



Protein degradation during reconsolidation as a mechanism for memory reorganization

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Memory is a reference formed from a past experience that is used to respond to present situations. However, the world is dynamic and situations change, so it is important to update the memory with new information each time it is reactivated in order to adjust the response in the future. Recent researches indicate that memory may undergo a dynamic process that could work as an updating mechanism. This process which is called reconsolidation involves destabilization of the memory after it is reactivated, followed by restabilization. Recently, it has been demonstrated that the initial destabilization process of reconsolidation requires protein degradation. Using protein degradation inhibition as a method to block reconsolidation, recent researches suggest that reconsolidation, especially the protein degradation-dependent destabilization process is necessary for memory reorganization.

Keywords: protein degradation, reconsolidation, memory reorganization, updating mechanism

INTRODUCTION

Memory can be stored for either a relatively short or a long period of time. For a memory to be stored long-term, it has to be “consolidated.” Through the protein synthesis-dependent consolidation process, the information is stabilized as a long-term memory that is relatively insensitive to disruption. Maintaining a memory, without retrieval, does not critically involve transient protein synthesis. After it is retrieved, however, it requires protein synthesis within a specific period, in order to recover the memory from a labile state. Without proper transient protein synthesis, the memory seems to be impaired, suggesting that protein synthesis is required to recover the memory from a certain state where the consolidated memory is destabilized. This post-retrieval, protein-dependent process is termed reconsolidation (Misanin et al., 1968; Nader et al., 2000). The early studies on reconsolidation focused on the consolidation-like restabilization process. In this article, however, we will focus on recent studies exploring the mechanism of destabilization induced by reactivation of a previously consolidated memory and show how these findings can be used to demonstrate that reconsolidation can work as an updating mechanism. We will also discuss on the physiological role of this process for memory updating and reorganization.

RETRIEVAL-INDUCED DESTABILIZATION

The post-retrieval destabilization process of reconsolidation may involve active loss of components maintaining the memory. Lee et al. (2008) have demonstrated that this active destabilization process requires ubiquitin–proteasome-dependent protein degradation. Inhibition of proteasome activity in the hippocampus after retrieval of contextual fear memory prevented becoming labile, possibly by blocking the destabilization process. Proteasome inhibitor treated together with protein synthesis inhibitor prevented the amnesic effect of protein synthesis inhibitor, preserving the fear memory. On the other hand, proteasome inhibitor itself had no effect on either reconsolidation or consolidation of contextual fear memory.

Polyubiquitinated protein level in the crude synaptosomal fraction has increased after retrieval of contextual fear memory. As the proteasome complex recognizes their target by the polyubiquitin, polyubiquitinated protein level indicates the extent of proteasome-dependent protein degradation. In addition, one of the polyubiquitinated synaptic proteins Shank is shown to be decreased through proteasome-dependent degradation after retrieval, reaching the lowest level 2 h after retrieval. The protein level is recovered to basal state 6 h after retrieval. Retrieval-induced protein degradation seems to be regulated in a target specific manner, as polyubiquitination of Shank and Guanylate kinase-associated protein (GKAP) increased after retrieval, while a postsynaptic density protein PSD-95 was not. Shank and GKAP are known to be regulated by synaptic activity in primary neuron culture (Ehlers, 2003; Hung et al., 2010), which is in good agreement with the destabilization process. These results suggest that specific synaptic proteins are destabilized after retrieval through the ubiquitin–proteasome-dependent degradation pathway. The fact that PSD-95, a scaffolding protein associated to receptors is not degraded while master scaffolding proteins Shank and GKAP are degraded may give an insight about how the master scaffolding structure is destabilized while the receptor complex itself is preserved, at least in terms of protein degradation. Although an important scaffolding protein PSD-95 may be preserved, the total scaffolding structure is at least partly destabilized, which is reconstructed afterward. There is still a possibility that proximal structure of the receptor complex may also be disassembled by other mechanisms.

