Karl Peter Giese · Kasia Radwanska
*Editors*

# Novel Mechanisms of Memory

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# Introduction

Understanding the mechanisms of memory is one of the biggest challenges of science. In order to face this challenge, new technologies are being intensively developed. Molecular biology tools, high-resolution microscopy, and optogenetics today allow for the precise analysis of signal transduction cascades within the living animal, cell, or single dendritic spine. Transgenic animals with very sophisticated gene manipulations and more accurate animal models of mental disorders are used to decipher the molecular basis of cognition. But still we do not know the answers to the very simple question: how and where is long-term memory stored? These basic gaps in our knowledge drive us to ask more and more detailed and precise questions about the molecular and cellular basis of cognition.

The main progress in the learning and memory field has been made with the investigation of simple animal models, such as Pavlovian fear conditioning studies with mice. During such training, an animal is exposed to a neutral conditioned stimulus (CS) (a new context, sound, or light signal) and an aversive unconditional stimulus (US) (e.g., electric shock). As a result of such experience, the conditioned stimulus acquires secondary aversive properties. The intensity of freezing response of the animal after the reexposure to the conditioned stimulus is used as a measure of fear memory strength. The early studies indicated several factors which prevent fear memory formation, and these included inhibitors of protein synthesis and transcription, antagonists of glutamatergic receptors, as well as inhibitors of intracellular cascades such as extracellular signal-regulated kinases 1 and 2 (ERK 1 and 2), α-isoform of calcium-/calmodulin-dependent kinase II (αCaMKII), or protein kinase A (PKA). These observations defined the basic molecular processes underlying memory formation and indicated directions of the research in the field. Thus recently, the model has been extensively developed. In the current book, we gathered some of the novel achievements of the field of the molecular basis of memory. These include ideas which span from the remodelling of the extracellular matrix, dynamic changes of the morphology and function of dendritic spines to epigenetic modifications occurring in the cell nucleus (Fig. 1). In nine chapters, new and important ideas related to learning and memory processes will be presented. In Chap. 1, the idea that memory after consolidation still can be dynamically remodelled

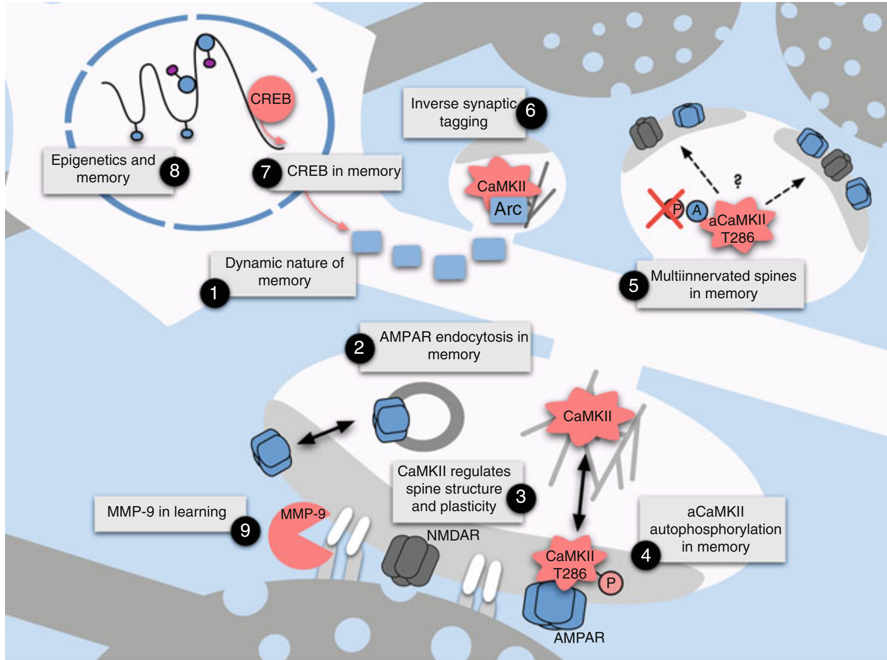


**Fig. 1** Novel mechanisms of memory drawn by Gosia Borczyk

will be introduced. This process involves the transport of AMPA receptors (Chap. 2). Furthermore, the role of CaMKII as a controller of synaptic function and information storage, which plays both enzymatic and structural roles, will be discussed (Chap. 3) together with the presentation of data supporting the critical role of alpha-CaMKII-T286 autophosphorylation in learning and memory (Chap. 4). The novel mechanism for memory formation involving the formation of big multi-innervated dendritic spines will be proposed in Chap. 5. We will also introduce the idea of the inverse synaptic tagging by Arc, the process which is suggested as a mechanism for the control of dendritic spine strength via the regulation of AMPA receptor surface expression by endocytosis (Chap. 6). Next, the possible role of CREB transcription factor as a universal memory enhancer in flies and mice (Chap. 7) and the function of chromatin modifications and DNA methylation in several forms of mammalian memory and associated synaptic plasticity (Chap. 8) will be critically discussed. Finally, a possible mechanistic role of an extracellular matrix metalloproteinase, MMP-9, in synaptic plasticity at the level of structural modulation of spine morphology will be proposed (Chap. 9).

We are aware of the fact that the topics covered in the book are just the tip of the iceberg of memory, but we believe that they are at the same time a great step toward understanding the complexity of the brain and the processes it governs. Therefore, we would like to express our gratitude for all contributing authors who were willing to share with us their exciting discoveries and ideas.

London, UK Karl Peter Giese
Warsaw, Poland Kasia Radwanska

# About the Editors

**Karl Peter Giese** studied biochemistry at the Ruhr University in Bochum, Germany, and earned a Ph.D. in neuroscience at the ETH Zurich in Switzerland. As a postdoctoral fellow at Cold Spring Harbor Laboratory, in the New York, USA, he began to investigate molecular and cellular mechanisms of memory. From 1998 until 2006, he worked as lecturer and reader at University College London, UK, and since 2006, he is Professor of Neurobiology of Mental Health at King's College London, UK. He has published more than 80 papers, and his H-index is currently 42.

**Kasia Radwanska** studied evolutionary ecology of freshwater snails at Warsaw University, Poland, and Bangor University, Wales. She earned her Ph.D. in neuroscience at Nencki Institute of Experimental Biology in Warsaw, where she began studies on the molecular basis of addiction. Next, she continued as a Marie Curie postdoctoral fellow at King's College London, UK in Prof. Karl Peter Giese's group studying molecular and cellular mechanisms of memory. Since 2013, she is a group leader at Nencki Institute of Experimental Biology working on molecular basis of alcohol addiction and memory.

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# Chapter 1
# Reconsolidation and the Dynamic Nature of Memory

**Karim Nader**

**Abstract** Research on the reconsolidation effect was greatly revitalized by the highly analytic demonstration of memory reconsolidation (Nader et al. 2000) in a well-defined behavioral protocol (auditory fear conditioning in the rat). Since this study, reconsolidation has been demonstrated in hundreds of studies over a range of species, tasks, and amnesic agents. Evidence for reconsolidation does not come solely from the behavioral level of analysis. Cellular and molecular correlates of reconsolidation have also been found. In this review I will first define the evidence on which reconsolidation is concluded to exist. I will then discuss some of the conceptual issues facing the field in determining when reconsolidation does and does not occur. Lastly I will explain the clinical implications of this effect.

**Keywords** Memory stabilization • Memory destabilization • Memory re-stabilization • Memory maintenance

## 1.1 Introduction

This is an exciting time in the study of learning and memory. Typically, in any learning and memory study, scientists broadly differentiate between certain phases of learning and memory. There is the learning phase in which information is acquired, stabilization phase in which specific mechanisms are engaged to stabilize initially unstable new information [referred to as synaptic consolidation (Glickman 1961; McGaugh 1966)], maintenance phase during which other mechanisms are involved to maintain the memory, and retrieval phase in which specific mechanisms will permit a memory to be retrieved (Miller and Springer 1973; Spear 1973). Prior to the year 2000, from a neurobiological perspective, only acquisition and memory stabilization (Martin et al. 2000; Kandel 2001; Dudai 2004) were considered to be active phases, in the sense that neurons had to perform certain computation or synthesize new RNA and proteins in order for these phases of memory processing to be

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carried out successfully. After acquisition and stabilization, all other phases were implicitly thought to be passive readout of changes in the circuits mediating the long-term memory (LTM).

Since the publication of Nader and colleagues' (2000) study demonstrating that a consolidated LTM memory can become un-stored and restored, a process coined "reconsolidation" (Nader et al. 2000), about 680 research papers have been published with this term in the title. There are now cellular and molecular models of this time-dependent memory phase.

This review will first describe the logic of the findings that brought the existence of the consolidation process into light. I will then describe how we concluded that a consolidated memory undergoes reconsolidation in a well-defined behavioral protocol (auditory fear conditioning in the rat). I will then discuss the range of species, tasks and treatments in which reconsolidation have been reported. One aspect of reconsolidation that has attracted experimental attention involves the finding that there seem to be conditions that facilitate or inhibit reconsolidation from occurring. While this is an extremely exciting aspect of the phenomenon, controversy surrounds the experimental procedures that have been employed to investigate these conditions. I present a logical approach that could help to identify the veracity of such conditions. Lastly, I will discuss clinical implications of reconsolidation and briefly review some of the results of the published clinical studies to date.

## 1.2 Consolidation: The Dominant Model of Memory Storage

Consolidation is defined as a time-dependent stabilization process leading eventually to the permanent stabilization of newly acquired memory (Ebbinghaus 1885; Müller and Pilzecker 1900; Glickman 1961; McGaugh 1966) (Fig. 1.1Ai). At the synaptic level of analysis, this process referred to as synaptic consolidation is thought to be a universal property of neurons.

The existence of the consolidation process has been shown from various lines of evidence demonstrating the presence of a post-acquisition time interval during which new memories are labile/unstable and sensitive to challenges (Fig. 1.1Ai). First, performance can be impaired by amnesic treatments, such as electroconvulsive shock (Duncan 1949), protein synthesis inhibitors (Flexner et al. 1965), or by new learning (Gordon and Spear 1973). Second, retention can be enhanced by administration of certain compounds, such as strychnine (McGaugh and Krivanek 1970). Crucially, these manipulations are only effective when administered shortly after new learning, but not when given after a few hours. These types of results led to the conclusion that memory exists in two states: when susceptible to enhancement or impairment, memory resides in a labile state, but if it is insensitive to these treatments, memory is stable and, by definition, consolidated (McGaugh 1966; Dudai 2004).

This same logic was employed by Schafe and colleagues to test for the existence of a consolidation process in the lateral and basal amygdala (LBA) for auditory fear memory. When the protein-synthesis inhibitor anisomycin is infused into the LBA

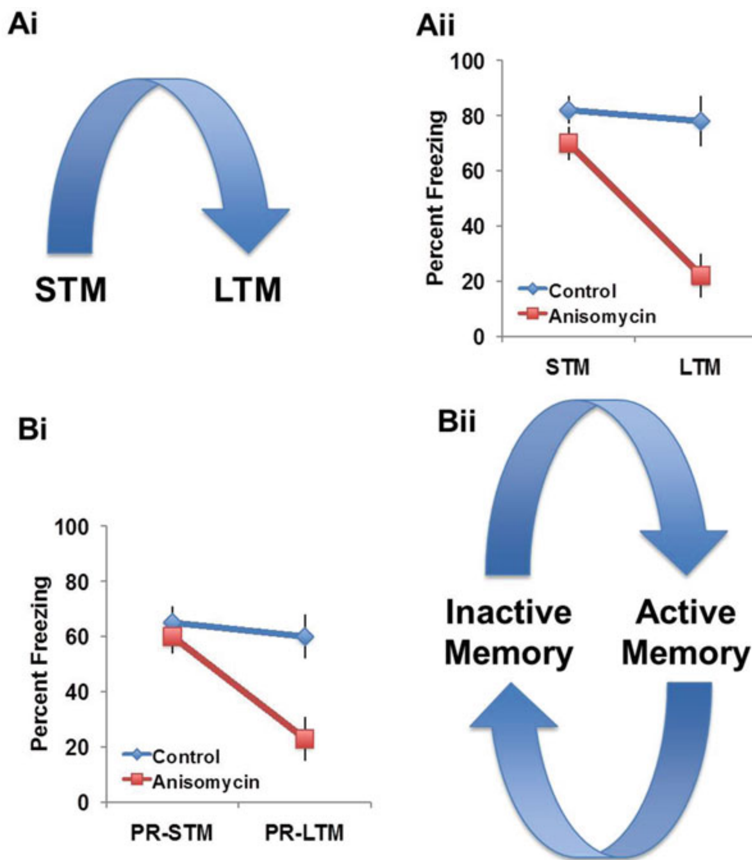


**Fig. 1.1** (**Ai**) text-book account of consolidation demonstrating that memories consolidate over time into LTM. The critical point is to show that once a memory is in LTM it is thought to remain fixed or permanent (Glickman 1961; McGaugh 1966). (**Aii**) A typical demonstration of a consolidation blockade (Schafe and LeDoux 2000). Intact STM and impaired LTM a pattern of impairment which defines a consolidation impairment (Dudai 2004; McGaugh 2004). (**Bi**) A typical demonstration of a reconsolidation blockade. Intact post-reactivation STM (PR-STM) and impaired LTM (PR-LTM), meeting the definitions for a consolidation blockade (Dudai 2004; McGaugh 2004). (**Bii**) An alternate model of memory that incorporates the findings of consolidation and reconsolidation datasets proposed by Lewis (1979). Consolidation Theory cannot explain the reconsolidation dataset. New and reactivated memories are in an Active state and then over time they stabilized and exist in an Inactive memory state. When a memory in an inactive memory state is remembered it returns to an active memory state

shortly after training, short-term memory (STM) is intact but LTM is impaired (Schafe and LeDoux 2000) (Fig. 1.1Aii); however, LTM remains intact when the infusion is delayed for 6 h. This pattern of results conforms to the operational definition of consolidation in the sense that the aspect of fear-conditioning memory that requires protein synthesis within the LBA is consolidated within at most 6 h after learning. In addition, we assume that the experimental manipulation induced amnesia

for those computations that the LBA supposedly mediates, i.e., the association between the conditioned (the tone; CS) and the unconditioned stimulus (the foot shock; US) (Rodrigues et al. 2009).

One of the basic tenets of the cellular consolidation model is that learning induces changes in synaptic efficacy, suggesting that the physiological "unit" of cellular consolidation is the synapse. Two main candidate mechanisms that were postulated to implement these changes are long-term potentiation (LTP) and long-term depression (LTD) (Malenka and Nicoll 1999; Martin et al. 2000). In parallel to the distinction of STM and LTM, with the latter being consolidated by a protein synthesis-dependent process, LTP is also divided into an early transient phase (E-LTP) and a stabilized, RNA and protein-synthesis-dependent late phase (L-LTP) (Goelet et al. 1986).

## 1.3 Behavioral Evidence for a Reconsolidation Process

The existence of a reconsolidation process in the LBA for consolidated, i.e., long-term, auditory fear memory has been concluded from a study that in logic and design followed those for consolidation as described in Schafe and colleagues (2000). One day after conditioning, at a time when, according to the results from the consolidation study, memory should be fully stabilized and immune to the amnesic agent, we reminded animals of the conditioning session by exposing them again to the CS, i.e., the tone (Nader et al. 2000). Anisomycin, at the same dose, concentration, and rate as in the Schafe and colleagues' consolidation study (Schafe and LeDoux 2000) was then either immediately or later infused into the LBA. When anisomycin was administered immediately, anisomycin-treated animals show intact post-reactivation-STM (PR-STM) but impaired PR-LTM (Fig. 1.1Bi), a pattern of results that is identical to what is found when blocking consolidation (Schafe and LeDoux 2000) (Fig. 1.1Aii). However, if the post-reactivation infusion was delayed by 6 h, anisomycin had no effect, demonstrating that the reactivation-induced instability/lability was transient. Importantly, animals that were not reminded prior to anisomycin infusions had intact memory.

Staying strictly within the commonly accepted consolidation framework, and applying only the definitions on which this framework is based, the following four conclusions can be drawn from the results of these experiments. First, the observation that the memory was insensitive to anisomycin when it was not reactivated demonstrates that it was "consolidated" 24 h after training—at least with regards to the specific amnesic treatment applied. Second, that only the reactivated memory was sensitive to disruption demonstrates that memory was in a labile state after reactivation. Third, the observation that the anisomycin-treated animals showed intact STM and impaired LTM after reactivation implies that a consolidation-like process is triggered by reactivation. And finally, given the amnesic treatment was ineffective 6 h after reactivation, this post-reactivation re-stabilization process is, like consolidation, a time-dependent process. Taken together, these four conclusions yield the interpretation that reactivation of a consolidated memory returns it again to a labile

state from which the memory has to undergo stabilization (i.e., reconsolidate) over time (Nader et al. 2000).

Consolidation and reconsolidation are thus both deduced from the evidence of a transient period of instability. In the case of consolidation, this window is initiated after acquisition of new information; in the case of reconsolidation, it is initiated after reactivation of an existing, consolidated memory representation. As is the case for consolidation, only during the reconsolidation phase can memory be enhanced by "memory enhancers" (Gordon 1977b; Rodriguez et al. 1993; Horne et al. 1997; Rodriguez et al. 1999), or impaired by amnesic treatments (Misanin et al. 1968) and interfering new learning (Gordon 1977a). These treatments are ineffective when reconsolidation is complete, which is also the case for consolidation.

The term "reconsolidation" was introduced as early as 1973, in the context of a discussion on memory retrieval. Spear asked "…how will the dynamic aspects of memory [will] be handled, that is, with successive learning trials or related successive experiences does the entire memory reconsolidate anew or merely the new information?" p. 188 (Spear 1973). As a consequence of the perceived inability of the consolidation hypothesis to account for reconsolidation, new memory models were developed that treated new and reactivated consolidated memories in similar ways (Spear 1973; Lewis 1979) (Fig. 1.1Bii).

Since the Nader and colleagues (2000)'s report, reconsolidation has been shown across a variety of species, tasks, and amnesic treatments (Table 1.1). In light of this evidence, it is therefore postulated that reconsolidation represents a fundamental memory process (Nader and Hardt 2009).

One of the most striking findings in this literature is a study by Lee (2008), who devised specific tools to block consolidation or reconsolidation mechanisms (Lee 2008). Most students of memory would assume that presenting additional learning trials to a consolidated memory would engage consolidation mechanisms, which will make the memory stronger. However, the evidence from Lee (2008)'s study suggests that a memory has to undergo reconsolidation to be strengthened. Moreover, memory strengthening by new learning was mediated by reconsolidation and not consolidation mechanisms. This evidence suggests that a recently acquired memory will be mediated by consolidation mechanisms within a time window of approximately 5 h. However, for the rest of the memory's lifetime, the memory will engage reconsolidation mechanisms. Therefore, based on this evidence, consolidation but not reconsolidation can be considered as the atypical memory process (Lee 2009).

This table lists some examples from various experimental paradigms, treatments, and species for studies reporting evidence for a reconsolidation process since the year 2000.

## 1.4 Alternative Interpretations

Reconsolidation, as we discussed above, has been defined by applying the very standards that define consolidation. Therefore, certain non-specific interpretations of the reconsolidation hypothesis pose the same challenges to the consolidation

**Table 1.1** Some of the paradigms in which reconsolidation has been reported

| | |
|---|---|
| Experimental paradigm | Habituation (Rose and Rankin 2006) |
| | Auditory fear conditioning (Nader et al. 2000) |
| | Contextual fear conditioning (Debiec et al. 2002) |
| | Instrumental Learning (Sangha et al. 2003), but see Hernandez and Kelley (2004) |
| | Inhibitory avoidance (Anokhin et al. 2002; Milekic and Alberini 2002) Conditioned aversion learning (Eisenberg et al. 2003) |
| | Motor sequence learning (Walker et al. 2003) |
| | Incentive learning (Wang et al. 2005) |
| | Object recognition (Kelly et al. 2003) |
| | Spatial memory (Suzuki et al. 2004; Morris et al. 2006) |
| | Memory for drug reward (Lee et al. 2005; Miller and Marshall 2005; Valjent et al. 2006) |
| | Episodic memory (Hupbach et al. 2007) |
| Treatment | Protein-synthesis inhibition (Nader et al. 2000) |
| | RNA synthesis inhibition (Sangha et al. 2003) |
| | Inhibition of kinase activity (Kelly et al. 2003; Duvarci et al. 2005) |
| | Protein-knockout mice (Bozon et al. 2003) Anti-sense (Taubenfeld et al. 2001; Lee et al. 2004) |
| | Inducible knockout mice (Kida et al. 2002) |
| | Receptor antagonists (Przybyslawski et al. 1999; Debiec and Ledoux 2004; Suzuki et al. 2004) |
| | Interference by new learning (Walker et al. 2003; Hupbach et al. 2007) |
| | Potentiated reconsolidation by increase in kinase activity (Tronson et al. 2006) |
| Species | Aplysia (Cai et al. 2012; Lee et al. 2012) |
| | Nematodes (Rose and Rankin 2006) |
| | Honeybees (Stollhoff et al. 2005) |
| | Snails (Sangha et al. 2003) |
| | Sea slugs (Child et al. 2003) |
| | Fish (Eisenberg et al. 2003) |
| | Crabs (Pedreira et al. 2002) |
| | Chicks (Anokhin et al. 2002) |
| | Mice (Kida et al. 2002) |
| | Rats (Nader et al. 2000); rat pups (Gruest et al. 2004) |
| | Humans (Walker et al. 2003; Hupbach et al. 2007; Kindt et al. 2009; Schiller et al. 2010) |

hypothesis, a consequence that is rarely acknowledged. The complexity of the data poses a problem for alternative interpretations, which should not merely provide new explanations for the reconsolidation dataset, but need to allow for predictions that are different from those offered by the reconsolidation model. For this reason, we will not address all the previous alternative interpretations here. A detailed discussion of these alternative interpretations including facilitation of extinction, transient retrieval impairment, non-specific effects, state-dependent learning, and new learning is presented in Nader and Hardt (2009).

## 1.5 Evidence for Reconsolidation Across Levels of Analysis

Evidence for reconsolidation does not come solely from the behavioral level of analysis. A cellular phenomenon akin to reconsolidation was shown for L-LTP (Fonseca et al. 2006). In this study, the authors report that when anisomycin is added 2 h after the induction of L-LTP it has no effect on L-LTP maintenance. If, however, the potentiated pathway is reactivated by administering test pulses that inhibit protein-synthesis, the potentiation is intact shortly after reactivation but becomes impaired over time. This suggests that reactivation of stabilized L-LTP returns its substrate to a labile state, in which it can be disrupted by inhibiting protein synthesis. This is consistent with the suggestion that the mechanisms mediating plasticity are stabilized (Finnie and Nader 2012) over time, just like consolidation (Goelet et al. 1986). Other evidence includes reports that reconsolidation blockade reverses increases in field potentials induced by fear conditioning in the LA in intact animals (Doyere et al. 2007). In sum, this evidence suggests the presence of a cellular correlate of the behaviorally-demonstrated reconsolidation impairment.

More recently, two papers using classic paradigm of *Aplysia* to study sensitization and long-term facilitation (LTF) reported that reconsolidation affects these kinds of processes. Indeed, when reconsolidation was blocked, the sensory-motor synaptic enhancement typically observed after LTF was reversed (Cai et al. 2012; Lee et al. 2012).

At the molecular level, interfering with reconsolidation can, in a time-dependent manner, remove molecular correlates of memory induced by learning and subsequent consolidation. Miller and Marshall (2005) showed that place-preference learning activates the extra-cellular signal-regulated kinase (ERK) in the nucleus accumbens (Miller and Marshall 2005). Blocking the activated ERK in the nucleus accumbens after reactivation results in intact PR-STM but impaired PR-LTM. In these amnesic animals, this also leads to the absence of activated form of ERK and its downstream transcription factors in the nucleus accumbens (see also Valjent et al. 2006 (Valjent et al. 2006) who demonstrate reduction in ERK and GluA1 phosphorylation using a similar procedure). Studying mechanisms of long-term habituation in *C. elegans*, Rose and Rankin (2006) showed that administering heat-shock or the non-NMDA glutamatergic antagonist, DMQX, after reactivation of a consolidated memory dramatically returns expression of AMPA receptors in the mechano-sensory neuron to a level typical for naïve animals (Rose and Rankin 2006). Importantly, the reconsolidation effects in all of these studies were contingent on memory reactivation—in the absence of a reminder the amnesic treatments were ineffective.

Another study by Kaang's group described, at the level of post-synaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic (AMPA) receptors, the biochemical process that destabilizes a consolidated memory and the subsequent reconsolidation process (Hong et al. 2013). Learning is thought to lead to AMPA receptor trafficking: calcium-permeable AMPA receptors are inserted into the post-synaptic density (PSD), then over time replaced by calcium-impermeable receptors (Rumpel et al. 2005).

Kaang's group asked what the AMPA receptor dynamics would be when a memory is destabilized and then reconsolidated. These authors reported that memory destabilization is associated with calcium-permeable AMPA receptors. Indeed, blocking the introduction of calcium-permeable AMPA receptors into the PSD prevented the memory from being unstored (Hong et al. 2013). Thus, they found that the replacement of calcium-impermeable AMPA receptors by calcium-permeable AMPA receptors mediated the process of reconsolidation.

These studies are a small sample of the dataset which provides striking evidence for the existence of a transient post-reactivation period of memory plasticity, i.e., memory reconsolidation, on the behavioral, physiological, and molecular levels of analysis.

## 1.6 Can Mechanisms Mediating Pre-synaptic Plasticity Undergo Reconsolidation?

Synapses usually have presynaptic and post-synaptic compartments. The electrical signal is conducted from the pre-synaptic to the post-synaptic compartment. One theory on the locus of memory posits that pre-synaptic changes are critical for LTM and L-LTP (Bliss and Collingridge 1993). These pre-synaptic changes are thought to increase the probability of vesicle release.

In all the studies that examined cellular or molecular correlates of consolidation or reconsolidation, blocking the respective memory processes reversed the learning-induced molecular/cellular correlates. For example, in a study, Bailey and colleagues (1993) reported that the blockade of consolidation in an Aplysia preparation with a protein-synthesis inhibitor prevented the increase in the number of synapses to the point where this number of synapses compared to levels of synapses in naïve animals. The same pattern of results has been shown in reconsolidation studies, as can be seen in the previous section.

Tsvetkov and colleagues (2002) have previously demonstrated that auditory fear conditioning induces predominantly pre-synaptic enhancements in both inputs to the lateral amygdala thought to mediate fear learning (Tsvetkov et al. 2002). Recently, this group assessed what would happen to these learning-induced pre-synaptic enhancements after blocking reconsolidation with rapamycin, a protein-synthesis inhibitor. They reported that these pre-synaptic enhancements were not reduced, but that a reduction in post-synaptic AMPA receptors correlated with the behavioral impairments (Li et al. 2013). This finding suggests that the post-synaptic mechanisms must detect how much potential exists on the pre-synaptic terminals and reduce the post-synaptic AMPA receptors below baseline PSD levels.

There are two theoretical implications of these findings for reconsolidation. First, perhaps, pre-synaptic mechanisms of long-term plasticity are independent of reconsolidation. This would entail that only the post-synaptic mechanisms of long-term memory could be susceptible to reconsolidation blockade. The second possibility is that pre-synaptic mechanisms are affected by reconsolidation, but the

amnesic treatment used, a protein synthesis inhibitor (PSI), was not appropriate to target the pre-synaptic mechanisms mediating reconsolidation. We know that pre-synaptic enhancements are not affected by PSIs. Therefore, a tool transiently challenging the mechanisms mediating long-term pre-synaptic efficacy would be needed to test this hypothesis.

## 1.7 Reconsolidation Is Not Universal

The fact that memory reconsolidation has been found across levels of analysis does not imply that reconsolidation is universal, i.e., observed under any circumstance. Another variation of the theme that reconsolidation is not a universal property of memory is the concept of constraints on this phenomenon, or "boundary conditions". These are situations of physiological, environmental, or psychological nature, in which memory that normally would reconsolidate no longer does. Several boundary conditions have been proposed, such as extinction consolidation (Eisenberg et al. 2003; Pederia and Maldonado 2003; Suzuki et al. 2004), memory age (Milekic and Alberini 2002; Suzuki et al. 2004), predictability of the reactivation stimulus (Pedreira et al. 2004; Morris et al. 2006) and training intensity (Suzuki et al. 2004). Others, however, have not identified similar boundary conditions in other protocols (for extinction, Stollhoff et al. 2005; Duvarci et al. 2006), old memories (Debiec et al. 2002; Lee et al. 2005), predictability of the reactivation stimulus (Pedreira et al. 2002; Bozon et al. 2003; Sangha et al. 2003; Valjent et al. 2006) or strength of training (Debiec et al. 2002; Lee et al. 2005). Whether additional parameters moderate boundary conditions remains to be seen.

The observed inconsistencies in the identification of the boundary conditions might be due to the absence of agreed-upon, standard experimental parameters required to test the presence of such boundary conditions. For example, if memory disruption is not observed within a set of experimental parameters, then it is concluded that the memory does not undergo reconsolidation under those conditions. A number of reports, however, have demonstrated that a memory may undergo reconsolidation only under specific reactivation conditions (De Vietti and Holiday 1972; Bozon et al. 2003; Suzuki et al. 2004). The implication of these findings is that it is extremely difficult to conclude based on behavioral studies that a memory never undergoes reconsolidation. Therefore the question remains whether the negative effects upon which the boundary conditions are based imply that a given memory never undergoes reconsolidation under those conditions, or the memory is still capable of undergoing reconsolidation with another reactivation protocol (Fig. 1.2). Given that the parameter space of possible reactivation procedures is essentially infinite, a real boundary condition is very difficult to prove at the behavioral level. This is likely part of why there is inconsistency in the field of boundary conditions.

Wang and colleagues (2009) took a complementary approach to identify some of the molecular mechanisms that are induced by boundary conditions to inhibit the occurrence of reconsolidation (Wang et al. 2009). If molecular or cellular indicators

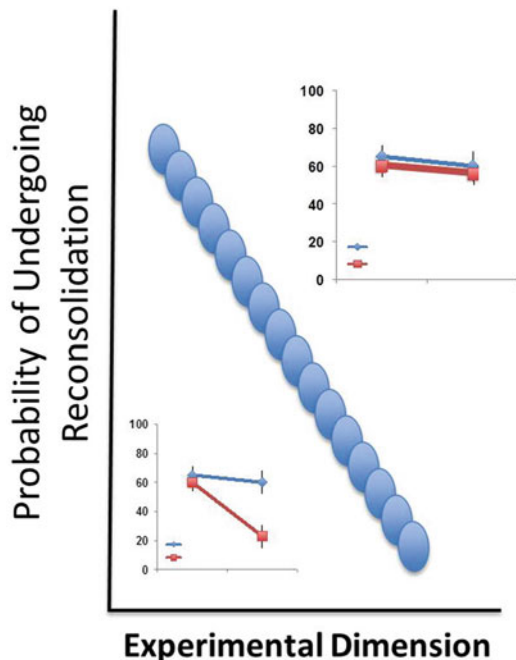


**Fig. 1.2** Possible functions describing the constraint's on reconsolidation. It is still an open question if the functions are linear or exponent. Different experimental conditions may produce different functions. The experimental space to the left of the curve is determined by examples in which the memory undergoes reconsolidation as demonstrated in the schematic behavioral impairment. The evidence for constraints on reconsolidation is derived from negative findings as shown in the schematic on the *right*. That is a logical limitation of the behavioral approach to this issue. Therefore, we suggested that a complementary approach to help resolve this issue would be to identify a molecular correlate for the absence of reconsolidation. This would act as positive evidence for the that the existence of the constraint

for when memories stop undergoing reconsolidation were identified, then we could make strong predictions concerning when we should see these mechanisms expressed (Fig. 1.3). Specifically, if strong memories, old memories, or extinction represent real boundary conditions, then the putative mechanisms mediating boundary conditions should be fully expressed within the respective memory system. Conversely, under conditions when a memory does undergo reconsolidation (e.g. weak training, little extinction, or young memories), then the mechanism mediating boundary conditions should be minimized. This strategy would significantly complement the behavioral studies described above in their search for true boundary conditions and help resolve some of the conflicting findings in the field.

An understanding of how boundary conditions are mediated across levels of analysis is critical because targeting reconsolidation of traumatic memories has been proposed to be a potential treatment for post-traumatic stress disorder (PTSD) (Przybyslawski and Sara 1997; Debiec et al. 2002; Schiller et al. 2010). Specifically, blocking the reconsolidation of traumatic memories might weaken the long-term

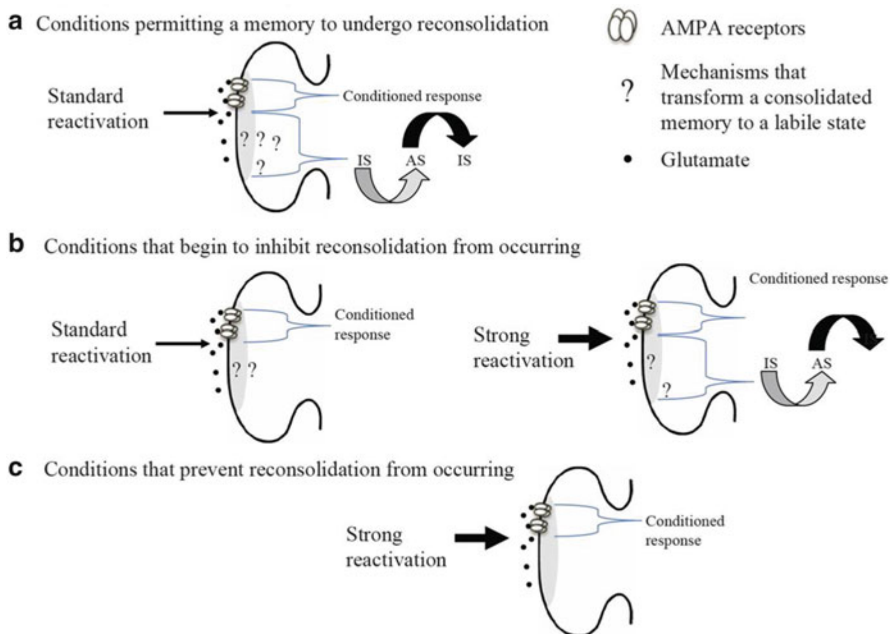


**Fig. 1.3** Conceptual diagram demonstrating how boundary conditions could inhibit memories from undergoing reconsolidation across memories types and memory systems. (**a**) Under experimental conditions when a memory undergoes reconsolidation, the mechanisms allowing a memory to be transformed from a consolidated to an labile active state (AS), must be present and functional at the synapse ("?" in figure). These mechanisms, of course, will involve more than surface receptors and will likely include a number of molecular processes that have yet to be identified. (**b**) Experimental conditions that begin to inhibit memories from undergoing reconsolidation may lead to a partial reduction in a mechanism that is critical for the induction of reconsolidation. The partial reduction might be sufficient to prevent the induction of reconsolidation when a standard protocol is used. However, there may still be sufficient amounts of this mechanism to permit the memory to undergo reconsolidation when a stronger reactivation is used. (**c**) Under conditions when the memory does not undergo reconsolidation, a boundary condition, a necessary mechanism for the induction of reconsolidation is reduced to the point that alternative reactivation protocols cannot induce the memory to undergo reconsolidation

maintenance of these traumatic memories, in turn, reducing PTSD pathology. However, if strong aversive experiences act as boundary conditions on reconsolidation (Suzuki et al. 2004), then this would suggest that the traumatic memories in PTSD patients may be resistant to undergoing reconsolidation thereby negating reconsolidation as a potential therapeutic target. Therefore, understanding boundary conditions, such as strength of training, is critical to ensure that we know if it is possible to target reconsolidation of very strong fear memories, and if so, what the optimal conditions are to allow an extremely strong fear memory to undergo reconsolidation.

To this end, Wang and colleagues (2009) found that strong auditory training produced memories that initially did not undergo reconsolidation but they did so over time on the order of 1 month. This suggests that boundary condition induced by strong training is transient (Fig. 1.4a). This in itself is striking, as the implicit

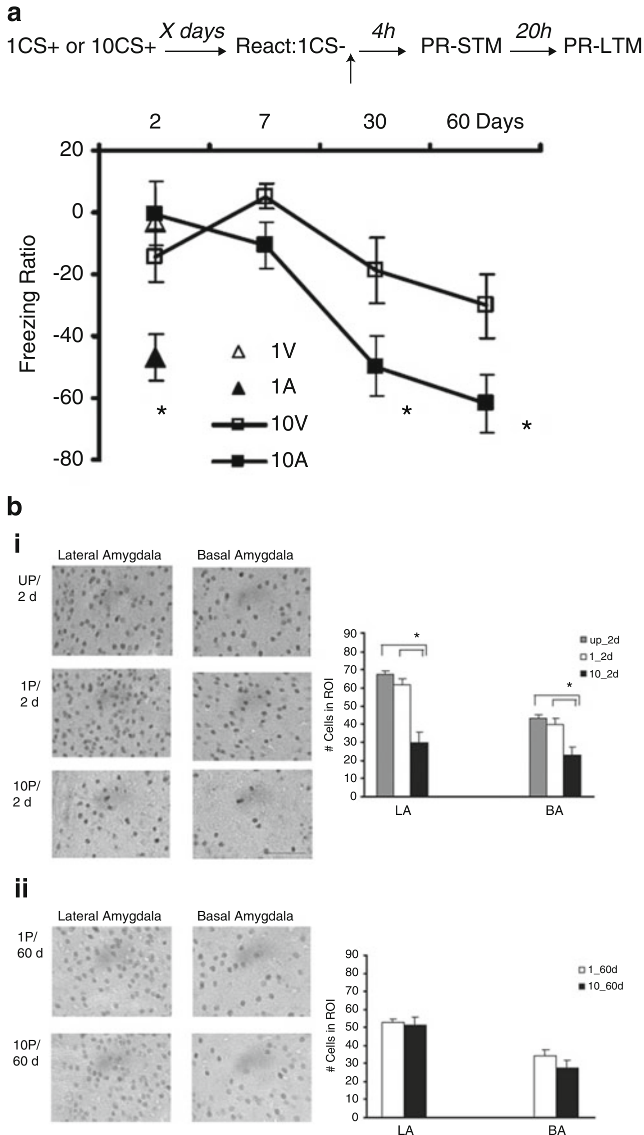


**Fig. 1.4** (**a**) Strong memories undergo reconsolidation at 30 and 60, but not 7, days after training (taken from Wang et al. 2009). The *top panel* represents the behavior protocol. Separate groups of animals were LBA-cannulated and trained with ten tone-footshock pairings. The memory was reactivated at 7, 30, or 60 days after training. The freezing ration was computed as ((PR-LTM – PR-STM)/PR-STM) × 100 %. Intra-LBA anisomycin infusion impaired the PR-LTM only when the strong memory was reactivated at 30- and 60-day after training. The *asterisk* (*) indicates significant group differences. (**b**) NR2B-subunit levels, assessed by immunohistochemistry (IHC), are inversely related to the ability of the strong memories to undergo reconsolidation over time. (*i*) Animals received ten tone-footshock pairings (10P), one pairing (1P), or one footshock followed by an unpaired tone (UP). They were sacrificed 2 days after training, a time when

assumption is that once a memory stops undergoing reconsolidation it will never begin again. This was the first demonstration that a putative boundary condition could be transient (Wang et al. 2009).

Wang and colleagues (2009) hypothesized that one principle that could mediate boundary conditions is to down-regulate the mechanisms that allow memories to undergo reconsolidation. What could be the molecular mechanism to inhibit reconsolidation of strong memories for up to 30 days after training in the LBA? Ben Mamou and colleagues (2006) demonstrated the NMDA receptors antagonists for the NR2B subunits are necessary in reactivation-induced destabilization, but that this destabilization does not get expressed in behavioral level (Ben Mamou et al. 2006). Specifically, pre-reactivation application of ifenprodil (a NR2B antagonist) prevented the memory from being impaired by post-reactivation anisomycin, however, it had no effect on the expression of freezing. New strong memories show similar properties: normal expression of freezing during reactivation but insensitivity to post-reactivation anisomycin. Ben Mamou and colleagues (2006) reasoned that strong training may down-regulate NR2B expression in the LBA, thereby making the memory insensitive to post-reactivation anisomycin infusions but capable of being expressed normally. It was hypothesized that NR2B expression in the LBA should be reduced under conditions when memories did not undergo reconsolidation but should remain normal when memories underwent reconsolidation. That was exactly what was observed. NR2B levels were normal when the memory underwent reconsolidation, but drastically reduced under the conditions in which the memory did not undergo reconsolidation (Fig. 1.4b). The reduction was subunit-selective, with NR1 subunits constant at all time-points.

The suggested role of the NR2B subunits in regulating when fear memory in the LBA will undergo reconsolidation may not generalize to all memory systems or types of memory. Currently, there are four studies that have examined the mechanisms involved in transforming a consolidated memory to a labile state. While we have demonstrated that NR2B subunit is critical for memories to return to a labile state within the LBA for fear conditioning (Ben Mamou et al. 2006), NMDA receptors in the hippocampus and within the amygdala for appetitive memories are thought to play a role in re-stabilization process (Milton et al. 2008; Suzuki et al. 2008).

**Fig. 1.4** (continued) the memory does not undergo reconsolidation, and their brains were later processed for IHC. The *left panel* represents the actual staining in regions of interest (ROI) in lateral and basal amygdala (LA, BA) in individual groups ($n=4$/group). The graph shows the quantification of NR2B-positive cell numbers in each ROI. While 1P and UP animals showed similar level of NR2B-immunostained cells, 10P animals showed significantly less stained cells in either LA or BA. The *asterisk* (*) indicates significant group differences. (*ii*) Animals received either 10P or 1P. They were sacrificed 60 days after training, a time when the memory does undergo reconsolidation, and their brains were later processed for IHC. Both groups show similar level of NR2B-positive cells in LA and BA. The scalar bar represents 80 μm. All pictures in the *left panel* are in the same scale. Each data point is represented in mean ± SEM

Milton et al. (2013) have shown that GluN2A is important for reconsolidation, whereas GluN2B is required for destabilization In the hippocampus, voltage-gated calcium channels (VGCC) (Suzuki et al. 2008) and protein-degradation (Lee et al. 2008) are critical for a memory to return to a labile state.

## 1.8 Does Reconsolidation Implies an Exact Recapitulation of Consolidation?

An important but somewhat neglected aspect of this debate is that the protocols used to study reconsolidation are different from those used to study consolidation, which renders direct comparison of results problematic. For example, in auditory fear conditioning, both CS and US are presented, leading to activation of afferents that relay auditory and pain information to the amygdala. Neurons that are thought to be the site of plasticity in the LBA are proposed to receive concurrent activation by these afferents (Blair et al. 2001). As a consequence, a series of second messenger systems are activated that are thought to lead to transcription and translation of proteins required for consolidation (Maren 2001; Schafe et al. 2001). In reconsolidation studies, however, typically only the CS is presented to reactive and induce plasticity in consolidated memory. Thus, consolidation studies examine the neurobiological changes after a CS and US are presented together, while reconsolidation studies examine neurobiological changes that happen after presentation of a CS alone. For this reason, at the brain systems/circuits and molecular level, consolidation and reconsolidation must be different, as only the former directly activates the pathways that relay US information to the amygdala, which are not directly activated in reconsolidation studies. Therefore, the demonstration of differences in brain regions or circuits mediating consolidation and reconsolidation may be rather trivial (Nader et al. 2005). It remains unclear which of the reported differences between consolidation and reconsolidation actually reflect genuine differences between the two processes as opposed to differences in the protocols used to induce them. A study in which differences between reconsolidation and consolidation were not attributable to differences in the protocols is the first to shed some light on this issue (Lee et al. 2004). The authors reported a double dissociation, separating the mechanisms mediating consolidation from those that mediate reconsolidation (Lee et al. 2004) (see also the work by Giese and colleagues (von Hertzen and Giese 2005)).

## 1.9 Clinical Implications

Consolidation and reconsolidation are processes ubiquitous to all neurons (not just those in memory systems) (Kandel 2001). The finding that consolidated memories return to a labile state and have to be restored has significant implications for a

number of clinical conditions such as post-traumatic stress disorder (PTSD), addiction, obsessive-compulsive disorder (OCD) or delusions/hallucinations. An understanding of the mechanisms mediating reconsolidation could provide the basis for developing new or refining old therapeutic tools to successfully manage if not cure some of these conditions. As an example of how this could be applied, imagine a patient with PTSD whose symptoms were resistant to both drugs and psychotherapy. A new way of treating this condition could be to reactivate the patient's traumatic memory and block its reconsolidation. Theoretically, this should lead to a "cure" within a single session. Although finding a cure in the removal of a memory in a single session may sound worthy of a fictional reality, early studies on humans using electroconvulsive therapy (ECT) demonstrates that this possibility may not be incompatible with real life.

Franks and colleagues (Rubin et al. 1969; Rubin 1976) treated patients suffering from either hallucinations, delusions, major depression, or OCD. In contrast to other studies that administered ECT when the subjects were anaesthetized, Rubin and colleagues kept the patients awake and directed them to focus on the objects of their compulsions or hallucinations. This experimental procedure reactivated the neural mechanisms mediating those memories when the ECT was delivered. All of the subjects were reportedly "cured" of their condition, even though some had had up to 30 previous ECT treatments while under anesthesia. The majority remained symptom-free for the 2 year period between the treatment and the publication of the manuscript. The fact that ECT was effective only when the memories were reactivated, but not when the memory reactivation was omitted (i.e., when the patient was anesthetized), suggests in principle that reconsolidation occurs in humans. Furthermore, this study provides evidence that the possibility of curing someone by removing a memory in a single session may not be so remote.

Today's treatments tend to be less intrusive than ECT. For example, beta-adrenergic antagonists such as propranolol have few side effects and are known to block reconsolidation of aversive and appetitive memories preferentially stored in the amygdala. The first attempt to target reconsolidation in patients with enduring PTSD symptoms reported a reduction in the strength of traumatic memories after a 15 min intervention (Brunet et al. 2008). It is important to note that some of these patients had been suffering from these PTSD symptoms for close to 30 years. Furthermore, it is remarkable that a single reactivation caused an old and consolidated memory to become un-stored again. Drug craving (Xue et al. 2012; Saladin et al. 2013) and PTSD (Brunet et al. 2008; Menzies 2012) are two clinical conditions in which it has been demonstrated that targeting their underlying maintaining mechanisms through reconsolidation can lead to significant clinical improvement. For a more extended discussion of these issues please see a recent review by Nader and colleagues (2013).

For other clinical conditions, such as OCD/major depression, that may involve multiple maintaining mechanisms, which may not be mediated by a single brain area, a new form of ECT has been shown to block reconsolidation (Kroes et al. 2014).

## 1.10 Conclusion

Reconsolidation as a memory process is a relatively recent entry in the domain of memory research. Reconsolidation has changed our view of memory from a passive to an active neurobiological process. The evidence for reconsolidation comes from a spectrum of species, amnesic agents, and reinforcers, spanning all levels of analysis from molecular, physiological, and behavioral levels, thereby suggesting that reconsolidation is a fundamental property of memory. What is more indicative of the status of reconsolidation in memory research is that evidence from both human and rodent studies has grown exponentially in the recent years.

Reconsolidation remains a topic of intensive research. One area of investigation that is being studied involves the identification of boundary conditions in reconsolidation. I have described a major limitation of the current approach to identify such boundary conditions and suggested a complementary approach to help resolve this important issue. Specifically, identifying a molecular or cellular indicator of when memories undergo reconsolidation represents this complementary approach.

There is a growing interest in utilizing reconsolidation blockage as a therapeutic tool in several clinical conditions, most importantly PTSD. Although most of the studies to date do not report significant clinical effects from targeting reconsolidation, evidence from these studies are proof-of-concept demonstrations of the usefulness of reconsolidation paradigm in clinical research. Particularly, the evidence demonstrating the effectiveness of blocking reconsolidation of traumatic memories as old as 30 years is a cause for optimism. Nonetheless future clinical research will undoubtedly benefit from advances in basic research, amongst others an increased understanding of the boundary conditions of reconsolidation.

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## References

Anokhin KV, Tiunova AA, Rose SP (2002) Reminder effects—reconsolidation or retrieval deficit? Pharmacological dissection with protein synthesis inhibitors following reminder for a passive-avoidance task in young chicks. Eur J Neurosci 15:1759–1765

Bailey CH, Kandel ER (1993) Structural changes accompanying memory storage. Annu Rev Physiol 55:397–426

Ben Mamou C, Gamache K, Nader K (2006) NMDA receptors are critical for unleashing consolidated auditory fear memories. Nat Neurosci 9:1237–1239

Blair HT, Schafe GE, Bauer EP, Rodrigues SM, LeDoux JE (2001) Synaptic plasticity in the lateral amygdala: a cellular hypothesis of fear conditioning. Learn Mem 8:229–242

Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361:31–39

Bozon B, Davis S, Laroche S (2003) A requirement for the immediate early gene zif268 in reconsolidation of recognition memory after retrieval. Neuron 40:695–701

Brunet A, Orr SP, Tremblay J, Robertson K, Nader K, Pitman RK (2008) Effect of post-retrieval propranolol on psychophysiologic responding during subsequent script-driven traumatic imagery in post-traumatic stress disorder. J Psychiatr Res 42:503–506
Cai D, Pearce K, Chen S, Glanzman DL (2012) Reconsolidation of long-term memory in aplysia. Curr Biol 22:1783–1788
Child FM, Epstein HT, Kuzirian AM, Alkon DL (2003) Memory reconsolidation in hermissenda. Biol Bull 205:218–219
De Vietti T, Holiday JH (1972) Retrograde amnesia produced by electroconvulsive shock after reactivation of a consolidated memory trace: a replication. Psychonomic Sci 29:137–138
Debiec J, Ledoux JE (2004) Disruption of reconsolidation but not consolidation of auditory fear conditioning by noradrenergic blockade in the amygdala. Neuroscience 129:267–272
Debiec J, LeDoux JE, Nader K (2002) Cellular and systems reconsolidation in the hippocampus. Neuron 36:527–538
Doyere V, Debiec J, Monfils MH, Schafe GE, LeDoux JE (2007) Synapse-specific reconsolidation of distinct fear memories in the lateral amygdala. Nat Neurosci 10:414–416
Dudai Y (2004) The neurobiology of consolidations, or, how stable is the engram? Annu Rev Psychol 55:51–86
Duncan CP (1949) The retroactive effect of electroconvulsive shock. J Comp Physiol Psychol 42:32–44
Duvarci S, Nader K, Ledoux JE (2005) Activation of extracellular signal-regulated kinase-mitogen-activated protein kinase cascade in the amygdala is required for memory reconsolidation of auditory fear conditioning. Eur J Neurosci 21:283–289
Duvarci S, Mamou CB, Nader K (2006) Extinction is not a sufficient condition to prevent fear memories from undergoing reconsolidation in the basolateral amygdala. Eur J Neurosci 24:249–260
Ebbinghaus M (1885) Über das Gedächtnis. K. Buehler, Leipzig
Eisenberg M, Kobilo T, Berman DE, Dudai Y (2003) Stability of retrieved memory: inverse correlation with trace dominance. Science 301:1102–1104
Finnie PS, Nader K (2012) The role of metaplasticity mechanisms in regulating memory destabilization and reconsolidation. Neurosci Biobehav Rev 36:1667–1707
Flexner LB, Flexner JB, Stellar E (1965) Memory and cerebral protein synthesis in mice as affected by graded amounts of puromycin. Exp Neurol 13:264–272
Fonseca R, Nagerl UV, Bonhoeffer T (2006) Neuronal activity determines the protein synthesis dependence of long-term potentiation. Nat Neurosci 9:478–480
Glickman S (1961) Preservative neural processes and consolidation of the memory trace. Psychol Bull 58:218–233
Goelet P, Castellucci VF, Schacher S, Kandel ER (1986) The long and short of long-term memory—a molecular framework. Nature 322:419–422
Gordon WC (1977a) Similarities of recently acquired and reactivated memories in interference. Am J Psychol 90:231–242
Gordon WC (1977b) Susceptibility of a reactivated memory to the effects of strychnine: a time-dependent phenomenon. Physiol Behav 18:95–99
Gordon WC, Spear NE (1973) Effect of reactivation of a previously acquired memory on the interaction between memories in the rat. J Exp Psychol 99:349–355
Gruest N, Richer P, Hars B (2004) Memory consolidation and reconsolidation in the rat pup require protein synthesis. J Neurosci 24:10488–10492
Hernandez PJ, Kelley AE (2004) Long-term memory for instrumental responses does not undergo protein synthesis-dependent reconsolidation upon retrieval. Learn Mem 11:748–754
Hong I, Kim J, Lee S, Ko HG, Nader K, Kaang BK, Tsien RW, Choi S (2013) AMPA receptor exchange underlies transient memory destabilization on retrieval. Proc Natl Acad Sci U S A 110:8218–8223
Horne CA, Rodriguez WA, Wright TP, Padilla JL (1997) Time-dependent effects of fructose on the modulation of a reactivated memory. Prog Neuropsychopharmacol Biol Psychiatry 21:649–658
Hupbach A, Gomez R, Hardt O, Nadel L (2007) Reconsolidation of episodic memories: a subtle reminder triggers integration of new information. Learn Mem 14(1–2):47–53

Kandel ER (2001) The molecular biology of memory storage: a dialogue between genes and synapses. Science 294:1030–1038
Kelly A, Laroche S, Davis S (2003) Activation of mitogen-activated protein kinase/extracellular signal-regulated kinase in hippocampal circuitry is required for consolidation and reconsolidation of recognition memory. J Neurosci 23:5354–5360
Kida S, Josselyn SA, de Ortiz SP, Kogan JH, Chevere I, Masushige S, Silva AJ (2002) CREB required for the stability of new and reactivated fear memories. Nat Neurosci 5:348–355
Kindt M, Soeter M, Vervliet B (2009) Beyond extinction: erasing human fear responses and preventing the return of fear. Nat Neurosci 12:256–258
Kroes MC, Tendolkar I, van Wingen GA, van Waarde JA, Strange BA, Fernandez G (2014) An electroconvulsive therapy procedure impairs reconsolidation of episodic memories in humans. Nat Neurosci 17:204–206
Lee JL (2008) Memory reconsolidation mediates the strengthening of memories by additional learning. Nat Neurosci 11:1264–1266
Lee JL (2009) Reconsolidation: maintaining memory relevance. Trends Neurosci 32:413–420
Lee JL, Everitt BJ, Thomas KL (2004) Independent cellular processes for hippocampal memory consolidation and reconsolidation. Science 304:839–843
Lee JL, Di Ciano P, Thomas KL, Everitt BJ (2005) Disrupting reconsolidation of drug memories reduces cocaine-seeking behavior. Neuron 47:795–801
Lee SH, Choi JH, Lee N, Lee HR, Kim JI, Yu NK, Choi SL, Lee SH, Kim H, Kaang BK (2008) Synaptic protein degradation underlies destabilization of retrieved fear memory. Science 319:1253–1256
Lee SH, Kwak C, Shim J, Kim JE, Choi SL, Kim HF, Jang DJ, Lee JA, Lee K, Lee CH, Lee YD, Miniaci MC, Bailey CH, Kandel ER, Kaang BK (2012) A cellular model of memory reconsolidation involves reactivation-induced destabilization and restabilization at the sensorimotor synapse in Aplysia. Proc Natl Acad Sci U S A 109:14200–14205
Lewis DJ (1979) Psychobiology of active and inactive memory. Psychol Bull 86:1054–1083
Li Y, Meloni EG, Carlezon WA Jr, Milad MR, Pitman RK, Nader K, Bolshakov VY (2013) Learning and reconsolidation implicate different synaptic mechanisms. Proc Natl Acad Sci U S A 110:4798–4803
Malenka RC, Nicoll RA (1999) Long-term potentiation—a decade of progress? Science 285:1870–1874
Maren S (2001) Neurobiology of Pavlovian fear conditioning. Annu Rev Neurosci 24:897–931
Martin SJ, Grimwood PD, Morris RG (2000) Synaptic plasticity and memory: an evaluation of the hypothesis. Annu Rev Neurosci 23:649–711
McGaugh JL (1966) Time-dependent processes in memory storage. Science 153:1351–1358
McGaugh JL (2004) The amygdala modulates the consolidation of memories of emotionally arousing experiences. Annu Rev Neurosci 27:1–28
McGaugh JL, Krivanek JA (1970) Strychnine effects on discrimination learning in mice: effects of dose and time of administration. Physiol Behav 5:1437–1442
Menzies RP (2012) Propranolol, traumatic memories, and amnesia: a study of 36 cases. J Clin Psychiatry 73:129–130
Milekic MH, Alberini CM (2002) Temporally graded requirement for protein synthesis following memory reactivation. Neuron 36(3):521–525
Miller CA, Marshall JF (2005) Molecular substrates for retrieval and reconsolidation of cocaine-associated contextual memory. Neuron 47:873–884
Miller RR, Springer AD (1973) Amnesia, consolidation, and retrieval. Psychol Rev 80:69–79
Milton AL, Lee JL, Butler VJ, Gardner R, Everitt BJ (2008) Intra-amygdala and systemic antagonism of NMDA receptors prevents the reconsolidation of drug-associated memory and impairs subsequently both novel and previously acquired drug-seeking behaviors. J Neurosci 28:8230–8237
Milton AL, Merlo E, Ratano P, Gregory BL, Dumbreck JK, Everitt BJ (2013) Double dissociation of the requirement for GluN2B- and GluN2A-containing NMDA receptors in the destabilization and restabilization of a reconsolidating memory. J Neurosci 33:1109–1115

Misanin JR, Miller RR, Lewis DJ (1968) Retrograde amnesia produced by electroconvulsive shock after reactivation of a consolidated memory trace. Science 160:203–204

Morris RG, Inglis J, Ainge JA, Olverman HJ, Tulloch J, Dudai Y, Kelly PA (2006) Memory reconsolidation: sensitivity of spatial memory to inhibition of protein synthesis in dorsal hippocampus during encoding and retrieval. Neuron 50:479–489

Müller GE, Pilzecker A (1900) Experimentelle beitrage zur lehre vom gedachtnis. Z Psychol Suppl. no.1:1

Nader K, Hardt O (2009) A single standard for memory: the case for reconsolidation. Nat Rev Neurosci 10:224–234

Nader K, Schafe GE, Le Doux JE (2000) Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. Nature 406:722–726

Nader K, Hardt O, Wang SH (2005) Response to Alberini: right answer, wrong question. Trends Neurosci 28:346–347

Nader K, Hardt O, Lanius R (2013) Memory as a new therapeutic target. Dialogues Clin Neurosci 15:475–486

Pederia ME, Maldonado H (2003) Protein synthesis subserves reconsolidation or extinction depending on reminder duration. Neuron 38:863–869

Pedreira ME, Perez-Cuesta LM, Maldonado H (2002) Reactivation and reconsolidation of long-term memory in the crab Chasmagnathus: protein synthesis requirement and mediation by NMDA-type glutamatergic receptors. J Neurosci 22:8305–8311

Pedreira ME, Perez-Cuesta LM, Maldonado H (2004) Mismatch between what is expected and what actually occurs triggers memory reconsolidation or extinction. Learn Mem 11:579–585

Przybyslawski J, Sara SJ (1997) Reconsolidation of memory after its reactivation. Behav Brain Res 84:241–246

Przybyslawski J, Roullet P, Sara SJ (1999) Attenuation of emotional and nonemotional memories after their reactivation: role of beta adrenergic receptors. J Neurosci 19:6623–6628

Rodrigues SM, LeDoux JE, Sapolsky RM (2009) The influence of stress hormones on fear circuitry. Annu Rev Neurosci 32:289–313

Rodriguez WA, Rodriguez SB, Phillips MY, Martinez JL Jr (1993) Post-reactivation cocaine administration facilitates later acquisition of an avoidance response in rats. Behav Brain Res 59:125–129

Rodriguez WA, Horne CA, Padilla JL (1999) Effects of glucose and fructose on recently reactivated and recently acquired memories. Prog Neuro-Psychopharmacol Biol Psychiatry 23:1285–1317

Rose JK, Rankin CH (2006) Blocking memory reconsolidation reverses memory-associated changes in glutamate receptor expression. J Neurosci 26:11582–11587

Rubin RD (1976) Clinical use of retrograde amnesia produced by electroconvulsive shock: a conditioning hypothesis. Can Psychiatr Assoc J 21:87–90

Rubin RD, Fried R, Franks CM (1969) New application of ECT. In: Rubin RD, Franks C (eds) Advances in behavior therapy, 1968. Academic, New York, pp 37–44

Rumpel S, LeDoux J, Zador A, Malinow R (2005) Postsynaptic receptor trafficking underlying a form of associative learning. Science 308:83–88

Saladin ME, Gray KM, McRae-Clark AL, Larowe SD, Yeatts SD, Baker NL, Hartwell KJ, Brady KT (2013) A double blind, placebo-controlled study of the effects of post-retrieval propranolol on reconsolidation of memory for craving and cue reactivity in cocaine dependent humans. Psychopharmacology (Berl) 226:721–737

Sangha S, Scheibenstock A, Lukowiak K (2003) Reconsolidation of a long-term memory in Lymnaea requires new protein and RNA synthesis and the soma of right pedal dorsal 1. J Neurosci 23:8034–8040

Schafe GE, LeDoux JE (2000) Memory consolidation of auditory pavlovian fear conditioning requires protein synthesis and protein kinase A in the amygdala. J Neurosci 20:RC96

Schafe GE, Nader K, Blair HT, LeDoux JE (2001) Memory consolidation of Pavlovian fear conditioning: a cellular and molecular perspective. Trends Neurosci 24:540–546

Schiller D, Monfils MH, Raio CM, Johnson DC, Ledoux JE, Phelps EA (2010) Preventing the return of fear in humans using reconsolidation update mechanisms. Nature 463:49–53

Spear N (1973) Retrieval of memory in animals. Psychol Rev 80:163–194

Stollhoff N, Menzel R, Eisenhardt D (2005) Spontaneous recovery from extinction depends on the reconsolidation of the acquisition memory in an appetitive learning paradigm in the honeybee (Apis mellifera). J Neurosci 25:4485–4492

Suzuki A, Josselyn SA, Frankland PW, Masushige S, Silva AJ, Kida S (2004) Memory reconsolidation and extinction have distinct temporal and biochemical signatures. J Neurosci 24:4787–4795

Suzuki A, Mukawa T, Tsukagoshi A, Frankland PW, Kida S (2008) Activation of LVGCCs and CB1 receptors required for destabilization of reactivated contextual fear memories. Learn Mem 15:426–433

Taubenfeld SM, Milekic MH, Monti B, Alberini CM (2001) The consolidation of new but not reactivated memory requires hippocampal C/EBPbeta. Nat Neurosci 4:813–818

Tronson NC, Wiseman SL, Olausson P, Taylor JR (2006) Bidirectional behavioral plasticity of memory reconsolidation depends on amygdalar protein kinase A. Nat Neurosci 9:167–169

Tsvetkov E, Carlezon WA, Benes FM, Kandel ER, Bolshakov VY (2002) Fear conditioning occludes LTP-induced presynaptic enhancement of synaptic transmission in the cortical pathway to the lateral amygdala. Neuron 34:289–300

Valjent E, Aubier B, Corbille AG, Brami-Cherrier K, Caboche J, Topilko P, Girault JA, Herve D (2006) Plasticity-associated gene Krox24/Zif268 is required for long-lasting behavioral effects of cocaine. J Neurosci 26:4956–4960

von Hertzen LS, Giese KP (2005) Memory reconsolidation engages only a subset of immediate-early genes induced during consolidation. J Neurosci 25:1935–1942

Walker MP, Brakefield T, Hobson JA, Stickgold R (2003) Dissociable stages of human memory consolidation and reconsolidation. Nature 425:616–620

Wang SH, Ostlund SB, Nader K, Balleine BW (2005) Consolidation and reconsolidation of incentive learning in the amygdala. J Neurosci 25:830–835

Wang SH, de Oliveira AL, Nader K (2009) Cellular and systems mechanisms of memory strength as a constraint on auditory fear reconsolidation. Nat Neurosci 12:905–912

Xue YX, Luo YX, Wu P, Shi HS, Xue LF, Chen C, Zhu WL, Ding ZB, Bao YP, Shi J, Epstein DH, Shaham Y, Lu L (2012) A memory retrieval-extinction procedure to prevent drug craving and relapse. Science 336:241–245

# Chapter 2
# AMPA Receptor Plasticity in Retrieval, Reconsolidation and Post-retrieval Extinction of Memories

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**Abstract** Learning and memory are evolutionary evolved 'survival factors'. They are fundamental in nature, as most species use stored information related to experience to adapt to constantly changing environments. When experiences are aversive (fearful), they should be avoided upon renewed contact. Thus, coupling environmental stimuli to aversive events enables the individual to respond adequately later in time. This involves implicit (non-declarative) learning in which the resulting associative memories can be recalled reflexively and may have lifetime persistence. Sometimes this learning can be maladaptive, resulting in a hyper-responsive system, in which stimulus-induced recall of memory leads to excessive fear and anxiety. In order to develop new therapeutic strategies to treat such disorders, it is important to understand the neural mechanisms of the capacity to encode, store, consolidate, retrieve and erase information. In this chapter we will touch upon synaptic plasticity mechanisms that underlie memory formation, and focus on the role of AMPA receptor (AMPAR) plasticity in the amygdala and hippocampus during the retrieval, destabilization and subsequent restabilization of memory.

**Keywords** Hippocampus • Amygdala • Contextual fear conditioning • Aversive memory • Glutamate receptor trafficking • Synaptic plasticity

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## 2.1 Distinct Processes of Fear Memory Formation and Retrieval

Over the years it has become increasingly clear that the process of memory formation and storage, including the phase following memory recall, is a dynamic and temporally defined encoding process that includes stabilization and destabilization of a memory not only at the level of behavioral expression, but also at the synaptic and cellular level (Nader 2003; Tronson and Taylor 2007).

Initially a labile short-term memory (STM) is transformed into a stable and persistent long-term memory (LTM) by the process of consolidation. For fear conditioning, a paradigm in which animals learn to associate an otherwise neutral stimulus (after learning referred to as conditioned stimulus; CS) with a foot-shock (unconditioned stimulus; US) this process typically takes 6 h, after which it is insensitive to pharmacological disruption (Muller and Pilzecker 1900; Hebb 1949; Glickman 1961; McGaugh 1966; Nader 2003). At the cellular level, at shorter time scales, molecular or synaptic learning takes place at synapses of particular brain areas, such as the hippocampus and amygdala, followed on a longer time scale by a gradual involvement of other brain regions in memory processing, leading to a 'reorganization' of memory *content* across different brain regions and systems, a process referred to as systems consolidation (Nader et al. 2000b).

The first hint that memory formation is not a linear process, but in fact dynamic in nature comes from early gross systemic amnestic manipulations given around the time of memory recall, which resulted in a loss of memory in subsequent retrieval tests (Misanin et al. 1968; Mactutus et al. 1979; Sara 2000) (Fig. 2.1). In order to better characterize this phenomenon, more sensitive studies from the laboratory of LeDoux and others found that disrupting the same cellular processes that act during initial consolidation, such as protein synthesis, activation of the CREB pathway and kinase activity, produced amnesia that was contingent on memory retrieval (Tronson and Taylor 2007; Nader and Hardt 2009). This showed that memory returns to a labile state after retrieval and requires a process of re-stabilization in order to persist further (Nader et al. 2000a; Schafe et al. 2001; Nader and Hardt 2009; Nader and Einarsson 2010). This active destabilization of synaptic memory and the process of re-stabilization that follows have been termed as 'reconsolidation' and are crucial to memory processing based on further experience (Nader et al. 2000a; Tronson and Taylor 2007). Reconsolidation is fundamental, but not ubiquitous (Tronson and Taylor 2007; Nader and Hardt 2009) and there appear to be boundary conditions or limiting factors, such as age and strength of the memory, and length of the reactivation trial, that determine the occurrence of reconsolidation (Wang et al. 2009; Nader and Einarsson 2010).

