

Molecular mechanisms of memory acquisition, consolidation and retrieval

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Memory is often considered to be a process that has several stages, including acquisition, consolidation and retrieval. Memory can be modified further through reconsolidation and performance can change during extinction trials while the original memory remains intact. Recent studies of the molecular basis of these processes have found that many signaling molecules are involved in several stages of memory but, in some cases, molecular pathways may be selectively recruited only during certain stages of memory.

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Abbreviations

AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
CaMK	Ca ²⁺ /calmodulin-dependent kinase
CREB	cAMP response element binding protein
CS	conditioned stimulus
ERK	extracellular signal-regulated kinase
GABA	γ -aminobutyric acid
LTP	long-term potentiation
MAP	mitogen-activated protein
NMDA	<i>N</i> -methyl-D-aspartate
PKA	protein kinase A
PKC	protein kinase C
US	unconditioned stimulus

Introduction

Memory, as measured by changes in an animal's behavior some time after learning, reflects many processes including acquisition, consolidation, retention, retrieval and performance. Molecular accounts of memory have focused mainly on the mechanisms that underlie acquisition. This emphasis is due, in large part, to the success of cellular models of learning, including forms of synaptic plasticity such as long-term potentiation (LTP). Although there are many unanswered questions about the role of LTP as a cellular mechanism of memory storage [1], it is clear that the study of LTP has provided a way to identify and characterize molecular mechanisms that potentially underlie memory storage [2]. On a molecular level, studies of LTP in hippocampal area CA1 have focused on the NMDA receptor and intracellular signaling events downstream of Ca²⁺ influx through the NMDA receptor (Figure 1). In this review, we explore recent advances in some of the neural systems and molecular processes that may mediate acquisition, consolidation and retrieval of memories for spatial learning and fear conditioning. We also explore some of

the ways that memories change after they have been retrieved during reconsolidation and extinction.

