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Mechanisms of memory stabilization and de-stabilization

C. M. Alberini*, M. H. Milekic and S. Tronel

Department of Neuroscience, Box 1065, Mount Sinai School of Medicine, New York, NY 10029 (USA),
Fax: +1 212 849 2611, e-mail: Cristina.Alberini@mssm.edu

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Abstract. Memories become stabilized through a time-dependent process that requires gene expression and is commonly known as consolidation. During this time, memories are labile and can be disrupted by a number of interfering events, including electroconvulsive shock, trauma and other learning or the transient effect of drugs such as protein synthesis inhibitors. Once consolidated, memories are insensitive to these disruptions. However, they can again become fragile if recalled or reactivated. Reactivation creates another time-dependent process, known as reconsolidation, during which the memory is restabilized. Here we discuss some of the questions currently debated in the field of memory consolidation and reconsolidation, the molecular and anatomical requirements for both processes and, finally, their functional relationship.

Keywords. Memory, consolidation, reconsolidation, molecular mechanisms, CREB, C/EBP, protein synthesis, hippocampus, amygdala.

'Memory is … neither perception or conception, but a state or affection of one of these, conditioned by lapse of time. As already observed, there is no such thing as memory of the present while present, for the present is object only of perception and the future of expectation, but the object of memory is the past'

ARISTOTLE, 350 BC

Introduction

How is memory maintained over time, and how does the passage of time influence memory?
These fascinating questions are currently receiving a great deal of attention, because recent findings have challenged some of the classical beliefs of how memories are stabilized and maintained. The classical view that once a long-term memory is formed, it does not become labile again, is no longer accurate. The arguments against the stable nature of long-lasting memories stem from the recent rediscovery of an important feature of the memory process: established memories are not permanently stable but can again become fragile when recalled or reactivated [1–3]. Thus, a stabilized memory is not fixed, and although it can persist for a long time, sometimes even for a lifetime, it can, under certain conditions, return to a labile state. This suggests that memories are highly malleable and dynamic, even the very strong ones that may control our daily lives.

How does a new memory become stable? The initial phase of consolidation and the dilemma of consolidation and reconsolidation

According to their temporal stability, memories can be distinguished as either short- or long-term. Short-term memory lasts for seconds to minutes, while long-term memory lasts for days, weeks, years and even a lifetime. A memory becomes long-lasting through a process known as consolidation, which transforms newly learned information into stable modifications, the nature of which still remains to be fully discovered. During the initial phase of consolidation, memories are labile and can be disrupted by interference such as trauma, other learning, seizure and administration of drugs, including protein and RNA synthesis inhibitors [4–7]. Once the process of consolidation is completed, consolidated memories are stable and insensitive to these interferences. Thus, the classical view

* Corresponding author.

of consolidation states that memories stabilize only once through a process that requires new gene expression. Once stabilized, they cannot be 'undone' and are lost only if their site of storage is physically damaged or if they decay by forgetting.

The term 'consolidation' was coined over 100 years ago by Muller and Pilzecker [8] in an attempt to explain their findings on so-called retroactive interference in humans, showing that the memory of a recently acquired list of words can be disrupted if the subject has to learn a second list of words in short succession. In contrast, the two lists of words are well remembered if they are presented temporally spaced by a relatively short interval (2–3 h). The authors proposed that a memory is initially labile and becomes stable through a time-dependent process of consolidation, and that once the memory is consolidated, it seems to be stored in a stable form that is not readily disrupted.

This hypothesis was later supported by clinical observations of the phenomenon of retrograde amnesia (RA) following head trauma and insults or epileptic seizures [9–13]. These conditions produce a loss of memories of events preceding the trauma or seizure, and the memory deficits are greater for information from recent as opposed to remote, suggesting the existence of a gradual long-term consolidation process. Duncan [14] extended these findings to animal models and showed that the closer in time an electroconvulsive shock is to the learning event, the greater the memory impairment is. This gradual loss of memories according to their age has become known as the gradient of RA. Interestingly, a gradient of RA is most often associated with damage to areas of the medial temporal lobe, particularly structures of the hippocampal formation [15, 16]. In contrast, a number of studies have failed to observe a temporal gradient of RA, particularly when the neocortex [17] or the amygdala [18, 19] was lesioned, suggesting that these areas play a permanent role in memory expression and/or storage.