Like initial consolidation, this process of post-retrieval restabilization also appears to involve different phases, like STM and LTM and is thought to engage many of the same cellular mechanisms (Schafe et al. 2001) and brain regions (Suzuki et al. 2004; Mamiya et al. 2009). However, reconsolidation is not merely a recapitulation of consolidation (Schafe et al. 2001; Lee et al. 2004; Tronson and

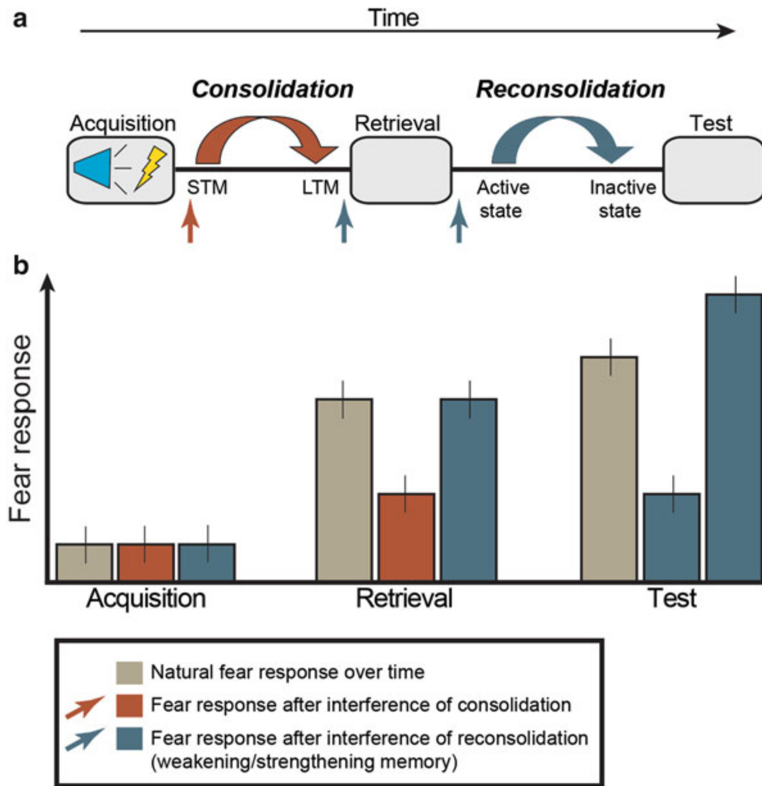


**Fig. 2.1** Memory processing is dynamic in nature. (**a**) Conditioning an animal to form an association between a CS (context and/or tone) and a US (foot shock) results in the consolidation of memory over a period of time, from an unstable short-term memory (STM) into a long-term memory (LTM) that is more stable and enduring. A short retrieval of memory returns it to an active labile state that requires the temporal process of reconsolidation to convert it to an inactive state that is persistent over time. (**b**) *Taupe*: During conditioning, prior to receiving a foot shock, animals show very low levels of fear responding as measured by freezing. However after consolidation, this fear response is significantly higher when measured during a retrieval test. Following this, the labile fear memory is reconsolidated and maintained after retrieval and expressed in subsequent retrieval tests. *Red*: Pharmacological infusions in the first hours after conditioning, disrupts the consolidation of memory and a loss of fear response on retrieval. *Blue*: Pharmacological infusions immediately prior to or in the first hours after retrieval disrupt the normal reconsolidation of memory and can result in a weakened (*light blue*) or strengthened (*dark blue*) fear memory depending on the type of intervention used. This modified fear memory is expressed as either a decrease or an increase in fear responding in subsequent retrieval tests (Color figure online)

Taylor 2007; Lee 2009), which is exemplified by the double dissociation between brain-derived neurotrophic factor (BDNF) and transcription factor early growth response 1 (Egr1/Zif268). Whereas the first is selectively required for consolidation in the hippocampus, Zif268 is selectively required for reconsolidation of contextual fear memory (Lee et al. 2004). Besides these, a number of other genes have been

found that are required for reconsolidation and have been described in comprehensive review by Tronson and Taylor in 2007 (Tronson and Taylor 2007).

Thus, reconsolidation is a retrieval-induced time-dependent process that is not merely a reiteration of consolidation and must have its own functional significance (Tronson and Taylor 2007; Lee 2009). Over the years, this post retrieval process has been exploited to understand the stability of memory and has recently come into focus for its adaptive nature that makes it a potential target for therapeutic purposes to treat disorders, such as post-traumatic stress disorder (PTSD) (Tronson et al. 2006; Tronson and Taylor 2007; Lee 2009, 2010; Nader and Hardt 2009; Schiller et al. 2010). Fear memories are often retrieved in the presence of additional information related to CS and US contingencies, and adaptive modification of a memory after reactivation maintains the predictive relevance of the memory to guide future behavior (Lee 2009). This adaptive nature of reconsolidation can be used to modify both the strength and content of memory (Lee 2008, 2009, 2010) and it is thus important to understand the molecular mechanisms that underlie this process.

Over the years, substantial work has been done in elucidating the synaptic mechanisms that underlie the consolidation of fear memories, whereas work on the process of reconsolidation has lacked behind. A number of neurotransmitter systems including the glutamatergic system (Maren 2005; Johansen et al. 2011), GABAergic (Berlau and McGaugh 2006; Makkar et al. 2010) and serotonergic systems (Roberts and Hedlund 2012; Woods et al. 2012) have been implicated in the regulation of synaptic plasticity and memory processing. Furthermore, it is believed that neuromodulation of synaptic plasticity by metabotropic glutamate receptors (Rodrigues et al. 2002; Johansen et al. 2011) and various monoamines, such as norepinephrine (Tully and Bolshakov 2010) and dopamine (Pezze and Feldon 2004), is critical to promote the formation of emotional memories (Bailey et al. 2000; Tully and Bolshakov 2010; Johansen et al. 2011). Recently, synaptic plasticity processes themselves, i.e. trafficking of ionotropic glutamate receptors, have been studied in the context of memory consolidation and reconsolidation. First, we will describe this recent work in terms of the molecular mechanism of AMPAR trafficking in reconsolidation (Sect. 2.2), before turning to the adaptive nature of reconsolidation and underlying mechanism (Sect. 2.3). Finally, we will discuss possible boundary conditions of reconsolidation as an adaptive process to modify memory strength and content (Sect. 2.4).

## 2.2 AMPA Receptor Trafficking, Synaptic Plasticity and the Processing of Memories

Changes in the strength of synaptic connections between neurons are widely believed to be mechanistically involved in the processing of memories in the brain. Experience generates neural activity, which in turn alters neural circuit function resulting in a modification of behavior. This activity-dependent change in strength and efficacy of excitatory synaptic function is termed synaptic plasticity, and is

thought to be the neural basis of a plethora of normal, adaptive and pathological brain function (Citri and Malenka 2008). The main excitatory connections in the brain are glutamatergic synapses that contain two main types of ionotropic glutamate receptors; the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) responsible for fast signal transmission and the *N*-methyl-D-aspartate receptors (NMDARs) for long-term changes in synaptic transmission, namely long-term potentiation (LTP) and long-term depression (LTD) (Malenka 1994, 2003; Malinow and Malenka 2002; Malenka and Bear 2004). Depression or enhancement of synaptic transmission can span temporal domains ranging from the short to the very long term, and is critical to incorporate transient experiences into permanent memory traces (Citri and Malenka 2008). At excitatory synapses, the different forms of plasticity that are expressed can be regulated from the pre- or postsynaptic site. Whereas modulation of presynaptic transmitter release is mostly implicated in brief memory, specifically postsynaptic mechanisms come into play when pertaining to persistent forms of memory (Roberts and Glanzman 2003; Kessels and Malinow 2009). Here, we will focus on postsynaptic changes in receptor numbers that underlie synaptic plasticity (Huganir and Nicoll 2013) and fear memory processing during retrieval and reconsolidation of memory.

AMPARs are mainly comprised of heterotetrameric complexes made up of at least a combination of two out of four subunits, designated as glutamate receptor GluA1–4 (Wenthold et al. 1996; Collingridge et al. 2009), that confer specific physiological properties of AMPAR channel function, such as kinetics, conductance and permeability. Most notably, GluA2-containing AMPARs are impermeable to divalent cations (calcium impermeable AMPA receptors (CI-AMPARs)), whereas those lacking the GluA2 subunit are calcium ($Ca^{2+}$) permeable (calcium permeable AMPA receptors (CP-AMPARs)) (Isaac et al. 2007) and have an inward rectification. AMPARs have been shown to underlie activity-dependent changes in excitatory synaptic function during different forms of learning (Kessels and Malinow 2009), with (1) the insertion of AMPARs into synapses resulting in synaptic strengthening and (2) the removal or endocytosis of AMPARs from synapses resulting in synaptic weakening (Kessels and Malinow 2009).

The synaptic localization of AMPARs is mainly regulated by the phosphorylation of specific amino acid residues in their carboxyl terminal tail (C-tail) (Malinow and Malenka 2002) and interactions with a wide range of binding partners (Malenka 2003; Sumioka 2013). The GluA1, GluA4 and GluA2L have long intracellular C-tails, whereas the GluA2, GluA3 and GluA4c have short ones (Kessels and Malinow 2009). The activity-driven synaptic incorporation of AMPARs is mainly mediated by long-tailed AMPARs (Hayashi et al. 2000; Harms et al. 2005; Kessels and Malinow 2009), and the activity dependent removal of AMPARs from synapses that results in synaptic weakening and LTD is mainly dependent on the phosphorylation of the GluA2 C-terminal tail at S880 by PKC and dephosphorylation of GluA1 at S845 or S831 (Chung et al. 2000; Lee et al. 2000, 2003; Perez et al. 2001; Seidenman et al. 2003; Kessels and Malinow 2009). Furthermore, three tyrosine residues in the GluA2 C-terminal tail are required for regulated endocytosis of GluA2-containing AMPARs. A mimetic peptide ($GluA2_{3Y}$) that spans the nine

amino acids harboring these three tyrosine residues specifically abolishes regulated NMDAR-dependent endocytosis of synaptic AMPARs (Ahmadian et al. 2004) through substrate competition, and serves as a plasticity blocker for the activity-dependent removal of synaptic AMPARs (Brebner et al. 2005).

Cellular processes of Long-Term Depression (LTD) are known to play a critical role in the consolidation of spatial memory (Ge et al. 2010) and have a debated role in cerebellar learning (Mauk et al. 2000). In the past, this form of plasticity was not considered to be crucial for the consolidation of either contextual or auditory fear memories, and amygdala neurons were shown to exhibit decreased firing to a CS not explicitly paired to a US, pointing to a role for LTD in limiting synaptic transmission in CS-pathways not correlated with the US (Collins and Pare 2000; Maren 2001). However in recent years, it has been suggested that LTD-like processes could serve as an adaptive response to reverse previously induced synaptic plasticity which would allow new information to be added to the same synapses through subsequent LTP-like mechanisms (Massey and Bashir 2007).

Two of the canonical brain regions crucial to the processing of fear memories are the hippocampus and the amygdala. The role of synapse strengthening resulting from the synaptic insertion of GluA1 and GluA2 subunits to synapses in the amygdala and the hippocampus after fear learning has been well described (Rumpel et al. 2005; Whitlock et al. 2006; Matsuo et al. 2008; Zhou et al. 2009). However, the putative role of AMPAR endocytosis in the retrieval and reconsolidation of memory has only recently come to light. This cellular process in the hippocampus and amygdala appears to mirror the retrieval and reconsolidation of contextual and auditory fear conditioning, respectively (Clem and Huganir 2010; Rao-Ruiz et al. 2011). For this form of synaptic plasticity to be relevant to behavior, it is important that (1) it follows the temporal dynamics of retrieval and reconsolidation, (2) it does not occur in the absence of retrieval and (3) the manipulation of AMPAR trafficking should disrupt the adaptive nature of reconsolidation to modify memory strength and content.

### *2.2.1 Role of AMPAR Dynamics Post-retrieval of Memory in the Amygdala*

In synapses of thalamic afferents to lateral amygdala neurons, two elegant studies have shown a role for AMPAR endocytosis in the retrieval of auditory fear memory. Both these studies showed that increased expression of AMPARs that corresponds to the consolidation of long-term memory is altered by the retrieval of auditory fear memory. Whereas the first study by Clem and colleagues (Clem and Huganir 2010) focused on AMPAR dynamics that underlies behavioral manipulation during reconsolidation, the second study by Hong and colleagues (Hong et al. 2013) focused on AMPAR plasticity underlying retrieval and reconsolidation. Taken together, these studies provide the first evidence that AMPAR trafficking is essential for retrieval and subsequent reconsolidation of fear memory in the amygdala.

Clem and Huganir (2010) described a role for AMPAR trafficking during the consecutive consolidation and reconsolidation windows (Fig. 2.2). The authors

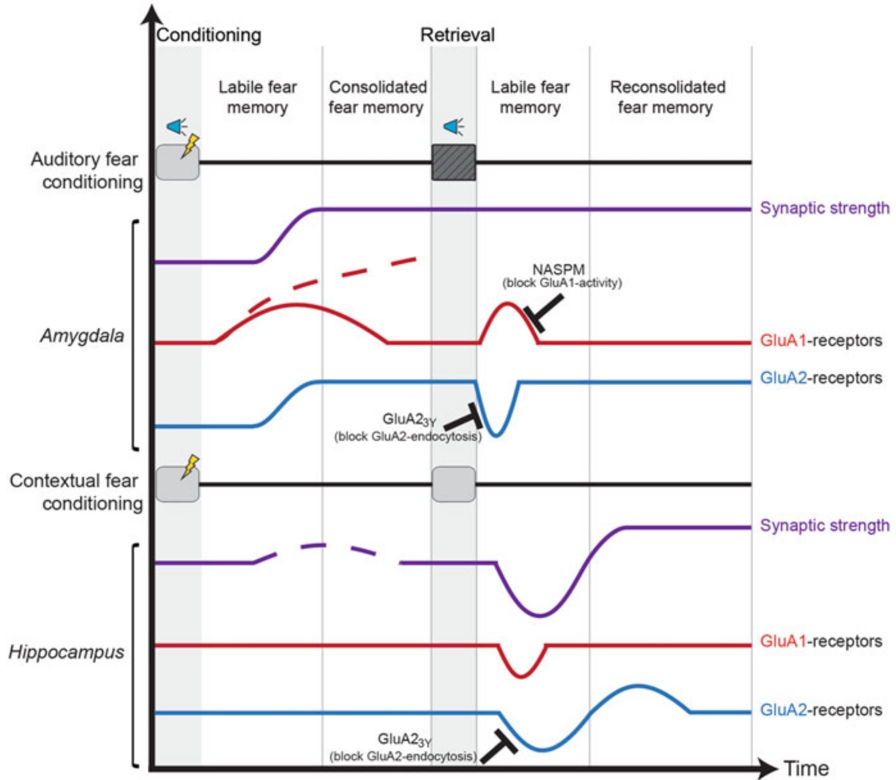


**Fig. 2.2** Model of synaptic mechanisms underlying the processing of fear memories in the lateral amygdala and hippocampus after auditory and contextual fear conditioning respectively. Consolidation of auditory fear memories (*upper panel*) results in a persistent increase in lateral amygdala (LA) synaptic strength up to 1 week after training, which is initially through GluA1-containing receptors (Clem and Huganir 2010; Hong et al. 2013) followed by the insertion and maintenance of GluA2-containing receptors at the synapse (Hong et al. 2013). In the hippocampus (*lower panel*) increased spontaneous transmission and synaptic strength has been observed at recent time points (3 h) after fear conditioning, but not 24 h later (Zhou et al. 2009). No conclusive evidence exist of increased expression of glutamate receptors at hippocampal synapses either 1–4 or 24 h after contextual learning. Within the LA, memory retrieval 8 days after training, induces the endocytosis of GluA2-containing receptors followed closely by subsequent insertion of GluA1-containing receptors (Hong et al. 2013) causing an unstable state of synaptic potentiation. These newly inserted GluA1-contaning receptors are gradually reverted back to GluA2-containing receptors over the course of reconsolidation (Hong et al. 2013). In the hippocampus, memory retrieval 24 h after training, initially results in a decrease in synaptic strength and an LTD-like state due to the endocytosis of GluA1, GluA2 and GluA3 receptors (Rao-Ruiz et al. 2011). This is followed by re-insertion of GluA1-containing receptors 4 h after retrieval (Rao-Ruiz et al. 2011). At 7 h after retrieval, synaptic strength is once again increased due to the insertion and maintenance of GluA2-containing receptors at the hippocampal synapse (Rao-Ruiz et al. 2011). NASPM and GluA2$_{3Y}$ are agents that have been used to block the activity or synaptic trafficking of AMPA receptor subunits (Rao-Ruiz et al. 2011; Hong et al. 2013). *Dashed lines* represent changes that have been observed in some studies (Zhou et al. 2009; Clem and Huganir 2010) that vary from the changes shown in *bold lines* (Rao-Ruiz et al. 2011; Hong et al. 2013)

(Clem and Huganir 2010) showed that the incorporation of calcium permeable AMPARs (CP-AMPARs) into synapses 24 h after tone-conditioning increased the capacity of synaptic weakening mediated by mGluR1-dependent LTD (Clem and Huganir 2010). This insertion of receptors occurred during a temporal window after retrieval that mirrors reconsolidation and during which fear memory can be altered and degraded by behavioral experience. The consolidation-specific incorporation was dependent on phosphorylation of GluA1 at S845, as GluA1 S845A mutants (He et al. 2009) did not show this CP-AMPAR surface stability (Clem and Huganir 2010). Retrieval and subsequent extinction training of the fear-associated tone, but not the context, normalized the level of CP-AMPARs, as well as AMPAR/NMDAR current ratios.

Hong and colleagues (2013) confirmed that for lateral amygdala synapses, just the retrieval of memory induces an abrupt exchange in surface expression of AMPARs, in which a switch in AMPAR subunits contribute to the plasticity observed before and after retrieval. However, they showed that rectification, and hence the incorporation of CP-AMPARs, is increased in a short time interval (1–2 h) after retrieval of auditory fear memory (Fig. 2.2). This time-dependent aspect has likely been missed by the initial study of Clem and Huganir (2010) due to the extinction training given after retrieval. Despite the increase in CP-AMPARs after retrieval, the increase in synaptic strength, in the form of increased AMPAR/NMDAR current ratios, was maintained over a period of a week post-conditioning and hence a lasting result of the consolidated memory, as shown before (Clem and Huganir 2010). Together this suggests that retrieval induces the synaptic removal of CI-AMPARs probably through endocytosis involving the C-terminal tail of GluA2 (Collingridge et al. 2010). Furthermore, blockade of either CI-AMPAR endocytosis, by use of the GluA$2_{3Y}$ peptide, or NMDA receptor activity during memory retrieval, precluded the exchange to CP-AMPARs and prevented memory destabilization. This indicates that the transient exchange of AMPARs may underlie the process by which a consolidated memory is converted to a labile unstable state during retrieval, as previously suggested for the hippocampus (Rao-Ruiz et al. 2011). Blocking the activity of CP-AMPARs immediately after retrieval disrupted reconsolidation and altered behavior. Finally, over the course of hours after retrieval the newly inserted CP-AMPARs were gradually exchanged back to CI-AMPARs that mirrored the time-course of reconsolidation. These studies (Hong et al. 2013) elegantly show that the endocytosis of CI-AMPARs and the transient re-insertion on CP-AMPARs is critical to retrieval and subsequent reconsolidation of a malleable memory into a more stable reconsolidated memory (Hong et al. 2013) (Fig. 2.2).

### *2.2.2 Role of AMPAR Dynamics Post-retrieval of Memory in the Hippocampus*

In the hippocampus, AMPAR endocytosis has been shown crucial for modulation of contextual fear memories, an event occurring specifically after recall of the memory (Rao-Ruiz et al. 2011). A short non-reinforced retrieval of contextual fear resulted

in a temporal wave of synaptic weakening, mediated by the removal of AMPARs and decreased synaptic strength (Fig. 2.2). More specifically, 1 h after retrieval of contextual fear a down regulation of the GluA1, GluA2 and GluA3 subunits was observed. These changes in receptor expression were specific to retrieval of contextual fear with no changes observed in the (1) absence of retrieval and (2) retrieval of a non-aversive context memory. These changes in receptor expression were measured in synaptic membrane fractions, and were confirmed by electrophysiological measurements of mEPSCs, indicating that retrieval of contextual fear results in the removal or endocytosis of AMPARs from hippocampal synapses. Indeed, blocking the regulated endocytosis of CI-AMPARs by the hippocampal infusion of $GluA2_{3Y}$ peptide prevented the down regulation of both the GluA2 and GluA3 subunits (Fig. 2.2). This wave of synaptic weakening and GluA1 synaptic re-insertion was followed by increased GluA2 expression 7 h after retrieval that was dependent on the first wave of endocytosis, and was attenuated if the first wave was blocked. This bi-phasic wave of AMPAR trafficking phase (Fig. 2.2) mirrored the period of reconsolidation and was critical to the adaptive nature of reconsolidation to modify both memory strength and content (see below). Related to memory strength, blocking the retrieval-induced endocytosis with intrahippocampal $GluA2_{3Y}$ peptide injections had an immediate effect on the expression of fear memory in a second retrieval session, without affecting initial retrieval. This exemplifies that the reconsolidation process, starting with activation of the memory, could be dependent on endocytosis of AMPAR in the hippocampus, similar to that in the amygdala (Hong et al. 2013). Moreover, the fact that memory was strengthened upon blocking retrieval-induced endocytosis in the hippocampus only (Rao-Ruiz et al. 2011; Hong et al. 2013) adds to the idea that the hippocampus exerts a constraint on memory processes (Rao-Ruiz et al. 2011), among which those in the amygdala (Wang et al. 2009).

This form of synaptic plasticity does not seem to be limited to the reconsolidation of fear memories and has been observed to play a role in expression of memory during retrieval in the perirhinal cortex (Cazakoff and Howland 2011). In the object recognition memory paradigm, local infusion of the $GluA2_{3Y}$ peptide did not alter the encoding and consolidation phases of memory, but specifically disrupted object recognition memory when administered prior to retrieval (Cazakoff and Howland 2011). This condition is different from that in the hippocampus and amygdala, in which pre-test injection of the $GluA2_{3Y}$ peptide did not alter expression of the first retrieval (Rao-Ruiz et al. 2011; Hong et al. 2013).

Thus, although mediated by different subunits, retrieval of fear memory in the amygdala (Clem and Huganir 2010; Hong et al. 2013) and hippocampus (Rao-Ruiz et al. 2011) appears to cause the endocytosis of AMPARs and subsequent synaptic weakening (Fig. 2.2). In other brain areas AMPAR trafficking might be a consequence of a consolidated memory, as blocking endocytosis in the perirhinal cortex affected the first retrieval of memory (Cazakoff and Howland 2011). The endocytosis of receptors is normally followed by the reinsertion of AMPARs into the affected synapse (Rao-Ruiz et al. 2011; Hong et al. 2013). This form of AMPAR plasticity mirrors the time course of retrieval, memory destabilization and subsequent restabilization. Furthermore, this endocytosis is critical to post-retrieval maintenance of both memory strength and content. Taken together, AMPAR endocytosis appears to

be a novel synaptic process by which a memory can be destabilized by retrieval and permanently manipulated with the addition of new information during subsequent reconsolidation.

## 2.3 The Adaptive Nature of Reconsolidation and the Role of AMPAR Trafficking

Reconsolidation is a time-dependent dynamic process that is not solely a replay of consolidation processes, but must have its own functional implications (Tronson and Taylor 2007). The adaptive nature of reconsolidation can be explained by the possibility to update memory *strength* and *content* (Lee 2008, 2009, 2010). Both fear memories (Nader and Hardt 2009) and drug-related memories (Sorg 2012) can undergo destabilization and require restabilization for maintenance during reconsolidation, which makes this an intriguing process to study from a therapeutic perspective.

With respect to modification of memory *strength* in reconsolidation, the most widely known example is loss of fear response (amnesia) due to intervention with protein synthesis inhibitor (PSI) (Debiec et al. 2002). However, the opposite has been shown as well; re-exposure to the conditioning context prevents the decrease in fear responses by ‘forgetting’ (Rohrbaugh and Riccio 1970; Gordon and Spear 1973). Therefore, it has been suggested that brief recall and hence reactivation of the memory could gradually increase memory stability, thereby maintaining its relevance. This natural process has been mimicked with applications of activators of PKA (Tronson et al. 2006) and NMDARs (Lee et al. 2006) prior to or immediately after a short retrieval, as this resulted in an enhanced memory that was persistent over time. Enhancement of memory strength by reconsolidation gives credence to the hypothesis of reconsolidation as a storage process (Tronson and Taylor 2007), in which memories could be strengthened or weakened depending on the cellular process that is interfered with. Therefore, the increase in memory strength after blocking AMPAR-endocytosis in the hippocampus indicates that this form of synaptic plasticity is part of the reconsolidation process that has a role in constraining memory strengthening (Rao-Ruiz et al. 2011). Reconsolidation of contextual fear memories engages both the hippocampus and amygdala (Mamiya et al. 2009). However, it has been found that for strong auditory fear memories, reconsolidation in the amygdala only takes place for remote memories, i.e., 28 days after training (Wang et al. 2009). Hence, it may be speculated that the hippocampus plays a gating role in the adaptive nature of reconsolidation in maintaining memory relevance.

With respect to modification of memory *content* in reconsolidation, it has been shown that complementary, additional information present during a retrieval trial, such as incorporation of a foot-shock can be incorporated into the malleable memory trace during reconsolidation (Monfils et al. 2009; Lee 2010), the paradigm of which is referred to as reconsolidation update, or post-retrieval extinction learning (Fig. 2.3).

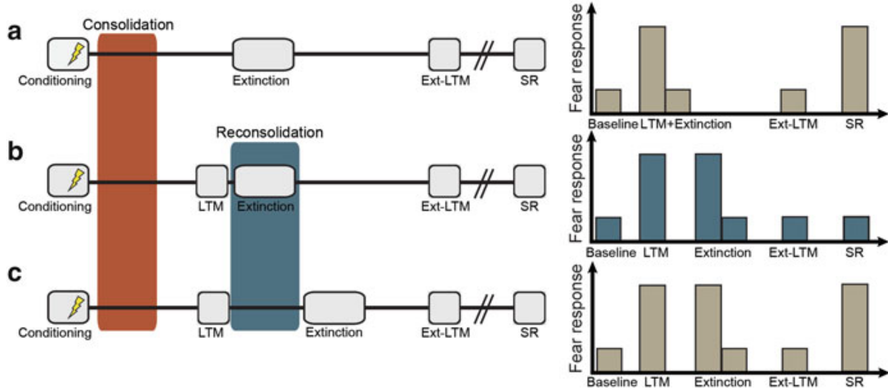


**Fig. 2.3** Post-retrieval extinction therapy (reconsolidation update) of a contextual fear memory permanently suppresses its expression in the long-term. (**a**) Prolonged exposure to the CS (context) in the form of a long retrieval session results in the formation of a new extinction memory that results in a decrease in fear responding as observed both during the extinction session as well as in a long-term extinction memory test (Ext-LTM) 24 h later. However, since extinction results in the formation of a new inhibitory memory rather than erasing the original fear memory, uncovering mechanisms, such as the passive passage of time, result in the spontaneous recovery (SR) of fear and its expression (*taupe*). (**b**) An extinction session given after retrieval *during* the period of memory lability has been shown to permanently suppress the expression of fear and no spontaneous recovery is observed with the passage of time (*blue*). (**c**) If this post-retrieval extinction session is given *after* the memory is reconsolidated, then a normal extinction memory is formed, which is expressed during an extinction long-term memory test, but not with the passage of time (*taupe*) (Color figure online)

### 2.3.1 Role of AMPAR Dynamics in Post-retrieval Extinction in Amygdala

In 2009, Monfils et al. (2009) for the first time showed that extinction training, the repetitive exposure to the CS that results in a blunted response to the CS, after retrieval during the reconsolidation window prevents the return of conditioned fear in rats. Over the last years, extinction training has been studied extensively in the attempt to develop therapies to suppress fear memory (reviewed by Maren et al. (2013)). The major limitation of this approach is that instead of updating the original memory trace, extinction training merely induces the formation of a neutral memory that suppresses the expression of fear. Eventually the balance between these separate memories may switch by passage of time (spontaneous recovery), presentation of the unconditioned stimulus (reinstatement) or changes of context (renewal), resulting in return of expression of the memory. However, combining reconsolidation and extinction by introducing the extinction training after activation of the memory, i.e. during the 6 h reconsolidation window, changes the content of a fear memory, altering fear responses on the long term (Monfils et al. 2009) (Fig. 2.3).

This phenomenon is of particular interest for therapeutic purposes, and this form of behavior therapy has also been used in humans, with a loss of fear response for up to 1 year (Schiller et al. 2010) (see Sect. 2.4). At the cellular level in the lateral amygdala, trafficking of calcium-permeable AMPARs is thought to underlie this process of post-retrieval extinction training or reconsolidation update therapy (Clem and Huganir 2010).

Clem and Huganir (2010) hypothesized that the reversal of fear-dependent synaptic potentiation of CP-AMPARs observed during initial consolidation could underlie reconsolidation update therapy (Monfils et al. 2009), which in turn would suppress the fear memory. In line with this, extinction therapy (twice 30 min of only CS presentation) immediately after, but not 6 h after retrieval, resulted in a long-lasting erasure of fear and a reversal of fear-mediated synaptic strengthening. Furthermore, due to prior removal of CP-AMPARs, additional synaptic depression was occluded in these animals in comparison with their controls (Clem and Huganir 2010).

With respect to a possible mechanism (Fig. 2.3), Monfils et al. (2009) found an increase in GluA1 phosphorylation at S845 both 3 min and 1 h after retrieval in the lateral amygdala. A second retrieval session 1 h, but not 3 min after the first retrieval session induced S845 GluA1-dephosphorylation. Given the fact that a second retrieval session within the reconsolidation window changes the phosphorylation state of the GluA1 subunit in the amygdala, this could well correspond with the observed differences in membrane expression of CP-AMPARs after retrieval only (Hong et al. 2013), or after post-retrieval extinction training (Clem and Huganir 2010) in the amygdala. The importance of this phosphorylation was shown by Clem and Huganir (2010), who demonstrated that animals with a substitution of the GluA1 phosphorylation site at S845 did not show this erasure of fear (Clem and Huganir 2010). The authors (Clem and Huganir 2010) thus concluded that behavioral manipulation could disrupt previously established fear by the induction of mGluR1-dependent LTD by CP-AMPAR trafficking, that reverses synaptic potentiation (Clem and Huganir 2010).

Retrieval-induced AMPAR trafficking in the hippocampus underlies memory strength (Rao-Ruiz et al. 2011), and a similar process occurs in amygdala, albeit at a different pace (Fig. 2.2). The question is whether this plasticity is also required for modification of memory *content* in reconsolidation of primarily hippocampus-dependent memories, i.e. in which the foreground stimulus is the context.

### 2.3.2 Role of AMPAR Dynamics in Post-retrieval Extinction in Hippocampus

Using a contextual fear conditioning paradigm, Rao-Ruiz et al. (2011) were able to show the applicability of post-retrieval extinction to suppress memories that would otherwise emerge by spontaneous recovery. In the hippocampus, a similar time-dependency of post-retrieval extinction seems to play a role as in the amygdala. Extinction training within (after 2 h), but not outside (after 24 h), the

reconsolidation window prevented spontaneous recovery measured 2 weeks later (Rao-Ruiz et al. 2011).

Furthermore, the authors showed that blocking retrieval-induced endocytosis by hippocampal injection of the $GluA2_{3Y}$ peptide in the post-retrieval extinction paradigm resulted in re-emerge of fear (Rao-Ruiz et al. 2011). In light of the recent observation that the transient exchange of AMPARs in the amygdala may underlie the conversion to a unstable state during retrieval (Hong et al. 2013), these data indicate that the blockade of CI-AMPAR trafficking resembled a situation as if the retrieval session was never given. The effect of post-retrieval extinction on the persistence of contextual fear memory has been confirmed by Flavell et al. in rats (Flavell et al. 2011). Moreover, administration of a L-type voltage gated calcium channel blocker, which prevents destabilization of fear memories, after retrieval of the memory prevented the effect of the post-retrieval extinction (Flavell et al. 2011). Furthermore, this study showed that the process initiated by post-retrieval extinction has an amnesic effect. Animals that received post-retrieval extinction were unable to re-acquire a contextual memory for a weak stimulus, unlike rats that only received extinction training.

At this stage, the parallels between the hippocampus and the amygdala in the possible retrieval-induced switch in subunit expression, i.e., the replacement of CI-AMPAR by CP-AMPARs, is mainly given by the detailed temporal analysis of synaptic membrane fractions (Rao-Ruiz et al. 2011). In the hippocampus, at 4 h after retrieval, the initial downregulation of all GluA-subunits was perpetuated by decreased expression of the GluA2 and GluA3 subunits of the AMPARs, but not GluA1 (Rao-Ruiz et al. 2011). Based on this, one might speculate that GluA1 is re-inserted into the synapse at a much shorter time-window than GluA2, i.e. at 2 h post-retrieval, thereby causing only a transient increase in the presence of CP-AMPARs between 2 and 6 h after retrieval in the hippocampus.

Taken together, the amygdala and hippocampus share retrieval-induced trafficking mechanisms, although these differ in the participation of the type of GluA subunits, as well as their temporal pattern. Hence, it is highly likely that even in other brain areas, such as prefrontal or anterior cingulate cortex, both retrieval, and post-retrieval extinction, makes use of similar mechanisms. These studies in amygdala and hippocampus demonstrate that the molecular basis of reconsolidation can be detected and molecularly analyzed, which opens the possibility to study this process, as well as its boundary conditions, in the context of other associative memories and in other brain areas.

## 2.4 The Adaptive Nature of Reconsolidation: Generality and Boundary Conditions

In 2010, Schiller et al. (2010) introduced the concept of reconsolidation-update, i.e. post-retrieval extinction, in human studies in a laboratory setting using visual discrimination fear conditioning. This study formed a proof of principal for the

translational value of reconsolidation update therapy as memory interference strategy in humans. Very recently, the approach to extinguish conditioned fear memory using this form of behavioral therapy was taken further in an elegant study by Xue et al. (2012), which showed that this type of therapy was also effective for other associative learning-based psychiatric diseases, i.e. addiction memories. Despite the generality of post-retrieval extinction and its underlying mechanism in the form of AMPAR plasticity there seem to be boundary conditions for it to become effective.

### *2.4.1 Generality in Post-retrieval Extinction: From Mice to Men*

The translation of the reconsolidation-update paradigm from rodents (Monfils et al. 2009) to man (Schiller et al. 2010) showed a similar time-dependency for extinction training within the boundaries of the reconsolidation time-frame. Subjects underwent visual discrimination fear conditioning, followed by post-retrieval extinction with a 10 min or 6 h interval (Schiller et al. 2010). Only the first group exhibited extinction of the memory and prevention of spontaneous recovery up to 1 year after training, as measured by skin conductance response. In addition, this blockade of memory was specific for the retrieved conditioned stimulus and could not be generalized to other fear memories. The finding that reconsolidation-update can suppress reinforcement of conditioned fear by US presentation has been confirmed in a different study (Oyarzun et al. 2012) using auditory fear conditioning.

To underscore the therapeutic relevance of post-retrieval extinction, Xue et al. (2012) employed this approach in drug-related memories using a similar approach and experimental set-up for both operant heroin and cocaine self-administration in rats and human heroin addicts in a clinical setting. In humans, a short retrieval of drug-associated cues before extinction training increased the effectiveness of extinction when given 10 min, but not 6 h, before the extinction session. Cue-induced craving and blood pressure were decreased during retrieval sessions at 1 day, 30 and 180 days after reconsolidation update. A similar time-frame of post-retrieval extinction training was found for drug-primed reinstatement and spontaneous recovery using contingent (operant self-administration) and non-contingent (place preference test) heroin conditioning. Hereby, the authors demonstrated that extinction of drug craving and relapse can benefit from the post-retrieval extinction mechanisms, using the malleable state of the memory after its activation (Fig. 2.3).

Due to the generalization of post-retrieval extinction therapy across species and across different forms of memory, this approach has proven itself as a robust method to disrupt fearful or drug-related memories. On the other hand, reproducibility in several laboratories has proven to be difficult (Chan et al. 2010; Flavell et al. 2011; Soeter and Kindt 2011; Ma et al. 2012; Kindt and Soeter 2013). These discrepancies shed light on the boundary conditions that constrain this form of therapy, but also allow us to speculate on the molecular pathway, namely the role of AMPAR-mediated plasticity, underlying these conditions (Auber et al. 2013).

### 2.4.2 Boundary Conditions in Post-retrieval Extinction

Recently, several attempts to prevent the return of fear in rodents (Chan et al. 2010; Costanzi et al. 2011; Flavell et al. 2011) and humans (Soeter and Kindt 2011; Warren et al. 2014) by post-retrieval extinction have failed. Chan et al. (Chan et al. 2010) showed the capability of preventing spontaneous recovery but not reinstatement of fear. In contrast, others demonstrated the inability to prevent spontaneous recovery of fear-potentiated startle after extinction, but not reinstatement of memory expression as a result of reinforcement by presentation of the US (Warren et al. 2014). Methodological issues concerning conditioning, extinction, and read-out of extinction have emphasized the limitations of the post-retrieval extinction paradigm as a therapeutic strategy. Further insight into the boundary conditions and the molecular mechanisms of reconsolidation might explain the restrictions and possibilities.

Ma et al. (2012) investigated the effect of reconsolidation update on return of drug-related memories using CPP for morphine in rats. Surprisingly, the timing of the reconsolidation update did either support or suppress the extinction. Whereas a 10-min retrieval-extinction interval inhibited spontaneous recovery and initial reinstatement of the drug memory, a 3-h interval fully prevented extinction to take place. This is in contrast with the idea that the active phase during which the memory is prone to modification lasts for 6 h. For fear-related memories the effective interval between retrieval and extinction ranges between 10 min and 2 h (Monfils et al. 2009; Clem and Huganir 2010; Rao-Ruiz et al. 2011; Warren et al. 2014). The initial retrieval induced retraction of GluA2-containing receptors in the amygdala is restored after 3 h (Hong et al. 2013), and the retraction of receptors in the hippocampus is partially restored, with only GluA2 and GluA3 being downregulated at 4 h (Rao-Ruiz et al. 2011). Albeit addictive and fear associations requiring a different retrieval-extinction interval, the temporal pattern of amygdalar and hippocampal GluA-regulation in retrieval of fear memory could impede successful suppression of fear.

So far, studies that have successfully disrupted memory in fact suppress the return of expression of a single memory trace. Disorders such as PTSD evolve from strong memories that are complex in nature and involve symptoms like sleep disturbance, general anxiety and emotional numbing (Quirk et al. 2010). Moreover, traumatic memories are oftentimes not readily amenable to immediate treatments (Kearns et al. 2012), and it is known that remote memories differ from recent memories in terms of reconsolidation (Frankland et al. 2006; Wichert et al. 2011). Thus, the observation that some studies cannot replicate permanent suppression of memory might be attributed to an insufficient retrieval-induced destabilization of the memory. This could be either due to the structure, i.e., the time or mismatch of expectancy of the retrieval session (Sevenster et al. 2012; Warren et al. 2014) or by the finding that stronger fear memories are particularly resistant to undergo reconsolidation (Wang et al. 2009). Whereas Soeter and Kindt (2011) used fearful pictures as CS in their paradigm, Oyarzun et al. (2012) used neutral stimuli as reminders. This could have affected the initial consolidation phase, thereby rendering the memory less amenable to reconsolidation. Warren (Warren et al. 2014) has proposed that the use of on-line

expectancy ratings requires a cortical representation of the CS-US association. This could elicit a stronger representation of the memory trace in the lateral amygdala, which has been suggested to mediate the reconsolidation dependent update of fear memory (Monfils et al. 2009). Furthermore, the addition of loud tones (104 dB) during CS presentation (Soeter and Kindt 2011; Kindt and Soeter 2013) might have been perceived as stressful stimuli and thereby could alter consolidation (Leaton and Cranney 1990) or reconsolidation processes (Coccoz et al. 2011).

In addition, it has been observed that for *remote* memories, retrieval followed by extinction did not prevent the return of fear in a contextual fear paradigm in mice (Siegmund and Wotjak 2007; Costanzi et al. 2011), nor the rewarding memory of morphine in rats (Ma et al.). Memories are increasingly stable over time, and less prone to disruption by pharmacological intervention during reconsolidation (Frankland et al. 2006). The age of fear memories might therefore restrict the use of reconsolidation update for behavioral intervention. Recently, a study by Gräff et al. (2014) showed that epigenetic mechanisms could contribute to this resistance. The authors compared epigenetic manipulation of gene expression by acetylation of histone proteins in recent and remote memories. They found that controlling levels of HDAC2 S-nitrosylation is crucial to restore the susceptibility of remote memories for post-retrieval extinction therapy. Besides showing an essential contribution of epigenetic mechanisms in remote memories, their study revealed possible plasticity routes involved in the suppression of fear memory expression. Gräff et al. (2014) showed convincing evidence for S-nitrosylation of HDAC2. However S-nitrosylation is an important factor in the regulation of AMPAR trafficking by affecting GluA2 via S-nitrosylation of NSF (Huang et al. 2005), or affecting directly GluA1 channel conductance and endocytosis (Selvakumar et al. 2013). Thus, inhibition of S-Nitrosylation by L-NAME, which prevented suppression of recent memory, could have affected trafficking of GluA1 and GluA2. Thereby the normal retrieval-induced plasticity in hippocampus and amygdala might have been disturbed.

Taken together, it is clear that therapies that use post-retrieval extinction have the potential of being beneficial in suppressing unwanted associative memories that occur in diseases where maladaptive memories are formed, such as PTSD or addiction. However since boundary conditions exist for these kind of therapies at both the preclinical and clinical level for which the molecular underpinnings are not completely understood, there is still a way to go until this method can be applied as a successful therapy in patients. As AMPAR trafficking and its related plasticity in amygdala and hippocampus underlie the reconsolidation process (Monfils et al. 2009; Clem and Huganir 2010; Rao-Ruiz et al. 2011; Hong et al. 2013) of fear memories, a number of open questions relate to the identity of interacting neuronal molecules involved in retrieval-induced AMPAR plasticity, as well as how impairments in AMPAR trafficking contribute to maladaptive processing of memories. Despite a wealth of information on molecules and pathways possibly involved in extinction and reconsolidation (Flavell et al. 2013), little has been done to delineate the processes of post-retrieval extinction therapy. Therefore, future studies should focus on disentangling the molecular cascade that leads to successful repression of associative memories to overcome the current boundaries in post-retrieval extinction.

## References

Ahmadian G, Ju W, Liu L, Wyszynski M, Lee SH, Dunah AW, Taghibiglou C, Wang Y, Lu J, Wong TP, Sheng M, Wang YT (2004) Tyrosine phosphorylation of GluR2 is required for insulin-stimulated AMPA receptor endocytosis and LTD. EMBO J 23:1040–1050

Auber A, Tedesco V, Jones CE, Monfils MH, Chiamulera C (2013) Post-retrieval extinction as reconsolidation interference: methodological issues or boundary conditions? Psychopharmacology (Berl) 226:631–647

Bailey CH, Giustetto M, Huang YY, Hawkins RD, Kandel ER (2000) Is heterosynaptic modulation essential for stabilizing Hebbian plasticity and memory? Nat Rev Neurosci 1:11–20

Berlau DJ, McGaugh JL (2006) Enhancement of extinction memory consolidation: the role of the noradrenergic and GABAergic systems within the basolateral amygdala. Neurobiol Learn Mem 86:123–132

Brebner K, Wong TP, Liu L, Liu Y, Campsall P, Gray S, Phelps L, Phillips AG, Wang YT (2005) Nucleus accumbens long-term depression and the expression of behavioral sensitization. Science 310:1340–1343

Cazakoff BN, Howland JG (2011) AMPA receptor endocytosis in rat perirhinal cortex underlies retrieval of object memory. Learn Mem 18:688–692

Chan WY, Leung HT, Westbrook RF, McNally GP (2010) Effects of recent exposure to a conditioned stimulus on extinction of Pavlovian fear conditioning. Learn Mem 17:512–521

Chung HJ, Xia J, Scannevin RH, Zhang X, Huganir RL (2000) Phosphorylation of the AMPA receptor subunit GluR2 differentially regulates its interaction with PDZ domain-containing proteins. J Neurosci 20:7258–7267

Citri A, Malenka RC (2008) Synaptic plasticity: multiple forms, functions, and mechanisms. Neuropsychopharmacology 33:18–41

Clem RL, Huganir RL (2010) Calcium-permeable AMPA receptor dynamics mediate fear memory erasure. Science 330(6007):1108–1112

Coccoz V, Maldonado H, Delorenzi A (2011) The enhancement of reconsolidation with a naturalistic mild stressor improves the expression of a declarative memory in humans. Neuroscience 185:61–72

Collingridge GL, Olsen R, Peters JA, Spedding M (2009) Ligand gated ion channels. Neuropharmacology 56:1

Collingridge GL, Peineau S, Howland JG, Wang YT (2010) Long-term depression in the CNS. Nat Rev Neurosci 11:459–473

Collins DR, Pare D (2000) Differential fear conditioning induces reciprocal changes in the sensory responses of lateral amygdala neurons to the CS(+) and CS(−). Learn Mem 7:97–103

Costanzi M, Cannas S, Saraulli D, Rossi-Arnaud C, Cestari V (2011) Extinction after retrieval: effects on the associative and nonassociative components of remote contextual fear memory. Learn Mem 18:508–518

Debiec J, LeDoux JE, Nader K (2002) Cellular and systems reconsolidation in the hippocampus. Neuron 36:527–538

Flavell CR, Barber DJ, Lee JL (2011) Behavioural memory reconsolidation of food and fear memories. Nat Commun 2:504

Flavell CR, Lambert EA, Winters BD, Bredy TW (2013) Mechanisms governing the reactivation-dependent destabilization of memories and their role in extinction. Front Behav Neurosci 7:214

Frankland PW, Ding HK, Takahashi E, Suzuki A, Kida S, Silva AJ (2006) Stability of recent and remote contextual fear memory. Learn Mem 13:451–457

Ge Y, Dong Z, Bagot RC, Howland JG, Phillips AG, Wong TP, Wang YT (2010) Hippocampal long-term depression is required for the consolidation of spatial memory. Proc Natl Acad Sci U S A 107:16697–16702

Glickman SE (1961) Perseverative neural processes and consolidation of the memory trace. Psychol Bull 58:218–233

Gordon WC, Spear NE (1973) The effects of strychnine on recently acquired and reactivated passive avoidance memories. Physiol Behav 10:1071–1075

Graff J, Joseph NF, Horn ME, Samiei A, Meng J, Seo J, Rei D, Bero AW, Phan TX, Wagner F, Holson E, Xu J, Sun J, Neve RL, Mach RH, Haggarty SJ, Tsai LH (2014) Epigenetic priming of memory updating during reconsolidation to attenuate remote fear memories. Cell 156:261–276

Harms KJ, Tovar KR, Craig AM (2005) Synapse-specific regulation of AMPA receptor subunit composition by activity. J Neurosci 25:6379–6388

Hayashi Y, Shi SH, Esteban JA, Piccini A, Poncer JC, Malinow R (2000) Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. Science 287:2262–2267

He K, Song L, Cummings LW, Goldman J, Huganir RL, Lee HK (2009) Stabilization of Ca2+-permeable AMPA receptors at perisynaptic sites by GluR1-S845 phosphorylation. Proc Natl Acad Sci U S A 106:20033–20038

Hebb DO (1949) The organization of behavior. Wiley, New York

Hong I, Kim J, Lee S, Ko HG, Nader K, Kaang BK, Tsien RW, Choi S (2013) AMPA receptor exchange underlies transient memory destabilization on retrieval. Proc Natl Acad Sci U S A 110:8218–8223

Huang Y, Man HY, Sekine-Aizawa Y, Han Y, Juluri K, Luo H, Cheah J, Lowenstein C, Huganir RL, Snyder SH (2005) S-nitrosylation of N-ethylmaleimide sensitive factor mediates surface expression of AMPA receptors. Neuron 46:533–540

Huganir RL, Nicoll RA (2013) AMPARs and synaptic plasticity: the last 25 years. Neuron 80:704–717

Isaac JT, Ashby MC, McBain CJ (2007) The role of the GluR2 subunit in AMPA receptor function and synaptic plasticity. Neuron 54:859–871

Johansen JP, Cain CK, Ostroff LE, LeDoux JE (2011) Molecular mechanisms of fear learning and memory. Cell 147:509–524

Kearns MC, Ressler KJ, Zatzick D, Rothbaum BO (2012) Early interventions for PTSD: a review. Depress Anxiety 29:833–842

Kessels HW, Malinow R (2009) Synaptic AMPA receptor plasticity and behavior. Neuron 61:340–350

Kindt M, Soeter M (2013) Reconsolidation in a human fear conditioning study: a test of extinction as updating mechanism. Biol Psychol 92:43–50

Leaton RN, Cranney J (1990) Potentiation of the acoustic startle response by a conditioned stimulus paired with acoustic startle stimulus in rats. J Exp Psychol Anim Behav Process 16:279–287

Lee JL (2008) Memory reconsolidation mediates the strengthening of memories by additional learning. Nat Neurosci 11:1264–1266

Lee JL (2009) Reconsolidation: maintaining memory relevance. Trends Neurosci 32:413–420

Lee JL (2010) Memory reconsolidation mediates the updating of hippocampal memory content. Front Behav Neurosci 4:168

Lee HK, Barbarosie M, Kameyama K, Bear MF, Huganir RL (2000) Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. Nature 405:955–959

Lee HK, Takamiya K, Han JS, Man H, Kim CH, Rumbaugh G, Yu S, Ding L, He C, Petralia RS, Wenthold RJ, Gallagher M, Huganir RL (2003) Phosphorylation of the AMPA receptor GluR1 subunit is required for synaptic plasticity and retention of spatial memory. Cell 112:631–643

Lee JL, Everitt BJ, Thomas KL (2004) Independent cellular processes for hippocampal memory consolidation and reconsolidation. Science 304:839–843

Lee JL, Milton AL, Everitt BJ (2006) Reconsolidation and extinction of conditioned fear: inhibition and potentiation. J Neurosci 26:10051–10056

Ma X, Zhang JJ, Yu LC (2012) Post-retrieval extinction training enhances or hinders the extinction of morphine-induced conditioned place preference in rats dependent on the retrieval-extinction interval. Psychopharmacology (Berl) 221:19–26

Mactutus CF, Riccio DC, Ferek JM (1979) Retrograde amnesia for old (reactivated) memory: some anomalous characteristics. Science 204:1319–1320

Makkar SR, Zhang SQ, Cranney J (2010) Behavioral and neural analysis of GABA in the acquisition, consolidation, reconsolidation, and extinction of fear memory. Neuropsychopharmacology 35:1625–1652

Malenka RC (1994) Synaptic plasticity in the hippocampus: LTP and LTD. Cell 78:535–538

Malenka RC (2003) Synaptic plasticity and AMPA receptor trafficking. Ann N Y Acad Sci 1003:1–11

Malenka RC, Bear MF (2004) LTP and LTD: an embarrassment of riches. Neuron 44:5–21

Malinow R, Malenka RC (2002) AMPA receptor trafficking and synaptic plasticity. Annu Rev Neurosci 25:103–126

Mamiya N, Fukushima H, Suzuki A, Matsuyama Z, Homma S, Frankland PW, Kida S (2009) Brain region-specific gene expression activation required for reconsolidation and extinction of contextual fear memory. J Neurosci 29:402–413

Maren S (2001) Neurobiology of Pavlovian fear conditioning. Annu Rev Neurosci 24:897–931

Maren S (2005) Synaptic mechanisms of associative memory in the amygdala. Neuron 47:783–786

Maren S, Phan KL, Liberzon I (2013) The contextual brain: implications for fear conditioning, extinction and psychopathology. Nat Rev Neurosci 14:417–428

Massey PV, Bashir ZI (2007) Long-term depression: multiple forms and implications for brain function. Trends Neurosci 30:176–184

Matsuo N, Reijmers L, Mayford M (2008) Spine-type-specific recruitment of newly synthesized AMPA receptors with learning. Science 319:1104–1107

Mauk MD, Medina JF, Nores WL, Ohyama T (2000) Cerebellar function: coordination, learning or timing? Curr Biol 10:R522–R525

McGaugh JL (1966) Time-dependent processes in memory storage. Science 153:1351–1358

Misanin JR, Miller RR, Lewis DJ (1968) Retrograde amnesia produced by electroconvulsive shock after reactivation of a consolidated memory trace. Science 160:554–555

Monfils MH, Cowansage KK, Klann E, LeDoux JE (2009) Extinction-reconsolidation boundaries: key to persistent attenuation of fear memories. Science 324:951–955

Muller GE, Pilzecker A (1900) Experimentelle Beitrage zur Lehre von Gedächtnis. Z Psychol 1:1–300

Nader K (2003) Memory traces unbound. Trends Neurosci 26:65–72

Nader K, Einarsson EO (2010) Memory reconsolidation: an update. Ann N Y Acad Sci 1191:27–41

Nader K, Hardt O (2009) A single standard for memory: the case for reconsolidation. Nat Rev Neurosci 10:224–234

Nader K, Schafe GE, Le Doux JE (2000a) Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. Nature 406:722–726

Nader K, Schafe GE, LeDoux JE (2000b) The labile nature of consolidation theory. Nat Rev Neurosci 1:216–219

Oyarzun JP, Lopez-Barroso D, Fuentemilla L, Cucurell D, Pedraza C, Rodriguez-Fornells A, de Diego-Balaguer R (2012) Updating fearful memories with extinction training during reconsolidation: a human study using auditory aversive stimuli. PLoS One 7, e38849

Perez JL, Khatri L, Chang C, Srivastava S, Osten P, Ziff EB (2001) PICK1 targets activated protein kinase Calpha to AMPA receptor clusters in spines of hippocampal neurons and reduces surface levels of the AMPA-type glutamate receptor subunit 2. J Neurosci 21:5417–5428

Pezze MA, Feldon J (2004) Mesolimbic dopaminergic pathways in fear conditioning. Prog Neurobiol 74:301–320

Quirk GJ, Pare D, Richardson R, Herry C, Monfils MH, Schiller D, Vicentic A (2010) Erasing fear memories with extinction training. J Neurosci 30:14993–14997

Rao-Ruiz P, Rotaru DC, van der Loo RJ, Mansvelder HD, Stiedl O, Smit AB, Spijker S (2011) Retrieval-specific endocytosis of GluA2-AMPARs underlies adaptive reconsolidation of contextual fear. Nat Neurosci 14:1302–1308

Roberts AC, Glanzman DL (2003) Learning in Aplysia: looking at synaptic plasticity from both sides. Trends Neurosci 26:662–670

Roberts AJ, Hedlund PB (2012) The 5-HT(7) receptor in learning and memory. Hippocampus 22:762–771
Rodrigues SM, Bauer EP, Farb CR, Schafe GE, LeDoux JE (2002) The group I metabotropic glutamate receptor mGluR5 is required for fear memory formation and long-term potentiation in the lateral amygdala. J Neurosci 22:5219–5229
Rohrbaugh M, Riccio DC (1970) Paradoxical enhancement of learned fear. J Abnorm Psychol 75:210–216
Rumpel S, LeDoux J, Zador A, Malinow R (2005) Postsynaptic receptor trafficking underlying a form of associative learning. Science 308:83–88
Sara SJ (2000) Retrieval and reconsolidation: toward a neurobiology of remembering. Learn Mem 7:73–84
Schafe GE, Nader K, Blair HT, LeDoux JE (2001) Memory consolidation of Pavlovian fear conditioning: a cellular and molecular perspective. Trends Neurosci 24:540–546
Schiller D, Monfils MH, Raio CM, Johnson DC, Ledoux JE, Phelps EA (2010) Preventing the return of fear in humans using reconsolidation update mechanisms. Nature 463:49–53
Seidenman KJ, Steinberg JP, Huganir R, Malinow R (2003) Glutamate receptor subunit 2 Serine 880 phosphorylation modulates synaptic transmission and mediates plasticity in CA1 pyramidal cells. J Neurosci 23:9220–9228
Selvakumar B, Jenkins MA, Hussain NK, Huganir RL, Traynelis SF, Snyder SH (2013) S-nitrosylation of AMPA receptor GluA1 regulates phosphorylation, single-channel conductance, and endocytosis. Proc Natl Acad Sci U S A 110:1077–1082
Sevenster D, Beckers T, Kindt M (2012) Retrieval per se is not sufficient to trigger reconsolidation of human fear memory. Neurobiol Learn Mem 97:338–345
Siegmund A, Wotjak CT (2007) A mouse model of posttraumatic stress disorder that distinguishes between conditioned and sensitised fear. J Psychiatr Res 41:848–860
Soeter M, Kindt M (2011) Disrupting reconsolidation: pharmacological and behavioral manipulations. Learn Mem 18:357–366
Sorg BA (2012) Reconsolidation of drug memories. Neurosci Biobehav Rev 36:1400–1417
Sumioka A (2013) Auxiliary subunits provide new insights into regulation of AMPA receptor trafficking. J Biochem 153:331–337
Suzuki A, Josselyn SA, Frankland PW, Masushige S, Silva AJ, Kida S (2004) Memory reconsolidation and extinction have distinct temporal and biochemical signatures. J Neurosci 24:4787–4795
Tronson NC, Taylor JR (2007) Molecular mechanisms of memory reconsolidation. Nat Rev Neurosci 8:262–275
Tronson NC, Wiseman SL, Olausson P, Taylor JR (2006) Bidirectional behavioral plasticity of memory reconsolidation depends on amygdalar protein kinase A. Nat Neurosci 9:167–169
Tully K, Bolshakov VY (2010) Emotional enhancement of memory: how norepinephrine enables synaptic plasticity. Mol Brain 3:15
Wang SH, de Oliveira AL, Nader K (2009) Cellular and systems mechanisms of memory strength as a constraint on auditory fear reconsolidation. Nat Neurosci 12:905–912
Warren VT, Anderson KM, Kwon C, Bosshardt L, Jovanovic T, Bradley B, Norrholm SD (2014) Human fear extinction and return of fear using reconsolidation update mechanisms: the contribution of on-line expectancy ratings. Neurobiol Learn Mem 113:165–173
Wenthold RJ, Petralia RS, Blahos J II, Niedzielski AS (1996) Evidence for multiple AMPA receptor complexes in hippocampal CA1/CA2 neurons. J Neurosci 16:1982–1989
Whitlock JR, Heynen AJ, Shuler MG, Bear MF (2006) Learning induces long-term potentiation in the hippocampus. Science 313:1093–1097
Wichert S, Wolf OT, Schwabe L (2011) Reactivation, interference, and reconsolidation: are recent and remote memories likewise susceptible? Behav Neurosci 125:699–704
Woods S, Clarke NN, Layfield R, Fone KC (2012) 5-HT(6) receptor agonists and antagonists enhance learning and memory in a conditioned emotion response paradigm by modulation of cholinergic and glutamatergic mechanisms. Br J Pharmacol 167:436–449

Xue YX, Luo YX, Wu P, Shi HS, Xue LF, Chen C, Zhu WL, Ding ZB, Bao YP, Shi J, Epstein DH, Shaham Y, Lu L (2012) A memory retrieval-extinction procedure to prevent drug craving and relapse. Science 336:241–245

Zhou M, Conboy L, Sandi C, Joels M, Krugers HJ (2009) Fear conditioning enhances spontaneous AMPA receptor-mediated synaptic transmission in mouse hippocampal CA1 area. Eur J Neurosci 30:1559–1564

# Chapter 3
# CaMKII: A Master Functional and Structural Molecule in Synaptic Plasticity and Memory

**Magdalena Sanhueza and German Fernandez**

**Abstract** Learning and memory relies, at least in part, on activity-dependent synaptic plasticity. A major plasticity model at glutamatergic synapses is NMDA-receptor (NMDAR)-dependent long-term potentiation (LTP). $Ca^{2+}$/calmodulin (CaM)-dependent kinase II (CaMKII) is critical for LTP and several forms of learning. It is a major component of post-synaptic densities and dendritic spines. Kinase interactions with key proteins in these specializations are differentially modulated by activity and dynamically regulate holoenzyme activity. During LTP CaMKII is activated, autophosphorylated and persistently translocated to synapses through NMDAR binding. Pharmacological or genetic interference with these processes impair LTP and learning. CaMKII may cause potentiation by synaptic recruitment of AMPA-type receptors (AMPARs) through regulation of receptor binding to scaffolding proteins. Additionally, CaMKII-dependent phosphorylation increases AMPAR conductance. Interestingly, CaMKII is also involved in metaplasticity, as it can regulate the sign of synaptic modification (potentiation or depression). The advent of high-resolution optical techniques has allowed inspection of CaMKII localization and activity in spine microdomains, providing new insights on holoenzyme multifaceted involvement in activity-dependent functional and structural changes. Finally, evidence suggests a role of CaMKII interaction with NMDARs in the maintenance of synaptic strength and spine stability. Thus, CaMKII emerges as a critical and complex controller of synaptic function and information storage, playing both enzymatic and structural roles.

**Keywords** CaMKII • Synaptic plasticity • NMDA-receptor • Dendritic spines • CaMKIIN

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## 3.1 CaMKII Structure, Activity and Regulation

CaMKII is a serine/threonine kinase consisting of 12 subunits assembled in a double hexameric ring structure (Fig. 3.1). The dodecameric enzyme may contain combinations of four different subunit isoforms (CaMKIIα-δ; reviewed in Lisman et al. 2002; Shonesy et al. 2014) each displaying catalytic activity. In the brain the most abundant isoforms are α (54 kDa) and β (60 kDa), present in a 3:1 ratio in heteromeric holoenzymes in the forebrain. Each subunit can be individually activated by $Ca^{2+}$/CaM, resulting in a graded total holoenzyme enzymatic activity. Moreover, the activity of each subunit is subject to various modulation processes, including intra- and inter-subunit phosphorylation reactions and interactions with other proteins. This results in an exquisite and dynamical spatiotemporal regulation of the enzyme, that depends on the properties of $Ca^{2+}$ signals as well as kinase subcellular localization.

Each CaMKII subunit has a catalytic domain, a regulatory domain and an association or hub domain that allows binding among subunits, the last two domains are connected by a variable linker region (Figs. 3.1 and 3.2). In basal conditions the autoinhibitory segment of the regulatory domain occludes the substrate-binding site (S site) in the catalytic domain, inhibiting kinase enzymatic activity. When the $Ca^{2+}$ concentration rises, binding of $Ca^{2+}$/CaM to the regulatory segment removes autoinhibition, allowing substrate access to the active site (Fig. 3.2). Opening of this gate

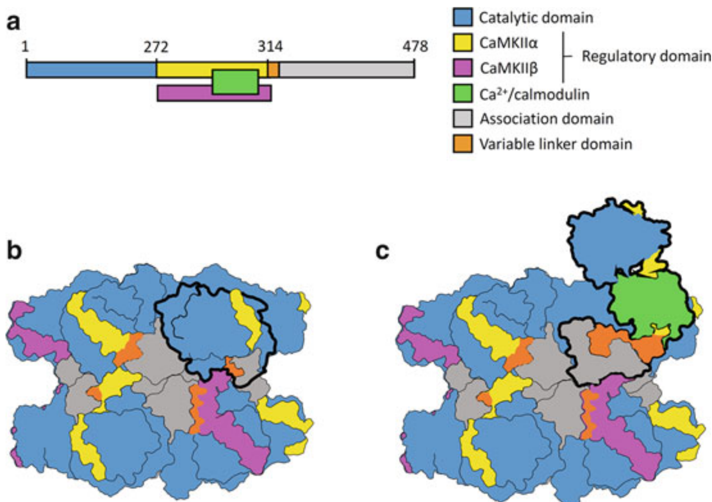


**Fig. 3.1** CaMKII holoenzyme structure and activation. (**a**) Schematic representation of αCaMKII and βCaMKII primary structure, illustrating the positions of catalytic, regulatory, linker and association domains, as well as the $Ca^{2+}$/CaM binding region. (**b**) Model of a lateral view of CaMKII holoenzyme in rest conditions. (**c**) Holoenzyme with a αCaMKII subunit activated by $Ca^{2+}$/CaM binding. Schemes here and in Fig. 3.2 are illustrative diagrams built from the structures published in the RCSB Protein Data Bank web site, using UCSF Chimera 10.1

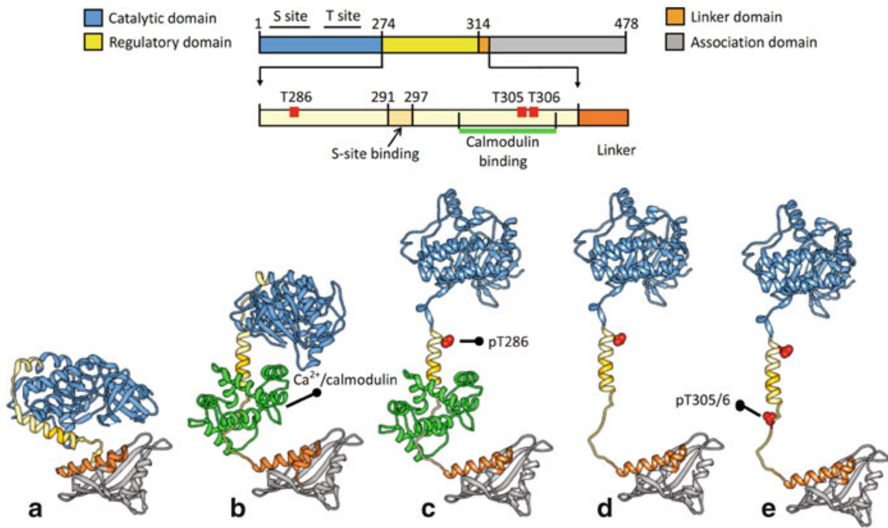


**Fig. 3.2** αCaMKII subunit in their different conformations. (**a**) Schematic of a αCaMKII subunit in close conformation highlighting the different domains colored as shown in the *inset*. *Inset*, subunit primary structure with detail of the regulatory and linker domains indicating the positions of T286 in the autoinhibitory segment and T306/306 in the $Ca^{2+}$/CaM binding segment. (**b**) Open conformation induced by $Ca^{2+}$/CaM binding. (**c**) After T286 phosphorylation by a neighboring activated subunit (not shown). (**d**) "Autonomous" subunit after dissociation of $Ca^{2+}$/CaM. (**e**) Inhibitory autophosphorylation at 305. Phosphorylated residues in **d** and **e** are shown in *red* (Color figure online)

also exposes the threonine residue T286 in the regulatory domain of αCaMKII (T287 in βCaMKII) that was basally attached to a region of the catalytic domain known as the T-site. Exposed T286/7 can be phosphorylated by an adjacent active subunit preventing complete gate closing and full inactivation after $Ca^{2+}$/CaM release. Therefore, trans-subunit autophosphorylation endows CaMKII with autonomous activity that persists after $Ca^{2+}$ has reached basal levels and lasts until the residue is dephosphorylated by phosphatases, thus acting as a $Ca^{2+}$-triggered molecular switch (Lisman 1985; Miller and Kennedy 1986; Lisman and Goldring 1988).

$Ca^{2+}$-dependent activation of CaMKII holoenzyme and trans-subunit autophosphorylation are highly cooperative processes (Chao et al. 2010). Moreover, T286 phosphorylation of a subunit dramatically decreases the $Ca^{2+}$/CaM dissociation rate (~10,000-fold) thus promoting its trapping (Meyer et al. 1992; Waxham et al. 1998). The consequence of these regulations is the temporal extension of holoenzyme activity. Interestingly, the high effective concentration of CaMKII subunits in specializations as the postsynaptic density (PSD) implicates that holoenzyme autophosphorylation can reduce availability of the highly regulated plasticity-related molecule CaM for other interactions. It should be noted, however, that a fully phosphorylated holoenzyme displays an autonomous activity that varies between 20 and 80 % of the maximal $Ca^{2+}$-dependent activity and it can be further

activated by $Ca^{2+}$/CaM (Coultrap et al. 2010). Interestingly, this percentage is substrate-dependent, with autonomy reaching higher values (~65 %) for T286 phosphorylation of neighboring subunits. Therefore trans-subunit autophosphorylation can render CaMKII partially independent of $Ca^{2+}$, thus prolonging the kinase active state. This property has key consequences on synaptic plasticity.

Due to autonomous activity, CaMKII can act as a frequency decoder of $Ca^{2+}$ oscillations, as demonstrated both in vitro as in single spines of living neurons (Fujii et al. 2013; De Koninck and Schulman 1998). At constant ATP concentration holoenzyme activity depends, in a graded way, not only on the magnitude but also on the frequency of the $Ca^{2+}$ spikes. This is because repetitive $Ca^{2+}$ spikes allow an increasing number of CaMKII subunits to become phosphorylated over time in a cooperative way: an autonomous subunit can phosphorylate an adjacent active subunit without the need of binding $Ca^{2+}$/CaM. Autonomous CaMKII activity increases exponentially with the frequency of $Ca^{2+}$ fluctuations and is modulated by the magnitude and duration of $Ca^{2+}$ transients, CaM availability and phosphatase activity.

CaMKII enzymatic activity can also be regulated by a secondary autophosphorylation process: after $Ca^{2+}$/CaM dissociates from a T286-phosphorylated subunit, this subunit can undergo autophosphorylation at the residues T305 and T306 in the $Ca^{2+}$/CaM binding segment (Fig. 3.2). The phosphorylation of either of these sites is sufficient to block $Ca^{2+}$/CaM association and thus $Ca^{2+}$-dependent activation, this reaction is therefore referred to as inhibitory autophosphorylation (Hanson and Schulman 1992). Even so, CaMKII autonomous activity is preserved until T286 becomes dephosphorylated. Secondary T305/T306 autophosphorylation takes place at ~100-fold slower rate than the T286/7 reaction, as it requires previous $Ca^{2+}$/CaM dissociation. In contrast to inter-subunit phosphorylation at T286, T305/306 phosphorylation can occur in basal $Ca^{2+}$ conditions in an intra-subunit manner and can render the kinase insensitive to $Ca^{2+}$ until these residues are dephosphorylated by protein phosphatases (Mukherji and Soderling 1994; Shonesy et al. 2014). This internal regulatory property of CaMKII has also important consequences on kinase activity and localization, with implications on synaptic plasticity and memory.