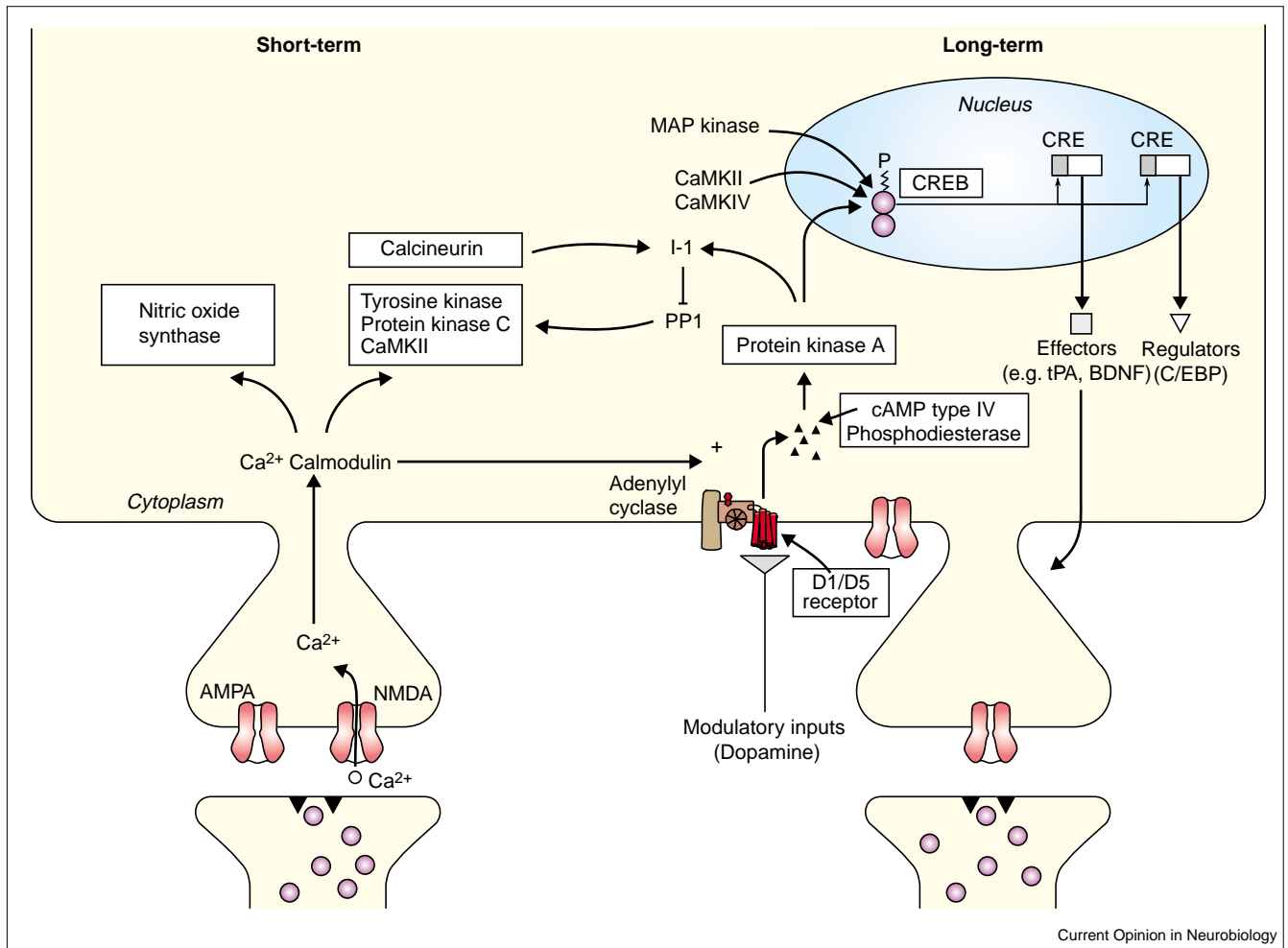
The use of pharmacological, genetic and lesion approaches has helped to define the brain systems and molecular processes important for different stages of memory, as illustrated by contextual fear conditioning (Figure 2). Memory acquisition occurs as the animal learns an association between a context and a shock. During consolidation, which can last from minutes to days, this memory is moved from a labile to a more fixed state. During retrieval, the animal is returned to the conditioning context, where memory for the context–shock association is assessed. It can be quite difficult to isolate experimentally the different stages of memory because experimental techniques potentially affect two or more stages of memory, depending on the time course of the manipulations (Figure 2). Short-lived treatments, however, can isolate consolidation independent of acquisition or retrieval. Advances in conditional genetic systems and reversible lesion techniques are bringing the time windows of these manipulations more in line with traditional pharmacological approaches [3,4].

Acquisition: the importance of temporal relations

Many neurobiological experiments examining the mechanisms that underlie associative learning have focused on delayed fear conditioning, in which a neutral conditioned stimulus (CS), such as a tone, is presented just before a footshock unconditioned stimulus (US). At the systems level, lesions of the amygdala block conditioned freezing to both the context and the tone, but lesions of the hippocampus block freezing to only the context [5]. Recent experiments have investigated the molecular mechanisms underlying trace conditioning, a variant of delayed conditioning in which an interval is inserted between the offset of the CS and the onset of the US. Several studies have shown that trace conditioning, studied in the form of trace eyeblink conditioning or trace auditory fear conditioning, is a form of episodic memory that, unlike delayed conditioning, is sensitive to lesions of the hippocampus [6–8].

Based on the role of the hippocampus in trace fear conditioning, one might expect that the same signal transduction pathways that have been implicated in other hippocampus-dependent tasks, such as context conditioning and spatial learning, would also be important for the acquisition of trace fear conditioning. At the cellular level, delayed fear conditioning appears to require the NMDA receptor [5], which acts as a coincidence detector because of its dual requirement for depolarization and glutamate [1]. Recently, Huerta *et al.* [9] have found deficits in trace auditory fear conditioning in genetically modified mice

