Chapter 10
Synaptic Protein Degradation in Memory Reorganization

Bong-Kiun Kaang and Jun-Hyeok Choi

Abstract The ubiquitin-proteasome system (UPS) is a ubiquitous, major pathway of protein degradation that is involved in most cellular processes by regulating the abundance of certain proteins. Accumulating evidence indicates a role for the UPS in specific functions of neurons. In this chapter, we first introduce the role of the UPS in neuronal function and the mechanism of UPS regulation following synaptic activity. Then, we focus on the recently revealed, distinct role of the UPS in the destabilization of a reactivated memory. Finally, we discuss the physiological role of this destabilization process. The reactivated memory may undergo modification from the initial memory depending on the context in which the memory is reactivated, which we will term memory reorganization. We will introduce the role of the *protein degradation–dependent destabilization process* for memory reorganization and suggest a hypothetical model combining the recent findings.

Keywords E3 ubiquitin ligase • Long-term memory • Spine • Ubiquitin proteasome system

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10.1 Introduction

The ubiquitin-proteasome system (UPS) is a ubiquitous, major pathway of protein degradation that governs the turnover of proteins, thereby inevitably affecting every process in which proteins are involved. In the UPS, the small protein ubiquitin is covalently conjugated to a substrate protein by the serial action of the E1 ubiquitin-activating enzyme, the E2 ubiquitin-conjugating enzyme, and the E3 ubiquitin ligase. After a serial reaction to produce a polyubiquitin chain on the substrate, the polyubiquitinated substrate is directed to a large proteasome complex that manages the degradation. E3 ubiquitin ligase seems to be the major component that determines substrate specificity (Fig. 10.1). Emerging evidence indicates the critical involvement of protein degradation in specialized functions of the neurons. Ubiquitin-proteasome-dependent degradation is known to play important roles in the regulation of synaptogenesis and the elimination of synapses in the development (DiAntonio et al. 2001; Ding et al. 2007; Liao et al. 2004; Schaefer et al. 2000; van Roessel et al. 2004; Wan et al. 2000), maintenance, and modulation of neurotransmission functions (Arancibia-Carcamo et al. 2009; Bedford et al. 2001; Burbea et al. 2002; Colledge et al. 2003; Dreier et al. 2005; Haas et al. 2007; Juo and Kaplan 2004; Kato et al. 2005; Patrick et al. 2003; Speese et al. 2003; Tada et al. 2010; van Roessel et al. 2004; Willeumier et al. 2006; Yao et al. 2007) and the structural remodeling of the synapse (Cartier et al. 2009; Colledge et al. 2003; Hoogenraad et al. 2007; Hung et al. 2010; Pak and Sheng 2003). Also, recent findings indicate that the UPS can be regulated by neuronal activity, suggesting a specific role for the UPS in plastic changes of synaptic strength (Ehlers 2003; Bingol et al. 2010;

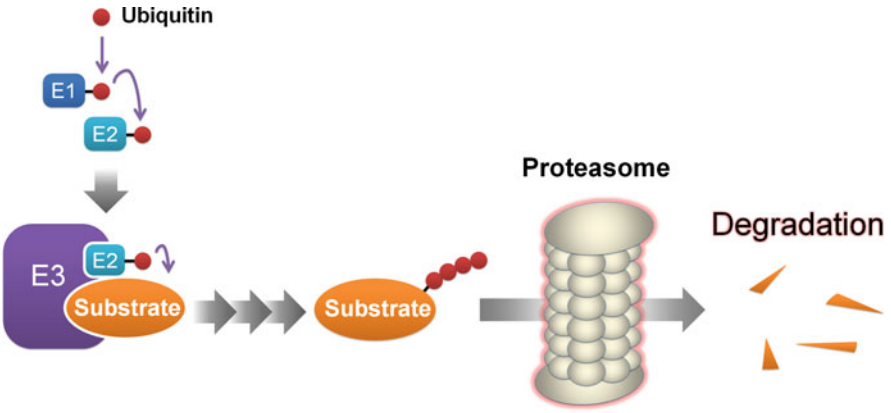

Fig. 10.1 *Mechanism of the ubiquitin–proteasome system.* Ubiquitin is first conjugated to E1 ubiquitin-activating enzyme (E1) in an ATP-dependent manner. The conjugated ubiquitin is then transferred to E2 ubiquitin-conjugating enzyme (E2). E3 ubiquitin ligase (E3) recognizes specific target proteins (substrates) and transfers and conjugates the ubiquitin from E2 to the substrate. E2 and E3 may also transfer the ubiquitin to a previously conjugated ubiquitin. After a serial reaction to produce a polyubiquitin chain on the substrate, the polyubiquitinated substrate is directed to a large proteasome complex that manages the degradation

Colledge et al. 2003; Deng and Lei 2007; Hou et al. 2006; Karpova et al. 2006; Kato et al. 2005; Pak and Sheng 2003; Patrick et al. 2003; Bingol and Schuman 2006; Djakovic et al. 2009; Fonseca et al. 2006; Shen et al. 2007).

In accordance with the findings on the role of the UPS in synaptic plasticity *in vitro*, recent *in vivo* studies show an involvement of the UPS in memory (Merlo and Romano 2007; Artinian et al. 2008; Wood et al. 2005; Lee et al. 2008; Lee 2008; Choi et al. 2010). Some of these findings suggest a distinct role of protein degradation in a specific step of reconsolidation (Lee et al. 2008; Lee 2008). Nader and colleagues (Nader et al. 2000) demonstrated that after a memory is retrieved, the previously consolidated memory becomes "labile" or sensitive to the amnesic effect of *protein synthesis inhibitors*, for a certain period of time. This indicates that the reactivated memory may have undergone an active destabilization process followed by a restabilization process, and this is termed reconsolidation. The early studies on reconsolidation focused on the consolidation-like restabilization process, which is mainly protein synthesis dependent (reviewed in Tronson and Taylor (2007), Nader and Hardt (2009), Dudai (2006)). However, the destabilization process is now demonstrated to rely on ubiquitin-proteasome-dependent degradation (Lee et al. 2008) (for a brief review, see Kaang et al. (2009)).

In this chapter, we will first discuss the specific role of the UPS in neuronal function and the mechanism for regulating the UPS following neuronal activity. Then, we will focus on recent studies exploring the distinct role of protein degradation as a mechanism of destabilization induced by the reactivation of a previously consolidated memory and also the significance of this process in memory reorganization.

10.2 The Ubiquitin-Proteasome System in Neurons

10.2.1 Regulation of Synapse Formation, Elimination, and Function by the UPS

Specific genes involved in the UPS are required for axon growth, synapse formation, and elimination. In *C. elegans*, Rpm-1, which is a subunit of the SCF ubiquitin ligase complex, is involved in axon growth and synaptogenesis (Schaefer et al. 2000). A mutant for this gene showed disorganized axon morphologies and presynaptic structures, while these phenotypes were rescued by expressing Rpm-1. FSN-1, another subunit of the SCF complex in *C. elegans,* was also shown to be involved in synapse formation (Liao et al. 2004). In the *Drosophila* neuromuscular junction (NMJ), a mutant for Highwire (a *Drosophila* homologue of Rpm-1) resulted in synapse outgrowth and expanded the extent of branches and the number of boutons (Wan et al. 2000). The overexpression of deubiquitinating protease fat facets resulted in a similar phenotype as the Highwire mutant in the *Drosophila* NMJ, suggesting that synapse formation may be regulated by the balance between positive and negative regulators of ubiquitination (DiAntonio et al. 2001). APC, another E3 ligase complex, has also been shown to be involved in synapse formation in the NMJ of *Drosophila* by

regulating the degradation of the scaffold protein liprin-α (van Roessel et al. 2004). Disrupting the functions of SCF complex subunits SKR-1, Cullin and SEL-10 in *C. elegans* also caused defects in synapse elimination. SKR-1-binding protein SYG-1 is shown to protect synapses from elimination by inhibiting the association between SKR-1 and SEL-10 (Ding et al. 2007).

