc. Therefore, it is not surprising that experiments in the field of molecular and cellular cognition are often different from apparently similar experiments in related fields. For example, an LTP study in a neurophysiology laboratory is

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likely to differ from a study of LTP in a laboratory in this new field. In a neurophysiology laboratory the goal may be to study mechanisms of synaptic plasticity, while in a molecular and cellular cognition laboratory the aim would be to determine how changes in these synaptic mechanisms affect learning and memory. These differences have important implications for the training of students, and for a plethora of other scientific and professional issues.

Finally, a word about the tool-box of molecular and cellular cognition. Molecular manipulations are as essential to this new field as neuroanatomical lesions are to behavioral neuroscience. The molecular tools used include transgenic manipulations (knock outs, knock ins, transgenics, viral vectors) and pharmacologic approaches. Unprecedented technical developments in genetic technology catalyzed the emergence of this field in the last 10 years. Below, I will summarize a number of transgenic studies focused on the role of synaptic plasticity in hippocampal-dependent learning and memory.

THE HIPPOCAMPUS, LTP, AND MEMORY

Patients with lesions of the hippocampal formation show a unique combination of amnesic symptoms: They include the inability to consolidate new memories and a temporally graded amnesia that affects recent memories more severely than remote ones. Remarkably, hippocampal lesions spare other cognitive functions, including intelligence, attention, empathy, emotion, etc. An important contribution of the pioneering studies with patient HM was the demonstration that memory is a process separate from other cognitive abilities and that the hippocampus has a prominent role in the consolidation of declarative memories. Following these studies, analogous experiments in monkeys, rats, and mice quickly showed that the key features of this amnesic syndrome were not uniquely human (for a recent review, see Eichenbaum, 2001).

Only two decades after the initial publication of the HM studies, a series of landmark experiments by Bliss, Lomo, and colleagues discovered that hippocampal synapses are capable of undergoing stable and long-lasting changes in synaptic strength referred to as LTP. Strikingly, they showed that this form of synaptic plasticity has properties strongly suggestive of a role in learning and memory (for a review see Malenka and Nicoll, 1999). For example, the mechanisms of LTP incorporate the specificity, associativity, reversibility, and cooperativity expected of a

memory mechanism. Additionally, computer simulations of parallel neuron networks, inspired by Hebbian-like LTP mechanisms, have been shown to process information in ways that are reminiscent of human and animal learning, suggesting that LTP-like phenomena could be mediating learning and memory. Later, a series of studies indicated that learning is accompanied by small but significant changes in synaptic plasticity. Despite some controversy surrounding the initial reports of synaptic changes triggered by learning, recent studies have confirmed these findings in the hippocampus (Moser et al., 1993) and amygdala (Rogan et al., 1997). But is synaptic plasticity necessary for hippocampal-dependent learning and memory?

One of the first manipulations used to address this question took advantage of blockers of the *N*-Methyl-D-Aspartate receptor (NMDAR), a synaptic glutamate receptor that regulates a calcium channel normally blocked by magnesium. To be activated, this receptor needs two separate events: binding of glutamate, and postsynaptic depolarization, which removes the magnesium block and allows calcium to go through the channel and induce LTP. Agents that block NMDARs cause severe deficits in hippocampal LTP and impair hippocampal-dependent learning, including spatial learning (Morris et al., 1986). However, NMDAR blockers have a number of other physiologic and behavioral effects that confound the interpretation of those studies (Cain et al., 1996; Saucier et al., 1996). Additionally, although NMDARs block all measurable LTP, they do not block all spatial learning. Indeed, spatial learning is observed under conditions that block all hippocampal LTP (Saucier and Cain, 1995). At the time that these pioneering experiments were published, there were few other manipulations that could be used to address the role of synaptic plasticity, including LTP, in learning. Fortunately, new advances in transgenic techniques provided the means to modify or delete any of the molecular components known to be involved in plasticity. The first of these components to be mutated in mice was the α -calcium calmodulin kinase II (α CaMKII).

α CAMKII: TRIGGERING THE MOLECULAR PROCESSES OF LTP AND LEARNING

α CaMKII is highly expressed in postnatal forebrain structures such as the hippocampus and cortex. Neuropharmacologic studies demonstrated that inhibiting the CaMKII family of kinases blocked the induction of LTP. This kinase is thought to potentiate synaptic

transmission by phosphorylating glutamate receptors. This is thought to increase their conductance, and may also increase the levels of these receptors in synaptic membranes (for a review see, Lisman and McIntyre, 2001). Transgenic studies showed that both a null mutation of α CaMKII (Silva et al., 1992a, 1992b) and a transgenic overexpression of a constitutively active form of this kinase (α CaMKIIT286D) (Bach et al., 1995; Mayford et al., 1995) impaired both the induction of LTP in the hippocampal CA1 region and hippocampal-dependent learning.