Subsequently, the cellular and molecular mechanisms of the consolidation process began to be uncovered in the 1960's, after it was shown that the administration of protein synthesis inhibitors immediately after learning prevents long-term memory formation without disrupting short-term memory [20, 21]. Later, it was shown that this is a fundamental, evolutionarily conserved requirement for memory formation, as it was found that it occurs in both invertebrate and vertebrate species and in different types of memories [22–24]. The deficit in long-term memory was always observed if protein or RNA synthesis was inhibited around the time of training. However, as the interval between training and injection was increased, memories became increasingly resistant to disruption. This suggested that the protein-RNA synthesis-dependent phase of consolidation is limited to only a few hours, a length of time undoubtedly shorter than the gradient of RA.

In the past decade, behavioral animal models and molecular biology techniques were instrumental in identifying a number of genes that are regulated after learning and required for long-term memory formation. The first insights about the nature of these genes came from invertebrate species, primarily *Aplysia californica* and *Drosophila melanogaster*. The great advantages offered by the simplicity of both systems allowed the discovery of the first molecular pathway essential for memory consolidation, that is the cyclic AMP (cAMP)-protein kinase A (PKA)-cAMP response element-binding protein (CREB)-CCAAT enhancer-binding protein (C/EBP)-dependent pathway [25–43]. Studies in both *Aplysia* and *Drosophila* systems led to the same conclusions after independently demonstrating that several components of the cAMP activation pathway converging on the transcription factor CREB are required for long-term memory formation, but dispensable for short-term memory. In addition, it was found that one of the first events that takes place downstream of CREB activation is the induction of members of yet another family of transcription factors, the C/EBPs. These factors were also reported to be required for the formation of long-term memory. Studies that targeted the same pathway in mammals demonstrated that the cAMP-PKA-CREB pathway is an evolutionarily conserved mechanism which is required for long-term memory consolidation of both explicit and implicit memories [44]. Numerous later works then confirmed and extended the understanding of the essential role of CREB in memory in various species, brain regions and learning systems [45–60]. Finally, as in *Aplysia*, the expression of C/EBP was found to be regulated following learning and essential for long-term memory formation in mammals [61–64].

In conclusion, a large body of evidence generated in the last 10 years indicates that the CREB-C/EBP pathway is evolutionarily conserved and plays a critical role during the formation of long-term memory.

The nature of the target genes regulated by CREB and C/EBPs still remains to be identified. However, because long-term memory is accompanied by synaptic morphological changes, which also depend on the activation of CREB [38, 65], it has been hypothesized that some of the target genes regulated by this pathway are involved in long-lasting structural modifications of synaptic contacts [66, 67].

The classical view of consolidation was challenged by the findings of a number of studies showing that established memories become labile when reactivated by recall and can be disrupted by the same agents and events [68–70] that disrupt the consolidation process, including protein synthesis inhibitors [71]. This protein synthesis-dependent process that is initiated after reactivation was called reconsolidation. The idea behind the definition is that the reconsolidation process is a reiteration of the original

consolidation phase. However, the fact that both new and reactivated memories are labile and dependent on protein synthesis to become stable does not reveal the nature of the underlying mechanisms and circuits. Thus, in the last few years, several laboratories studied and compared the features of both processes, in an attempt to understand their bases and relationships [70, 72].