As autophosphorylation of T286 and T305/306 residues critically modulates CaMKII properties, the availability of protein phosphatases at different kinase localizations, as well as their possible differential accessibility to the phosphorylation sites, will be crucial for holoenzyme regulation. At the cytoplasm CaMKII is mostly dephosphorylated by protein phosphatase 2A (PP2A), while at the PSD it is a substrate of phosphatase 1 (PP1) (Strack et al. 1997).

Finally, CaMKII activity can also be regulated by its interaction with other proteins. In consequence, at different subcellular domains kinase function will critically depend on the availability of specific binding partners. A particularly relevant case is the binding of αCaMKII to the cytoplasmic carboxy-terminus of the NMDAR subunit GluN2B. This interaction occurs at the kinase T-site and thus requires CaMKII to be in an open conformation attained by $Ca^{2+}$-dependent kinase activation or T286 phosphorylation. Binding prevents subunit inactivation after the $Ca^{2+}$ concentration returns to basal levels (Bayer and Schulman 2001; Leonard et al. 1999). Therefore, CaMKII binding of GluN2B constitutes an additional mechanism

to generate constitutive enzymatic activity. As T286 phosphorylation, αCaMKII-GluN2B binding also promotes $Ca^{2+}$/CaM trapping, but in contrast to T286 phosphorylation, it suppresses the inhibitory phosphorylation of T305/T306 even in the absence of CaM (Bayer et al. 2001). This form of autonomy does not by itself lead to T286 autophosphorylation because $Ca^{2+}$/CaM binding is required for full exposition of this residue, but it can facilitate $Ca^{2+}$-dependent T286 autophosphorylation as now binding of a single $Ca^{2+}$/CaM complex to a neighboring subunit is required. These properties, together with the increased affinity for $Ca^{2+}$/CaM, make the GluN2B-bound kinase pool particularly sensitive to $Ca^{2+}$ stimulation, and T286 autophosphorylation can thus occur in suboptimal $Ca^{2+}$ conditions. On the other hand, αCaMKII-GluN2B binding is strengthened by T286 phosphorylation (Bayer et al. 2001). Overall, this evidence indicates that CaMKII binding to NMDAR and T286 autophosphorylation can work synergistically in ruling holoenzyme activity and localization. Most of this evidence proceeds from in vitro studies, but consistent results in living cells will be discussed later.

The complex regulation of CaMKII is under intense investigation as it is considered critical for synaptic plasticity and memory.

## 3.2 NMDA Receptor-Dependent Synaptic Plasticity and Memory

Short trains of high-frequency presynaptic stimulation (~100 Hz) and other protocols causing coincident pre and postsynaptic firing within a narrow time window and in a proper sequence, trigger a long-term potentiation (LTP) of glutamatergic transmission by activation of NMDA receptors (NMDAR) and $Ca^{2+}$ influx to the stimulated dendritic spines (Nicoll and Roche 2013). LTP can be induced in different brain regions and constitutes a main model of long-lasting synaptic plasticity. While NMDARs do not participate in all forms of LTP in the mammalian brain (Nicoll and Malenka 1995), we will here mainly discuss NMDAR-dependent potentiation, with emphasis on CA1-hippocampal LTP. On the other hand, NMDAR-dependent long-term depression and other forms of persistent synaptic depression (LTD; Huang 2009) are also widespread brain phenomena that can be triggered by low frequency (~1 Hz) stimulation. Both forms of synaptic plasticity may contribute to memory. Pharmacological or genetic blockade of NMDAR or disruption of downstream signal transduction pathways impair different forms of learning (Anderson et al. 2007). NMDAR-dependent LTP-like processes are induced during spatial learning or novel sensory experience in the hippocampus and cortex, respectively, and in the amygdala during fear conditioning (Whitlock et al. 2006; Takahashi et al. 2003; Rumpel et al. 2005). Remarkably, recent work shows that LTD-inducing stimulation can inactivate a previously generated associative fear memory depending on the amygdala, and an LTP-inducing stimulus can reactivate this memory (Nabavi et al. 2014). Therefore, NMDAR-dependent LTP and LTD are considered key cellular mechanisms for memory formation and modification in the brain.

NMDARs are coincidence detectors of pre and postsynaptic activity, as they only conduce if glutamate is released from presynaptic terminals and the postsynaptic cell is concurrently depolarized to allow removal of basal $Mg^{2+}$ blockade. $Ca^{2+}$ entry through NMDARs can trigger multiple signaling cascades depending on the magnitude, duration and localization of the $Ca^{2+}$ signals. LTP induction relies on the activation of different kinases, with a central role of CaMKII (Lisman et al. 2012; Nicoll and Roche 2013). In turn, LTD has been mainly associated to dephosphorylation processes triggered by the activation of protein phosphatases instead of kinases (Malenka and Bear 2004; but see Coultrap et al. 2014). These different signaling pathways have opposite effects on the enrichment and function of synaptic AMPARs, leading to an enhancement or decrease in synaptic strength, respectively. NMDAR requirement of coincident activity, together with postsynaptic signaling compartmentalization in individual spines, allow LTP and LTD to be synapse-specific, following a Hebbian rule (Hebb 1949).

Functional synaptic plasticity is accompanied by structural changes in dendritic spines, typically modifications in size and morphology that can be induced at the single-synapse level. LTP is associated with a persistent increase in spine size and the stabilization of nascent spines (Hill and Zito 2013; Matsuzaki et al. 2004). In turn, LTD induction is thought to cause spine shrinkage (Zhou et al. 2004). Structural changes during synaptic plasticity may contribute to memory formation and storage (Segal 2005). The involvement of CaMKII as a key enzyme in LTP and associated structural changes is clearly established and will be described here in detail.

## 3.3 CaMKII Pivotal Role in NMDAR-Dependent LTP and Different Forms of Learning

While tens of molecules have been reported to participate in LTP, just a few of them are really essential and the vast majority just modulates this form of plasticity (Nicoll and Roche 2013). Two of the key proteins implicated in NMDAR-dependent LTP are the NMDAR itself and CaMKII.

### *3.3.1 CaMKII Activation Is Necessary and Sufficient to Induce Synaptic Potentiation and Contributes to Learning*

During LTP induction $Ca^{2+}$ entry through NMDARs induces CaMKII activation and trans-subunit autophosphorylation at T286/7 (Fukunaga et al. 1993, 1995). CaMKII activity and T286/7 autophosphorylation rendering the kinase partially autonomous are essential for LTP induction, as if these processes are blocked by pharmacological or genetic means, LTP is prevented or strongly impaired (Lisman et al. 2002; Nicoll and Roche 2013). Seminal work by Silva and coworkers revealed that mutant mice that do not express the major CaMKII isoform αCaMKII have deficient LTP

and impaired spatial learning, (Silva et al. 1992a, b). Residual LTP and learning capacity in knock-in (KI) mice likely relay on a compensatory translocation of the βCaMKII isoform into the PSD (Elgersma et al. 2002). Moreover, in transgenic mice expressing a non-phosphorylatable T286 site mutation (T286A) and thus lacking autophosphorylation-induced autonomous activity, LTP is absent and one-trial spatial learning is severely impaired (Giese et al. 1998). In line with these results, intracellular application of a CaMKII inhibitor blocks LTP induction (Malinow et al. 1989). Finally, in KI mice expressing a kinase-dead mutant form (K42R) of αCaMKII that can bind $Ca^{2+}$/CaM but does not display catalytic activity, LTP is absent and inhibitory avoidance learning is strongly impaired (Yamagata et al. 2009).

On the other hand, αCaMKII activity can by itself generate LTP. Postsynaptic injection or overexpression of a truncated version of αCaMKII displaying constitutive activity triggers potentiation and occludes further LTP induction (Hayashi et al. 2000; Lledo et al. 1995; Pettit et al. 1994; Poncer et al. 2002). However, unexpected results were obtained from transgenic mice expressing a full-length pseudo-phosphorylated form of αCaMKII that displays autonomous activity (T286D), as in this model LTP induced by high-frequency stimulation was normal (Mayford et al. 1995; Rotenberg et al. 1996). This apparent contradiction was clarified by recent work in slice cultures that thoroughly examined the effect on synaptic strength of different CaMKII phosphorylation states of residues T286/T305/T306, by introducing modified holoenzymes rather than subunit fragments (Pi et al. 2010). In this study, expression of full-length T286D αCaMKII potentiated transmission to a saturated level and occluded LTP induction as long as inhibitory autophosphorylation were prevented, i.e. if mutations that abolish phosphorylation of T305/6 (T305A/T306A) were also introduced. In turn, the triple mutation T286D and phosphomimetic T305/306 (T286D/T305/306D) fails to potentiate transmission, causing instead LTD. Indeed, in T286D-KI mice the frequency-response curve for plasticity is shifted to the right (Mayford et al. 1995). This is a form of metaplasticity, a higher-order type of plasticity, or "plasticity of synaptic plasticity", meaning that the differential induction of LTD or LTP by moderate protocols is modulated by previous synaptic activity. The frequency required to generate LTP instead of LTD in T286D-KI is larger than for wild type, but normal LTP can be induced by saturating protocols (Mayford et al. 1995), indicating an increase in the threshold for LTP induction. Consistent with a role of inhibitory phosphorylation in this regulation, in T305/T306A-KI mice metaplasticity is absent (Zhang et al. 2005). Therefore, in addition to the requirement of T286 autophosphorylation for LTP, inhibitory phosphorylation at T305/306 has a key regulatory function. Note also that for all mutant forms of autonomous αCaMKII and inhibitory phosphorylation, T286A/D and T305D/A, spatial learning was impaired (Elgersma et al. 2002; Giese et al. 1998; Rotenberg et al. 1996).

In summary, αCaMKII activation and T286 autophosphorylation are required for LTP and several forms of learning, and phospho-T286 can generate LTP or LTD depending on T305/306 phosphorylation that acts as a control point for metaplasticity and memory formation.

With respect to the β subunit, βCaMKII KO mice also present impaired LTP and hippocampal-dependent learning (Borgesius et al. 2011). However, this is most probably due to altered αCaMKII distribution and targeting to dendritic spines, for which a non-catalytic role of the β subunit is required. This and other evidence that will be discussed in detail later implicate CaMKII holoenzymes in both signaling and structural processes in dendritic spines (Hell 2014). Note that as holoenzymes normally present both types of subunits, modifications in one type may alter the availability and function of the other.

CaMKII activation and phosphorylation state have important consequences on its interaction with other proteins and thus on kinase localization. This allows a differential action on targets situated at specific spine microdomains. In turn, CaMKII binding to specific cytoplasmic and PSD proteins can regulate kinase activity, as is the case of the NMDAR subunit GluN2B. These properties allow an exquisite activity-dependent and spatiotemporal regulation of CaMKII and their targets in spines.

### *3.3.2 Different Pools of CaMKII Exist in Dendritic Spines in Basal Conditions*

CaMKII is highly abundant in spine cytoplasm and it is also a major component of the PSD (Hell 2014). Early biochemical, EM and optical studies suggested the existence of different CaMKII pools in dendritic spines, depending on various protein–protein interactions and subject to regulation by synaptic activity. Nowadays, the development of new optical methodologies allows unraveling CaMKII distribution, interactions, mobility and regulation by activity in dendrites and spines, including high-resolution spatiotemporal molecular tracking at a microdomain scale.

The steady-state turnover of overexpressed or endogenous CaMKII in spines was initially measured in hippocampal cell and slice cultures by quantitative optical microscopy techniques as confocal fluorescence recovery after photobleaching (FRAP) or two-photon (2p)-laser photoactivation (PA) and microscopy (Okamoto et al. 2004; Sharma et al. 2006; Asrican et al. 2007; Sturgill et al. 2009). CaMKII subpopulations with different basal turnover rates were detected in these studies: a major fraction (~65–80 %) leaves spines in a few minutes, which is longer than expected for simple diffusion, suggesting that at least part of this pool requires previous dissociation from binding partners at spines. In turn, a small but detectable fraction (~15–20 %) remains unchanged after 30 min, indicating the existence of a strongly-bound fraction (but see Shen and Meyer 1999). More recently developed methodologies as photo-activated localization microscopy (PALM) and stochastic optical reconstruction microscopy (STORM), have allowed high-resolution monitoring of CaMKII mobility in individual spines by single-molecule tracking (Lu et al. 2014). According to their kinetic properties, at least three different pools of αCaMKII can be detected in spines; a larger fraction is regulated by the actin cytoskeleton and ~10 % can be considered as immobile (no change after 30 min).

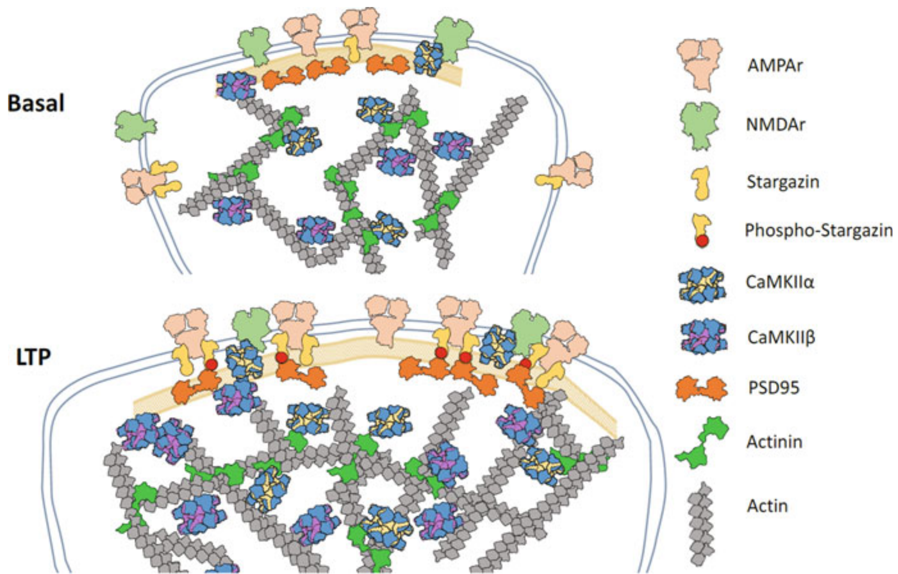


**Fig. 3.3** Model of the functional and structural changes in a dendritic spine after LTP induction. Cartoon illustrating the general spine structure and main protein–protein interactions in PSD and actin cytoskeleton before and after LTP induction. CaMKII holoenzymes containing either α or β subunits are shown to highlight their differential interactions with spine proteins. Note the increase in CaMKII-NMDAR complex at the synapse and stargazin phosphorylation promoting PSD-95 binding and AMPAR trapping. Actin-cytoskeleton with CaMKII holoenzymes crosslinking the filaments and their restructuration after LTP are also shown

In basal conditions, a major proportion of CaMKII in spines interacts with the cytoskeleton. This mainly relies on the direct binding of βCaMKII to F-actin, through a segment in the variable linker domain that is absent in αCaMKII (Fink et al. 2003; Lu et al. 2014; Shen et al. 1998). Interestingly, this association to actin filaments confers the holoenzyme a key role in the maintenance of dendritic spine structure. Through the stoichiometric interaction mediated by the β subunit, CaMKII acts as an F-actin bundling protein that stabilizes spine cytoskeleton (Okamoto et al. 2007; Fig. 3.3). In agreement with this, downregulation of βCaMKII reduced the size and number of mature spines and increased filopodial structures in hippocampal organotypic slices (Okamoto et al. 2004). Actin filament crosslinking by CaMKII depends on the β subunit in a non-enzymatic manner, as this is not affected by mutations that abolish kinase catalytic activity. Therefore, in addition to CaMKII actions as a signaling molecule, in the basal state it plays a structural role as a cytoskeletal component. Moreover, βCaMKII interaction with F-actin is required for CaMKII targeting to spines: normal CaMKII distribution in spines relies on the presence of βCaMKII but not on its activity, as mutant mice that do not express the β subunit display holoenzyme misslocalization, but transgenic mice with a mutated β subunit that cannot be activated by $Ca^{2+}$/CaM, retain actin-binding properties and show normal CaMKII enrichment in spines (Borgesius et al. 2011).

CaMKII is also highly enriched at the PSD (Kennedy et al. 1983), where a key binding partner is GluN2B. This interaction is critical for synaptic plasticity (reviewed in Sanhueza and Lisman 2013) and spine stabilization (Hill and Zito 2013). Other αCaMKII PSD-binding partners include α-actinin, densin-180, the scaffolding protein Shank, $Ca^{2+}$ channels, the NMDAR subunit GluN1, as well as other CaMKII holoenzymes (Petersen et al. 2003).

The binding of α subunits to synaptic proteins and β to actin may provide a physical link between PSD and cytoskeleton. Moreover, the interaction of αCaMKII with the F-actin-binding protein α-actinin constitutes an indirect mechanism for holoenzyme association to spine cytoskeleton (Hell 2014). α-actinin cross-links F-actin and it also binds to the GluN1 subunit of NMDAR. Thus, these interactions may strengthen the structural connection between PSD and spine actin cytoskeleton.

It is known that spine head volume in CA1 pyramidal neurons is positively correlated with both PSD size and synaptic strength in individual spines (Harris and Stevens 1989; Matsuzaki et al. 2001). Remarkably, a correlation was also found between the bound-amount of αCaMKII and both spine size and synaptic strength (Asrican et al. 2007). This applies to the non-soluble pool that may include the PSD-associated subset, the fraction bound to actin and the self-associated holoenzymes (Hudmon et al. 2005; Petersen et al. 2003). As CaMKII can simultaneously interact with multiple PSD proteins (Robison et al. 2005) as well as with actin cytoskeleton, holoenzymes may play a structural role at synapses and spines by clustering molecular complexes and thus contributing to maintain synaptic strength and spine stability. Moreover, as these protein–protein interactions depend on kinase activation state, it is expected that CaMKII may constitute a dynamic organizer of synaptic complexes, involved in the structural changes occurring during the induction of plasticity. Experimental evidence supporting this claim will be discussed in Sect. 3.4.

Ultrastructure studies have widely reported the existence of a large and variable pool of CaMKII holoenzymes at the cytoplasmic side of the PSD (Dosemeci et al. 2001; Petersen et al. 2003). Recent high-resolution negative stain EM tomography allowed 3D mapping of the ring-like structures deep in the PSD, detecting the presence of scattered holoenzymes also in the PSD core (Fera et al. 2012). Further studies are needed to disclose if the CaMKII populations at the PSD matrix and membrane fringe constitute different functional pools.

With regard to estimations of the basal amount of CaMKII holoenzymes at synapses, it is known that anoxic/ischemic stress during acute brain slice preparation and tissue homogenization causes post-mortem kinase translocation to PSD (Suzuki 1994). Thus, many studies probably overestimated the amount of PSD-associated holoenzymes in basal conditions. Recently, Ding and coworkers (Ding et al. 2013) confirmed this prediction by comparing immuno-electron microscopy observations from quickly-fixed tissue with that prepared after a delay of a few minutes. Even so, this study still found a substantial pool of αCaMKII localized to the cytoplasmic fringe of PSD and a smaller but consistently visible group within the PSD core, mainly concentrated at the lateral edges. Intriguingly, a significant amount of CaMKII localized to the proximal cytoplasmic region, close to spine neck.

Basal enrichment of CaMKII varies considerably among individual spines and PSD and this variability is much higher than for PSD-95, member of the

membrane-associated guanylate kinase (MAGUK) family and the most abundant scaffold protein at synapses (Asrican et al. 2007; Fera et al. 2012; Petersen et al. 2003). This suggests a dynamical regulation of PSD CaMKII content and is consistent with an information storage role of the enzyme. Indeed, CaMKII undergoes redistribution in dendrites and spines due to the activity-dependent regulation of its interactions with other proteins.

### *3.3.3 CaMKII Activation and Persistent Translocation to PSD During LTP*

Time-lapse fluorescence microscopy studies have allowed live monitoring of activity-dependent CaMKII redistribution in dendrites, spines and PSD, adding to initial biochemical results. Early imaging work reported a transient translocation of GFP-αCaMKII from dendrites to spines and from an F-actin-attached state to a presumed synaptic location in cultured hippocampal cells (Shen and Meyer 1999). Synaptic stimulation triggers holoenzyme detachment from spine actin cytoskeleton and its synaptic translocation by association to GluN2B (Leonard et al. 1999) (Fig. 3.3). Dissociation of β-CaMKII from F-actin can be induced by either $Ca^{2+}$/CaM binding to βCaMKII or Thr287 autophosphorylation (Hell 2014). Moreover, as $Ca^{2+}$/CaM interacts with αCaMKII at the same region that α-actinin, $Ca^{2+}$ entry to spines should also disrupt this association. Interestingly, the duration of CaMKII translocation to synaptic puncta increases with stimulation strength (Bayer et al. 2006). While both CaMKII translocation and GluN2B binding do not require T286 phosphorylation but only kinase activation, autophosphorylation at this site stabilizes the interaction and prolongs kinase activity-induced accumulation at postsynaptic sites in living cells (Bayer et al. 2006; Meyer and Shen 2000; Shen and Meyer 1999).

Remarkably, live confocal/2p imaging and immunogold EM microscopy work showed that after demonstrated induction of LTP in hippocampal slice cultures, activated CaMKII undergoes a persistent accumulation at spines and PSD (Otmakhov et al. 2004; Strack et al. 1997). In agreement with this, FRAP studies indicated a long-lasting reduction in the mobility of αCaMKII in spines after LTP induction (Sharma et al. 2006). Interestingly, single-molecule tracking of αCaMKII by PALM and STORM techniques revealed that besides activity-dependent immobilization at the PSD, it also accumulates at regions far from synapsis (Lu et al. 2014), presumably close to spine neck, supporting a CaMKII role in spine structural plasticity (see below).

The induction of LTP in single spines by optical methods has demonstrated that both CaMKII activation and translocation is specific for the stimulated spines (Lee et al. 2009; Zhang et al. 2008). This suggests that translocation of active CaMKII to synapses by binding to the NMDAR allows the synapse-specificity of LTP.

CaMKII permanence at PSD is differentially modulated by their autophosphorylation processes. The pseudo-phosphorylated form T286D prolongs kinase retention time at synaptic sites and the non-phosphorylatable version T286A is targeted to

synapses but to a lower level than wild type (Bayer et al. 2006; Gustin et al. 2011; Meyer and Shen 2000). In turn, αCaMKII T305/306 inhibitory autophosphorylation promotes kinase dissociation from synapses. In vitro studies showed that T305/306 autophosphorylation reduced binding to PSD (Strack et al. 1997). Moreover, KI mice with non-phosphorytable T305/306 display increased PSD-attached CaMKII in vivo, whereas pseudo-phosphorylated T305/306 dramatically decrease binding (Elgersma et al. 2002). In summary, autonomy-inducing and inhibitory autophosphorylations regulates in opposing ways CaMKII translocation and persistence at synapses.

### *3.3.4 CaMKII and Structural Plasticity of Dendritic Spines*

In addition to synaptic functional changes, CaMKII is involved in spine structural plasticity (Okamoto et al. 2009). The long-lasting increase in spine size during LTP results from the reorganization of actin cytoskeleton (Murakoshi and Yasuda 2012). CaMKII activation is required for persistent spine enlargement as the latter is blocked by either CaMKII pharmacological or genetic inhibition (Matsuzaki et al. 2004; Yamagata et al. 2009).

$Ca^{2+}$ increase during LTP induction promotes the dissociation of βCaMKII from F-actin, destabilizing holoenzyme-dependent filament crosslinking and causing cytoskeleton disassembly (Okamoto et al. 2007). At later stages, the amount of F-actin in spines increases (Okamoto et al. 2004), generating more binding sites for CaMKII accumulation, which may stabilize structural changes by bundling F-actin and slowing down actin turnover in spines. On the other hand, CaMKII may regulate cytoskeleton reorganization by the modulation of the small-GTPases RhoA and Cdc42 pathways that promote actin depolymerization and polymerization, respectively. These GTPases may contribute in a spatiotemporal complex way to the initial actin restructuration and the maintenance phase of spine enlargement (Colgan and Yasuda 2014). Moreover, during LTP induction CaMKII regulates the confinement of an F-actin "enlargement pool" at the stimulated spine, a process required for persistent spine expansion (Honkura et al. 2008). Finally, spine size can be increased by αCaMKII T286 autophosphorylation through a structural action not requiring catalytic activity (Pi et al. 2010). The underlying mechanism of this spine change is not clear, but is different from that of synaptic strengthening and can be dissociated from it.

Therefore, by playing both enzymatic and structural roles, CaMKII mediates functional synaptic plasticity and spine morphological changes.

### *3.3.5 CaMKII Binding to NMDAR and the Mechanisms of CaMKII-Mediated Synaptic Strengthening*

The complex formed by CaMKII and NMDAR is present in basal conditions in PSD (Leonard et al. 1999; Strack and Colbran 1998) and increases after NMDAR stimulation. Activity-dependent αCaMKII-GluN2B binding is critical for LTP induction

and is triggered by strong synaptic stimulation that activates CaMKII in spines. Genetic interference with this interaction abolishes or reduces LTP induction and impairs memory (Barria and Malinow 2005; Zhou et al. 2007; Halt et al. 2012; Stein et al. 2014;). Therefore, CaMKII translocation to PSD by direct binding to NMDARs is required for synaptic strengthening, presumably because it allows kinase close proximity to critical synaptic targets.

The mechanism of synaptic potentiation by CaMKII is not fully understood, but is thought to rely on the regulation of AMPAR function and localization (reviewed in Lisman et al. 2012; Nicoll and Roche 2013). A first proposed mechanism involves the phosphorylation of S831 residue in the C-tail of the AMPAR GluA1 subunit, a modification that increases single-channel conductance of GluA1 homomers by enhancing the gating of individual subunits (reviewed in Derkach et al. 2007). A similar modulation occurs for native GluA1-GluA2 heteromers associated to transmembrane AMPAR-regulatory proteins (TARPs; Kristensen et al. 2011). However, the proportion of AMPARs undergoing phosphorylation at S831 was recently shown to be very small, thus questioning the relevance of this mechanism for LTP (Hosokawa et al. 2015). On the other hand, it has long been known that during the induction of LTP AMPARs are inserted into synapses in a CaMKII-dependent manner (Hayashi et al. 2000).

New available methodologies are contributing to shed light on the implications of activity-dependent CaMKII translocation to PSD and the molecular basis of synaptic enhancement. FRAP studies and 2p microscopy using pH-sensitive tags allow monitoring the population of surface receptors and their dynamics in neurons from slice cultures. Moreover, the trajectory of individual surface AMPARs and the translocation of single CaMKII molecules can now be tracked by high-resolution microscopic techniques as receptor labelling with semiconductor quantum dots (QD) and FLIM/STORM imaging. The use of these tools has modified the way synapses are conceived, from a view in which neurotransmitter receptors and other specialized proteins are very stable at synapses, to an idea of synapses as highly dynamic structures allowing permanent in and out receptor trafficking (Choquet 2010).

In basal conditions, AMPARs continuously cycle between the plasma membrane and intracellular compartments, maintaining a pool of surface receptors that can be recruited to synapses. Stable synaptic transmission represents a state of dynamic equilibrium in the highly active AMPARs traffic. Receptor abundance at synapses depends on the complex regulation of different processes: receptor exocytosis and endocytosis (for degradation or recycling), surface diffusion and synaptic trapping (Newpher and Ehlers 2008; Opazo and Choquet 2011). During the induction of synaptic plasticity this equilibrium is altered by modification of one or more of these steps. Synaptic potentiation is associated with an increase in both AMPAR exocytosis and endocytosis, thought to occur mainly at extrasynaptic sites (Choquet 2010). However, imaging of recombinant surface-fluorescent receptors suggests that synaptic recruitment of AMPARs during LTP induction mainly involves trapping of laterally diffusing receptors (Makino and Malinow 2009; Patterson et al. 2010), at least during early stages (Lledo et al. 1998). Incorporation of AMPARs to the plasma membrane by a mechanism depending on the small GTPase Ras-ERK pathway may contribute to later LTP phases (Patterson et al. 2010; Zhu et al. 2002).

NMDAR activation induces AMPAR immobilization at PSD, as inferred from single-molecule tracking. Translocation of active CaMKII to synapses by binding to GluN2B is required to induce the diffusional trapping of AMPARs by a process that is not well understood, but may involve the generation of new synaptic binding sites or ‘slots’ for receptor recruitment (Lisman et al. 2012). Overexpression of a mutant form of αCaMKII that does not bind to GluN2B abolishes both CaMKII translocation and AMPAR trapping at synapses (Opazo et al. 2010; Tomita et al. 2005). On the other hand, the catalytically-dead αCaMKII K42R that adopts the open conformation after binding $Ca^{2+}$/CaM but does not exhibit enzymatic activity, preserves the ability to translocate to synapses in an activity-dependent way, but fails to induce receptor trapping (Opazo et al. 2010). In agreement with this, K42R knock-in mice display normal CaMKII synaptic translocation, but LTP, structural plasticity and learning are severely impaired (Yamagata et al. 2009).

Overall, these results suggest that activity-dependent AMPAR targeting to synapses requires both CaMKII binding to the NMDAR and kinase enzymatic activity. A widely studied CaMKII phosphorylation target at synapses is stargazin, an AMPAR auxiliary subunit and member of the TARP family that regulates both channel gating and trafficking (Díaz 2010). This reaction enhances the binding of stargazin to PSD-95 through PDZ domains, promoting the diffusional trapping of stargazin-associated receptors at synapses (Opazo et al. 2010). Stargazin phosphorylation depends on the pool of CaMKII bound to the NMDAR (Tsui and Malenka 2006). This mechanism may promote the generation of new synaptic slots for receptor recruitment during early LTP.

In line with this evidence, a mutation that mimics stargazin phosphorylation at the CaMKII target S9 (S9D) enhances transmission and occludes further LTP induction in hippocampal slice cultures. On the other hand, preventing stargazin phosphorylation by introducing a non-phosphorylatable form (S9A) blocks LTP. Besides S9, CaMKII phosphorylates a series of serine residues at stargazin C-tail (Tomita et al. 2005). Unphosphorylated stargazin associates to lipid bilayer through electrostatic interactions and phosphorylation causes tail release from membrane, allowing binding to PSD-95 (Sumioka et al. 2010). During LTP this complex may recruit AMPARs by providing new anchoring sites at synapses (Fig. 3.3). In this model, phosphorylated stargazin and PSD-95 are the minimal components of the synaptic trapping slots (Opazo et al. 2011). In contrast to this view, a recent study proposes that the minimal requirement for receptor synaptic insertion during LTP is the existence of a sufficiently large pool of extrasynaptic glutamate receptors (Granger et al. 2013).

CaMKII switch-like properties inspired the theory that the holoenzyme may not only trigger LTP, but also sustain it through a long-lasting enzymatic effect on their synaptic targets, thus working as a molecular memory that stores information on previous synaptic activity (reviewed in Lisman et al. 2002). Biochemical studies supported this proposal showing that after induction T286 becomes phosphorylated and remains in this state for at least 1 h (Fukunaga et al. 1995; Lengyel et al. 2004). Intriguingly, kinase autonomous activity was found to last only a few minutes (Lengyel et al. 2004), challenging the proposal that persistent CaMKII enzymatic

action could maintain potentiation. This issue was further addressed by a study in which LTP and ongoing CaMKII activation were simultaneously examined in single dendritic spines by high-resolution optical methods (Lee et al. 2009). LTP was induced by glutamate uncaging and CaMKII spatiotemporal activation was monitored by the αCaMKII activity sensor Camuiα, based on fluorescence resonance energy transfer (FRET). Study showed that αCaMKII activation during LTP lasted for ~1 min. Still, authors acknowledge that a remaining autonomous activity generated by the small fraction of αCaMKII subunits bound to GluN2B (estimated to be ~0.2 % of spine CaMKII; Feng et al. 2011) may be below the resolution limit of this methodology (Fujii et al. 2013; Lee et al. 2009). Interestingly, new evidence with Camuiα indicates that kinase deactivation is not due to T286 dephosphorylation (Otmakhov et al. 2015), consistent with a prolonged phosphorylation of this site. The role of this persistent phosphorylated state as well as the mechanism by which autonomous CaMKII activity is lost, remain to be determined. Possible mechanisms are discussed later.

Overall, evidence suggests that, at least during early stages of LTP, CaMKII-activity and binding to NMDAR is required for AMPAR trapping at synapses, presumably acting through TARP phosphorylation. In turn, experimental results to date do not support a role of T286-dependent autonomous activity in the maintenance phase of LTP (Lee et al. 2009), suggesting the involvement of other mechanisms at later stages. CaMKII activity can be transduced into the synaptic dispersion of SynGAP, which inactivates the Ras pathway, thus supporting AMPAR incorporation and spine enlargement during late LTP (Araki et al. 2015).

A different line of evidence suggests that the CaMKII-GluN2B complex can play a role in late-LTP and the persistent control of synaptic strength (Sanhueza et al. 2011; Lisman et al. 2012), possibly by a structural action. Interestingly, the possibility arises that long-lasting T286 phosphorylation after LTP might stabilize this interaction.

## 3.4 CaMKII-NMDAR Binding in the Maintenance of Synaptic Strength: A Memory Storage Mechanism at Synapses?

It has long been known that NMDAR-bound CaMKII exists in basal conditions in the PSDs of living cells (Leonard et al. 1999; Strack and Colbran 1998), but the functional implications of this association are just beginning to be unraveled. In addition to a role in LTP induction, this complex may be involved in the control of synaptic strength. Key evidence in support of this idea comes from electrophysiological, biochemical and imaging work in acute and organotypic brain slices, as well as single-molecular tracking of surface AMPA receptors in cell cultures upon genetic manipulations of αCaMKII and its binding to GluN2B.

The development of synthetic peptides derived from CaMKIIN, an endogenous and selective CaMKII inhibitor protein (Chang et al. 1998, 2001), has allowed

investigation of the relationship between basal CaMKII-GluN2B binding and synaptic function. These peptides (CN peptides) were developed from the fraction of CaMKIIN protein required for kinase inhibition, and bind to αCaMKII at a region that include the GluN2B-binding site (T-site). Accordingly, CN peptides block $Ca^{2+}$-induced binding of αCaMKII to GluN2B in vitro (Vest et al. 2007). As for GluN2B, CN interaction with CaMKII can only take place when the kinase is in the open conformation due to $Ca^{2+}$/CaM binding or T286 autophosphorylation. Interestingly, the transient application of CN peptide to hippocampal slices in basal conditions persistently disrupted CaMKII-GluN2B interaction and caused a long-lasting synaptic depression (Sanhueza et al. 2011). Concurrently, the bound amount of αCaMKII in spines was persistently decreased, with no evident change in spine size. CN-induced depression is postsynaptic, is different from LTD and correlates with age-dependent average basal synaptic enrichment of CaMKII (Sanhueza et al. 2011; Gouet et al. 2012; Petralia et al. 2005). Moreover, CN increased AMPAR basal surface diffusion, as did genetically disruption of CaMKII binding to GluN2B (Opazo et al. 2010).

LTP induction is blocked in the presence of CN peptides, as expected for a CaMKII inhibitor, but surprisingly, CN-treated slices display enhanced LTP when induced after drug removal (Sanhueza et al. 2007, 2011). This does not appear to be a metaplasticity phenomenon, as is observed for protocols that saturate LTP. Most relevant, transient CN application allowed LTP re-induction in synaptic pathways that previously underwent saturated LTP. To note, the persistent effect on transmission and subsequent LTP induction is observed for drug concentrations that dissociate the CaMKII-GluN2B complex, but not for lower concentrations causing only kinase inhibition. An attractive interpretation of these results is that CN peptides partially reverse an LTP maintenance mechanism at synapses by causing dissociation of CaMKII from GluN2B. While this hypothesis requires direct demonstration, the following indirect evidence supports it: (a) CaMKII binding to GluN2B is required for LTP induction; (b) this complex exists at synapses at basal levels of activity and its disruption is associated with synaptic depression and enhanced subsequent LTP; and (c) CNs reverse LTP saturation, allowing its re-induction. Alternatively, the observed modulation of synaptic strength and of the further induction of plasticity may indicate that agents causing CaMKII-GluN2B dissociation trigger a homeostatic regulation of synaptic function.

Interestingly, basal transmission and synaptic confinement of AMPARs also require binding of stargazin to PSD-95 or other MAGUKs (Bats et al. 2007; Schnell et al. 2002; Sainlos et al. 2011). It is not clear whether the stargazin pool basally bound to MAGUKs is phosphorylated, but this cannot be discarded as basal phosphorylation of TARPs exists at PSDs (Inamura et al. 2006), suggesting this may be involved in basal receptor confinement. Indeed, Tomita et al. (2005) proposed that a balance between stargazin phosphorylation/dephosphorylation processes may exist at basal levels of activity. The similarity in the effects of targeting CaMKII-NMDAR complex and TARP-PSD-95 interaction is intriguing. More experiments are needed to determine a possible relationship among them.

A role of the CaMKII-NMDAR complex in the maintenance of synaptic strength may rely on protein–protein interactions allowing receptor clustering at synapses (Lisman et al. 2012; MacGillavry et al. 2013). Still, the involvement of CaMKII autonomous activity of the small but strategically-located GluN2B-bound pool cannot be discarded.

The CaMKII-NMDAR complex may constitute a mechanism for LTP maintenance or other information storage mechanism at synapses, whose regulation would allow maintaining synaptic strength and plasticity in dynamic ranges, avoiding their saturation.

## 3.5 CaMKIIN, a Putative CaMKII-Regulating Protein in the Brain

The endogenous protein CaMKIIN specifically inhibits CaMKII enzymatic activity by binding to the T-site and sterically occluding substrate access to the neighboring active site (Chang et al. 1998; Vest et al. 2007). CaMKIIN and related peptides interfere with $Ca^{2+}$-dependent kinase binding to GluN2B in vitro (Chang et al. 1998; Vest et al. 2007) and causes the dissociation of basally existent complex in living cells (Sanhueza et al. 2011). To date, two isoforms have been identified, CaMKIINα and β (also known as CaMK2N1 and 2), showing wide but not identical distributions in the brain (Chang et al. 2001; Radwańska et al. 2010). CaMKIIN isoforms are found in CaMKII-containing cells and were first reported to be soluble proteins of around 80 amino acids (but see Saha et al. 2007) and 70 % identity. CaMKIIN mRNA is rapidly (<30 min) expressed and protein transiently up-regulated by novelty or fear learning in an isoform- and region-specific manner (Lepicard et al. 2006; Radwańska et al. 2010). CaMKIIN mRNA rapid and transient expression after learning resembles immediate-early genes such as c-Fos, Zif268 or Arc/Arg3.1 (Guzowski 2002). Therefore, CaMKIINα, β proteins are putative plasticity-related proteins. It was hypothesized that they may be part of a negative feedback mechanism, inhibiting further CaMKII activity or its association with NMDARs (Lucchesi et al. 2011). Interestingly, they could underlie the transient kinase activation after LTP induction. Both CaMKIIN isoforms inhibit CaMKII with the same potency and specificity. CaMKIINα mRNA displays a cell- and time-specific regulation by an antisense transcript expressed at lower levels and in specific brain regions, including neocortex, caudoputamen and piriform cortex (Ling et al. 2011). These authors showed that the antisense transcript can form cytoplasmic double-stranded RNA aggregates with the CaMKIINα mRNA, potentially inhibiting its expression. Moreover, both sense and antisense transcripts are upregulated in adult stages compared to embryos/P1 animals. Overall, this evidence suggests a complex gene expression and post-transcriptional regulation of CaMKIINs during development and learning.

The function of these proteins in the intact brain is unknown. As discussed, a transient increase may depress synaptic strength and reverse LTP saturation in the hippocampus. As CaMKIIN inhibits both $Ca^{2+}$-dependent and independent CaMKII

activity and also interferes with kinase interaction with GluN2B, its regulation of synaptic function may be quite complex. The modulation of plasticity would depend on whether CaMKIIN increases are chronic or transient: sustained upregulation of CaMKIIN may inhibit or impair the induction of LTP in a graded way and the opposite may happen for its downregulation. On the other hand, transient increases in protein -such as those occurring after learning- would have the opposite effect, i.e., to facilitate LTP induction after CaMKIIN returns to baseline (Sanhueza et al. 2007, 2011). Possible roles in the selective erasure of previously acquired memories, as a homeostatic regulator of synaptic function or as a mechanism to stop CaMKII activity after LTP, remain to be directly evaluated. In any case, CaMKIIN would allow keeping synaptic function in a dynamic range.

## 3.6 Concluding Remarks

CaMKII intricate regulation by $Ca^{2+}$ signals, autophosphorylation and interactions with other proteins allow an exquisite spatiotemporal regulation of the enzyme activity and localization, determining its key role in several aspects of synaptic dynamics. Within holoenzyme large repertoire of functions, probably the most exciting is its participation in synaptic plasticity and memory. While the development of new methodologies in recent years has allowed learning much about these processes, there are many questions that are still open. While CaMKII is long known to be critical for LTP, it is surprising that we have not yet achieved a clear understanding of what are the mechanisms coupling its activation with synaptic transmission enhancement.

Here we reviewed some of the best known aspects of CaMKII contributions to synaptic plasticity and structure, and outlined fundamental questions that have not yet been resolved. Important aspects had to be left out, as the proposed role in homeostatic synaptic plasticity, LTD, GABAergic synaptic function, among others. The fact that holoenzyme roles are so diverse is perhaps what makes it more difficult to dissect their role in memory. CaMKII is emerging as master molecule playing both enzymatic and structural roles in synaptic signaling and information storage the synapse, with broader implications than previously thought.

## References

Anderson P, Morris R, Amaral D, Bliss T, O'Keefe J (2007) The hippocampus book. Oxford University Press, Oxford

Araki Y, Zeng M, Zhang M, Huganir RL (2015) Rapid dispersion of SynGAP from synaptic spines triggers AMPA receptor insertion and spine enlargement during LTP. Neuron 85:173–189

Asrican B, Lisman J, Otmakhov N (2007) Synaptic strength of individual spines correlates with bound Ca2+-calmodulin-dependent kinase II. J Neurosci 27:14007–14011

Barria A, Malinow R (2005) NMDA receptor subunit composition controls synaptic plasticity by regulating binding to CaMKII. Neuron 48:289–301

Bats C, Groc L, Choquet D (2007) The interaction between stargazin and PSD-95 regulates AMPA receptor surface trafficking. Neuron 53:719–734

Bayer KU, Schulman H (2001) Regulation of signal transduction by protein targeting: the case for CaMKII. Biochem Biophys Res Commun 289:917–923

Bayer KU, De Koninck P, Leonard AS, Hell JW, Schulman H (2001) Interaction with the NMDA receptor locks CaMKII in an active conformation. Nature 411:801–805

Bayer KU, LeBel E, McDonald GL, O'Leary H, Schulman H, De Koninck P (2006) Transition from reversible to persistent binding of CaMKII to postsynaptic sites and NR2B. J Neurosci 26:1164–1174

Borgesius NZ, van Woerden GM, Buitendijk GHS, Keijzer N, Jaarsma D, Hoogenraad CC, Elgersma Y (2011) βCaMKII plays a nonenzymatic role in hippocampal synaptic plasticity and learning by targeting αCaMKII to synapses. J Neurosci 31:10141–10148

Chang BH, Mukherji S, Soderling TR (1998) Characterization of a calmodulin kinase II inhibitor protein in brain. Proc Natl Acad Sci U S A 95:10890–10895

Chang BH, Mukherji S, Soderling TR (2001) Calcium/calmodulin-dependent protein kinase II inhibitor protein: localization of isoforms in rat brain. Neuroscience 102:767–777

Chao LH, Pellicena P, Deindl S, Barclay LA, Schulman H, Kuriyan J (2010) Intersubunit capture of regulatory segments is a component of cooperative CaMKII activation. Nat Struct Mol Biol 17:264–272

Choquet D (2010) Fast AMPAR trafficking for a high-frequency synaptic transmission. Eur J Neurosci 32:250–260

Colgan LA, Yasuda R (2014) Plasticity of dendritic spines: subcompartmentalization of signaling. Annu Rev Physiol 76:365–385

Coultrap SJ, Buard I, Kulbe JR, Dell'Acqua ML, Bayer KU (2010) CaMKII autonomy is substrate-dependent and further stimulated by Ca2+/calmodulin. J Biol Chem 285: 17930–17937

Coultrap SJ, Freund RK, O'Leary H, Sanderson JL, Roche KW, Dell'Acqua ML, Bayer KU (2014) Autonomous CaMKII mediates both LTP and LTD using a mechanism for differential substrate site selection. Cell Rep 6:431–437

De Koninck P, Schulman H (1998) Sensitivity of CaM kinase II to the frequency of Ca2+ oscillations. Science 279:227–230

Derkach VA, Oh MC, Guire ES, Soderling TR (2007) Regulatory mechanisms of AMPA receptors in synaptic plasticity. Nat Rev Neurosci 8:101–113

Díaz E (2010) Regulation of AMPA receptors by transmembrane accessory proteins. Eur J Neurosci 32:261–268

Ding JD, Kennedy MB, Weinberg RJ (2013) Subcellular organization of CaMKII in rat hippocampal pyramidal neurons. J Comp Neurol 521:3570–3583

Dosemeci A, Tao-Cheng JH, Vinade L, Winters CA, Pozzo-Miller L, Reese TS (2001) Glutamate-induced transient modification of the postsynaptic density. Proc Natl Acad Sci U S A 98:10428–10432

Elgersma Y, Fedorov NB, Ikonen S, Choi ES, Elgersma M, Carvalho OM, Giese KP, Silva AJ (2002) Inhibitory autophosphorylation of CaMKII controls PSD association, plasticity, and learning. Neuron 36:493–505

Feng B, Raghavachari S, Lisman J (2011) Quantitative estimates of the cytoplasmic, PSD, and NMDAR-bound pools of CaMKII in dendritic spines. Brain Res 1419:46–52

Fera A, Dosemeci A, Sousa AA, Yang C, Leapman RD, Reese TS (2012) Direct visualization of CaMKII at postsynaptic densities by electron microscopy tomography. J Comp Neurol 520:4218–4225

Fink CC, Bayer KU, Myers JW, Ferrell JE, Schulman H, Meyer T (2003) Selective regulation of neurite extension and synapse formation by the beta but not the alpha isoform of CaMKII. Neuron 39:283–297

Fujii H, Inoue M, Okuno H, Sano Y, Takemoto-Kimura S, Kitamura K, Kano M, Bito H (2013) Nonlinear Decoding and Asymmetric Representation of Neuronal Input Information by CaMKIIα and Calcineurin. Cell Rep 3:978–987

Fukunaga K, Stoppini L, Miyamoto E, Muller D (1993) Long-term potentiation is associated with an increased activity of Ca2+/calmodulin-dependent protein kinase II. J Biol Chem 268:7863–7867

Fukunaga K, Muller D, Miyamoto E (1995) Increased phosphorylation of Ca2+/calmodulin-dependent protein kinase II and its endogenous substrates in the induction of long term potentiation. J Biol Chem 270:6119–6124

Giese KP, Fedorov NB, Filipkowski RK, Silva AJ (1998) Autophosphorylation at Thr286 of the alpha calcium-calmodulin kinase II in LTP and learning. Science 279:870–873

Gouet C, Aburto B, Vergara C, Sanhueza M (2012) On the mechanism of synaptic depression induced by CaMKIIN, an endogenous inhibitor of CaMKII. PLoS One 7, e49293

Granger AJ, Shi Y, Lu W, Cerpas M, Nicoll R (2013) LTP requires a reserve pool of glutamate receptors independent of subunit type. Nature 493:495–500

Gustin RM, Shonesy BC, Robinson SL, Rentz TJ, Baucum AJ, Jalan-Sakrikar N, Winder DG, Stanwood GD, Colbran RJ (2011) Loss of Thr286 phosphorylation disrupts synaptic CaMKIIα targeting, NMDAR activity and behavior in pre-adolescent mice. Mol Cell Neurosci 47:286–292

Guzowski JF (2002) Insights into immediate-early gene function in hippocampal memory consolidation using antisense oligonucleotide and fluorescent imaging approaches. Hippocampus 12:86–104

Halt AR, Dallapiazza RF, Zhou Y, Stein IS, Qian H, Juntti S, Wojcik S, Brose N, Silva AJ, Hell JW (2012) CaMKII binding to GluN2B is critical during memory consolidation. EMBO J 31:1203–1216

Hanson PI, Schulman H (1992) Neuronal Ca2+/calmodulin-dependent protein kinases. Annu Rev Biochem 61:559–601

Harris KM, Stevens JK (1989) Dendritic spines of CA 1 pyramidal cells in the rat hippocampus: serial electron microscopy with reference to their biophysical characteristics. J Neurosci 9:2982–2997

Hayashi Y, Shi SH, Esteban JA, Piccini A, Poncer JC, Malinow R (2000) Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. Science 287:2262–2267

Hebb DO (1949) The organization of behavior: a neuropsychological theory. Wiley, New York

Hell JW (2014) CaMKII: claiming center stage in postsynaptic function and organization. Neuron 81:249–265

Hill TC, Zito K (2013) LTP-induced long-term stabilization of individual nascent dendritic spines. J Neurosci 33:678–686

Honkura N, Matsuzaki M, Noguchi J, Ellis-Davies GCR, Kasai H (2008) The subspine organization of actin fibers regulates the structure and plasticity of dendritic spines. Neuron 57:719–729

Hosokawa T, Mitsushima D, Hosokawa T, Mitsushima D, Kaneko R, Hayashi Y (2015) Stoichiometry and phosphoisotypes of hippocampal report stoichiometry and phosphoisotypes of hippocampal AMPA-type glutamate receptor phosphorylation. Neuron 85(1):60–67

Huang Z (2009) Molecular and cellular neuroscience molecular regulation of neuronal migration during neocortical development. Mol Cell Neurosci 42:11–22

Hudmon A, Lebel E, Roy H, Sik A, Schulman H, Waxham MN, De Koninck P (2005) A mechanism for Ca2+/calmodulin-dependent protein kinase II clustering at synaptic and nonsynaptic sites based on self-association. J Neurosci 25:6971–6983

Inamura M, Itakura M, Okamoto H, Hoka S, Mizoguchi A, Fukazawa Y, Shigemoto R, Yamamori S, Takahashi M (2006) Differential localization and regulation of stargazin-like protein, gamma-8 and stargazin in the plasma membrane of hippocampal and cortical neurons. Neurosci Res 55:45–53

Kennedy MB, Bennett MK, Erondu NE (1983) Biochemical and immunochemical evidence that the "major postsynaptic density protein" is a subunit of a calmodulin-dependent protein kinase. Proc Natl Acad Sci U S A 80:7357–7361

Kristensen AS, Jenkins MA, Banke TG, Schousboe A, Makino Y, Johnson RC, Huganir R, Traynelis SF (2011) Mechanism of Ca2+/calmodulin-dependent kinase II regulation of AMPA receptor gating. Nat Neurosci 14:727–735

Lee S-JR, Escobedo-Lozoya Y, Szatmari EM, Yasuda R (2009) Activation of CaMKII in single dendritic spines during long-term potentiation. Nature 458:299–304
Lengyel I, Voss K, Cammarota M, Bradshaw K, Brent V, Murphy KPSJ, Giese KP, Rostas JAP, Bliss TVP (2004) Autonomous activity of CaMKII is only transiently increased following the induction of long-term potentiation in the rat hippocampus. Eur J Neurosci 20:3063–3072
Leonard AS, Lim IA, Hemsworth DE, Horne MC, Hell JW (1999) Calcium/calmodulin-dependent protein kinase II is associated with the N-methyl-D-aspartate receptor. Proc Natl Acad Sci U S A 96:3239–3244
Lepicard EM, Mizuno K, Antunes-Martins A, von Hertzen LSJ, Giese KP (2006) An endogenous inhibitor of calcium/calmodulin-dependent kinase II is up-regulated during consolidation of fear memory. Eur J Neurosci 23:3063–3070
Ling K-H, Hewitt CA, Beissbarth T, Hyde L, Cheah P-S, Smyth GK, Tan S-S, Hahn CN, Thomas T, Thomas PQ et al (2011) Spatiotemporal regulation of multiple overlapping sense and novel natural antisense transcripts at the Nrgn and Camk2n1 gene loci during mouse cerebral corticogenesis. Cereb Cortex 21:683–697
Lisman J (1985) A mechanism for memory storage insensitive to molecular turnover: a bistable autophosphorylating kinase. Proc Natl Acad Sci U S A 82:3055–3057
Lisman JE, Goldring MA (1988) Feasibility of long-term storage of graded information by the Ca2+/calmodulin-dependent protein kinase molecules of the postsynaptic density. Proc Natl Acad Sci U S A 85:5320–5324
Lisman J, Schulman H, Cline H (2002) The molecular basis of CaMKII function in synaptic and behavioural memory. Nat Rev Neurosci 3:175–190
Lisman J, Yasuda R, Raghavachari S (2012) Mechanisms of CaMKII action in long-term potentiation. Nat Rev Neurosci 13:169–182
Lledo PM, Hjelmstad GO, Mukherji S, Soderling TR, Malenka RC, Nicoll RA (1995) Calcium/calmodulin-dependent kinase II and long-term potentiation enhance synaptic transmission by the same mechanism. Proc Natl Acad Sci U S A 92:11175–11179
Lledo PM, Zhang X, Südhof TC, Malenka RC, Nicoll RA (1998) Postsynaptic membrane fusion and long-term potentiation. Science 279:399–403
Lu HE, MacGillavry HD, Frost NA, Blanpied TA (2014) Multiple spatial and kinetic subpopulations of CaMKII in spines and dendrites as resolved by single-molecule tracking PALM. J Neurosci 34:7600–7610
Lucchesi W, Mizuno K, Giese KP (2011) Novel insights into CaMKII function and regulation during memory formation. Brain Res Bull 85:2–8
MacGillavry H, Song Y, Raghavachari S, Blanpied T (2013) Nanoscale scaffolding domains within the postsynaptic density concentrate synaptic AMPA receptors. Neuron 78:615–622
Makino H, Malinow R (2009) AMPA receptor incorporation into synapses during LTP: the role of lateral movement and exocytosis. Neuron 64:381–390
Malenka RC, Bear MF (2004) LTP and LTD: an embarrassment of riches. Neuron 44:5–21
Malinow R, Schulman H, Tsien RW (1989) Inhibition of postsynaptic PKC or CaMKII blocks induction but not expression of LTP. Science 245:862–866
Matsuzaki M, Ellis-Davies GC, Nemoto T, Miyashita Y, Iino M, Kasai H (2001) Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. Nat Neurosci 4:1086–1092
Matsuzaki M, Honkura N, Ellis-Davies GCR, Kasai H (2004) Structural basis of long-term potentiation in single dendritic spines. Nature 429:761–766
Mayford M, Wang J, Kandel ER, O'Dell TJ (1995) CaMKII regulates the frequency-response function of hippocampal synapses for the production of both LTD and LTP. Cell 81:891–904
Meyer T, Shen K (2000) In and out of the postsynaptic region: signalling proteins on the move. Trends Cell Biol 10:238–244
Meyer T, Hanson PI, Stryer L, Schulman H (1992) Calmodulin trapping by calcium-calmodulin-dependent protein kinase. Science 256:1199–1202
Miller SG, Kennedy MB (1986) Regulation of brain type II Ca2+/calmodulin-dependent protein kinase by autophosphorylation: a Ca2+-triggered molecular switch. Cell 44:861–870

Mukherji S, Soderling TR (1994) Regulation of Ca2+/calmodulin-dependent protein kinase II by inter- and intrasubunit-catalyzed autophosphorylations. J Biol Chem 269:13744–13747
Murakoshi H, Yasuda R (2012) Postsynaptic signaling during plasticity of dendritic spines. Trends Neurosci 35:135–143
Nabavi S, Fox R, Proulx CD, Lin JY, Tsien RY, Malinow R (2014) Engineering a memory with LTD and LTP. Nature 511:348–352
Newpher TM, Ehlers MD (2008) Glutamate receptor dynamics in dendritic microdomains. Neuron 58:472–497
Nicoll RA, Malenka RC (1995) Contrasting properties of two forms of long-term potentiation in the hippocampus. Nature 377:115–118
Nicoll RA, Roche KW (2013) Long-term potentiation: peeling the onion. Neuropharmacology 74:18–22
Okamoto K-I, Nagai T, Miyawaki A, Hayashi Y (2004) Rapid and persistent modulation of actin dynamics regulates postsynaptic reorganization underlying bidirectional plasticity. Nat Neurosci 7:1104–1112
Okamoto K-I, Narayanan R, Lee SH, Murata K, Hayashi Y (2007) The role of CaMKII as an F-actin-bundling protein crucial for maintenance of dendritic spine structure. Proc Natl Acad Sci U S A 104:6418–6423
Okamoto K, Bosch M, Hayashi Y (2009) The roles of CaMKII and F-actin in the structural plasticity of dendritic spines: a potential molecular identity of a synaptic tag? Physiology (Bethesda) 24:357–366
Opazo P, Choquet D (2011) A three-step model for the synaptic recruitment of AMPA receptors. Mol Cell Neurosci 46:1–8
Opazo P, Labrecque S, Tigaret CM, Frouin A, Wiseman PW, De Koninck P, Choquet D (2010) CaMKII triggers the diffusional trapping of surface AMPARs through phosphorylation of stargazin. Neuron 67:239–252
Opazo P, Sainlos M, Choquet D (2011) Regulation of AMPA receptor surface diffusion by PSD-95 slots. Curr Opin Neurobiol 22:453–460
Otmakhov N, Tao-Cheng JJ-H, Carpenter S, Asrican B, Dosemeci A, Reese TS, Lisman J (2004) Persistent accumulation of calcium/calmodulin-dependent protein kinase II in dendritic spines after induction of NMDA receptor-dependent chemical long-term potentiation. J Neurosci 24:9324–9331
Otmakhov N, Regmi S, Lisman JE (2015) Fast decay of CaMKII FRET sensor signal in spines after LTP induction is not due to its dephosphorylation. PLoS One 10, e0130457
Patterson MA, Szatmari EM, Yasuda R (2010) AMPA receptors are exocytosed in stimulated spines and adjacent dendrites in a Ras-ERK-dependent manner during long-term potentiation. Proc Natl Acad Sci U S A 107:15951–15956
Petersen JD, Chen X, Vinade L, Dosemeci A, Lisman JE, Reese TS (2003) Distribution of postsynaptic density (PSD)-95 and Ca. Interface 23:11270–11278
Petralia RS, Sans N, Wang YX, Wenthold RJ (2005) Ontogeny of postsynaptic density proteins at glutamatergic synapses. Mol Cell Neurosci 29:436–452
Pettit DL, Perlman S, Malinow R (1994) Potentiated transmission and prevention of further LTP by increased CaMKII activity in postsynaptic hippocampal slice neurons. Science 266:1881–1885
Pi HJ, Otmakhov N, Lemelin D, De Koninck P, Lisman J (2010) Autonomous CaMKII can promote either long-term potentiation or long-term depression, depending on the state of T305/T306 phosphorylation. J Neurosci 30:8704–8709
Poncer JC, Esteban JA, Malinow R (2002) Multiple mechanisms for the potentiation of AMPA receptor-mediated transmission by alpha-Ca2+/calmodulin-dependent protein kinase II. J Neurosci 22:4406–4411
Radwańska K, Tudor-Jones AA, Mizuno K, Pereira GS, Lucchesi W, Alfano I, Łach A, Kaczmarek L, Knapp S, Giese KP (2010) Differential regulation of CaMKII inhibitor beta protein expression after exposure to a novel context and during contextual fear memory formation. Genes Brain Behav 9:648–657

Robison AJ, Bass MA, Jiao Y, MacMillan LB, Carmody LC, Bartlett RK, Colbran RJ (2005) Multivalent interactions of calcium/calmodulin-dependent protein kinase II with the postsynaptic density proteins NR2B, densin-180, and alpha-actinin-2. J Biol Chem 280:35329–35336

Rotenberg A, Mayford M, Hawkins RD, Kandel ER, Muller RU (1996) Mice expressing activated CaMKII lack low frequency LTP and do not form stable place cells in the CA1 region of the hippocampus. Cell 87:1351–1361

Rumpel S, LeDoux J, Zador A, Malinow R (2005) Postsynaptic receptor trafficking underlying a form of associative learning. Science 308:83–88

Saha S, Datta K, Rangarajan P (2007) Characterization of mouse neuronal Ca2+/calmodulin kinase II inhibitor α. Brain Res 1148:38–42

Sainlos M, Tigaret C, Poujol C, Olivier NB, Bard L, Breillat C, Thiolon K, Choquet D, Imperiali B (2011) Biomimetic divalent ligands for the acute disruption of synaptic AMPAR stabilization. Nat Chem Biol 7:81–91

Sanhueza M, Lisman J (2013) The CaMKII/NMDAR complex as a molecular memory. Mol Brain 6:10

Sanhueza M, McIntyre CC, Lisman JE (2007) Reversal of synaptic memory by Ca2+/calmodulin-dependent protein kinase II inhibitor. J Neurosci 27:5190–5199

Sanhueza M, Fernandez-Villalobos G, Stein IS, Kasumova G, Zhang P, Bayer KU, Otmakhov N, Hell JW, Lisman J (2011) Role of the CaMKII/NMDA receptor complex in the maintenance of synaptic strength. J Neurosci 31:9170–9178

Schnell E, Sizemore M, Karimzadegan S, Chen L, Bredt DS, Nicoll RA (2002) Direct interactions between PSD-95 and stargazin control synaptic AMPA receptor number. Proc Natl Acad Sci U S A 99:13902–13907

Segal M (2005) Dendritic spines and long-term plasticity. Nat Rev Neurosci 6:277–284

Sharma K, Fong DK, Craig AM (2006) Postsynaptic protein mobility in dendritic spines: long-term regulation by synaptic NMDA receptor activation. Mol Cell Neurosci 31:702–712

Shen K, Meyer T (1999) Dynamic control of CaMKII translocation and localization in hippocampal neurons by NMDA receptor stimulation. Science 284:162–166

Shen K, Teruel MN, Subramanian K, Meyer T (1998) CaMKIIβ functions as an F-actin targeting module that localizes CaMKIIα/β heterooligomers to dendritic spines. Neuron 21:593–606

Shonesy BC, Jalan-Sakrikar N, Cavener VS, Colbran RJ (2014) CaMKII: a molecular substrate for synaptic plasticity and memory. Prog Mol Biol Transl Sci 122:61–87

Silva A, Stevens C, Tonegawa S, Wang Y (1992a) Deficient hippocampal long-term potentiation in alpha-calcium-calmodulin kinase II mutant mice. Science 257:201–206

Silva AJ, Paylor R, Wehner JM, Tonegawa S (1992b) Impaired spatial learning in alpha-calcium-calmodulin kinase II mutant mice. Science 257:206–211

Stein IS, Donaldson MS, Hell JW (2014) CaMKII binding to GluN2B is important for massed spatial learning in the Morris water maze. F1000Res 3:193

Strack S, Colbran RJ (1998) Autophosphorylation-dependent targeting of calcium/calmodulin-dependent protein kinase II by the NR2B subunit of the N-methyl-D-aspartate receptor. J Biol Chem 273:20689–20692

Strack S, Choi S, Lovinger DM, Colbran RJ (1997) Translocation of autophosphorylated calcium/calmodulin-dependent protein kinase II to the postsynaptic density. J Biol Chem 272:13467–13470

Sturgill JF, Steiner P, Czervionke BL, Sabatini BL (2009) Distinct domains within PSD-95 mediate synaptic incorporation, stabilization, and activity-dependent trafficking. J Neurosci 29:12845–12854

Sumioka A, Yan D, Tomita S (2010) TARP phosphorylation regulates synaptic AMPA receptors through lipid bilayers. Neuron 66:755–767

Suzuki T (1994) Protein kinases involved in the expression of long-term potentiation. Int J Biochem 26:735–744

Takahashi T, Svoboda K, Malinow R (2003) Experience strengthening transmission by driving AMPA receptors into synapses. Science 299:1585–1588

Tomita S, Stein V, Stocker TJ, Nicoll RA, Bredt DS (2005) Bidirectional synaptic plasticity regulated by phosphorylation of stargazin-like TARPs. Neuron 45:269–277
Tsui J, Malenka RC (2006) Substrate localization creates specificity in calcium/calmodulin-dependent protein kinase II signaling at synapses. J Biol Chem 281:13794–13804
Vest RS, Davies KD, O'Leary H, Port JD, Bayer KU (2007) Dual mechanism of a natural CaMKII inhibitor. Mol Biol Cell 18:5024–5033
Waxham MN, Tsai AL, Putkey JA (1998) A mechanism for calmodulin (CaM) trapping by CaM-kinase II defined by a family of CaM-binding peptides. J Biol Chem 273:17579–17584
Whitlock JR, Heynen AJ, Shuler MG, Bear MF (2006) Learning induces long-term potentiation in the hippocampus. Science 313:1093–1097
Yamagata Y, Kobayashi S, Umeda T, Inoue A, Sakagami H, Fukaya M, Watanabe M, Hatanaka N, Totsuka M, Yagi T et al (2009) Kinase-dead knock-in mouse reveals an essential role of kinase activity of Ca2+/calmodulin-dependent protein kinase IIalpha in dendritic spine enlargement, long-term potentiation, and learning. J Neurosci 29:7607–7618
Zhang L, Kirschstein T, Sommersberg B, Merkens M, Manahan-Vaughan D, Elgersma Y, Beck H (2005) Hippocampal synaptic metaplasticity requires inhibitory autophosphorylation of Ca2+/calmodulin-dependent kinase II. J Neurosci 25:7697–7707
Zhang Y, Holbro N, Oertner TG (2008) Optical induction of plasticity at single synapses reveals input-specific accumulation of alphaCaMKII. Proc Natl Acad Sci U S A 105:12039–12044
Zhou Q, Homma KJ, Poo M (2004) Shrinkage of dendritic spines associated with long-term depression of hippocampal synapses. Neuron 44:749–757
Zhou Y, Takahashi E, Li W, Halt A, Wiltgen B, Ehninger D, Li G-D, Hell JW, Kennedy MB, Silva AJ (2007) Interactions between the NR2B receptor and CaMKII modulate synaptic plasticity and spatial learning. J Neurosci 27:13843–13853
Zhu JJ, Qin Y, Zhao M, Van Aelst L, Malinow R (2002) Ras and Rap control AMPA receptor trafficking during synaptic plasticity. Cell 110:443–455

# Chapter 4
# CaMKII Autophosphorylation-Dependent Learning and Memory

**Fabio A.B. Vigil and Karl Peter Giese**

**Abstract** Calcium/calmodulin-dependent kinase II is the most abundant protein in the post-synaptic density. It has been proposed to play an important role on learning and memory due to its autophosphorylation ability. Once phosphorylated in the right position CaMKII remains active even after the initial stimulus has finished. Although the model seems quite logical and straight forward the function of CaMKII autophosphorylation in learning and memory is still a matter of discussion. While its importance in learning is well established there isn't still enough data to reach a conclusion on memory. In this chapter we will discuss CaMKII autophosphorylation theory, its importance for LTP, learning, memory and possible relevance in different diseases.

**Keywords** Synaptic plasticity • LTP • Signalling • Memory • Hippocampus

## 4.1 Biochemistry of CaMKII

Calcium/calmodulin-dependent kinase II (CaMKII) is the major post-synaptic density (PSD) protein and counts for 1–2 % of total protein in the brain (Erondu and Kennedy 1985). CaMKII is a serine/threonine kinase activated by binding of the calcium/calmodulin complex (CaM). This kinase has a wide range of substrates and is involved in many aspects of cellular function, such as the regulation of ion channel function, neurotransmitter release, gene transcription, cytoskeletal organization, protein degradation and intracellular calcium homeostasis (Hudmon and Schulman 2002; Lisman et al. 2002, 2012; Bingol et al. 2010; Lucchesi et al. 2011). CaMKII is composed of an auto-inhibitory regulatory domain, an N-terminal catalytic domain and a C-terminal association domain (Chao et al. 2011; Hell 2014). In mammals four different isoforms of this enzyme are expressed: α, β, γ and δ isoforms (Gaertner et al. 2004). The most common isoforms in the brain are α and β

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CaMKII. These kinases differ in their kinetic of interaction with CaM, the ability to bind to cytoskeleton elements and their expression patterns (De Koninck and Schulman 1998; Thiagarajan et al. 2002; Cheng et al. 2006; Okamoto et al. 2009; Lucchesi et al. 2011). Nevertheless, those isoforms are usually associated with each other creating a holoenzyme composed of 12 CaMKII subunits organized into two hexameric rings (Hoelz et al. 2003; Rosenberg et al. 2005). The 12 subunits mainly include α and β CaMKII, creating heteromers, but homomers consisting of only αCaMKII have been found (Bronstein et al. 1988). The organization of this enzyme in a complex of subunits facilitates the occurrence of autophosphorylation between the different subunits. The most studied autophosphorylation site of this enzyme is at threonine in position 286 (T286) present in αCaMKII. In βCaMKII this threonine is in position 287. The phosphorylation at T286 allows CaMKII to remain in an active state even when free CaM levels have returned to baseline as it also dramatically enhances CaM binding to the enzyme (Hanson et al. 1989; Meyer et al. 1992; Irvine et al. 2006).