In addition to attempts to increase the regional and temporal specificity of mutations, efforts were also made to improve their biochemical specificity. For example, a point-mutation that substituted threonine for alanine at position 286 (T286A), prevented the autophosphorylation of α CaMKII at this site. This autophosphorylation allows the kinase to be active in the absence of calcium. Lisman and colleagues proposed that phosphorylation at this site reflected a biochemical "memory" of the enzyme's recent history of activity, and may therefore have a role in plasticity and learning. Indeed, the T286A mutation resulted in profound impairments in hippocampal LTP and learning (Giese et al., 1998). Importantly, the electrophysiologic effects of the T286A mutants are more specific than in the other α CaMKII mutants described above. For example, presynaptic plasticity was not affected in the T286A mutants, demonstrating that the autophosphorylation of this kinase is critical for post-, but not for presynaptic function (Giese et al., 1998). α CaMKII, however, is known to affect activity-dependent structural plasticity, and therefore, mutations of this kinase could affect the later stages of hippocampal development. Consequently, the learning deficits of the three mutants described above could be due to deficits in hippocampal development.

To study this possibility, the tetracycline-controlled transactivator system (tTA) was used to regulate the expression of a constitutively active form of α CaMKII (α CaMKIIT286D) (Mayford et al., 1996). With the tTA-system, a tetracycline analogue (doxycycline) is used to repress the expression of the regulated gene. Thus, with this temporally controlled system it was possible to repress the α CaMKIIT286D transgene during development and simply lift the repression at appropriate experimental times. The results of these studies were consistent with the idea that α CaMKII is vital for hippocampal LTP and learning (Mayford et al., 1996). Although these experiments represented an important step forward in transgenic studies of learning, they also raised questions concerning the physiologic meaningfulness of overexpressing a kinase with wide substrate specificity. Be-

cause the substrate specificity of α CaMKII may be in large part restricted by the localization of the enzyme, the phenotype of these mice may have been due to the nondiscriminate and unphysiologic phosphorylation of proteins.

To circumvent this problem, a new inducible strategy was used to further explore the role of α CaMKII in learning (Ohno et al., 2001). This novel strategy (synomics) takes advantage of synergies between pharmacological and genetic manipulations. In this case, α CaMKII function was regulated by combining an α CaMKII T286A heterozygous mutation with a 5-mg/kg dose of an NMDAR blocker (CPP). Unlike the homozygous T286A mutation described above, the heterozygous mutation does not have an effect on learning. Similarly, although 10 mg/kg of CPP given 30 min before training blocks a hippocampal dependent task (contextual conditioning), 5 mg/kg of this drug does not. Thus, only the combination of these two treatments (T286A mutation and CPP) can induce a contextual deficit, indicating that NMDAR-dependent activation of α CaMKII is critical for learning and memory (Ohno et al., 2001). Similar results were also obtained for hippocampal LTP (Ohno et al., 2002). Nevertheless, it is unclear how exactly these two manipulations (T286A mutation and CPP) interact to cause the phenotypes described. Although each of the experiments presented above has clear limitations, taken together the results summarized above demonstrate a role for α CaMKII in LTP and learning.

NMDAR: A MOLECULAR COINCIDENCE DETECTOR

As described above, the NMDAR is thought to be a molecular coincidence detector because its activation requires two events: binding of glutamate and neuronal depolarization (presumably driven by other inputs to the same neuron or by back-propagating spikes). Depolarization is thought to remove the magnesium that normally blocks this channel. Interestingly, a number of transgenic NMDAR manipulations were shown to affect hippocampal synaptic plasticity and learning. Deleting the NMDAR epsilon subunit, which is normally expressed only in postnatal forebrain, results in deficits in hippocampal LTP and spatial learning (Sakimura et al., 1995). Furthermore, a point mutation in the glycine site of the NMDAR1 subunit (glycine binding potentiates receptor function) also disrupts hippocampal LTP and learning (Kew et al., 2000). However, these two subunits are expressed in a wide range of cell types and brain regions, which complicates the interpretation of the