Figure 1



Molecular events that underlie the early and late phases of long-term potentiation [2]. Stimulation of NMDA-type glutamate receptors, as a result of postsynaptic depolarization through AMPA receptors and the binding of glutamate, allows Ca^{2+} to enter the postsynaptic neuron. Among the immediate effects of Ca^{2+} are the activation of CaMKII, PKC and calcineurin. Long-lasting LTP occurs when adenylyl cyclase is activated by Ca^{2+} or by modulatory inputs, which stimulate adenylyl cyclase through G-protein-coupled receptors. This leads to increases in cAMP levels, which activate PKA, which then translocates into the nucleus where it phosphorylates CREB. Other protein kinases, such

as CaMKII, CaMKIV and MAP kinase, also regulate gene expression, and it is now understood that there is extensive crosstalk among these different kinase pathways [61]. Experiments examining the molecular basis of memory have found many of these signaling molecules to be important for acquisition and consolidation, but their role in retrieval, reconsolidation and extinction remains unclear. Adapted with permission from [2]. BDNF, brain-derived neurotrophic factor; C/EBP, CCAAT enhancer binding protein; CRE, cAMP response element; I-1, protein phosphatase inhibitor-1; PP1, protein phosphatase-1; tPA, tissue plasminogen activator.

with selective deletions of the gene encoding the R1 subunit of the NMDA receptor in hippocampal area CA1. Other recent evidence suggests that the inhibition of protein kinase A (PKA) in the hippocampus also impairs trace auditory fear conditioning, just as it interferes with contextual fear conditioning [10].

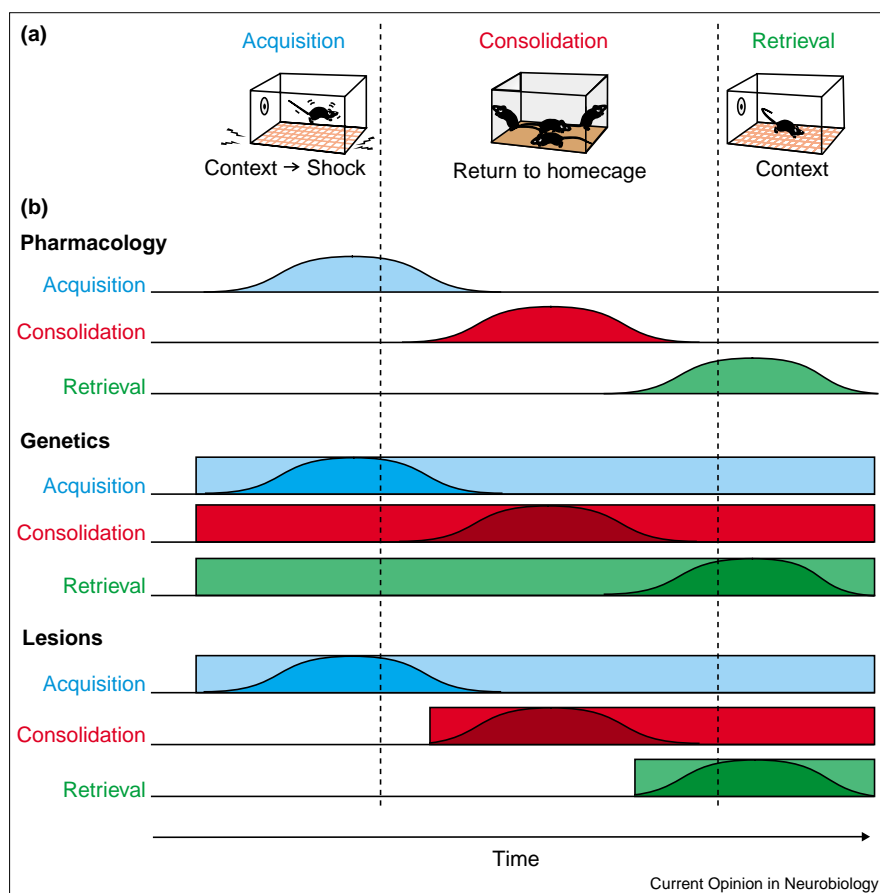
There are several ways in which the NMDA receptor, PKA and the hippocampus might be involved in contextual and trace fear conditioning. One possibility is that the hippocampus is involved in maintaining an active representation of the CS during the trace interval [8], allowing that CS trace representation to be paired with the

US despite the trace interval. Another possibility comes from the finding that hippocampal lesions lead to context deficits, and CA1 NMDA receptor knockout mice have deficits in contextual fear conditioning [11••]. Thus, the association between the CS and the US may be mediated by contextual processing that bridges the trace interval.