There is evidence that NMDA receptor, L-type voltage gated calcium channel (LVGCC), and cannabinoid receptor 1 (CB1 receptor) are also required for retrieval-induced destabilization. NMDA receptor antagonist treated locally in amygdala prevented the amnesic effect of anisomycin in cued fear memory (Ben Mamou et al., 2006). Inhibitor of LVGCC and CB1 receptor treated either systemically or locally in hippocampus blocked the amnesic effect of anisomycin in contextual fear memory (Suzuki et al.,

2008). Considering the nature of these molecules as channels and receptors, these might work as upstream molecules of retrieval-induced protein degradation. Interestingly, CaMKII which can be activated by NMDA receptor and LVGCC are known to phosphorylate a subunit of proteasome complex, which leads to increased activity (Djakovic et al., 2009). CaMKII α is also known to work as a scaffold protein that translocate proteasome from the dendritic shaft to the synaptic spines induced by synaptic activity in primary neuron culture (Bingol et al., 2010). These two researches support the hypothesis that NMDA receptor and LVGCC are activated at the initial stage of retrieval-induced destabilization, regulating protein degradation. However, more researches are required to directly demonstrate the linkage between these molecules in destabilization *in vivo*.

UPDATING INFORMATION INDUCES RECONSOLIDATION

As reconsolidation is a dynamic process induced by reactivation of the memory, it has been hypothesized that this might work as an updating mechanism for the reactivated memory (Dudai and Eisenberg, 2004). Related to this idea, there is a body of evidence indicating that reconsolidation occurs only when there is updating information.

TASTE ASSOCIATED MEMORY

Efforts to demonstrate that reconsolidation works as an updating mechanism first produced numerous reports showing that reconsolidation is induced by updating information. Intra-cortical blockade of protein synthesis in the insular cortex after retrieval of taste-recognition memory disrupted memory only when there was updating information (Rodriguez-Ortiz et al., 2005). Animals have an innate aversion to a novel taste, resulting in reduced consumption. However, when the novel taste is repeatedly exposed, the animal shows a gradual increment of consumption that eventually reaches a plateau. During this period of gradual increase in consumption, intra-cortical anisomycin treatment in the insular cortex after exposure to the taste impairs the memory, not only blocking the incremental increase, but also impairing the previous consumption level. However, when the consumption reaches a plateau, likely when there is no more updating information, animals were tolerant to anisomycin treatment. Another type of taste memory called conditioned taste aversion, where a novel taste is associated with an aversive stimulus, was also impaired by simultaneous anisomycin treatment in the insular cortex and central amygdala after additional conditioned taste aversion trials, while there was no effect after the aversion reached the asymptote where there is no more updating information (Garcia-DeLaTorre et al., 2009).

SPATIAL MEMORY

In the Morris water maze, a spatial memory task where the animal has to learn the position of a hidden platform in a water filled maze in order to escape from the water, anisomycin treatment during a reminder trial of intermediately trained animals led to impairment in the probe test, while there was no effect in well-trained animals (Rodriguez-Ortiz et al., 2008). A modified version of the water maze, where the platform position is changed day-to-day and new information is acquired every day, was performed by another group (Morris et al., 2006). Intra-hippocampal anisomycin treatment after

a reactivation trial following 6 days of training impaired the next day's performance, while anisomycin treatment after reactivation of the normal water maze when the animal was fully trained did not produce an effect.

OBJECT RECOGNITION MEMORY

Object recognition memory is a memory for object identity. After exposure to two objects, one of the objects is changed to a novel one. If the animal remembers the first two objects, it can discriminate those from the novel object. Intra-hippocampal treatment with anisomycin after reactivating the memory by presenting the initial two objects again did not yield any effect. However, treatment after reactivating the memory by presenting one of the initial objects together with a novel object led to impairment of memory for the reactivated initial object (Rossato et al., 2007). This means that object recognition memory undergoes reconsolidation only when the familiar object is presented together with a novel object. Another group has shown that under conditions where presenting the initial objects does not induce reconsolidation, presenting those same objects with salient, novel contextual information induced reconsolidation of the memory (Winters et al., 2009).

Although these studies show a correlation in which reconsolidation occurs specifically and exclusively when there is updating information under certain conditions, they do not directly demonstrate that reconsolidation is required to update a previously formed memory.

RECONSOLIDATION IS REQUIRED FOR MEMORY REORGANIZATION

In order to demonstrate that reconsolidation is required to update a previously formed memory, we have to show that blocking reconsolidation prevents incorporation of the updating information. However, many treatments that block reconsolidation are also known to block consolidation, thus making it hard to discriminate whether the failure to incorporate updating information is due to the treatment effect on reconsolidation of the previously formed memory or on consolidation of the new information. Additionally, even a treatment that impair reconsolidation but not consolidation has limitation when it works on the reestablishment step, as this would leave the initial memory to be destabilized. With the initial memory destabilized, the incorporation of the updating information would fail even if this process does not directly require reconsolidation. However, treatments that block the destabilization step of reconsolidation may be a good method to demonstrate the requirement of reconsolidation for memory updating, as reconsolidation is blocked while the initial memory itself would be intact.