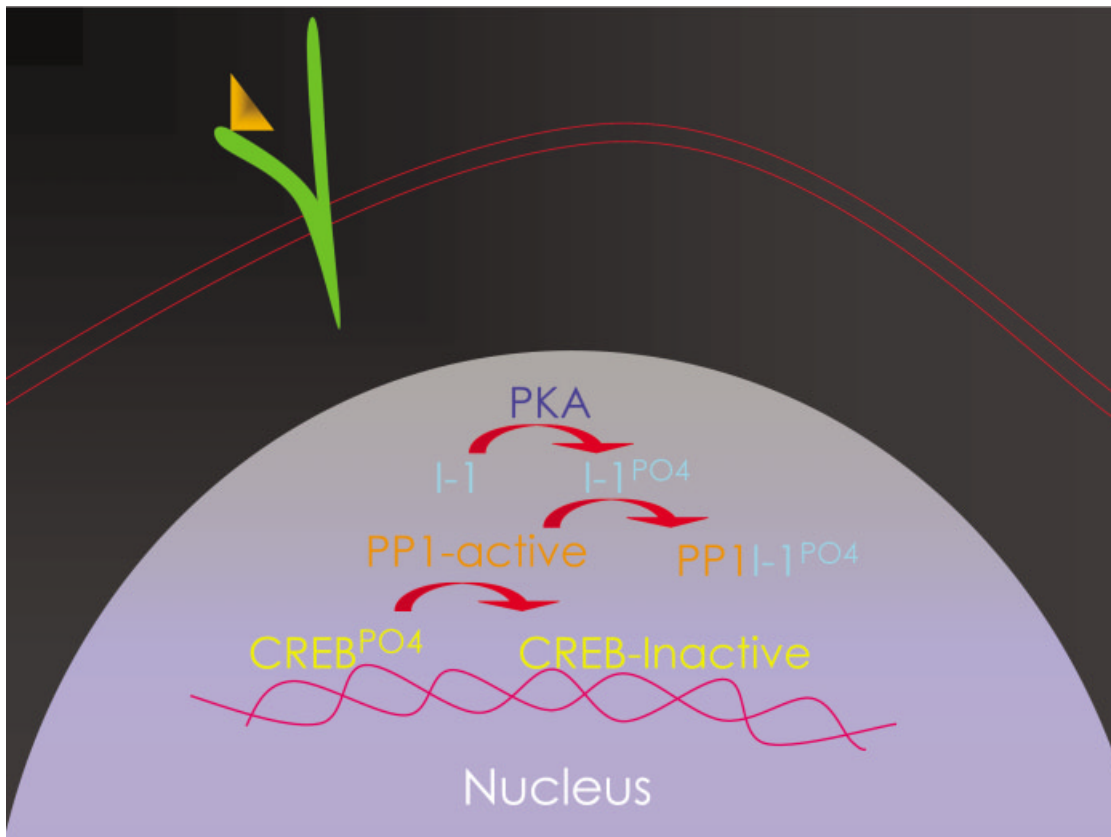


Figure 1 The antagonistic interactions between PKA and PP1. Modulatory pathways such as the adrenergic and dopaminergic receptor systems (indicated as transmembrane receptors) are thought to affect the levels of cAMP during learning and thus modulate PKA activity. Increases in PP1 activity increase the dephosphorylation of CREB, which would be expected to block memory, while increases in PKA activity drive the phosphorylation of Inhibitor-1 (I-1), which then should bind and inactivate PP1. This may allow phosphorylated CREB to promote gene expression required for the stability of synaptic changes and memory. A similar antagonism also may regulate the stability of phosphorylation of CaMKII substrates required for the induction of synaptic changes and for learning.

findings. For example, NMDARs play a key role in synaptic transmission in the neocortex. Thus, it could be that the spatial learning deficits of the two NMDAR mutants mentioned are due to problems in cortical sensory processing and not to deficits in hippocampal LTP.

To address this problem, the Cre/LoxP recombination system was used to delete the NMDAR1 subunit only in hippocampal CA1 pyramidal neurons (Tsien et al., 1996). In this transgenic system, genes flanked by LoxP sites can be deleted by the phage Cre recombinase. To restrict the deletion of the NMDAR1, Tsien and colleagues used the α CaMKII promoter to control the expression of Cre recombinase. This promoter is normally activated in postnatal excitatory neurons of the forebrain. However, the exact site where the α CaMKII-Cre transgene is inserted in the

genomic DNA can affect the range of tissues expressing the Cre recombinase. Interestingly, expression analysis showed that one of the lines used by Tsien and colleagues expressed this recombinase almost exclusively in the CA1 region of the hippocampus. By mating this line with another mouse line where the NMDAR1 was flanked by LoxP sites, these investigators were able to derive mutant mice with a CA1 specific deletion of the NMDAR1. Although LTP was disrupted only in the CA1 region, these mutants showed profound spatial learning deficits, indicating that NMDAR function in this hippocampal subregion is critical for learning (Tsien et al., 1996).

More recently, a similar approach was used to delete the NMDAR1 in the CA3 region of the hippocampus. Neurophysiologic studies of these mice revealed impaired LTP in the recurrent commissural/

associational (C/A) synapses of the hippocampus, but not in other synapses. These mutant mice appeared to perform normally in the Morris water maze under traditional training conditions, but showed clear deficits when some of the spatial guiding cues were removed from the experimental room; a result consistent with the idea that CA3 is critical for “filling in the blanks” (pattern completion) after partial cue removal (Nakazawa et al., 2002). These elegant findings demonstrated the critical role of NMDAR in synaptic plasticity and learning in both the CA3 and CA1 regions. They also suggest a novel strategy to determine the role of specific synapses in a complex memory system. However, the specificity of the gene deletions derived with this method appear to be sensitive to both age and genetic background, and therefore, when using this Cre-dependent methodology it is essential to confirm the regional specificity of the gene deletions in the animals studied, a difficult and time-consuming task.

GATING PLASTICITY AND LEARNING

Another pathway involved in learning is the cAMP-signaling pathway (Fig. 1). A number of elegant electrophysiologic studies suggested that a balance between the activities of cAMP dependent protein kinase A (PKA) and the phosphatases, PP1 and calcineurin, gate the stability of synaptic changes (Blitzer et al., 1998). At low levels of cAMP, the Ca^{2+} /CaM-dependent phosphatase calcineurin is thought to inactivate the PP1 phosphatase inhibitor 1 (I-1), activating PP1 and *closing the gate* to synaptic plasticity. In contrast, high cAMP levels (i.e., produced by Ca^{2+} stimulated adenylyl cyclases) turn on PKA. This kinase phosphorylates proteins required for LTP including I-1. Phosphorylated I-1 inhibits PPI, thus *opening the gate* to plasticity (Blitzer et al., 1998). This elegant model has been tested with a variety of mutant mice, and the results suggest that this kinase/phosphatase gate is critical for LTP and learning.