The observation that a presumably consolidated, and thus stable, memory can again become labile and sensitive to disruption when reactivated was initially made approximately 35 years ago. A study by Misanin and colleagues [68] first demonstrated that, if an electroconvulsive shock (ECS) is administered immediately after the recall of a passive avoidance memory 24 h after learning, when consolidation is presumed to have already occurred, the memory is lost. The authors ruled out the possibility that this impairment was due to a general interference with the consolidation process, as ECS delivered at the same time point after learning but in the absence of reactivation had no effect on future recalls of that memory. The idea that a memory can be disrupted after recall was controversial, and some scientists were unable to replicate the original experiments. Dawson and McGaugh [73] tried to replicate the original findings of Misanin et al. [68], but were unable to observe an effect of the ECS treatment when given after passive avoidance memory reactivation. In addition, others reported that the amnesia following reactivation was only transient [69]. Nevertheless, a number of studies confirmed the initial findings of Misanin et al. [68] using different memory tasks and amnesic agents, including hypothermia and protein synthesis inhibitors [71, 74, 75]. A basic model that came from these experiments was that it is not the time elapsed since encoding that determines the susceptibility of the memory trace, but the functional state of the trace itself. Lewis [1] proposed that memories exist in either an active or an inactive state. New memories are active during the original learning phase. Stored memories generally remain in an inactive state, however, when recalled, they again return to an active state; When a memory is in an active state, it can be modulated in different ways and therefore can become sensitive to disruptions, whereas inactive traces are insensitive to such manipulations.

For some reason, this area of research became silent and was almost forgotten in the field until 10 years ago, when the issue of memory stability after recall began to resurface. A number of studies showed that tetrodotoxin (TTX) inactivation of specific brain areas [76] and pharmacological interference after memory recall [77–79] disrupt those memories. Around the same time, Nader et al. [80] reported the same phenomenon using auditory fear conditioning and inhibiting protein synthesis in a specific area of the brain that mediates fear memory formation and expression, the basolateral amygdala (BLA). The consolidation of an auditory fear memory is blocked by the administration of protein synthesis inhibitors directly into BLA immediately after training [81]. Nader et al. [80] showed that, when protein synthesis inhibitors are injected directly into the BLA immediately after the recall of a previously consolidated fear memory either 24 h or 14 days after the original learning, the memory is impaired on a subsequent test. The authors concluded that, similarly to what was suggested by Lewis [1], every time a memory is reactivated, or is in an active state, it requires another phase of protein synthesis-dependent consolidation. Thus, in line with earlier suggestions, the authors proposed that memories undergo a process of reconsolidation following recall.

One of the major questions that has emerged from these findings is whether and for what reason a process such as the initial phase of consolidation occurs each time a memory is reactivated; in other words, is reconsolidation a recapitulation of the original consolidation or is it a distinct process? Why does reconsolidation occur? One could argue that it is expected that reconsolidation is not an exact copy of the consolidation process, as new learning is distinct from a reactivation process, which often occurs through only a partial re-experience (i.e. reactivation by recall, in which only CS presentation occurs). However, for the precise reason that reactivation is generally different than training, it is important that we address how similar or different the underlying mechanisms of stabilization actually are.

Mechanisms and circuitry of reconsolidation

A number of laboratories including our own have investigated whether reconsolidation requires the same molecular mechanisms and/or brain regions as consolidation. Several studies concluded that a number of mechanisms and brain regions are commonly used by both consolidation and reconsolidation, while others have shown that the two processes have distinctive features: molecules that are regulated and required for the consolidation of a particular memory are not necessarily engaged or required during its reconsolidation [70, 72, 82]. von Herzen and Giese [82] analyzed the expression levels of two specific context-shock transcripts, the serum and glucocorticoid-induced kinase 3 (SGK3) and nerve growth factor-inducible gene B (NGFI-B), which they found to be upregulated in the mouse hippocampus following contextual fear conditioning. While both genes are regulated during consolidation, only the former was found to be regulated following reactivation, suggesting that reconsolidation is only a partial recapitulation of consolidation. Furthermore, Lee et al. [83] have proposed that the molecular mechanisms required by the hippocampus during either consolidation or reconsolidation are distinct. They reported that antisense-mediated disruption of zif268 in