## 4.2 The T286 Autophosphorylation Theory

Due to this ability to switch from a CaM-dependent to a CaM-independent state of activation (bistability), CaMKII has been suggested to act as a memory molecule preserving "memories" of strong calcium signals (Lisman 1994). Before the publication of his paper in 1994 Lisman used mathematical models to study if CaMKII bistability was a good candidate to store long-term memory (Lisman and Goldring 1988a, b). His models suggested that CaMKII autonomous activity could be stable enough to maintain long-term potentiation (LTP) and memories for a lifetime. However, a more recent model proposes that this might not be the case. Using analysis of the stochastic transition between the ON and OFF states of CaMKII as a consequence of T286 phosphorylation, Miller et al. (2005) have reported the autophosphorylation to be stable for only a few years. Although these models are unlikely to be correct, as illustrated below, they inspired many studies on the role of CaMKII autophosphorylation including the generation of knock-in mutant mice that contain a single point mutation in position 286, inhibiting the autophosphorylation by replacing threonine for alanine (T286A) (Giese et al. 1998).

## 4.3 T286 Autophosphorylation and LTP

There has been a considerable amount of effort on studying the relationship between T286 phosphorylation and the occurrence of LTP, a form of synaptic plasticity thought to underlie memory formation and storage (Bliss and Collingridge 1993). The study of T286A mutant mice revealed that the autophosphorylation of CaMKII is required for the induction of *N*-methyl-D-aspartate (NMDA) receptor-dependent

LTP at excitatory hippocampal CA1 synapses (Giese et al. 1998). Interestingly, in the first 2 weeks of postnatal life CaMKII autophosphorylation is not required for CA1 LTP (Yasuda et al. 2003). There is a developmental switch changing CA1 LTP from protein kinase A (PKA)- to CaMKII-dependence. Further, LTP in dentate gyrus (DG), a brain area where constantly new neurons are born, does not require CaMKII autophosphorylation. Wu et al. (2006) observed that the pharmacological block of CaMKII activity does not impair DG LTP. It is necessary to block PKA or MAPK/ERK kinase (MEK) together with CAMKII to achieve impairment in LTP. This suggested an additional mechanism of LTP regulation present in these cells. The same results were observed in T286A DG cells. The mutation alone is not capable of blocking LTP. LTP is impaired only when PKA or MEK is also blocked in these cells (Cooke et al. 2006). In accordance with this observation, in obese Zucker rats, an obesity model, and also in a rat hypothyroidism model CaMKII T286 autophosphorylation is reduced and CA1 LTP is impaired. On the other hand, the same models do not have impaired DG LTP (Alzoubi et al. 2005; Gerges et al. 2005). Regarding neocortical cells, *in vitro* and *in vivo* experiments, using T286A animals, demonstrated a dependency on CaMKII T286 autophosphorylation for LTP induction (Hardingham et al. 2003). It is important to consider that even in a specific area of the hippocampus, like CA1, different types of synapses have different dependency of CaMKII T286 autophosphorylation for LTP formation. The results presented earlier which show a dependency of CaMKII autophosphorylation for LTP occurrence in CA1 area were conducted at excitatory synapses in pyramidal cells (Giese et al. 1998), but inhibitory synapses of the neuron can induce NMDA receptor-dependent LTP in the absence of T286 autophosphorylation (Lamsa et al. 2007).

Despite being necessary for LTP induction at some synapses, stimulus-induced elevation of CaMKII activity appears to be a transient phenomenon. Using electrophysiological high-frequency stimulation to induce LTP, Lengyel et al. (2004) did not detect any changes in autonomous CaMKII activity after electrical stimulation. According to the authors, this might be due to the fact that a change in autonomous CaMKII activity is too transient to be detectable, too small to be measured or it may not occur. Therefore, the same authors used a chemical stimulation to induce LTP and short-term potentiation (STP) and they noticed that both protocols produce very similar effects on CaMKII autonomous activity. These stimulations led only to a transient increase in CaMKII autonomous activity that lasts up to 5 min, while T286 autophosphorylation remained elevated for longer. STP would be accompanied by an increase in T286 autophosphorylation that disappeared after 10 min. After LTP induction T286 autophosphorylation remained increased even after 60 min (Lengyel et al. 2004). Other groups also found that LTP induction leads only to transient increase of CaM-independent activity, by about 1 min, using two-photon glutamate uncaging pulses to induce LTP and fluorescence resonance energy transfer (FRET)-based sensor to study CaMKII activity at the level of single synapses (Lee et al. 2009; Fujii et al. 2013). Taken together, functional studies have established that the T286 autophosphorylation has a fundamental role for the induction of some forms of NMDA receptor-dependent LTP, and it is unlikely that autonomous CaMKII activity maintains LTP, as this activity lasts only for a very short duration.

## 4.4 Molecular Bases for the Roles of T286/287 Autophosphorylation in LTP

Lisman et al. (2012) proposed a model for the role of CaMKII in LTP based on the modulation of glutamate receptor activity. In short, CaM is produced by calcium influx through NMDA receptors. CaM activates CaMKII holoenzymes that are present in the synapse, therefore, initiating T286 and T287 autophosphorylation. The T287 phosphorylation of βCaMKII subunits and CaM binding releases the holoenzyme from binding to F-actin, allowing CaMKII to freely diffuse in the synapse. Once CaMKII reaches the PSD area, its localization is stabilized by binding to the NMDA receptor. CaMKII can actually bind to two different sites of the NMDA receptor. One site depends on CaMKII's association to CaM (within residues 1120–1480 of the NMDA receptor) and a second binding site depends on T286 phosphorylation (residues 839–1120) (Bayer et al. 2001). Bayer et al. (2001) even showed that, besides being anchored in the PSD area by the interaction with the NMDA receptor, this interaction might actually create an autonomous activity state of CaMKII, independent of CaM and also independent of autophosphorylation of its subunits. Although this is a very interesting finding by Bayer et al. (2001), the data were obtained from *in vitro* experiments and such mode of activation still hasn't been shown to occur *in vivo*. Nevertheless, once in the PSD autophosphorylated CaMKII was suggested to increase glutamatergic transmission by phosphorylation of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subunit GluA1 at serine 831. This phosphorylation increases AMPA receptor conductance (Derkach et al. 1999). Furthermore, CaMKII can also increase the number of AMPA receptors in the PSD area by phosphorylation of the protein stargazin. Stargazin is an AMPA receptor binding protein and once it is phosphorylated it also binds to the scaffold protein PSD-95, enhancing the number of AMPA receptors in the PSD area (Tomita et al. 2005; Opazo et al. 2010). This way, the network effect of an initial burst of calcium into the cell would lead to an increase in glutamatergic transmission. Such mechanism could be important for the induction of LTP. However, if we consider the turnover and dephosphorylation of proteins this mechanism is most likely transient and it cannot account for LTP maintenance.

Another possible molecular explanation for a role of CaMKII autophosphorylation in LTP emerges from the synaptic tag hypothesis. The synaptic tag theory proposes that during early LTP, a short lasting form of LTP, synapses can be "tagged". Upon future stimulation of the neuron plasticity-related proteins (PRP) can be synthesized in the soma, which then freely diffuse into the dendrites but will only be taken up by previously tagged synapses to induce long lasting LTP (Frey and Morris 1997). Redondo et al. (2010) have shown that although CaMKII is not necessary for production of PRP it is necessary for the tagging of the synapses. Although very interesting, Redondo et al. (2010)'s observation has a limitation. KN-93, a pharmacological blocker was used to block CaMKII function. As shown in their article, this inhibitor is not specific for CaMKII, so the authors analysed different doses of KN-93 and decided to use a concentration of 1 μM. At this concentration KN-93 has

a high affinity for CaMKII but there is still some inhibition of calcium/cAMP-response element binding protein (CREB) activity by other CaM-dependent kinases such as CaMKIV. So the effects observed in synapse tagging by KN-93 treatment can't be considered to be dependent of CaMKII activity only. The lack of specificity of drugs like KN-93 or KN-62 is not due to an upstream inhibition of CaMKII, but rather due to binding competition with CaM (Mochizuki et al. 1993; Enslen and Soderling 1994; Wayman et al. 2008). For a more complete discussion on the matter of the lack of specificity of KN-93 see Wayman et al. (2008).

## 4.5 Other Effects of T286/T287 Autophosphorylation in Neurons

Autophosphorylation at T286 is not only involved in LTP. It also impacts on other electrophysiological features of neurons. For example, as mentioned above, the occurrence of STP produces an increase in T286 phosphorylation and this increase disappears after 10 min (Lengyel et al. 2004). Accordingly, T286A mutants lack STP at excitatory hippocampal CA1 synapses (Giese et al. 1998). But the function of CaMKII autophosphorylation goes beyond synaptic potentiation. The state of activation of a neuron is defined by a balance between excitation and inhibition, between LTP and long-term depression (LTD). This balance is taken in account in the metaplasticity theory and CaMKII could play an important role in metaplasticity. Metaplasticity, which is commonly defined as "plasticity of synaptic plasticity", involves the alteration of the threshold for LTP and/or LTD induction in response to the previous history of synaptic activity of a specific synapse. In other words, metaplasticity is a shift in the balance between LTP and LTD due to a trigger of activity (priming). Such mechanism can be important to prevent the saturation of LTP in stimulated synapses as to filter off subthreshold priming activities at synapses (Abraham and Tate 1997). This learning mechanism has been proposed to be CaMKII autophosphorylation-dependent by Mayford et al. (1995). Those authors have suggested a role for CaMKII autonomous activity in metaplasticity by creating a different mutation on the T286 position of αCaMKII. Mayford et al. (1995) expressed a transgene that had the threonine in this site changed to an aspartic acid (T286D) mimicking the autophosphorylation. The transgene was expressed in addition to the endogenous CaMKII gene. As a result a metaplasticity phenotype was caused, favouring LTD over LTP. However, a later study conducted by Bejar et al. (2002) with the T286D mutants revealed that these mutants have altered expression of many proteins, including the inhibitory neuropeptide Y. Because of this, the metaplasticity phenotype in these mutants cannot be assigned directly to altered autonomous activity of CaMKII. Furthermore, Li et al. (2014) have observed that for a priming effect to occur a shift from a CaMKII dependent mechanism to a protein kinase Mzeta dependent mechanism may have to occur. Although it is still too early to fully understand the role of CaMKII in metaplasticity phenomenon, the

study by Mayford et al. (1995) was a very important first step. More recently, Coultrap et al. (2014) have shown that T286 phosphorylation is important for both LTP and LTD. In their experiment it was observed that T286 phosphorylation was required for the induction of LTP and LTD. While in case of LTP the phosphorylated enzyme phosphorylates the AMPA receptor subunit GluA1 at S831 increasing its conductance, after LTD induction CaMKII phosphorylates GluA1 at S567, a phosphorylation site known to reduce synaptic GluA1 localization (Coultrap et al. 2014). Considering T286 autophosphorylation as a metaplasticity switch, its control of the balance between LTP and LTD should respond to the history of stimulation of the neuron. Indeed continuous stimulation of CA1 cells from T286A mutant mice showed a decrease in the slow component of post-burst after-hyperpolarization. As a consequence of continued stimulation wild-type cells have an increased after-hyperpolarization, which is a negative feedback for neuronal firing. Since T286A neurons have a reduced after-hyperpolarization response, this suggests that T286 autophosphorylation might be a mechanism for downregulating the excitability of neurons as a response to a history of repetitive stimulation (Sametsky et al. 2009). Regarding weak stimulation, T286A cortical neurons have a higher threshold for intensity of the stimulus required to elicit a post-synaptic response (Pattinson et al. 2006).

Besides control of the electrophysiological features of neurons, autophosphorylation of CaMKII at T286/T287 site is also important to regulate the morphology of these cells. βCaMKII is capable of binding to F-actin. This binding occurs on a site situated between the regulatory and association domains of βCaMKII, and αCaMKII lacks this binding site. Unbinding is dependent on autophosphorylation of T287 and/or calcium-calmodulin binding (Shen and Meyer 1999; Hell 2014). Okamoto et al. (2009) has suggested a role for F-actin and βCaMKII interaction on spine enlargement and stabilization. Their model goes as follow, in a basal state of activation F-actin is bundled by CaMKII. This interaction maintains the stability of spine structure. Upon stimulation of the spine complexes of CaMKII are activated and the T287 site is phosphorylated releasing F-actin from βCaMKII interaction. This unbundling of F-actin allows the polymerization of this cytoskeleton element creating a spine enlargement. Afterwards the T287 site is dephosphorylated, CaMKII complex binds to F-actin again and stabilizes the spine in this enlarged size (Okamoto et al. 2009). Another example of a role for CaMKII autophosphorylation on structural changes of the synapse can be found in Jourdain et al. (2003). These authors have exposed hippocampal CA1 cultured cells to calyculin A, a phosphatase inhibitor, which markedly enhances autophosphorylation of CaMKII (Fukunaga et al. 1993). As a result of this treatment, they observed growth of filopodia and appearance of new spines in dendrites of the cells. The same results were obtained by the authors by injecting activated α and β CaMKII in the cultures. Activation of these enzymes was achieved *in vitro* by exposing a purified solution of these enzymes to CaM in optimum conditions. In a bigger scale even the dendrites that hold these synapses might have their structure regulated by CaMKII autophosphorylation. T286A mutants have abnormal dendritic branching in spinal cord neurons,

with significantly increased numbers of dendritic branches and total dendritic length compared with wild-type littermates (Pattinson et al. 2006).

Sanhueza and Lisman (2013) proposed a model for the molecular basis of CaMKII in memory that combines the kinase activity of CaMKII and the structural role of the enzymatic complex. Once again it all starts with calcium influx through NMDA receptors, which creates CaM that activates CaMKII. Activated CaMKII passes through autophosphorylation at T286 and T287 and migrates to the PSD where it binds to NMDA receptors. This first part accounts for the need of kinase activity. When bound to the NMDA receptor in the PSD the CaMKII complex would work like a scaffolding protein, interacting with the NMDA receptor and actinin in the PSD area. This complex would then bind to densin, delta-catenin, AMPA receptor-binding protein (ABP) and N-cadherin in an even larger complex. The ABP protein could anchor AMPA receptors in the PSD area, while N-cadherin creates a physical trans-synaptic bound that enlarges synapses both pre- and post-synaptically (Sanhueza and Lisman 2013). This hypothesis combines the effects of CaMKII in the glutamatergic system and in synapse size regulation.

Figure 4.1 illustrates a schematic representation of possible molecular targets of T286-autophosphorylated CaMKII heteromers in a dendritic spine.

## 4.6 T286 Autophosphorylation in Learning and Memory

The T286A mutant mice have severe learning impairments in the Morris water maze task (Giese et al. 1998; Need and Giese 2003). Specifically, the loss of T286 autophosphosporylation does not affect use-of-platform learning, but it blocks strategy and spatial learning. Interestingly, environmental enrichment and handling do not overcome the spatial learning deficits in the T286A mutants (Need and Giese 2003). Thus, the lack of T286 autophosphorylation leads to one of the most severe learning deficits ever described for mice. Place cell recordings in T286A mutants have shown that the autophosphorylation is required for place cell stability (Cho et al. 1998; Cacucci et al. 2007). The T286A mutants are also impaired in memory formation in passive avoidance, as well as cued and context fear conditioning (Irvine et al. 2005). In these tasks one-trial memory formation requires T286 autophosphorylation, whereas massed training can lead to memory formation in the absence of the autophosphorylation. Memory formation in absence of T286 autophosphorylation is hippocampus-dependent (Irvine et al. 2011), indicating that hippocampal memory can be maintained without T286 autophosphorylation.

Gokcek-Sarac et al. (2013) reported that animals with “good” performance in the 12-arm radial maze have higher levels of T286-autophosphorylated CaMKII in comparison with animals with “poor” performance and this difference is not simply the result of altered αCaMKII levels. In fact, T286 autophosphorylation is widely accepted as important biological substrate for learning so that it is used as a tool to study the neuroanatomical substrates of learning. For example, Cox et al. (2014) have used the T286 phosphorylation to assess how unrestricted and restricted

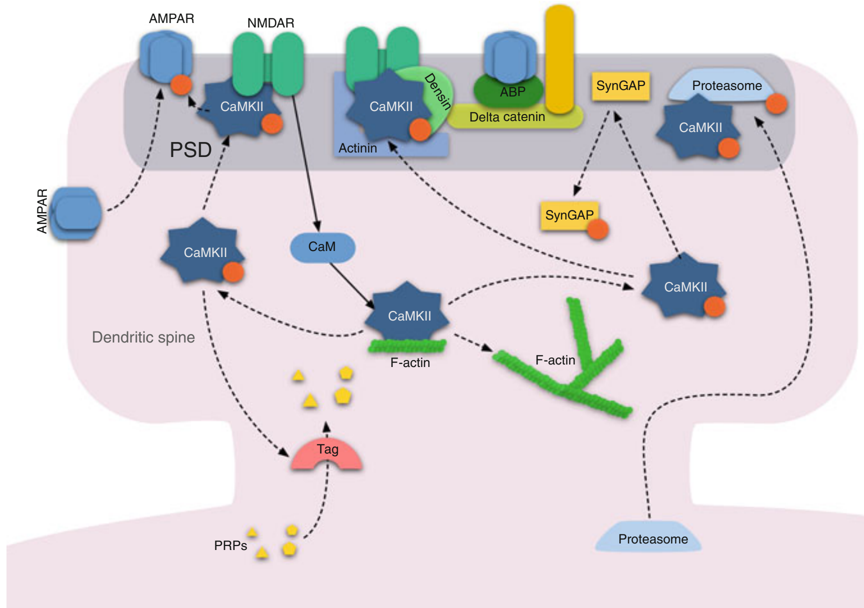


**Fig. 4.1** A schematic representation of possible molecular targets of T286-autophosphorylated CaMKII heteromers in the dendritic spine. After activation of the holoenzyme by calcium/calmodulin (CaM), T286 autophosphorylation occurs and the enzyme dissociates from F-actin. This allows the diffusion of autophosphorylated CaMKII into the post-synaptic density (PSD) and it enables F-actin remodelling. Autophosphorylated CaMKII can bind to the NMDA receptor (NMDAR) and phosphorylate AMPA receptors (AMPAR). The phosphorylation of the AMPAR will increase its conductance and stabilize its localization in the PSD. The CaMKII complex might also form a bigger complex involving different proteins that would localize AMAPRs in the PSD as well as include cell adhesion molecules to connect pre- and post-synaptic terminals. Another possible effect of autophosphorylated CaMKII in the spine could be the regulation of SynGAP localization and state of phosphorylation. Although the effects of this regulation have not yet been well defined, CaMKII activity might cause an inhibition of the ERK pathway by stimulating SynGAP activity. The final result of this ERK inhibition is a decrease of protein synthesis. CaMKII autophosphorylation may also be important for localizing proteasome to the PSD. Finally, CaMKII might be involved synaptic tagging, which allows the uptake of newly synthesized plasticity-related proteins (PRPs) into the activated spine. CaMKII could be the tag itself or it regulates the tag

learning of a new environment leads to different patterns of activation in the hippocampus.

Although the relevance of CaMKII T286 phosphorylation in learning/memory formation has been established and widely accepted, its role in memory maintenance is still a matter of discussion. In fact despite all the cognitive impairments found in the T286A mutants, Ohno et al. (2005) observed that these animals were able to learn trace eyeblink conditioning normally. This result indicates a hippocampus-dependent mechanism of memory formation that is

CaMKII autophosphorylation-independent. It is worth noticing that trace eyeblink conditioning requires a long training protocol. The T268A animals were also capable of forming memories of contextual and cued fear conditioning once they were exposed to prolonged training protocols (Irvine et al. 2005, 2011). This memory formation mechanism might be dependent on the generation of multi-innervated dendritic spines (Radwanska et al. 2011). This mechanism will be discussed in more detail in another chapter of this book. Moreover, Buard et al. (2010) even showed that blocking CaMKII activity after fear conditioning training had no effect on memory storage.

At this point we can't conclude if CaMKII autophosphorylation is or is not relevant for memory maintenance. Although some studies seem to indicate that CaMKII activity is not relevant for long-term memories, other studies suggest different conclusions. Kimura et al. (2008) reported that partial reduction of CaMKII autophosphorylation in heterozygous T286A mutants impairs extinction of contextual fear memory. However, extinction is not forgetting; rather it is a form of new learning, where the animal learns to suppress the conditioned response. Furthermore, it has been suggested that CaMKII activity is important for degradation (Bingol et al. 2010) and synthesis of plasticity-related proteins (von Hertzen and Giese 2005). Both processes are crucial for memory maintenance after retrieval. Retrieval can destabilize memory, which involves degradation of synaptic proteins, followed by reconsolidation, a protein synthesis-dependent process (Kelly et al. 2003; Lee et al. 2004, 2008; Lee 2008). CaMKII activity could be important for both, destabilization and reconsolidation. In terms of destabilization, CaMKII activity might be relevant since it has been observed that T286 autophosphorylation increases CaMKII's affinity to proteasome and promotes proteasome recruitment to spines. CaMKII can also phosphorylate proteasome subunit Rpt6 on serine 120 and increase its activity (Djakovic et al. 2009; Bingol et al. 2010; Jarome et al. 2013). CaMKII phosphorylates also the protein CYLD. Once phosphorylated, CYLD is activated and facilitates proteasomal degradation of protein by removing K63-linked polyubiquitins (Thein et al. 2014). Regarding synthesis of proteins, CaMKII interacts with the synaptic GTPase-activating protein (SynGAP). Through a cascade of signals, which includes the extracellular signal-regulated kinases (ERK) pathway, SynGAP's interaction with CaMKII inhibits protein synthesis. This signalling pathway is critical for synaptic plasticity (Wang et al. 2013). Besides activating SynGAP, CaMKII also regulates SynGAP distribution to the PSD area (Yang et al. 2013). The role of CaMKII in retrieval of a memory is still a hypothesis that needs testing and even less can be concluded about the relevance of the T286/287 phosphorylation in this mechanism. It was also shown that different polymorphisms in the αCaMKII gene are associated with working memory abilities in humans (Easton et al. 2013a).

Next to playing a role in learning and memory, T286 autophosphorylation also appears to regulate locomotor reactivity to novel environments that are potentially threatening (Easton et al. 2013c). Additionally, it controls the establishment of ethanol drinking behaviour in mice and possibly humans (Easton et al. 2013b). A detailed list of the behavioural changes observed in the T286A mutants can be found in Table 4.1.

**Table 4.1** Behavioural phenotypes of T286A mutants

| Behavioural test | Phenotype | Reference |
|---|---|---|
| Morris water maze | – Increased escape latency in training trials | Giese et al. (1998) |
| | – During probe trial did not search selectively in the target quadrant and did not selectively cross the platform site | |
| | – Pre-training with visible platform in fixed location does not overcome spatial learning deficits | |
| | – No swimming speed deficits | |
| | – Training with visual platform showed mild impairment only during blocks 2 and 3 out of 6 blocks | |
| Morris water maze | – Increased escape latency and path length during training trials | Need and Giese (2003) |
| | – Environmental enrichment had no effect on the impairment | |
| | – During visual platform test no difference in latency, swim speed or path length | |
| | – During probe trial both, naive and enriched mutants, did not search selectively in the target quadrant and did not selectively cross the platform site | |
| | – During probe trial enriched mutants swam faster than non-enriched | |
| | – Mutants had an increase in floating behaviour that was rescued by enrichment | |
| | – Both enriched and non-enriched T286A mutants showed more thigmotaxis swimming than wild type animals | |
| Morris water maze | – Heterozygote mutants show normal latency reduction during training | Kimura et al. (2008) |
| | – During first probe trial heterozygote mutants show normal memory of the platform location | |
| | – Heterozygote mutants have impaired memory extinction [4 probe trials of 6 min] | |
| | – During extinction trials heterozygote mutants have normal swim speed and floating time | |
| Passive avoidance test | – No avoidance of the dark compartment after only one training session | Irvine et al. (2005) |
| | – When trained repetitive times, until a 120 s criteria, memory was formed and maintained | |
| | – Significant higher number of trials to reach criteria | |
| Contextual fear conditioning | – Severely impaired contextual freezing on memory test 24 h after training with 1 or 3 shocks | Irvine et al. (2005) |
| | – When trained with 5 shocks show decreased fear response during the shocks, but normal contextual freezing scores in memory test 24 h later | |

(continued)

**Table 4.1** (continued)

| Behavioural test | Phenotype | Reference |
|---|---|---|
| Contextual fear conditioning | – Impaired short term memory when tested 2 h after 3 shocks training | Irvine et al. (2011) |
| | – Normal short term memory after 5 shocks training | |
| | – Training with 2 trials of 1 shock with 1 h inter-trial interval was not sufficient to create long term memory [tested 24 h after training] | |
| | – Hippocampal post-training lesion impairs long term memory after 5 shocks training [tested 8 days after training] | |
| | – Normal freezing scores when tested 30 days after 5 shocks training | |
| | – Aged mutants have impaired memory when tested 24 h after 5 shocks training | |
| Contextual fear conditioning | – Heterozygote mutants show normal freezing scores 24 h after 1 shock training | Kimura et al. (2008) |
| | – Heterozygote mutants show impaired extinction after 5 days of 6 min exposures to conditioning chamber | |
| | – Heterozygote mutants have impaired extinction after 30 min re-exposure to the chamber 24 h after training | |
| | – Heterozygote mutants have normal levels of extinction when tested 21 days after 30 min re-exposure to the chamber 15 min after training | |
| Cued fear conditioning | – Severely impaired freezing scores in memory test 48 h after 1 shock training | Irvine et al. (2005) |
| | – Normal freezing scores during memory test 48 h after 3 shocks training. | |
| Cued fear conditioning | – Training with 2 trials of 1 shock with 1 h inter-trial interval was not sufficient to create long term memory [tested 48 h after training] | Irvine et al. (2011) |
| | – Normal freezing scores when tested 31 days after 5 shocks training | |
| | – Aged mutants have normal memory when tested 48 h after 5 shocks training | |
| Trace eyeblink conditioning | – Normal learning during training [two sessions of 30 trials per day for 3 days] with normal percentage of conditioned response (CR), CR onset latency, latency to the peak of CR and normal duration of CR | Ohno et al. (2005) |
| | – Hippocampal lesion impaired mutant's percentage of conditioned response | |

(continued)

**Table 4.1** (continued)

| Behavioural test | Phenotype | Reference |
|---|---|---|
| Taste preference test | – No difference in the preference for 0.45 % sucrose solution | Easton et al. (2013c) |
| | – Less preference for 5 % sucrose solution in the homozygote mutants but no effect on heterozygote | |
| | – Heterozygote mutants avoid more the 10 mg/dL quinine solution | |
| | – No effect of mutation on the avoidance of 20 mg/dL quinine solution | |
| Two-bottle free-choice drinking paradigm | – Drank significantly less alcohol then naive animals | Easton et al. (2013c) |
| Loss of righting reflex | – No effect of mutation on the loss of righting reflex due to acute or subchronic alcohol treatment | Easton et al. (2013c) |
| Alcohol-induced conditioned place preference | – Conditioning was established faster in homozygote mutants than wild type animals | Easton et al. (2013a) |
| | – Heterozygote mutants did not develop a significant conditioning | |
| | – Place preference was extinguished in homozygote mutants on the third test | |
| | – Homozygote mutants presented hyperactive/hyper-aroused to the test arena | |
| | – Hyperactivity/hyper-arousal induced by the test environment in homozygote mutants was normalised by alcohol | |

## 4.7 T286 Autophosphorylation and Diseases

Alterations in T286 autophosphorylation could be very relevant in many diseases. Pathological changes in T286 may be caused by genetic mutations in CaMKII gene, dephosphorylation increase or impairment and/or changes in NMDA receptor activity.

Regarding NMDA receptor abnormalities, Ohno et al. (2001) observed that neither heterozygous T286A mutation nor a subthreshold dose of an NMDA receptor blocker had any effect on contextual memory formation, but the combination of the two impaired memory formation. Thus, deficits in T286 phosphorylation combined with NMDA impairment can have negative impact on learning. However, such synergetic effect has not yet been observed in diseases.

Recently, abnormal T286 autophosphorylation has been linked with α-thalassemia X-linked mental retardation (ATRX) (Shioda et al. 2011). In an ATRX mouse model T286- and T287-autophosphorylation and CaMKII autonomous activity are increased in medial prefrontal cortex (mPFC). Possibly the increased autophosphorylations are due to decreased levels of protein phosphatase 1, an enzyme that dephosphorylates at the autophosphorylation sites. The increased CaMKII activity appears specific, as the activities of CaMKI, CaMKIV, ERK and PKCα are not

changed in the ATRX mouse model. Interestingly, the increased T286 autophosphorylation in mPFC is in contrast to decreased T286 autophosphorylation in the hippocampus of the same mouse model (Nogami et al. 2011).

As for mutations in the αCaMKII gene, Easton et al. (2013b) reported a correlation between alcohol dependence and seven single nucleotide polymorphisms (SNP) in the αCaMKII gene of male humans. Additionally, a correlation between SNPs in this CaMKII gene of healthy humans and their working memory performance in spatial and no-spatial tasks was also observed (Easton et al. 2013a).

Most of the correlations between changes in T286 autophosphorylation and diseases come from models that don't have a direct, straightforward association with CaMKII. For example, obese Zucker rats, an obesity model, and also rats with hypothyroidism have reduced T286 autophosphorylation in hippocampal area CA1 as well as impaired LTP (Alzoubi et al. 2005; Gerges et al. 2005). Another example is rats exposed to heated-air early in life. These animals have hyperthermia-induced seizures during the exposure. This is used as a model for febrile seizure, a common cause of neurological emergency in human childhood. These animals present deficits in spatial learning and memory in the Morris water maze later in their life. At this stage they also show an increased translocation of CaMKII from the PSD to the cytosol, decreased levels of T286 phosphorylation and increased levels of phosphorylation on the threonine 305 (T305) of CaMKII (Xiong et al. 2014). Additionally, in prefrontal cortex of rat model of Attention-deficit/hyperactivity disorder (ADHD) T286/287 autophosphorylations are elevated. In this model GluA1 phosphorylation at the Ser-831, a known substrate of CaMKII phosphorylation, is also increased. Furthermore, those animals have an attention deficit in the Y-maze task and are impaired in a novel object recognition test. Interestingly, acute treatment with methylphenidate reverts the behavioural phenotypes and restores T286/T287 and GluA1 phosphorylation to normal levels (Yabuki et al. 2014).

Reese et al. (2011) observed that the distribution of T286-phosphorylated CaMKII changes from dendritic arborizations to neural perikarya in samples of patients with mild cognitive impairment and Alzheimer's disease. CaMKII localization was correlated with cognitive assessment scores. The same authors also treated animals with soluble amyloid beta (Aβ) oligomers resulting in a loss of T286-autophosphorylated CaMKII from synaptic spines in primary hippocampal neurons (Reese et al. 2011). Corroborating with the relevance of T286 autophosphorylation in Alzheimer's disease, it was observed that two different treatments capable of reducing the effects of Aβ also affect T286 autophosphorylation levels (Zeng et al. 2010; Ding et al. 2013).

A list of diseases that lead to impairment in T286 autophosphorylation can be found in Table 4.2.

Besides the T286 autophosphorylation other phosphorylation sites of CaMKII may also be relevant in some diseases. The function of autophosphorylations at T305 as well as T306 is still to be determined but they have been accepted as inhibitory phosphorylations (Lucchesi et al. 2011; Lisman et al. 2012). Phosphorylations of these two sites have been shown to be deregulated in a model of Angelman syndrome, a form of mental retardation. This model has memory deficits in the Morris

**Table 4.2** T286 autophosphorylation impairment in diseases

| Disease | Disease model | T286 impairment | Reference |
|---|---|---|---|
| α-Thalassemia X-linked mental retardation | Mutant mice lacking exon 2 of the gene that encodes ATRX, a chromatin remodeling protein of the sucrose-nonfermenting 2 family (ATRX$^{\Delta E2}$ mice) | – Increased T286 autophosphorylation in medial prefrontal cortex | Shioda et al. (2011) |
| α-Thalassemia X-linked mental retardation | ATRX$^{\Delta E2}$ mice | – Decreased T286 autophosphorylation in hippocampus | Nogami et al. (2011) |
| Obesity | Obese Zucker rats, a genetic model of obesity with noninsulin-dependent diabetes and hypertension | – Reduced levels of T286 autophosphorylation in the CA1 area of hippocampus | Alzoubi et al. (2005) |
| | | – Normal levels of T286 autophosphorylation in the DG area of hippocampus | |
| Hypothyroidism | Surgical remove of the thyroid gland (thyroidectomy) in rats | – Decreased levels of T286 autophosphorylation in the CA1 area of hippocampus | Gerges et al. (2005) |
| | | – Normal T286 autophosphorylation in the DG area of hippocampus | |
| Febrile seizure | Hyperthermia-induced seizures due to early-life exposure to heated-air | – Decreased levels of T286 autophosphorylation in the PSD in hippocampus | Xiong et al. (2014) |
| Attention-deficit/ hyperactivity disorder | Juvenile male stroke-prone spontaneously hypertensive rats | – Increased T286 autophosphorylation in medial prefrontal cortex | Yabuki et al. (2014) |
| | | – Normal T286 autophosphorylation in the CA1 area of hippocampus | |
| Alzheimer's disease | Human brain tissue | – Change in the distribution of T286 autophosphorylated CaMKII from dendritic arborizations to neural perikarya in the hippocampus | Reese et al. (2011) |
| Alzheimer's disease | Primary rat hippocampal cultures treated with Aβ oligomers | – Reduction on T286 autophosphorylated CaMKII levels at synaptic spines | Reese et al. (2011) |

(continued)

**Table 4.2** (continued)

| Disease | Disease model | T286 impairment | Reference |
|---|---|---|---|
| Alzheimer's disease | Rats injected with $A\beta_{1-42}$ into the lateral cerebral ventricle | – Decreased levels of T286 autophosphorylated CaMKII in whole brain tissue | Ding et al. (2013) |
| Alzheimer's disease | Rat's hippocampal slices treated with Aβ oligomers | – Reduction in LTP-induced T286 autophosphorylated CaMKII levels | Zeng et al. (2010) |

water maze and contextual fear conditioning and they are reversed by the introduction of a mutation on the T305/306 site of CaMKII, blocking its inhibitory phosphorylations (van Woerden et al. 2007).

## 4.8 Conclusion

At this point we can conclude that the T286 autophosphorylation of αCaMKII responds to neuronal activity and it is fundamentally important for synaptic strengthening and memory formation. Further, in several diseases the T286 autophosphorylation is impaired, but so far no disease is known that directly impacts on this phosphorylation. Given the prominence of the T286 autophosphorylation in synaptic strengthening and memory formation, still only little is known about why this autophosphorylation is so important. For example, GluA1 phosphorylation at S831 can only be one of several targets, as corresponding GluA1 knock-in mutants have a rather mild impairment in memory formation in comparison with T286A mutants (compare, Giese et al. (1998) and Lee et al. (2003)). Moreover, GluA1 knockout mice also have a much milder impairment in memory formation that T286A mutants (Sanderson et al. 2008). Consistent with the idea that autophosphorylated CaMKII has multiple targets in spines, it was shown that NMDA receptor-activated CaMKII is located not only in the PSD but also at distant points from the PSD (Lu et al. 2014).

Although we have progressed a great deal in understanding the T286 phosphorylation site in the α-isoform of CaMKII very little is known about the T287 site, present in the β isoform of this enzyme. A more complete understanding of the function of both phosphorylation sites might help understand the control of CaMKII activity, localization and function as an enzymatic complex and/or structural molecule.

A bigger challenge will be to conciliate the interactions between these two phosphorylation sites and phosphorylation at T305/306, the effect of this interaction for subcellular localization and activity of CaMKII and specially the network effect of this complex CaMKII regulation.

Future research should also integrate the regulation of CaMKII by phosphorylation with different mechanisms of CaMKII regulation. A good example is provided by Coultrap and Bayer (2014) showing that αCaMKII is regulated by nitrosylation at C280/289. These nitric oxide-induced nitrosylations at C280 and C289 generate an autonomous state of CaMKII activity, which is independent of T286 autophosphorylation and which actually reduces T286 autophosphorylation. Interestingly, this mechanism has no impact on T305 autophosphorylation (Coultrap and Bayer 2014).

In terms of behaviour all the studies indicate that the phosphorylation of the T286 site is an important biological substrate for learning/memory formation, but its relevance for memory maintenance is still to be determined. Data obtained until now indicate that T286 autophosphorylation is not necessary for memory maintenance. One example is Buard et al. (2010) observation that inhibition of CaMKII after fear conditioning training has no effect on memory maintenance. Another indication that memory maintenance is not dependent on T286 autophosphorylation comes from different studies showing that the learning impairment of T286A animals could be overcome with more intensive training and once the memory is formed these animals present the same memory scores then wild type animals (Irvine et al. 2005, 2011; Ohno et al. 2005; Radwanska et al. 2011).

However, based on independent observations (Djakovic et al. 2009; Bingol et al. 2010; Jarome et al. 2013; Wang et al. 2013; Thein et al. 2014) and some preliminary data from our group (not published), we have raised the possibility that CaMKII activity might play a role in retrieval-dependent memory maintenance. But it is unclear whether the T286 autophosphorylation is required. This might not be the case as the T286A mutant mutants do not show a phenotype that corroborates with this idea.

Finally, the presence of impairments and deregulations in CaMKII on different neurological diseases has just started to be explored and there is still a lot to be studied. But the data acquired until this point show some promising perspectives.

## References

Abraham WC, Tate WP (1997) Metaplasticity: a new vista across the field of synaptic plasticity. Prog Neurobiol 52:303–323

Alzoubi KH, Aleisa AM, Alkadhi KA (2005) Impairment of long-term potentiation in the CA1, but not dentate gyrus, of the hippocampus in Obese Zucker rats: role of calcineurin and phosphorylated CaMKII. J Mol Neurosci 27:337–346

Bayer KU, De Koninck P, Leonard AS, Hell JW, Schulman H (2001) Interaction with the NMDA receptor locks CaMKII in an active conformation. Nature 411:801–805

Bejar R, Yasuda R, Krugers H, Hood K, Mayford M (2002) Transgenic calmodulin-dependent protein kinase II activation: dose-dependent effects on synaptic plasticity, learning, and memory. J Neurosci 22:5719–5726

Bingol B, Wang CF, Arnott D, Cheng D, Peng J, Sheng M (2010) Autophosphorylated CaMKIIalpha acts as a scaffold to recruit proteasomes to dendritic spines. Cell 140:567–578

Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361:31–39

Bronstein JM, Wasterlain CG, Farber DB (1988) A retinal calmodulin-dependent kinase: calcium/calmodulin-stimulated and -inhibited states. J Neurochem 50:1438–1446

Buard I, Coultrap SJ, Freund RK, Lee YS, Dell'Acqua ML, Silva AJ, Bayer KU (2010) CaMKII "autonomy" is required for initiating but not for maintaining neuronal long-term information storage. J Neurosci 30:8214–8220

Cacucci F, Wills TJ, Lever C, Giese KP, O'Keefe J (2007) Experience-dependent increase in CA1 place cell spatial information, but not spatial reproducibility, is dependent on the autophosphorylation of the alpha-isoform of the calcium/calmodulin-dependent protein kinase II. J Neurosci 27:7854–7859

Chao LH, Stratton MM, Lee IH, Rosenberg OS, Levitz J, Mandell DJ, Kortemme T, Groves JT, Schulman H, Kuriyan J (2011) A mechanism for tunable autoinhibition in the structure of a human Ca2+/calmodulin-dependent kinase II holoenzyme. Cell 146:732–745

Cheng D, Hoogenraad CC, Rush J, Ramm E, Schlager MA, Duong DM, Xu P, Wijayawardana SR, Hanfelt J, Nakagawa T, Sheng M, Peng J (2006) Relative and absolute quantification of postsynaptic density proteome isolated from rat forebrain and cerebellum. Mol Cell Proteomics 5:1158–1170

Cho YH, Giese KP, Tanila H, Silva AJ, Eichenbaum H (1998) Abnormal hippocampal spatial representations in alphaCaMKIIT286A and CREBalphaDelta-mice. Science 279:867–869

Cooke SF, Wu J, Plattner F, Errington M, Rowan M, Peters M, Hirano A, Bradshaw KD, Anwyl R, Bliss TV, Giese KP (2006) Autophosphorylation of alphaCaMKII is not a general requirement for NMDA receptor-dependent LTP in the adult mouse. J Physiol 574:805–818

Coultrap SJ, Bayer KU (2014) Nitric oxide induces Ca2+-independent activity of the Ca2+/calmodulin-dependent protein kinase II (CaMKII). J Biol Chem 289(28):19458–19465

Coultrap SJ, Freund RK, O'Leary H, Sanderson JL, Roche KW, Dell'Acqua ML, Bayer KU (2014) Autonomous CaMKII mediates both LTP and LTD using a mechanism for differential substrate site selection. Cell Reports 6:431–437

Cox CD, Rex CS, Palmer LC, Babayan AH, Pham DT, Corwin SD, Trieu BH, Gall CM, Lynch G (2014) A map of LTP-related synaptic changes in dorsal hippocampus following unsupervised learning. J Neurosci 34:3033–3041

De Koninck P, Schulman H (1998) Sensitivity of CaM kinase II to the frequency of Ca2+ oscillations. Science 279:227–230

Derkach V, Barria A, Soderling TR (1999) Ca2+/calmodulin-kinase II enhances channel conductance of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate type glutamate receptors. Proc Natl Acad Sci U S A 96:3269–3274

Ding J, Xi YD, Zhang DD, Zhao X, Liu JM, Li CQ, Han J, Xiao R (2013) Soybean isoflavone ameliorates beta-amyloid 1-42-induced learning and memory deficit in rats by protecting synaptic structure and function. Synapse 67:856–864

Djakovic SN, Schwarz LA, Barylko B, DeMartino GN, Patrick GN (2009) Regulation of the proteasome by neuronal activity and calcium/calmodulin-dependent protein kinase II. J Biol Chem 284:26655–26665

Easton AC, Lourdusamy A, Loth E, Toro R, Giese KP, Kornhuber J, de Quervain DJ, Papassotiropoulos A, Fernandes C, Muller CP, Schumann G (2013a) CAMK2A polymorphisms predict working memory performance in humans. Mol Psychiatry 18:850–852

Easton AC, Lucchesi W, Lourdusamy A, Lenz B, Solati J, Golub Y, Lewczuk P, Fernandes C, Desrivieres S, Dawirs RR, Moll GH, Kornhuber J, Frank J, Hoffmann P, Soyka M, Kiefer F, Schumann G, Peter Giese K, Muller CP, Treutlein J, Cichon S, Ridinger M, Mattheisen P, Herms S, Wodarz N, Zill P, Maier W, Mossner R, Gaebel W, Dahmen N, Scherbaum N, Schmal C, Steffens M, Lucae S, Ising M, Muller-Myhsok B, Nothen MM, Mann K, Rietschel M (2013b) AlphaCaMKII autophosphorylation controls the establishment of alcohol drinking behavior. Neuropsychopharmacology 38:1636–1647

Easton AC, Lucchesi W, Mizuno K, Fernandes C, Schumann G, Giese KP, Muller CP (2013c) AlphaCaMKII autophosphorylation controls the establishment of alcohol-induced conditioned place preference in mice. Behav Brain Res 252:72–76

Enslen H, Soderling TR (1994) Roles of calmodulin-dependent protein kinases and phosphatase in calcium-dependent transcription of immediate early genes. J Biol Chem 269:20872–20877

Erondu NE, Kennedy MB (1985) Regional distribution of type II Ca2+/calmodulin-dependent protein kinase in rat brain. J Neurosci 5:3270–3277

Frey U, Morris RG (1997) Synaptic tagging and long-term potentiation. Nature 385:533–536

Fujii H, Inoue M, Okuno H, Sano Y, Takemoto-Kimura S, Kitamura K, Kano M, Bito H (2013) Nonlinear decoding and asymmetric representation of neuronal input information by CaMKIIalpha and calcineurin. Cell Reports 3:978–987

Fukunaga K, Stoppini L, Miyamoto E, Muller D (1993) Long-term potentiation is associated with an increased activity of Ca2+/calmodulin-dependent protein kinase II. J Biol Chem 268: 7863–7867

Gaertner TR, Kolodziej SJ, Wang D, Kobayashi R, Koomen JM, Stoops JK, Waxham MN (2004) Comparative analyses of the three-dimensional structures and enzymatic properties of alpha, beta, gamma and delta isoforms of Ca2+-calmodulin-dependent protein kinase II. J Biol Chem 279:12484–12494

Gerges NZ, Alzoubi KH, Alkadhi KA (2005) Role of phosphorylated CaMKII and calcineurin in the differential effect of hypothyroidism on LTP of CA1 and dentate gyrus. Hippocampus 15:480–490

Giese KP, Fedorov NB, Filipkowski RK, Silva AJ (1998) Autophosphorylation at Thr286 of the alpha calcium-calmodulin kinase II in LTP and learning. Science 279:870–873

Gokcek-Sarac C, Adali O, Jakubowska-Dogru E (2013) Hippocampal levels of ChAT, PKA, phospho-PKA and phospho-CaMKIIalpha but not CaMKIIalpha positively correlate with spatial learning skills in rats. Neurosci Lett 545:112–116

Hanson PI, Kapiloff MS, Lou LL, Rosenfeld MG, Schulman H (1989) Expression of a multifunctional Ca2+/calmodulin-dependent protein kinase and mutational analysis of its autoregulation. Neuron 3:59–70

Hardingham N, Glazewski S, Pakhotin P, Mizuno K, Chapman PF, Giese KP, Fox K (2003) Neocortical long-term potentiation and experience-dependent synaptic plasticity require alpha-calcium/calmodulin-dependent protein kinase II autophosphorylation. J Neurosci 23: 4428–4436

Hell JW (2014) CaMKII: claiming center stage in postsynaptic function and organization. Neuron 81:249–265

Hoelz A, Nairn AC, Kuriyan J (2003) Crystal structure of a tetradecameric assembly of the association domain of Ca2+/calmodulin-dependent kinase II. Mol Cell 11:1241–1251

Hudmon A, Schulman H (2002) Neuronal CA2+/calmodulin-dependent protein kinase II: the role of structure and autoregulation in cellular function. Annu Rev Biochem 71:473–510

Irvine EE, Vernon J, Giese KP (2005) AlphaCaMKII autophosphorylation contributes to rapid learning but is not necessary for memory. Nat Neurosci 8:411–412

Irvine EE, von Hertzen LS, Plattner F, Giese KP (2006) AlphaCaMKII autophosphorylation: a fast track to memory. Trends Neurosci 29:459–465

Irvine EE, Danhiez A, Radwanska K, Nassim C, Lucchesi W, Godaux E, Ris L, Giese KP (2011) Properties of contextual memory formed in the absence of alphaCaMKII autophosphorylation. Mol Brain 4:8

Jarome TJ, Kwapis JL, Ruenzel WL, Helmstetter FJ (2013) CaMKII, but not protein kinase A, regulates Rpt6 phosphorylation and proteasome activity during the formation of long-term memories. Front Behav Neurosci 7:115

Jourdain P, Fukunaga K, Muller D (2003) Calcium/calmodulin-dependent protein kinase II contributes to activity-dependent filopodia growth and spine formation. J Neurosci 23: 10645–10649

Kelly A, Laroche S, Davis S (2003) Activation of mitogen-activated protein kinase/extracellular signal-regulated kinase in hippocampal circuitry is required for consolidation and reconsolidation of recognition memory. J Neurosci 23:5354–5360

Kimura R, Silva AJ, Ohno M (2008) Autophosphorylation of alphaCaMKII is differentially involved in new learning and unlearning mechanisms of memory extinction. Learn Mem 15:837–843

Lamsa K, Irvine EE, Giese KP, Kullmann DM (2007) NMDA receptor-dependent long-term potentiation in mouse hippocampal interneurons shows a unique dependence on Ca(2+)/calmodulin-dependent kinases. J Physiol 584:885–894

Lee JL (2008) Memory reconsolidation mediates the strengthening of memories by additional learning. Nat Neurosci 11:1264–1266

Lee HK, Takamiya K, Han JS, Man H, Kim CH, Rumbaugh G, Yu S, Ding L, He C, Petralia RS, Wenthold RJ, Gallagher M, Huganir RL (2003) Phosphorylation of the AMPA receptor GluR1 subunit is required for synaptic plasticity and retention of spatial memory. Cell 112:631–643

Lee JL, Everitt BJ, Thomas KL (2004) Independent cellular processes for hippocampal memory consolidation and reconsolidation. Science 304:839–843

Lee SH, Choi JH, Lee N, Lee HR, Kim JI, Yu NK, Choi SL, Kim H, Kaang BK (2008) Synaptic protein degradation underlies destabilization of retrieved fear memory. Science 319: 1253–1256

Lee SJ, Escobedo-Lozoya Y, Szatmari EM, Yasuda R (2009) Activation of CaMKII in single dendritic spines during long-term potentiation. Nature 458:299–304

Lengyel I, Voss K, Cammarota M, Bradshaw K, Brent V, Murphy KP, Giese KP, Rostas JA, Bliss TV (2004) Autonomous activity of CaMKII is only transiently increased following the induction of long-term potentiation in the rat hippocampus. Eur J Neurosci 20:3063–3072

Li Q, Rothkegel M, Xiao ZC, Abraham WC, Korte M, Sajikumar S (2014) Making synapses strong: metaplasticity prolongs associativity of long-term memory by switching synaptic tag mechanisms. Cereb Cortex 24:353–363

Lisman J (1994) The CaM kinase II hypothesis for the storage of synaptic memory. Trends Neurosci 17:406–412

Lisman J, Goldring M (1988a) Evaluation of a model of long-term memory based on the properties of the Ca2+/calmodulin-dependent protein kinase. J Physiol 83:187–197

Lisman JE, Goldring MA (1988b) Feasibility of long-term storage of graded information by the Ca2+/calmodulin-dependent protein kinase molecules of the postsynaptic density. Proc Natl Acad Sci U S A 85:5320–5324

Lisman J, Schulman H, Cline H (2002) The molecular basis of CaMKII function in synaptic and behavioural memory. Nat Rev Neurosci 3:175–190

Lisman J, Yasuda R, Raghavachari S (2012) Mechanisms of CaMKII action in long-term potentiation. Nat Rev Neurosci 13:169–182

Lu HE, MacGillavry HD, Frost NA, Blanpied TA (2014) Multiple spatial and kinetic subpopulations of CaMKII in spines and dendrites as resolved by single-molecule tracking PALM. J Neurosci 34:7600–7610

Lucchesi W, Mizuno K, Giese KP (2011) Novel insights into CaMKII function and regulation during memory formation. Brain Res Bull 85:2–8

Mayford M, Wang J, Kandel ER, O'Dell TJ (1995) CaMKII regulates the frequency-response function of hippocampal synapses for the production of both LTD and LTP. Cell 81:891–904

Meyer T, Hanson PI, Stryer L, Schulman H (1992) Calmodulin trapping by calcium-calmodulin-dependent protein kinase. Science 256:1199–1202

Miller P, Zhabotinsky AM, Lisman JE, Wang XJ (2005) The stability of a stochastic CaMKII switch: dependence on the number of enzyme molecules and protein turnover. PLoS Biol 3, e107

Mochizuki H, Ito T, Hidaka H (1993) Purification and characterization of Ca2+/calmodulin-dependent protein kinase V from rat cerebrum. J Biol Chem 268:9143–9147

Need AC, Giese KP (2003) Handling and environmental enrichment do not rescue learning and memory impairments in alphaCamKII(T286A) mutant mice. Genes Brain Behav 2:132–139

Nogami T, Beppu H, Tokoro T, Moriguchi S, Shioda N, Fukunaga K, Ohtsuka T, Ishii Y, Sasahara M, Shimada Y, Nishijo H, Li E, Kitajima I (2011) Reduced expression of the ATRX gene, a chromatin-remodeling factor, causes hippocampal dysfunction in mice. Hippocampus 21: 678–687

Ohno M, Frankland PW, Chen AP, Costa RM, Silva AJ (2001) Inducible, pharmacogenetic approaches to the study of learning and memory. Nat Neurosci 4:1238–1243

Ohno M, Tseng W, Silva AJ, Disterhoft JF (2005) Trace eyeblink conditioning requires the hippocampus but not autophosphorylation of alphaCaMKII in mice. Learn Mem 12:211–215

Okamoto K, Bosch M, Hayashi Y (2009) The roles of CaMKII and F-actin in the structural plasticity of dendritic spines: a potential molecular identity of a synaptic tag? Physiology (Bethesda) 24:357–366

Opazo P, Labrecque S, Tigaret CM, Frouin A, Wiseman PW, De Koninck P, Choquet D (2010) CaMKII triggers the diffusional trapping of surface AMPARs through phosphorylation of stargazin. Neuron 67:239–252

Pattinson D, Baccei M, Karadottir R, Torsney C, Moss A, McCutcheon J, Giese KP, Fitzgerald M (2006) Aberrant dendritic branching and sensory inputs in the superficial dorsal horn of mice lacking CaMKIIalpha autophosphorylation. Mol Cell Neurosci 33:88–95

Radwanska K, Medvedev NI, Pereira GS, Engmann O, Thiede N, Moraes MF, Villers A, Irvine EE, Maunganidze NS, Pyza EM, Ris L, Szymanska M, Lipinski M, Kaczmarek L, Stewart MG, Giese KP (2011) Mechanism for long-term memory formation when synaptic strengthening is impaired. Proc Natl Acad Sci U S A 108:18471–18475

Redondo RL, Okuno H, Spooner PA, Frenguelli BG, Bito H, Morris RG (2010) Synaptic tagging and capture: differential role of distinct calcium/calmodulin kinases in protein synthesis-dependent long-term potentiation. J Neurosci 30:4981–4989

Reese LC, Laezza F, Woltjer R, Taglialatela G (2011) Dysregulated phosphorylation of Ca(2+)/calmodulin-dependent protein kinase II-alpha in the hippocampus of subjects with mild cognitive impairment and Alzheimer's disease. J Neurochem 119:791–804

Rosenberg OS, Deindl S, Sung RJ, Nairn AC, Kuriyan J (2005) Structure of the autoinhibited kinase domain of CaMKII and SAXS analysis of the holoenzyme. Cell 123:849–860

Sametsky EA, Disterhoft JF, Ohno M (2009) Autophosphorylation of alphaCaMKII downregulates excitability of CA1 pyramidal neurons following synaptic stimulation. Neurobiol Learn Mem 92:120–123

Sanderson DJ, Good MA, Seeburg PH, Sprengel R, Rawlins JN, Bannerman DM (2008) The role of the GluR-A (GluR1) AMPA receptor subunit in learning and memory. Prog Brain Res 169:159–178

Sanhueza M, Lisman J (2013) The CaMKII/NMDAR complex as a molecular memory. Mol Brain 6:10

Shen K, Meyer T (1999) Dynamic control of CaMKII translocation and localization in hippocampal neurons by NMDA receptor stimulation. Science 284:162–166

Shioda N, Beppu H, Fukuda T, Li E, Kitajima I, Fukunaga K (2011) Aberrant calcium/calmodulin-dependent protein kinase II (CaMKII) activity is associated with abnormal dendritic spine morphology in the ATRX mutant mouse brain. J Neurosci 31:346–358

Thein S, Tao-Cheng JH, Li Y, Bayer KU, Reese TS, Dosemeci A (2014) CaMKII mediates recruitment and activation of the deubiquitinase CYLD at the postsynaptic density. PLoS One 9, e91312

Thiagarajan TC, Piedras-Renteria ES, Tsien RW (2002) Alpha- and betaCaMKII. Inverse regulation by neuronal activity and opposing effects on synaptic strength. Neuron 36:1103–1114

Tomita S, Stein V, Stocker TJ, Nicoll RA, Bredt DS (2005) Bidirectional synaptic plasticity regulated by phosphorylation of stargazin-like TARPs. Neuron 45:269–277

van Woerden GM, Harris KD, Hojjati MR, Gustin RM, Qiu S, de Avila FR, Jiang YH, Elgersma Y, Weeber EJ (2007) Rescue of neurological deficits in a mouse model for Angelman syndrome by reduction of alphaCaMKII inhibitory phosphorylation. Nat Neurosci 10:280–282

von Hertzen LS, Giese KP (2005) Alpha-isoform of Ca2+/calmodulin-dependent kinase II autophosphorylation is required for memory consolidation-specific transcription. Neuroreport 16:1411–1414

Wang CC, Held RG, Hall BJ (2013) SynGAP regulates protein synthesis and homeostatic synaptic plasticity in developing cortical networks. PLoS One 8, e83941

Wayman GA, Lee YS, Tokumitsu H, Silva AJ, Soderling TR (2008) Calmodulin-kinases: modulators of neuronal development and plasticity. Neuron 59:914–931

Wu J, Rowan MJ, Anwyl R (2006) Long-term potentiation is mediated by multiple kinase cascades involving CaMKII or either PKA or p42/44 MAPK in the adult rat dentate gyrus in vitro. J Neurophysiol 95:3519–3527

Xiong Y, Zhou H, Zhang L (2014) Influences of hyperthermia-induced seizures on learning, memory and phosphorylative state of CaMKIIalpha in rat hippocampus. Brain Res 1557:190–200

Yabuki Y, Shioda N, Maeda T, Hiraide S, Togashi H, Fukunaga K (2014) Aberrant CaMKII activity in the medial prefrontal cortex is associated with cognitive dysfunction in ADHD model rats. Brain Res 1557:90–100

Yang Y, Tao-Cheng JH, Bayer KU, Reese TS, Dosemeci A (2013) CaMKII-mediated phosphorylation regulates distributions of Syngap-alpha1 and -alpha2 at the postsynaptic density. PLoS One 8, e71795

Yasuda H, Barth AL, Stellwagen D, Malenka RC (2003) A developmental switch in the signaling cascades for LTP induction. Nat Neurosci 6:15–16

Zeng Y, Zhao D, Xie CW (2010) Neurotrophins enhance CaMKII activity and rescue amyloid-beta-induced deficits in hippocampal synaptic plasticity. J Alzheimers Dis 21:823–831

# Chapter 5
# An Alternative Mechanism for Long-Term Memory Formation

**Kasia Radwanska and Karl Peter Giese**

**Abstract** Long-term memory (LTM) formation has been linked with functional strengthening of existing synapses as well as other processes including *de novo* synaptogenesis. However, it is unclear whether synaptogenesis can contribute to LTM formation. Using alpha-calcium/calmodulin kinase II autophosphorylation-deficient (T286A) mutants, we demonstrate that when functional strengthening is severely impaired contextual LTM formation is linked with training-induced PSD95 upregulation followed by persistent generation of multiinnervated spines (MIS), a type of synapse that is characterized by several presynaptic terminals contacting the same postsynaptic spine. In the chapter, the molecular processes involved in generation of MIS and properties of MIS-dependent memory will be discussed.

**Keywords** αCaMKII autophosphorylation • Multiinnervated spines • PSD-95 • Fear memory • Hippocampus

## 5.1 Introduction

The ability to form, store and update long-term memory (LTM) is fundamental for brain function. Research in recent decades has provided some insights into LTM mechanisms, although current knowledge is limited because the vast majority of studies have considered only the role of long-term potentiation (LTP) (Giese 2012). LTP is an experimentally induced, long-lasting enhancement of chemical synaptic

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transmission. Depending on the stimulation protocol, distinct versions of LTP can be obtained. The NMDA receptor-dependent version(s) is believed to model endogenous LTP that is induced after behavioural training in a memory task (Gruart et al. 2006; Whitlock et al. 2006). Consistent with the idea that NMDA receptor-dependent LTP (NMDAR-LTP) is important, an impairment of this synaptic plasticity correlates with affected LTM formation or maintenance in many studies (e.g., Silva 2003; Sacktor 2011). However, many investigations have also shown that there can be LTM formation despite LTP deficits (e.g., Irvine et al. 2005, 2011). Therefore, it has become clear that LTP is not the sole LTM mechanism; there is at least one alternative mechanism. This raises the question, what other cellular mechanism(s) can enable LTM formation when LTP is impaired. We have used an NMDAR-LTP-deficient mutant mouse line, T286A knockin mutants which lack the T286 autophosphorylation of the α-isoform of calcium/calmodulin-dependent kinase II (αCaMKII), to address this question (Radwanska et al. 2011).

## 5.2 LTP and Contextual Fear Memory Phenotype of T286A Mutants

αCaMKII is a major synaptic kinase, which is expressed postnatally, exclusively in glutamatergic neurons (more details on αCaMKII and its T286 autophosphorylation are in Chapter 4). In agreement with the late expression during postnatal development, the lack of T286 autophosphorylation does not impair LTP in early postnatal life (Yasuda et al. 2003). However, in adulthood T286A mutants have fully blocked NMDA receptor-dependent LTP at excitatory synapses in hippocampal area CA1 as established with a multitude of stimulation protocols (Giese et al. 1998; Irvine et al. 2005; Cooke et al. 2006; Radwanska et al. 2011). The LTP deficit is specific as the T286A mutation does not affect basal synaptic transmission, paired-pulse facilitation and post-tetanic potentiation (Giese et al. 1998). Nonetheless, a recent study has shown that the T286A have also impaired long-term depression (LTD) at hippocampal CA1 synapses (Coultrap et al. 2014).

Despite the loss of NMDA receptor-dependent LTP (and LTD) at hippocampal CA1 synapses, the T286A mutants form hippocampus-dependent contextual fear LTM (Irvine et al. 2005, 2011). Specifically, the T286A mutants form contextual fear LTM after five massed training trials, whilst they are impaired in one-trial LTM formation and LTM formation after two spaced training trials (Irvine et al. 2011). The contextual fear LTM after massed training lasts at least 30 days and it requires the hippocampus as demonstrated by post-training lesion experiments (Irvine et al. 2011). Further, immediate-early gene imaging after massed training shows that the T286A do not form contextual fear LTM as a result of brain system compensation (Radwanska et al. 2011).

## 5.3 T286A Mutants as Tool to Discover a Novel LTM Mechanism

We used the T286A mice as tool to identify a LTM mechanism that is distinct from LTP, since these mutants have fully blocked NMDA receptor-dependent CA1-LTP and they form hippocampus-dependent contextual fear LTM. LTP is thought to result from strengthening of existing synapses. Thus, in principle *de novo* synaptogenesis may be a LTM mechanism which is alternate to LTP. Consistent with this idea, an increase in dendritic spine density has been linked with LTM formation (e.g.Leuner et al. 2003; Restivo et al. 2009; Xu et al. 2009; Yang et al. 2009). We used electron microscopy to analyse changes in synapse number after contextual fear conditioning in T286A mutants. Specifically, we analysed synapses in stratum radiatum of area CA1 in the dorsal hippocampus, a critical brain area for contextual fear LTM formation (Rampon et al. 2000; Hunsaker et al. 2008). We found that 2 h after conditioning the total synapse number increases in T286A mutants and wild-type littermates (Radwanska et al. 2011). However, this increased synaptogenesis was not detected 24 h after conditioning (Radwanska et al. 2011). Thus, there is only transient synaptogenesis after conditioning in the T286A mutants (and wild-type littermates). The function of this transient synaptogenesis is unclear but it is unlikely to be the mechanism that stores contextual fear LTM in the T286A mutants.

Not all synapses in the hippocampus consist of one presynaptic terminal and one postsynaptic dendritic spine. There are also multi-spine boutons (MSB) (Toni et al. 1999; Geinisman et al. 2001), where a presynaptic terminal contacts multiple spines, and multi-innervated dendritic spines (MIS), where a spine is contacted by more than one presynaptic terminal (Fiala et al. 1998; Nikonenko et al. 2003; Petrak et al. 2005; Popov et al. 2005). These synapse types are relatively rare in the hippocampus (1–5 % of all synapses). MSB generation is associated with LTP in cultured hippocampal slices (Toni et al. 1999) and occurs after trace eyeblink conditioning in CA1 stratum radiatum (Geinisman et al. 2001). This trace conditioning requires up to 800 training trials and increased MSB number lasting for at least 24 h after conditioning. However, the functional impact of MSB generation is not known. The generation of MIS is dependent on NMDA receptor activation in hippocampal slice cultures (Nikonenko et al. 2003) but before our study (Radwanska et al. 2011) a function for MIS generation in memory formation/storage was not known.

Using an electron microscopic investigation of the T286A mutants, we tested whether a change in rare synapse number could be associated with contextual fear LTM formation. We found that conditioning of the T286A mutants leads to an increase in MIS number that was detected after 2 h and which lasts at least 24 h (Radwanska et al. 2011) (Fig. 5.1). Contextual fear conditioning-induced MIS generation was not observed in wild-type mice, suggesting that CaMKII autophosphorylation-dependent signalling normally suppresses this type of synaptogenesis. Our result suggested that MIS generation enable LTM formation when LTP is impaired.

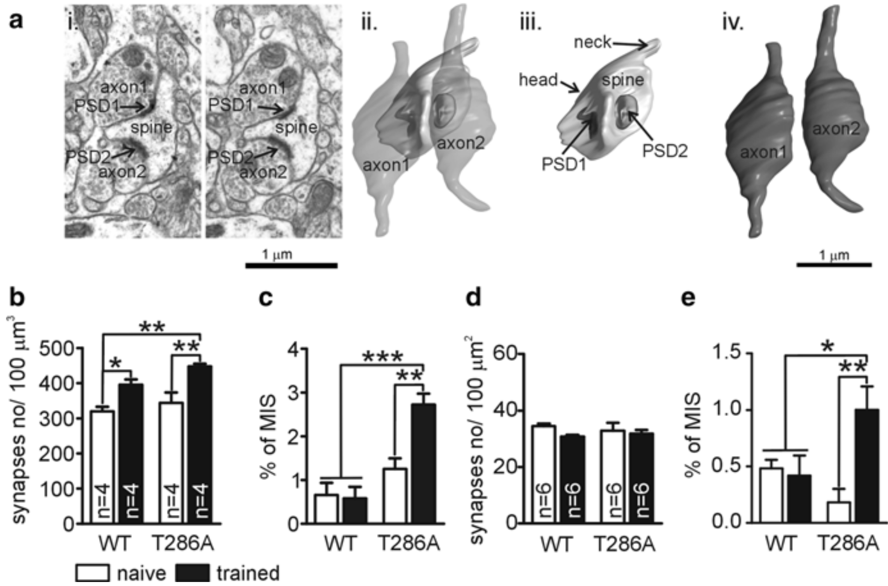


**Fig. 5.1** Training-induced generation of MIS in T286A mutants, but not in WT mice. Synapse density and MIS were analyzed 2 h after conditioning in stratum radiatum of hippocampal area CA1 using three-dimensional (3-D) electron microscopy (**b**, **c**) and 24 h after training using two-dimensional (2-D) electron microscopy (**d**, **e**). (**a**) (*i*) Two serial electron micrographs with a single spine innervated by 2 axonal boutons (axon1 and axon2) each with single post-synaptic density (PSD1 and PSD2). (*ii*) 3-D reconstructions of this MIS. The red color indicates the PSDs contacting the spine. (*iii*) The spine without the axonal boutons. (*iv*) Two axonal boutons. Reprinted from Radwanska et al. (2011)

## 5.4 Is MIS Generation Required for Contextual Fear LTM Formation in T286A Mutants?

To establish that MIS generation is required for contextual fear LTM in T286A mutants it is necessary to specifically block this type of synaptogenesis. So far only little is known about the molecular mechanisms underlying MIS generation. The induction of MIS generation requires NMDA receptor activation (Nikonenko et al. 2003). PSD-95 overexpression is sufficient to induce MIS generation (Nikonenko et al. 2008). This overexpression leads to activation of neuronal nitric oxide synthase that produces a retrograde messenger to attract a presynaptic terminal onto the dendritic spine (Nikonenko et al. 2008). PSD-95 protein is locally translated in dendritic spines from pre-existing stores of mRNA after synaptic activation (Kelleher et al. 2004; Lee et al. 2005; Swiech et al. 2008; Belelovsky et al. 2009). Accordingly PSD-95 protein expression is not sensitive to block of transcription (Lee et al. 2005; Belelovsky et al. 2009) but is regulated by the activity of mammalian target of rapamycin (mTOR) signaling followed by protein synthesis (Lee et al. 2005; Belelovsky et al. 2009).