Transgenic mice expressing R(AB), an inhibitory form of the regulatory subunit of PKA, show unstable LTP and memory, as if overexpression of this inhibitor of PKA *closes the gate* to synaptic changes required for memory (Abel et al., 1997). This *gate* appears also to be *closed* by transgenic expression of a constitutively active form of calcineurin (Winder et al., 1998). Calcineurin is the neuronal form of the ubiquitous Ca/CaM-dependent Ser/Thr phosphatase 2B (PP-2B). Type 2B phosphatases are heterodimers composed of the catalytic A subunit and a regulatory B subunit. Transgenic overexpression of a truncated

catalytic subunit results in unstable LTP (Winder et al., 1998) and memory (Mansuy et al., 1998a). Importantly, repression of this calcineurin transgene under the control of the tTA system reverses the long-term memory deficits of the mutants demonstrating that these effects are not due to the developmental expression of the calcineurin transgene (Mansuy et al., 1998b). Consistent with the *gate* model proposed above, the LTP deficits of transgenic mice expressing a truncated catalytic calcineurin subunit could be partially rescued by PP1 inhibitors and by pharmacologic activation of the PKA pathway (Winder et al., 1998).

The role of calcineurin in LTP and learning was also confirmed with a modified tetracycline-inducible system. In this system, the transactivator (tTA) is mutated so that instead of repressing transcription, the mutant tTA activates it. With this reverse transactivator (rtTA), doxycycline is used to induce (not repress) the transgene, and therefore, there is no need to maintain the animals on doxycycline as is the case for the tTA system. Doxycycline may affect behavior, and is difficult to wash out of the body because it is stored in muscle and bone. Inducible overexpression of calcineurin with the rtTa system also resulted in unstable LTP and memory, indicating that the previous findings were not due to indirect effects of prolonged exposure to doxycycline (Mansuy et al., 1998). The rtTA system was also used to overexpress a COOH-terminal autoinhibitory domain that represses calcineurin function (rtTA-CN inhibitor mice) (Malleret et al., 2001). Consistent with the *gate* model, inducible overexpression of this inhibitory domain results in enhanced early and late phases of CA1 LTP. Long-term depression (LTD), on the other hand, seemed normal. Similar measurements performed *in vivo* in both anesthetized and awake mice confirmed the LTP enhancement of the rtTA-CN inhibitor mice, demonstrating that this enhancement is not an artifact of slice preparation. Interestingly, the LTP enhancement observed in the rtTA-CN inhibitor mice was observed soon after induction. Similarly, even short-term memory was enhanced in the rtTA-CN inhibitor mice (Malleret et al., 2001). In contrast, the transgenic increases in calcineurin function described above resulted in LTP and memory impairments that did not affect the earlier stages of these two processes. This indicates that the earlier stages of LTP and learning are less sensitive to calcineurin activity.

Interestingly, a recent study indicated that deleting the calcineurin regulatory subunit B (CNB KO) resulted in enhanced LTP (Zeng et al., 2001), a result consistent with the overexpression of the autoinhibitory domain of the catalytic subunit in the rtTA-CN inhibitor mice. The B subunit is needed for full acti-

vation of the heterodimeric calcineurin, and thus this deletion should result in lower calcineurin activity. Unlike the rtTA-CN inhibitor mice, the CNB KO showed impaired LTD, a finding that agrees with the hypothesis that calcineurin is required for LTD. Reflecting their different electrophysiologic phenotypes, the behavior of the rtTA-CN inhibitor mice also differ from that observed in the CNB KO. Unlike the rtTA-CN inhibitor mice, the CNB KO mice did not show enhanced learning (Zeng et al., 2001). It is possible that the loss of LTD in the CNB KO blocked this learning enhancement. Instead, these mice showed a specific deficit in spatial working memory. Other forms of learning and memory, however, appeared normal. These results suggest that working memory may be especially sensitive to deficits in LTD, perhaps because this form of plasticity may be needed for circuits that store and quickly discard information (Zeng et al., 2001). These results also demonstrate the benefits of using different genetic approaches in studying the function of a particular gene product. In addition to adding convergent data, each approach can also reveal unique facets of the function of a gene. Therefore, as new and more specific genetic technologies become available, it is important to know that they do not replace traditional approaches. Instead, these exciting new technologies allow the testing of ever more specific and intricate ideas about information processing in the brain.

ERK SIGNALING

The results summarized above demonstrate that NMDAR-dependent activation of α CaMKII and PKA/PP1/calcineurin signaling are critical for LTP and learning. However, there are other signaling mechanisms involved in these phenomena, including the ERK pathway. Both the induction of LTP and behavioral training (i.e., contextual fear conditioning) activate ERK (English and Sweatt, 1996). ERK activation can be documented with antibodies that are specific to the phosphorylated or active conformation of this kinase. Behavioral experiments with specific inhibitors against MEK, a kinase that phosphorylates and activates ERK, have indicated that this kinase is involved in LTP (English and Sweatt, 1997) and learning (Atkins et al., 1998). Recently, a study of a knockout mutant of ERK1 showed that the deletion of this kinase leads to the upregulation of ERK2 and to a subsequent enhancement in striatal LTP and striatal-dependent learning and memory (Mazzucchelli et al., 2002). This is one of the first studies of the biochem-

ical mechanisms required for striatal-dependent learning and memory (Mazzucchelli et al., 2002).

Additionally, a number of mutations of upstream regulators of ERK signaling, including Ras (Ohno et al., 2001), the Neurofibromin GTPase Activating Protein (NF1) (Silva et al., 1997; Costa et al., 2001, 2002), the guanine-nucleotide-releasing-factor (GRF) (Brambilla et al., 1997; Giese et al., 2001), and the B-Raf kinase disrupt LTP, learning, and memory. It is important to note that these signaling events may modulate both excitatory and inhibitory synaptic function.