the rat hippocampus impairs reconsolidation but not consolidation of contextual fear conditioning, whereas, conversely, antisense-mediated disruption of brain-derived neurotrophin factor (BDNF) impairs consolidation but not reconsolidation. Although this study did not investigate the temporal profiles of the requirement for zif268 and BDNF, and therefore cannot exclude that the same transcripts are required for both processes at other timepoints, it at least indicates that molecular requirements during consolidation and reconsolidation have distinct temporal dynamics. In addition, distinct neural circuits have been shown to be activated and required during consolidation and reconsolidation in a variety of memories [62, 84–88]. For example, we have investigated inhibitory avoidance (IA) consolidation and reconsolidation in the rat to determine whether molecules required for consolidation are also essential during reconsolidation in the same brain regions. In IA, the animal is exposed to a context in a shuttlebox that is divided into a safe and a shock compartment. Upon entering the shock compartment, the animal receives a foot shock, and therefore learns to avoid this compartment during future re-exposures (tests). Memory of this experience is measured by recording the latency of the animal to reenter the shock compartment at desired timepoints after training. IA learning and memory formation require an intact hippocampus and amygdala [89], as, indeed, this memory represents an association between the representation of contextual cues and the fear induced by the shock. As described above, an evolutionarily conserved, fundamental molecular pathway required for memory consolidation is mediated by the transcription factors CREB and C/EBP. The regulation and expression of CREB, C/EBPβ and C/EBPδ in various brain regions following IA training revealed that, in the hippocampus, learning results in a rapid increase in CREB phosphorylation at the residue Ser133 (pCREB), an event critical for CREB activation [90], and this increase remains significantly elevated for at least 20 h. At later timepoints, between 9 and 20 h after training, the expression of C/EBPβ and C/EBPδ is significantly induced and, like that of pCREB, remains elevated for a relatively long time, that is, for more than 28 h. Importantly, the disruption of C/EBPβ by injection of specific antisense oligodeoxynucleotides (β-ODNs) in the hippocampus, 5 h after training, abolishes long-term memory, indicating that this transcription factor plays an essential role during IA consolidation [62]. When we investigated the C/EBPβ requirement in the hippocampus after IA memory reactivation, we found a different scenario. Testing IA retention 48 h after training (Test 1), which reactivated the memory, followed by β-ODN hippocampal injections 5 h later, has no effect on memory retention measured 48 h later (96 h after training, Test 2). Moreover, this result was strengthened by the observation that protein synthesis inhibitors injected into the hippocampus immediately after recall also fails to affect memory retention at Test 2. Nevertheless, systemic administration of protein synthesis inhibitors immediately after recall demonstrated that IA undergoes reconsolidation and requires protein synthesis in some brain regions in order to be maintained [62, 91], suggesting that, if the hippocampus is not critical for reconsolidation, other regions must be. Another potential candidate region that could be involved in the molecular processing of IA reconsolidation is the amygdala. When we investigated the role and the molecular requirements of this region, we found that while protein synthesis is required for both consolidation and reconsolidation, C/EBPβ plays an opposite role compared with that found in the hippocampus. β-ODN injections into the BLA disrupt the reconsolidation, but not the consolidation of IA [92, M. H. Milekic and C. A. Alberini, unpublished]. Thus, like several others authors [70, 72], we found evidence that consolidation is distinct from reconsolidation mainly because it engages distinct circuits. On the other hand, in different brain regions, both processes engage common molecular pathways.

This conclusion seems, at first, to disagree with that of Nader et al. [80], who having found that amygdala protein synthesis is required for both consolidation and reconsolidation of auditory fear conditioning, proposed that the two processes anatomically overlap. However, as the participation of more brain regions is investigated in several tasks, it seems to emerge that the circuits underlying these two processes overlap only partially, but that the amygdala in particular is often involved in both consolidation and reconsolidation of aversive as well as incentive types of memories [80, 86, 93–99].

In summary, from the studies thus far reported, the two processes of consolidation and reconsolidation appear to overlap only partially in terms of molecular mechanisms and brain areas engaged. Moreover, based on two additional observations described below, it further appears that initial consolidation and reconsolidation are distinct processes, although they serve a common purpose: stabilizing memory.

Reconsolidation and the passage of time

Another point that has been strongly debated is whether amnesia produced by disrupting reconsolidation is persistent. Some groups have reported that, whereas amnesia caused by protein synthesis inhibition after training is long-lasting, amnesia resulting from inhibition of protein synthesis after reactivation spontaneously recovers over time [100–102] or recovers after exposure to a reminder [103]. The question of the persistence of amnesia induced by protein synthesis inhibitors after reconsolidation may be related to the degree and duration of protein synthesis inhibition. This issue was under debate for the consolida-

tion process a few decades ago [104], and a possible explanation for the temporary effect was that protein synthesis was only partially inhibited or inhibited for an insufficient length of time. Indeed, studies by Flood et al. [105, 106] and Davis [107] found that stronger memories require longer (stronger) inhibition of protein synthesis to be permanently disrupted. Moreover, partial inhibition of protein synthesis during the consolidation or reconsolidation phase results in temporary impairment that recovers at later times [108].