Consolidation: memory modulation and storage

One of the hallmarks of long-term memory storage is that newly learned information is sensitive to disruption after acquisition, a property that gives rise to the phenomenon of retrograde amnesia [12]. This labile state following training suggests that a period of consolidation occurs that

Figure 2



Stages of memory. (a) Three different stages of memory for contextual Pavlovian fear conditioning. During the acquisition stage, a memory for the context and shock is established. During the consolidation stage, the mouse is returned to its home cage, where the memory is consolidated. During retrieval, the mouse is returned to the conditioning context and memory is assessed by scoring conditioned freezing behavior. Fear conditioning often results in a robust memory for the context–shock association after a single shock presentation, which allows these stages of memory to be isolated in time. (b) The time course of different experimental manipulations used to study stages of memory. Manipulations designed to assess acquisition, consolidation and retrieval are shown in blue, red and green, respectively. The colored area represents the time course of the manipulation. Traditional genetic manipulations result in the gene of interest being present or absent throughout each stage of memory. Lesions are present during the stage of interest, as well as during subsequent stages. Pharmacological approaches offer the highest temporal specificity because they can be applied and removed from the system within a relatively short time window. Even with pharmacology, however, manipulations before acquisition will affect early stages of consolidation, and manipulations before retrieval may affect late stages of consolidation or retention. The recent use of temporally regulated transgenes and reversible lesions (shown as shaded curves) brings the temporal specificity of genetic and lesion approaches closer to that of traditional pharmacological manipulations.

may last for hours or even days. Memories can be impaired or enhanced during the consolidation period, suggesting that this labile state may have evolved as a way to allow memory to be integrated with new experience [12]. In the long term, consolidation even appears to involve the transfer of information to other brain regions, as revealed by the time-limited nature of the retrograde amnesia that occurs after lesions of the hippocampus [13^{••},14^{••}].

Although post-conditioning lesion studies are informative in disentangling effects on acquisition and consolidation, the permanence of many types of lesions makes it difficult to determine precisely the effects of a lesion on consolidation and retrieval. Thus, it is important that short-lived treatments be administered after acquisition to study consolidation processes unconfounded by effects on retrieval processes (Figure 2). By using a pharmacological approach to inactivate the hippocampus with an AMPA receptor antagonist, Riedel *et al.* [4] found that long-term inactivation during consolidation disrupted performance in a retention test. Thus, the hippocampus is important in the consolidation of spatial memories, independent of its role in acquisition or retrieval.

A similar technique has been used to investigate the role of the lateral amygdala in acquisition and consolidation. Wilensky *et al.* [15] found that temporary inactivation of the lateral amygdala by muscimol, a GABA_A agonist, immediately before conditioning blocked auditory fear conditioning, but inactivation immediately after conditioning did not. This argues that the lateral amygdala has a specific role in memory acquisition but not in memory consolidation for Pavlovian fear conditioning.

Reversible lesion studies have helped to clarify the role of the hippocampus and amygdala in consolidation. At a molecular level, recent studies have focused on specific time windows during consolidation when certain molecular processes are required. During consolidation of contextual fear memories, there are two time periods of sensitivity to inhibitors of PKA and protein synthesis: one immediately after conditioning, and another four hours later [16]. The first sensitive period may be mediated by NMDA receptor activation, whereas the second may be mediated by the action of dopaminergic systems [17]. The observation that the sensitive periods for PKA and protein

synthesis inhibition coincide supports the idea that PKA, perhaps acting through the transcription factor CREB [18], plays a central role in the cascade of events leading to the induction of gene expression and new protein synthesis both during memory acquisition and consolidation (Figure 1). Thus, there appear to be waves of consolidation that might correspond to waves of protein synthesis and gene expression [19].

In parallel to these pharmacological studies, recent biochemical experiments have shown that different signal transduction pathways are activated with distinct time courses during the consolidation period [17]. Biochemical experiments complement behavioral experiments because transient changes in kinase activity or gene expression can provide correlative evidence about the role of specific molecular pathways in consolidation. Recent biochemical studies of the ERK/MAP kinase cascade, which appears to be important for LTP and for memories for fear conditioning, have found that this cascade is selectively activated in the amygdala 60 min after auditory fear conditioning [20]. It is, however, unclear how this finding relates to the finding of Wilensky *et al.* [15] that post-training amygdala inactivation does not impair fear conditioning.