The studies described above indicate that NMDAR-dependent activation of CaMKII, cAMP and the ERK signaling pathways play a critical role in events that trigger or gate plasticity and learning. But, what are the mechanisms responsible for the consolidation or perpetuation of synaptic changes and memory?

TRANSCRIPTIONAL REGULATION AND MEMORY

Studies in a number of organisms have demonstrated a universal requirement for transcription and translation in long-term memory (for a review see Davis and Squire, 1984). For example, protein synthesis inhibitors block later stages of LTP and memory without affecting earlier stages of these phenomena. Pioneering studies in *Aplysia* showed that the transcription factor cAMP Responsive Element Binding protein (CREB) is required for the stability of synaptic changes (Dash et al., 1990). Interestingly, a null mutation of the CREB α and δ isoforms (CREB $^{\alpha\delta-}$) in the 129/C57B16 genetic background disrupted the stability of hippocampal LTP. This same mutation also impaired long-term memory (but not short-term memory) tested in a wide range of tasks (Bourtchuladze et al., 1994; Kogan et al., 1997; Gass et al., 1998; Falls et al., 2000). Additionally, transgenic expression of a dominant-negative form of CREB that heterodimerizes with CREB, CREM, and ATF1 but inhibits DNA binding, also impaired hippocampal-dependent long-term memory. This transgenic manipulation also impaired forms of LTP that were thought to be sensitive to CREB manipulations, such as forskolin-induced and dopamine-regulated LTP (Pittenger et al., 2002).

In addition to evidence demonstrating that lower levels of CREB can result in impairments in memory, there are also results that indicate that higher levels of CREB may facilitate memory formation. For example, injection of herpes simplex virus carrying a CREB gene into the amygdala, a structure with a critical role in fear conditioning, enhanced this form

of memory (Josselyn et al., 2001). Similarly, overexpressing CREB in mice enhances LTP, suggesting that the levels of this transcription factor correlate with the ease of induction of synaptic changes underlying memory formation (Barco et al., 2002).

To better define when and where CREB is required for memory in mice, an inducible system was used to regulate the function of a transgenic CREB repressor (Kida et al., 2002). This repressor was fused to a mutant ligand-binding domain (LBD) of the estrogen receptor that binds tamoxifen instead of estrogen, a critical property that keeps circulating estrogen from confounding the experiments with this inducible system (Logie and Stewart, 1995). Importantly, this fusion protein is fully inducible in less than 6 h, and the induction is fully reversible in less than 24 h (Kida et al., 2002). By comparison, the tTA system requires days or weeks for full induction and repression. However, unlike the tTA system, which can be potentially used to regulate the expression of any gene, the LBD approach can only be used with proteins whose function can be regulated in fusions with LBD (Feil et al., 1996).

Studies with the LBD-CREB repressor mice demonstrated that this transcription factor has a critical role in memory consolidation, but not in the early stages of memory formation, because inducing the transgenic CREB-repressor blocked long-term memory, without affecting short-term memory. Induction of this transgenic repressor before testing memory recall did not affect the retrieval of memories, but it did affect the stability of the recalled memories (Kida et al., 2002). There is considerable evidence that suggests that the process of memory recall places memories in a labile state, presumably because at this stage they can be changed or edited (Sara, 2000). The studies with the transgenic CREB repressor suggest that this transcription factor is engaged during memory recall, and that its function is required for the stabilization of remembered memories (Kida et al., 2002).

In addition to experiments demonstrating a requirement for CREB function in memory, there is also evidence showing that learning activates CREB-dependent transcription. For example, experiments with antibodies specific for the phosphorylated form of CREB showed that behavioral training, as well as memory retrieval, can induce the phosphorylation (activation) of CREB (Hall et al., 2001). Additionally, experiments with a transgenic β -galactosidase gene under the regulation of a CREB-dependent promoter showed that both LTP and learning can activate CREB-dependent transcription (Impey et al., 1996). A large number of other studies have shown that CREB is involved in memory in a variety of other organisms tested (for a review, see Silva et al., 1998).

For example, elegant genetic studies in flies demonstrated a direct correlation between CREB and memory (Yin et al., 1994).

CREB is thought to regulate the expression of Zif268, a transcription factor whose expression is triggered by LTP and learning. Studies of a Zif268 null-mutant mouse showed that this transcription factor is needed for the stability of plasticity and memory (Jones et al., 2001). The Zif268 mutants show unstable LTP measured in the dentate gyrus of freely behaving mice, and impaired long-term memory. Just as the CREB mutants, the memory deficits of these mutants affected a wide range of tasks, and could be rescued by spacing the interval between training trials. These data suggest that CREB transcription leads to the synthesis of Zif268, and that the synthesis of this transcription factor leads to the transcription of genes required for memory (Jones et al., 2001). But what regulates CREB activation during memory formation?

There is extensive evidence that cAMP signaling can activate CREB (for a review, see Silva et al., 1998). Indeed, deleting both calcium/calmodulin induced adenylyl cyclases (1 and 8) destabilizes both CA1 LTP and hippocampal memory, without having a pronounced effect in the early stages of these two phenomena. Activating adenylyl cyclases with forskolin rescues the LTP and memory deficits of this double mutant, demonstrating that the effects of the mutation were not due to developmental deficits (Wong et al., 1999).