Using proteasome inhibitor to block reconsolidation, a recent research has demonstrated that retrieval-induced destabilization of the previously formed memory is required to further strengthen the memory (Lee, 2008). After the first contextual fear conditioning, additional conditioning at the same context the next day further strengthen the fear response. The author showed that the strengthening mechanism resembles reconsolidation rather than consolidation, by inhibiting distinct molecular requirements for consolidation and reconsolidation. Then, the author showed that proteasome inhibitor locally treated in hippocampus after the

second conditioning impaired further strengthening of the memory, leaving memory strength unchanged from the initial memory. Active weakening of the memory, or extinction, has also been demonstrated to be impaired by proteasome inhibitor (Lee et al., 2008). Local treatment of proteasome inhibitor in hippocampus after contextual fear memory extinction trials impaired the decrement of fear response, leaving the fear response level similar to the initial level. There is also evidence that proteasome activity is required for partially modifying the content of the memory rather than modifying the quantitative strength of the memory (Choi et al., 2010). Using an object–place associative memory, where positional memory for four objects is formed and two of the objects are inter-switched the next day in order to partially modify the memory, the authors demonstrated that proteasome inhibition treated locally in hippocampus after the second day exposure impaired successful modification of the memory.

These researches demonstrate not only that reconsolidation is required for incorporating updating information to the previously formed memory, but also show that retrieval-induced destabilization is required to reorganize the reactivated memory. Memory strengthening and memory weakening, as well as typical reconsolidation where the memory is recovered to a similar level as the original memory, seem to be initiated by destabilization of the initial memory. These facts give insight for a model of memory reorganization. When the memory is reactivated, it first undergoes a protein degradation-dependent destabilization process. The next step depends on the context of reactivation. When the memory is no longer valid, the destabilized memory either stays in the destabilized state or actively encodes the extinction information. When the memory is further reinforced by additional training, the destabilized memory is restabilized to a stronger state than the initial memory. When the reactivating context is not effective to either weaken or strengthen the initial memory, the destabilized memory is restabilized to a similar state as the initial memory. This alternative destination of the memory after reactivation may also agree with the “dominance of the trace” theory that a certain trace conflicting with another trace (i.e., the “excitatory” original CS–US trace, and an “inhibitory” or new CS–no US) gains control over behavior after the retrieval, which also shows transient sensitivity to consolidation blocker. The restabilization process after the destabilization process is protein synthesis-dependent and could resemble the consolidation process in many aspects.

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CONCLUSION AND FUTURE DIRECTIONS

Reconsolidation has been hypothesized to be an updating mechanism that incorporates the new information to the previously formed memory. However, it was not possible to directly demonstrate that reconsolidation is required to update the memory. Recent studies focusing on the destabilization process of reconsolidation not only has provided understanding of the mechanism of reconsolidation, but also gave a breakthrough to demonstrate the requirement of reconsolidation for memory updating. It also provided insights for the model of memory reorganization, including memory weakening, maintaining, and strengthening after it is reactivated.

There is evidence showing that UPS plays an important role in *in vitro* model of learning and memory, such as long-term potentiation (LTP) and long-term depression (LTD; Colledge et al., 2003; Fonseca et al., 2006; Hou et al., 2006; Karpova et al., 2006; Deng and Lei, 2007; Cai et al., 2010). Whereas protein degradation did not show a critical role in the consolidation of contextual fear memory, another study showed that protein degradation is required for both consolidation and reconsolidation of spatial memory (Artinian et al., 2008). In contrast with the absolute requirement of protein synthesis for both long-term synaptic plasticity and memory, the involvement of protein degradation may differ between memory types and experimental conditions. There is also a possibility that protein degradation plays more critical role in specific situations, such as in destabilization of the reactivated memory than in general learning and memory process.

Although it is shown that protein degradation is required for the destabilization of reactivated memory, more studies are required to fully understand the molecular mechanism of the destabilization process, especially upstream pathway that activates and regulates protein degradation and downstream target proteins in the pre- and postsynaptic area. A variety of molecular and cell biological approaches will be helpful to this end and may overcome certain limitations caused possibly by the complex effects of proteasome inhibitors. In addition, it is important to apply these findings to different types of memory, and also to varying situations of memory reorganization.

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