There are also studies demonstrating that the UPS modulates presynaptic neurotransmission function. In the *Drosophila* NMJ, the UPS components are shown to regulate the level of the presynaptic and essential synaptic vesicle-priming protein DUNC-13. An inhibition of proteasome activity resulted in an accumulation of DUNC-13 and an increased presynaptic efficacy (Speese et al. 2003). Pharmacological inhibition of proteasome activity has demonstrated that the UPS also plays an important role in regulating synaptic transmission in mammalian presynaptic terminals. Using a fluorescent dye in a hippocampal neuron culture, it was shown that a 2-hour inhibition of proteasome activity increased the recycling pool of vesicles by 76%, with no change in the rate or total amount of dye release (Willeumier et al. 2006). SCRAPPER, a synapse-localized E3 ubiquitin ligase, was shown to bind and ubiquitinate RIM1, a modulator of presynaptic plasticity. Neurons from SCRAPPER-knockout mice showed an increased frequency of miniature excitatory postsynaptic currents that was rescued by the expression of exogenous SCRAPPER or the knockdown of RIM1 (Yao et al. 2007). A novel ubiquitin ligase, Fbxo45, selectively expressed in the nervous system, was demonstrated to regulate neurotransmission, likely by modulating the synaptic vesicle-priming factor Munc13-1 at the synapse (Tada et al. 2010).

Several studies have demonstrated that the level of GLR-1 glutamate receptor is regulated by the UPS in *C. elegans* (Burbea et al. 2002; Dreier et al. 2005; Juo and Kaplan 2004; van Roessel et al. 2004). By expressing the dominant-negative form of proteasome subunits postsynaptically in the *Drosophila* NMJ, it was shown that the proteasome regulates the abundance of GluRIIB-containing glutamate receptors, limiting the synaptic strength (Haas et al. 2007). Agonist-induced AMPA receptor internalization was also regulated by the ubiquitin-proteasome-dependent degradation of PSD-95 in mammalian neurons (Patrick et al. 2003; Colledge et al. 2003). By expressing a dominant-negative form of Fbx2 that directs the ubiquitination of NR1 in hippocampal neuron, increased NR1 levels and NMDA receptor currents were seen in an activity-dependent manner, suggesting that the UPS is involved in the homeostatic control of synaptic NR1 (Kato et al. 2005). There is also evidence showing that the level of $GABA_A$ receptor, the key receptor for inhibitory transmission, is regulated by the UPS (Arancibia-Carcamo et al. 2009; Bedford et al. 2001).

Besides the regulation of receptors that directly mediates synaptic transmission, UPS also regulates the architectural components of the synapse. Serum-inducible kinase (SNK) was induced in hippocampal neurons by synaptic activity and was targeted to dendritic spines. Then SNK phosphorylated spine-associated Rap guanosine triphosphatase–activating protein (SPAR, a postsynaptic actin regulatory protein), which was then subjected to ubiquitin-proteasome-dependent degradation, thereby affecting the morphological change in the spines. The activation of SNK was dependent on the activities of the NMDA receptor, the AMPA receptor, and the

L-type voltage-gated calcium channel (LVGCC) (Pak and Sheng 2003). The activity of ubiquitin C-terminal hydrolase L1 (UCH-L1), a deubiquitinating enzyme, was rapidly regulated by NMDA receptor activation, affecting the synaptic protein distribution and spine morphology, size, and density, indirectly showing that the UPS is involved in activity-dependent structural remodeling (Cartier et al. 2009). Also, scaffolding proteins such as *Shank, GKAP, AKAP79/150, PSD-95, and liprin-α* have been demonstrated to be regulated by the UPS in an activity-dependent manner (Ehlers 2003; Colledge et al. 2003; Hoogenraad et al. 2007). Among these proteins, the specific E3 ligases for GKAP and PSD-95 were identified as TRIM3 and Mdm2, respectively (Colledge et al. 2003; Hung et al. 2010). Given the role of these scaffolding proteins in mediating multiple protein-protein interactions in synapse architecture and function, the UPS may be one of the pathways regulating activity-driven synapse remodeling.

10.2.2 Synaptic Activity–Dependent Regulation of the UPS

Long-lasting synaptic plasticity requires the incorporation of newly synthesized proteins. Protein degradation provides another mechanism for regulating the protein profile in activated neurons. Chronic inhibition or upregulation of synaptic activity in cultured neurons results in a changed protein profile: the levels of some proteins increase with the upregulation of activity and decrease upon the inhibition of activity, some are inversely regulated, and some are maintained at stable levels (Ehlers 2003). Some of the changes in synapse structure and function mediated by the UPS, as mentioned in the previous section, were induced in an activity-dependent manner (Colledge et al. 2003; Patrick et al. 2003; Kato et al. 2005; Pak and Sheng 2003; Cartier et al. 2009). In addition, the UPS also modulates rapid, activity-induced plasticity, long-term potentiation (LTP), and long-term depression (LTD) (Hou et al. 2006; Colledge et al. 2003; Fonseca et al. 2006; Karpova et al. 2006; Deng and Lei 2007).

Polyribosomes are transported to dendritic spines during LTP, and there is a body of evidence showing that proteins are locally synthesized in the activated sites (Aakalu et al. 2001; Ostroff et al. 2002; Pfeiffer and Huber 2006). Similar to this local protein synthesis, several studies demonstrated that proteasomes are transported from the dendritic shaft to the synaptic spines after synaptic activity, suggesting the possibility of local protein degradation (Bingol and Schuman 2006; Shen et al. 2007; Bingol et al. 2010). Synaptic activity enhanced the proteasome entry rate by ~1.5-fold while dramatically reducing the exit rate by at least sixfold, likely induced by an association with the actin cytoskeleton (Bingol and Schuman 2006). Another report has shown that NAC1, a cocaine-regulated transcriptional protein that associates with subunits of the proteasome complex, is cotranslocated with the proteasome from the nucleus into the dendritic spines by enhanced synaptic activity (Shen et al. 2007). Translocation of the proteasome can be blocked either by the depletion of NAC1 or by the expression of a dominant-negative mutant lacking the proteasome binding domain. A recent report demonstrated that

calcium-/calmodulin-dependent protein kinase IIα (CaMKIIα) acts as a scaffold responsible for the activity-dependent translocation of the proteasome to dendritic spines (Bingol et al. 2010). CaMKIIα showed a biochemical association with the proteasome in the brain and also showed colocalization with the proteasome in a hippocampal culture. Activity-dependent translocation of CaMKIIα in hippocampal culture was necessary and sufficient for the translocation of the proteasome. This process required autophosphorylation of CaMKIIα, while kinase activity itself was not necessary. This evidence supports the possibility of activity-dependent local protein degradation, which may serve as one of the mechanisms controlling the local protein composition at synapses after stimulation (Fig. 10.2).