Calcium/calmodulin kinase IV (CaMKIV) is also known to activate CREB. A dominant-negative form of CaMKIV (dnCaMKIV) expressed in postnatal forebrain affected late, but not early stages of CA1 LTP. Similarly, this transgene disrupted long-, but not short-term memory tested in spatial learning and contextual fear conditioning tasks (Kang et al., 2001). Importantly, expression studies demonstrated that the dnCaMKIV transgenic mice showed lower CREB phosphorylation levels after learning, suggesting that this kinase may be involved in CREB activation during learning (Kang et al., 2001).

All of the studies reviewed so far pertain to neuronal mechanisms required for memory consolidation in the hippocampus. However, there is a growing body of data that suggests that this structure has a time-limited role in memory, and that remote memories are stored in neocortical sites (Squire et al., 2001).

REMOTE MEMORY FORMATION

A number of recent studies provided further evidence for the hypothesis that the hippocampus is only tem-

porarily involved in memory, and that eventually memories are stored in the neocortex (Squire et al., 2001). Nevertheless, both sites presumably require synaptic plasticity to store memories either temporarily (hippocampus) or permanently (neocortex). Recently, mutant mice have been used to test these ideas. Heterozygous α CaMKII mutants show deficient cortical LTP, but have seemingly intact hippocampal LTP (Frankland et al., 2001). Consistent with the hypothesis introduced above, these mutants have normal spatial memory for 3 days after training, but show a clear amnesia when tested 10–50 days after training (Frankland et al., 2001).

This time course is consistent with that revealed by previous deoxyglucose studies of hippocampal and neocortical activity: the hippocampus is activated during spatial training and in recall tests given within a few days of the end of training (5 days) (Bontempi et al., 1999). In contrast, in recall tests given 25 days after the end of training, neocortical sites, but not the hippocampus, are activated (Bontempi et al., 1999). Additionally, experiments with hippocampal-lesioned mice showed that these mice were severely impaired in spatial retention tests of information learned 3 days or 2 weeks prior to the hippocampal surgery, but remembered normally spatial information acquired between 4 and 6 weeks before surgery.

The consistency among all of these very different experiments is remarkable and it suggests that remote memory is disrupted in the heterozygous α CaMKII mutants because they lack LTP in neocortical sites required for permanent memory storage. A complementary pattern of findings was observed with mice carrying a CA1 inducible and reversible genetic lesion of the NMDA receptor (Shimizu et al., 2000). Disrupting the NMDA receptor gene in CA1 anytime within a week of training disrupted both spatial and contextual fear memory. However, disruptions at later times did not affect these two forms of memory (Shimizu et al., 2000). Interestingly, recent studies with a presenilin KO mouse suggest the provocative hypothesis that hippocampal neurogenesis in the adult may be involved in *clearing* unused memory traces from the hippocampus (Feng et al., 2001). Together, these findings shed new light onto the mechanisms required for remote memory storage.

The key strength of the studies described in the last two sections is that short-term memory is normal in the mutant mice studied, indicating that the processes required for memory acquisition (sensory processing, motivation, etc.) are unaffected in those mice, thus simplifying the interpretation of the results. However, in all of the cases presented so far, the connection between LTP and learning has been inferred from

experiments that impaired LTP. However, the history of biology is full of examples of the danger of inferring function solely from loss-of-function experiments. Therefore, it is important to ask whether molecular changes that enhance LTP also enhance learning and memory?

TRANSGENETIC MANIPULATIONS THAT ENHANCE LTP AND LEARNING

Transgenic manipulations that result in either the overexpression of genes known to be required for LTP, or the mutation of genes that normally down-regulate synaptic plasticity, can result in enhancements in LTP and learning. For example, a null mutation of the nociceptin receptor facilitates hippocampal LTP and spatial learning (Manabe et al., 1998). Activation of the nociceptin receptor leads to inhibition of adenylate cyclase and consequently to lower cAMP levels. Thus, the absence of this negative regulator of adenylate cyclase should lead to higher levels of cAMP during LTP-like events associated with learning. This could lead to increases in neuronal excitability, through the repression of calcium-dependent potassium channels, or to the potentiation of PKA activation (see above), either of which could potentially enhance learning.

The overexpression of Tissue Plasminogen Activator (TPA) also enhances LTP and learning (Madani et al., 1999). TPA is an extracellular protease that is thought to be involved in synaptic remodeling triggered by plasticity and learning. Interestingly, a TPA null mutation impairs LTP and learning (Huang et al., 1996), suggesting that inhibiting this protease leads to impairments in synaptic remodeling required for learning and memory. These results also suggest that overexpressing TPA enhances learning by promoting synaptic remodeling (Madani et al., 1999).

Similar to the TPA transgenics, a transgenic overexpression of the NMDAR subunit 2B enhances the opening time of the NMDAR, potentiates LTP and enhances learning (Tang et al., 1999). Thus, deficits in NMDA receptor function lead to impairments in LTP and learning, while mutations that increase the activity of this receptor potentiate LTP and learning. Experiments involving the deletion and the overexpression of a gene are strong tests of that gene's function. There are several other transgenic and KO manipulations that facilitate LTP and learning, such as the transgenic expression of the presynaptic Growth Associated Protein 43 (Routtenberg et al., 2000), and the mutation of a telencephalon-specific cell adhesion molecule (Nakamura et al., 2001).