A complete analysis of the role of ERK/MAP kinase cascade in memory consolidation will require appropriate post-training pharmacological or genetic manipulations of this signal transduction pathway. PKA and ERK both target CREB [21], and studies have found increases in hippocampal PKA activity and phospho-CREB levels immediately, three and six hours after training [17]. Given the importance of PKA and CREB for fear conditioning, it will be particularly useful to determine the identity of those genes induced by learning and also whether there are distinct subsets of genes induced and proteins synthesized during the different critical periods of consolidation [19]. One of these genes might be the early growth response gene 1 (*egr1*), because expression of this gene is induced in the lateral amygdala after context conditioning [22].

Genetic approaches are particularly powerful because they provide molecular specificity as well as the potential for cell-type and regional specificity. One of the difficulties in interpreting conventional genetic experiments, however, is distinguishing effects on acquisition from effects on consolidation (Figure 2). Many investigators have interpreted a specific effect of a mutation on long-term memory as implying an involvement of that process in memory consolidation. Such an assumption is based on the idea that short-term and long-term memory are serial processes [23], but there is evidence that these processes may, in part, be parallel [24–26]. Thus, normal short-term memory does not necessarily imply that a long-term memory deficit is attributable to effects on the consolidation of long-term memory. Furthermore, there are also potential effects of these manipulations on retention rather than consolidation.

To study consolidation with genetic approaches, therefore, it is important to be able to restrict temporally the expression of a gene to the consolidation period. Recent genetic approaches have exploited the tetracycline system to achieve temporal regulation [3]. In this system, gene expression can be regulated by the administration of doxycycline, a tetracycline analog, in water or food. This system therefore has the potential to isolate consolidation, although it does not yet have the temporal specificity required to examine small time windows.

Using this inducible, spatially restricted transgenic system, Shimuzi *et al.* [11**] switched NMDA receptor function off in hippocampal area CA1 during the consolidation period. The selective suppression of NMDA receptor function for the first week after spatial training resulted in impairments in a retention test given 15 days after training. In contrast, suppression of NMDA receptor expression for the one week before retrieval did not impair performance. Similar results were obtained in studies of long-term retention of contextual fear conditioning. Interestingly, pharmacological blockade of the NMDA receptor after conditioning does not disrupt consolidation of contextual fear conditioning [27], suggesting that longer term blockade of NMDA receptor function may be necessary to inhibit consolidation, although transient blockade is sufficient to impair acquisition [5].

The finding that memory consolidation depends on the NMDA receptor is surprising because the NMDA receptor does not appear to be involved in basal synaptic transmission in hippocampal area CA1 [1]. This suggests that correlated firing of hippocampal neurons during the post-training period may be necessary for memory consolidation [28]. Alternatively, the NMDA receptor may selectively mediate basal synaptic transmission in hippocampal area CA1 during consolidation, as it appears to do in the amygdala and during a specific developmental time period in the striatum [29,30].

Retrieval: distinct molecular mechanisms?

During a test for memory, a stimulus is presented and the subject must remember what it learned previously about that stimulus. There are various neurobiological and behavioral theories about the mechanisms that underlie this retrieval process [31–33]. Analyses of retrieval are faced with the challenge of disentangling the effects of a manipulation on retrieval from its effects on performance of the behavioral task, a problem that might be particularly difficult in studies of the hippocampus [34**]. In the absence of controls that closely match the conditions for performance, it is difficult to make inferences about the role of a neurobiological process in retrieval.

Riedel *et al.* [4] have addressed the role of the hippocampus in retrieval by training animals to form a spatial preference with normal hippocampal function. When the hippocampus was temporarily inactivated before a spatial

memory test, retrieval was impaired. Animals that had temporary hippocampal inactivation, however, showed a defined but inaccurate spatial preference, suggesting that they could perform the behavior necessary to demonstrate a spatial preference. These data indicate that at a systems level the hippocampus, which is critical for memory consolidation, may also play an important role in memory retrieval (see also [35]). Retrieval of remote memories may be less dependent on the hippocampus [13•,14•].