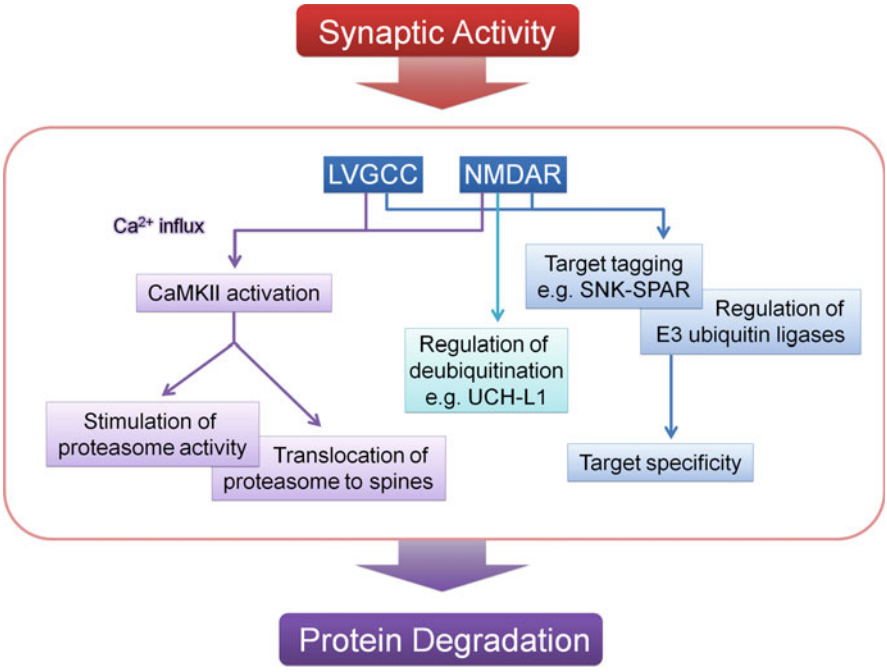

Fig. 10.2 *Regulation of the UPS by synaptic activity*. Roughly three pathways that affect protein degradation are regulated by synaptic activity. When there is a synaptic activity, NMDA receptors and LVGCCs are activated, resulting in an influx of external calcium ions. These calcium ions in turn activate CaMKII, which may then phosphorylate a proteasome complex subunit, thereby upregulating general proteasome activity. Autophosphorylated CaMKII also works as a scaffold for the translocation of the proteasome from the dendritic shaft to the synaptic spines. As postsynaptic proteins seem to be differentially regulated by neuronal activity in vitro or by retrieval in vivo, another pathway should regulate this target-specific differential turnover ratio. Although the upstream members of this pathway are not well characterized, the pathway is likely to involve regulation of either target tagging or specific E3 ligases' activity, which governs the target-specific protein degradation by regulating polyubiquitination. For example, SNK phosphorylates a specific protein SPAR, leading to the degradation of this protein. This process is dependent on NMDA receptor and LVGCC. Deubiquitination, an opposite process of ubiquitination, is also regulated by synaptic activity. UCH-L1 is a deubiquitinating enzyme regulated by NMDA receptor activation

A recent study revealed one of the upstream pathways that may regulate neuronal activity-induced proteasome stimulation (Djakovic et al. 2009). Blockade or upregulation of neuronal activity induced rapid inhibition or enhancement of proteasome activity, respectively. This regulation of proteasome activity is dependent on NMDA receptors and LVGCCs and also requires CaMKII activity, which phosphorylates a subunit of the proteasome complex, Rpt6. As external calcium entry and CaMKII activation are crucial molecular requirements for synaptic plasticity, the regulation of proteasome activity by this pathway may provide a mechanism for remodeling the synaptic composition and strength via protein degradation. However, as many synaptic molecules are differentially regulated, i.e., some are increased by an upregulation of activity, while others are decreased (Ehlers 2003), there should be mechanisms to differentially regulate the degradation of each protein, probably by differentially regulating various E3 ligases. This synaptic activity–induced regulation of specific E3 ligases is largely unknown so far (Fig. 10.2).

10.3 Role of the UPS in the Destabilization of Retrieved Memory

10.3.1 Protein Degradation as a Mechanism of Postretrieval Destabilization

Recently, Lee et al. suggested protein degradation as a mechanism of destabilizing memory after it is activated (Lee et al. 2008). Overall, polyubiquitination of synaptic proteins in the hippocampus was specifically increased after the retrieval of consolidated contextual fear conditioning, which induces *protein synthesis–dependent reconsolidation*. As polyubiquitination is a key step of the ubiquitin-proteasome-dependent protein degradation pathway, this result suggests that total ubiquitin-proteasome-dependent protein degradation of synaptic proteins is increased under this condition. This result is in accordance with reports showing that activity regulates postsynaptic protein composition through the ubiquitin-proteasome system mentioned in the previous section (Ehlers 2003), likely providing a mechanism for the activity-driven functional reorganization of synapses in culture systems.

The retrieval-induced degradation of synaptic proteins seems to be target specific. For example, the polyubiquitination of specific synaptic proteins, including Shank and GKAP, was increased, whereas that of PSD-95 was stable. This pattern resembles the results acquired in culture systems. Notably, the endogenous level of Shank in the synaptosomal fraction of the hippocampus decreased after retrieval, reaching the lowest level 2 h after retrieval and recovering to basal levels at 6 h after retrieval. This retrieval-induced decrease in the endogenous Shank level was

blocked by clasto-lactacystin-β-lactone (β-lactone), a specific proteasome inhibitor, strongly suggesting that specific synaptic proteins are destabilized after retrieval through the ubiquitin-proteasome-dependent degradation pathway.

The inhibition of proteasome activity in the hippocampus after retrieval seems to prevent the destabilization of memory. Postretrieval anisomycin treatment leads to impairment of the previously formed memory. However, local treatment of proteasome inhibitor β-lactone along with anisomycin in the hippocampus after the retrieval of contextual fear memory prevented the amnesic effect of anisomycin. β-lactone treatment alone did not affect memory. These results suggest that ubiquitin-proteasome-dependent protein degradation underlies the destabilization of a previously formed memory after it is retrieved. On the other hand, β-lactone treatment immediately after conditioning did not prevent the amnesic effect of anisomycin on consolidation. This result demonstrates that β-lactone does not have a critical role in the consolidation process of this fear memory and that the effect of β-lactone cannot be attributed to a direct compensation of the effects of anisomycin. This supports the hypothesis that protein degradation plays a critical role in the destabilization of previously formed memories after retrieval, rather than in the consolidation-like restabilization process. However, another study demonstrated that both consolidation and reconsolidation of spatial memory in a water maze task were impaired by the inhibition of proteasome activity (Artinian et al. 2008), and the consolidation of learning in the crab *Chasmagnathus* was also interfered with by UPS inhibition (Merlo and Romano 2007). These indicate that the involvement of proteasome-dependent degradation may differ between species and memory types.

There are also reports suggesting a critical role for proteasome activity in LTP (Karpova et al. 2006; Fonseca et al. 2006), though the treatment of proteasome inhibitor started more than 30 min before LTP induction and might possibly have affected the protein profile before the induction of LTP (which is different from the research of Lee et al., where the drug was injected after the memory task) (Lee et al. 2008). It is also possible that the effect of proteasome inhibition on consolidation was simply not detected in the relatively strong conditioning protocol in the research of Lee et al. Meanwhile, the involvement of the UPS in LTD might have some relationship with the role of the UPS in the destabilization of reactivated memory (Colledge et al. 2003; Deng and Lei 2007; Hou et al. 2006). This destabilization process shows a similar outcome as depotentiation, the reversal of potentiation that shares some mechanisms with LTD.

10.3.2 Molecules Involved in Postretrieval Destabilization

Several molecules, including the NMDA receptor, are also involved in the destabilization of reactivated memory (Ben Mamou et al. 2006). NMDA receptor antagonist AP5, as well as NR2B selective inhibitor ifenprodil, locally applied in the amygdala before the retrieval of cued fear conditioning prevented the amnesic effect of postretrieval anisomycin injection. On the other hand, AMPA receptor

antagonist CNQX did not interfere with the blocking effect of anisomycin. However, several studies have shown that the NMDA receptor antagonist itself has an amnesic effect when the previously formed memory is retrieved (Brown et al. 2008; Itzhak 2008; Lee and Everitt 2008; Milton et al. 2008; Suzuki et al. 2004; Lee et al. 2006). Systemic treatment with the NMDA antagonist MK-801 produced an amnesic effect on the reconsolidation of contextual and cued fear conditioning, odor-reward association, and drug-associated memories. Intra-amygdala NMDA receptor antagonism by AP5 also prevented the reconsolidation of drug-associated memory. These results demonstrate that the effect of NMDA receptor inhibition differs among various memory paradigms and treatment methods and also that NMDA receptors may be required for the restabilization of destabilized memory under certain conditions.

LVGCC and central cannabinoid receptor 1 (CB1 receptor) are also involved in the destabilization of reactivated contextual fear memory (Suzuki et al. 2008). Systemic and hippocampal treatments of LVGCC or CB1 receptor inhibitors prevented the amnesic effect of anisomycin after the retrieval of contextual fear memory. Systemic blockade of LVGCCs also protected reactivated memories against the amnesic effects of CREB activity inhibition. As LVGCCs and CB1 receptors are also required for memory extinction (Suzuki et al. 2004, 2008), there may be overlap between the initial destabilization mechanisms during reconsolidation and extinction.