It is also important to establish whether, as hypothesized, reconsolidation occurs every time a memory is reactivated, or whether the memory becomes independent of such a process over time. As discussed above, the assumption of the reconsolidation process is that every time a memory is reactivated, it becomes susceptible to disruption by protein synthesis inhibitors. However, the risk of losing a memory every time it is recalled seems to be highly disadvantageous. An alternative hypothesis which could explain the results reported by several groups in the last few years on reconsolidation is that the recalled memories being abolished via protein synthesis inhibition were recently acquired and not yet fully consolidated. In fact, most studies reporting the reconsolidation phenomenon investigated the effect of reactivation on very recent – one- or two-day-old – memories, but not on older ones. Thus, older, more consolidated memories may not become fragile when recalled. We tested this hypothesis by investigating whether the degree of vulnerability of a recalled memory changes as a function of the time elapsing between initial learning and recall. When the IA memory of rats was reactivated at either 2, 7, 14 or 28 days following training, it showed an increasingly strong resistance to protein synthesis inhibitors: while young, 2- and 7-day-old memories became fragile and were disrupted by protein synthesis inhibitors administered after recall, older, 14- and 28-day-old memories were resistant to the same treatment [91]. This showed that the passage of time influences the stability of a memory and that an older memory is less susceptible to disruption following its reactivation (Fig. 1).

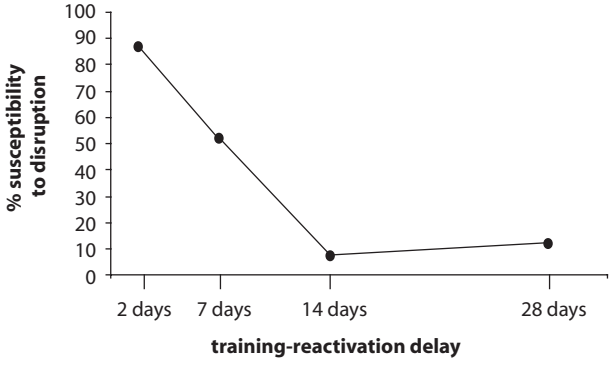

Figure 1. The requirement for protein synthesis following IA reactivation is temporally graded. The percent (%) susceptibility to disruption by anisomycin of IA reactivated memory at different timepoints after training is represented. Reprinted with permission from [91]. Copyright 2002 Elsevier.

As we described above, because we had previously found that protein synthesis and C/EBPβ are required in the BLA for the reconsolidation of a 2-day-old IA memory, we asked whether both requirements in the amygdala have a temporal gradient similar to the one seen with systemic inhibition of protein synthesis. This was exactly the case. Rats trained on IA that underwent reactivation 2 weeks after training, followed by injections of either protein synthesis inhibitors or β-ODN into the BLA, had an intact memory on later tests [M. H. Milekic and C. M. Alberini, unpublished]. Thus, these data show that the requirement for protein synthesis and C/EBPβ in the amygdala after memory reactivation is limited to relatively young memories.

Similar effects due to age of memory were reported in contextual fear conditioning in both mice [109] and Medaka fish [110]. Interestingly, Suzuki et al. [109] found that, whereas protein synthesis inhibitors could disrupt a 24-h to 3-week-old contextual fear memory after reactivation in mice, an 8-week-old memory was insensitive to disruption by a similar reactivation session. However, if the reactivation session was prolonged, even the older memory could be disrupted. They also showed that weak (1 shock) versus strong (3 shocks) training protocols correlate with more or less susceptibility to disruption after reactivation, respectively. These results showed that reexposure duration, age of memory and strength of memory interact to influence the degree of vulnerability of a reactivated memory.

In conclusion, as the time interval since training lengthens, there is increasing resistance to post-reactivation disruption. Thus, remote, well-consolidated memories do not return to a labile state after reactivation, and recall does not place stable memories in a complete state of vulnerability. Conversely, recently acquired memories, although already insensitive to protein synthesis inhibition, become unstable if reactivated and do require protein synthesis to be later recalled.