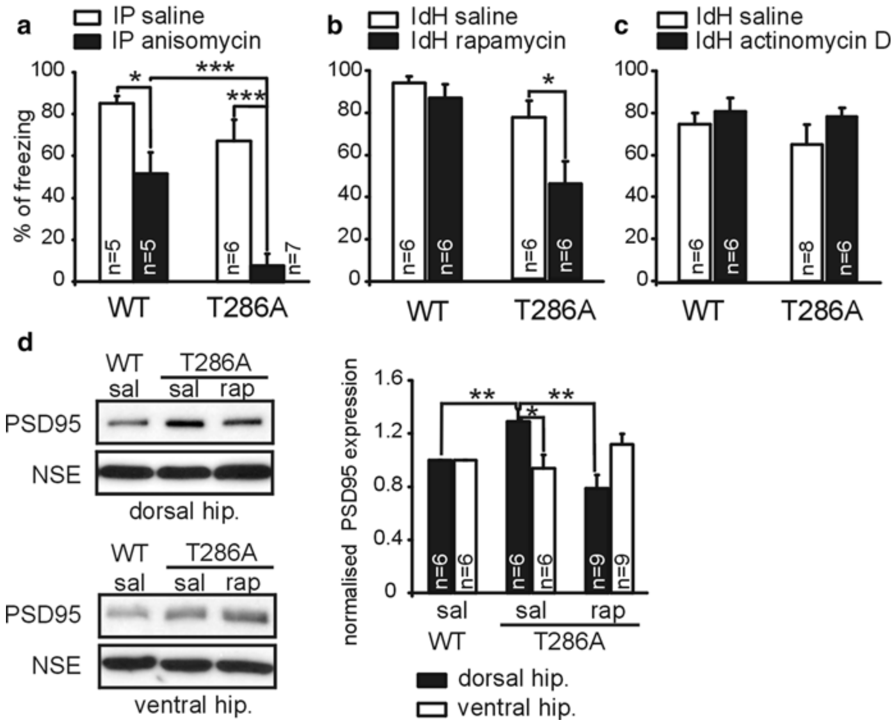


**Fig. 5.2** Contextual LTM formation requires mTOR signaling and protein synthesis in the dorsal hippocampus in the T286A mutants. WT mice and T286A mutants were trained with massed foreground conditioning. (**a**) Anisomycin (225 mg/kg) was intraperitoneally (IP) injected immediately after training. Contextual LTM was tested 24 h later. (**b**) Rapamycin (2.5 μg/side) or (**c**) actinomycin D (10 ng/side) were bilaterally injected into dorsal hippocampus (IdH) immediately after training and 24 h after conditioning the animals were tested for contextual LTM. (**d**) Training-induced PSD95 overexpression requires mTOR signaling in the dorsal hippocampus in the T286A mutants. Rapamycin (2.5 μg/side) was bilaterally injected into dorsal hippocampus (IdH) immediately after conditioning. The ventral and dorsal hippocampi were collected 2 h after training and lysates were assayed by immunoblotting. Reprinted from Radwanska et al. (2011)

We analysed the regulation of PSD-95 expression in hippocampal area CA1 after contextual fear conditioning in the T286A mutants (Radwanska et al. 2011). Only the training conditions that lead to LTM formation cause an upregulation of PSD-95. Further, we found that the same amount of massed training does not upregulate PSD-95 expression in wild-type mice, as expected as these mice do not generate MIS. Thus, we found a perfect correlation between MIS generation and PSD-95 overexpression. Further, we showed that the PSD-95 upregulation is blocked by rapamycin, a specific blocker of mTOR signalling (Radwanska et al. 2011). Finally, post-training administration of rapamycin also blocks contextual LTM in T286A mutants (Radwanska et al. 2011) (Fig. 5.2). Taken together, these studies strongly suggest that MIS generation is required for contextual LTM in T286A mutants. As shown in Fig. 5.3, we propose a new model of LTM formation when LTP is impaired.

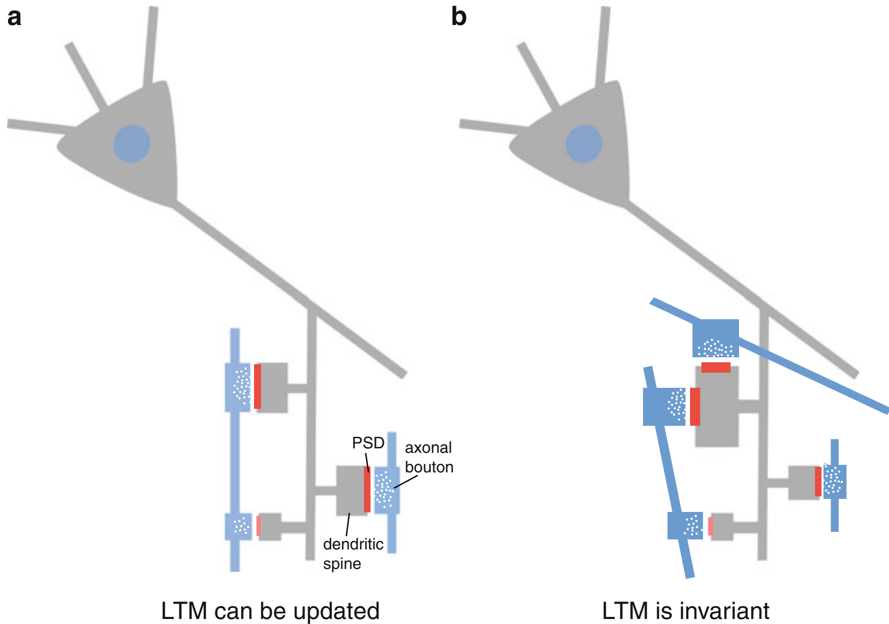


**Fig. 5.3** Two models of LTM formation. Panel (**a**) shows LTM formation in the presence of LTP, as indicated in *brown*. It is likely that the strengthened synapses are clustered (Govindarajan et al. 2006). Panel (**b**) shows a model of LTM formation when LTP is impaired. In this case, MIS are generated as indicated in *brown*. Depotentiation of LTP is a relatively simple molecular process (involves endocytosis of AMPA receptors), whereas loss of MIS is expected to be more complicated as various adhesion molecules keep the MIS together. Consequently, MIS-dependent LTM may be less modifiable than LTP-dependent LTM. Evidence supporting this idea is discussed in the main text (Color figure online)

## 5.5 What Is the Downside of MIS-Dependent LTM?

Memory storage in the brain is a dynamic process. The memories which were consolidated, i.e. went through protein synthesis-dependent phase after training, still can be remodeled while recalled. The memory recall involves destabilization of memory trace which in order to be further stored needs to be reconsolidated in a protein synthesis- and transcription-dependent process (Nader et al. 2000). LTM which is based in LTP-like plasticity is easily updatable because AMPA receptor endocytosis is sufficient for depotentiation of the strengthened synapses and memory destabilization (Migues et al. 2010). The question emerged whether MIS-dependent LTM can also be destabilized by retrieval. Since MIS are structural synapses that adhere together, it was expected that retrieval would have to induce a multitude of molecular processes to destabilize MIS, which seemed unlikely. Retrieval-induced memory destabilization can be assessed by blocking reconsolidation with inhibitors of protein synthesis or gene transcription. Using this experimental design, we found

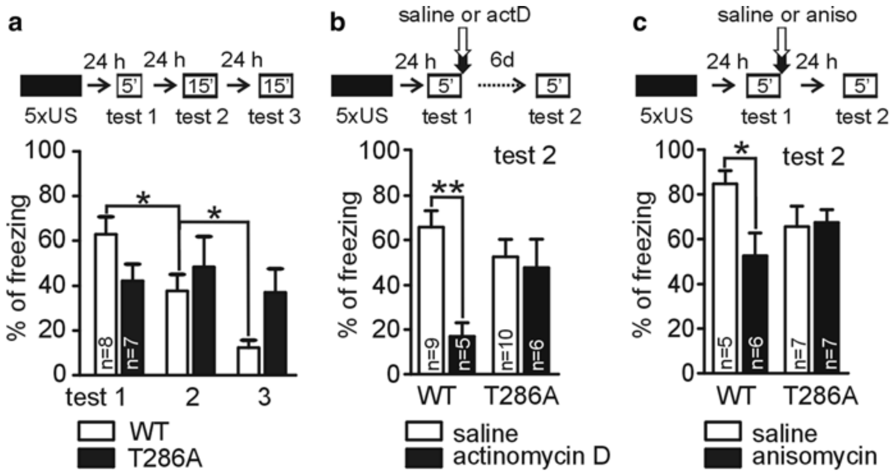


**Fig. 5.4** Contextual LTM in T286A mutants is less flexible than in WT mice. WT mice and T286A mutants were trained with massed foreground conditioning and then they underwent extinction training (**a**) or were tested for memory destabilization after memory reactivation, using actinomycin D (actD) (**b**) or anisomycine (**c**) injection to block memory reconsolidation. Reprinted from Radwanska et al. (2011)

retrieval does not destabilize contextual fear LTM in the T286A (Fig. 5.4). Therefore, MIS-dependent LTM may be less flexible than LTP-dependent LTM. An alternative interpretation of the impaired contextual LTM flexibility in T286A mutants is that αCaMKII autophosphorylation is essential for destabilization of LTM as it has been suggested to regulate ubiquitination at the synapse that appears to be a critical process (Bingol et al. 2010; Lee et al. 2008).

## 5.6 Conclusion

Eleven years ago Nikonenko and colleagues showed that NMDA receptor activation could induce MIS generation in hippocampal slice cultures (Nikonenko et al. 2003). This was the first evidence that MIS might be involved in brain plasticity. However, Nikonenko and colleagues did not study MIS generation in intact brain; for example, before and after training in a behavioral task. It is only recently that we have shown that MIS generation may occur during memory formation when synaptic strengthening is impaired (Radwanska et al. 2011). At the same time it is unknown whether MIS-generation can be a more general mechanism for memory formation also in other physiological or pathological conditions. Based on our understanding of MIS-linked memory, it can be hypothesized that generation of multi-innervated dendritic spines, is the mechanism of development of addiction or hippocampal memory formation in old age and that the engagement of this mechanism not only

requires more training trials but that it also impairs the updating of newly acquired memories.

In old age memory abilities decline, an individual needs more practice to learn new things. This is primarily due to hippocampal dysfunction impairing spatial and contextual learning and memory (Small et al. 2011). The ageing process impacts on the physiology of hippocampal neurons, resulting in a deficit in the induction of LTP, the prime mechanism underlying memory formation (e.g., Murphy et al. 2004). However, despite LTP impairment new memories can be formed in old age. Currently, the 'alternate mechanism' that enables memory formation in old age is not known. The engagement of this alternate mechanism might require more training trials and it might impair the flexibility of newly acquired memory. Taking into account the requirements of MIS-linked memory described in T286A mutants, such as intensive training, it is an intriguing hypothesis that the same mechanism can be used by an ageing brain when hippocampal LTP is impaired. Furthermore, it can be hypothesized that MIS may be involved in particular in formation of long-lasting and inflexible memories and behaviors. These criteria are fulfilled in particular by such brain dysfunctions as drug addiction (Koob 2009).

## References

Belelovsky K, Kaphzan H, Elkobi A, Rosenblum K (2009) Biphasic activation of the mTOR pathway in the gustatory cortex is correlated with and necessary for taste learning. J Neurosci 29:7424–7431

Bingol B, Wang CF, Arnott D, Cheng D, Peng J, Sheng M (2010) Autophosphorylated CaMKIIalpha acts as a scaffold to recruit proteasomes to dendritic spines. Cell 140:567–578

Cooke SF, Wu J, Plattner F, Errington M, Rowan M, Peters M, Hirano A, Bradshaw KD, Anwyl R, Bliss TV, Giese KP (2006) Autophosphorylation of alphaCaMKII is not a general requirement for NMDA receptor-dependent LTP in the adult mouse. J Physiol 574:805–818

Coultrap SJ, Freund RK, O'Leary H, Sanderson JL, Roche KW, Dell'Acqua ML, Bayer KU (2014) Autonomous CaMKII mediates both LTP and LTD using a mechanism for differential substrate site selection. Cell Rep 6:431–437

Fiala JC, Feinberg M, Popov V, Harris KM (1998) Synaptogenesis via dendritic filopodia in developing hippocampal area CA1. J Neurosci 18:8900–8911

Geinisman Y, Berry RW, Disterhoft JF, Power JM, Van der Zee EA (2001) Associative learning elicits the formation of multiple-synapse boutons. J Neurosci 21:5568–5573

Giese KP, Fedorov NB, Filipkowski RK, Silva AJ (1998) Autophosphorylation at Thr286 of the alpha calcium-calmodulin kinase II in LTP and learning. Science 279:870–873

Giese, KP (2012) Long-term potentiation and memory. In: Memory Mechanisms in Health and Disease, KP Giese, ed., World Scientific, Singapore 1–17

Govindarajan A, Kelleher RJ, Tonegawa S (2006) A clustered plasticity model of long-term memory engrams. Nat Rev Neurosci 7:575–583

Gruart A, Munoz MD, Delgado-Garcia JM (2006) Involvement of the CA3-CA1 synapse in the acquisition of associative learning in behaving mice. J Neurosci 26:1077–1087

Hunsaker MR, Lee B, Kesner RP (2008) Evaluating the temporal context of episodic memory: the role of CA3 and CA1. Behav Brain Res 188:310–315

Irvine EE, Vernon J, Giese KP (2005) AlphaCaMKII autophosphorylation contributes to rapid learning but is not necessary for memory. Nat Neurosci 8:411–412

Irvine EE, Danhiez A, Radwanska K, Nassim C, Lucchesi W, Godaux E, Ris L, Giese KP (2011) Properties of contextual memory formed in the absence of alphaCaMKII autophosphorylation. Mol Brain 4:8

Kelleher RJ 3rd, Govindarajan A, Tonegawa S (2004) Translational regulatory mechanisms in persistent forms of synaptic plasticity. Neuron 44:59–73

Koob GF (2009) Neurobiological substrates for the dark side of compulsivity in addiction. Neuropharmacology 56(Suppl 1):18–31

Lee CC, Huang CC, Wu MY, Hsu KS (2005) Insulin stimulates postsynaptic density-95 protein translation via the phosphoinositide 3-kinase-Akt-mammalian target of rapamycin signaling pathway. J Biol Chem 280:18543–18550

Lee SH, Choi JH, Lee N, Lee HR, Kim JI, Yu NK, Choi SL, Lee SH, Kim H, Kaang BK (2008) Synaptic protein degradation underlies destabilization of retrieved fear memory. Science 319:1253–1256

Leuner B, Falduto J, Shors TJ (2003) Associative memory formation increases the observation of dendritic spines in the hippocampus. J Neurosci 23:659–665

Migues PV, Hardt O, Wu DC, Gamache K, Sacktor TC, Wang YT, Nader K (2010) PKMzeta maintains memories by regulating GluR2-dependent AMPA receptor trafficking. Nat Neurosci 13:630–634

Murphy GG, Fedorov NB, Giese KP, Ohno M, Friedman E, Chen R, Silva AJ (2004) Increased neuronal excitability, synaptic plasticity, and learning in aged Kvbeta1.1 knockout mice. Curr Biol 14:1907–1915

Nader K, Schafe GE, Le Doux JE (2000) Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. Nature 406:722–726

Nikonenko I, Jourdain P, Muller D (2003) Presynaptic remodeling contributes to activity-dependent synaptogenesis. J Neurosci 23:8498–8505

Nikonenko I, Boda B, Steen S, Knott G, Welker E, Muller D (2008) PSD-95 promotes synaptogenesis and multiinnervated spine formation through nitric oxide signaling. J Cell Biol 183:1115–1127

Petrak LJ, Harris KM, Kirov SA (2005) Synaptogenesis on mature hippocampal dendrites occurs via filopodia and immature spines during blocked synaptic transmission. J Comp Neurol 484:183–190

Popov VI, Deev AA, Klimenko OA, Kraev IV, Kuźminykh SB, Medvedev NI, Patrushev IV, Popov RV, Rogachevskii VV, Khutsiyan SS, Stewart MG, Fesenko EE (2005) Three-dimensional reconstruction of synapses and dendritic spines in the rat and ground squirrel hippocampus: new structural-functional paradigms for synaptic function. Neurosci Behav Physiol 35:333–341

Radwanska K, Medvedev NI, Pereira GS, Engmann O, Thiede N, Moraes MF, Villers A, Irvine EE, Maunganidze NS, Pyza EM, Ris L, Szymanska M, Lipinski M, Kaczmarek L, Stewart MG, Giese KP (2011) Mechanism for long-term memory formation when synaptic strengthening is impaired. Proc Natl Acad Sci U S A 108:18471–18475

Rampon C, Tang YP, Goodhouse J, Shimizu E, Kyin M, Tsien JZ (2000) Enrichment induces structural changes and recovery from nonspatial memory deficits in CA1 NMDAR1-knockout mice. Nat Neurosci 3:238–244

Restivo L, Vetere G, Bontempi B, Ammassari-Teule M (2009) The formation of recent and remote memory is associated with time-dependent formation of dendritic spines in the hippocampus and anterior cingulate cortex. J Neurosci 29:8206–8214

Sacktor TC (2011) How does PKMzeta maintain long-term memory? Nat Rev Neurosci 12:9–15

Silva AJ (2003) Molecular and cellular cognitive studies of the role of synaptic plasticity in memory. J Neurobiol 54:224–237

Small SA, Schobel SA, Buxton RB, Witter MP, Barnes CA (2011) A pathophysiological framework of hippocampal dysfunction in ageing and disease. Nat Rev Neurosci 12:585–601

Swiech L, Perycz M, Malik A, Jaworski J (2008) Role of mTOR in physiology and pathology of the nervous system. Biochim Biophys Acta 1784:116–132

Toni N, Buchs PA, Nikonenko I, Bron CR, Muller D (1999) LTP promotes formation of multiple spine synapses between a single axon terminal and a dendrite. Nature 402:421–425

Whitlock JR, Heynen AJ, Shuler MG, Bear MF (2006) Learning induces long-term potentiation in the hippocampus. Science 313:1093–1097

Xu T, Yu X, Perlik AJ, Tobin WF, Zweig JA, Tennant K, Jones T, Zuo Y (2009) Rapid formation and selective stabilization of synapses for enduring motor memories. Nature 462:915–919

Yang G, Pan F, Gan WB (2009) Stably maintained dendritic spines are associated with lifelong memories. Nature 462:920–924

Yasuda H, Barth AL, Stellwagen D, Malenka RC (2003) A developmental switch in the signaling cascades for LTP induction. Nat Neurosci 6:15–16

# Chapter 6
# Inverse Synaptic Tagging by Arc

**Hiroyuki Okuno, Anna Araki, and Keiichiro Minatohara**

**Abstract** Long-term synaptic plasticity and memory formation require activity-dependent gene expression. However, it remains unknown how such activity-induced gene products are targeted to proper cellular compartments including synapses. Investigation of the targeting of the memory related-protein Arc from the soma to the synapses have elucidated a novel "inverse" synaptic tagging mechanism that enables Arc to specifically target the un-potentiated synapses. The findings provide new mechanistic insights into how the contrast between strong and weak synapses is maintained during long-term synaptic plasticity. Furthermore, they facilitate a better understanding of the molecular basis of biochemical memory at individual synapses and ultimately learning and memory.

**Keywords** Arc/Arg3.1 • CaMKII • Synaptic tagging and capture • Inverse synaptic tagging • Synaptic plasticity • Immediate-early genes

## 6.1 Introduction

The formation and sculpting neuronal circuits in the brain are not specific to the course of development but occur continuously throughout life. Synapses, connection sites between neurons, are added or removed from neuronal circuits daily (Bhatt et al. 2009; Holtmaat and Svoboda 2009). Synapses also undergo both positive and negative changes with respective to shape, size, and the efficiency of information transmission depending on the patterns of synaptic inputs (Bliss and Collingridge 2013; Kasai et al. 2003). These synaptic changes can be last for hours, days, months, or even throughout life (Abraham et al. 1985, 2002; Bliss and Lomo 1973; Dudek and Bear 1993; Hofer et al. 2009). These plastic properties of synapses are thought to be the fundamental cellular basis of our cognitive functions including learning and memory (Hebb 1949; Neves et al. 2008; Whitlock et al. 2006).

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Experience-evoked gene expression, or more generally, neuronal activity-dependent gene expression in the brain is a prominent cellular event that occurs during various memory formation processes. Activity-dependent gene expression is indispensable for memory consolidation, reconsolidation, updates, and extinction (Kandel 2001; Nader and Hardt 2009; Okuno 2011; Silva et al. 1998) and is also critical for long-lasting synaptic modifications including long-term potentiation (LTP), long-term depression (LTD), and synaptic morphological changes (Bliss and Collingridge 2013; Govindarajan et al. 2006; Redondo and Morris 2011). This further corroborates the fundamental importance of these cellular processes in long-term memory formation and storage. In recent decades, a great deal of effort has been invested in identifying and charactering activity-dependent genes that critically regulate the synaptic and cellular events underlying memory formation and cognitive processing (Bourtchuladze et al. 1994; Brakeman et al. 1997; Flavell et al. 2008; Greer et al. 2010; Lanahan et al. 1997; Nedivi et al. 1993; Qian et al. 1993).

The neuronal immediate early gene *Arc* (also called *Arg*3.1) is among the most promising candidate memory regulatory genes (Bramham et al. 2010; Korb and Finkbeiner 2011; Shepherd and Bear 2011) because of its highly dynamic expression that is correlated with augmented neuronal activity required for cognitive processes such as spatial learning and memory consolidation (Guzowski et al. 1999; Kawashima et al. 2009; Ramirez-Amaya et al. 2005). This chapter provides an overview of the current knowledge of *Arc* function in synaptic plasticity. The inverse tagging model of Arc as a novel molecular mechanism for memory formation is also introduced. Finally, the *in vivo* relevance of inverse tagging mechanisms will be discussed.

## 6.2 The Plasticity-Related, Activity-Regulated Gene Arc

*Arc/Arg*3.1 was first isolated from rat seizure-induced hippocampal cDNA libraries (Link et al. 1995; Lyford et al. 1995). However, *Arc* has emerged as one of the most dynamically regulated and most sensitive genes in response to a wide variety of physiological stimuli including sensory inputs (Carpenter-Hyland et al. 2010; Tagawa et al. 2005) and cognitive burdens related to learning and memory (Fig. 6.1a) (Guzowski et al. 1999, 2000; Ramirez-Amaya et al. 2005). Because of its high responsiveness, *Arc* transcripts and Arc protein are now widely used as reliable activity markers for visualizing neuronal ensembles activated during specific behaviors or cognitive paradigms (Chawla et al. 2005; Mamiya et al. 2009; Ploski et al. 2008; Tse et al. 2011; Wintzer et al. 2014).

*Arc* is evolutionally conserved in vertebrates, and has no paralogue in genomes, suggesting that it has unique but critical roles. *Arc* encodes an approximately 400-amino acid protein that has no catalytic or other known functional motifs. Because Arc protein directly or indirectly interacts with many proteins, it is likely function as a scaffold/anchor protein (Fig. 6.1b). A large part of Arc's function is thought to occur postsynaptically. Biochemical and electron microscopy studies demonstrate

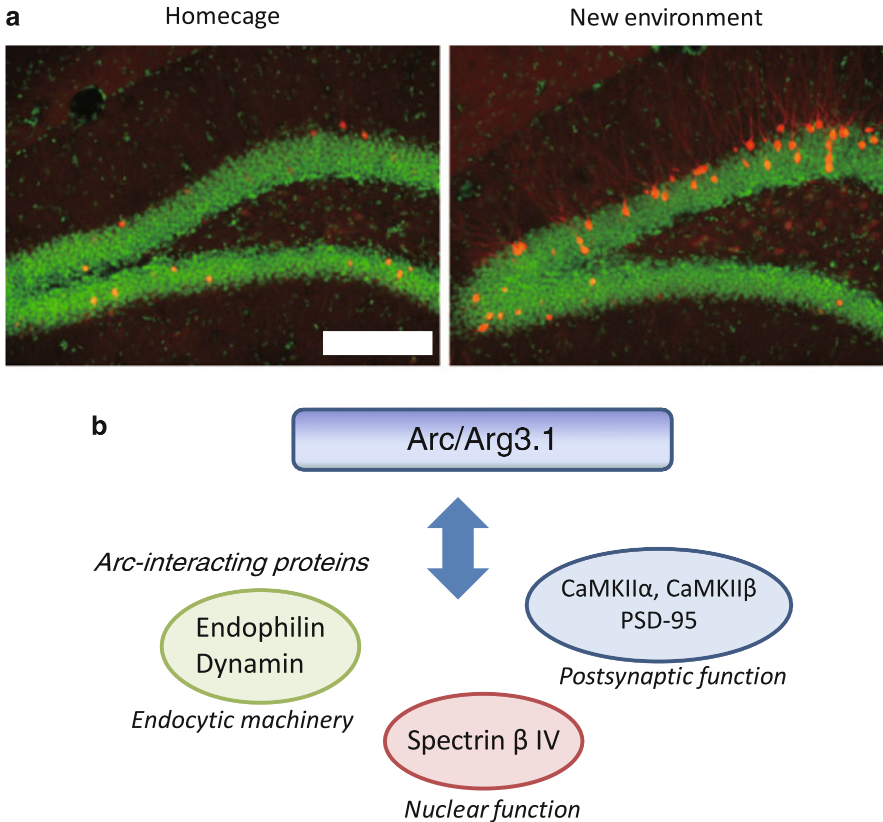


**Fig. 6.1** Neuronal immediate-early gene Arc/Arg3.1. (**a**) Experience-induced Arc expression in the hippocampus. When mice are exposed to a new environment, Arc protein (*red*) is rapidly induced in the dentate gyrus of the hippocampus within a few hours (*right*). Much fewer neurons express Arc under the homecage condition (*left*). Nuclear staining is shown in *green*. Bar = 200 μm. (**b**) Arc protein and its binding partners. Arc protein is shown to interact with several proteins with different functions. Only some of known Arc-interacting proteins are shown (Color figure online)

that Arc protein is present in the postsynaptic density of activated neurons (Chowdhury et al. 2006; Moga et al. 2004; Rodriguez et al. 2008). At the postsynaptic site, Arc interacts with the endocytic proteins endophilin and dynamin to facilitate the removal of AMPA-type glutamate receptors (AMPARs) from the plasma membrane (Fig. 6.2a) (Chowdhury et al. 2006; Rial Verde et al. 2006; Shepherd et al. 2006). This biological function together with the activity-dependent expression of *Arc* accounts for the physiological roles of *Arc* in several forms of protein translation-dependent LTD (Park et al. 2008; Plath et al. 2006; Smith-Hicks et al. 2010; Waung et al. 2008) and homeostatic plasticity/synaptic scaling (Fig. 6.2b) (Beique et al. 2011; Chowdhury et al. 2006; Rial Verde et al. 2006; Shepherd

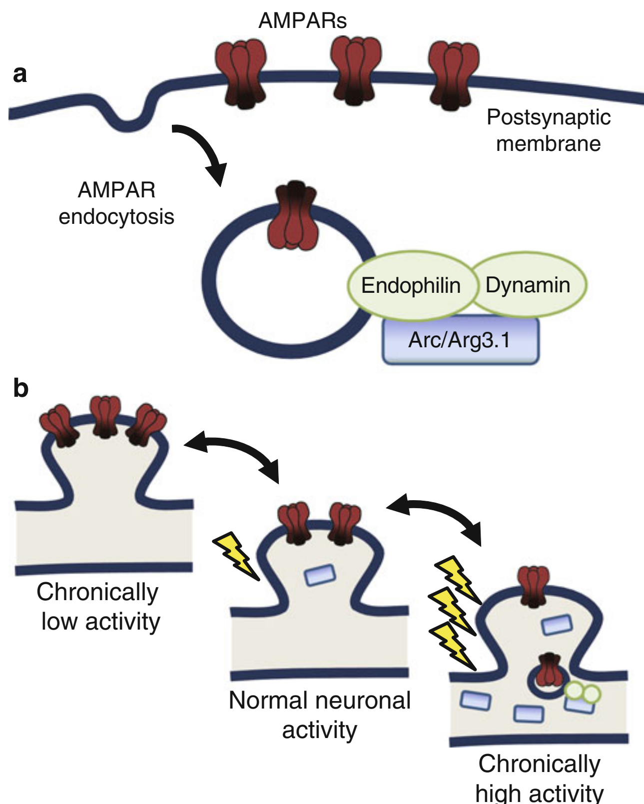


**Fig. 6.2** Arc/Arg3.1 function at the postsynaptic site. (**a**) Role of Arc in AMPAR trafficking. Arc forms a complex with endophilin and dynamin. This endocytic machinery complex facilitates the removal of AMPARs from the postsynaptic membrane. (**b**) A model of Arc's role in homeostatic synaptic scaling. The activity-regulated dynamic expression of Arc together with Arc-dependent AMPAR endocytosis provides a favorable mechanism for homeostatic modulation of the surface AMPAR expression. (*Left*) Under conditions of chronically low neuronal activity, Arc expression is reduced and Arc has little effect on AMPA-receptor. (*Middle*) With normal neuronal activity, Arc molecules play a role to maintain AMPAR trafficking. (*Right*) Arc expression is augmented with persistent high neuronal activity, facilitating the Arc-dependent endocytosis of AMPARs

et al. 2006). However, these pivotal roles of *Arc* in the cell-wide weakening of glutamatergic synaptic strength are irreconcilable with a large body of evidence indicating that *Arc* is most strongly induced by stimuli that evoke LTP (Link et al. 1995; Rodriguez et al. 2008; Ying et al. 2002) and that both *Arc* mRNA and protein accumulate in the dendritic areas that receive high-frequency synaptic inputs (Moga et al. 2004; Steward et al. 1998; Steward and Worley 2001; Wallace et al. 1998). Arc protein is also enriched in the nuclei of neuronal cells, although its function there remains unclear at the moment (Bloomer et al. 2007, 2008; Korb et al. 2013).

## 6.3 Roles of Arc in Long-Term Synaptic Plasticity and Homeostatic Plasticity

Long-lasting synaptic enhancement (i.e., LTP) and suppression (i.e., LTD) are two major forms of synaptic plasticity; they function as the principal cellular mechanisms underlying memory formation and storage (Bliss and Collingridge 1993; Morris et al. 2003; Nabavi et al. 2014). *Arc* is induced effectively in neurons by various stimuli that evoke LTP and LTD (Link et al. 1995; Messaoudi et al. 2002; Waung et al. 2008). Importantly, the fact that LTP and LTD are impaired in *Arc*-deficient mice directly suggests the involvement of *Arc* in both forms of synaptic plasticity (Park et al. 2008; Plath et al. 2006). In particular, compelling evidence indicates that *Arc* plays crucial roles in various LTD processes. In the hippocampal CA1 region, *Arc* is upregulated in approximately 40 % of pyramidal cells after rodents are exposed to a novel environment (Guzowski et al. 1999; Ramirez-Amaya et al. 2005). Interestingly, LTD is preferentially induced in cells expressing *Arc* upon novelty exposure but not in *Arc*-negative neurons (Jakkamsetti et al. 2013). *Arc* mRNA is present in the dendritic regions, and its local translation to Arc protein is implicated in a site-specific LTD mechanism (Waung et al. 2008). Furthermore, rapid induction of Arc after LTD-inducing stimulation plays a role in cerebellar LTD in Purkinje cells (Smith-Hicks et al. 2010). Several studies show that *Arc* is also involved in LTP induction or maintenance, although the molecular mechanisms underlying synaptic enhancement remain obscure (Messaoudi et al. 2007; Plath et al. 2006).

*Arc* is also implicated in other types of synaptic adaptive changes, termed synaptic homeostasis. Synaptic scaling is a cell-wide homeostatic mechanism that changes the strength of all synapses in response to the perturbation of synaptic inputs (Turrigiano 2008, 2012). Through this mechanism, when the synaptic activity of neurons in the neuronal network is chronically suppressed, for example by the sodium channel blocker tetrodotoxin, the strength of neuronal synapses is globally augmented. Meanwhile, opposite situation such as chronic enhancement of synaptic activity with GABA receptor antagonists result in globally reduced synaptic efficiency. This bidirectional change at each synapse occurs in proportion to the initial strength (i.e., a multiplicative function), thus termed synaptic scaling. Synaptic scaling is generally thought to be advantageous for maintaining neuronal excitability within a certain dynamic range without affecting relative balance between strong and weak synapses (Turrigiano 2008). Activity-regulated Arc expression together with Arc's role in promoting AMPAR endocytosis constitutes a cell-autonomous mechanism that accounts for the homeostatic control of AMPAR surface expression levels (Shepherd and Huganir 2007). If all synaptic activity is chronically suppressed, neurons will shut down activity-dependent Arc expression. This reduction of Arc expression would favor the enhancement of synaptic strength, leading to the augmentation of cellular excitability. Conversely, if neurons continuously receive strong inputs to a majority of synapses, Arc will be robustly induced and accumulate throughout the neuronal somata and dendrites. In turn, this augmented Arc

expression would reduce overall synaptic strength within the activated neurons, decreasing responsiveness to excitatory inputs. In this way, cellular responsiveness or excitability is homeostatically controlled, at least in part, by activity-regulated Arc expression (Fig. 6.2b). Consistent with this model, synaptic scaling of AMPARs is lost in neurons lacking *Arc* (Beique et al. 2011; Shepherd et al. 2006).

## 6.4 Inverse Synaptic Tagging of Arc

The maintenance of long-term synaptic plasticity requires *de novo* synthesis of transcripts and proteins during a particular time window after synapses receive plasticity-inducing stimuli (Cooke and Bliss 2005; Kandel 2001; Morris et al. 2003; Okuno 2011). During this time window, newly synthesized plasticity-related proteins are thought to act at postsynaptic sites to facilitate the establishment and maintenance of long-term synaptic modifications (Redondo and Morris 2011; Rogerson et al. 2014). However, a fundamental question remains: how do the plasticity-related proteins, which are synthesized at the soma and dendrites, selectively target the synapses undergoing plastic changes? The synaptic tagging and capture hypothesis adopts a conceptual framework in which activity-triggered local changes at synaptic sites (i.e., synaptic tagging) permit the use of activity-induced plasticity-related proteins (i.e., capture) to stabilize and maintain changes in synaptic efficacy (Frey and Morris 1997; Martin et al. 2000; Morris 2006). The synaptic tagging and capture hypothesis succinctly explains how protein synthesis-dependent LTP reconciles input-specific synaptic modifications with the broad cellular distribution of newly synthesized proteins required for LTP maintenance (Govindarajan et al. 2006; Redondo and Morris 2011).

An opposite but non-mutually exclusive hypothesis is the *inverse* synaptic tagging model, which assumes the existence of synaptic tags that mark *non-potentiated* synapses and are involved in processes to prevent these synapses from being strengthened. A recent study indicates that Arc is involved in such an inverse synaptic tagging process (Okuno et al. 2012). The framework of the inverse synaptic tagging of Arc is described below.

### *6.4.1 Activity-Induced Arc Is Preferentially Accumulated in Inactive Synapses*

As described above, *Arc* mRNA and protein expressions are tightly regulated by synaptic activity; LTP-inducing stimulation is the one of the most effective stimuli for inducing *Arc* in the hippocampus (Link et al. 1995; Moga et al. 2004; Steward et al. 1998). Furthermore, *Arc* mRNA and protein accumulate around the dendritic areas where strong inputs are delivered (Moga et al. 2004; Steward and Worley 2001; Wallace et al. 1998). Based on these observations, it is widely speculated that

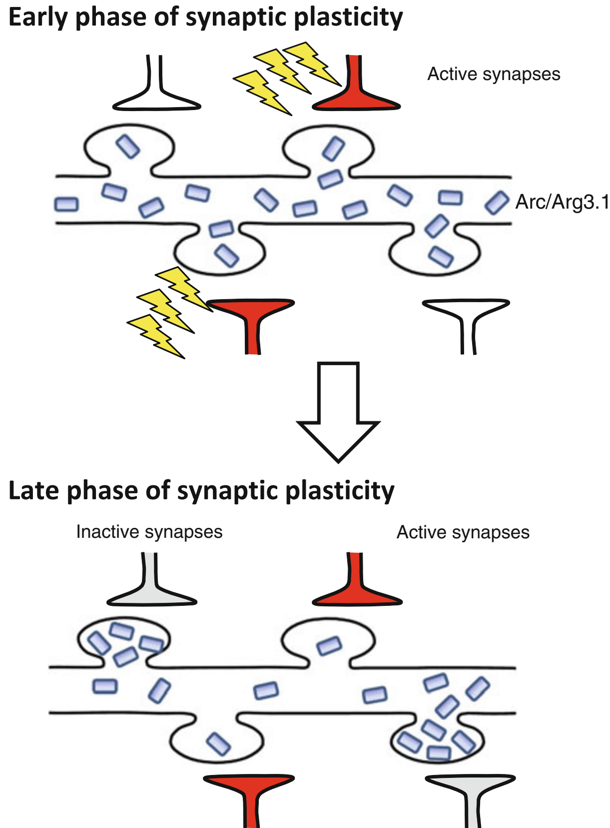


**Fig. 6.3** Selective accumulation of activity-induced Arc/Arg3.1 in inactive synapses during the late phase of synaptic plasticity. Upon receiving plasticity-inducing synaptic inputs, newly synthesized Arc is delivered to the dendrites (*top*). Arc is then gradually lost from active synapses but accumulates in inactive synapses during a following period (*bottom*). This Arc accumulation in the inactive synapses relies upon selective interaction with the inactive form of CaMKIIβ

Arc protein directly regulates LTP induction and/or maintenance at LTP-expressing synapses (Bramham et al. 2010). However, recent experimental evidence suggests otherwise; Arc protein accumulates at the postsynapse when neuronal activity is pharmacologically suppressed both *in vitro* and *in vivo* (Kim et al. 2012; Okuno et al. 2012). Consistently, the synapse-specific presynaptic inhibition with tetanus toxin results in the site-specific accumulation of Arc protein at the postsynapses opposed to the inhibited presynapses. These experimental results suggest that Arc preferentially targets postsynapses under synaptically inactivated conditions (Fig. 6.3). If so, how does newly synthesized Arc behave in dendrites when synapses are activated, for example, by LTP-inducing stimulation? Administration of strong synaptic inputs leads to volume expansion in a subset of postsynapses, which reliably represents synaptic enhancement in individual synapses (Harvey and Svoboda 2007; Matsuzaki et al. 2004). Live imaging of Arc dynamics at dendrites shows that

newly synthesized Arc at the soma is less incorporated in expanded synapses, which are presumably active LTP-induced sites, compared to non-expanded inactive synapses (Okuno et al. 2012). These findings collectively indicate the preferential targeting of activity-induced Arc protein into inactive rather than active synapses (Fig. 6.3).

### *6.4.2 Molecular Mechanisms of Arc Accumulation in Inactive Synapses: Dynamic Interaction between Arc and CaMKIIβ*

Arc protein possesses several domains involved in protein-protein interactions; biochemical screening has indeed unveiled several proteins that interact with Arc (Fig. 6.1b) (Bloomer et al. 2007; Chowdhury et al. 2006; Donai et al. 2003; Korb et al. 2013). Some Arc-interacting proteins are related to endocytic processes, while others are synaptic or nucleic proteins. Among those, so far reported, calcium/calmodulin dependent kinase type II (CaMKII) is the only protein whose interaction with Arc is dynamically regulated (Okuno et al. 2012).

CaMKII is one of the most abundant proteins associated with the postsynaptic density and acts as a key signaling mediator that may decode the spatiotemporal patterns of $Ca^{2+}$ mobilization into kinase activity (Hudmon and Schulman 2002; Kennedy 2000; Lisman et al. 2002). CaMKII dynamically changes its conformation upon $Ca^{2+}$ and calmodulin binding. Under $Ca^{2+}$/CaM-unbound conditions, the catalytic domain of CaMKII is masked by its regulatory domain (i.e., autoinhibitory domain), thus suppressing its kinase activity (Giese et al. 1998; Irvine et al. 2006). This state of CaMKII is referred to as its inactive form. Upon $Ca^{2+}$/CaM-binding, the autoinhibitory domain moves away from the catalytic domain; in this state, CaMKII exerts kinase activity on its substrates (i.e., active form). Two neuron-specific isoforms, CaMKIIα and CaMKIIβ, which are both expressed at synapses, have been suggested to have distinct biochemical properties and physiological roles in the regulation of synaptic functions (Fink et al. 2003; O'Leary et al. 2006; Okamoto et al. 2007; Thiagarajan et al. 2002). Interestingly, despite the overall high similarity between these isoforms, CaMKIIα and CaMKIIβ act differently on Arc protein. The inactive form of CaMKIIβ has a higher affinity for Arc than its active form, while the active form of CaMKIIα binds more readily to Arc than its inactive form (Okuno et al. 2012). Furthermore, CaMKIIβ has greater affinity for Arc than CaMKIIα. Therefore, CaMKIIβ rather than CaMKIIα likely plays a dominant role in Arc regulation at neuronal synapses. Because the activity state of CaMKII is mainly regulated by synaptic activity at the postsynaptic site (Fujii et al. 2013; Lee et al. 2009), Arc's synaptic localization at inactive synapses is possibly regulated through the strongest interaction between inactive CaMKIIβ and Arc, especially under synaptically suppressed conditions. Indeed, CaMKIIβ knockdown or knockout results in a dramatic decrease in Arc enrichment at synapses both *in vitro* and *in vivo* (Okuno et al. 2012).

CaMKIIβ is structurally distinct from CaMKIIα in that it has a unique amino acid sequence essential for specific binding to F-actin (O'Leary et al. 2006; Okamoto et al. 2007; Shen et al. 1998). Furthermore, Arc binds indirectly to F-actin (Lyford et al. 1995). Therefore, the inactive form of CaMKIIβ could serve as a molecular scaffold linking F-actin and Arc at the postsynaptic density. Interestingly, the F-actin/CaMKIIβ complex is disrupted in the presence of $Ca^{2+}$/CaM (Fink et al. 2003; O'Leary et al. 2006; Okamoto et al. 2007), similar to the destabilization of the Arc interaction with active CaMKIIβ. Therefore, sustained low $Ca^{2+}$ concentration during synaptic inactivity would favor the stabilization of the F-actin/CaMKIIβ/Arc complex. However, it remains unclear whether the F-actin/CaMKIIβ/Arc complex accounts for Arc's effect on dendritic spine morphology (Peebles et al. 2010).

### *6.4.3 Arc in Inactive Synapses Reduces AMPAR Expression*

At the postsynapse, Arc reduces the surface expression of AMPARs by facilitating endocytic processes (Fig. 6.2) (Chowdhury et al. 2006; Rial Verde et al. 2006). Thus, the preferential targeting of Arc to less active synapses via interaction with inactive CaMKIIβ would provide a mechanism for the synapse-specific control of AMPAR trafficking according to the history of local synaptic activity and inactivity. Indeed, the concentration of Arc at synapses during inactivity is negatively correlated with the surface expression levels of the GluA1 subunit of AMPARs at individual synapses (Fig. 6.4a) (Okuno et al. 2012). These findings indicate that the degree of maintenance of newly synthesized Arc in the synaptic pool quantitatively determines GluA1 turnover in an input-specific manner. Furthermore, the downregulation of GluA1 surface expression in Arc-containing synapses but not Arc-lacking synapses is consistent with the notion that an inactivity-modulated concentration gradient of Arc plays a role in the clearance of initially upregulated GluA1 from less active synapses during the late phase of LTP (Kim et al. 2012). Because the number of glutamate receptors at the postsynaptic surface directly determines synaptic strength between neurons, Arc's role in AMPAR regulation critically contributes to maintaining weak synapses weak, while allowing strong, essential synapses to remain strong (Fig. 6.4b).

### *6.4.4 Inverse Synaptic Tagging*

Although it is postulated and widely accepted that the tag-captured plasticity-related protein interaction provides the basis for the persistence of enhanced synaptic strength at stimulated inputs, the Arc dynamics described above suggest an alternative scenario (Fig. 6.5). In this scenario, some "inverse tags" may be specifically generated to sort newly synthesized plasticity-related proteins to inactive synapses via a molecular inactivity-sensing mechanism, thereby facilitating the shunting of

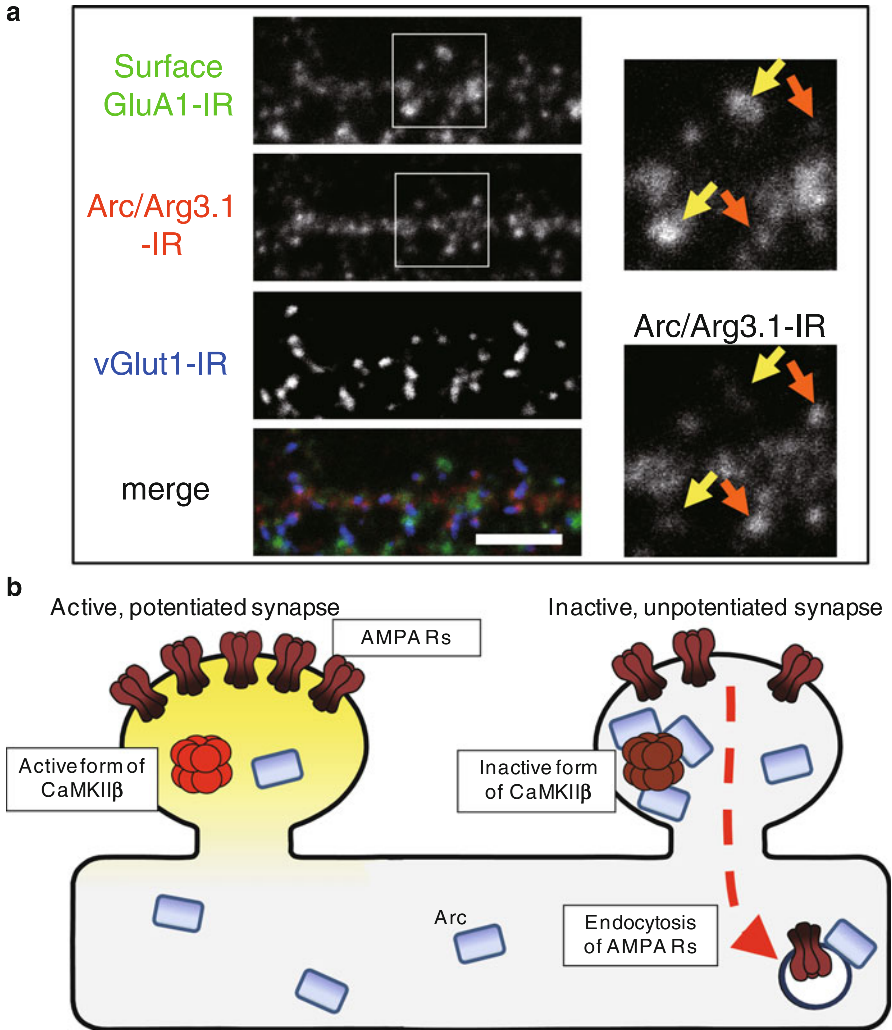


**Fig. 6.4** Synapse-specific regulation of AMPARs by Arc/Arg3.1. (**a**) Synaptic Arc concentration determines surface expression levels of GluA1 in individual synapses. Representative triple immunostaining of a dendritic segment from hippocampal neurons in which Arc is induced and accumulates in synapses during synaptic inactivation. Framed areas are expanded on the *right*. Some spines contained high surface GluA1 signals but low Arc signals (*yellow arrows*), whereas others displayed the opposite pattern (*orange arrows*). Scale bar, 5 μm. Figures are reproduced from Okuno et al. (2012) with permission of Elsevier. (**b**) A model of Arc function on AMPAR clearance at active and inactive synapses. The surface expression of AMPARs is augmented at the synapses that receive strong inputs, which also trigger Arc induction. During the late phase of post stimulation, Arc is differently maintained in the synapses depending on the amount and history of synaptic activity. In the synapses that receive frequent inputs (active synapses), CaMKIIβ more likely to be activated, and thus its interaction with Arc is often weakened. As a result, Arc tends to flow out from the synapses. In contrast, synapses with low activity (inactive synapses) are more likely to contain the inactive form of CaMKIIβ, which provides a scaffold for Arc at the synapse. The CaMKIIβ-stabilized Arc has a greater chance to form a complex with the endocytic machinery, which subsequently promotes AMPA-R clearance from the synapse. Through such an activity-dependent control of synaptic localization, Arc may contribute to synaptic homeostasis while active, potentiated synapses remain unaffected (Color figure online)

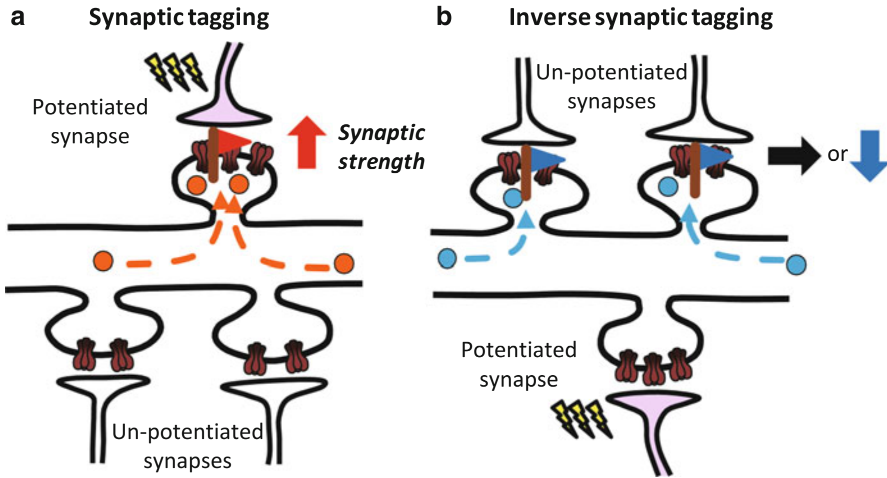


**Fig. 6.5** Synaptic tagging and inverse synaptic tagging models. (**a**) Synaptic tagging. During the induction phase of LTP, plasticity-inducing stimuli develop a putative signature (tag) within the activated synapses (a *red flag*). This "synaptic tag" serves as a mark to distinguish the synapses to be potentiated from other non-stimulated synapses, and stimulus-induced plasticity-related proteins (*red circles*) are then captured based on the presence of the synaptic tags. The plasticity-related proteins are required to maintain the synaptic enhancement during the late phase of LTP. (**b**) Inverse synaptic tagging. In contrast to the synaptic tagging, the inverse synaptic tag (*blue flags*) is created in synapses other than the synapse to be potentiated. The stimulus-induced negative plasticity-related proteins (*blue circles*) are captured in the non-potentiated synapses, and play a role in preventing un-desired synaptic augmentation during the late phase of LTP (Color figure online)

weak inputs. In other words, some plasticity-related proteins might be excluded from active synapses that contain an active synaptic tag and would instead be captured by neighboring inactive synapses expressing an inverse tag such as inactive CaMKIIβ. The selective avoidance of actively tagged synapses by a negative plasticity factor such as Arc via a preferential interaction with an inverse tag may be considered the conceptual opposite of the classical notion of "synaptic tag and capture," and could thus be termed an "inverse synaptic tagging" process (Fig. 6.5). However, in principle, these two mechanisms could well function in parallel, thereby ensuring that the contrast between strong and weak inputs remains stable over time. This novel working model of the synapse-specific role of activity-induced Arc at less active synapses may well reconcile the requirement for Arc during the late phase of various forms of long-term synaptic plasticity as well as Arc's demonstrated role as a cell-wide mediator of AMPAR endocytosis during homeostatic plasticity and synaptic scaling (Shepherd and Bear 2011). Furthermore, the model provides new mechanistic insights on how the contrast between strong and weak synapses is maintained during long-term synaptic plasticity.

### 6.4.5 Synaptic Elimination

During postnatal development, neuronal circuits in the brain are initially formed through highly active synaptogenesis around birth. Subsets of synapses are subsequently selected and strengthened in a usage-dependent and activity-regulated manner, while many excess unnecessary synapses are weakened and eventually eliminated (Katz and Shatz 1996; Lichtman and Colman 2000). Many distinct molecular and cellular mechanisms appear to be involved in the synaptic elimination processes depending on the brain region and neuronal circuit. One of the most well-studied synaptic elimination occurs in the developing cerebellum, wherein Purkinje cells initially innervated by multiple climbing fibers are later dominated by a single climbing fiber during the course of postnatal development (Kano and Hashimoto 2009); *Arc* was recently reported to be critically involved in this process (Kawata et al. 2014; Mikuni et al. 2013). The studies show the Purkinje cell-specific knockdown of *Arc* both *in vitro* and *in vivo* significantly interfere with surplus climbing fiber elimination. Together with the evidence that Purkinje cell activation induces *Arc* mRNA and protein expression in the developing cerebellum (Mikuni et al. 2013; Smith-Hicks et al. 2010) and that weak climbing fiber synapses are preferentially subjected to synapse elimination (Hashimoto et al. 2009; Hashimoto and Kano 2003), these observations are concordant with the notion that Arc plays a role in suppressing synaptic strength at inactive, weak synapses through the inverse synaptic tagging mechanism.

In the mature brain, although the rate is substantially reduced, synaptogenesis and synaptic elimination still occur continuously (Bhatt et al. 2009; Holtmaat and Svoboda 2009). Recent advances in imaging technologies and molecular genetics clearly demonstrate that synaptic structural dynamics is correlated with cognitive functions including memory formation and storage (Fu and Zuo 2011; Kasai et al. 2003). A recent study shows that during fear memory formation, synapse elimination is selectively observed in active hippocampal neurons in contextual fear-conditioned mice (Sanders et al. 2012); such synaptic changes could represent a compensatory adaptation to enhanced synaptic strength in a subset of synapses during learning although whether or not these eliminated spines were functional remains unclear in the study. Provided that active neurons express Arc in the hippocampus, the synapse elimination observed in that study is also consistent with Arc's role in inverse synaptic tagging.

### 6.4.6 Role of Arc in Memory Formation

Several independent lines of Arc-null mice exhibit a wide range of cognitive deficits in tasks related to spatial memory, fear memory, taste aversion, and object recognition (Peebles et al. 2010; Plath et al. 2006). Consistently, Arc knockdown by antisense oligonucleotides in rats results in spatial and fear memory impairment

(Czerniawski et al. 2011; Guzowski et al. 2000; Ploski et al. 2008). In addition, cortical plasticity and neuronal representation are abnormal in *Arc*-knockout mice (Gao et al. 2010; McCurry et al. 2010; Wang et al. 2006). These lines of evidence strongly indicate *Arc* is indispensable for long-term memory formation and normal cortical responsiveness in the brain. Such Arc-dependent memory formation and cortical plasticity could be realized through inverse synaptic tagging of Arc, which functions to stabilize the contrast between strong and weak inputs over time by preventing undesired synaptic enhancement at weak synapses while sparing potentiated synapses.

Behavioral tagging is a phenomenon that could be interpreted as *in vivo* evidence of the synaptic tagging demonstrated in brain slices or cultured cells (Viola et al. 2014). In this paradigm, a weak training protocol that normally only produces short-term memory can be transformed to create long-term memories if the training is combined with a novel experience during a critical time window around the training (Ballarini et al. 2009; Moncada et al. 2011; Wang et al. 2010). *Arc* knockdown by antisense-oligonucleotides was recently reported to interfere behavioral tagging processes (Martínez et al. 2012). However, how *Arc*'s role in the inverse tagging process contributes to memory enhancement in the context of behavioral tagging remains to be investigated.

## 6.5 Conclusions

This chapter summarizes the function of the neuronal immediate early gene *Arc* and its possible roles in the inverse synaptic tagging process. The inverse synaptic tagging of Arc presents new mechanistic insights on how the contrast between strong and weak synapses may be maintained during long-term synaptic plasticity, and shed light on the fundamental role of the targeting of activity-induced gene products to synapses as a molecular basis of memory allocation at individual synapses. During the past decade, Arc has become recognized as a key regulator of synaptic plasticity and cognitive functions in the brain (Bramham et al. 2010; Okuno 2011; Shepherd and Bear 2011). As discussed above, the dysregulation of *Arc* caused abnormal neuronal circuit refinement, deficits in neuronal plasticity, and impaired memory function. Animal models implicate *Arc* in various neurodevelopmental disorders including fragile X syndrome (Park et al. 2008) and autism (Auerbach et al. 2011) as well as neurodegenerative diseases such as Alzheimer disease (Rudinskiy et al. 2012; Wu et al. 2011). Furthermore, recent large-scale human genetic studies suggest that mutations in patients with Schizophrenia are overrepresented in genes associated with the Arc signaling complex (Fromer et al. 2014; Kirov et al. 2012; Purcell et al. 2014). Therefore, further investigation of *Arc*'s role in the inverse synaptic tagging process is required to advance our understanding of how the brain manages memory and cognitive functions under normal and pathological conditions.

## References

Abraham WC, Bliss TV, Goddard GV (1985) Heterosynaptic changes accompany long-term but not short-term potentiation of the perforant path in the anaesthetized rat. J Physiol 363:335–349

Abraham WC, Logan B, Greenwood JM, Dragunow M (2002) Induction and experience-dependent consolidation of stable long-term potentiation lasting months in the hippocampus. J Neurosci 22:9626–9634

Auerbach BD, Osterweil EK, Bear MF (2011) Mutations causing syndromic autism define an axis of synaptic pathophysiology. Nature 480:63–68

Ballarini F, Moncada D, Martinez MC, Alen N, Viola H (2009) Behavioral tagging is a general mechanism of long-term memory formation. Proc Natl Acad Sci U S A 106:14599–14604

Beique JC, Na Y, Kuhl D, Worley PF, Huganir RL (2011) Arc-dependent synapse-specific homeostatic plasticity. Proc Natl Acad Sci U S A 108:816–821

Bhatt DH, Zhang S, Gan WB (2009) Dendritic spine dynamics. Annu Rev Physiol 71:261–282

Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361:31–39

Bliss TV, Collingridge GL (2013) Expression of NMDA receptor-dependent LTP in the hippocampus: bridging the divide. Mol Brain 6:5

Bliss TV, Lomo T (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol 232:331–356

Bloomer WA, VanDongen HM, VanDongen AM (2007) Activity-regulated cytoskeleton-associated protein Arc/Arg3.1 binds to spectrin and associates with nuclear promyelocytic leukemia (PML) bodies. Brain Res 1153:20–33

Bloomer WA, VanDongen HM, VanDongen AM (2008) Arc/Arg3.1 translation is controlled by convergent N-methyl-D-aspartate and Gs-coupled receptor signaling pathways. J Biol Chem 283:582–592

Bourtchuladze R, Frenguelli B, Blendy J, Cioffi D, Schutz G, Silva AJ (1994) Deficient long-term memory in mice with a targeted mutation of the cAMP-responsive element-binding protein. Cell 79:59–68

Brakeman PR, Lanahan AA, O'Brien R, Roche K, Barnes CA, Huganir RL, Worley PF (1997) Homer: a protein that selectively binds metabotropic glutamate receptors. Nature 386:284–288

Bramham CR, Alme MN, Bittins M, Kuipers SD, Nair RR, Pai B, Panja D, Schubert M, Soule J, Tiron A, Wibrand K (2010) The Arc of synaptic memory. Exp Brain Res 200:125–140

Carpenter-Hyland EP, Plummer TK, Vazdarjanova A, Blake DT (2010) Arc expression and neuroplasticity in primary auditory cortex during initial learning are inversely related to neural activity. Proc Natl Acad Sci U S A 107:14828–14832

Chawla MK, Guzowski JF, Ramirez-Amaya V, Lipa P, Hoffman KL, Marriott LK, Worley PF, McNaughton BL, Barnes CA (2005) Sparse, environmentally selective expression of Arc RNA in the upper blade of the rodent fascia dentata by brief spatial experience. Hippocampus 15:579–586

Chowdhury S, Shepherd JD, Okuno H, Lyford G, Petralia RS, Plath N, Kuhl D, Huganir RL, Worley PF (2006) Arc/Arg3.1 interacts with the endocytic machinery to regulate AMPA receptor trafficking. Neuron 52:445–459

Cooke SF, Bliss TV (2005) Long-term potentiation and cognitive drug discovery. Curr Opin Investig Drugs 6:25–34

Czerniawski J, Ree F, Chia C, Ramamoorthi K, Kumata Y, Otto TA (2011) The importance of having Arc: expression of the immediate-early gene Arc is required for hippocampus-dependent fear conditioning and blocked by NMDA receptor antagonism. J Neurosci 31:11200–11207

Donai H, Sugiura H, Ara D, Yoshimura Y, Yamagata K, Yamauchi T (2003) Interaction of Arc with CaM kinase II and stimulation of neurite extension by Arc in neuroblastoma cells expressing CaM kinase II. Neurosci Res 47:399–408

Dudek SM, Bear MF (1993) Bidirectional long-term modification of synaptic effectiveness in the adult and immature hippocampus. J Neurosci 13:2910–2918

Fink CC, Bayer KU, Myers JW, Ferrell JE Jr, Schulman H, Meyer T (2003) Selective regulation of neurite extension and synapse formation by the beta but not the alpha isoform of CaMKII. Neuron 39:283–297

Flavell SW, Kim TK, Gray JM, Harmin DA, Hemberg M, Hong EJ, Markenscoff-Papadimitriou E, Bear DM, Greenberg ME (2008) Genome-wide analysis of MEF2 transcriptional program reveals synaptic target genes and neuronal activity-dependent polyadenylation site selection. Neuron 60:1022–1038

Frey U, Morris RG (1997) Synaptic tagging and long-term potentiation. Nature 385:533–536

Fromer M, Pocklington AJ, Kavanagh DH, Williams HJ, Dwyer S, Gormley P, Georgieva L, Rees E, Palta P, Ruderfer DM, Carrera N, Humphreys I, Johnson JS, Roussos P, Barker DD, Banks E, Milanova V, Grant SG, Hannon E, Rose SA, Chambert K, Mahajan M, Scolnick EM, Moran JL, Kirov G, Palotie A, McCarroll SA, Holmans P, Sklar P, Owen MJ, Purcell SM, O'Donovan MC (2014) De novo mutations in schizophrenia implicate synaptic networks. Nature 506:179–184

Fu M, Zuo Y (2011) Experience-dependent structural plasticity in the cortex. Trends Neurosci 34:177–187

Fujii H, Inoue M, Okuno H, Sano Y, Takemoto-Kimura S, Kitamura K, Kano M, Bito H (2013) Nonlinear decoding and asymmetric representation of neuronal input information by CaMKIIalpha and calcineurin. Cell Reports 3:978–987

Gao M, Sossa K, Song L, Errington L, Cummings L, Hwang H, Kuhl D, Worley P, Lee HK (2010) A specific requirement of Arc/Arg3.1 for visual experience-induced homeostatic synaptic plasticity in mouse primary visual cortex. J Neurosci 30:7168–7178

Giese KP, Fedorov NB, Filipkowski RK, Silva AJ (1998) Autophosphorylation at Thr286 of the alpha calcium-calmodulin kinase II in LTP and learning. Science 279:870–873

Govindarajan A, Kelleher RJ, Tonegawa S (2006) A clustered plasticity model of long-term memory engrams. Nat Rev 7:575–583

Greer PL, Hanayama R, Bloodgood BL, Mardinly AR, Lipton DM, Flavell SW, Kim TK, Griffith EC, Waldon Z, Maehr R, Ploegh HL, Chowdhury S, Worley PF, Steen J, Greenberg ME (2010) The Angelman Syndrome protein Ube3A regulates synapse development by ubiquitinating arc. Cell 140:704–716

Guzowski JF, McNaughton BL, Barnes CA, Worley PF (1999) Environment-specific expression of the immediate-early gene Arc in hippocampal neuronal ensembles. Nat Neurosci 2: 1120–1124

Guzowski JF, Lyford GL, Stevenson GD, Houston FP, McGaugh JL, Worley PF, Barnes CA (2000) Inhibition of activity-dependent arc protein expression in the rat hippocampus impairs the maintenance of long-term potentiation and the consolidation of long-term memory. J Neurosci 20:3993–4001

Harvey CD, Svoboda K (2007) Locally dynamic synaptic learning rules in pyramidal neuron dendrites. Nature 450:1195–1200

Hashimoto K, Kano M (2003) Functional differentiation of multiple climbing fiber inputs during synapse elimination in the developing cerebellum. Neuron 38:785–796

Hashimoto K, Ichikawa R, Kitamura K, Watanabe M, Kano M (2009) Translocation of a "winner" climbing fiber to the Purkinje cell dendrite and subsequent elimination of "losers" from the soma in developing cerebellum. Neuron 63:106–118

Hebb DO (1949) The organization of behaviour. Wiley, New York

Hofer SB, Mrsic-Flogel TD, Bonhoeffer T, Hubener M (2009) Experience leaves a lasting structural trace in cortical circuits. Nature 457:313–317

Holtmaat A, Svoboda K (2009) Experience-dependent structural synaptic plasticity in the mammalian brain. Nat Rev 10:647–658

Hudmon A, Schulman H (2002) Neuronal CA2+/calmodulin-dependent protein kinase II: the role of structure and autoregulation in cellular function. Annu Rev Biochem 71:473–510

Irvine EE, von Hertzen LS, Plattner F, Giese KP (2006) alphaCaMKII autophosphorylation: a fast track to memory. Trends Neurosci 29:459–465

Jakkamsetti V, Tsai NP, Gross C, Molinaro G, Collins KA, Nicoletti F, Wang KH, Osten P, Bassell GJ, Gibson JR, Huber KM (2013) Experience-induced Arc/Arg3.1 primes CA1 pyramidal neurons for metabotropic glutamate receptor-dependent long-term synaptic depression. Neuron 80:72–79

Kandel ER (2001) The molecular biology of memory storage: a dialogue between genes and synapses. Science 294:1030–1038

Kano M, Hashimoto K (2009) Synapse elimination in the central nervous system. Curr Opin Neurobiol 19:154–161

Kasai H, Matsuzaki M, Noguchi J, Yasumatsu N, Nakahara H (2003) Structure-stability-function relationships of dendritic spines. Trends Neurosci 26:360–368

Katz LC, Shatz CJ (1996) Synaptic activity and the construction of cortical circuits. Science 274:1133–1138

Kawashima T, Okuno H, Nonaka M, Adachi-Morishima A, Kyo N, Okamura M, Takemoto-Kimura S, Worley PF, Bito H (2009) Synaptic activity-responsive element in the Arc/Arg3.1 promoter essential for synapse-to-nucleus signaling in activated neurons. Proc Natl Acad Sci U S A 106:316–321

Kawata S, Miyazaki T, Yamazaki M, Mikuni T, Yamasaki M, Hashimoto K, Watanabe M, Sakimura K, Kano M (2014) Global scaling down of excitatory postsynaptic responses in cerebellar Purkinje cells impairs developmental synapse elimination. Cell Reports 8:1119–1129

Kennedy MB (2000) Signal-processing machines at the postsynaptic density. Science 290: 750–754

Kim R, Okuno H, Bito H (2012) Deciphering the molecular rules governing synaptic targeting of the memory-related protein Arc. Commun Integr Biol 5:496–498

Kirov G, Pocklington AJ, Holmans P, Ivanov D, Ikeda M, Ruderfer D, Moran J, Chambert K, Toncheva D, Georgieva L, Grozeva D, Fjodorova M, Wollerton R, Rees E, Nikolov I, van de Lagemaat LN, Bayes A, Fernandez E, Olason PI, Bottcher Y, Komiyama NH, Collins MO, Choudhary J, Stefansson K, Stefansson H, Grant SG, Purcell S, Sklar P, O'Donovan MC, Owen MJ (2012) De novo CNV analysis implicates specific abnormalities of postsynaptic signalling complexes in the pathogenesis of schizophrenia. Mol Psychiatry 17:142–153

Korb E, Finkbeiner S (2011) Arc in synaptic plasticity: from gene to behavior. Trends Neurosci 34:591–598

Korb E, Wilkinson CL, Delgado RN, Lovero KL, Finkbeiner S (2013) Arc in the nucleus regulates PML-dependent GluA1 transcription and homeostatic plasticity. Nat Neurosci 16:874–883

Lanahan A, Lyford G, Stevenson GS, Worley PF, Barnes CA (1997) Selective alteration of long-term potentiation-induced transcriptional response in hippocampus of aged, memory-impaired rats. J Neurosci 17:2876–2885

Lee SJ, Escobedo-Lozoya Y, Szatmari EM, Yasuda R (2009) Activation of CaMKII in single dendritic spines during long-term potentiation. Nature 458:299–304

Lichtman JW, Colman H (2000) Synapse elimination and indelible memory. Neuron 25:269–278

Link W, Konietzko U, Kauselmann G, Krug M, Schwanke B, Frey U, Kuhl D (1995) Somatodendritic expression of an immediate early gene is regulated by synaptic activity. Proc Natl Acad Sci U S A 92:5734–5738

Lisman J, Schulman H, Cline H (2002) The molecular basis of CaMKII function in synaptic and behavioural memory. Nat Rev 3:175–190

Lyford GL, Yamagata K, Kaufmann WE, Barnes CA, Sanders LK, Copeland NG, Gilbert DJ, Jenkins NA, Lanahan AA, Worley PF (1995) Arc, a growth factor and activity-regulated gene, encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites. Neuron 14:433–445

Mamiya N, Fukushima H, Suzuki A, Matsuyama Z, Homma S, Frankland PW, Kida S (2009) Brain region-specific gene expression activation required for reconsolidation and extinction of contextual fear memory. J Neurosci 29:402–413

Martin SJ, Grimwood PD, Morris RG (2000) Synaptic plasticity and memory: an evaluation of the hypothesis. Annu Rev Neurosci 23:649–711

Martínez M, Alen N, Ballarini F, Moncada D, Viola H (2012) Memory traces compete under regimes of limited Arc protein synthesis: implications for memory interference. Neurobiol Learn Mem 98:165–173

Matsuzaki M, Honkura N, Ellis-Davies GC, Kasai H (2004) Structural basis of long-term potentiation in single dendritic spines. Nature 429:761–766

McCurry CL, Shepherd JD, Tropea D, Wang KH, Bear MF, Sur M (2010) Loss of Arc renders the visual cortex impervious to the effects of sensory experience or deprivation. Nat Neurosci 13:450–457

Messaoudi E, Ying SW, Kanhema T, Croll SD, Bramham CR (2002) Brain-derived neurotrophic factor triggers transcription-dependent, late phase long-term potentiation in vivo. J Neurosci 22:7453–7461

Messaoudi E, Kanhema T, Soule J, Tiron A, Dagyte G, da Silva B, Bramham CR (2007) Sustained Arc/Arg3.1 synthesis controls long-term potentiation consolidation through regulation of local actin polymerization in the dentate gyrus in vivo. J Neurosci 27:10445–10455