These molecules may work as upstream factors in the protein degradation pathway after memory is reactivated. As mentioned in the previous section, NMDA receptor and LVGCC-dependent external calcium entry, and the resulting activation of CaMKII, constitute a pathway that regulates proteasome activity *in vitro* (Djakovic et al. 2009). Autophosphorylation of CaMKIIα and its translocation are also responsible for the regulation of proteasome translocation. Studies of the relationships among these molecules and the protein degradation induced by memory reactivation are required to fully understand the mechanism of destabilization induced by memory reactivation.

10.4 Memory Reorganization

10.4.1 Weakening the Reactivated Memory

Although memory can be stably stored for a long time, it sometimes has to be updated as circumstances change. The idea that reconsolidation may be an updating mechanism was hypothesized years ago (Dudai and Eisenberg 2004), and accumulating evidence suggests that this is indeed the case (Garcia-DeLaTorre et al. 2009; Lee 2008; Lee et al. 2008; Morris et al. 2006; Rodriguez-Ortiz et al. 2005; Rodriguez-Ortiz et al. 2008; Rossato et al. 2007; Winters et al. 2009).

The idea that reconsolidation is required for reorganization assumes that new information is incorporated during the labile state, leading to the stabilization of new information together with restabilization of the reactivated initial memory. Although there are some differences between the restabilization process of the reactivated memory and the *consolidation process* of the initially encoded memory, these two share many molecular mechanisms. The pharmacological treatments that can block restabilization of the reactivated memory usually also block the consolidation of a new memory. Even if these treatments block the incorporation of new information after the previously formed memory is reactivated, the results can be interpreted as the inhibition of either the independent consolidation of new information or the reconsolidation-based updating mechanism. Furthermore, even if there were treatments that exclusively impaired reconsolidation, such treatments would also lead to impaired initial memory. As the new information is related to the initial memory, it is hard to determine whether the incorporation of new information is actually impaired or whether it is simply not expressed due to an impairment of the initial memory that may be required for the expression of the updated component. Pioneering studies of the destabilization mechanism after the reactivation of a previously formed memory have provided a breakthrough regarding the role of reconsolidation as an updating mechanism. If the destabilization process is critical for the incorporation of the new information into the previously formed memory, pharmacological treatments that block the destabilization of the reactivated memory should impair the updating procedure while preserving the previous memory.

The strength of a previously formed memory may be weakened as one realizes that the memory of the initial situation is no longer valid. Extinction is an example of this kind of learning paradigm. In extinction of classical conditioning, for example, the subjects are extensively exposed to the conditioned stimulus (CS) without unconditioned stimulus (US), leading to a weaker conditioned response (CR) to the CS (Fig. 10.3a). This type of learning paradigm may be considered the modification and reorganization of the original memory in conjunction with the new information, i.e., that the CS is no longer associated with the US. In the paper reporting protein degradation as a mechanism of destabilization in reactivated memory, Lee et al. also confirmed that blocking protein degradation results in impaired contextual fear memory extinction (Lee et al. 2008). Local treatment with protein degradation inhibitors in the hippocampus after the extinction trial blocked the decrement of freezing the next day, whereas the vehicle group showed normal memory extinction. This result indicates that *protein degradation–dependent destabilization* of the reactivated memory is required for further reorganization or specifically for weakening of the initial memory. Similar results were found when considering the putative upstream molecules of destabilization, the LVGCCs and CB1 receptors (Suzuki et al. 2008). Other than their role in destabilization within the reconsolidation process, these molecules are also required for extinction (Suzuki et al. 2004; Suzuki et al. 2008). These results are in accordance with the findings on protein degradation inhibition, although the possibility remains that these molecules have unique roles in extinction learning.

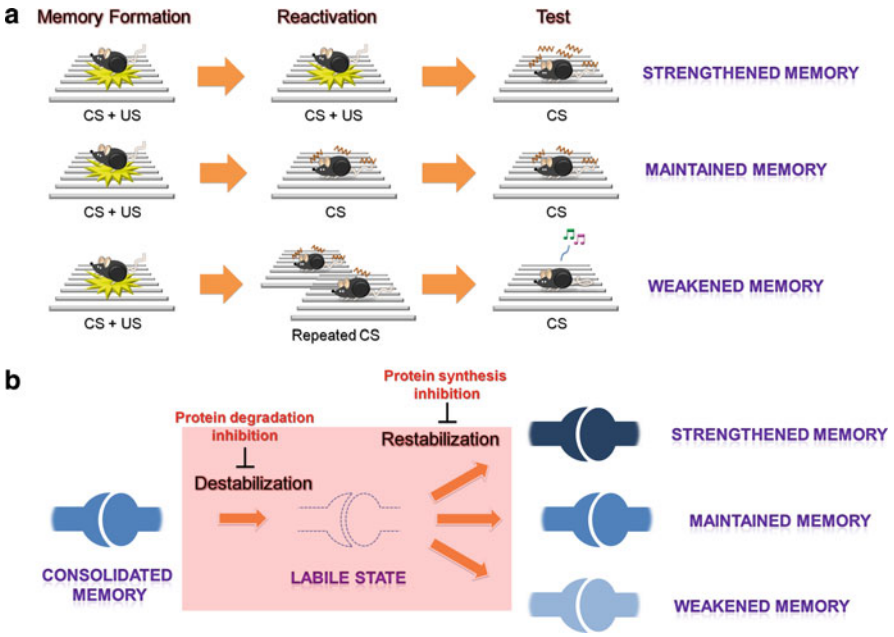

Fig. 10.3 *A model for memory reorganization – strengthening, maintaining, and weakening*. (**a**) Cartoons of the behavioral scheme used to reveal the mechanism underlying memory strengthening, maintaining, and weakening. After the original contextual fear conditioning, the memory is reactivated in various situations. In the scheme for memory strengthening, the animal receives an additional US shock. In the scheme for memory maintaining, it is exposed to the training context (CS) for a few minutes. In the scheme for memory weakening, it is repeatedly exposed to the training context (CS). Drugs are applied after memory reactivation, and the memory level is tested on the next day. (**b**) The diagram represents the state of the memory during the strengthening, maintaining, and weakening of the consolidated memory. Although the diagrams are shown with a single synapse, note that this is a simple symbolic representation

Although reconsolidation and extinction have been considered distinct processes thus far, the results described above demonstrate that reconsolidation and extinction share a common molecular mechanism, at least in the initial stages after the reactivation of the memory. Based on this interpretation, it may be possible to consider reconsolidation and extinction under a unified model in the reorganization of preexisting memory. After the consolidated memory is reactivated, it undergoes a destabilization process, which involves active degradation of scaffolding proteins such as Shank and GKAP in the spines, followed by restabilization either to recover the initial memory (reconsolidation) via protein synthesis or to maintain the destabilized state (extinction) with either minimal protein synthesis or active suppressive memory formation. Although some reports support the "unlearning" paradigm of extinction (Kim et al. 2007, 2009), active relearning of the CS-"no US" association (which is dependent on protein synthesis) is also a well-known

mechanism of extinction (reviewed in (Lattal et al. 2006; Quirk and Mueller 2008)). It is not yet clear whether the *protein degradation–dependent destabilization* process is the initial part of either the unlearning or the relearning mechanism of extinction. It is also possible that different independent mechanisms cooperatively work toward the result of extinction.

10.4.2 Strengthening the Reactivated Memory

In some learning paradigms, one learning trial leads to robust memory that can be saturated, but in most cases, repeated learning leads to a gradual strengthening of memory. Several experiments utilized this gradual strengthening of memory to demonstrate that reconsolidation occurs when there is new information. Additionally, several studies have indicated that the application of certain drugs during reconsolidation can enhance the strength of memory, suggesting that reconsolidation can be potentially associated with an increase in memory strength (Lee et al. 2006; Tronson et al. 2006). However, these studies do not provide direct evidence for the hypothesis that the reconsolidation process is required for updating and increasing memory strength.