It is important to note, however, that enhancements in LTP do not always result in the facilitation of learning and memory. There could be many reasons for this, including the possibility of other unknown physiologic changes, such as deficits in LTD or shifts in the thresholds between LTP and LTD (Migaud et al., 1998; Zeng et al., 2001). There are also examples of mutants with deficient hippocampal LTP but no obvious deficits in hippocampal-dependent learning. Initial studies reported that the KO of the Glutamate Receptor 1 (GluR1) results in deficits in CA1 LTP, but seemingly normal spatial learning (Zamanillo et al., 1999). However, follow-up studies found that more physiologic stimuli were able to induce nearly normal LTP in these mutants. Analogously, although the deletion of the Thy-1 gene disrupted LTP in the dentate gyrus but did not affect spatial learning (Nosten-Bertrand et al., 1996), later LTP recordings *in vivo* revealed significant levels of potentiation in the Thy-1 mutants (Errington et al., 1997).

The connections between complex biologic phenomena, such as plasticity and learning, are almost always more intricate than originally thought. For example, epigenetic phenomena, such as genomic imprinting, seemed to violate the rule that genes underlie inherited traits. However, extensive studies showed that these phenomena reflect the complexity of mechanisms that regulate gene expression during development (Surani, 2001). Similarly, future studies may reveal that glutamate receptors are not essential for the expression of all synaptic-specific changes underlying learning and memory. Perhaps other classes of channels may also be able to mediate these changes, and therefore support learning in the presence of severe glutamate receptor mutations. Therefore, some of the apparent violations of the LTP-learning hypothesis may reflect the complexity of the relation between these two phenomena. The results presented above provide strong evidence for the idea that the molecular and cellular processes required for synaptic plasticity have a key role in learning and memory. But what is the role of plasticity in hippocampal learning and memory?

IMAGING CIRCUIT AND SYSTEMS FUNCTION IN THE BRAIN

It is important to note that although the studies reported above make a compelling case for the role of synaptic plasticity in hippocampal learning and memory, most of them do not address *how* these synaptic mechanisms modulate learning. Hippocampal *in vivo* single unit recordings in mutant mice have been used

to determine how changes in plasticity affect learning. Hippocampal circuits are known to process spatial information, and *in vivo* electrophysiologic studies of hippocampal neuronal firing have shown that pyramidal neurons can fire in specific locations of the animal's immediate environment called place fields (Shapiro and Eichenbaum, 1999). The α CaMKII286D transgenics (Rotenberg et al., 1996), the T286A α CaMKII mutants, the CREB ^{$\alpha\delta^-$} mice (Cho et al., 1998), and the PKA R(AB) transgenics (Rotenberg et al., 2000), that have deficits in hippocampal LTP and spatial learning, all show unstable place fields. Additionally, pharmacologic blockade of NMDAR function also results in deficits in LTP, place cell stability, and spatial learning (Kentros et al., 1998). Importantly, the severity of the LTP and learning deficits observed in the studies mentioned above correlate with the degree of place field instability. These findings provide compelling evidence that similar mechanisms are involved in LTP and place field stability, and they suggest a connection between these two phenomena. The manipulations described above, however, did not eliminate place fields, suggesting that the ability of hippocampal circuits to generate place fields depends on mechanisms other than LTP. Interestingly, deleting NMDAR1 subunits specifically in either the hippocampal CA1 or the CA3 regions eliminated LTP in those regions and resulted in very specific alterations in the properties of place fields (McHugh et al., 1996). The place fields of the NMDAR1-CA1 mutants were more diffuse and their firing less organized. Usually, pyramidal neurons with overlapping fields show coordinated firing. However, CA1 neurons in the NMDAR1 mutants showed lower levels of correlated firing suggesting that NMDAR-dependent synaptic plasticity in the CA1 region is required for coordinating the firing patterns of these neurons (McHugh et al., 1996). Hence, it is not surprising that a deficit in the orchestration of firing of neurons in this critical region result in spatial learning deficits.

Interestingly, deletion of the NMDAR1 in the CA3 region resulted in a different place field phenotype. Just as with spatial learning, the place fields of the mutant mice were much more sensitive to partial removal of spatial cues than in controls. These results suggest that NMDAR1-synaptic plasticity in CA3 networks is critical for pattern completion of spatial information (Nakazawa et al., 2002).

Recent advances have allowed the imaging of brain systems in the mouse, a technique that shows a great deal of promise for molecular and cellular studies of cognitive function (Small et al., 2000). This magnetic resonance imaging approach relies on a

resting, rather than dynamic, T2 signals, and consequently is capable of higher resolutions that enable the study of brain structures such as the mouse hippocampus. A proof of principle study with this approach showed that the PKA R(AB) transgenics (see above), which had previously been shown to have decreased hippocampal PKA activity, show a pronounced decrease in the intensity of normalized hippocampal resting T2 signals. Importantly, neuroanatomical analysis did not detect any abnormalities, suggesting that the biochemical deficits are responsible for these signal differences (Small et al., 2000). Thus, this method may be able to detect and localize pure physiological brain lesions, a property that will be invaluable in multisystem analysis of mutant mice. An important component of the technology is the exciting possibility of carrying out parallel human and mouse studies, specifically in mouse models of cognitive disorders.