Mikuni T, Uesaka N, Okuno H, Hirai H, Deisseroth K, Bito H, Kano M (2013) Arc/Arg3.1 is a postsynaptic mediator of activity-dependent synapse elimination in the developing cerebellum. Neuron 78:1024–1035

Moga DE, Calhoun ME, Chowdhury A, Worley P, Morrison JH, Shapiro ML (2004) Activity-regulated cytoskeletal-associated protein is localized to recently activated excitatory synapses. Neuroscience 125:7–11

Moncada D, Ballarini F, Martinez MC, Frey JU, Viola H (2011) Identification of transmitter systems and learning tag molecules involved in behavioral tagging during memory formation. Proc Natl Acad Sci U S A 108:12931–12936

Morris RG (2006) Elements of a neurobiological theory of hippocampal function: the role of synaptic plasticity, synaptic tagging and schemas. Eur J Neurosci 23:2829–2846

Morris RG, Moser EI, Riedel G, Martin SJ, Sandin J, Day M, O'Carroll C (2003) Elements of a neurobiological theory of the hippocampus: the role of activity-dependent synaptic plasticity in memory. Philos Trans R Soc London B Biol Sci 358:773–786

Nabavi S, Fox R, Proulx CD, Lin JY, Tsien RY, Malinow R (2014) Engineering a memory with LTD and LTP. Nature 511:348–352

Nader K, Hardt O (2009) A single standard for memory: the case for reconsolidation. Nat Rev 10:224–234

Nedivi E, Hevroni D, Naot D, Israeli D, Citri Y (1993) Numerous candidate plasticity-related genes revealed by differential cDNA cloning. Nature 363:718–722

Neves G, Cooke SF, Bliss TV (2008) Synaptic plasticity, memory and the hippocampus: a neural network approach to causality. Nat Rev 9:65–75

O'Leary H, Lasda E, Bayer KU (2006) CaMKIIbeta association with the actin cytoskeleton is regulated by alternative splicing. Mol Biol Cell 17:4656–4665

Okamoto K, Narayanan R, Lee SH, Murata K, Hayashi Y (2007) The role of CaMKII as an F-actin-bundling protein crucial for maintenance of dendritic spine structure. Proc Natl Acad Sci U S A 104:6418–6423

Okuno H (2011) Regulation and function of immediate-early genes in the brain: beyond neuronal activity markers. Neurosci Res 69:175–186

Okuno H, Akashi K, Ishii Y, Yagishita-Kyo N, Suzuki K, Nonaka M, Kawashima T, Fujii H, Takemoto-Kimura S, Abe M, Natsume R, Chowdhury S, Sakimura K, Worley PF, Bito H (2012) Inverse synaptic tagging of inactive synapses via dynamic interaction of Arc/Arg3.1 with CaMKIIbeta. Cell 149:886–898

Park S, Park JM, Kim S, Kim JA, Shepherd JD, Smith-Hicks CL, Chowdhury S, Kaufmann W, Kuhl D, Ryazanov AG, Huganir RL, Linden DJ, Worley PF (2008) Elongation factor 2 and fragile X mental retardation protein control the dynamic translation of Arc/Arg3.1 essential for mGluR-LTD. Neuron 59:70–83

Peebles CL, Yoo J, Thwin MT, Palop JJ, Noebels JL, Finkbeiner S (2010) Arc regulates spine morphology and maintains network stability in vivo. Proc Natl Acad Sci U S A 107: 18173–18178

Plath N, Ohana O, Dammermann B, Errington ML, Schmitz D, Gross C, Mao X, Engelsberg A, Mahlke C, Welzl H, Kobalz U, Stawrakakis A, Fernandez E, Waltereit R, Bick-Sander A, Therstappen E, Cooke SF, Blanquet V, Wurst W, Salmen B, Bosl MR, Lipp HP, Grant SG, Bliss TV, Wolfer DP, Kuhl D (2006) Arc/Arg3.1 is essential for the consolidation of synaptic plasticity and memories. Neuron 52:437–444

Ploski JE, Pierre VJ, Smucny J, Park K, Monsey MS, Overeem KA, Schafe GE (2008) The activity-regulated cytoskeletal-associated protein (Arc/Arg3.1) is required for memory consolidation of pavlovian fear conditioning in the lateral amygdala. J Neurosci 28:12383–12395

Purcell SM, Moran JL, Fromer M, Ruderfer D, Solovieff N, Roussos P, O'Dushlaine C, Chambert K, Bergen SE, Kahler A, Duncan L, Stahl E, Genovese G, Fernandez E, Collins MO, Komiyama NH, Choudhary JS, Magnusson PK, Banks E, Shakir K, Garimella K, Fennell T, DePristo M, Grant SG, Haggarty SJ, Gabriel S, Scolnick EM, Lander ES, Hultman CM, Sullivan PF, McCarroll SA, Sklar P (2014) A polygenic burden of rare disruptive mutations in schizophrenia. Nature 506:185–190

Qian Z, Gilbert ME, Colicos MA, Kandel ER, Kuhl D (1993) Tissue-plasminogen activator is induced as an immediate-early gene during seizure, kindling and long-term potentiation. Nature 361:453–457

Ramirez-Amaya V, Vazdarjanova A, Mikhael D, Rosi S, Worley PF, Barnes CA (2005) Spatial exploration-induced Arc mRNA and protein expression: evidence for selective, network-specific reactivation. J Neurosci 25:1761–1768

Redondo RL, Morris RG (2011) Making memories last: the synaptic tagging and capture hypothesis. Nat Rev 12:17–30

Rial Verde EM, Lee-Osbourne J, Worley PF, Malinow R, Cline HT (2006) Increased expression of the immediate-early gene arc/arg3.1 reduces AMPA receptor-mediated synaptic transmission. Neuron 52:461–474

Rodriguez JJ, Davies HA, Errington ML, Verkhratsky A, Bliss TV, Stewart MG (2008) ARG3.1/ARC expression in hippocampal dentate gyrus astrocytes: ultrastructural evidence and co-localization with glial fibrillary acidic protein. J Cell Mol Med 12:671–678

Rogerson T, Cai DJ, Frank A, Sano Y, Shobe J, Lopez-Aranda MF, Silva AJ (2014) Synaptic tagging during memory allocation. Nat Rev 15:157–169

Rudinskiy N, Hawkes JM, Betensky RA, Eguchi M, Yamaguchi S, Spires-Jones TL, Hyman BT (2012) Orchestrated experience-driven Arc responses are disrupted in a mouse model of Alzheimer's disease. Nat Neurosci 15:1422–1429

Sanders J, Cowansage K, Baumgartel K, Mayford M (2012) Elimination of dendritic spines with long-term memory is specific to active circuits. J Neurosci 32:12570–12578

Shen K, Teruel MN, Subramanian K, Meyer T (1998) CaMKIIbeta functions as an F-actin targeting module that localizes CaMKIIalpha/beta heterooligomers to dendritic spines. Neuron 21:593–606

Shepherd JD, Bear MF (2011) New views of Arc, a master regulator of synaptic plasticity. Nat Neurosci 14:279–284

Shepherd JD, Huganir RL (2007) The cell biology of synaptic plasticity: AMPA receptor trafficking. Annu Rev Cell Dev Biol 23:613–643

Shepherd JD, Rumbaugh G, Wu J, Chowdhury S, Plath N, Kuhl D, Huganir RL, Worley PF (2006) Arc/Arg3.1 mediates homeostatic synaptic scaling of AMPA receptors. Neuron 52:475–484

Silva AJ, Kogan JH, Frankland PW, Kida S (1998) CREB and memory. Annu Rev Neurosci 21:127–148

Smith-Hicks C, Xiao B, Deng R, Ji Y, Zhao X, Shepherd JD, Posern G, Kuhl D, Huganir RL, Ginty DD, Worley PF, Linden DJ (2010) SRF binding to SRE 6.9 in the Arc promoter is essential for LTD in cultured Purkinje cells. Nat Neurosci 13:1082–1089

Steward O, Worley PF (2001) A cellular mechanism for targeting newly synthesized mRNAs to synaptic sites on dendrites. Proc Natl Acad Sci U S A 98:7062–7068

Steward O, Wallace CS, Lyford GL, Worley PF (1998) Synaptic activation causes the mRNA for the IEG Arc to localize selectively near activated postsynaptic sites on dendrites. Neuron 21:741–751

Tagawa Y, Kanold PO, Majdan M, Shatz CJ (2005) Multiple periods of functional ocular dominance plasticity in mouse visual cortex. Nat Neurosci 8:380–388

Thiagarajan TC, Piedras-Renteria ES, Tsien RW (2002) Alpha- and betaCaMKII. Inverse regulation by neuronal activity and opposing effects on synaptic strength. Neuron 36:1103–1114

Tse D, Takeuchi T, Kakeyama M, Kajii Y, Okuno H, Tohyama C, Bito H, Morris RG (2011) Schema-dependent gene activation and memory encoding in neocortex. Science 333:891–895

Turrigiano GG (2008) The self-tuning neuron: synaptic scaling of excitatory synapses. Cell 135:422–435

Turrigiano G (2012) Homeostatic synaptic plasticity: local and global mechanisms for stabilizing neuronal function. Cold Spring Harb Perspect Biol 4:a005736

Viola H, Ballarini F, Martinez MC, Moncada D (2014) The tagging and capture hypothesis from synapse to memory. Prog Mol Biol Transl Sci 122:391–423

Wallace CS, Lyford GL, Worley PF, Steward O (1998) Differential intracellular sorting of immediate early gene mRNAs depends on signals in the mRNA sequence. J Neurosci 18:26–35

Wang KH, Majewska A, Schummers J, Farley B, Hu C, Sur M, Tonegawa S (2006) In vivo two-photon imaging reveals a role of arc in enhancing orientation specificity in visual cortex. Cell 126:389–402

Wang SH, Redondo RL, Morris RG (2010) Relevance of synaptic tagging and capture to the persistence of long-term potentiation and everyday spatial memory. Proc Natl Acad Sci U S A 107:19537–19542

Waung MW, Pfeiffer BE, Nosyreva ED, Ronesi JA, Huber KM (2008) Rapid translation of Arc/Arg3.1 selectively mediates mGluR-dependent LTD through persistent increases in AMPAR endocytosis rate. Neuron 59:84–97

Whitlock JR, Heynen AJ, Shuler MG, Bear MF (2006) Learning induces long-term potentiation in the hippocampus. Science 313:1093–1097

Wintzer ME, Boehringer R, Polygalov D, McHugh TJ (2014) The hippocampal CA2 ensemble is sensitive to contextual change. J Neurosci 34:3056–3066

Wu J, Petralia RS, Kurushima H, Patel H, Jung MY, Volk L, Chowdhury S, Shepherd JD, Dehoff M, Li Y, Kuhl D, Huganir RL, Price DL, Scannevin R, Troncoso JC, Wong PC, Worley PF (2011) Arc/Arg3.1 regulates an endosomal pathway essential for activity-dependent beta-amyloid generation. Cell 147:615–628

Ying SW, Futter M, Rosenblum K, Webber MJ, Hunt SP, Bliss TV, Bramham CR (2002) Brain-derived neurotrophic factor induces long-term potentiation in intact adult hippocampus: requirement for ERK activation coupled to CREB and upregulation of Arc synthesis. J Neurosci 22:1532–1540

# Chapter 7
# CREB-Mediated Memory Enhancement

**Anne Tanenhaus, Jiabin Zhang, and Jerry C.P. Yin**

**Abstract** CREB-responsive transcription is a conserved, important mechanism employed during memory formation. In both *Drosophila* and mice, overexpression of the activator isoform can enhance this process. However, in both systems, some conflicting data exists, providing a lingering doubt about its universality. In this review, we consider how molecular, cellular and systems parameters affect the fly dCREB2 gene during memory formation. This multi-level analysis provides plausible explanations for some of the discrepant data that exists in the fly system, and perhaps can inform mouse behavioral analysis as well.

**Keywords** CREB • Long-term memory • Memory enhancement • Intrinsic excitability • Synaptic plasticity • Drosophila • cAMP signaling • Transcription • Systems neuroscience

## 7.1 Genes and Behavior

How complex behavior is regulated in organisms is a timeless question, spanning multiple scientific fields. With the age of genetics, science gained a molecular foothold on many of the fundamental questions in biology. This led to a rapid expansion in our understanding of how individual genes can guide cellular and organism-wide processes, and ultimately, remarkably, behavior.

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The application of *Drosophila* genetics to the study of development led to the dramatic isolation and understanding of homeotic mutants (Lewis 1994). The fine structure mapping and ultimate sequencing of these mutations showed that strategically positioned, single gene mutations could have large effects on the complex program of animal development (Bender et al. 1983). This key insight encouraged the subsequent search for master regulatory genes in the development of the body plan (Nusslein-Volhard and Wieschaus 1980). Ultimately, these advances emboldened the application of this approach to more complex processes in the brain. In the early 1970s, the isolation of *period* mutations in Seymour Benzer's laboratory showed that single gene changes could in fact have dramatic effects on circadian rhythmicity, a conserved, emergent neurobiological behavior. Mutations in *period* were characterized that could abolish, lengthen or shorten the circadian cycle (Konopka and Benzer 1971). This striking discovery raised the core question: how could a single molecule guide the behavior of an entire organism? What role does a given molecule play in a cell, and how is this cellular role integrated in a complex nervous system? The past 50 years of research has confirmed that *period* is a master regulatory gene in a cell autonomous context, though how these cellular oscillations are coordinated within the many neurons that comprise the central clock, and how organismal periodicity emerges from the independent cycling of peripheral clocks located in all cells remains an active area of research.

Soon after the successes in dissecting the circadian system, the Benzer lab identified the cAMP pathway as a critical player in a simple learning and memory task, olfactory avoidance behavior. Mutations were isolated in genes that were involved in the synthesis and degradation of cAMP, *dunce/rutabaga* (Dudai et al. 1976, 1983; Davis and Kiger 1981). In parallel, discoveries pioneered in the Kandel and Schwartz labs characterized a role for the cAMP pathway in learning and memory in *Aplysia*, arguing for the universal importance of this signal transduction pathway in learning and memory formation (Castelluci et al. 1982; Bernier et al. 1982).

The cAMP signaling system is a foundational, core signal transduction pathway (Skalhegg and Tasken 2000; Sassone-Corsi 2012). The most extensive and best-characterized regulator of this pathway is the large G-protein coupled receptor family. Upon ligand binding to its cognate receptor, the GTPase exchange activity of the receptor leads to the dissociation of the heterotrimeric G-protein complex, and the free Gs or Gi subunit in turn activates or inhibits specific members of the large, adenylyl cyclase family of proteins. The subsequent increase or decrease in the rate of cAMP synthesis leads to the dissociation or (increased association) of the cAMP protein kinase (PKA) catalytic subunit. The free, dissociated PKA catalytic subunit can then phosphorylate substrate proteins, including the nuclear transcription factor CREB (or dCREB2 in flies) (Montminy 1997). Like many transcription factors, CREB proteins also interact with co-activators and co-repressors in the nucleus. CBP and CRTC (TORCS) are the two best-known co-activators that interact with all CREB proteins (Chrivia et al. 1993; Kwok et al. 1994; Altarejos and Montminy 2011). The interaction between all CREBs and CBP is phosphorylation-dependent, and involves the well-characterized S133 residue (S231 in *Drosophila*) and known

kinase pathways involving cAMP/PKA, MAPK, calcium (CaMKs) and possibly protein kinase Cs (PKCs) (Johannessen and Moens 2007). Other modifications that occur on conserved residues may contribute to altering the affinity of the CREB/CBP interaction, import/export of CREB from the nucleus, and CREB dimerization (Sun et al. 1994; Sun and Maurer 1995; Wu and McMurray 2001; Shanware et al. 2007, 2010; Liu et al. 2014). The details of the functional relationship between the CBP and CRTC (TORCS) co-activators and CREB-responsive transcription remain unclear.

Experiments using very different approaches in diverse organisms implicated CREB activity in long-term memory (LTM) formation (Dash et al. 1990; Bourtchuladze et al. 1994; Yin et al. 1994; Bartsch et al. 1995). Upon behavioral training, this family of proteins is phosphorylated on the conserved serine 133 residue, and this is a generally considered a necessary step for protein:protein interaction with CBP, downstream transcription, and memory formation (but see also Briand et al. 2015 which challenges this view, S133A mutant mice have no impairment). This mechanism is recruited broadly for many adaptive changes in neurons and different cell types, and in all complex organisms (Shaywitz and Greenberg 1999).

## 7.2 CREB and Memory Enhancement

In 1995, it was demonstrated that acute induction of a dCREB2-encoding transgene could enhance memory formation in *Drosophila* (Yin et al. 1995a, b). Flies are capable of associative learning, which is most commonly studied in the context of the aversive olfactory classical conditioning paradigm. In this behavior, a single presentation of an odor paired with a train of electric shocks will produce a robust avoidance response to that odor, which persists for hours. Repeated presentations of the shock-odor pairing can produce two genetically dissociable forms of consolidated memory. Repetitive massed training results in ARM (anesthesia resistant memory), which does not require *de novo* protein synthesis around the time of training (Tully et al. 1994; Yin et al. 1994). Repetitive spaced (distributed) training produces dCREB2-dependent LTM, which can last for at least 7 days (Tully et al. 1994; Yin et al. 1994, 1995a, b). When a heat shock-driven dCREB2 isoform (C28) was expressed (Fig. 7.1), a single training trial was shown to be sufficient to produce LTM (Yin et al. 1995a, b). This behavioral enhancement was surprising, since most neuroscientists would not have predicted that manipulating a single molecule, much less one that is present in all cells, would have such striking effects. Shortly thereafter, it was also shown in *Aplysia* that increasing the function of CREB reduced the threshold for formation of long-term facilitation (LTF) (Bartsch et al. 1995). In these experiments, inhibition of the repressor isoform CREB1 permitted formation of LTF following a single application of serotonin, which normally produces only short-term facilitation (Bartsch et al. 1995). While surprising, these findings were consistent with the emerging model of CREB recruitment as a molecular switch

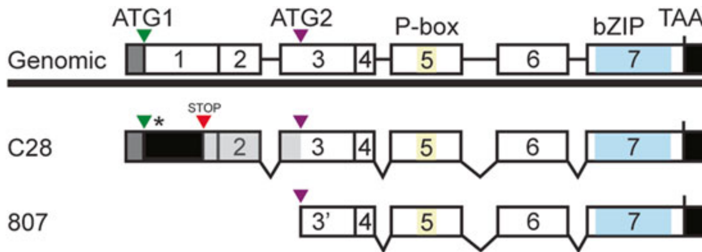


**Fig. 7.1** *Drosophila* dCREB2 genes and transgenes involved in memory enhancement. A cartoon of the dCREB2 genomic region is shown at the *top*. The coding exons are numbered, and the well-conserved P-box (which contains most of the conserved phosphorylation sites) and bZIP domains are indicated. The longest open reading frame begins with ATG1, and the first internal start codon (ATG2) and the TAA stop codon at the end of the gene are shown. The C28 transgene is shown in the *middle* cartoon, with the site of the frame shift (*asterisk*) and the resulting STOP codon indicated. ATG2 is located downstream of the STOP, and can be used for translation re-initiation, or internal initiation. 807 is the artificially truncated transgene which forces high-level initiation from ATG2

point for memory consolidation. The demonstration of synaptic tagging and capture mechanisms showed how cell-wide transcriptional activation could result in specific synaptic facilitation, and provided a viable mechanism for memory enhancement by CREB activation (Frey and Morris 1997; Martin et al. 1997; Casadio et al. 1999). Finally, CREB enhancement of memory formation was demonstrated in mammals when it was shown that viral overexpression of CREB in the rat basolateral amygdala (BLA) reduced the behavioral threshold for consolidated fear memory (Josselyn et al. 2001).

However, a number of findings emerged that complicated our understanding of gain-of-function roles of CREB in memory formation. First, the initial euphoria regarding dCREB2-mediated memory enhancement was dampened when it became apparent that behavioral enhancement in *Drosophila* was somewhat stochastic, and did not occur in every experiment. Perazzona and colleagues added to this confusion when they reported a sequence rearrangement in the C28 transgene (represented by an * in Fig. 7.1), and their inability to recapitulate behavioral enhancement (Perazzona et al. 2004). It has also become clear that CREB can play multiple roles in memory formation, regulating both synaptic and intrinsic cellular plasticity (Benito and Barco 2010), and that several of these roles may be involved in CREB-mediated memory enhancement. Finally, it is emerging both in *Drosophila* and in mammals, that the functions of CREB within systems mechanisms of memory consolidation are much more complex than previously assumed.

While some of these challenges are beginning to be resolved, gain-of function roles for CREB in memory formation largely remain poorly understood. This chapter reviews the current state of some of this research, drawing mostly from published work in rodent and *Drosophila* studies. For the most part, we treat CREB's role in both systems to be nearly identical. The work in the two systems is complementary, and results in one sometimes shed light on uncertainties in the other. There

are a number of excellent reviews that touch on CREB's role in memory enhancement, and these provide alternative perspectives (Scott et al. 2002; Barco et al. 2003; Alberini and Chen 2012; Stern and Alberini 2013; Kida and Serita 2014). Future studies will likely reveal a nuanced picture of CREB function that reflects its molecular complexity, its diversity of function at a cellular level, and its function within the intricate spatio-temporal environment of neural systems.

## 7.3 Molecular Mechanisms of CREB-Mediated Memory Formation and Enhancement

The fly dCREB2 gene undergoes alternative splicing to produce a variety of mRNA molecules (Yin et al. 1995b), but the biological significance of most of these different mRNAs is still unknown. As in all organisms, different dCREB2 products can act as transcriptional activators, or transcriptional repressors (Yin et al. 1995b; Alberini 2009). The gene also utilizes internal translation initiation to produce truncated proteins, one of which functions as an activator of CRE-mediated transcription (Tubon et al. 2013). The original sequence rearrangement in the C28 transgene (see Fig. 7.1) produced a frame shift in the longest open reading frame, thus prematurely terminating the protein at the STOP codon shown in Fig. 7.1 (Yin et al. 1995a, b; Perazzona et al. 2004). However, a downstream, in-frame methionine (ATG2) can be used in mammalian cells and flies to produce a transgenic activator protein. When translation is forced to start at ATG2, the 807 protein binds CRE sequences, up-regulates CRE-luciferase and enhances memory formation. Mutating the ATG2 codon blocks this enhancement (Tubon et al. 2013). Further analysis suggests that the endogenous *Drosophila* dCREB2 gene also uses internal translation initiation to make an activator protein (Tubon et al. 2013; Fropf et al. 2013). The use of an in-frame, internal initiation codon is not limited to the dCREB2 gene. The original knockout of the mouse CREB gene (which occurred at the N-terminal end of the longest open reading frame) resulted in up-regulation of a minor, alternatively spliced mRNA isoform, and the use of an internal, downstream, in-frame ATG codon to initiate translation (Hummler et al. 1994; Blendy et al. 1996; Lemberger et al. 2008). This compensatory molecular mechanism produced a protein with decreased specific activity that nonetheless functioned as a transcriptional activator (Bourtchuladze et al. 1994; Blendy et al. 1996; Kogan et al. 1997).

In addition to coding differences introduced through alternative splicing and internal translation initiation, the dCREB2 proteins contain all of the molecular motifs that have been more extensively characterized on the mammalian proteins. There are a large number of conserved sites for post-translational modifications: phosphorylation (up to eight different serine or threonine residues), oxidation, acetylation, SUMOylation, and O-glycosylation (Sun et al. 1994; Goren et al. 2001; Lu et al. 2003; Comerford et al. 2003; Johannessen and Moens 2007; Endo et al. 2011; Rexach et al. 2012; Chen et al. 2014). Immunocytochemistry and biochemical

analysis also demonstrates that different subpools of protein are localized in different parts of cells (Cammarota et al. 1999; Bevilaqua et al. 1999; Ryu et al. 2005; Lee et al. 2005; Kuramoto et al. 2005; Chalovich et al. 2006; Garat et al. 2006; Cox et al. 2008; Takahashi et al. 2013; Ahmed et al. 2013; Liu et al. 2014), suggesting that coding differences and post-translational modifications likely contribute to determining the complex pattern of subcellular localization. The ultimate regulation of dCREB2-dependent gene expression results from the convergence of signaling pathways acting at the transcriptional, translational, and post-translational levels to affect protein expression, activity, and subcellular localization. Several mechanisms for CREB gain-of-function have been shown to produce sub-threshold memory formation: suppression of blocker isoforms in *Aplysia* (Bartsch et al. 1995), viral-mediated overexpression of the wild type protein in flies (Yin et al. 1995a, b; Tubon et al. 2013) and in mice (Josselyn et al. 2001; Brightwell et al. 2007; Han et al. 2007), or overexpression of two different constitutively active mutant proteins in mice (Restivo et al. 2009; Suzuki et al. 2011).

## 7.4 Cellular Mechanisms of CREB-Mediated Memory Enhancement

Across many contexts, CREB mediates cell-wide adaptive responses. CREB-dependent gene expression plays a role in a plethora of cellular processes, including cell survival, proliferation, differentiation, metabolic regulation, synaptic plasticity, growth, and regulation of neural excitability (Shaywitz and Greenberg 1999; West et al. 2001; Mayr and Montminy 2001; Conkright et al. 2003; Dragunow 2004; Conkright and Montminy 2005; Wen et al. 2010; Benito and Barco 2010; Altarejos and Montminy 2011; Kandel 2012). Hundreds of genes, if not more, contain CRE elements in their promoter regions, and CREB regulates several downstream transcriptional cascades (Greenberg et al. 1992; Zhang et al. 2005; Lesiak et al. 2013; Hansen et al. 2014). Therefore, at a cellular level, the mechanism for how gain-of-function in dCREB2 activity leads to memory enhancement is not straightforward. To date, several CREB-responsive target genes have been identified that contribute to memory formation (e.g. *c-fos*, *BDNF*, *arc*; Sheng et al. 1991; Finkbeiner et al. 1997; Kawashima et al. 2009). However, the global nature of the cellular adaptations that underlie CREB-mediated memory enhancement is incompletely characterized, and likely to involve multiple cellular processes.

### *7.4.1 Synaptic Plasticity*

Prevailing models of memory formation indicate that long-term modulation of synaptic function underlies memory formation and stability. It is generally accepted that CREB is a key component in mediating the molecular stabilization of these

synaptic changes (Kandel 2001). While neurophysiological changes are difficult to measure in *Drosophila*, work in other model systems lends insight into the mechanisms of CREB-mediated memory enhancement. As with LTM, sustained long-term potentiation (L-LTP) requires protein synthesis (Frey et al. 1993; Huang and Kandel 1994). Therefore, one possibility is that supplementing CREB activity reduces the threshold for behavioral memory by bypassing the requirement for the *de novo* gene expression that supports synaptic plasticity. Elegant studies demonstrating synaptic tagging suggest that this is a viable explanation (Frey and Morris 1997). In support of this possibility, injection of phosphorylated ApCREB-1 itself is sufficient to induce LTF (Bartsch et al. 1998; Casadio et al. 1999), and blocking the repressor form of CREB (ApCREB-2) enhances LTF (Bartsch et al. 1995) in *Aplysia*. Similarly, expression of the constitutively active VP16-CREB lowers the threshold for induction of L-LTP in CA1 neurons, and bypasses the requirement for new protein synthesis in mammals (Barco et al. 2002). Likewise, LTP and L-LTP in CA1 neurons are both enhanced in transcriptionally overactive $CREB^{Y134F}$ mice, which also show enhanced performance in spatial, social, and contextual fear memory tasks (Suzuki et al. 2011). Interestingly, transcriptionally overactive CREB-DIEDML mutant mice show enhancement in both long-term memory, and in several forms of short-term memory, though the threshold and molecular mechanisms for short-term memory and LTM enhancement are dissociable (Suzuki et al. 2011). Together, these studies suggest that CREB is sufficient for the stabilization of long-term synaptic potentiation, and that these changes correlate with enhanced behavioral performance. CREB also mediates morphological synaptic changes that support memory formation. In *Aplysia*, the formation of LTF is accompanied new synaptic growth, that occurs downstream of CREB activity (Kandel 2001). Likewise, viral expression of an activated form of CREB, CREB-CA promotes the formation of silent synapses, and increases dendritic spine density in CA1 pyramidal neurons (Marie et al. 2005). In addition, CREB overexpression increases dendritic spine density in amygdala neurons (Sargin et al. 2013). These studies indicate that CREB is also sufficient to support morphological synaptic plasticity that contributes to memory formation.

Pharmacological and genetic manipulations that affect epigenetic marks can also enhance memory formation through effects on CREB and synaptic plasticity (Vecsey et al. 2007). This greatly increases the number and type of factors that can affect CREB activity since epigenetic marks are known to be points of integration for many different stimuli.

### *7.4.2 Cellular Excitability*

Recent studies in mammals have identified an additional conserved role for CREB in the regulation of intrinsic excitability, and it has been proposed that CREB-mediated modulation of excitability also contributes to memory processing (Benito and Barco 2010). In general, increasing CREB activity in neurons will increase their

tendency to fire action potentials (Dong et al. 2006; Viosca et al. 2009; Zhou et al. 2009; Lopez de Armentia et al. 2007; Jancic et al. 2009). While the details of this mechanism have not been completely characterized, this effect can be attributed to an decrease in potassium conductance as well as an increase in sodium conductance (Dong et al. 2006). Therefore, it is also possible that gain-of-function in CREB activity leads to a state in which long-term memory is able to form by increasing excitability, thereby lowering the behavioral input threshold for cellular consolidation. In the lateral amygdala, overexpression of CREB in certain cells, which results in increased excitability in those neurons, favors the recruitment of those cells into new fear memory traces, and enhances memory (Han et al. 2007; Zhou et al. 2009). Furthermore, increasing neuronal excitability in a random subset of neurons in the amygdala either by expression of dominant negative potassium channels, through the activation of DREADD receptors, or by optogenetic stimulation will also favor the recruitment of these cells to memory traces. Importantly, any of these stimulations (increasing excitability, increasing CREB activity) delivered immediately before training will enhance memory formation (Yiu et al. 2014). This is not due to increased participation of cells in the memory trace, as the overall number of cells that are labeled during recall is not changed (Han et al. 2007; Yiu et al. 2014). Therefore, the memory enhancement appears to be due to intracellular effects of elevated excitability. Together, these studies show that elevated intrinsic excitability is sufficient to enhance memory formation, and therefore provide an additional mechanism for CREB-mediated memory enhancement.

Currently, it is possible that either or both of these mechanisms contribute to CREB-mediated memory enhancement. Further characterization of CREB target genes, and which of these genes are responsible for memory enhancement will shed light on these issues. However, it seems most likely that changes in both synaptic and intrinsic excitability together contribute to an overall ***cellular state*** in which the threshold for synaptic consolidation is lowered. Therefore, regardless of the exact mechanism for enhancement, it is useful to conceptualize CREB activation as producing a general permissive state that allows for long-term memory formation.

## 7.5 Systems Influences on CREB-Mediated Memory Enhancement

Besides for molecular and cellular parameters that can influence memory enhancement, there are systems factors that can contribute to variability in behavior. In particular, these systems level parameters can affect the pre-training state of the fly, thereby contributing to whether memory enhancement occurs, or not. Given the role of CREB in determining neuronal excitability, it seems likely that these systems parameters work at least partially through effects on neuronal excitability, thus affecting the “starting state” of the animal.

### 7.5.1 Metabolic State

Recent work in flies has demonstrated that the metabolic state of the flies prior to training can contribute to memory enhancement. CRTC (TORCs) is one of the two co-activators that are known to interact with CREB family members. Mild fasting enhances memory formation in a CRTC- and dCREB2-dependent manner (Hirano et al. 2013). These experiments demonstrate that the pre-training state (fasting versus sated) of the fly influences the ability to enhance memory formation, and that the recruitment of dCREB2 and CRTC are necessary for this process. In the resting (sated) state, CRTC resides in the cytoplasm under the control of phosphorylation-mediated tethering. When cAMP/calcium increases, and during mild starvation, phosphatase activity removes key phosphorylation events, and the protein enters the nucleus (Altarejos and Montminy 2011). In the nucleus, it interacts with dCREB2 to enhance memory formation. In rodents, caloric restriction is generally thought of as a process that can delay the effects of aging and neurodegeneration, and perhaps even enhance cognition. There is at least one report of a role for CREB in this protective pathway, suggesting that it can function in the brain as a metabolic and nutrient sensor (Fusco et al. 2012).

Other metabolic factors can enhance hippocampal-dependent memory formation in mammals. Early reports showed that glucose injected into the brain could enhance memory formation (Korol and Gold 1998; Salinas and Gold 2005), and more recent ones document the effects of insulin-like growth factor II (Chen et al. 2011; Stern et al. 2014). While detailed mechanistic information remains under investigation, it is interesting that CRTC has been shown to act as an enhancing protein when augmented in the dentate gyrus (Sekeres et al. 2012). These independent avenues of research show that the metabolic state at the time of training is an important part of mammalian memory formation, although their direct connection with CREB remains unclear, and their involvement in flies is untested.

Another parameter (which may be related to metabolism) that can enhance memory formation in mammals is physical exercise. Over the years, there is a substantial literature demonstrating a CREB-dependent enhancing effect of exercise on memory formation (Shen et al. 2001; Vaynman et al. 2004; Yang et al. 2014). The molecular and cellular mechanisms that are recruited remain under active investigation, but could include general effects on adult neurogenesis. Environmental enrichment is another systems level parameter that can enhance memory formation, at least partially through a CREB-dependent manner (Williams et al. 2001; Huang et al. 2006; Correa et al. 2012). Regardless of the similarities or differences in the mechanisms that are recruited, this work on metabolism, exercise, and environmental enrichment demonstrates that systems parameters can affect memory enhancement, and that this enhancement can involve CREB.

### 7.5.2 Temporal Factors

It has been shown in a variety of organisms that the time-of-day affects learning and/or memory formation (Lyons et al. 2006; Ruby et al. 2008; Eckel-Mahan et al. 2008; Gerstner et al. 2009; Fropf et al. 2014). Under normal conditions, endogenous dCREB2 activity, protein subcellular localization, and abundance of different protein isoforms varies over the diurnal cycle (Belvin et al. 1999; Tanenhaus et al. 2012; Fropf et al. 2014). Most prominent is the oscillation in dCREB2 activity, as measured in transgenic flies where luciferase expression is placed under the control of reiterated cyclic AMP-Response Element (CRE) DNA sequences (Belvin et al. 1999). In live flies, reporter activity oscillates under circadian control, peaking once in the middle of the daytime (ZT=6), and again in the middle of the nighttime (ZT=18) (Fig. 7.2). Originally it could not be determined if oscillations occurred broadly, in many tissues, or if it was limited to certain tissue. Using a new spatially restricted version of the reporter, it was discovered that this oscillatory pattern in CRE-luciferase activity appears to occur in most, if not all, cells in the nervous system (Tanenhaus et al. 2012). This oscillatory pattern includes the regions of the brain that are likely to be involved in memory formation (Tanenhaus et al. 2012; Zhang et al. 2015), suggesting a possible mechanism for the effect of training time on LTM formation. Based on the oscillatory nature of the reporter transgene, the simplest prediction would be that performance would be best when animals are trained around the peaks in reporter activity, and worst when trained during the troughs. When tested, the time-of-day when behavioral training occurs does affect

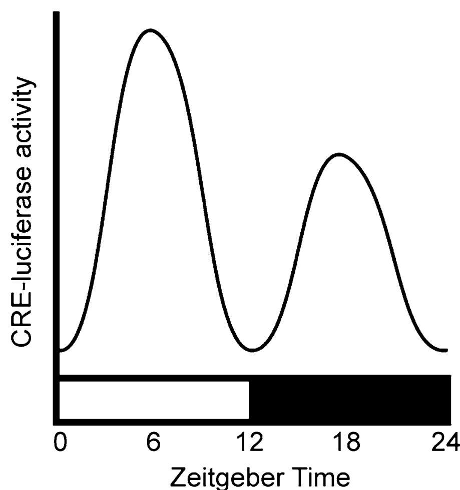


**Fig. 7.2** dCREB2- and CRE-dependent reporter activity oscillates. Both ubiquitously expressed CRE-luciferase (Belvin et al. 1999) and spatially restricted (Tanenhaus et al. 2012) CRE-luciferase transgenic reporter flies show oscillations in reporter activity. Flies entrained to a 12-h lights on 12-h lights off schedule are measured hourly for luciferase activity in live behaving flies. Most, if not all cells exhibit this pattern of activity across the daytime (*white bar*) and nighttime (*black bar*)

24-h memory. The nighttime peak and trough in behavioral performance match very well the molecular oscillations that can be detected using western blotting, and the general pattern of dCREB2 activity (Fropf et al. 2014). However, the daytime peak in dCREB2 activity does not result in an increase in memory formation.

Interestingly, there is also an interaction between time-of-day and dCREB2-mediated enhancement of olfactory memory. When the dCREB2-activator (807 see Fig. 7.1) is expressed under the control of a heat-shock promoter and induced immediately before training, a sub-threshold training condition will produce robust LTM when animals are trained (and tested) at ZT = 11 (late-day), but not when trained at ZT = 6 (Tubon et al. 2013; see Fig. 7.3). Surprisingly, behavioral enhancement correlates best with a trough in endogenous dCREB2 activity, rather than a peak. Although the data do not all line up perfectly with our simplest of expectations, it is clear that the time-of-day when training occurs has marked effects on memory formation, and is a factor in determining the probability of dCREB2-mediated memory enhancement.

### 7.5.3 Anatomical Factors

One of the major differences between CREB-related behavioral experiments in rodents and flies is the anatomical specificity of the manipulations. In rodents, the use of forebrain-specific promoters to drive inducible transgenes, or site-directed

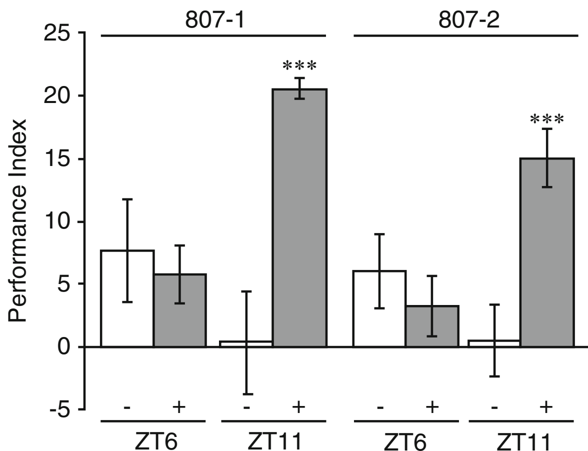


**Fig. 7.3** The time of day affects memory enhancement. Two different lines of flies containing the 807 transgene (807-1 and 807-2) were tested for their ability to enhance memory formation. Transgenic flies kept under circadian entrainment were either heat-shock induced (+) or not (−), at the indicated zeitgeber times, allowed to recover for 90 min, and then trained with a single training trial. All flies were assayed for 24-h memory. The Performance Index (corrects − incorrects/total # of flies × 100) is plotted as a function of treatment and training time. ***p < 0.01, *T*-test, N = 8

cannulation and viral injections, has limited the areas of the brain where CREB activity is altered to defined regions. In particular, the hippocampus and the amygdala have been the most intensively targeted and studied areas. In contrast, the original fly enhancement experiments utilized the heat-shock promoter, which is ubiquitously expressed. Surprisingly, though the requirement for dCREB2 in LTM formation is firmly accepted, and the neural circuitry of olfactory memory formation is understood in some detail, it is still unclear where dCREB2 is required in olfactory LTM.

The areas of the brain that have been implicated in LTM are shown in cartoon form in Fig. 7.4. NMDA receptor activity is required in the R2/R4m neurons of the ellipsoid body for LTM formation (Wu et al. 2007). These neurons form synapses in characteristic "ring" structures shown as green and purple concentric circles. The dorso-anterior-lateral (DAL) neurons (shown in red) are a pair of cells that innervate the mushroom body. Detailed analysis shows that dCREB2-dependent, *de novo* protein synthesis is needed in these cells for LTM formation (Chen et al. 2012). However, the major area of the brain that is involved in the association of odor and shock is the mushroom body, and most researchers believe that long-term memory will at least partially reside in a sparse circuit in this structure. The three major axonal projections from the mushroom body neurons are shown in blue (the αβ lobe), brown (the α′β′ lobe), or yellow (the γ lobe). The calyx region, which contains the dendrites of these neurons, is partially obscured by the cell bodies. In spite of the simpler brain anatomy, the existing data suggests that systems consolidation, or reorganization of the learned information, does occur during *Drosophila* memory formation (Dubnau and Chiang 2013).

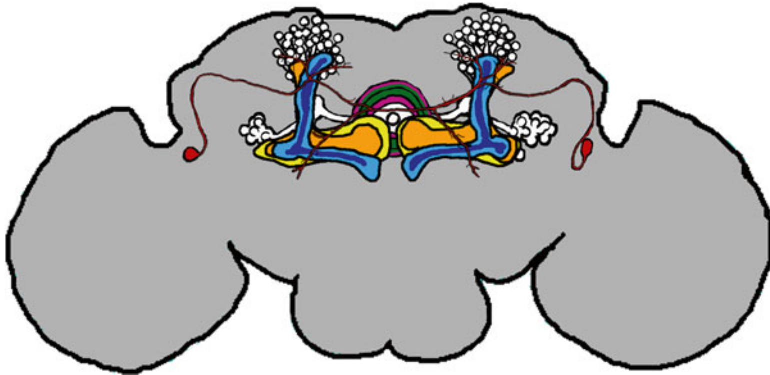

**Fig. 7.4** Relevant brain anatomy. A cartoon frontal view of the adult head and brain of the fruit fly shows some of the anatomical regions involved in memory formation. All cell bodies are shown as *white circles*. The neuropil region of the ellipsoid body neurons is shown as a series of concentric rings. The R2/R4m neurons form synapses in the *green* and *purple rings*. The bilaterally symmetrical mushroom bodies receive synaptic information in the calyx region, which is partially obscured. These neurons project their axons through the characteristic lobes, which are shown using different colors. The αβ lobes are shown in *blue* and *dark blue*, the α′β′ lobes in *brown*, and the γ lobes in *yellow* (Color figure online)

Recent experiments that ask about the requirement for dCREB2 activity in the mushroom body during memory formation have produced discrepant results. When a dCREB2 blocker transgene is induced throughout the mushroom body, there is either no effect, or disruption, of 24-h memory (Chen et al. 2012; Hirano et al. 2013). One possible difference between the two experiments is the gene dosage of the dCREB2 blocker transgene (Hirano et al. 2013). However, data gathered using the new, spatially restricted transgenic CRE-reporter fly suggests another possible resolution to the differing results (Zhang et al. 2015). When this reporter is used to survey dCREB2 activity in different brain regions during memory consolidation, a complex pattern of changes occurs. These experiments utilize two groups of flies, ones that receive repetitive, forward-paired, spaced training and genetically identical ones that receive repetitive, backwards-paired, spaced training. Only flies that receive the forward-paired training produce long-term memory. Across different experiments, reporter activity can be measured in different anatomical regions. Interestingly, in different lobes of the mushroom bodies, and even sub-lobes within one region, there are different patterns in the response of the reporter to memory formation (Zhang et al. 2015). In certain sub-regions of the αβ lobes (those driven by the c772/c747 drivers), there is an ***increase*** in dCREB2 activity immediately after the end of training (relative to the control, backwards-paired group), but the differences disappear about 12 h after the end of training (N. B. In the tissues which have been tested, both forward- and backwards-paired groups show increases in activity over an untrained control group [J. Zhang, personal communication]). In other regions of the αβ lobes (driven by the c739 driver), and in the γ lobe (driven by 1471), reporter activity is substantially ***suppressed*** for about two days in the memory group, before increases emerge in the forward-pairing group in the third and fourth days (Zhang et al. 2015). In the α′β′ region, there are no changes between the two groups. These bidirectional changes in dCREB2 activity in different regions of the mushroom body lobes could contribute to the experimental differences previously described (Chen et al. 2012; Hirano et al. 2013). When the blocker is overexpressed in all regions of the mushroom body, it would be predicted to have opposite effects in different regions. If the net effects are equivalent, they would cancel each other out, and there would be no effect on LTM formation (Chen et al. 2012). If, however, the effect in the c772/c747 region is more dominant, then the blocker would be predicted to impair LTM (Hirano et al. 2013). The "state" of the fly at the time of training might have been different in the two experiments, and tipped the balance one way or the other.

Outside of the mushroom body, dCREB2 activity is increased in the R2/R4m neurons and remains elevated for several days (Zhang et al. 2015). Therefore, in the fly brain, dCREB2 activity increases and decreases in different brain regions after training, depending upon the area, and the time point that is measured after the end of training. Notably, these changes occur over a relatively long timescale (hours to days), distinct from acute CREB recruitment, which is measured on the scale of minutes (Fropf et al. 2013). Based on the mammalian work on CREB and intrinsic excitability (see above), an appealing interpretation is that these activity curves represent the excitability state of different populations of neurons. Figure 7.5 presents

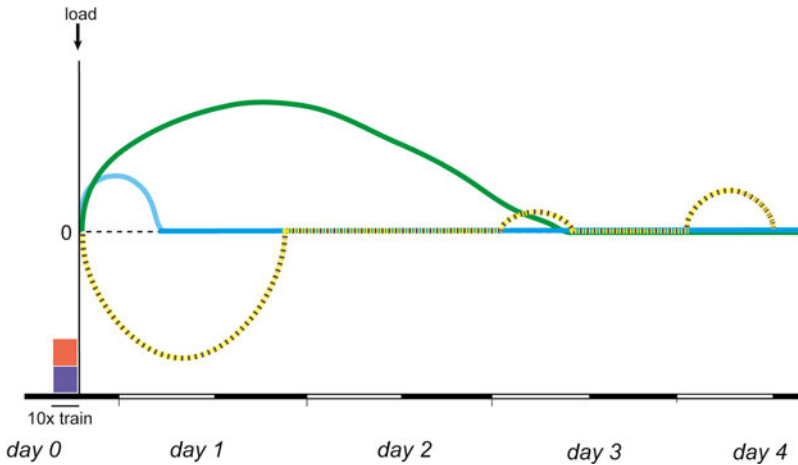


**Fig. 7.5** Cartoon summary of reporter activity in certain brain regions during consolidation. The change in reporter activity ([forward-paired] – [backwards-paired]/backwards-paired) is plotted as a function of time after the end of training for three different regions of the brain. This change in the c747/c772-driven parts of the αβ lobes of the mushroom body is shown in *blue*. The change in the c739-driven αβ sublobe neurons of the mushroom body is shown in *yellow/black*. The change in the R2/R4m neurons of the ellipsoid body is shown in *green*. Changes are bidirectional, and occur over a long time frame. For all groups, the changes correlate not only with forward pairing, but also with the requirement for rest intervals (Zhang et al. 2015) (Color figure online)

a cartoon summary of the changes in reporter activity (presumably excitability) in the forward-paired flies relative to the backwards-paired flies for each different region. The three different patterns of change during memory consolidation suggest that ubiquitous manipulations of gain- or loss-of-function in dCREB2 activity may have counteractive effects in different brain regions, making it hard to predict their effects on memory formation. Although the changes are complex, they are strikingly similar to previous functional imaging data describing associative changes in calcium responsiveness upon re-presentation of a shock-paired odor. The different lobes of the mushroom body showed differences in their onset kinetics and duration of the increased calcium response (Yu et al. 2006; Akalal et al. 2010, 2011). The αβ and γ lobes exhibited long-lasting changes, with γ lobe signals persisting out to 4 days after pairing, and αβ lobe signals correlating with mutations that affect LTM formation.

Importantly, this reporter data also suggests that the process of systems consolidation requires 3–4 days before it is complete. In the field, the convention of using 24-h memory as an indicator of long-term memory is not likely to provide a complete picture of what is happening at the systems level. Together, these experiments suggest that dCREB2 may play multiple roles in memory formation, and that multiple constraints are likely to shape the eventual set point for memory formation.

## 7.6 Summary: The Variability in Memory Enhancement

The interconnectivity of the molecular, cellular and systems aspects of dCREB2 activity reveal many different reasons why memory enhancement can be inconsistent. The complexity of the dCREB2 gene, and the redundancy in the mammalian CREB superfamily (consisting of the CREB, CREM and ATF-1 genes), clearly show that compensatory molecular mechanisms exist, and are deployed, to contribute to a "molecular homeostasis" in CREB-mediated transcription. This molecular flexibility likely means that dCREB2-responsive transcription never deviates too far from a baseline state, and that any transgenic molecular perturbation will result in pushback at the molecular level through the endogenous gene(s).

It is currently believed that CREB and dCREB2 can contribute to two different cellular mechanisms involved in memory formation: synaptic plasticity and intrinsic excitability (which likely shapes memory allocation). When considering the inconsistency in behavioral enhancement, intrinsic excitability is a likely choice for a source of experimental variability. The pre-training behavioral state of the fly can affect baseline excitability, and this could either increase or decrease the range over which transgenic manipulations can have their effects. Since memory formation in flies has a ceiling, it seems likely that the greatest enhancement will occur when the excitable state of the fly (at the time of training) is at a minimum.

dCREB2-dependent memory enhancement occurs sporadically, and is an all-or-nothing phenomenon. Overall, there appear to be a set of conditional states that can affect excitability and together determine whether enhancement occurs. These include pre-training parameters that are usually not well controlled in fly behavioral experiments, such as metabolic state, satiety and exercise (locomotor activity), all of which are generally assumed to be equivalent when different groups of flies are trained. The time-of-day when training occurs, or transgene induction happens, also affects memory formation, and can determine whether behavioral enhancement occurs or not. Finally, the complex anatomical pattern of dCREB2 activation during memory processing likely contributes to variable responses in different brain regions to ubiquitous dCREB2 transgene induction. Since these responses can be opposite in directionality, the pre-training factors could bias competition between different sub-regions, thus affecting whether enhancement occurs or not. In addition to these factors, it is likely that many of the conditional states that restrict or permit memory enhancement are still unidentified. Further characterization of these states will provide significant insight into the phenomenon of memory enhancement, and into the gating of memory formation in general.

Although CREB-mediated memory enhancement in the mouse system is less controversial than in flies, the earlier data demonstrating the necessity of CREB activity in memory formation (using loss-of-function approaches) had its share of disagreement (Gass et al. 1998; Balschun et al. 2003). While it is always possible that the details of lab-to-lab genetic backgrounds or behavioral protocols differ in subtle but important ways, it seems equally possible that some of the systems-level issues that affect fly memory enhancement could affect the experiments testing

CREB's role in memory formation. Since the loss-of-function genetic manipulations involved gene knock-outs, the pressure for genetic compensation (to allow for development) increases, and modulatory factors (like the systems-level parameters mentioned previously) become more critical. Because this signaling pathway is positioned to respond to molecular, cellular and systems factors, compensation at one or more of these levels can contribute to unexpected results.

## 7.7 Genes, Neurons and Circuits

Since the initial discoveries in *Drosophila* and *Aplysia* that individual genes could function as regulators of complex behavior, there has been a fundamental shift in the way that genes are viewed. This shift has bridged the fields of molecular genetics and behavioral neuroscience, and has uncovered the challenge of how specific molecular events fit into larger neural systems. Among these first behavior genes, *CREB* and *period* stand out as ancient core transcriptional regulators whose behavioral function is conserved in the most basic and in the most complicated nervous systems. *Period* is now understood to function cell autonomously (to set cellular transcription rhythms), within and between the central clock cells (to set a central rhythm), and in peripheral clocks to synchronize central and peripheral rhythms. This work serves as a case study for how a cell autonomous molecular role can be coherently integrated within a whole organism. Like Period, the cAMP/PKA/CREB signaling pathway functions within cells, responds to changes between cells, and is involved with altering the state of the organism in response to systems-level changes. However, how this transduction system affects events throughout these levels, and how this information is ultimately integrated into a functional system is yet to be fully understood. The phenomenon of CREB-mediated memory enhancement is particularly remarkable, given the diverse cellular and systems-level functions that contribute to memory formation itself. That CREB itself *can* enhance memory formation, and that this capability is conserved across species suggests a certain inherent unity in its role in memory formation. Nonetheless, the picture that is emerging suggests that CREB is a multi-functional molecule, with varied inputs, outputs, and roles among different neurons. Future research promises to reveal a set of processes as intricate, but also as congruent as all biological systems.

## References

Ahmed BY, Husnain O, Stafford R, Howard M, Gujar AS, Moradiya V, Patel KK, Sihotra S (2013) Hyperphosphorylation of CREB in human dopaminergic neurons: a kinetic study of cellular distribution of total CREB and phosphor-CREB following oxidative stress. NeuroReport 24:757–762

Akalal DB, Yu D, Davis RL (2010) A late-phase, long-term memory trace forms in the γ neurons of Drosophila mushroom bodies after olfactory classical conditioning. J Neurosci 30: 16699–16708

Akalal DB, Yu D, Davis RL (2011) The long-term memory trace formed in the Drosophila α/β mushroom body neurons is abolished in long-term memory mutants. J Neurosci 31: 5643–5647
Alberini CM (2009) Transcription factors in long-term memory and synaptic plasticity. Physiol Rev 89:121–145
Alberini CM, Chen DY (2012) Memory enhancement: consolidation, reconsolidation and insulin-like growth factor 2. Trends Neurosci 35:274–283
Altarejos JY, Montminy M (2011) CREB and the CRTC co-activators: sensors for hormonal and metabolic signals. Nat Rev Mol Cell Biol 12:141–151
Balschun D, Wolfer DP, Gass P, Mantamadiotis T, Wetzl H, Schutz G, Frey JU, Lipp HP (2003) Does cAMP response element-binding protein have a pivotal role in hippocampal synaptic plasticity and hippocampus-dependent memory? J Neurosci 23:6304–6314
Barco A, Alarcon JM, Kandel ER (2002) Expression of constitutively active CREB protein facilitates the late phase of long-term potentiation by enhancing synaptic capture. Cell 108: 689–703
Barco A, Pittenger C, Kandel ER (2003) CREB, memory enhancement and the treatment of memory disorders: promises, pitfalls and prospects. Expert Opin Ther Targets 7:101–114
Bartsch D, Ghirardi M, Skehel PA, Karl KA, Herder SP, Chen M, Bailey CH, Kandel ER (1995) *Aplysia* CREB2 represses long-term facilitation; relief of repression converts transient facilitation into long-term functional and structural change. Cell 83:979–992
Bartsch D, Casadio A, Karl KA, Serodio P, Kandel ER (1998) CREB1 encodes a nuclear activator, a repressor, and a cytoplasmic modulator that form a regulatory unit critical for long-term facilitation. Cell 95:211–223
Belvin MP, Zhou H, Yin JC (1999) The *Drosophila* dCREB2 gene affects the circadian clock. Neuron 22:777–787
Bender W, Akam M, Karch F, Beachy PA, Peifer M, Spierer P, Lewis EB, Hogness DS (1983) Molecular genetics of the bithorax complex in *Drosophila melanogaster*. Science 221:23–29
Benito E, Barco A (2010) CREB's control of intrinsic and synaptic plasticity: implications for CREB-dependent memory models. Trends Neurosci 33:230–240
Bernier L, Castellucci VF, Kandel ER, Schwartz JH (1982) Facilitatory transmitter causes a selective and prolonged increase in adenosine 3′, 5′-monophosphate in sensory neurons mediating the gill and siphon withdrawal reflex in *Aplysia*. J Neurosci 2:1682–1691
Bevilaqua LR, Cammarota M, Paratcha G, de Stein ML, Izquierdo I, Medina JH (1999) Experience-dependent increase in cAMP-responsive element binding protein in synaptic and nonsynaptic mitochondria of the rat hippocampus. Eur J Neurosci 11:3753–3756
Blendy JA, Kaestner KH, Schmid W, Gass P, Schutz G (1996) Targeting of the CREB gene leads to up-regulation of a novel CREB mRNA isoform. EMBO J 15:1098–1106
Bourtchuladze R, Frenguelli B, Blendy J, Cioffi D, Schutz G, Silva AJ (1994) Deficient long-term memory in mice with a targeted mutation of the cAMP-responsive element-binding protein. Cell 79:59–68
Briand LA, Lee BG, Lelay J, Kaestner KH, Blendy JA (2015) Serine 133 phosphorylation is not required for hippocampal CREB-mediated transcription and behavior. Learn Mem 22: 109–115
Brightwell JJ, Smith CA, Neve RL, Colombo PJ (2007) Long-term memory for place learning is facilitated by expression of cAMP response element-binding protein in the dorsal hippocampus. Learn Mem 14:195–199
Cammarota M, Paratcha G, Bevilaqua LR, Levi de Stein M, Lopez M, Pellegrino de Iraldi A, Izquierdo I, Medina JH (1999) Cyclic AM-responsive element binding protein in brain mitochondria. J Neurochem 72:2272–2277
Casadio A, Martin KC, Giustetto M, Zhu H, Chen M, Bailey CH, Kandel ER (1999) A transient, neuron-wide form of CREB-mediated long-term facilitation can be stabilized at specific synapses by local protein synthesis. Cell 99:221–237
Castelluci VF, Nairn A, Greengard P, Schwartz JH, Kandel ER (1982) Inhibitor of adenosine 3′,5′-monophosphate-dependent protein kinase blocks presynaptic facilitation in Alysia. J Neurosci 2:1673–1681

Chalovich EM, Zhu J, Caltagarone J, Bowser R, Chu CT (2006) Functional repression of cAMP response element in 6-hydroxydopamine-treated neuronal cells. J Biol Chem 281: 17870–17881

Chen DY, Stern SA, Garcia-Osta A, Saunier-Rebori B, Pollonini G, Bambah-Mukku D, Blitzer RD, Alberini CM (2011) A critical role for IGF-II in memory consolidation and enhancement. Nature 469:491–497

Chen CC, Wu JK, Lin HW, Pai TP, Fu TF, Wu CL, Tully T, Chiang AS (2012) Visualizing long-term memory formation in two neurons of the *Drosophila* brain. Science 335:678–685

Chen YC, Hsu WL, Ma YL, Tai DJ, Lee EH (2014) CREB SUMOylation by the E3 ligase PIAS1 enhances spatial memory. J Neurosci 34:9574–9589

Chrivia JC, Kwok RP, Lamb N, Hagiwara M, Montminy MR, Goodman RH (1993) Phosphorylated CREB binds specifically to the nuclear protein CBP. Nature 365:855–859

Comerford KM, Leonard MO, Karhausen J, Carey R, Colgan SP, Taylor CT (2003) Small ubiquitin-related modifier-1 modification mediates resolution of CREB-dependent responses to hypoxia. Proc Natl Acad Sci U S A 100:986–991

Conkright MD, Montminy M (2005) CREB: the unindicted cancer co-conspirator. Trends Cell Biol 15:457–459

Conkright MD, Guzman E, Flechner L, Su AI, Hogenesch JB, Montminy M (2003) Genome-wide analysis of CREB target genes reveals a core promoter requirement for cAMP responsiveness. Mol Cell 11:1101–1108

Correa SA, Hunter CJ, Palygin O, Wauters SC, Martin KJ, McKenzie C, McKelvey K, Morris RG, Pankratov Y, Arthur JS, Frenguelli BG (2012) MSK1 regulates homeostatic and experience-dependent synaptic plasticity. J Neurosci 32:13039–13051

Cox LJ, Hengst U, Gurskaya NG, Lukyanov KA, Jaffrey SR (2008) Intra-axonal translation and retrograde trafficking of CREB promotes neuronal survival. Nat Cell Biol 10:149–159

Dash PK, Hochner B, Kandel ER (1990) Injection of the cAMP-responsive element into the nucleus of *Aplysia* sensory neurons blocks long-term facilitation. Nature 345:718–721

Davis RL, Kiger JA Jr (1981) *Dunce* mutants of *Drosophila melanogaster*: mutants defective in the cyclic AMP phosphodiesterase enzyme system. J Cell Biol 90:101–107

Dong Y, Green T, Saal D, Marie H, Neve R, Nestler EJ, Malenka RC (2006) CREB modulates excitability of nucleus accumbens neurons. Nat Neurosci 9:475–477

Dragunow M (2004) CREB and neurodegeneration. Front Biosci 9:100–103

Dubnau J, Chiang A-S (2013) Systems memory consolidation in *Drosophila*. Curr Opin Neurobiol 23:84–91

Dudai Y, Jan YN, Byers D, Quinn WG, Benzer S (1976) *Dunce*, a mutant of *Drosophila* deficient in learning. Proc Natl Acad Sci U S A 73:1694–1698

Dudai Y, Uzzan A, Benzer S (1983) Abnormal activity of adenylate cyclase in the *Drosophila* memory mutant *rutabaga*. Neurosci Lett 42:207–212

Eckel-Mahan KL, Phan T, Han S, Wang H, Chan GC, Scheiner ZS, Storm DR (2008) Circadian oscillation of hippocampal MAPK activity and cAMP: implications for memory persistence. Nat Neurosci 11:1074–1082

Endo A, Sumi D, Iwamoto N, Kumagai Y (2011) Inhibition of DNA binding activity of cAMP response element-binding protein by 1,2-naphthoquinone through chemical modification of cys-286. Chem Biol Interact 192:272–277

Finkbeiner S, Tavazoie SF, Maloratsky A, Jacobs KM, Harris KM, Greenberg ME (1997) CREB: a major mediator of neuronal neurotrophin responses. Neuron 19:1031–1047

Frey U, Morris RG (1997) Synaptic tagging and long-term potentiation. Nature 385:533–536

Frey U, Huang YY, Kandel ER (1993) Effects of cAMP simulate a late stage of LTP in hippocampal CA1 neurons. Science 260:1661–1664

Fropf R, Tubon TC Jr, Yin JC (2013) Nuclear gating of a *Drosophila dCREB2* activator is involved in memory formation. Neurobiol Learn Mem 106:258–267

Fropf R, Zhang J, Tanenhaus AK, Fropf WJ, Siefkes E, Yin JC (2014) Time of day influences memory formation and dCREB2 proteins in *Drosophila*. Front Syst Neurosci 8:43

Fusco S, Ripoli C, Podda MV, Ranieri SC, Leone L, Toietta G, McBurney MW, Schutz G, Riccio A, Grassi C, Galeotti T, Pani G (2012) A role for neuronal cAMP responsive-element binding (CREB)-1 in brain responses to calorie restriction. Proc Natl Acad Sci U S A 109:621–626

Garat CV, Fankell D, Irickson PF, Reusch JEB, Bauer NN, McMurtry IF, Klemm DJ (2006) Platelet-derived growth factor BB induces nuclear export and proteosomal degradation of CREB via phosphatidylinositol 3-kinase/Akt signaling in pulmonary artery smooth muscle cells. Mol Cell Biol 26:4934–4938

Gass P, Wolfer DP, Balschun D, Rudolph D, Frey U, Lipp HP, Schutz G (1998) Deficits in memory tasks of mice with CREB mutations depend on gene dosage. Learn Mem 5:274–288

Gerstner JR, Lyons LC, Wright KP Jr, Loh DH, Rawashdeh O, Eckel-Mahan KL, Roman GW (2009) Cycling behavior and memory formation. J Neurosci 29:12824–12830

Goren I, Tavor E, Goldblum A, Honigman A (2001) Two cysteine residues in the DNA-binding domain of CREB control binding to CRE and CREB-mediated gene expression. J Mol Biol 313:695–709

Greenberg ME, Thompson MA, Sheng M (1992) Calcium regulation of immediate early gene transcription. J Physiol Paris 86:99–108

Han JH, Kushner SA, Yiu AP, Cole CJ, Matynia A, Brown RA, Neve RL, Guzowski JF, Silva AJ, Josselyn SA (2007) Neuronal competition and selection during memory formation. Science 316:457–460

Hansen KF, Sakamoto K, Pelz C, Impey S, Obrietan K (2014) Profiling status epilepticus-induced changes in hippocampal RNA expression using high-throughput RNA sequencing. Sci Rep 4:6930

Hirano Y, Masuda T, Naganos S, Matsuno M, Ueno K, Miyashita T, Horiuchi J, Saitoe M (2013) Fasting launches CRTC to facilitate long-term memory formation in *Drosophila*. Science 339:443–446

Huang YY, Kandel ER (1994) Recruitment of a long-lasting and protein kinase A-dependent long-term potentiation in the CA1 region of hippocampus requires repeated tetanization. Learn Mem 1:74–82

Huang FL, Huang KP, Wu J, Boucheron C (2006) Environmental enrichment enhances neurogranin expression and hippocampal learning and memory but fails to rescue the impairments of neurogranin null mutant mice. J Neurosci 26:6230–6237

Hummler E, Cole TJ, Blendy JA, Ganss R, Aguzzi A, Schmid W, Beermann F, Schutz G (1994) Targeted mutation of the CREB gene: compensation within the CREB/ATF family of transcription factors. Proc Natl Acad Sci U S A 91:5647–5651

Jancic D, Lopez de Armentia M, Valor LM, Olivares R, Barco A (2009) Inhibition of cAMP response element-binding protein reduces neuronal excitability and plasticity, and triggers neurodegeneration. Cereb Cortex 19:2535–2547

Johannessen M, Moens U (2007) Multisite phosphorylation of the cAMP response element-binding protein (CREB) by a diversity of protein kinases. Front Biosci 12:1814–1832

Josselyn SA, Shi C, Carlezon WA Jr, Neve RL, Nestler EJ, Davis M (2001) Long-term memory is facilitated by cAMP response element-binding protein overexpression in the amygdala. J Neurosci 21:2404–2412

Kandel E (2001) The molecular biology of memory storage: a dialogue between genes and synapses. Science 294:1030–1038

Kandel E (2012) The molecular biology of memory: cAMP, PKA, CRE, CREB-1, CREB-2 and CPEB. Mol Brain 5:14

Kawashima T, Okuno H, Nonaka M, Adachi-Morishima A, Kyo N, Okamura M, Takemoto-Kimura S, Worley PF, Bito H (2009) Synaptic activity-responsive element in the *Arc/Arg3.1* promoter essential for synapse-to-nucleus signaling in activated neurons. Proc Natl Acad Sci U S A 106:316–321

Kida S, Serita T (2014) Functional roles of CREB as a positive regulator in the formation and enhancement of memory. Brain Res Bull 105:17–24

Kogan JH, Frankland PW, Blendy JA, Coblentz J, Marowitz Z, Schutz G, Silva AJ (1997) Spaced training induces normal long-term memory in CREB mutant mice. Curr Biol 7:1–11

Konopka RJ, Benzer S (1971) *Clock* mutants of *Drosophila melanogaster*. Proc Natl Acad Sci U S A 69:2332–2336

Korol DL, Gold PE (1998) Glucose, memory, and aging. Am J Clin Nutr 67:764S–771S

Kuramoto N, Kubo K, Ogita K, Platenik J, Balcar VJ, Takarada T, Nakamichi N, Yoneda Y (2005) Nuclear condensation of cyclic adenosine monophosphate responsive element-binding protein in discrete murine brain structures. J Neurosci Res 80:667–676

Kwok RP, Lundblad JR, Chrivia JC, Richards JP, Bachinger HP, Brennan RG, Roberts SG, Green MR, Goodman RH (1994) Nuclear protein CBP is a coactivator for the transcription factor CREB. Nature 370:223–226

Lee J, Kim CH, Simon DK, Aminova LR, Andreyev AY, Kushnareva YE, Murphy AN, Lonze BE, Kim KS, Ginty DD, Ferrante RJ, Ryu H, Ratan RR (2005) Mitochondrial cyclic AMP response element-binding protein (CREB) mediates mitochondrial gene expression and neuronal survival. J Biol Chem 280:40398–40401

Lemberger T, Parkitna JR, Chai M, Schutz G, Engblom D (2008) CREB has a context-dependent role in activity-regulated transcription and maintains neuronal cholesterol homeostasis. FASEB J 22:2872–2879

Lesiak A, Pelz C, Ando H, Zhu M, Davare M, Lambert TJ, Hansen KF, Obrietan K, Appleyard SM, Impey S, Wayman GA (2013) A genome-wide screen of CREB occupancy identifies the RhoA inhibitors Par6C and Rnd3 as regulators of BDNF-induced synaptogenesis. PLoS One 8, e64658

Lewis EB (1994) Homeosis: the first 100 years. Trends Genet 10:341–343

Liu Y, Sun L-Y, Singer DV, Ginnan R, Singer HA (2014) CaMKII delta-dependent inhibition of cAMP-response element binding protein activity in vascular smooth muscle. J Biol Chem 288:33519–33529

Lopez de Armentia M, Jancic D, Olivares R, Alarcon JM, Kandel ER, Barco A (2007) cAMP response element-binding protein-mediated gene expression increases the intrinsic excitability of CA1 pyramidal neurons. J Neurosci 27:13909–13918

Lu Q, Hutchins AE, Doyle CM, Lundblad JR, Kwok RP (2003) Acetylation of cAMP-responsive element-binding protein (CREB) by CREB-binding protein enhances CREB-dependent transcription. J Biol Chem 278:15727–15734

Lyons LC, Collado MS, Khabour O, Green CL, Eskin A (2006) The circadian clock modulates core steps in long-term memory formation in *Aplysia*. J Neurosci 26:8662–8671

Marie H, Morishita W, Yu X, Calakos N, Malenka RC (2005) Generation of silent synapses by acute in vivo expression of CaMKIV and CREB. Neuron 45:741–752

Martin KC, Casadio A, Zhu H, Yaping E, Rose JC, Chen M, Bailey CH, Kandel ER (1997) Synapse-specific, long-term facilitation of *aplysia* sensory to motor synapses: a function for local protein synthesis in memory storage. Cell 91:927–938

Mayr B, Montminy M (2001) Transcriptional regulation by the phosphorylation-dependent factor CREB. Nat Rev Mol Cell Biol 2:599–609

Montminy M (1997) Transcriptional regulation by cyclic AMP. Annu Rev Biochem 2:599–609

Nusslein-Volhard C, Wieschaus E (1980) Mutations affecting segment number and polarity in *Drosophila*. Nature 287:795–801

Perazzona B, Isabel G, Preat T, Davis RL (2004) The role of cAMP response element-binding protein in *Drosophila* long-term memory. J Neurosci 24:8823–8828

Restivo L, Tafi E, Ammassari-Teule M, Marie H (2009) Viral-mediated expression of a constitutively active form of CREB in hippocampal neurons increases memory. Hippocampus 19:228–234

Rexach JE, Clark PM, Mason DE, Neve RL, Peters EC, Hsieh-Wilson LC (2012) Dynamic O-GlcNAc modification regulates CREB-mediated gene expression and memory formation. Nat Chem Biol 8:253–261

Ruby NF, Hwang CE, Wessells C, Fernandez F, Zhang P, Sapolsky R, Heller HC (2008) Hippocampal-dependent learning requires a functional circadian system. Proc Natl Acad Sci U S A 105:15593–15598

Ryu H, Lee J, Impey S, Ratan RR, Ferrante RJ (2005) Antioxidants modulate mitochondrial PKA and increase CREB binding to D-loop DNA of the mitochondrial genome in neurons. Proc Natl Acad Sci U S A 102:13915–13920

Salinas JA, Gold PE (2005) Glucose regulation of memory for reward reduction in young and aged rats. Neurobiol Aging 26:45–52

Sargin D, Mercaldo V, Yiu AP, Higgs G, Han JH, Frankland PW, Josselyn SA (2013) CREB regulates spine density of lateral amygdala neurons: implications for memory allocation. Front Behav Neurosci 7:209

Sassone-Corsi P (2012) The cyclic AMP pathway. Cold Spring Har Perspect Biol 4:a011148

Scott R, Bourtchuladze R, Gossweiler S, Dubnau J, Tully T (2002) CREB and the discovery of cognitive enhancers. J Mol Neurosci 19:171–177

Sekeres MJ, Mercaldo V, Richards B, Sargin D, Mahadevan V, Woodin MA, Frankland PW, Josselyn SA (2012) Increasing CRTC2 function in the dentate gyrus during memory formation or reactivation increases memory strength without compromising memory quality. J Neurosci 32:17857–17868

Shanware NP, Trinh AT, Williams LM, Tibbetts RS (2007) Coregulated ataxia telangiectasia-mutated and casein kinase sites modulate cAMP-response element-binding protein-coactivator interactions in response to DNA damage. J Biol Chem 282:6283–6291

Shanware NP, Zhan L, Hutchinson JA, Kim SH, Williams LM, Tibbetts RS (2010) Conserved and distinct modes of CREB/ATF transcription factor regulation by PP2A/B56gamma and genotoxic stress. PLoS One 5:e12173. doi:10.1371/journal.pone.0012173

Shaywitz AJ, Greenberg ME (1999) CREB: a stimulus-induced transcription factor activated by a diverse array of extracellular signals. Annu Rev Biochem 68:821–861

Shen H, Tong L, Balazs R, Cotman CW (2001) Physical activity elicits sustained activation of the cyclic AMP response element-binding protein and mitogen-activated protein kinase in the rat hippocampus. Neuroscience 107:219–229

Sheng M, Thompson MA, Greenberg ME (1991) CREB: a Ca2+-regulated transcription factor phosphorylated by calmodulin-dependent kinases. Science 252:1427–1430

Skalhegg BS, Tasken K (2000) Specificity in the cAMP/PKA signaling pathway. Differential expression, regulation, and subcellular localization of subunits of PKA. Front Biosci 5: D678–D693

Stern SA, Alberini CM (2013) Mechanisms of memory enhancement. Wiley Interdiscip Rev Syst Biol Med 5:37–53

Stern SA, Chen DY, Alberini CM (2014) The effect of insulin and insulin-like growth factors on hippocampus- and amydgala-dependent long-term memory formation. Learn Mem 21: 556–563

Sun P, Enslen H, Myung PS, Maurer RA (1994) Differential activation of CREB by Ca2+/ calmodulin-dependent protein kinases type II and type IV involves phosphorylation of a site that negatively regulates activity. Genes Dev 8:2527–2539

Sun P, Maurer RA (1995) An inactivating point mutation demonstrates that interaction of cAMP response element binding protein (CREB) with the CREB binding protein is not sufficient for transcriptional activation. J Biol Chem 270:7041–7044.