Following the report that protein degradation underlies the weakening of reactivated memory, another study demonstrated that destabilization of reactivated memory is also required for the strengthening of contextual fear memory (Lee 2008). The author first demonstrated that contextual fear memory can be further strengthened by repeated conditioning with a relatively weak, aversive US (Fig. 10.3a). Given that the consolidation and reconsolidation of contextual fear have different molecular requirements (Lee et al. 2004), Lee showed that the strengthening of a consolidated memory that occurs upon second training does not match the molecular mechanism of consolidation, as the treatment that has an amnesic effect exclusively upon consolidation had no effect. The author also demonstrated that a treatment that has an amnesic effect exclusively on reconsolidation could impair the strengthening as well as the reactivated memory. However, the amnesic treatment of reconsolidation impairs and ablates the original reactivated memory and thus also impairs strengthening, no matter whether the strengthening mechanism actually relies on reconsolidation. To more directly demonstrate the requirement for the reconsolidation mechanism in memory strengthening, the author locally applied a *protein degradation inhibitor* to the hippocampus after the second training. If *protein degradation–dependent destabilization* was required to strengthen the reactivated memory, the *protein degradation inhibitor* would block further enhancement of the memory, leaving it at the level of initially consolidated memory. This was what the author observed (Lee 2008).

10.4.3 Hypothetical Model for Memory Reorganization

The fact that strengthening reactivated memories requires *protein degradation–dependent destabilization*, together with the evidence that reconsolidation and extinction partly share a common mechanism, indicates that the maintenance, weakening, and strengthening of a reactivated original memory may be interpreted under a unified model of reorganization (Fig. 10.3b). After a memory is consolidated, it can be retrieved by certain situations that include one or more components related to the original memory. These situations may be quite diverse and can determine the fate of the retrieved memory. In some cases, the memory seems to be maintained without being reactivated. When the memory retrieval is very brief, or when the memory is saturated by overtraining, it is not susceptible to the amnesic effect of *protein synthesis inhibitors*, even though the memory is well retrieved (Suzuki et al. 2004; Rodriguez-Ortiz et al. 2005, 2008; Garcia-DeLaTorre et al. 2009; Wang et al. 2009). On the other hand, when a memory, usually unsaturated, is retrieved for more than a very brief period, it can be reactivated and reorganized. The reactivated memory first becomes destabilized by a mechanism that is likely initiated by the NMDA receptor, LVGCC, or CB1 receptor and involves protein degradation. The fate of the destabilized memory depends on the incoming information specific to the situation. In cases where the original memory is no longer valid, the destabilized memory will either passively remain in a destabilized state or the extinction information will be actively encoded, weakening the memory. In cases where the original memory should be strengthened by additional training, the destabilized memory is restabilized into a stronger memory. Finally, in cases where there is no additional training, but there is not sufficient information to conclude that the original memory is no longer valid, the destabilized memory is restabilized to a similar level as the original memory.

The underlying molecular pathway of this reorganization mechanism is still under investigation. The destabilization process seems to be initiated by activation of NMDA receptors, LVGCC, and CB1 receptors (Suzuki et al. 2008; Ben Mamou et al. 2006). The UPS seems to have a critical role in this process, though the direct links of the upstream molecules have not been demonstrated *in vivo*. In vitro studies show the possibility that NMDA receptors and LVGCC can activate CaMKII, which in turn activates and translocates the proteasome to the synaptic spines along with the autophosphorylated CaMKII (Bingol et al. 2010; Bingol and Schuman 2006). The increase of degradation in the synaptosomal fraction can be well explained by this pathway (Lee et al. 2008). However, the pathway that links synaptic activity to the specificity of the substrate for degradation is unknown. Two substrates demonstrated to be actively degraded during the destabilization step are Shank and GKAP, both of which have been proven to be regulated by synaptic activity *in vitro* (Ehlers 2003), where GKAP is especially ubiquitinated by TRIM3 ubiquitin ligase (Hung et al. 2010). Given the role of these proteins as scaffolding proteins of the synaptic spine, in which Shank specifically acts as a "master" scaffolding protein that holds together intermediate scaffolding proteins such as

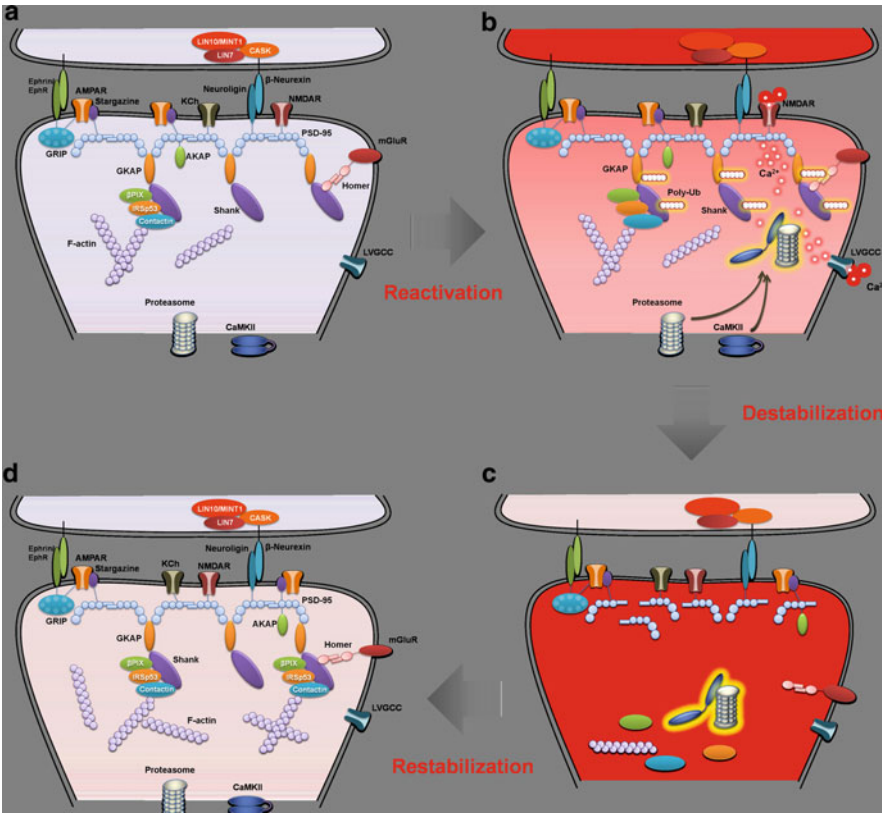

Fig. 10.4 *A model for memory reorganization – synaptic remodeling*. (**a**) Structure of a synapse encoding memory. (**b**) When the memory is reactivated, NMDA receptor and LVGCC are opened, allowing calcium influx to the spine. These calcium ions activate CaMKII, which then phosphorylates the proteasome to increase the activity. The activated CaMKII may undergo autophosphorylation and can associate with and translocate the proteasome from the dendritic shaft to the spine. Meanwhile, target proteins are polyubiquitinated by the specific action of E3 ligases and other proteins. The known proteins that undergo polyubiquitination after memory reactivation are Shank and GKAP, as indicated. (**c**) The recruited active proteasomes degrade these specifically polyubiquitinated targets. Since the targets here are scaffolding proteins, it is a likely consideration that this spine undergoes structural remodeling. (**d**) A protein synthesis–dependent process restabilizes the synapse either to a state similar to the initial state or to a modified state

GKAP and PSD-95, and also considering the fact that the UPS is involved in activity-dependent synaptic remodeling (Pak and Sheng 2003; Cartier et al. 2009), it seems that during reconsolidation, reactivated synapses undergo synaptic remodeling, first being disassembled during the destabilization step and then being recovered to a state similar to the initial one or becoming stabilized as a modified state. This process might accompany morphological changes as well. Restabilization is basically protein synthesis dependent and shares many molecular

mechanisms with the original consolidation, although some differences exist. The process of restabilization may be the key step that governs the fate of the memory (Fig. 10.4). The reorganization process investigated so far is focused on the postsynaptic site. The role of protein degradation on the presynaptic site is largely unknown.

The model here is based on the reorganization of memory strength. However, it is noteworthy that there are other types of reorganization where the memory content is changed rather than the quantitative extent of the memory. A recent study demonstrated that partial modification of an object-place associative memory requires both protein synthesis and degradation (Choi et al. 2010). One day after the animal had initially formed object-place associative memory for four objects placed in a context, it was exposed to a context where two of the objects positions were changed. Without any treatment, the animal would reorganize the initial memory in order to learn the changed position of the objects. However, when either a *protein synthesis inhibitor* or a *proteasome inhibitor* was treated right after the second exposure, the animal could not appropriately reorganize the memory. Although more research is required to clearly reveal the memory reorganization process in this situation, the requirement of both protein synthesis and degradation matches the suggested model.