At a cellular level, there is evidence that the NMDA receptor, which is critical for memory acquisition, is not involved in retrieval of previously established memories. Steele and Morris [36] have found that blocking NMDA receptors in the hippocampus has no effect on the retrieval of a previously established spatial memory. Thus, whereas the NMDA receptor is critical for acquisition and perhaps consolidation, it may not be involved in retrieving memories.

Similarly, PKA and protein kinase C (PKC) play important roles in acquisition, and PKA is critically involved in consolidation, but neither appears to be necessary for retrieving fear memories [16,37]. In addition, protein synthesis, which is required for consolidation, is not necessary for retrieval of conditioned fear [16,38]. These findings, coupled with the finding of NMDA-receptor-independent retrieval of spatial learning, suggest that the molecular processes involved in consolidation and retrieval might differ.

Conditional transgenic experiments, however, suggest that retrieval may share a requirement for certain signaling molecules with acquisition and consolidation. Using the tetracycline-controlled transactivator (rtTA) system, in which doxycycline turns transgene expression off, Mayford *et al.* [39] found that overexpressing a constitutively active form of CaMKII α impaired performance on a memory test. This might be attributable to effects on consolidation or retrieval, because the transgene was switched on immediately after training.

Using the reverse tetracycline-controlled transactivator (rtTA) system, in which doxycycline turns gene expression on, Mansuy *et al.* [40] were able to selectively investigate retrieval. They found that transiently overexpressing a constitutively active form of calcineurin, a serine/threonine phosphatase, led to impairments in retrieval of spatial learning. The use of overexpression experiments to study CaMKII α and calcineurin, however, does not address directly the issue of whether these proteins are normally involved in retrieval; thus, it will be particularly interesting to use regulated knockouts of these genes to examine the role of these signal transduction proteins in retrieval.

Beyond retrieval: extinction and reconsolidation

Retrieval tests result not only in reactivating the memory from acquisition, but also in establishing new memories for the events that occur during the retrieval tests themselves. During retrieval trials in contextual fear conditioning, an

animal is placed into the context in the absence of shock (Figure 2). These types of trials constitute extinction trials, which result in the diminishment of fear evoked by the context as the animal learns that the context no longer predicts shock. Many findings have demonstrated that although performance in the presence of the extinguished stimulus is diminished, the originally established association remains strong [33,41]. These experiments show that extinction is an active learning process that suppresses rather than erases the original learning. Thus, new memories are formed during extinction and one might expect that the systems involved in establishing memories for acquisition also would be involved in establishing memories for extinction. Although there is evidence that the hippocampus may be important for mediating context-dependent effects of extinction ([42,43•]; but see also [44]), its role in extinction itself remains unclear.

One structure that has been proposed to be involved in extinction is the prefrontal cortex [5,45•], although the evidence is mixed [45•,46,47]. Lesions of the ventromedial prefrontal cortex may cause increases in fear after acquisition, which complicates the interpretation of any extinction effect. Quirk *et al.* [45•] found that lesions of the ventromedial prefrontal cortex had no effect on acquisition of cued fear conditioning or on the short-term extinction of conditioned fear. When tested the next day, however, lesioned rats showed more fear, suggesting that memories for extinction were not retained from the previous day. Although lesions of the ventromedial prefrontal cortex may increase freezing, this finding suggests that the prefrontal cortex may be involved in the consolidation or retrieval of memories for extinction.

At a cellular level, there is evidence that memories established during acquisition and extinction share a requirement for the NMDA receptor. Blocking the NMDA receptor interferes with extinction of context-evoked freezing [48] and fear-potentiated startle [49]. Transgenic mice that overexpress NMDA receptor 2B show enhanced acquisition and an enhanced rate of extinction of fear conditioning [50]. This suggests that signal transduction pathways activated by Ca²⁺ influx through the NMDA receptor might mediate extinction just as they mediate acquisition. Other recent findings show that protein synthesis, which is critical for memories for contextual fear and spatial locations, is not required for memories established during extinction [38]. This is a surprising finding that suggests that acquisition and extinction may be mediated by different intracellular signaling mechanisms, although both may require NMDA receptor function.