Suzuki A, Fukushima H, Mukawa T, Toyoda H, Wu LJ, Zhao MG, Xiu H, Shang Y, Endoh K, Iwamoto T, Mamiya N, Okano E, Hasegawa S, Mercaldo V, Zhang Y, Maeda R, Ohta M, Josselyn SA, Kida S (2011) Upregulation of CREB-mediated transcription enhances both short- and long-term memory. J Neurosci 31:8786–8802

Takahashi M, Obayashi M, Ishiguro T, Sato N, Niimi Y, Ozaki K, Mogushi K, Mahmut Y, Tanaka H, Tsuruta F, Dolmetsch R, Yamada M, Takahashi H, Kato T, Mori O, Eishi Y, Mizusawa H, Ishikawa K (2013) Cytoplasmic location of alpha1A voltage-gated calcium channel C-terminal fragment ($Ca_v2.1$-CTF) aggregate is sufficient to cause cell death. PLoS One 8:e50121

Tanenhaus AK, Zhang J, Yin JC (2012) In vivo circadian oscillation of dCREB2 and NF-kappaB activity in the *Drosophila* nervous system. PLoS One 7:45130. doi:10.1371/journal.pone.0045130

Tubon TC Jr, Zhang J, Friedman EL, Jin H, Gonzales ED, Zhou H, Drier D, Gerstner JR, Paulson EA, Fropf R, Yin JC (2013) dCREB2-mediated enhancement of memory formation. J Neurosci 33:7475–7487

Tully T, Preat T, Boynton SC, Del Vecchio M (1994) Genetic dissection of consolidated memory in *Drosophila*. Cell 79:35–47

Vaynman S, Ying Z, Gomez-Pinilla F (2004) Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. Eur J Neurosci 20:2580–2590

Vecsey CG, Hawk JD, Lattal MK, Stein JM, Fabian SA, Attner MA, Cabrera SM, McDonough CB, Brindle PK, Abel T, Wood MA (2007) Histone deacetylase inhibitors enhance memory and synaptic plasticity via CREB:CBP-dependent transcriptional activation. J Neurosci 27:6128–6140

Viosca J, Lopez de Armentia M, Jancic D, Barco A (2009) Enhanced CREB-dependent gene expression increases the excitability of neurons in the basal amygdala and primes the consolidation of contextual and cued fear memory. Learn Mem 16:193–197

Wen AY, Sakamoto KM, Miller LS (2010) The role of the transcription factor CREB in immune function. J Immunol 185:6413–6419

West AE, Chen WG, Dalva MB, Dolmetsch RE, Kornhauser JM, Shaywitz AJ, Takasu MA, Tao X, Greenberg ME (2001) Calcium regulation of neuronal gene expression. Proc Natl Acad Sci U S A 98:11024–11031

Williams BM, Luo Y, Ward C, Redd K, Gibson R, Kuczaj SA, McCoy JG (2001) Environmental enrichment: effects on spatial memory and hippocampal CREB immunoreactivity. Physiol Behav 73:649–658

Wu X, McMurray CT (2001) Calmodulin kinase II attenuation of gene transcription by preventing cAMP response element-binding protein (CREB) dimerization and binding of the CREB-binding protein. J Biol Chem 276:1735–1741

Wu CL, Xia S, Fu TF, Wang H, Chen YH, Leong D, Chiang AS, Tully T (2007) Specific requirement of NMDA receptors for long-term memory consolidation in *Drosophila* ellipsoid body. Nat Neurosci 10:1578–1586

Yang JL, Lin YT, Chuang PC, Bohr VA, Mattson MP (2014) BDNF and exercise enhance neuronal DNA repair by stimulating CREB-mediated production of apurinic/apyrimidinic endonuclease 1. Neuromolecular Med 16:161–174

Yin JC, Wallach JS, Del Vecchio M, Wilder EL, Zhou H, Quinn WG, Tully T (1994) Induction of a dominant negative CREB transgene specifically blocks long-term memory in *Drosophila*. Cell 79:49–58

Yin JC, Del Vecchio M, Zhou H, Tully T (1995a) CREB as a memory modulator: induced expression of a dCREB2 activator isoform enhances long-term memory in *Drosophila*. Cell 81:107–115

Yin JC, Wallach JS, Wilder EL, Klingensmith J, Dang D, Perrimon N, Zhou H, Tully T, Quinn WG (1995b) A *Drosophila CREB/CREM* homolog encodes multiple isoforms, including a cyclic AMP-dependent protein kinase-responsive transcriptional activator and antagonist. Mol Cell Biol 15:123–130

Yiu AP, Mercaldo V, Yan C, Richards B, Rashid AJ, Hsiang HL, Pressey J, Mahadevan V, Tran MM, Kushner SA, Woodin MA, Frankland PW, Josselyn SA (2014) Neurons are recruited to a memory trace based on relative neuronal excitability immediately before training. Neuron 83:722–735

Yu D, Akalal DB, Davis RL (2006) Drosophila alpha/beta mushroom body neurons form a branch-specific, long-term cellular memory trace after spaced olfactory conditioning. Neuron 52:845–855

Zhang X, Odom DT, Conkright MD, Canettieri G, Best J, Chen H, Jenner R, Herbolsheimer E, Jacobsen E, Kadam S, Ecker JR, Emerson B, Hogenesch JB, Unterman T, Young RA, Montminy M (2005) Genome-wide analysis of cAMP-response element binding protein occupancy, phosphorylation, and target gene activation in human tissues. Proc Natl Acad Sci U S A 102:4459–4464

Zhang J, Tanenhaus AK, Davis JC, Hanlon BM, Yin JC (2015) Spatio-temporal *in vivo* recording of dCREB2 dynamics in *Drosophila* long-term memory processing. Neurobiol Learn Mem 118:80–88

Zhou Y, Won J, Karlsson MG, Zhou M, Rogerson T, Balaji J, Neve R, Poirazi P, Silva AJ (2009) CREB regulates excitability and the allocation of memory to subsets of neurons in the amygdala. Nat Neurosci 12:1438–1443

# Chapter 8
# Epigenetics and Memory

**Glenn E. Schafe**

**Abstract** Interest in the role of 'epigenetic' modifications in learning and memory processes has escalated rapidly in recent years, due, in part, to the promise of answering long-standing basic science questions about how memories are acquired, stored and maintained over time in the brain. Epigenetic mechanisms, including modifications of chromatin structure and DNA methylation, have long been studied in the context of developmental biology. Recent studies, however, have shown that many of these same mechanisms are conserved in the adult nervous system, where they continue to influence gene expression in the context of long-lasting behavioral change. Within the field of memory research, it has recently been suggested that environmentally-driven alterations in epigenetic processes may represent an ideal candidate mechanism for long-term memory storage and maintenance. In this chapter, I review the role of both chromatin modifications and DNA methylation in several different forms of mammalian memory and associated synaptic plasticity, including a discussion of their involvement in distinct memory-related processes such as consolidation, reconsolidation and extinction. The study of epigenetics in the context of memory holds potential promise for the treatment of neurological conditions that are characterized by memory impairment, as well as psychological conditions such as drug addiction and post-traumatic stress disorder (PTSD) that are characterized by persistent unwanted memories.

**Keywords** Epigenetics • Memory • Consolidation • Reconsolidation • Extinction

## 8.1 Introduction

Traditional views of memory formation and consolidation have emphasized the importance of NMDA receptor (NMDAR)-driven alterations in protein kinase signaling cascades, the activation of transcription factors, and associated changes in gene expression and *de novo* protein synthesis that are thought to be critical for

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long-term memory and synaptic plasticity (Milner et al. 1998; Barco et al. 2006). Within the last decade, however, it has become increasingly clear that 'epigenetic' mechanisms play an additional critical role in transcriptional regulation, synaptic plasticity, and long-term memory formation (Levenson and Sweatt 2005; Levenson et al. 2006; Barrett and Wood 2008; Jiang et al. 2008). Broadly speaking, the field of epigenetics is concerned with alterations in gene expression that are caused by mechanisms other than changes in the underlying DNA sequence itself. Epigenetic mechanisms, including modifications of chromatin structure and DNA methylation, have long been studied in the context of developmental biology (Levenson and Sweatt 2005; Day and Sweatt 2010), where they are thought to promote enduring alterations in the way genes are expressed (or not expressed) during the process of cellular differentiation. Recent studies, however, have shown that many of these same mechanisms are conserved in the adult nervous system, where they continue to influence gene expression in the context of long-lasting behavioral change. Within the field of memory research, it has recently been suggested that environmentally-driven alterations in epigenetic processes may represent an ideal candidate mechanism for long-term memory storage and maintenance (Day and Sweatt 2010, 2011a, b, c). In this chapter, I will review the role of both chromatin modifications and DNA methylation in several different forms of mammalian memory and associated synaptic plasticity, including a discussion of their involvement in distinct memory-related processes such as consolidation, reconsolidation and extinction.

## 8.2 Chromatin Modifications, Synaptic Plasticity and Memory Consolidation

Modifications of chromatin structure have been by far the most widely studied epigenetic modification in the context of learning and memory. Chromatin consists of strands of DNA packaged tightly around a core of eight histone proteins, two each of histones H2A, H2B, H3, and H4. Collectively, this arrangement of DNA and histone proteins is known as the 'nucleosome' (Fig. 8.1a). Each histone protein is associated with a 'tail' on its N-terminus which projects out of the nucleosome complex and serves as the principal site by which histones, and therefore chromatin, may be regulated in a post-translational manner. In its unmodified state, chromatin is highly condensed as the result of tight binding of histones to DNA via positively charged lysine residues on the N-terminal tails of histone proteins (Fig. 8.1b). As a consequence, unmodified chromatin, which is sometimes referred to as 'heterochromatin', is typically not permissive to transcription (Levenson and Sweatt 2005; Tsankova et al. 2007). Chromatin, however, may be dynamically regulated in a post-translational manner via a variety of modifications, including acetylation, methylation and phosphorylation, with histone acetylation by far the most widely studied in the context of memory research. The acetylation of histones on their N-terminal tails via enzymes known as histone acetyltransferases (HATs)

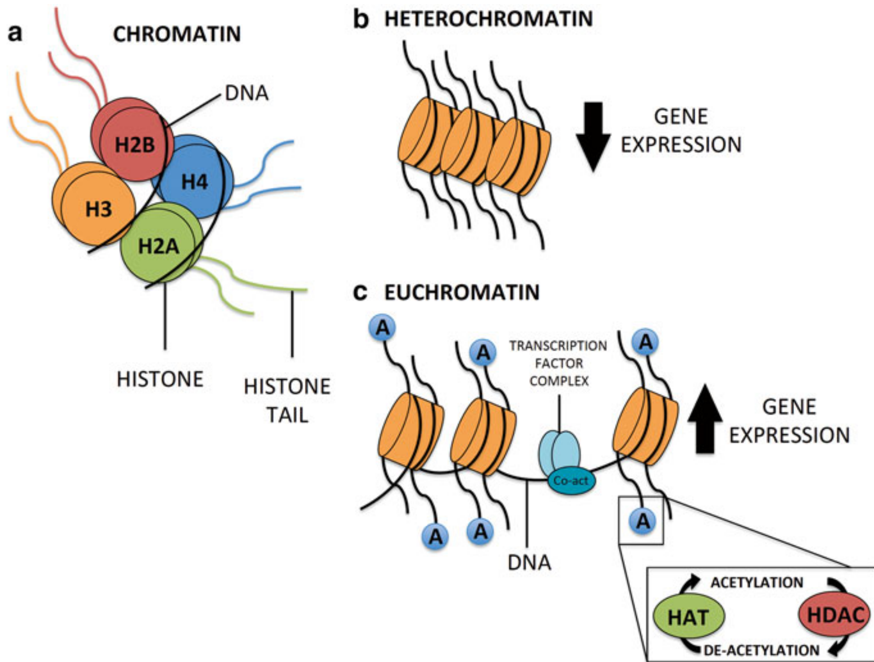


**Fig. 8.1** Modification of chromatin structure by histone acetylation. (**a**) Chromatin consists of strands of DNA packaged tightly around a core of eight histone proteins, two each of histones H2A, H2B, H3, and H4. (**b**) In its unmodified state, chromatin is highly condensed. As a consequence, unmodified chromatin, which is sometimes referred to as 'heterochromatin', is typically not permissive to transcription. (**c**) The acetylation of histones on their N-terminal tails via enzymes known as histone acetyltransferases (HATs) neutralizes the positive charge on the lysine residue, causing chromatin structure to relax. In this uncondensed 'euchromatin' state, chromatin is permissive to the recruitment of transcription factors and to enhanced transcription. Histone de-acetylases (HDACs) can reverse this process

neutralizes the positive charge on the lysine residue, causing chromatin structure to relax. In this uncondensed 'euchromatin' state, chromatin is permissive to the recruitment of transcription factors and to enhanced transcription of memory-related genes (Fig. 8.1c). A number of proteins have been identified to possess HAT activity, including the CREB binding protein (CBP), the E1A-associated protein (also known as 'p300' for its molecular weight), and the p300-CBP associated factor (PCAF) (Korzus et al. 2004; Kouzarides 2007; Barrett and Wood 2008; Barrett et al. 2011). Histone acetylation may also be reversed by a second class of enzymes known as histone deacetylases (HDACs) (Varga-Weisz and Becker 1998; Turner 2002; Yang and Seto 2007), of which several families and 11 known isoforms exist in the rat (Fischer et al. 2010; Broide et al. 2007). "Class I" HDACs, which include HDAC 1 and HDAC 2, are widely distributed in the central nervous system where they are believed to serve as potent negative regulators of synaptic plasticity and memory formation (Guan et al. 2009).

Modifications in chromatin structure have been widely implicated in synaptic plasticity and in several different types of memory. One of the earliest studies to implicate histone and/or lysine acetylation in memory formation was published in 2001 by Michael Swank and David Sweatt (2001). Using a novel taste learning paradigm in mice, they showed that exposure to a novel taste induces a long-lasting increase in lysine acetylation within the insular (gustatory) cortex (Swank and Sweatt 2001), a cortical region that is known to be critical for long-lasting taste memories (Rosenblum et al. 1993; Berman et al. 1998, 2000). In subsequent work from the Sweatt lab, contextual fear conditioning was observed to increase acetylation of histone H3 in area CA1 of the hippocampus, an effect which was downstream of NMDAR activation and signaling via the extracellular signal-regulated kinase (ERK) (Levenson et al. 2004). Further, pharmacological inhibition of HDAC activity with the compounds trichostatin A (TSA) or sodium butyrate (NaB), which both prevent de-acetylation, was observed to enhance long-term potentiation (LTP) in hippocampal area CA1 in a transcription-dependent manner. In behavioral experiments, systemic administration of NaB prior to fear conditioning was observed to enhance the consolidation of contextual fear memory; that is, long-term memory, tested 24 h after training, was significantly impaired, while short-term memory, tested 1 h after training, was not affected (Levenson et al. 2004).

These initial findings implicating HDAC activity in the consolidation of contextual fear memory have been subsequently extended to include other hippocampal-dependent memory tasks, including spatial memory and object recognition (Guan et al. 2009; Stefanko et al. 2009; Zhao et al. 2010). Using a combined pharmacological and molecular genetic approach, Li-Huei Tsai's group, for example, showed that treatment with the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA), which selectively targets HDAC1 and HDAC2, enhances LTP in hippocampal area CA1 as well as contextual fear conditioning (Guan et al. 2009). Further, genetic over-expression of HDAC2, but not HDAC1, was observed to impair LTP in hippocampal area CA1 as well as spatial learning in the Morris water maze and contextual fear conditioning (Guan et al. 2009). Conversely, mice that lacked HDAC2 exhibited enhanced contextual fear conditioning and LTP in area CA1 (Guan et al. 2009). That same year, Marcelo Wood's group showed that pharmacological inhibition of HDAC activity using systemic administration of NaB enhanced long-term memory for object recognition (tested 24 h after training) using a version of the task that normally results in very little memory retention (Stefanko et al. 2009). Remarkably, this effect persisted for at least 7 days. Short-term memory, tested within hours after training, was not affected (Stefanko et al. 2009). These results paralleled those of a previous study from the same group that showed that HDAC inhibition can elicit a transcription-dependent late-phase LTP using a stimulation protocol that normally produces only short-lasting LTP (Vecsey et al. 2007). Similar findings have been reported in the crab *Chasmagnathus,* where treatment with the HDAC inhibitor NaB was observed to promote long-term memory formation in a version of the context-signal memory task that typically produces only short-lasting memory (Federman et al. 2009).

Histone de-acetylation has also been implicated in amygdala-dependent 'cued' fear conditioning. A relatively early study from Po Wu Gean's lab observed that forskolin-induced LTP at cortical inputs to the lateral amygdala (LA) is enhanced by bath application of the HDAC inhibitor TSA (Yeh et al. 2004). Further, intra-amygdala infusion of TSA was observed to enhance long-term retention of fear-potentiated startle to a visual conditioned stimulus, while short-term memory was not affected (Yeh et al. 2004). Consistent with these findings, Tim Bredy and Mark Barad showed that systemic administration of the HDAC inhibitor valproic acid enhances amygdala-dependent auditory fear conditioning (Bredy and Barad 2008). Most recently, work from my own lab has shown that auditory fear conditioning leads to an increase in histone H3 acetylation in the LA. Further, bath application of the HDAC inhibitor TSA to amygdala slices enhances stimulation-induced LTP at both cortical and thalamic inputs to the LA, and intra-LA infusion of TSA enhances the consolidation of an auditory fear memory; short-term memory, tested within hours of training, is not affected, while long-term memory, tested 24 h after training, is significantly enhanced (Monsey et al. 2011).

The role of HAT activity in synaptic plasticity and memory formation has also been extensively studied. To date, the majority of studies examining the requirement of specific HATs in memory processes have focused on CBP and p300 using molecular genetic models in the context of hippocampal-dependent memory paradigms, including novel object recognition, spatial memory and contextual fear memory (Korzus et al. 2004; Wood et al. 2005, 2006; Oliveira et al. 2007, 2011; Barrett et al. 2011; Valor et al. 2011). The methods used in these studies to manipulate CBP and p300 range considerably from forebrain and tissue-specific knockout to transgenic approaches, and the findings are equally variable. Hippocampal LTP using traditional high-frequency stimulation protocols has been observed to be impaired in some (Alarcon et al. 2004), but not all studies (Wood et al. 2005). Further, at the behavioral level the most consistent impairment observed has been for object memory (Alarcon et al. 2004; Korzus et al. 2004; Wood et al. 2005, 2006; Oliveira et al. 2007, 2011; Barrett et al. 2011; Valor et al. 2011), and specifically long-term, but not short-term object memory (Alarcon et al. 2004; Wood et al. 2006; Barrett et al. 2011; Oliveira et al. 2011). At least one study from Ted Abel's lab using a transgenic mouse that over-expressed a truncated form of CBP that lacked the HAT binding domain has observed impairments in both spatial learning and contextual fear memory (Wood et al. 2005), while other studies from the same group targeting p300 have observed impairments in contextual fear memory but not spatial learning (Oliveira et al. 2007, 2011). Other groups targeting CBP have observed impairments in spatial learning, but not context fear memory (Korzus et al. 2004) or no effect on either task (Oike et al. 1999; Valor et al. 2011). Further, only two studies have to date reported a deficit in amygdala-dependent cued fear memory formation in a CBP knockout (Oike et al. 1999; Alarcon et al. 2004), while most other studies that have targeted either CBP or p300 have found no effect (Korzus et al. 2004; Wood et al. 2005, 2006; Oliveira et al. 2007, 2011; Valor et al. 2011). Interestingly, the Oike et al., study observed a significant effect on the consolidation of cued fear memory (impaired long-term, but intact short-term memory), but no effect on either

contextual fear or spatial memory (Oike et al. 1999). The reasons for these discrepant findings are not entirely clear, but can likely be accounted for, in part, by the different types of molecular genetic manipulations involved in these studies and the regions of the brain they affect, as well as subtle variations in behavioral protocols. Further, given that both CBP and p300 share ~90 % sequence homology (Wang et al. 2008) it has been suggested that each of these HATs may compensate for one another during the formation of certain types of memory formation (Oliveira et al. 2011). If true, this might explain the lack of consistent findings between different types of memory in studies that have targeted specific HATs.

More recently, studies have taken advantage of the commercial availability of broad-spectrum pharmacological inhibitors of HAT activity. While these studies generally lack the ability to make claims about the role of a specific HAT in learning and memory processes, they have the advantage of acutely and broadly targeting HAT activity in specific brain regions independently of potential developmental confounds or concerns about compensatory mechanisms (Marek et al. 2011; Zhao et al. 2012; Maddox et al. 2013a, b). Direct infusion of the broad spectrum HAT inhibitor garcinol into the hippocampus or the amygdala has been shown to impair both object recognition (Zhao et al. 2012) and auditory fear memory (Maddox et al. 2013a, b), respectively. Further, direct intra-amygdala infusion of c646, a small molecule HAT inhibitor that more selectively targets p300/CBP (Marek et al. 2011), has been recently observed to significantly impair the acetylation of histone H3 in the LA following auditory fear conditioning and, in parallel, the consolidation of an auditory fear memory; short-term memory is intact, while long-term memory is significantly impaired (Maddox et al. 2013a, b). Further, intra-LA infusion of either garcinol or c646 significantly impairs the consolidation of training-related neural plasticity in the LA; training-related enhancements in tone-evoked neural activity in the LA are intact during a test of short-term memory given within hours after fear conditioning, but are impaired, along with the fear memory, 24 h later during a test of long-term memory (Maddox et al. 2013a, b). Collectively, these findings suggest that HAT activity plays a critical role in the consolidation of amygdala-dependent cued fear memories and associated neural plasticity in the LA.

While histone acetylation has received the most attention in the learning and memory literature, other histone modifications, including histone methylation, have also been implicated in long-term memory formation. Like histone acetylation, histone methylation occurs at lysine residues on the N-terminal tails of histones. Histone methylation can either positively or negatively regulate transcription depending upon the lysine residue that is methylated and the number of methyl groups that are added to it. Both dimethylation and trimethylation of histone 3 at lysine 9 (H3K9) are typically associated with heterochromatin formation and gene silencing, while trimethylation of histone 3 at lysine 4 (H3K4) is associated with euchromatin and gene activation (Sims et al. 2003; Martin and Zhang 2005). In an elegant study Farah Lubin and her colleagues found that contextual fear conditioning increases H3K4 trimethylation in hippocampal area CA1, specifically around the Zif-268 and BDNF gene promoters, two immediately early genes that have been widely implicated in contextual fear learning in the hippocampus (Poirier et al. 2008; Gupta et al. 2010; Penke et al. 2014). Further, mice lacking certain histone

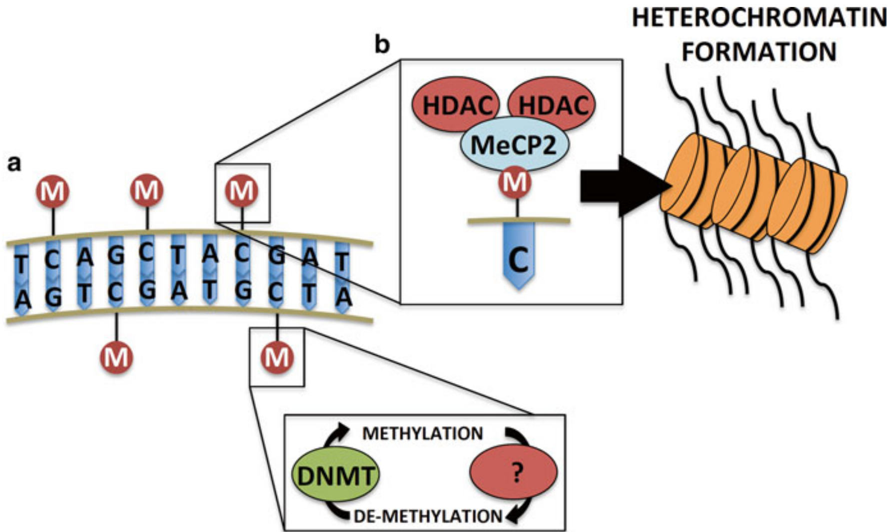


**Fig. 8.2** Modification of chromatin structure by DNA methylation. (**a**) Methylation of cytosine residues on strands of DNA is catalyzed by DNA methyltransferases (DNMTs). (**b**) Cytosine methylation recruits methyl-DNA binding proteins, including MeCP2. The binding of MeCP2, in turn, recruits HDACs to the site of methylation, which promote the condensation of chromatin around the methylation site. The methylation of cytosine residues on DNA via DNMTs is thus typically thought to negatively regulate transcription via promoting heterochromatin formation and preventing the binding of transcription factors

de-methylating enzymes, such as *Mll*, a specific H3K4 methyltransferase, had impaired context fear conditioning (Gupta et al. 2010). These findings suggest that a complete understanding of the role of chromatin modifications in memory will require attention to multiple histone modifications and the role each plays in regulating memory-related gene expression.

## 8.3 DNA Methylation, Synaptic Plasticity and Memory Consolidation

The methylation of DNA is a second major source of epigenetic modification that has been implicated in learning and memory. In contrast to histone acetylation, DNA methylation has typically been associated with transcriptional repression, a process which is catalyzed by DNA methyltransferases (DNMTs) (Miranda and Jones 2007). Several different isoforms of DNMTs exist, including DNMT1, which is widely considered a 'maintenance' DNMT, as well as DNMT3A and DNMT3B, which have both been shown to be critical for *de novo* methylation (Siedlecki and Zielenkiewicz 2006). Methylation of cytosines by DNMTs at cytosine-phosphodiester-guanine (CpG) dinucleotides on the promoter region of genes

recruits methyl-DNA binding proteins, such as MeCP2 (Chahrour et al. 2008; Roth and Sweatt 2009; Fig. 8.2a). The binding of MeCP2, in turn, recruits HDACs to the site of methylation, which promote the condensation of chromatin around the methylation site (Razin 1998; Fig. 8.2b). The methylation of cytosine residues on DNA via DNMTs is thus typically thought to negatively regulate transcription via promoting heterochromatin formation and preventing the binding of transcription factors (Levenson and Sweatt 2005; Levenson et al. 2006; Miller and Sweatt 2007; Miller et al. 2010). In development, this process has been associated with gene silencing and cellular differentiation, and is known to be a long-lasting, static epigenetic modification (Levenson and Sweatt 2005; Miranda and Jones 2007). Post-mitotic neurons of the CNS, however, are known to express high levels of DNMT mRNA into adulthood, which has raised the intriguing possibility that dynamic regulation of DNA methylation may be critical for neuronal function, including synaptic plasticity and memory formation.

David Sweatt's lab was the first to systematically implicate DNA methylation in synaptic plasticity and memory formation. They observed that bath application of phorbol 12,13-diacetate (PDA), an activator of PKC, significantly increases DNMT3A mRNA expression and promotes a significant decrease in DNA methylation (or de-methylation) on the plasticity-promoting gene *reelin* in hippocampal area CA1 (Levenson et al. 2006). Further, bath application of the DNMT inhibitors zebularine or 5-AZA was observed to impair LTP in area CA1 induced by theta-burst stimulation (Levenson et al. 2006). In follow-up experiments aimed at extending these findings to memory, Courtney Miller and David Sweatt showed that contextual fear conditioning leads to a significant increase in DNMT3A and DNMT3B mRNA expression in area CA1 and that intra-hippocampal infusions of either zebularine or 5-AZA significantly impairs contextual fear memory (Miller and Sweatt 2007). Further, contextual fear conditioning, like PKC activation, was observed to lead to a significant de-methylation of the memory-promoting *reelin* gene and a corresponding increase in the expression of *reelin* mRNA in area CA1. Conversely, fear conditioning was observed to increase methylation of the plasticity and memory-suppressing gene protein phosphatase 1 (PP1), which was accompanied by a decrease in PP1 mRNA expression in area CA1 (Miller and Sweatt 2007). In a companion study, the same lab showed that contextual fear conditioning leads to increased expression of BDNF mRNA in area CA1, specifically on Exon 4, that was accompanied by an increase in histone H3 acetylation and a decrease in CpG methylation (de-methylation) of the BDNF exon 4 promoter (Lubin et al. 2008). Intra-CA1 infusion of the DNMT inhibitor zebularine was observed to impair contextual fear conditioning. Further, zebularine was observed to prevent training-related increases in histone H3 acetylation, de-methylation on the BNDF exon 4 promoter and increases in BDNF mRNA expression in area CA1 (Lubin et al. 2008). More recently, a study from Karl Peter Giese's lab observed long-lasting changes (up to 24 h) in BDNF gene expression and DNA methylation on the BDNF gene following contextual fear conditioning (Mizuno et al. 2012). Collectively, these exciting findings suggest that dynamic changes in the methylation (and de-methylation) of genes are critical for promoting memory formation in the

hippocampus. More recent work has extended these findings to other forms of hippocampal-dependent memory, including spatial learning (Feng et al. 2010) and cocaine-induced conditioned place preference (Han et al. 2010).

DNA methylation has also been implicated in amygdala-dependent cued fear conditioning. Experiments in my own lab, for example, have shown that auditory fear conditioning leads to a significant increase in the protein expression of DNMT3A in the LA (Monsey et al. 2011). Further, bath application of the DNMT inhibitor 5-AZA impairs LTP at thalamic and cortical inputs to the LA (Monsey et al. 2011) and intra-LA infusion of the pharmacologically distinct DNMT inhibitors 5-AZA or RG108 impairs both memory consolidation of auditory fear conditioning and, in parallel, the consolidation of training-related neural plasticity in the LA; both fear memory and training-related increases in tone-evoked activity in the LA are intact in the short-term, but significantly impaired in the long-term (Monsey et al. 2011; Maddox et al. 2014).

Given that DNA methylation is thought to negatively regulate transcription, and therefore should prevent memory formation, the precise mechanism by which DNMT *inhibition* impairs memory consolidation in both hippocampus and amygdala remains, at present, poorly understood. Several observations, however, exist that can potentially explain this paradoxical effect. First, the findings of Miller and Sweatt (2007) suggest that the methylation of memory suppressor genes, such as PP1, may play a critical role in memory formation. As described previously, they observed that contextual fear conditioning leads to an increase in the methylation of the PP1 gene and a corresponding decrease in PP1 gene expression in the hippocampus during the period in which contextual fear memories are being consolidated (Miller and Sweatt 2007). Further, that same study observed that treatment with the DNMT inhibitor 5-AZA at doses that effectively impair contextual fear memory prevents training-related increases in PP1 methylation and significantly increases PP1 levels in area CA1 (Miller and Sweatt 2007). Thus, it appears that one mechanism by which DNMT inhibitors impair synaptic plasticity and memory is by promoting the expression of memory suppressor genes that would otherwise be suppressed by training (Miller and Sweatt 2007; Miller et al. 2008; Roth and Sweatt 2009).

Interestingly, a second mechanism by which DNMT inhibition may regulate memory formation and synaptic plasticity is by influencing histone acetylation (Miller et al. 2008; Roth and Sweatt 2009). For example, intra-CA1 infusion of a DNMT inhibitor has been shown to impair both contextual fear memory and the training-related increase in histone H3 acetylation in the hippocampus (Lubin et al. 2008; Miller et al. 2008). Further, pre-treatment with an HDAC inhibitor has been shown to rescue the contextual fear memory deficit induced by a DNMT inhibitor (Miller et al. 2008). A similar pattern of findings has been observed in the amygdala. Experiments in my lab, for example, have shown that intra-LA infusion of 5-AZA not only impairs fear memory consolidation but also significantly attenuates the training-related increase in H3 acetylation following auditory fear conditioning. Further, pre-treatment with the HDAC inhibitor TSA was observed to rescue the memory consolidation deficit induced by the DNMT inhibitor 5-AZA (Monsey

et al. 2011). A similar pattern of findings was observed in our neurophysiology experiments, where co-perfusion of TSA to amygdala slices completely reversed the 5-AZA-infuced LTP deficit at both thalamic and cortical inputs to the LA (Monsey et al. 2011). Thus, both histone acetylation and DNA methylation appear to work in concert to regulate memory formation and synaptic plasticity.

A third possibility involves the differential effect of methyl binding proteins, such as MeCP2, across different subsets of genes. As mentioned previously, MeCP2 is typically associated with heterochromatin formation via the recruitment of HDACs around methylated sites on the promoter regions of genes (Razin 1998). Recent findings, however, have suggested that MeCP2 can interact with the transcription factor CREB1 to regulate the activation of CREB-mediated genes, suggesting that DNA methylation and the binding MeCP2 at CRE sites is associated with transcriptional activation rather than repression (Chahrour et al. 2008). Thus, it is quite possible that the methylation of CREB-mediated genes in both the hippocampus and amygdala is required for memory consolidation, which would explain, in part, why DNMT inhibitors impair both types of memory. Consistent with this notion, Farah Lubin's lab has shown that contextual fear conditioning leads to an increase in both DNA methylation and the binding of MeCP2 on the Zif-268 promoter, effects which were correlated with increased Zif-268 gene expression (Gupta et al. 2010). Whether a similar process occurs in the amygdala during cued fear conditioning, and whether it might be blocked by a DNMT inhibitor, remains to be examined, but it is worth noting that auditory fear conditioning has been shown to regulate Zif-268 expression in the LA (Malkani and Rosen 2000; Maddox et al. 2011) and that knockdown of Zif-268 protein in the LA impairs the consolidation of both contextual and auditory fear conditioning (Malkani et al. 2004; Maddox et al. 2011).

## 8.4 Long-Lasting Modifications in DNA Methylation in 'Remote' Memory Consolidation and Maintenance

One of the major challenges in contemporary memory research is to understand how long-lasting memories are formed and stored in the brain, especially given that most molecular events that have been studied in the context of memory formation, including protein kinase activation, the activation of transcription factors and the expression of memory-related genes, have been shown to be transient. From the outset, epigenetics was an exciting new area of memory research because modifications in chromatin structure and DNA methylation represented two potential mechanisms by which the expression of memory-related genes might be altered in a long-term manner, thus serving as a potential molecular substrate for long-term memory maintenance (Day and Sweatt 2010, 2011a, b, c). Interestingly, however, studies that have examined memory-related modifications in both chromatin structure and DNA methylation in the brain, and particularly in the hippocampus, have

found them to be transient as well (Barrett and Wood 2008; Day and Sweatt 2010). For example, in their 2007 study, Miller and Sweatt observed that contextual fear conditioning was associated with alterations in DNA methylation on memory-related genes in area CA1 within 1 h after fear conditioning that were not evident 24 h later (Miller and Sweatt 2007). Given that during development and during the process of cellular differentiation DNA methylation is considered to be highly stable, this finding was somewhat surprising and suggested that DNA methylation is a more dynamic, reversible process in the adult nervous system (Miller and Sweatt 2007).

An exception to this rule was observed in a relatively recent study by Courtney Miller, David Sweatt and colleagues that examined DNA methylation in regions of the prefrontal cortex in hippocampal-dependent contextual fear memory (Miller et al. 2010). Beginning with the pioneering work of Brenda Milner and William Scoville with patient H.M. in the 1950s, it has long been appreciated that the hippocampus is not a site of long-term memory storage, but rather a region where immediate (declarative) memories are formed and then slowly stabilized over time in regions of the cortex during a process known as “systems consolidation” (Milner et al. 1998). Consistent with this idea, lesions of the hippocampus in rats made 1 day after training have been observed to reliably impair contextual fear conditioning, while lesions made 28 days after training have no significant effect (Kim and Fanselow 1992; see, however, Sparks et al. 2013). Presumably, at this point the contextual memory is no longer dependent on the hippocampus and stored in the cortex. Subsequent work from Paul Frankland, Alcino Silva and colleagues suggested that the anterior cingulate cortex (ACC) is a site of storage of long-term, ‘remote’ contextual fear memories. In their work, pharmacological inactivation of the ACC was observed to impair the retrieval of an 18–36 day old remote contextual fear memory, but had no effect on a 1–3 day old context fear memory (Frankland et al. 2004; Frankland and Bontempi 2005). Inspired by these findings, Miller and colleagues asked whether contextual fear conditioning might regulate long-lasting epigenetic modifications in the ACC, and thus provide a kind of molecular signature for long-term, remote memory storage. They observed a significant increase in the methylation of the memory-suppressor gene calcineurin (CaN) in the prefrontal cortex that emerged 1 day after contextual fear conditioning and remained elevated for at least 30 days after training (Miller et al. 2010). This long-lasting increase in CaN promoter methylation in the prefrontal cortex was associated with a significant decrease in CaN mRNA and protein expression, and was dependent upon NMDAR receptor activation *in the hippocampus* at the time of fear conditioning (Miller et al. 2010). Further, infusion of DNMT inhibitors into the ACC 30 days after fear conditioning was observed to significantly impair training-related CaN hypermethylation, increase the expression of CaN mRNA, and disrupt the retrieval of remote contextual fear memory (Miller et al. 2010). Infusion of DNMT inhibitors into the ACC 1 day after fear conditioning, at a time point prior to when the ACC becomes involved in the memory trace, was observed to have no effect (Miller et al. 2010). Collectively, these findings suggest that the long-term storage of memories that are initially dependent upon the hippocampus are ultimately maintained in the cortex by

persistent alterations in DNA methylation on memory-related genes. The implications of these findings are very exciting, because they suggest that altered patterns of DNA methylation and the resultant expression (or suppression) of memory-related genes may represent a self-perpetuating molecular signal which might be ‘captured’ by the synapse to maintain memory over time. It remains currently unknown whether similarly long-lasting changes in DNA methylation underlie memory maintenance in other regions of the brain, such as the amygdala and cerebellum, that are thought to be critical loci of long-lasting memories.

## 8.5 Epigenetic Mechanisms and Memory Reconsolidation

As we’ve seen, newly acquired memories are thought to be inherently unstable, acquiring stability over time as they are ‘consolidated’ into long-term representations in the brain (Milner et al. 1998). It has long been appreciated that memories, even long-term memories, are not fixed but rather highly dynamic (Loftus 1979, 2005). The mere act of retrieving (recalling) a long-term memory, for example, has been shown to trigger a new phase of instability for a brief window of time during which the memory may be updated (e.g. strengthened or weakened) prior to being re-stabilized in a process known as ‘reconsolidation’ (Sara 2000; Nader 2003). In the laboratory, reconsolidation is typically studied by having an experimental animal recall a well-established memory (the memory ‘reactivation’ trial) followed by a pharmacological treatment designed to either impair or enhance the reconsolidation process. Memory is then tested again shortly after retrieval (the ‘post-reactivation short-term memory’ test; PR-STM) and then again 24 h later (the ‘post-reactivation long-term memory’ test; PR-LTM). Reconsolidation is said to be impaired if PR-LTM is impaired relative to PR-STM. Conversely, reconsolidation is said to be enhanced if PR-LTM is enhanced relative to PR-STM. The study of the memory reconsolidation process has important theoretical implications for how memories are structured in the brain in addition to clinical applications for the treatment of psychiatric disorders ranging from post-traumatic stress disorder (PTSD) to drug addiction that are characterized by unusually strong and persistent memories (Pitman et al. 2000; Tronson and Taylor 2007).

Within the last decade, considerable progress has been made at defining the cellular and molecular mechanisms underlying memory reconsolidation in the mammalian brain (Dudai and Eisenberg 2004; Tronson and Taylor 2007). With notable exceptions (Alberini 2005), findings have suggested that the reconsolidation process shares many of its core molecular features with that of initial memory consolidation, including the involvement of the NMDA receptor and the activation of NMDAR-driven protein kinase signaling cascades (Duvarci et al. 2005; Ben Mamou et al. 2006; Tronson et al. 2006; Milton et al. 2008), the recruitment of transcription factors (Kida et al. 2002), *de novo* mRNA and protein synthesis (Nader et al. 2000; Da Silva et al. 2008; Duvarci et al. 2008), and the involvement of immediate early genes (Lee et al. 2005; Maddox et al. 2011; Maddox and Schafe 2011a). While the

importance of *de novo* mRNA transcription in memory reconsolidation has been well established (Nader et al. 2000; Kida et al. 2002; Da Silva et al. 2008; Duvarci et al. 2008; but see Parsons et al. 2006), few studies have until recently examined the role of epigenetics in memory reconsolidation.

Farah Lubin and David Sweatt were the first to implicate chromatin modifications in memory reconsolidation. Using a hippocampus-dependent contextual fear conditioning paradigm, they showed that retrieval of a contextual fear memory promotes a significant increase in histone H3 acetylation in hippocampal area CA1 and a corresponding increase in the association of acetylated histone H3 with the Zif-268 gene promoter, two effects that were each mediated via the IKK/NFκB signaling pathway (Lubin and Sweatt 2007). A year later, Tim Bredy and Mark Barad showed that systemic administration of the HDAC inhibitor valproic acid enhances the reconsolidation of amygdala-dependent auditory fear conditioning; that is, PR-STM is not affected, while PR-LTM is significantly enhanced (Bredy and Barad 2008). Consistent with these findings, my lab has observed that retrieval of an auditory fear memory is associated with an increase in histone H3 acetylation in the LA (Maddox and Schafe 2011a, b). Further, intra-LA infusion of the HDAC inhibitor TSA enhances the reconsolidation of an auditory fear memory (Maddox and Schafe 2011a, b). In a more recent study, we observed that intra-LA infusion of either the broad spectrum HAT inhibitor garcinol or the more selective p300/CBP inhibitor c646 impairs retrieval-related histone H3 acetylation in the LA (Maddox et al. 2013a, b). Further, both garcinol and c646 were observed to significantly impair the reconsolidation of an auditory fear memory; PR-LTM was impaired, while PR-STM was not affected (Maddox et al. 2013a, b). This effect of HAT inhibition in the LA was observed only for reactivated fear memories; infusion of the drugs in the absence of fear memory retrieval had no effect on subsequent memory recall. It was also observed to be time-limited; the drugs had to be given shortly after retrieval of the fear memory in order to significantly disrupt fear memory reconsolidation. Further, we showed that inhibition of HAT activity following retrieval can impair even older, well-established (2 week old) auditory fear memories. Finally, we observed that memory-related neural plasticity in the LA was disrupted by intra-LA infusion of a HAT inhibitor following retrieval; training-related enhancements in tone-evoked neural activity in the LA were intact during a test of PR-STM, but are significantly impaired, along with the fear memory, 24 h later during a test of PR-LTM (Maddox et al. 2013a, b). Collectively, these findings suggest that HAT activity plays a critical role in the reconsolidation of amygdala-dependent cued fear memories and associated neural plasticity in the LA.

The role of DNA methylation has been less widely studied in the context of reconsolidation. Inhibition of DNMT activity in the LA shortly after retrieval has been observed to impair the reconsolidation of an auditory fear memory, an effect which was observed to be specific to reactivated fear memories (Maddox and Schafe 2011a, b). Further, intra-LA infusion of the pharmacologically distinct DNMT inhibitors 5-AZA or RG108 was observed to impair memory-related neural plasticity in the LA; both fear memory and training-related increases in tone-evoked activity in the LA were intact during the PR-STM test, but significantly impaired during

the PR-LTM test (Maddox et al. 2014). Interestingly, DNMT inhibition in the LA was observed to impair retrieval-related increases in histone H3 acetylation. Further, pre-treatment with the HDAC inhibitor TSA was observed to prevent the reconsolidation impairment induced by DNMT inhibition (Maddox and Schafe 2011a, b). Thus, similar to what has been observed previously in studies of memory consolidation, histone acetylation and DNA methylation appear to work in concert to regulate memory reconsolidation.

The specific genes that are targeted for epigenetic modification during the reconsolidation process in the amygdala have yet to be explored and will be an important area of study in future experiments. It will also be of interest to study how persistent alterations in DNA methylation that accompany long-term memory maintenance in areas of the prefrontal cortex and other brain regions are regulated by reconsolidation. Are these long-term epigenetic modifications temporarily de-stabilized with memory recall? Do they re-stabilize after reconsolidation is complete? Does interference with the reconsolidation process lead to a removal of these epigenetic marks on DNA? This latter question would be particularly interesting to ask in light of findings which suggest that memories that are impaired due to interference with the reconsolidation process do not recover with time or following experimental treatments that typically result in memory recovery after amnesia (Duvarci and Nader 2004).

## 8.6 Epigenetic Mechanisms and Memory Extinction

In classical or Pavlovian conditioning, extinction refers to a gradual reduction in conditioned responding after the conditioned stimulus (CS) is repeatedly presented in the absence of the unconditioned stimulus (US) (Pavlov 1927). In the classically conditioned salivary response, for example, an experimental animal is first presented with pairings of an arbitrary cue (the CS) that is paired with delivery of food (the US). Over repeated pairings, the CS comes to elicit a conditioned response (CR) of salivation. After conditioning, repeated non-reinforced presentations of the CS (e.g. without the accompanying US) results in a gradual decrease in the conditioned salivary response to the CS. For nearly a century, it has been widely appreciated that extinction does not promote 'erasure' of a memory, but rather represents a kind of (inhibitory) learning and memory that serves to suppress the expression of the original memory (Pavlov 1927).

The neural mechanisms of extinction have been studied in considerable detail in several memory systems, most notably that of the conditioned eyeblink reflex (Robleto et al. 2004). In recent years, the neural and molecular mechanisms underlying the extinction of both contextual and cued Pavlovian fear memories have attracted the most interest, due in part to the promise of discovering novel treatments for fear-based psychological disorders (Milad et al. 2006; Myers and Davis 2007). In brief, the extinction of fear memories is believed to involve plasticity within the medial prefrontal (infralimbic; IL) cortex (Milad and Quirk 2002; Santini

et al. 2004; Peters et al. 2010), a region of the prefrontal cortex which projects to GABAergic intercalated cell masses in the amygdala, populations of GABAergic cells that serve to gate information flow within and between amygdala subnuclei and that have been shown to be critical for the expression of fear extinction (Quirk et al. 2003; Likhtik et al. 2008).

Inspired by the potential clinical benefits of facilitating the acquisition and/or consolidation of fear extinction memories, several labs have shown that either systemic or intra-IL administration of inhibitors of HDAC activity can facilitate extinction of an auditory fear memory (Lattal et al. 2007; Bredy and Barad 2008; Fujita et al. 2012; Itzhak et al. 2012), particularly under conditions of weak extinction learning (Stafford et al. 2012). Further, work from Tim Bredy's lab has shown that pharmacological inhibition of HAT activity in the IL, specifically that of the histone acetyltransferase PCAF, significantly impairs the consolidation of fear extinction memory and LTP in the IL (Wei et al. 2012). Conversely, pharmacological activation of PCAF within the IL enhances fear extinction (Wei et al. 2012). At a molecular level, fear extinction was observed to promote an increase in binding of the transcriptional repressor ATF4 (CREB2) on the Zif-268 promoter and a corresponding decrease in Zif-268 expression in the IL, and each of these effects was prevented by inhibition of PCAF activity (Wei et al. 2012). These findings collectively suggest that HAT activity within the IL, and specifically that of PCAF, recruits transcriptional repressors and negatively regulates memory-related genes in the IL during fear extinction. Interestingly, a follow-up study from the same group showed that intra-IL infusion of the p300/CBP HAT inhibitor c646 paradoxically facilitates fear extinction memory and enhances LTP in the IL (Marek et al. 2011). These findings suggest that a remarkable degree of specificity exists between the actions of specific HATs in the IL during the formation of an extinction memory. Future studies will be required to further explore the role of these HATs and the genes they target in the IL during fear extinction.

## 8.7 Conclusions

Interest in the role of epigenetic modifications in learning and memory processes has escalated rapidly in recent years, due, in part, to the promise of answering long-standing basic science questions about how memories are acquired, stored and maintained over time in the brain. From a clinical perspective, the study of epigenetic modifications in memory also holds potential promise for the treatment of neurological conditions that are characterized by memory impairment (Kosik et al. 2012; Rudenko and Tsai 2014a, b), as well as psychological conditions such as drug addiction and PTSD that are characterized by persistent unwanted memories (Tsankova et al. 2007; Renthal and Nestler 2008; Schafe 2014). Many outstanding questions remain, however, for future research. It remains poorly understood, for example, how specific epigenetic tags on memory-related genes (or subsets of genes) are selectively targeted during learning to promote long-lasting memories.

We also still know very little about the extent to which these epigenetic modifications within specific memory circuits are transient or long-lasting. Further, it remains unclear how such lasting epigenetic modifications on memory-related genes, which are presumably cell-wide, are selectively recruited in a synapse-specific manner within a memory circuit. Each of these important questions await further study.

## References

Alarcon JM, Malleret G, Touzani K, Vronskaya S, Ishii S, Kandel ER, Barco A (2004) Chromatin acetylation, memory, and LTP are impaired in CBP+/− mice: a model for the cognitive deficit in Rubinstein-Taybi syndrome and its amelioration. Neuron 42(6):947–959

Alberini CM (2005) Mechanisms of memory stabilization: are consolidation and reconsolidation similar or distinct processes? Trends Neurosci 28(1):51–56

Barco A, Bailey CH, Kandel ER (2006) Common molecular mechanisms in explicit and implicit memory. J Neurochem 97(6):1520–1533

Barrett RM, Wood MA (2008) Beyond transcription factors: the role of chromatin modifying enzymes in regulating transcription required for memory. Learn Mem 15(7):460–467

Barrett RM, Malvaez M, Kramar E, Matheos DP, Arrizon A, Cabrera SM, Lynch G, Greene RW, Wood MA (2011) Hippocampal focal knockout of CBP affects specific histone modifications, long-term potentiation, and long-term memory. Neuropsychopharmacology 36(8):1545–1556

Ben Mamou C, Gamache K, Nader K (2006) NMDA receptors are critical for unleashing consolidated auditory fear memories. Nat Neurosci 9(10):1237–1239

Berman DE, Hazvi S, Rosenblum K, Seger R, Dudai Y (1998) Specific and differential activation of mitogen-activated protein kinase cascades by unfamiliar taste in the insular cortex of the behaving rat. J Neurosci 18(23):10037–10044

Berman DE, Hazvi S, Neduva V, Dudai Y (2000) The role of identified neurotransmitter systems in the response of insular cortex to unfamiliar taste: activation of ERK1-2 and formation of a memory trace. J Neurosci 20(18):7017–7023

Bredy TW, Barad M (2008) The histone deacetylase inhibitor valproic acid enhances acquisition, extinction, and reconsolidation of conditioned fear. Learn Mem 15(1):39–45

Broide RS, Redwine JM, Aftahi N, Young W, Bloom FE, Winrow CJ (2007) Distribution of histone deacetylases 1–11 in the rat brain. J Mol Neurosci 31:47–58

Chahrour M, Jung SY, Shaw C, Zhou X, Wong ST, Qin J, Zoghbi HY (2008) MeCP2, a key contributor to neurological disease, activates and represses transcription. Science 320(5880):1224–1229

Da Silva WC, Bonini JS, Bevilaqua LR, Medina JH, Izquierdo I, Cammarota M (2008) Inhibition of mRNA synthesis in the hippocampus impairs consolidation and reconsolidation of spatial memory. Hippocampus 18(1):29–39

Day JJ, Sweatt JD (2010) DNA methylation and memory formation. Nat Neurosci 13(11): 1319–1323

Day JJ, Sweatt JD (2011a) Cognitive neuroepigenetics: a role for epigenetic mechanisms in learning and memory. Neurobiol Learn Mem 96(1):2–12

Day JJ, Sweatt JD (2011b) Epigenetic mechanisms in cognition. Neuron 70(5):813–829

Day JJ, Sweatt JD (2011c) Epigenetic modifications in neurons are essential for formation and storage of behavioral memory. Neuropsychopharmacology 36(1):357–358

Dudai Y, Eisenberg M (2004) Rites of passage of the engram: reconsolidation and the lingering consolidation hypothesis. Neuron 44(1):93–100

Duvarci S, Nader K (2004) Characterization of fear memory reconsolidation. J Neurosci 24(42):9269–9275

Duvarci S, Nader K, LeDoux JE (2005) Activation of extracellular signal-regulated kinase-mitogen-activated protein kinase cascade in the amygdala is required for memory reconsolidation of auditory fear conditioning. Eur J Neurosci 21(1):283–289

Duvarci S, Nader K, LeDoux JE (2008) De novo mRNA synthesis is required for both consolidation and reconsolidation of fear memories in the amygdala. Learn Mem 15(10):747–755

Federman N, Fustinana MS, Romano A (2009) Histone acetylation is recruited in consolidation as a molecular feature of stronger memories. Learn Mem 16(10):600–606

Feng J, Zhou Y, Campbell SL, Le T, Li E, Sweatt JD, Silva AJ, Fan G (2010) Dnmt1 and Dnmt3a maintain DNA methylation and regulate synaptic function in adult forebrain neurons. Nat Neurosci 13(4):423–430

Fischer A, Sananbenesi F, Mungenast A, Tsai LH (2010) Targeting the correct HDAC(s) to treat cognitive disorders. Trends Pharmacol Sci 31(12):605–617

Frankland PW, Bontempi B (2005) The organization of recent and remote memories. Nat Rev Neurosci 6(2):119–130

Frankland PW, Bontempi B, Talton LE, Kaczmarek L, Silva AJ (2004) The involvement of the anterior cingulate cortex in remote contextual fear memory. Science 304(5672):881–883

Fujita Y, Morinobu S, Takei S, Fuchikami M, Matsumoto T, Yamamoto S, Yamawaki S (2012) Vorinostat, a histone deacetylase inhibitor, facilitates fear extinction and enhances expression of the hippocampal NR2B-containing NMDA receptor gene. J Psychiatr Res 46(5):635–643

Guan JS, Haggarty SJ, Giacometti E, Dannenberg JH, Joseph N, Gao J, Nieland TJ, Zhou Y, Wang X, Mazitschek R, Bradner JE, DePinho RA, Jaenisch R, Tsai LH (2009) HDAC2 negatively regulates memory formation and synaptic plasticity. Nature 459(7243):55–60

Gupta S, Kim SY, Artis S, Molfese DL, Schumacher A, Sweatt JD, Paylor RE, Lubin FD (2010) Histone methylation regulates memory formation. J Neurosci 30(10):3589–3599

Han J, Li Y, Wang D, Wei C, Yang X, Sui N (2010) Effect of 5-aza-2-deoxycytidine microinjecting into hippocampus and prelimbic cortex on acquisition and retrieval of cocaine-induced place preference in C57BL/6 mice. Eur J Pharmacol 642(1–3):93–98

Itzhak Y, Anderson KL, Kelley JB, Petkov M (2012) Histone acetylation rescues contextual fear conditioning in nNOS KO mice and accelerates extinction of cued fear conditioning in wild type mice. Neurobiol Learn Mem 97(4):409–417

Jiang Y, Langley B, Lubin FD, Renthal W, Wood MA, Yasui DH, Kumar A, Nestler EJ, Akbarian S, Beckel-Mitchener AC (2008) Epigenetics in the nervous system. J Neurosci 28(46):11753–11759

Kida S, Josselyn SA, de Ortiz SP, Kogan JH, Chevere I, Masushige S, Silva AJ (2002) CREB required for the stability of new and reactivated fear memories. Nat Neurosci 5(4):348–355

Kim JJ, Fanselow MS (1992) Modality-specific retrograde amnesia of fear. Science 256(5057):675–677

Korzus E, Rosenfeld MG, Mayford M (2004) CBP histone acetyltransferase activity is a critical component of memory consolidation. Neuron 42(6):961–972

Kosik KS, Rapp PR, Raz N, Small SA, Sweatt JD, Tsai LH (2012) Mechanisms of age-related cognitive change and targets for intervention: epigenetics. J Gerontol A Biol Sci Med Sci 67(7):741–746

Kouzarides T (2007) Chromatin modifications and their function. Cell 128(4):693–705

Lattal KM, Barrett RM, Wood MA (2007) Systemic or intrahippocampal delivery of histone deacetylase inhibitors facilitates fear extinction. Behav Neurosci 121(5):1125–1131

Lee JL, Di Ciano P, Thomas KL, Everitt BJ (2005) Disrupting reconsolidation of drug memories reduces cocaine-seeking behavior. Neuron 47(6):795–801

Levenson JM, Sweatt JD (2005) Epigenetic mechanisms in memory formation. Nat Rev Neurosci 6(2):108–118

Levenson JM, O'Riordan KJ, Brown KD, Trinh MA, Molfese DL, Sweatt JD (2004) Regulation of histone acetylation during memory formation in the hippocampus. J Biol Chem 279(39):40545–40559

Levenson JM, Roth TL, Lubin FD, Miller CA, Huang IC, Desai P, Malone LM, Sweatt JD (2006) Evidence that DNA (cytosine-5) methyltransferase regulates synaptic plasticity in the hippocampus. J Biol Chem 281(23):15763–15773

Likhtik E, Popa D, Apergis-Schoute J, Fidacaro GA, Pare D (2008) Amygdala intercalated neurons are required for expression of fear extinction. Nature 454(7204):642–645
Loftus EF (1979) The malleability of human memory. Am Sci 67(3):312–320
Loftus EF (2005) Planting misinformation in the human mind: a 30-year investigation of the malleability of memory. Learn Mem 12(4):361–366
Lubin FD, Sweatt JD (2007) The IkappaB kinase regulates chromatin structure during reconsolidation of conditioned fear memories. Neuron 55(6):942–957
Lubin FD, Roth TL, Sweatt JD (2008) Epigenetic regulation of BDNF gene transcription in the consolidation of fear memory. J Neurosci 28(42):10576–10586
Maddox SA, Schafe GE (2011a) The activity-regulated cytoskeletal-associated protein (Arc/Arg3.1) is required for reconsolidation of a Pavlovian fear memory. J Neurosci 31(19):7073–7082
Maddox SA, Schafe GE (2011b) Epigenetic alterations in the lateral amygdala are required for reconsolidation of a Pavlovian fear memory. Learn Mem 18(9):579–593
Maddox SA, Monsey MS, Schafe GE (2011) Early growth response gene 1 (Egr-1) is required for new and reactivated fear memories in the lateral amygdala. Learn Mem 18(1):24–38
Maddox SA, Watts CS, Schafe GE (2013a) p300/CBP histone acetyltransferase activity is required for newly acquired and reactivated fear memories in the lateral amygdala. Learn Mem 20(2):109–119
Maddox SA, Watts CW, Schafe GE (2013b) A naturally-occurring histone acetyltransferase inhibitor derived from *Garcinia indica* impairs newly acquired and reactivated fear memories. PLoS One 8(1), e54463
Maddox SA, Watts CS, Schafe GE (2014) DNA methyltransferase activity is required for memory-related neural plasticity in the lateral amygdala. Neurobiol Learn Mem 107:93–100
Malkani S, Rosen JB (2000) Specific induction of early growth response gene 1 in the lateral nucleus of the amygdala following contextual fear conditioning in rats. Neuroscience 97(4):693–702
Malkani S, Wallace KJ, Donley MP, Rosen JB (2004) An egr-1 (zif268) antisense oligodeoxynucleotide infused into the amygdala disrupts fear conditioning. Learn Mem 11(5):617–624
Marek R, Coelho CM, Sullivan RK, Baker-Andresen D, Li X, Ratnu V, Dudley KJ, Meyers D, Mukherjee C, Cole PA, Sah P, Bredy TW (2011) Paradoxical enhancement of fear extinction memory and synaptic plasticity by inhibition of the histone acetyltransferase p300. J Neurosci 31(20):7486–7491
Martin C, Zhang Y (2005) The diverse functions of histone lysine methylation. Nat Rev Mol Cell Biol 6(11):838–849
Milad MR, Quirk GJ (2002) Neurons in medial prefrontal cortex signal memory for fear extinction. Nature 420(6911):70–74
Milad MR, Rauch SL, Pitman RK, Quirk GJ (2006) Fear extinction in rats: implications for human brain imaging and anxiety disorders. Biol Psychol 73(1):61–71
Miller CA, Sweatt JD (2007) Covalent modification of DNA regulates memory formation. Neuron 53(6):857–869
Miller CA, Campbell SL, Sweatt JD (2008) DNA methylation and histone acetylation work in concert to regulate memory formation and synaptic plasticity. Neurobiol Learn Mem 89(4):599–603
Miller CA, Gavin CF, White JA, Parrish RR, Honasoge A, Yancey CR, Rivera IM, Rubio MD, Rumbaugh G, Sweatt JD (2010) Cortical DNA methylation maintains remote memory. Nat Neurosci 13(6):664–666
Milner B, Squire LR, Kandel ER (1998) Cognitive neuroscience and the study of memory. Neuron 20(3):445–468
Milton AL, Lee JL, Butler VJ, Gardner R, Everitt BJ (2008) Intra-amygdala and systemic antagonism of NMDA receptors prevents the reconsolidation of drug-associated memory and impairs subsequently both novel and previously acquired drug-seeking behaviors. J Neurosci 28(33):8230–8237
Miranda TB, Jones PA (2007) DNA methylation: the nuts and bolts of repression. J Cell Physiol 213(2):384–390

Mizuno K, Dempster E, Mill J, Giese KP (2012) Long-lasting regulation of hippocampal Bdnf gene transcription after contextual fear conditioning. Genes Brain Behav 11:651–659
Monsey MS, Ota KT, Akingbade IF, Hong ES, Schafe GE (2011) Epigenetic alterations are critical for fear memory consolidation and synaptic plasticity in the lateral amygdala. PLoS One 6(5), e19958
Myers KM, Davis M (2007) Mechanisms of fear extinction. Mol Psychiatry 12(2):120–150
Nader K (2003) Memory traces unbound. Trends Neurosci 26(2):65–72
Nader K, Schafe GE, Le Doux JE (2000) Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. Nature 406(6797):722–726
Oike Y, Hata A, Mamiya T, Kaname T, Noda Y, Suzuki M, Yasue H, Nabeshima T, Araki K, Yamamura K (1999) Truncated CBP protein leads to classical Rubinstein-Taybi syndrome phenotypes in mice: implications for a dominant-negative mechanism. Hum Mol Genet 8(3):387–396
Oliveira AM, Wood MA, McDonough CB, Abel T (2007) Transgenic mice expressing an inhibitory truncated form of p300 exhibit long-term memory deficits. Learn Mem 14(9):564–572
Oliveira AM, Estevez MA, Hawk JD, Grimes S, Brindle PK, Abel T (2011) Subregion-specific p300 conditional knock-out mice exhibit long-term memory impairments. Learn Mem 18(3):161–169
Parsons RG, Gafford GM, Baruch DE, Riedner BA, Helmstetter FJ (2006) Long-term stability of fear memory depends on the synthesis of protein but not mRNA in the amygdala. Eur J Neurosci 23(7):1853–1859
Pavlov IP (1927) Conditioned reflexes. Oxford University Press, London
Penke Z, Morice E, Veyrac A, Gros A, Chagneau C, LeBlanc P, Samson N, Baumgartel K, Mansuy IM, Davis S, Laroche S (2014) Zif268/Egr1 gain of function facilitates hippocampal synaptic plasticity and long-term spatial recognition memory. Philos Trans R Soc Lond B Biol Sci 369(1633):20130159
Peters J, Dieppa-Perea LM, Melendez LM, Quirk GJ (2010) Induction of fear extinction with hippocampal-infralimbic BDNF. Science 328(5983):1288–1290
Pitman RK, Shalev AY, Orr SP (2000) Posttraumatic stress disorder: emotion, conditioning, & memory. In: Gazzaniga MS (ed) The new cognitive neurosciences. MIT Press, Cambridge
Poirier R, Cheval H, Mailhes C, Garel S, Charnay P, Davis S, Laroche S (2008) Distinct functions of egr gene family members in cognitive processes. Front Neurosci 2(1):47–55
Quirk GJ, Likhtik E, Pelletier JG, Pare D (2003) Stimulation of medial prefrontal cortex decreases the responsiveness of central amygdala output neurons. J Neurosci 23(25):8800–8807
Razin A (1998) CpG methylation, chromatin structure and gene silencing-a three-way connection. EMBO J 17(17):4905–4908
Renthal W, Nestler EJ (2008) Epigenetic mechanisms in drug addiction. Trends Mol Med 14(8):341–350
Robleto K, Poulos AM, Thompson RF (2004) Brain mechanisms of extinction of the classically conditioned eyeblink response. Learn Mem 11(5):517–524
Rosenblum K, Meiri N, Dudai Y (1993) Taste memory: the role of protein synthesis in gustatory cortex. Behav Neural Biol 59(1):49–56
Roth TL, Sweatt JD (2009) Regulation of chromatin structure in memory formation. Curr Opin Neurobiol 19(3):336–342
Rudenko A, Tsai LH (2014a) Epigenetic modifications in the nervous system and their impact upon cognitive impairments. Neuropharmacology 80:70–82
Rudenko A, Tsai LH (2014b) Epigenetic regulation in memory and cognitive disorders. Neuroscience 264:51–63
Santini E, Ge H, Ren K, Pena de Ortiz S, Quirk GJ (2004) Consolidation of fear extinction requires protein synthesis in the medial prefrontal cortex. J Neurosci 24(25):5704–5710
Sara SJ (2000) Retrieval and reconsolidation: toward a neurobiology of remembering. Learn Mem 7(2):73–84
Schafe GE (2014) Epigenetics: a new frontier for post-traumatic stress disorder? Epigenomics 6(2):157–159

Siedlecki P, Zielenkiewicz P (2006) Mammalian DNA methyltransferases. Acta Biochim Pol 53(2):245–256

Sims RJ 3rd, Nishioka K, Reinberg D (2003) Histone lysine methylation: a signature for chromatin function. Trends Genet 19(11):629–639

Sparks FT, Spanswick SC, Lehmann H, Sutherland RJ (2013) Neither time nor number of context-shock pairings affect long-term dependence of memory on hippocampus. Neurobiol Learn Mem 106:309–315

Stafford JM, Raybuck JD, Ryabinin AE, Lattal KM (2012) Increasing histone acetylation in the hippocampus-infralimbic network enhances fear extinction. Biol Psychiatry 72(1):25–33

Stefanko DP, Barrett RM, Ly AR, Reolon GK, Wood MA (2009) Modulation of long-term memory for object recognition via HDAC inhibition. Proc Natl Acad Sci U S A 106(23):9447–9452

Swank MW, Sweatt JD (2001) Increased histone acetyltransferase and lysine acetyltransferase activity and biphasic activation of the ERK/RSK cascade in insular cortex during novel taste learning. J Neurosci 21(10):3383–3391

Tronson NC, Taylor JR (2007) Molecular mechanisms of memory reconsolidation. Nat Rev Neurosci 8(4):262–275

Tronson NC, Wiseman SL, Olausson P, Taylor JR (2006) Bidirectional behavioral plasticity of memory reconsolidation depends on amygdalar protein kinase A. Nat Neurosci 9(2):167–169

Tsankova N, Renthal W, Kumar A, Nestler EJ (2007) Epigenetic regulation in psychiatric disorders. Nat Rev Neurosci 8(5):355–367

Turner BM (2002) Cellular memory and the histone code. Cell 111(3):285–291

Valor LM, Pulopulos MM, Jimenez-Minchan M, Olivares R, Lutz B, Barco A (2011) Ablation of CBP in forebrain principal neurons causes modest memory and transcriptional defects and a dramatic reduction of histone acetylation but does not affect cell viability. J Neurosci 31(5):1652–1663

Varga-Weisz PD, Becker PB (1998) Chromatin-remodeling factors: machines that regulate? Curr Opin Cell Biol 10(3):346–353

Vecsey CG, Hawk JD, Lattal KM, Stein JM, Fabian SA, Attner MA, Cabrera SM, McDonough CB, Brindle PK, Abel T, Wood MA (2007) Histone deacetylase inhibitors enhance memory and synaptic plasticity via CREB: CBP-dependent transcriptional activation. J Neurosci 27(23):6128–6140

Wang L, Tang Y, Cole PA, Marmorstein R (2008) Structure and chemistry of the p300/CBP and Rtt109 histone acetyltransferases: implications for histone acetyltransferase evolution and function. Curr Opin Struct Biol 18(6):741–747

Wei W, Coelho CM, Li X, Marek R, Yan S, Anderson S, Meyers D, Mukherjee C, Sbardella G, Castellano S, Milite C, Rotili D, Mai A, Cole PA, Sah P, Kobor MS, Bredy TW (2012) p300/CBP-associated factor selectively regulates the extinction of conditioned fear. J Neurosci 32(35):11930–11941

Wood MA, Kaplan MP, Park A, Blanchard EJ, Oliveira AM, Lombardi TL, Abel T (2005) Transgenic mice expressing a truncated form of CREB-binding protein (CBP) exhibit deficits in hippocampal synaptic plasticity and memory storage. Learn Mem 12(2):111–119

Wood MA, Attner MA, Oliveira AM, Brindle PK, Abel T (2006) A transcription factor-binding domain of the coactivator CBP is essential for long-term memory and the expression of specific target genes. Learn Mem 13(5):609–617

Yang XJ, Seto E (2007) HATs and HDACs: from structure, function and regulation to novel strategies for therapy and prevention. Oncogene 26(37):5310–5318

Yeh SH, Lin CH, Gean PW (2004) Acetylation of nuclear factor-kappaB in rat amygdala improves long-term but not short-term retention of fear memory. Mol Pharmacol 65(5):1286–1292

Zhao Z, Fan L, Frick KM (2010) Epigenetic alterations regulate estradiol-induced enhancement of memory consolidation. Proc Natl Acad Sci U S A 107(12):5605–5610

Zhao Z, Fan L, Fortress AM, Boulware MI, Frick KM (2012) Hippocampal histone acetylation regulates object recognition and the estradiol-induced enhancement of object recognition. J Neurosci 32(7):2344–2351

# Chapter 9
# Matrix Metalloproteinase 9 (MMP-9) in Learning and Memory

**Ewelina Knapska and Leszek Kaczmarek**

**Abstract** **Matrix metalloproteinase 9** (MMP-9) is a protein only recently recognized as pivotal for neuronal plasticity, learning, and memory. MMP-9, together with its endogenous inhibitor TIMP-1, compose a perisynaptically operating enzymatic system involved in the dynamic remodeling of the extracellular matrix via cleavage of numerous extracellular substrates, including growth factor precursors, cell surface receptors, and adhesion molecules. In this chapter we present an overview of the data available on MMP-9 involvement in long term potentiation (LTP, a model of neuronal plasticity), learning and memory. The data show that MMP-9 is required for formation of late LTP, although not in all pathways that were studied. Moreover, MMP-9 activation in specific brain structures following learning of different behavioral tasks has been shown. Studies with inhibitors and genetic ablation of MMP-9 demonstrate that it contributes to the mechanisms underlying memory formation. However, its involvement differ between the tasks with respect to the anatomical location and the temporal pattern, suggesting specific role of MMP-9 in learning and memory. In particular, MMP-9 has been found indispensable for appetitive and spatial memory formation, whereas aversive learning was normal in mice missing MMP-9 activity. Notably, hippocampal LTP (implicated in spatial learning) was disturbed by MMP-9 inhibition, similarly to LTP evoked in the basal and central nuclei of the amygdala, supposedly supporting appetitive memory. In contrast, no deficit in lateral amygdala LTP was observed under conditions of impaired MMP-9 activity. Thus, an interesting avenue of research arises and a more detailed investigation of various molecular mechanisms operating within various brain structures is required.