10.5 Conclusions and Future Directions

As for many basic cellular processes, neuronal functions are also under the influence of the UPS. Recent studies indicate that the UPS can be regulated in response to synaptic activity, suggesting a role for the UPS in synaptic plasticity and memory. The role of protein degradation in the destabilization step of reconsolidation shows that the UPS may serve a very specific role, more than simply maintaining proteins at an appropriate level.

Since reconsolidation was first demonstrated, many studies have focused on the mechanism of the restabilization step of the full process. However, the destabilization that occurs prior to restabilization is also a unique and important process. Recent studies focusing on the destabilization process of reactivated memory have not only revealed the underlying mechanism of this process but also given insight into important aspects of the fate of reactivated memory. In contrast to the *protein synthesis–dependent restabilization process*, destabilization of reactivated memory seems to be dependent on protein degradation. There are several molecules that may work in a putative upstream pathway to regulate protein degradation. As synaptic protein turnover rates are differentially regulated by neuronal activity, more studies are required to elucidate the target-specific regulation of each protein. Using a proteasome inhibitor as a tool to block destabilization of reactivated memory, it was shown that this reactivation-induced destabilization is required for the reorganization of the reactivated memory, a process that includes maintaining, weakening, and strengthening the memory. These results suggest a

unified model of reorganization, beginning with the destabilization of reactivated memory and followed by stabilization of appropriate information, depending on the situation.

The studies based on culture systems and molecular analyses after behavioral processes suggest that the protein degradation–based mechanism may work on a synaptic level. However, there is no direct evidence as to whether each synapse that is involved in the memory behaves according to the memory state, i.e., destabilization followed by restabilization, which is an important issue. Another important issue is whether this protein degradation–dependent reorganization mechanism can be applied to systems-level changes such as systems consolidation and systems reconsolidation (Debiec et al. 2002; Frankland and Bontempi 2005). More studies are required to reveal the details of the mechanism and also to apply studies on *in vitro* systems to the *in vivo* destabilization process. Studies utilizing more selective targeting of a specifically regulated proteasome function would also be valuable compared to those using a general, pharmacological inhibition of proteasome activity. It is also important to determine the range of memory tasks and animal models to which this model can be applied.

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References

Aakalu, G., Smith, W. B., Nguyen, N., Jiang, C., & Schuman, E. M. (2001). Dynamic visualization of local protein synthesis in hippocampal neurons. *Neuron, 30*(2), 489–502. doi:S0896-6273(01)00295-1 [pii].

Arancibia-Carcamo, I. L., Yuen, E. Y., Muir, J., Lumb, M. J., Michels, G., Saliba, R. S., Smart, T. G., Yan, Z., Kittler, J. T., & Moss, S. J. (2009). Ubiquitin-dependent lysosomal targeting of GABA(A) receptors regulates neuronal inhibition. *Proceedings of the National Academy of Sciences of the United States of America, 106*(41), 17552–17557. doi:0905502106 [pii] 10.1073/pnas.0905502106.

Artinian, J., McGauran, A. M., De Jaeger, X., Mouledous, L., Frances, B., & Roullet, P. (2008). Protein degradation, as with protein synthesis, is required during not only long-term spatial memory consolidation but also reconsolidation. *The European Journal of Neuroscience, 27*(11), 3009–3019. doi:EJN6262 [pii] 10.1111/j.1460-9568.2008.06262.x.

Bedford, F. K., Kittler, J. T., Muller, E., Thomas, P., Uren, J. M., Merlo, D., Wisden, W., Triller, A., Smart, T. G., & Moss, S. J. (2001). GABA(A) receptor cell surface number and subunit stability are regulated by the ubiquitin-like protein Plic-1. *Nature Neuroscience, 4*(9), 908–916. doi:10.1038/nn0901-908 nn0901-908 [pii].

Ben Mamou, C., Gamache, K., & Nader, K. (2006). NMDA receptors are critical for unleashing consolidated auditory fear memories. *Nature Neuroscience, 9*(10), 1237–1239. doi:nn1778 [pii] 10.1038/nn1778.

Bingol, B., & Schuman, E. M. (2006). Activity-dependent dynamics and sequestration of proteasomes in dendritic spines. *Nature, 441*(7097), 1144–1148. doi:nature04769 [pii] 10.1038/nature04769.

Bingol, B., Wang, C. F., Arnott, D., Cheng, D., Peng, J., & Sheng, M. (2010). Autophosphorylated CaMKIIalpha acts as a scaffold to recruit proteasomes to dendritic spines. *Cell, 140*(4), 567–578. doi:S0092-8674(10)00059-0 [pii] 10.1016/j.cell.2010.01.024.

Brown, T. E., Lee, B. R., & Sorg, B. A. (2008). The NMDA antagonist MK-801 disrupts reconsolidation of a cocaine-associated memory for conditioned place preference but not for self-administration in rats. *Learning and Memory, 15*(12), 857–865. doi:15/12/857 [pii] 10.1101/lm.1152808.

Burbea, M., Dreier, L., Dittman, J. S., Grunwald, M. E., & Kaplan, J. M. (2002). Ubiquitin and AP180 regulate the abundance of GLR-1 glutamate receptors at postsynaptic elements in *C. elegans*. *Neuron, 35*(1), 107–120. doi:doi:S0896627302007493 [pii].

Cartier, A. E., Djakovic, S. N., Salehi, A., Wilson, S. M., Masliah, E., & Patrick, G. N. (2009). Regulation of synaptic structure by ubiquitin C-terminal hydrolase L1. *The Journal of Neuroscience, 29*(24), 7857–7868. doi:29/24/7857 [pii] 10.1523/JNEUROSCI.1817-09.2009.

Choi, J. H., Kim, J. E., & Kaang, B. K. (2010). Protein synthesis and degradation are required for the incorporation of modified information into the pre-existing object-location memory. *Molecular Brain, 3*, 1. doi:1756-6606-3-1 [pii] 10.1186/1756-6606-3-1.

Colledge, M., Snyder, E. M., Crozier, R. A., Soderling, J. A., Jin, Y., Langeberg, L. K., Lu, H., Bear, M. F., & Scott, J. D. (2003). Ubiquitination regulates PSD-95 degradation and AMPA receptor surface expression. *Neuron, 40*(3), 595–607. doi:S0896627303006871 [pii].

Debiec, J., LeDoux, J. E., & Nader, K. (2002). Cellular and systems reconsolidation in the hippocampus. *Neuron, 36*(3), 527–538. doi:S0896627302010012 [pii].

Deng, P. Y., & Lei, S. (2007). Long-term depression in identified stellate neurons of juvenile rat entorhinal cortex. *Journal of Neurophysiology, 97*(1), 727–737. doi:01089.2006 [pii] 10.1152/jn.01089.2006.

DiAntonio, A., Haghighi, A. P., Portman, S. L., Lee, J. D., Amaranto, A. M., & Goodman, C. S. (2001). Ubiquitination-dependent mechanisms regulate synaptic growth and function. *Nature, 412*(6845), 449–452. doi:10.1038/35086595 35086595 [pii].

Ding, M., Chao, D., Wang, G., & Shen, K. (2007). Spatial regulation of an E3 ubiquitin ligase directs selective synapse elimination. *Science, 317*(5840), 947–951. doi:1145727 [pii] 10.1126/science.1145727.

Djakovic, S. N., Schwarz, L. A., Barylko, B., DeMartino, G. N., & Patrick, G. N. (2009). Regulation of the proteasome by neuronal activity and calcium/calmodulin-dependent protein kinase II. *The Journal of Biological Chemistry, 284*(39), 26655–26665. doi:M109.021956 [pii] 10.1074/jbc.M109.021956.

Dreier, L., Burbea, M., & Kaplan, J. M. (2005). LIN-23-mediated degradation of beta-catenin regulates the abundance of GLR-1 glutamate receptors in the ventral nerve cord of *C. elegans*. *Neuron, 46*(1), 51–64. doi:doi:S0896-6273(05)00161-3 [pii] 10.1016/j.neuron.2004.12.058.