MOUSE MODELS OF COGNITIVE DISORDERS

The inherent promise of the human genome project is that the identification of genes underlying inherited disorders would provide key tools for developing animal models that would bring us closer to understanding the causes of, and developing treatments for, these disorders. Indeed, recent studies have demonstrated the potential of this approach to the study of human cognitive disorders. For example, following the identification of the Ras GTPase Activating (Ras-GAP) gene responsible for Neurofibromatosis type I (NF1), several animal models were generated that have been used to investigate this inherited disorder. Mice heterozygous for the neurofibromin gene showed deficits in hippocampal LTP and hippocampal-dependent learning, perhaps due to enhanced GABA inhibition (Costa et al., 2002). Remarkably, various molecular manipulations that decrease Ras function rescue the inhibition, LTP, and learning deficits of these mutants, suggesting a compelling connection between the enhanced inhibition and decreased LTP and learning (Costa et al., 2002). Nevertheless, the increased inhibition in these mice could also have altered other physiologic processes relevant to learning, such as the propagation of information in dendritic trees and in neuronetworks. These findings suggest that drugs that decrease GABA-mediated inhibition or Ras function (FTIs) may be useful in treating the learning disabilities associated with this disorder.

Another cognitive disorder that has been modeled extensively in mice is Alzheimer's disease (AD) (for

a review, see Ashe, 2001). Human genetic studies demonstrated that mutations in the presenilin and amyloid precursor protein (APP) genes increase the formation of $A\beta_{42}$ peptide deposited in plaques ubiquitously found in Alzheimer's brains. Mice with a variety of transgenic mutations on these two genes also develop plaques with striking similarity to those found in AD. Interestingly, these mice show behavioral deficits reminiscent of the human condition (for a review, see Ashe, 2001). Immunization with an $A\beta_{42}$ peptide decreases both the β -amyloid deposition and the learning impairments of the AD model mice tested, suggesting that similar treatments may be effective in humans (Janus et al., 2000; Morgan et al., 2000)! Also, studies in mouse models of AD have helped to forward the hypothesis that the cognitive deficits present in AD may not necessarily be the consequence of neurodegeneration observed more prominently in later stages of this disorder, because various mouse AD models show clear cognitive deficits in the absence of any neurodegeneration.

Besides their usefulness as clinical research tools, animal models of cognitive disorders may also prove critical in bridging the divide between animal and human studies of cognitive mechanisms. It is often very difficult to evaluate whether findings from animal studies really apply to human cognition. Genes may be an important link in bringing closer together studies of animal and human cognition.

SCOPE, STRUCTURE, AND FUTURE OF THE FIELD

Despite the success of this young field, the debate continues concerning the intellectual validity of some of the basic assumptions of molecular and cellular cognition. One of the most hotly debated issues concerns the validity of integrating findings from molecular, electrophysiologic, and behavioral studies of mutant mice. Some argue that the amount of knowledge at each of these levels of analysis is inadequate to allow for successful integration across levels. Although more in-depth knowledge of the phenomena involved at each of these levels (membrane receptors, LTP, place fields, etc.) would enrich the models proposed, history demonstrates that significant integrative discoveries may be made without complete understanding of the levels being integrated. For example, the discovery of DNA had an enormous impact on our understanding of heredity, even though the developmental molecular and cellular mechanisms that translate gene action into dynamic three-dimensional biologic structures are still unknown. Like

memory, development involves the complex interplay between molecular, cellular, systems and regional mechanisms. Therefore, there is no reason to suspect that the integrated approach that served biology so well in studies of development will not be equally applicable to studies of the molecular and cellular basis of cognitive function. The studies summarized here demonstrate this very point. Indeed, in the last 10 years findings from this field have provided key contributions towards establishing the idea that stable, long-lasting changes in synaptic function underlie learning and memory. Importantly, the field has already started to go beyond its focus on the role of synaptic plasticity in learning and memory. Current work is exploring the relevance to memory formation of other physiologic mechanisms, such as those controlling inhibition, excitability, and synaptic scaling. These mechanisms may determine where and when memories are encoded and stored in neuronal networks. Genetic manipulations specific to regions or cell types within these networks will be critical to work out the mechanisms of these processes.

There is also a growing emphasis in the field on improving the biochemical, temporal, and brain-region specificity of the mutations studied. This greater precision will allow researchers to study phenomena previously overshadowed by blunter and coarser manipulations. However, this does not mean that more traditional technologies will become obsolete. Instead, many of the findings mentioned in this review demonstrate that different methods reveal different facets of the function of a gene. For example, if all we knew about the NMDAR1 gene came from the elegant CA3 studies mentioned above, we would conclude that this molecule is not critical for the process that lays down memories.

As the specificity of the genetic manipulations increases, it is also essential to improve the sophistication of the molecular, electrophysiologic, and behavioral tools used in the analysis of the mutant mice. Similarly, the field must also improve the modeling tools currently used to interpret results and plan future experiments. Thus, as in its first 10 years, the future of the field of molecular and cellular cognition will continue to rest on the ability to attract scientists with diverse expertise from other areas of cognitive research, including neurophysiology, psychology, modeling, and cognitive science. Undoubtedly, the scientific convergence, derived from this crossdisciplinary integrative process, will continue to fuel the heady excitement that characterized the first 10 years of this young field.

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