**Keywords** Matrix metaloproteinase 9 (MMP-9) • LTP • Behavioral Training • Appetitive learning • Amygdala • Hippocampus

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## 9.1 Introduction

Despite a myriad of molecules proposed to play a role in the brain plasticity, little is known about extracellular matrix (ECM), and cell adhesion molecules (CAMs) as well as the activity of extracellular enzymes, involved in, among others, proteolysis that allow for ECM/CAMs modifications. The ECM has been recognized as a critical factor affecting synapses, as it ensheathes them, forming a fourth synaptic element (in addition to pre- and post-synapse, and glial invaginations). The ECM/CAMs also span the synaptic cleft, providing a transsynaptic adhesive apparatus. It has been suggested that steric constraints imposed by the ECM/CAMs limit synaptic dynamics (Fawcett 2009; Gundelfinger et al. 2010; Tsien 2013). However, most recent studies argue for a more multi-faceted view. Specifically, proteolytic activity may arise from the ECM/CAMs cryptic ligand(s) that activate cell surface receptors and initiate intracellular signaling cascade(s) (Dityatev et al. 2010; Rivera et al. 2010; Sonderegger and Matsumoto-Miyai 2014).

These data are especially prominent in the case of matrix metalloproteinases (MMPs) and their endogenous inhibitors, tissue inhibitors of metalloproteinases (TIMPs), and in particular MMP-9 and TIMP-1. MMPs are pericellularly acting endopeptidases that play an essential role in the dynamic remodeling of the ECM via cleavage of numerous extracellular substrates, including growth factor precursors, cell surface receptors, and adhesion molecules (Nagase and Woessner 1999; Sternlicht and Werb 2001; Mott and Werb 2004; Ethell and Ethell 2007; Michaluk and Kaczmarek 2007).

It should also be emphasized that MMP-9 is a subject to a very complex regulation at the level of transcription, mRNA translocation, translation and activation, as it is released extracellularly in a latent, pro-form with the enzymatic site covered by a propeptide that has to be cleaved off. It is of great interest that at the gene expression level, MMP-9 as well as TIMP-1 are controlled by transcription factor AP-1, including the c-Fos protein (Jaworski et al. 1999; Kaczmarek et al. 2002; Rylski et al. 2009; Ganguly et al. 2013; Kuzniewska et al. 2013), whose expression and function in learning and memory has been well documented (Kaczmarek 1993; Kaczmarek et al. 2002; Ramirez et al. 2013).

In neurons, MMP-9 mRNA, protein and enzymatic activity can be detected at the dendritic spines that harbor postsynaptic domains of excitatory synapses (Konopacki et al. 2007; Sbai et al. 2008; Wilczynski et al. 2008; Gawlak et al. 2009). Importantly, MMP-9 may affect the spine morphology, being involved in a transition of small spines, possibly carrying silent synapses to convert them initially to elongated protrusions and then towards larger, mushroom spines and formation of the spine head protrusions (Wang et al. 2008; Bilousova et al. 2009; Michaluk et al. 2011; Szepesi et al. 2013, 2014). Furthermore, MMP-9 mRNA has been found to be translocated towards dendrites in a glutamate-dependent manner and then locally translated (Szklarczyk et al. 2002; Konopacki et al. 2007; Konopka et al. 2010; Dziembowska et al. 2012; Janusz et al. 2013). Following neuronal stimulation MMP-9 is locally released to reveal its enzymatic activity towards such extracullular (including

extrasynaptic) protein targets as β-dystroglycan, neuroligin-1 and ICAM-5 (Michaluk et al. 2007; Tian et al. 2007; Sbai et al. 2008; Conant et al. 2011; Peixoto et al. 2012). It is also of note that MMP-9 increases lateral dendritic mobility of NMDA receptors and their physiological properties (Michaluk et al. 2009; Gorkiewicz et al. 2010). Surprisingly, excessive exogenous MMP-9 does not affect gross appearance of the ECM (Michaluk et al. 2009). In addition, its effects on NMDAR mobility, as well as the ability to affect spine morphology, are abolished by the integrin β1 blocking antibody (Michaluk et al. 2009, 2011), which suggests that integrin-dependent signaling, rather than massive alteration of the ECM, is responsible for mediating MMP-9 functions at the synapse.

## 9.2 Quality of the Experimental Tools and Methods

To fully appreciate the meaning of the results implicating MMP-9 in synaptic plasticity, learning and memory, it is helpful to consider the experimental toolbox available to study this enzyme. It should be stressed that MMPs, similarly to other proteases form a very evolutionally divergent family. Therefore, it is difficult to apply from one animal species to another, without careful testing, such tools as antibodies directed against MMP-9. This is of particular concern considering that many antibodies have been developed towards human protein but are often not of sufficient specificity for rodents. Fortunately, zymography approaches allow the specific detection of enzymatic activity of MMP-9. Cleaving gelatin has been of particular use in this regard, as this capacity is apparently shared only with MMP-2 among the MMPs active in the brain. Moreover, gelatin gel zymography permits detection of molecular species with defined size, thus differentiating MMP-9 from MMP-2.

Notably, MMP-9 is just 1 of over 20 enzymes with similar protein structures and greatly overlapping activities. Hence, it has been a daunting, and not yet satisfactorily solved task, to produce specific inhibitors, that act solely against MMP-9. Other molecular blockers, such as anti-sense oligonucleotides are notorious for their off-target side effects, whereas RNAi technology has not apparently been efficiently applied in the MMP-9 brain studies. Thus, genetic ablation (MMP-9 knockout, KO) appears to be the most specific approach to prove MMP-9 involvement in the phenomenon under study. Nevertheless, only global, unconditional, non-inducible knockouts are available for MMP-9 and compensatory effects should be taken into consideration.

## 9.3 MMP-9 Activity and Long-Term Potentiation

Long-term potentiation (LTP) is suggested as a mechanism underlying memory acquisition and storage. This term refers to the phenomenon of the activity-dependent increase in synaptic efficacy. Brief strong and repeated (e.g., tetanic)

stimulation results in an enhancement of the response to subsequent stimuli with respect to the response to the same stimuli before stimulation. There are several forms of LTP which differ as far as their location, stimulation paradigms and pharmacological properties are concerned (Bliss and Collingridge 1993). Three major categories of LTP with respect to its duration are distinguished: LTP1 with an average decay constant of approximately 2 h, LTP2 with a time constant of approximately 4 days and LTP3 with an average decay constant of approximately 23 days (the latter two are often collectively called late- or L-LTP in contrast to early- or E-LTP; Racine et al. 1983; Abraham and Otani 1991).

Extensive studies on LTP evoked in hippocampal slices by stimulation of Schaffer collaterals projecting from pyramidal neurons of CA3 to CA1 revealed distinct cellular and molecular mechanisms that underlie different temporal phases of hippocampal LTP. The induction phase (E-LTP), which involves combined activation of Src family tyrosine kinases, protein kinase A, protein kinase C, MAPK and, in particular, $Ca^{2+}$/calmodulin-dependent protein kinase II, results in phosphorylation of several synaptic proteins, e.g., glutamate receptor-gated ion channels, and the enhancement of the subsequent postsynaptic current (Soderling and Derkach 2000). Longer-lasting forms of LTP require gene transcription and protein synthesis (Krug et al. 1984; Otani et al. 1989; Kaczmarek 1992).

The role of MMP-9 in induction and maintenance of LTP has been investigated mainly at synapses formed between Schaffer collaterals and area CA1 dendrites in the hippocampus, in rat and mouse brain slices (Nagy et al. 2006), and in rats in vivo (Bozdagi et al. 2007; Nagy et al. 2007), see Table 9.1. The studies showed that stimulation that induces protein synthesis-dependent L-LTP in the CA1 area increased active MMP-9 protein levels and proteolytic activity in this structure. Moreover, the increase in proteolytic activity was abolished by either the general MMP inhibitor GM6001 or MMP-2/9 inhibitor (Nagy et al. 2006). Notably, no changes in MMP-9 mRNA level or distribution were observed 90 min following induction of LTP (Bozdagi et al. 2007). On the other hand, however, Dziembowska et al. (2012) reported that LTP evoked by the perforant path stimulation in the hippocampal dentate gyrus resulted in MMP-9 mRNA accumulation in the dendritic tree of the granule neurons. This discrepancy could be explained by longer time exploited by Dziembowska and co-workers, consistent with MMP-9 gene regulation by AP-1 (see also Ganguly et al. 2013; Kuzniewska et al. 2013), as well as by different locations of the recorded LTP. Importantly, pharmacological inhibition of MMP-9 (in slices with GM6001, a broad spectrum MMP inhibitor or MMP-2/-9 inhibitor, in vivo with MMP-2/-9 inhibitor or MMP-9 blocking antibodies) prevented induction of late-phase LTP (Nagy et al. 2006, Bozdagi et al. 2007, Wang et al. 2008). Moreover, chemical LTP, protein synthesis-dependent potentiation evoked by chemical stimulation with Sp-cAMPS, was abolished by pre-incubation with GM6001 or MMP-2/-9 inhibitors (Nagy et al. 2006). Furthermore, Meighan et al. (2006) observed that in rat slices treated with FN-439, a MMP inhibitor, administered 10 min before tetanization and continued throughout measurement of LTP induction and maintenance, the initial robust potentiation returned to baseline levels within 60 min. Conant et al. (2010) observed reduced potentiation for 30 min following

**Table 9.1** MMP-9 activity and long-term potentiation

| LTP pathway | Preparation | MMP-9 activity manipulation | Observed effects | Reference |
|---|---|---|---|---|
| Schaffer collaterals–CA1 area | Rat brain slices | | ⇑ Active MMP-9 protein levels and proteolytic activity | Nagy et al. (2006), Bozdagi et al. (2007) |
| | Rats *in vivo* | | No changes in MMP-9 mRNA level or distribution | Bozdagi et al. (2007) |
| | Rats *in vivo* | | | |
| | Rat brain slices | Blocked by GM6001, FN-439 or MMP-2/9 inhibitors | Blocked induction of L-LTP, early potentiation decrease with time | Nagy et al. (2006), Meighan et al. (2006), Wang et al. (2008) |
| | Mouse brain slices | Blocked by NNGH inhibitor | Lower E-LTP | Conant et al. (2010) |
| | Rats *in vivo* | Blocked by MMP-2/9 inhibitor or MMP-9 blocking antibodies | Blocked induction of L-LTP | Bozdagi et al. (2007), Nagy et al. (2007) |
| | Mouse brain slices | Null mutants | ⇓ Magnitude and duration of LTP | Nagy et al. (2006) |
| | | Transgenic mice overexpressing MMP-9 | ⇑ Magnitude and duration of L-LTP | Fragkouli et al. (2012) |
| Mossy fiber (MF)–CA3 area | Rat brain slices | | ⇑ Pro- and active- forms of MMP-9 and proteolytic activity | Wiera et al. (2012) |
| | Rats *in vivo* | | ⇑ MMP-9 mRNA | Dziembowska et al. (2012) |
| | Rat brain slices | Blocked by FN-439 inhibitor | Disruption of L-LTP formation | Wójtowicz and Mozrzymas (2010) |
| | | Blocked by FN-439 or NNGH inhibitors | Attenuation of LTP induction and disruption of L-LTP formation | Wójtowicz and Mozrzymas (2014) |
| | | Blocked by MMP-2/-9 inhibitor | Disruption of L-LTP formation | |
| | Mouse brain slices | Null mutants | disruption of L-LTP formation, rescued by auto active MMP-9 | Wiera et al. (2013) |
| | Rat brain slices | Transgenic rats overexpressing MMP-9 | Disruption of L-LTP formation | |
| Hippocampus-prefrontal cortex | Rats *in vivo* | Adenovirally driven | Blocked formation of L-LTP, early potentiation intact | Okulski et al. (2007) |
| | Rat brain slices | Overexpression of TIMP-1 | Blocked formation of L-LTP | |
| | | S24994 inhibitor | | |
| Chemical LTP | Hippocampal neuronal cultures | Blocked by GM6001 or | ⇑ MMP-9 activity measured by zymography and beta-dystroglycan cleavage | Szepesi et al. (2013) |
| | | MMP-2/9 inhibitors | ⇓ Neuronal activity evoked by cLTP | Niedringhaus et al. (2012) |

GM6001, FN-439, NNGH, TIMP-1—inhibitors of MMPs; MMP-2/9—inhibitor of MMP-2 and MMP-9; S24994—inhibitor of MMP-9

tetanic stimulation in mouse hippocampal slices treated with MMP inhibitor NNGH. It should also be noted that proteolytically active MMP-9 induced potentiation in the CA1 area (Nagy et al. 2006), and this potentiation mutually occluded, and was occluded by, tetanically evoked potentiation (Bozdagi et al. 2007). In contrast, in slices from MMP-9 null-mutant mice, LTP in the CA1 was impaired both in magnitude and duration, and adding recombinant active MMP-9 to null-mutant slices restored the magnitude and duration of LTP to the wild-type levels (Nagy et al. 2006). On the other hand, there were no effects on MMP-9 activation or effects of blocking of MMP-9 activity with inhibitors in other forms of short-term synaptic potentiation (such as early phase of LTP and paired-pulse facilitation) or long-lasting synaptic depression (LTD) (Nagy et al. 2006, Bozdagi et al. 2007). There were also no effects of adding MMP-2/9 inhibitor to potentiated slices 60–90 min after induction of LTP by either chemical or tetanic stimulation (Nagy et al. 2006). On the other hand, Fragkouli et al. (2012) showed that transgenic mice overexpressing MMP-9 show increased late phase of LTP, in both magnitude and duration.

In summary, all of the studies described above show that MMP-9 is required for formation of late LTP in the CA1 field of the hippocampus. Moreover, it has been shown, by monitoring excitatory postsynaptic potentials (EPSPs) and spine size, that MMP-9 is involved concomitantly in synaptic potentiation and spine enlargement (Wang et al. 2008). When MMP activity was eliminated during theta-stimulation-induced LTP by applying MMP-2/9 or GM6001 inhibitors, both spine enlargement and synaptic potentiation were transient, suggesting that MMP-mediated extracellular remodeling during LTP plays a crucial role in establishing persistent modifications in both synapse structure and function, and thus is critical for learning and memory (Wang et al. 2008).

Besides the Schaffer collaterals–CA1 pathway, the role of MMP-9 in LTP has been studied in the mossy fiber (MF)–CA3 pathway in rat and mouse brain slices. The mechanisms of induction and expression of LTP in the CA3, in contrast to LTP in the CA1, are largely presynaptic. It has been shown that LTP induction in the MF–CA3 pathway correlated with increased expression and activity of MMP-9 (Wiera et al. 2012). In situ zymography showed that LTP induction was associated with increased gelatinase activity in the cytoplasm of the hilar and CA3 neurons. Further analysis demonstrated that this effect was due to synthesis and activation of MMP-9. In contrast, MMP-2 was not affected by LTP induction. Wójtowicz and Mozrzymas (2010), using broad spectrum MMP inhibitor, FN439, showed that inhibition of MMP proteolytic activity before and up to 30 min, but not 60 min, after LTP induction resulted in disruption of the late phase of LTP. The paired-pulse facilitation ratio, post-tetanic potentiation and burst-like pattern of mossy fiber stimulation were not changed in the presence of FN-439, suggesting that the temporal pattern of presynaptic transmitter release was not significantly affected by MMPs inhibition (Wójtowicz and Mozrzymas 2010). In the following study, Wójtowicz and Mozrzymas (2014), using different MMP inhibitors, showed that inhibition of MMP by broad spectrum inhibitors, FN439 or NNGH, but not by more specific MMP-2/-9 inhibitor, interfered also with LTP induction. They also observed that MMP-2/-9 inhibitor impaired formation of late LTP in the CA3 when LTP was induced by the paired activity of the mossy fibers and recurrent collateral synapses

but not when it was induced by the tetanization protocol. Another study (Wiera et al. 2013) has shown that formation of L-LTP in the MF–CA3 pathway was significantly impaired in the brain slices from MMP-9 knock-out mice and transgenic rats overexpressing MMP-9. In contrast, both transgenic models showed a normal basal synaptic transmission and short-term plasticity. Presence of exogenous, active MMP-9 restored LTP in knock-out mice, whereas in the wild-type mice MMP-9 excess impaired LTP. Importantly, auto-active MMP-9 induced potentiation in the MF–CA3 pathway in slices form wild-type mice. To sum up, the results show that MMP-9 is crucial for formation of L-LTP in the MF–CA3 pathway, though the detailed mechanisms of its involvement may differ from those observed in the CA3–CA1 projection.

The role of MMP-9 activity in LTP has been also studied at the hippocampal-prefrontal cortex pathway. Okulski et al. (2007) observed that adenovirally driven overexpression of TIMP-1, a MMP inhibitor, in the prelimbic part of the prefrontal cortex applied before the tetanic stimulation blocked late phase of LTP (6 h after the stimulation) in rats. In contrast, TIMP-1 overexpression did not affect early phase of LTP (up to 1 h after the stimulation). Moreover, S24994, a MMP-9 inhibitor, prevented late phase of LTP in the medial prefrontal rat slices when applied before but not after high frequency stimulation.

Moreover, MMP-9 dependency of LTP has been also studied in different pathways within the amygdala (Gorkiewicz et al. 2015). It has been shown that LTP in the basal and central but not in the lateral amygdala is affected by MMP-9 knock-out. These results were also confirmed in brain slices treated with a specific MMP-9 inhibitor. The data suggest that MMP-9 plays different roles in synaptic plasticity in different nuclei of the amygdala.

Recent studies have extended the role of MMPs, including MMP-9, to hippocampal cell cultures, by employing a chemical LTP protocol. Chemical LTP (cLTP) refers to a model system developed by Otmakhov et al. (2004) in hippocampal slice cultures that was also successfully applied for dissociated hippocampal neuronal cultures (Oh et al. 2006; Molnár 2011). Niedringhaus et al. (2012) have demonstrated an enhancement of neuronal activity within in vitro networks of hippocampal neurons subjected to the cLTP protocol and found that this effect was abrogated by treatment with MMP inhbitors (as well as by antibody directed against integrin β1). Szepesi et al. (2013) have shown that MMP-9, but not MMP-2, was activated during cLTP. Recently, Szepesi et al. (2014) provided evidence that cLTP produces increases in the spine volume, GluA1 receptor synaptic accumulation and immobilization and all those effects required MMP activity.

## 9.4 MMP-9 Expression and Activity following Behavioral Training

Many studies have shown MMP-9 activation in specific brain structures following learning of different behavioral tasks (see Table 9.2). Most of the studies employed behavioral trainings, such as the Morris water maze, inhibitory avoidance,

**Table 9.2** MMP-9 expression and activity following behavioral training

| Learning paradigm | Animal | Brain structures and MMP-9 level change | | References |
|---|---|---|---|---|
| | | Pro form | Active form | |
| Morris water maze task | Rat (male) | | Hippocampus (⇑), prefrontal cortex (⇑), cerebellum (⇔) | Wright et al. (2003) |
| | Rat (female) | Hippocampus (⇑), prefrontal cortex (⇓), piriform cortex (⇔) | Hippocampus (⇑), prefrontal cortex (⇑), piriform cortex (⇔) | Wright et al. (2004) |
| | Rat (male) | Hippocampus (⇓) | Hippocampus (⇑) | Meighan et al. (2006) |
| Object recognition task | Rat (female) | Hippocampus (⇔), prefrontal cortex (⇑), piriform cortex (⇔) | Hippocampus (⇑), prefrontal cortex (⇑), piriform cortex (⇑) | Wright et al. (2004) |
| Head-shake response habituation | Rat (female) | Hippocampus (⇔), prefrontal cortex (⇔), piriform cortex (⇔) | Hippocampus (⇑), prefrontal cortex (⇑), piriform cortex (⇔) | Wright et al. (2004) |
| | | Hippocampus (⇑), prefrontal cortex (⇔), piriform cortex (⇓), cerebellum (⇔) | Hippocampus (⇑), prefrontal cortex (⇓), piriform cortex (⇔), cerebellum (⇔) | Wright et al. (2006) |
| Inhibitory avoidance learning | Rat (male) | Hippocampus (⇑) | Hippocampus (⇑) | Nagy et al. (2007) |
| Contextual fear conditioning | Mouse (male) | | Prefrontal cortex (⇑), hippocampus (⇑), basolateral amygdala (⇑) | Ganguly et al. (2013) |
| Methamphetamine-induced behavioral sensitization | Rat (male) | Nucleus accumbens (⇑), frontal cortex (⇑) | Nucleus accumbens (⇑), frontal cortex (⇑) | Mizoguchi et al. (2007a, b) |
| Reinstatement of cocaine-primed conditioned place preference | Rat (male) | | Medial prefrontal cortex (⇑), dorsal hippocampus (⇔) | Brown et al. (2008) |
| Appetitively motivated place learning in IntelliCage | Mouse (female) | | Central amygdala (⇑) | Knapska et al. (2013) |

contextual fear conditioning, object exploration and response habituation, relying on the activity of the hippocampus. The studies have also provided some information on MMP-9 activation in the cerebral cortex. In all investigated tasks, increased expression of pro and/or active form of MMP-9 has been observed in the hippocampus.

However, significant between-studies differences in expression of two forms of MMP-9 in the hippocampus, as well as in the cortex have been observed. In the following paragraphs we will summarize the methods and results of these studies, in an attempt to find the possible reasons of the observed discrepancies.

MMP-9 expression and activity has been measured following the **Morris water maze** training. The Morris water maze is performed in a round pool of water in which a platform is submerged beneath the surface. When placed in the maze, the animal is expected to find the platform. The learning process can be assessed by measuring time it takes for each animal to find the platform over a number of trials and, once the platform has been removed, the percentage of time spent in the quadrant in which the platform was previously located (probe trial). In this task, the animal must learn the spatial position of the invisible platform based on cues from the surrounding environment. In the study of Wright et al. (2004) the rats were trained for 4 consecutive days (5 trials/day) and sacrificed 4 h following completion of the training. The levels of pro and active forms of MMP-9 were measured by Western blotting and compared to home cage controls. The level of pro form of MMP-9 was increased in the hippocampus and decreased in the prefrontal cortex, but no change in the piriform cortex was observed. In contrast, the levels of active form of MMP-9 were increased in both the hippocampus and the prefrontal cortex. Again, no change in the piriform cortex was observed. The results confirmed earlier observations of the study by Wright et al. (2003), in which rats were trained in the Morris water maze (5 trials/day) and sacrificed immediately after the final trial of the second, fourth or sixth day of the training. Comparing the level of expression of active form of MMP-9 (measured by zymography) to the home cage controls they found increased activity in both the hippocampus and prefrontal cortex, but no change in the cerebellum. In the study of Meighan et al. (2006), the rats were subjected to 1, 2, 3, or 4 days of the training in the Morris water maze (5 trials/day) and sacrificed 3 h after the final trial. The levels of expression of pro- and active forms of MMP-9 were measured in the hippocampus (by Western blotting) and compared to home cage controls and water maze-yoked animals (which swam for an equal amount of time in the maze in a darkened room without an escape platform). The levels of pro MMP-9 were significantly lower than those in controls throughout the training, whereas the levels of active MMP-9 (defined by the authors as two forms of the molecular weight much lower that typically considered to MMP-9) were increased only at the beginning of the training (day 1). The latter change was accompanied by increased MMP-9 mRNA level observed after the first day of the training. The behavioral protocols of the Morris water maze training used in the abovementioned studies were similar, thus differences in obtained results should be attributed to diverse MMP-9 measuring methods and the different delay periods between the behavioral training and harvesting of the brains.

Other hippocampus-dependent behavioral training used in the studies on MMP-9 involvement in learning is a **novel object recognition task**, in which animals explore objects located in the arena (usually either a square or a round box) and, after some delay, either one of the familiar objects is replaced with a novel object (the non-spatial, object-change task) or one of the familiar objects is moved to a new location in the arena (the spatial, place-change task). The time spent on exploration

of the novel and familiar objects was measured. In the study of Wright et al. (2004) the rats were tested on seven 6-min trials, separated by 3 min. intervals. Both spatial and non-spatial changes of object configuration were tested. The brains were harvested 4 h following the completion of the task. The levels of pro and active forms of MMP-9 were measured by Western blotting and compared to home cage controls. The level of pro form of MMP-9 was increased in the prefrontal cortex, while no changes in the hippocampus and piriform cortex were observed. In contrast, the levels of active form of MMP-9 were increased in both the hippocampus, and the prefrontal and piriform cortices.

MMP-9 expression and activity have also been measured following **habituation of head-shake response** (HSR). The HSR is a rapid rotation of the head in response to a mild air stimulus applied to the ear. When the air stimuli are presented repeatedly the HSR is habituated, i.e., its frequency decreases. Thus, it is an example of nonassociative learning, with memory traces presumably located in the hippocampus, cerebral cortex and cerebellum. In the experiments of Wright et al. (2004, 2006), female rats were subjected to two habituation sessions with different between-sessions intervals. With the increase of the delay between the sessions the animals displayed more HSRs, at the 24 h reaching the level not statistically different from their performance at the beginning of the training. In the first study the brains were removed 4 h following the second session of habituation (there were two sessions, 5 min apart) and the levels of active and pro form of MMP-9 were measured by Western blotting. There were no changes in the levels of pro form of MMP-9 in the hippocampus, as well as prefrontal and piriform cortices. In contrast, there were significant increases of active form of MMP-9 in the hippocampus and prefrontal cortex, but no change in the piriform cortex as compared to the home cage controls. In another study, Wright et al. (2006) measured levels of pro and active forms of MMP-9 in the hippocampus, prefrontal cortex, piriform cortex and cerebellum of female rats, which underwent habituation of the HSR. The rats were subjected to two sessions of HSR habituation with 5 min, 2 h, 6 h and 24 h intervals between them. The brains were harvested immediately following the second session of habituation. There were no differences in the level of MMP-9 expression (measured by Western blotting) when comparing the HSR habituated rats, yoked controls (which, during the habituation sessions, were in close proximity to the experimental animals but were not subjected to habituation procedure) and home cage controls across four time intervals for any of the four brain structures examined. In contrast, comparing the level of pro and active forms of MMP-9 (measured by zymography) of HSR habituated rats (two sessions with 2 h interval) and yoked controls, Wright and co-workers observed that active MMP-9 was increased in the hippocampus and decreased in the prefrontal cortex, and pro MMP-9 was increased in the hippocampus and decreased in the piriform cortex of the HSR habituated rats. The main differences between the two studies on MMP-9 activation following the HSR habituation seem to be the temporal pattern of the behavioral trainings and the delay between the sessions as well as methods of measurement of MMP-9 level.

The subsequent studies employed one-trial hippocampus-dependent learning paradigms to show the dynamics of MMP-9 expression/activation. Nagy et al.

(2007) investigated MMP-9 activation following **inhibitory avoidance learning**, a procedure in which an animal is supposed to learn to avoid one of the two compartments of the experimental cage because it was previously associated with a footshock. They observed that protein levels and proteolytic activity of MMP-9 became elevated in the hippocampus by 6 h, peaked at 12–24 h, and then declined to baseline values by 72 h following the training, as compared to the unpaired and home cage controls. The dynamics of MMP-9 activation has been also investigated following **contextual fear conditioning** (Ganguly et al. 2013), a training paradigm in which an animal receives an inescapable footshock or footshocks in an experimental cage and thus it learns to fear this cage. Comparing MMP-9 expression in the brains of mice exploring novel environment and mice exposed to a footshock in a novel context, they showed maximal increase of MMP-9 expression at 2–12 h following fear conditioning in the hippocampus, prefrontal cortex and basolateral amygdala. Furthermore, using an indirect method to measure MMP-9 activity (employing its ability to cleave its native substrate β-dystroglycan), they showed that MMP-9 activity paralleled its expression. The observed changes in MMP-9 expression and activity were accompanied by MMP-9 transcription.

Much less is known about MMP-9 activation following appetitively (positively) motivated behaviors. MMP-9 expression and activity levels have been studied in the animal models of drug dependence. For instance, Mizoguchi et al. (2007b) investigated MMP-9 activity following **behavioral sensitization induced by repeated methamphetamine treatment**. Methamphetamine, a drug of abuse, exerts both acute and long-lasting effects on psychomotor behavior. It has been proposed that the mechanisms underlying behavioral sensitization (that can be observed after subsequent injections of the drug, given at the same dose, as increased locomotor activity) involve synaptic plasticity. In this study, rats, which were given methamphetamine at a dose of 2 mg/kg for 5 days, were killed after 2 or 24 h after the final administration. Compared to controls, which received saline injections, the level of MMP-9 protein as well as its activity were increased in the frontal cortex and nucleus accumbens both after 2 and 24 h. Increased activity of MMP-9 has been also shown in the medial prefrontal cortex of rats subjected to the **reinstatement of cocaine-primed conditioned place preference** (CPP). In the CPP paradigm one of the compartments of the cage is associated with a reward, i.e., cocaine injections, then the learned preference for this compartment is extinguished by repeated presentations to this compartment without a reward, and finally and animal receives a remainder, i.e., is injected with cocaine. Such procedure results in reinstatement of the previously extinguished preference. MMP-9 activity was measured in rats that underwent training for cocaine-CPP followed by extinction sessions and either saline- or cocaine-priming injections (Brown et al. 2008). Cocaine-induced reinstatement significantly increased MMP-9 activity at 1, 3 and 24 h after injection, as compared with saline controls. No changes in MMP-9 occurred in the dorsal hippocampus. Since no changes in MMP-9 activity were observed 1 h after reinstatement in animals given no extinction sessions, the observed response seems to be specific to the reinstatement phenomenon. Moreover, it has been recently shown (Knapska et al. 2013) that beta-dystroglycan, native substrate of MMP-9, is cleaved in the central

amygdala 2 h following the appetitively, but not aversively motivated place learning. **Place preference** was obtained by serving sweetened water as a reward while for **place avoidance** an air-puff was used as a punishment associated with one compartment of the cage. The training was carried out **in the IntelliCage** system (for the detailed description of the tests see the following section).

To sum up, many studies have shown MMP-9 activation in specific brain structures following learning of different behavioral tasks. Interestingly, the anatomical locations and the temporal patterns differ with the tasks. The observed discrepancies in the results of the studies can be explained by the differences in MMP-9 measuring methods and the delay between the behavioral training and harvesting of the brains. Such studies, though correlative in nature, paved the way for investigation of brain structure-specific function of MMP-9.

## 9.5 Effects of Manipulation of MMP-9 Expression or Activity on Learning

Various techniques of blocking MMP-9 expression or activity showed its involvement in learning and memory (see Table 9.3). In the studies, which results are described in detail below, the MMP-9 knock-out mice, MMP-9 inhibitors and antisense oligonucleotides to MMP-9 has been used as methods of affecting MMP-9 activity.

The MMP-9 role in **context and cued fear conditioning** has been tested in MMP-9 knock-out mice. In a context fear conditioning, an animal receives an inescapable footshock(s) in an experimental cage and thus it associates the cage with the footshock. Then it is brought back to the home cage and after some delay it is again exposed to the same apparatus and tested without the footshock. The memory of the situation is examined by measuring the freezing reaction during the retention test. The procedure of cued fear conditioning is similar, but the original exposure to a footshock is accompanied by a clear sensory stimulus, e.g., a tone, and the testing is carried out in an experimental cage different from the training chamber, but in the presence of the conditional stimulus. In the study of Nagy et al. (2006) the mice were trained in one context (30 s tone co-terminating with a 2 s, 0.75 mA footshock) and tested 24 h later in the training context (context test), and 30 h later in another context, in which freezing response was measured to a 3 min tone presentation (cued test). The results showed an impairment of contextual fear memory in MMP-9 deficient mice, but no change in cued fear memory was observed in these animals as compared to wild-type littermates.

It has also been shown that infusions of FN-439, a broad spectrum MMP inhibitor, to the hippocampus attenuated learning in the **Morris water maze** (the procedure described in the previous section, Meighan et al. 2006). The inhibitor was infused into the lateral ventricle twice, 10 min before and 3 h after the training, to block basal, as well as training-induced MMP-9 activity. The infusions were made for 4 days of the training and resulted in increased latency to find the hidden platform throughout this period. In contrast, when the pre-training infusion was omitted on the second day of the training, commencement of the inhibitor infusions after the

**Table 9.3** Effects of manipulation of MMP-9 expression or activity on learning

| Learning paradigm | Animal | MMP-9 activity manipulation method | Brain structures affected | Effects on learning | References |
|---|---|---|---|---|---|
| *Inhibition of MMP-9 activity* | | | | | |
| Morris water maze task | Rat (male) | Broad spectrum MMP inhibitor FN-439 | Infused to the ventricle | Impaired | Meighan et al. (2006) |
| | | Antisense oligonucleotides to MMP-3 and MMP-9 | Dorsal hippocampus | | |
| | Rat (male) | Broad spectrum MMP inhibitor FN-439 | Infused to the ventricle or dorsal hippocampus | Impaired | Wright et al. (2007) |
| Inhibitory avoidance learning | Rat (male) | MMP-2/-9 inhibitor | Dorsal hippocampus | Impaired | Nagy et al. (2007) |
| Contextual fear conditioning | Mouse | MMP-9 null-mutant mice | Whole brain | Impaired | Nagy et al. (2006) |
| Cued fear conditioning | Mouse | MMP-9 null-mutant mice | Whole brain | Intact | Nagy et al. (2006) |
| Methamphetamine-induced behavioral sensitization and conditioned place preference | Mouse | MMP-9-null-mutant mice | Whole brain | Impaired | Mizoguchi et al. (2007a, b) |
| | Rat (male) | MMP-2/-9 inhibitor or doxycycline (MMP inhibitor) | Infusion pump in frontal cortex or right ventricle | Blocked | Mizoguchi et al. (2007a, b) |
| Appetitvely motivated place and discrimination learning | Mouse (female) | MMP-9 null-mutant mice/TIMP-1 nanoparticles | whole brain/ central amygdala | Impaired | Knapska et al. (2013) |
| Aversively motivated place and discrimination learning | Mouse (female) | MMP-9 null-mutant mice/TIMP-1 nanoparticles | Whole brain/ central amygdala | Intact | Knapska et al. (2013) |
| *MMP-9 overexpression* | | | | | |
| Morris water maze task | Mouse (male and female) | Overexpression of MMP9 | Whole brain | Improved | Fragkouli et al. (2012) |
| Novel object recognition task | Mouse (male and female) | Overexpression of MMP9 | Whole brain | Improved | Fragkouli et al. (2012) |

training on subsequent days 3 and 4 did not inhibit task acquisition any further, suggesting that MMPs are primarily involved during early stages of learning. Similarly, infusion of antisense oligonucleotides to MMP-3 and MMP-9 to the dorsal hippocampi of rats 12 h before the training (modified procedure, 15 trials for 1 day),

significantly attenuated learning (as measured by latency to find the hidden platform, Meighan et al. 2006). Very similar results were obtained by Wright et al. (2007), who infused rats with FN-439 inhibitor either intra-cerebroventricularly or to the dorsal hippocampus 20 min prior to and again 10 min after the behavioral training for 8 days of the training (5 trials/day) and observed attenuated learning in the Morris water maze regardless of the place of infusion.

The effect of MMP-9 inhibition has also been tested by Nagy et al. (2007), in the **inhibitory avoidance task**. The authors infused the rats with MMP-2/9-specific inhibitor to the dorsal hippocampus at certain time points following the training. Then they tested memory retention (measured as a latency to enter the compartment of the cage, in which the animals previously received a footshock) after 24 h, a time when animals normally exhibit strong memory of the task. They observed that infusions of the inhibitor at 1.5, 3.5 and 9.5 h significantly impaired inhibitory avoidance memory. Moreover, they showed that MMP inhibition at 1.5 and 9.5 h following the training resulted in persistent memory deficits, which could be observed at 72 h. Nagy and co-workers (2007) also demonstrated that the rats infused with the inhibitor relearned the task effectively when drug-free and subsequently displayed robust inhibitory avoidance memory, proving that hippocampal integrity was uncompromised by the inhibitor infusion. The time points after the training when the inhibitor was infused were chosen based on the dynamics of MMP-9 proteolytic activity induced by the inhibitory avoidance training (described in the previous section). Thus, taken together, these data suggest that MMP-9 in the hippocampus contributes to the mechanisms underlying inhibitory avoidance memory formation, as well as that MMP-9 is involved in Morris water maze learning.

Mizoguchi et al. (2007b) showed that both **behavioral sensitization induced by repeated methamphetamine treatment** and **methamphetamine induced conditioned place preference** were decreased in MMP-9 deficient mice as compared to wild-type mice. Moreover, they showed that the lack of MMP-9 gene affects methamphetamine induced dopamine release and uptake in the nucleus accumbens. Similar effects were observed when doxycycline (MMP inhibitor) or MMP-2/-9 inhibitor were infused through the osmotic pump connected to the cannula placed in the right ventricle or the frontal cortex (Mizoguchi et al. 2007a). The data suggest that MMP-9 may play a role in functional and, possibly, structural changes in the nucleus accumbens and the frontal cortex, which are related to methamphetamine induced behavioral sensitization and place preference learning.

It has recently been shown (Knapska et al. 2013) that genetic ablation of MMP-9 impaired appetitively motivated conditioning (i.e., **place preference and appetitively motivated discriminative learning in the IntelliCages**). On the other hand, lack of MMP-9 did not impair **place avoidance and aversively motivated discrimination** in the same apparatus. In this study an automated test system (IntelliCage, New-Behavior AG; http://www.newbehavior.com) that allows for assessing both spatial and operant behavior in group-housed mice, as well as for balancing appetitive and aversive conditions, was used. The IntelliCage consists of four operant learning chambers that fit into the corners of a large housing cage. Access into the chamber is provided via a tubular antenna reading the transponder

codes that allow recognition of individual animals. The chamber contains two openings permitting access to the ends of drinking tubes. Access to the tubes can be barred by small, motorized doors, which can be opened by nosepoking. Aversive stimulation can be delivered in the form of air-puffs directed to the head of the mouse through tubing controlled by electric valves. The whole setup is controlled by a microcomputer recognizing visits, nosepokes, and tube-lickings of individual mice, and delivering reward (by opening the access to water or sweetened water after a nosepoke) or punishment (an air-puff or access to quinine solution), according to preprogrammed schedules depending on the assignment of the mice to different test groups within the same cage. In the place preference test, the mice were supposed to learn that sweetened water was accessible by nosepoking in only one of the four corners within the large cage, whereas in the aversive training, they were learning to avoid a corner, in which nosepokes were punished with an air-puff. In another learning paradigm, less spatial in nature and relying on a different kind of aversive reinforcement, the mice had to discriminate between bottles (placed on two sides of the same corner) that contained either sweetened or tap water (i.e., an appetitive task) or bitter (quinine-adulterated) and tap water (i.e., an aversive task). Appetitively motivated but not aversively motivated tasks described above were impaired in MMP-9 knock-out mice as compared with wild type littermates. Importantly, these results were mirrored when extracellular MMP-9 activity was blocked in the central amygdala by nanoparticle-based release of its inhibitor, recombinant TIMP-1, showing that local MMP-9 activity in the central amygdala is important for the appetitive, but not aversive, learning.

Overexpression of MMP-9 in the brain has been also shown to affect neuronal plasticity and learning. Fragkouli et al. (2012) observed that transgenic mice overexpressing MMP-9 in the brain (full-length MMP-9 under the platelet-derived growth factor B-chain promoter) displayed enhanced discrimination between the novel and familiar objects in the **novel object recognition task**. The animals were familiarized with the objects in three 5-min trials with a 15-min inter-trial intervals and tested in the 5-min probe trial that took place 15 min after the last learning trial. Moreover, the transgenic MMP-9 mice outperformed the wild-type controls in the **Morris water maze**, finding either visible or hidden platform faster during acquisition and reversal learning (mice were trained for 3 or 4 consecutive days for acquisition and reversal phases, respectively, 5 trials/day). Moreover, transgenic mice spent more time than the control animals in the quadrant in which the platform was previously located during the probe trial that followed reversal learning. The probe trial took place 1 h after the last learning trial. The observed changes in behavior were accompanied by increased dendritic spine density in hippocampal and cortical pyramidal neurons in the transgenic mice subjected to behavioral training and prolonged maintenance of LTP in the CA1 field of the hippocampus in slices from the transgenic mice.

In summary, it has been shown that changing MMP-9 expression or activity affects learning and memory. Interestingly, the existing data suggest that the role of MMP-9 in learning is brain structure- and behavioral training-specific (Nagy et al. 2006; Knapska et al. 2013). For instance, Nagy et al. (2006) showed that MMP-9 null mutant mice were impaired in contextual (hippocampus-dependent)

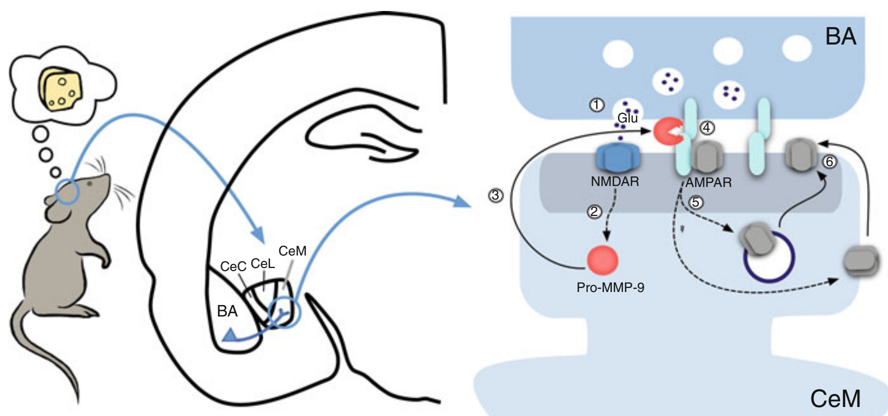


**Fig. 9.1** Involvement of MMP-9 differs between the tasks with respect to the anatomical location, suggesting specific role of MMP-9 in learning and memory. For instance, MMP-9 is activated in the central amygdala (Ce) by appetitively but not aversively motivated learning (Knapska et al. 2013), moreover also late-LTP established at the connection from the basal amygdala (BA) to Ce requires MMP-9 (Gorkiewicz et al. 2015). Possible molecular scenario for the excitatory synaptic plasticity in Ce, underlying the appetitive learning (shown on the left) is following: (*1*) glutamate (Glu) released at the BA axons activates NMDA receptors, NMDAR; (*2*) in result MMP-9 is locally produced (MMP-9 is dendritically/synaptically translated, Dziembowska et al. 2012) and (*3*) released in a pro-form into the synaptic cleft to (*4*) cleave postsynaptic cell adhesion molecules (e.g., beta-dystroglycan, neuroligin-1, nectin-3, see: Michaluk et al. 2007; Peixoto et al. 2012; van der Kooj et al. 2014) that form transsynaptic adhesive apparatus by linking to either neurexins or nectin-1. Next, AMPA receptors accumulate (GluA1 in particular), either through (*5*) exocytosis or (*6*) trafficking and immobilization (Szepesi et al. 2014)

fear conditioning. In contrast, the mice were not deficient in cued fear conditioning (which is more basolateral amygdala-dependent). Furthermore, Knapska et al. (2013) showed that both MMP-9 null mutation and specific blocking of MMP-9 activity with TIMP-1 inhibitor in the central amygdala result in an impairment of appetitively but not aversively motivated discrimination learning (Fig. 9.1). Such results suggest that, though MMP-9 can be abundantly upregulated by increased neuronal activity throughout the brain, its involvement in learning and memory is very specific.

## 9.6 Summary

MMP-9 is a protein only recently recognized as pivotal for neuronal plasticity, learning, and memory. Its activation in specific brain structures following learning of different behavioral tasks has been shown. Studies with inhibitors and genetic ablation of MMP-9 demonstrate that it contributes to the mechanisms underlying memory formation. Interestingly, its involvement differ between the tasks with respect to the anatomical location and the temporal pattern, suggesting specific role

of MMP-9 in learning and memory. Most recent studies on hippocampal neurons show a possible mechanistic role of MMP-9 in synaptic plasticity at the level of structural modulation of spine morphology.

Recent studies on gene polymorphisms that affect the MMP-9 expression as well as studies directly measuring MMP-9 levels pointed out the possible role of MMP-9 in psychiatric disorders, such as drug and alcohol addiction, major depression, bipolar disorder, and schizophrenia (Mash et al. 2007; Rybakowski et al. 2009a, b; Samochowiec et al. 2010; Domenici et al. 2010). The existing data suggest that the role of MMP-9 in learning is brain structure- and behavioral training-specific. Thus, an interesting avenue of research arises and a more detailed investigation of various molecular mechanisms operating within various brain structures in patients suffering from psychiatric disorders is required. Importantly, a large set of therapeutic inhibitors of matrix metalloproteinases was tested in clinical trials for anti-cancer therapy. Some of them (Baker et al. 2002; Vihinen et al. 2005) may probably be used in the treatment of psychiatric disorders as well.

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## References

Abraham WC, Otani S (1991) Macromolecules and the maintenance of long-term potentiation. In: Morrell F (ed) Kindling and synaptic plasticity. Birkhauser, Boston, pp 92–109

Baker AH, Edwards DR, Murphy G (2002) Metalloproteinase inhibitors: biological actions and therapeutic opportunities. J Cell Sci 115:3719–3727

Bilousova TV, Dansie L, Ngo M, Aye J, Charles JR, Ethell DW, Ethell IM (2009) Minocycline promotes dendritic spine maturation and improves behavioural performance in the fragile X mouse model. J Med Genet 46:94–102

Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361:31–39

Bozdagi O, Nagy V, Kwei KT, Huntley GW (2007) In vivo roles for matrix metalloproteinase-9 in mature hippocampal synaptic physiology and plasticity. J Neurophysiol 98:334–344

Brown TE, Forquer MR, Harding JW, Wright JW, Sorg BA (2008) Increase in matrix metalloproteinase-9 levels in the rat medial prefrontal cortex after cocaine reinstatement of conditioned place preference. Synapse 62:886–889

Conant K, Lonskaya I, Szklarczyk A, Krall C, Steiner J, Maguire-Zeiss K, Lim ST (2011) Methamphetamine-associated cleavage of the synaptic adhesion molecule intercellular adhesion molecule-5. J Neurochem 118:521–532

Conant K, Wang Y, Szklarczyk A, Dudak A, Mattson MP, Lim ST (2010) Matrix metalloproteinase-dependent shedding of intercellular adhesion molecule-5 occurs with long-term potentiation. Neuroscience 166:508–521

Dityatev A, Schachner M, Sonderegger P (2010) The dual role of the extracellular matrix in synaptic plasticity and homeostasis. Nat Rev Neurosci 11:735–746

Domenici E, Willé DR, Tozzi F, Prokopenko I, Miller S, McKeown A, Brittain C, Rujescu D, Giegling I, Turck CW, Holsboer F, Bullmore ET, Middleton L, Merlo-Pich E, Alexander RC, Muglia P (2010) Plasma protein biomarkers for depression and schizophrenia by multi analyte profiling of case-control collections. PLoS One 5, e9166

Dziembowska M, Milek J, Janusz A, Rejmak E, Romanowska E, Gorkiewicz T, Tiron A, Bramham CR, Kaczmarek L (2012) Activity-dependent local translation of matrix metalloproteinase-9. J Neurosci 32:14538–14547

Ethell IM, Ethell DW (2007) Matrix metalloproteinases in brain development and remodeling: synaptic functions and targets. J Neurosci Res 85:2813–2823

Fawcett JW (2009) Recovery from spinal cord injury: regeneration, plasticity and rehabilitation. Brain 132:1417–1418

Fragkouli A, Papatheodoropoulos C, Georgopoulos S, Stamatakis A, Stylianopoulou F, Tsilibary EC, Tzinia AK (2012) Enhanced neuronal plasticity and elevated endogenous sAPPα levels in mice over-expressing MMP9. J Neurochem 121:239–251

Ganguly K, Rejmak E, Mikosz M, Nikolaev E, Knapska E, Kaczmarek L (2013) Matrix metalloproteinase (MMP) 9 transcription in mouse brain induced by fear learning. J Biol Chem 288:20978–20991

Gawlak M, Górkiewicz T, Gorlewicz A, Konopacki FA, Kaczmarek L, Wilczynski GM (2009) High resolution in situ zymography reveals matrix metalloproteinase activity at glutamatergic synapses. Neuroscience 158:167–176

Gorkiewicz T, Szczuraszek K, Wyrembek P, Michaluk P, Kaczmarek L, Mozrzymas JW (2010) Matrix metalloproteinase-9 reversibly affects the time course of NMDA-induced currents in cultured rat hippocampal neurons. Hippocampus 20:1105–1108

Gorkiewicz T, Balcerzyk M, Kaczmarek L, Knapska E (2015) Matrix metalloproteinase (MMP-9) is indispensable for long term potentiation in the central and basal but not in the lateral nucleus of the amygdala. Front Cell Neurosci 9:73

Gundelfinger ED, Frischknecht R, Choquet D, Heine M (2010) Converting juvenile into adult plasticity: a role for the brain's extracellular matrix. Eur J Neurosci 31:2156–2165

Janusz A, Milek J, Perycz M, Pacini L, Bagni C, Kaczmarek L, Dziembowska M (2013) The Fragile X mental retardation protein regulates matrix metalloproteinase 9 mRNA at synapses. J Neurosci 33:18234–18241

Jaworski J, Biedermann IW, Lapinska J, Szklarczyk A, Figiel I, Konopka D, Nowicka D, Filipkowski RK, Hetman M, Kowalczyk A, Kaczmarek L (1999) Neuronal excitation-driven and AP-1-dependent activation of tissue inhibitor of metalloproteinases-1 gene expression in rodent hippocampus. J Biol Chem 274:28106–28112

Kaczmarek L (1992) Expression of c-fos and other genes encoding transcription factors in long-term potentiation. Behav Neural Biol 57:263–266

Kaczmarek L (1993) Molecular biology of vertebrate learning: is c-fos a new beginning? J Neurosci Res 34:377–381

Kaczmarek L, Lapinska-Dzwonek J, Szymczak S (2002) Matrix metalloproteinases in the adult brain physiology: a link between c-Fos, AP-1 and remodeling of neuronal connections? EMBO J 21:6643–6648

Knapska E, Lioudyno V, Kiryk A, Mikosz M, Górkiewicz T, Michaluk P, Gawlak M, Chaturvedi M, Mochol G, Balcerzyk M, Wojcik DK, Wilczynski GM, Kaczmarek L (2013) Reward learning requires activity of matrix metalloproteinase-9 in the central amygdala. J Neurosci 33:14591–14600

Konopacki FA, Rylski M, Wilczek E, Amborska R, Detka D, Kaczmarek L, Wilczynski GM (2007) Synaptic localization of seizure-induced matrix metalloproteinase-9 mRNA. Neuroscience 150:31–39

Konopka W, Kiryk A, Novak M, Herwerth M, Parkitna JR, Wawrzyniak M, Kowarsch A, Michaluk P, Dzwonek J, Arnsperger T, Wilczynski G, Merkenschlager M, Theis FJ, Köhr G, Kaczmarek L, Schütz G (2010) MicroRNA loss enhances learning and memory in mice. J Neurosci 30:14835–14842

Krug M, Lössner B, Ott T (1984) Anisomycin blocks the late phase of long-term potentiation in the dentate gyrus of freely moving rats. Brain Res Bull 13:39–42

Kuzniewska B, Rejmak E, Malik AR, Jaworski J, Kaczmarek L, Kalita K (2013) Brain-derived neurotrophic factor induces matrix metalloproteinase 9 expression in neurons via the serum response factor/c-Fos pathway. Mol Cell Biol 33:2149–2162

Mash DC, ffrench-Mullen J, Adi N, Qin Y, Buck A, Pablo J (2007) Gene expression in human hippocampus from cocaine abusers identifies genes which regulate extracellular matrix remodeling. PLoS One 2, e1187
Meighan SE, Meighan PC, Choudhury P, Davis CJ, Olson ML, Zornes PA, Wright JW, Harding JW (2006) Effects of extracellular matrix-degrading proteases matrix metalloproteinases 3 and 9 on spatial learning and synaptic plasticity. J Neurochem 96:1227–1241
Michaluk P, Kaczmarek L (2007) Matrix metalloproteinase-9 in glutamate-dependent adult brain function and dysfunction. Cell Death Differ 14:1255–1258
Michaluk P, Kolodziej L, Mioduszewska B, Wilczynski GM, Dzwonek J, Jaworski J, Gorecki DC, Ottersen OP, Kaczmarek L (2007) Beta-dystroglycan as a target for MMP-9, in response to enhanced neuronal activity. J Biol Chem 282:16036–16041
Michaluk P, Mikasova L, Groc L, Frischknecht R, Choquet D, Kaczmarek L (2009) Matrix metalloproteinase-9 controls NMDA receptor surface diffusion through integrin beta1 signaling. J Neurosci 29:6007–6012
Michaluk P, Wawrzyniak M, Alot P, Szczot M, Wyrembek P, Mercik K, Medvedev N, Wilczek E, De Roo M, Zuschratter W, Muller D, Wilczynski GM, Mozrzymas JW, Stewart MG, Kaczmarek L, Wlodarczyk J (2011) Influence of matrix metalloproteinase MMP-9 on dendritic spine morphology. J Cell Sci 124:3369–3380
Mizoguchi H, Yamada K, Mouri A, Niwa M, Mizuno T, Noda Y, Nitta A, Itohara S, Banno Y, Nabeshima T (2007a) Role of matrix metalloproteinase and tissue inhibitor of MMP in methamphetamine-induced behavioral sensitization and reward: implications for dopamine receptor down-regulation and dopamine release. J Neurochem 102:1548–1560
Mizoguchi H, Yamada K, Niwa M, Mouri A, Mizuno T, Noda Y, Nitta A, Itohara S, Banno Y, Nabeshima T (2007b) Reduction of methamphetamine-induced sensitization and reward in matrix metalloproteinase-2 and -9-deficient mice. J Neurochem 100:1579–1588
Molnár E (2011) Long-term potentiation in cultured hippocampal neurons. Semin Cell Dev Biol 22:506–513
Mott JD, Werb Z (2004) Regulation of matrix biology by matrix metalloproteinases. Curr Opin Cell Biol 16:558–564
Nagase H, Woessner JF Jr (1999) Matrix metalloproteinases. J Biol Chem 274:21491–21494
Nagy V, Bozdagi O, Matynia A, Balcerzyk M, Okulski P, Dzwonek J, Costa RM, Silva AJ, Kaczmarek L, Huntley GW (2006) Matrix metalloproteinase-9 is required for hippocampal late-phase long-term potentiation and memory. J Neurosci 26:1923–1934
Nagy V, Bozdagi O, Huntley GW (2007) The extracellular protease matrix metalloproteinase-9 is activated by inhibitory avoidance learning and required for long-term memory. Learn Mem 14:655–664
Niedringhaus M, Chen X, Dzakpasu R, Conant K (2012) MMPs and soluble ICAM-5 increase neuronal excitability within in vitro networks of hippocampal neurons. PLoS One 7, e42631
Oh MC, Derkach VA, Guire ES, Soderling TR (2006) Extrasynaptic membrane trafficking regulated by GluR1 serine 845 phosphorylation primes AMPA receptors for long-term potentiation. J Biol Chem 281:752–758
Okulski P, Jay TM, Jaworski J, Duniec K, Dzwonek J, Konopacki FA, Wilczynski GM, Sánchez-Capelo A, Mallet J, Kaczmarek L (2007) TIMP-1 abolishes MMP-9-dependent long-lasting long-term potentiation in the prefrontal cortex. Biol Psychiatry 62:359–362
Otani S, Marshall CJ, Tate WP, Goddard GV, Abraham WC (1989) Maintenance of long-term potentiation in rat dentate gyrus requires protein synthesis but not messenger RNA synthesis immediately post-tetanization. Neuroscience 28:519–526
Otmakhov N, Khibnik L, Otmakhova N, Carpenter S, Riahi S, Asrican B, Lisman J (2004) Forskolin-induced LTP in the CA1 hippocampal region is NMDA receptor dependent. J Neurophysiol 91:1955–1962
Peixoto RT, Kunz PA, Kwon H, Mabb AM, Sabatini BL, Philpot BD, Ehlers MD (2012) Transsynaptic signaling by activity-dependent cleavage of neuroligin-1. Neuron 76:396–409
Racine RJ, Milgram NW, Hafner S (1983) Long-term potentiation phenomena in the rat limbic forebrain. Brain Res 260:217–231

Ramirez S, Tonegawa S, Liu X (2013) Identification and optogenetic manipulation of memory engrams in the hippocampus. Front Behav Neurosci 7:226

Rivera S, Khrestchatisky M, Kaczmarek L, Rosenberg GA, Jaworski DM (2010) Metzincin proteases and their inhibitors: foes or friends in nervous system physiology? J Neurosci 30:15337–15357

Rybakowski JK, Skibinska M, Kapelski P, Kaczmarek L, Hauser J (2009a) Functional polymorphism of the matrix metalloproteinase-9 (MMP-9) gene in schizophrenia. Schizophr Res 109:90–93

Rybakowski JK, Skibinska M, Leszczynska-Rodziewicz A, Kaczmarek L, Hauser J (2009b) Matrix metalloproteinase-9 gene and bipolar mood disorder. Neuromolecular Med 11:128–132

Rylski M, Amborska R, Zybura K, Michaluk P, Bielinska B, Konopacki FA, Wilczynski GM, Kaczmarek L (2009) JunB is a repressor of MMP-9 transcription in depolarized rat brain neurons. Mol Cell Neurosci 40:98–110

Samochowiec A, Grzywacz A, Kaczmarek L, Bienkowski P, Samochowiec J, Mierzejewski P, Preuss UW, Grochans E, Ciechanowicz A (2010) Functional polymorphism of matrix metalloproteinase-9 (MMP-9) gene in alcohol dependence: family and case control study. Brain Res 1327:103–106

Sbai O, Ferhat L, Bernard A, Gueye Y, Ould-Yahoui A, Thiolloy S, Charrat E, Charton G, Tremblay E, Risso J-J, Chauvin J-P, Arsanto J-P, Rivera S, Khrestchatisky M (2008) Vesicular trafficking and secretion of matrix metalloproteinases-2, -9 and tissue inhibitor of metalloproteinases-1 in neuronal cells. Mol Cell Neurosci 39:549–568

Soderling TR, Derkach VA (2000) Postsynaptic protein phosphorylation and LTP. Trends Neurosci 23:75–80

Sonderegger P, Matsumoto-Miyai K (2014) Activity-controlled proteolytic cleavage at the synapse. Trends Neurosci 37(8):413–423

Sternlicht MD, Werb Z (2001) How matrix metalloproteinases regulate cell behavior. Annu Rev Cell Dev Biol 17:463–516

Szepesi Z, Bijata M, Ruszczycki B, Kaczmarek L, Wlodarczyk J (2013) Matrix metalloproteinases regulate the formation of dendritic spine head protrusions during chemically induced long-term potentiation. PLoS One 8, e63314

Szepesi Z, Hosy E, Ruszczycki B, Bijata M, Pyskaty M, Bikbaev A, Heine M, Choquet D, Kaczmarek L, Wlodarczyk J (2014) Synaptically released matrix metalloproteinase activity in control of structural plasticity and the cell surface distribution of GluA1-AMPA receptors. PLoS One 9, e98274

Szklarczyk A, Lapinska J, Rylski M, McKay RDG, Kaczmarek L (2002) Matrix metalloproteinase-9 undergoes expression and activation during dendritic remodeling in adult hippocampus. J Neurosci 22:920–930

Tian L, Stefanidakis M, Ning L, Van Lint P, Nyman-Huttunen H, Libert C, Itohara S, Mishina M, Rauvala H, Gahmberg CG (2007) Activation of NMDA receptors promotes dendritic spine development through MMP-mediated ICAM-5 cleavage. J Cell Biol 178:687–700

Tsien RY (2013) Very long-term memories may be stored in the pattern of holes in the perineuronal net. Proc Natl Acad Sci U S A 110:12456–12461

van der Kooj MA, Fantin M, Rejmak E, Grosse J, Zanoletti O, Fournier C, Ganguly K, Kalita K, Kaczmarek L, Sandi C (2014) Role for MMP-9 in stress-induced downregulation of nectin-3 in hippocampal CA1 and associated behavioural alterations. Nat Commun 5:4995

Vihinen P, Risto A-a, Kähäri V-M (2005) Matrix metalloproteinases as therapeutic targets in cancer. Curr Cancer Drug Targets 5:203–220

Wang X, Bozdagi O, Nikitczuk JS, Zhai ZW, Zhou Q, Huntley GW (2008) Extracellular proteolysis by matrix metalloproteinase-9 drives dendritic spine enlargement and long-term potentiation coordinately. Proc Natl Acad Sci U S A 105:19520–19525

Wiera G, Wójtowicz T, Lebida K, Piotrowska A, Drulis-Fajdasz D, Gomułkiewicz A, Gendosz D, Podhorska-OkołówM CM, Wilczyński G, Dzięgiel P, Kaczmarek L, Mozrzymas JW (2012) Long term potentiation affects intracellular metalloproteinases activity in the mossy fiber-CA3 pathway. Mol Cell Neurosci 50:147–159

Wiera G, Wozniak G, Bajor M, Kaczmarek L, Mozrzymas JW (2013) Maintenance of long-term potentiation in hippocampal mossy fiber-CA3 pathway requires fine-tuned MMP-9 proteolytic activity. Hippocampus 6:529–543

Wilczynski GM et al (2008) Important role of matrix metalloproteinase 9 in epileptogenesis. J Cell Biol 180:1021–1035

Wójtowicz T, Mozrzymas JW (2010) Late phase of long-term potentiation in the mossy fiber-CA3 hippocampal pathway is critically dependent on metalloproteinases activity. Hippocampus 8:917–921

Wójtowicz T, Mozrzymas JW (2014) Matrix metalloprotease activity shapes the magnitude of EPSPs and spike plasticity within the hippocampal CA3 network. Hippocampus 2:135–153

Wright JW, Masino AJ, Reichert JR, Turner GD, Meighan SE, Meighan PC, Harding JW (2003) Ethanol-induced impairment of spatial memory and brain matrix metalloproteinases. Brain Res 963:252–261

Wright JW, Murphy ES, Elijah IE, Holtfreter KL, Davis CJ, Olson ML, Muhunthan K, Harding JW (2004) Influence of hippocampectomy on habituation, exploratory behavior, and spatial memory in rats. Brain Res 1023:1–14

Wright JW, Meighan SE, Murphy ES, Holtfreter KL, Davis CJ, Olson ML, Benoist CC, Muhunthan K, Harding JW (2006) Habituation of the head-shake response induces changes in brain matrix metalloproteinases-3 (MMP-3) and -9. Behav Brain Res 174:78–85

Wright JW, Brown TE, Harding JW (2007) Inhibition of hippocampal matrix metalloproteinase-3 and -9 disrupts spatial memory. Neural Plast 2007:73813