Dudai, Y. (2006). Reconsolidation: The advantage of being refocused. *Current Opinion in Neurobiology, 16*(2), 174–178. doi:S0959-4388(06)00035-3 [pii] 10.1016/j.conb.2006.03.010.

Dudai, Y., & Eisenberg, M. (2004). Rites of passage of the engram: Reconsolidation and the lingering consolidation hypothesis. *Neuron, 44*(1), 93–100. doi:10.1016/j.neuron.2004.09.003 S0896627304005720 [pii].

Ehlers, M. D. (2003). Activity level controls postsynaptic composition and signaling via the ubiquitin-proteasome system. *Nature Neuroscience, 6*(3), 231–242. doi:10.1038/nn1013 nn1013 [pii].

Fonseca, R., Vabulas, R. M., Hartl, F. U., Bonhoeffer, T., & Nagerl, U. V. (2006). A balance of protein synthesis and proteasome-dependent degradation determines the maintenance of LTP. *Neuron, 52*(2), 239–245. doi:S0896-6273(06)00637-4 [pii] 10.1016/j.neuron.2006.08.015.

Frankland, P. W., & Bontempi, B. (2005). The organization of recent and remote memories. *Nature Reviews Neuroscience, 6*(2), 119–130. doi:nrn1607 [pii] 10.1038/nrn1607.

Garcia-DeLaTorre, P., Rodriguez-Ortiz, C. J., Arreguin-Martinez, J. L., Cruz-Castaneda, P., & Bermudez-Rattoni, F. (2009). Simultaneous but not independent anisomycin infusions in

insular cortex and amygdala hinder stabilization of taste memory when updated. *Learning and Memory, 16*(9), 514–519. doi:16/9/514 [pii] 10.1101/lm.1356509.

Haas, K. F., Miller, S. L., Friedman, D. B., & Broadie, K. (2007). The ubiquitin-proteasome system postsynaptically regulates glutamatergic synaptic function. *Molecular and Cellular Neuroscience, 35*(1), 64–75. doi:S1044-7431(07)00027-9 [pii] 10.1016/j.mcn.2007.02.002.

Hoogenraad, C. C., Feliu-Mojer, M. I., Spangler, S. A., Milstein, A. D., Dunah, A. W., Hung, A. Y., & Sheng, M. (2007). Liprinalpha1 degradation by calcium/calmodulin-dependent protein kinase II regulates LAR receptor tyrosine phosphatase distribution and dendrite development. *Developmental Cell, 12*(4), 587–602. doi:S1534-5807(07)00056-1 [pii] 10.1016/j.devcel.2007.02.006.

Hou, L., Antion, M. D., Hu, D., Spencer, C. M., Paylor, R., & Klann, E. (2006). Dynamic translational and proteasomal regulation of fragile X mental retardation protein controls mGluR-dependent long-term depression. *Neuron, 51*(4), 441–454. doi:S0896-6273(06)00545-9 [pii] 10.1016/j.neuron.2006.07.005.

Hung, A. Y., Sung, C. C., Brito, I. L., & Sheng, M. (2010). Degradation of postsynaptic scaffold GKAP and regulation of dendritic spine morphology by the TRIM3 ubiquitin ligase in rat hippocampal neurons. *PLoS One, 5*(3), e9842. doi:10.1371/journal.pone.0009842.

Itzhak, Y. (2008). Role of the NMDA receptor and nitric oxide in memory reconsolidation of cocaine-induced conditioned place preference in mice. *Annals of the New York Academy of Sciences, 1139*, 350–357. doi:NYAS1139051 [pii] 10.1196/annals.1432.051.

Juo, P., & Kaplan, J. M. (2004). The anaphase-promoting complex regulates the abundance of GLR-1 glutamate receptors in the ventral nerve cord of *C. elegans*. *Current Biology, 14*(22), 2057–2062. doi:doi:S0960982204008802 [pii] 10.1016/j.cub.2004.11.010.

Kaang, B. K., Lee, S. H., & Kim, H. (2009). Synaptic protein degradation as a mechanism in memory reorganization. *The Neuroscientist, 15*(5), 430–435. doi:1073858408331374 [pii] 10.1177/1073858408331374.

Karpova, A., Mikhaylova, M., Thomas, U., Knopfel, T., & Behnisch, T. (2006). Involvement of protein synthesis and degradation in long-term potentiation of Schaffer collateral CA1 synapses. *The Journal of Neuroscience, 26*(18), 4949–4955. doi:26/18/4949 [pii] 10.1523/JNEUROSCI.4573-05.2006.

Kato, A., Rouach, N., Nicoll, R. A., & Bredt, D. S. (2005). Activity-dependent NMDA receptor degradation mediated by retrotranslocation and ubiquitination. *Proceedings of the National Academy of Sciences of the United States of America, 102*(15), 5600–5605. doi:0501769102 [pii] 10.1073/pnas.0501769102.

Kim, J., Lee, S., Park, K., Hong, I., Song, B., Son, G., Park, H., Kim, W. R., Park, E., Choe, H. K., Kim, H., Lee, C., Sun, W., Kim, K., Shin, K. S., & Choi, S. (2007). Amygdala depotentiation and fear extinction. *Proceedings of the National Academy of Sciences of the United States of America, 104*(52), 20955–20960. doi:0710548105 [pii] 10.1073/pnas.0710548105.

Kim, J., Park, S., Lee, S., & Choi, S. (2009). Amygdala depotentiation ex vivo requires mitogen-activated protein kinases and protein synthesis. *Neuroreport, 20*(5), 517–520. doi:10.1097/WNR.0b013e328329412d.

Lattal, K. M., Radulovic, J., & Lukowiak, K. (2006). Extinction: [corrected] does it or doesn't it? The requirement of altered gene activity and new protein synthesis. *Biological Psychiatry, 60*(4), 344–351. doi:S0006-3223(06)00766-9 [pii] 10.1016/j.biopsych.2006.05.038.

Lee, J. L. (2008). Memory reconsolidation mediates the strengthening of memories by additional learning. *Nature Neuroscience, 11*(11), 1264–1266. doi:nn.2205 [pii] 10.1038/nn.2205.

Lee, S. H., Choi, J. H., Lee, N., Lee, H. R., Kim, J. I., Yu, N. K., Choi, S. L., Kim, H., & Kaang, B. K. (2008). Synaptic protein degradation underlies destabilization of retrieved fear memory. *Science, 319*(5867), 1253–1256. doi:1150541 [pii] 10.1126/science.1150541.

Lee, J. L., & Everitt, B. J. (2008). Appetitive memory reconsolidation depends upon NMDA receptor-mediated neurotransmission. *Neurobiology of Learning and Memory, 90*(1), 147–154. doi:S1074-7427(08)00031-2 [pii] 10.1016/j.nlm.2008.02.004.

Lee, J. L., Everitt, B. J., & Thomas, K. L. (2004). Independent cellular processes for hippocampal memory consolidation and reconsolidation. *Science, 304*(5672), 839–843. doi:10.1126/science.1095760 1095760 [pii].

Lee, J. L., Milton, A. L., & Everitt, B. J. (2006). Reconsolidation and extinction of conditioned fear: Inhibition and potentiation. *The Journal of Neuroscience, 26*(39), 10051–10056. doi:26/39/10051 [pii] 10.1523/JNEUROSCI.2466-06.2006.

Liao, E. H., Hung, W., Abrams, B., & Zhen, M. (2004). An SCF-like ubiquitin ligase complex that controls presynaptic differentiation. *Nature, 430*(6997), 345–350. doi:10.1038/nature02647 nature02647 [pii].

Merlo, E., & Romano, A. (2007). Long-term memory consolidation depends on proteasome activity in the crab Chasmagnathus. *Neuroscience, 147*(1), 46–52. doi:S0306-4522(07)00520-9 [pii] 10.1016/j.neuroscience.2007.04.022.

Milton, A. L., Lee, J. L., Butler, V. J., Gardner, R., & Everitt, B. J. (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. *The Journal of Neuroscience, 28*(33), 8230–8237. doi:28/33/8230 [pii] 10.1523/JNEUROSCI.1723-08.2008.