This analysis of extinction is made even more complicated by the suggestion that the original memory goes through another phase of consolidation when it is retrieved. During this reconsolidation, the original memory is placed in a labile state, making it subject to the effects of inhibition of the same cellular and molecular processes, such as NMDA

receptor activation and protein synthesis, that are critical for the original consolidation [51,52*]. The neurobiological analyses of extinction and reconsolidation raise interesting issues about the changes that occur in memory during retrieval tests. Determining how new memories that are established during extinction interact with reconsolidated memories from acquisition will be critical for defining the molecular characteristics of learning and memory.

Conclusions

The use of systems, pharmacological, biochemical and genetic methods has done much to define the nature of learning and how it is modified during the processes of consolidation, retrieval, reconsolidation and extinction. As shown in Figure 1, the molecular mechanisms of acquisition have been extensively studied, largely on the basis of our knowledge of the intracellular signaling pathways activated by the NMDA receptor. A key challenge for the field is to determine whether the processes of consolidation, retrieval and extinction share similar mechanisms or whether they are mediated by distinct cellular processes.

Experiments outlined in this review suggest that acquisition and consolidation share many of the same molecular mechanisms. On a broader level this is intriguing, because many of these molecular mechanisms involved in learning are coincidence detectors — that is, they respond to appropriate stimuli synergistically. In the case of the NMDA receptor, these stimuli are glutamate binding and depolarization of the postsynaptic cell; for activating the cAMP/PKA pathway, these stimuli include Ca^{2+} and G-protein activation of adenylyl cyclase. During acquisition, these processes are thought to be activated by stimuli in the environment. During consolidation, these external stimuli are no longer present. Thus, a critical issue is defining the mechanisms that mediate the consolidation process.

Several possible neurobiological mechanisms present themselves. One possibility at the cellular level is that acquisition sets into motion molecular processes that are active for long periods of time, such as the generation of prolonged PKA activation after serotonin treatment in *Aplysia* sensory neurons [53]. Another possibility is that acquisition initiates a cascade of molecular events that occurs for distinct time periods, such as happens after viral infection or in the patterns of genes induced following synaptic potentiation [53]. A third possibility at the systems level is that consolidation involves the ‘replaying’ of neuronal events that occur during training. Several studies have observed neuronal firing patterns during the post-training period, including in hippocampal place cells, which have a tendency to fire during sleep in the same pattern as they fired during training [28]. This systems hypothesis therefore might include a role for sleep in memory consolidation [54].

The molecular events underlying consolidation might thus be an extension of the processes that begin during acquisition. There also is some evidence from neuropsychological

experiments that retrieval may share similar mechanisms with acquisition; memory performance is enhanced if acquisition conditions match those of retrieval, and imaging experiments have suggested that similar brain regions are activated during retrieval and acquisition [55]. Nevertheless, pharmacological, genetic and biochemical experiments suggest that retrieval may recruit different molecular mechanisms than acquisition, although these mechanisms may overlap. Similarly, extinction may be mediated by distinct systems and molecular processes [38,45*].

Beyond the techniques outlined here, recent advances in genetic approaches, as well as the development of imaging techniques for rodents, promise to revolutionize our understanding of memory processes. Improvements to regulated genetic systems allow rapid temporal regulation in specific subsets of neurons, providing the temporal resolution necessary to define the molecular basis of these aspects of memory. These regulated genetic systems include the use of conditional temperature-sensitive alleles in *Drosophila* [56], the use of an improved version of the tetracycline-regulated system [57**], and the use of steroid hormone binding domains that are specifically recognized by exogenous steroids [58].

Imaging studies have been especially informative for the identification of human brain regions active during acquisition and retrieval because these approaches allow for non-invasive, repetitive studies of neural activity. New methods have emerged recently that use imaging techniques to visualize gene expression and neural activity in rodents [59,60]. These imaging approaches provide a means to explore the role of specific intracellular signaling pathways in the processes of acquisition, consolidation, retrieval and extinction by using appropriate reporter genes. The combination of these imaging techniques with the development of rapidly regulated genetic systems promises to define the molecular processes by which memory is modified during consolidation, retrieval and extinction.

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