Morris, R. G., Inglis, J., Ainge, J. A., Olverman, H. J., Tulloch, J., Dudai, Y., & Kelly, P. A. (2006). Memory reconsolidation: Sensitivity of spatial memory to inhibition of protein synthesis in dorsal hippocampus during encoding and retrieval. *Neuron, 50*(3), 479–489. doi:S0896-6273(06)00280-7 [pii] 10.1016/j.neuron.2006.04.012.

Nader, K., & Hardt, O. (2009). A single standard for memory: The case for reconsolidation. *Nature Reviews Neuroscience, 10*(3), 224–234. doi:nrn2590 [pii] 10.1038/nrn2590.

Nader, K., Schafe, G. E., & Le Doux, J. E. (2000). Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. *Nature, 406*(6797), 722–726. doi:10.1038/35021052.

Ostroff, L. E., Fiala, J. C., Allwardt, B., & Harris, K. M. (2002). Polyribosomes redistribute from dendritic shafts into spines with enlarged synapses during LTP in developing rat hippocampal slices. *Neuron, 35*(3), 535–545. doi:S0896627302007857 [pii].

Pak, D. T., & Sheng, M. (2003). Targeted protein degradation and synapse remodeling by an inducible protein kinase. *Science, 302*(5649), 1368–1373. doi:10.1126/science.1082475 1082475 [pii].

Patrick, G. N., Bingol, B., Weld, H. A., & Schuman, E. M. (2003). Ubiquitin-mediated proteasome activity is required for agonist-induced endocytosis of GluRs. *Current Biology, 13*(23), 2073–2081. doi:S0960982203007826 [pii].

Pfeiffer, B. E., & Huber, K. M. (2006). Current advances in local protein synthesis and synaptic plasticity. *The Journal of Neuroscience, 26*(27), 7147–7150. doi:26/27/7147 [pii] 10.1523/JNEUROSCI.1797-06.2006.

Quirk, G. J., & Mueller, D. (2008). Neural mechanisms of extinction learning and retrieval. *Neuropsychopharmacology, 33*(1), 56–72. doi:1301555 [pii] 10.1038/sj.npp. 1301555.

Rodriguez-Ortiz, C. J., De la Cruz, V., Gutierrez, R., & Bermudez-Rattoni, F. (2005). Protein synthesis underlies post-retrieval memory consolidation to a restricted degree only when updated information is obtained. *Learning and Memory, 12*(5), 533–537. doi:lm.94505 [pii] 10.1101/lm.94505.

Rodriguez-Ortiz, C. J., Garcia-DeLaTorre, P., Benavidez, E., Ballesteros, M. A., & Bermudez-Rattoni, F. (2008). Intrahippocampal anisomycin infusions disrupt previously consolidated spatial memory only when memory is updated. *Neurobiology of Learning and Memory, 89*(3), 352–359. doi:S1074-7427(07)00168-2 [pii] 10.1016/j.nlm.2007.10.004.

Rossato, J. I., Bevilaqua, L. R., Myskiw, J. C., Medina, J. H., Izquierdo, I., & Cammarota, M. (2007). On the role of hippocampal protein synthesis in the consolidation and reconsolidation of object recognition memory. *Learning and Memory, 14*(1), 36–46. doi:14/1/36 [pii] 10.1101/lm.422607.

Schaefer, A. M., Hadwiger, G. D., & Nonet, M. L. (2000). rpm-1, a conserved neuronal gene that regulates targeting and synaptogenesis in *C. elegans*. *Neuron, 26*(2), 345–356. doi:doi:S0896-6273(00)81168-X [pii].

Shen, H., Korutla, L., Champtiaux, N., Toda, S., LaLumiere, R., Vallone, J., Klugmann, M., Blendy, J. A., Mackler, S. A., & Kalivas, P. W. (2007). NAC1 regulates the recruitment of the proteasome complex into dendritic spines. *The Journal of Neuroscience, 27*(33), 8903–8913. doi:27/33/8903 [pii] 10.1523/JNEUROSCI.1571-07.2007.

Speese, S. D., Trotta, N., Rodesch, C. K., Aravamudan, B., & Broadie, K. (2003). The ubiquitin proteasome system acutely regulates presynaptic protein turnover and synaptic efficacy. *Current Biology, 13*(11), 899–910. doi:S0960982203003385 [pii].

Suzuki, A., Josselyn, S. A., Frankland, P. W., Masushige, S., Silva, A. J., & Kida, S. (2004). Memory reconsolidation and extinction have distinct temporal and biochemical signatures. *The Journal of Neuroscience, 24*(20), 4787–4795. doi:10.1523/JNEUROSCI.5491-03.2004 24/20/4787 [pii].

Suzuki, A., Mukawa, T., Tsukagoshi, A., Frankland, P. W., & Kida, S. (2008). Activation of LVGCCs and CB1 receptors required for destabilization of reactivated contextual fear memories. *Learning and Memory, 15*(6), 426–433. doi:15/6/426 [pii] 10.1101/lm.888808.

Tada, H., Okano, H. J., Takagi, H., Shibata, S., Yao, I., Matsumoto, M., Saiga, T., Nakayama, K. I., Kashima, H., Takahashi, T., Setou, M., & Okano, H. (2010). Fbxo45, a novel ubiquitin ligase, regulates synaptic activity. *The Journal of Biological Chemistry, 285*(6), 3840–3849. doi:M109.046284 [pii] 10.1074/jbc.M109.046284.

Tronson, N. C., & Taylor, J. R. (2007). Molecular mechanisms of memory reconsolidation. *Nature Reviews Neuroscience, 8*(4), 262–275. doi:nrn2090 [pii] 10.1038/nrn2090.

Tronson, N. C., Wiseman, S. L., Olausson, P., & Taylor, J. R. (2006). Bidirectional behavioral plasticity of memory reconsolidation depends on amygdalar protein kinase A. *Nature Neuroscience, 9*(2), 167–169. doi:nn1628 [pii] 10.1038/nn1628.

van Roessel, P., Elliott, D. A., Robinson, I. M., Prokop, A., & Brand, A. H. (2004). Independent regulation of synaptic size and activity by the anaphase-promoting complex. *Cell, 119*(5), 707–718. doi:S0092867404010967 [pii] 10.1016/j.cell.2004.11.028.

Wan, H. I., DiAntonio, A., Fetter, R. D., Bergstrom, K., Strauss, R., & Goodman, C. S. (2000). Highwire regulates synaptic growth in Drosophila. *Neuron, 26*(2), 313–329. doi:S0896-6273(00)81166-6 [pii].

Wang, S. H., de Oliveira, A. L., & Nader, K. (2009). Cellular and systems mechanisms of memory strength as a constraint on auditory fear reconsolidation. *Nature Neuroscience, 12*(7), 905–912. doi:nn.2350 [pii] 10.1038/nn.2350.

Willeumier, K., Pulst, S. M., & Schweizer, F. E. (2006). Proteasome inhibition triggers activity-dependent increase in the size of the recycling vesicle pool in cultured hippocampal neurons. *The Journal of Neuroscience, 26*(44), 11333–11341. doi:26/44/11333 [pii] 10.1523/JNEUROSCI.1684-06.2006.

Winters, B. D., Tucci, M. C., & DaCosta-Furtado, M. (2009). Older and stronger object memories are selectively destabilized by reactivation in the presence of new information. *Learning and Memory, 16*(9), 545–553. doi:16/9/545 [pii] 10.1101/lm.1509909.

Wood, M. A., Kaplan, M. P., Brensinger, C. M., Guo, W., & Abel, T. (2005). Ubiquitin C-terminal hydrolase L3 (Uchl3) is involved in working memory. *Hippocampus, 15*(5), 610–621. doi:10.1002/hipo.20082.

Yao, I., Takagi, H., Ageta, H., Kahyo, T., Sato, S., Hatanaka, K., Fukuda, Y., Chiba, T., Morone, N., Yuasa, S., Inokuchi, K., Ohtsuka, T., Macgregor, G. R., Tanaka, K., & Setou, M. (2007). SCRAPPER-dependent ubiquitination of active zone protein RIM1 regulates synaptic vesicle release. *Cell, 130*(5), 943–957. doi:S0092-8674(07)00902-6 [pii] 10.1016/j.cell.2007.06.052.