These results appear to be in disagreement with the findings of Nader et al. [80], who showed that the requirement for protein synthesis (within the amygdala) lasts much longer. Classical auditory conditioning memories reactivated 2 weeks after training are disrupted by post-testing injection of anisomycin into the amygdala. Using contextual fear conditioning, Debiec et al. [3] found that a memory reactivated even 45 days after learning could be disrupted by hippocampal lesions after reactivation. A possible explanation for the discrepancies between this and the findings described above is that, as suggested by Suzuki et al. [109], the intensity of training and reactivation and the nature of different tasks produce temporal requirements for protein synthesis after reactivation that

have different temporal evolutions. Notably, these conclusions do not exclude the possibility that reactivation of fully consolidated memories is accompanied by a phase of de novo protein synthesis. However, new protein synthesis that is induced by the reactivation of a fully consolidated memory does not appear to be essential for the maintenance of the memory.

Why does protein synthesis dependence of a reactivated memory decrease as time from the original training increases? One explanation may argue that this reflects the nature of the molecular changes underlying memory stabilization. A dominant cellular/molecular view of memory storage hypothesizes that the consolidation of a new memory is accompanied by the growth of new synapses [66, 111–114]. Thus, it is believed that, as time from the original training elapses and consolidation proceeds, the number of newly formed synapses increases until it reaches a plateau. One could speculate that when a memory is reactivated, a given number of the same newly formed synapses is reengaged and, therefore, destabilized and reorganized in order to incorporate the new information. As a result, if memory reactivation occurs soon after training, it can potentially destabilize a large part (perhaps most) of the new synapses. On the other hand, if reactivation occurs later, the proportion of the synapses to be reorganized decreases. Hence, over time, the vulnerability of that memory will progressively diminish.

Another hypothesis to consider is that both the initial consolidation and, for a limited time, reconsolidation physically share a process of encoding, the former because it encodes new memory traces, and the latter because it replays the recalled memory traces in order to strengthen them. In support of this hypothesis, training-driven, time-dependent changes in the topography of firing activity, possibly related to memory consolidation, have been described in rabbit avoidance learning by Freeman and Gabriel [115]. Similarly, Ambrogi-Lorenzini [116] reported that different brain structures are required during different temporal phases of memory formation in rat. Thus, as found in several temporal lobe-dependent memories, it is possible that the initial phase of consolidation is driven by modification of encoding circuits (e.g. which include the hippocampus), which, over time, may lead to long-lasting changes in physically distinct storage circuits (which exclude the hippocampus) [117]. Therefore, encoding of a reactivated memory would interfere with the stability of that memory only if the initial phase of consolidation is active, that is, when the same encoding circuits are still engaged.

Because the time scale that reconsolidation requires for becoming insensitive to protein synthesis inhibitors is much longer than initial memory vulnerability (e.g. weeks versus hours/days for IA), it is possible that what is critical for the extended consolidation of a memory is not the original protein synthesis-dependent phase of newly acquired information but rather the further integration of this information into system representations [4, 118]. Such a process may be mediated by modulatory hormonal and/or neuronal pathways and involve different areas of the brain [5, 119].

Functions of memory reconsolidation

Why would a memory become labile following reactivation? It has been proposed that the protein synthesis induced by memory reactivation allows for the incorporation of new information into pre-existing memories [1, 2], and, indeed, it seems intuitively obvious that memories need to be continuously updated with new learning. However, our data described above showed that, if this is the case, editing a young memory is different from editing a completely consolidated one. But that requires determining whether or not one function of reconsolidation is to mediate the formation of associations between new information and reactivated memories.

To investigate this question, we used a second-order conditioning paradigm. Whereas first-order, classical Pavlovian conditioning involves the formation of an association between the representations of the stimuli paired during training [pairing between a conditioned stimulus (CS) with an unconditioned stimulus (US)], second-order conditioning promotes the formation of associations between the second-order CS (S2) and the conditioned response elicited by the first-order CS (S1) [120, 121]. Thus, the stimulus-response learning that occurs during second-order conditioning represents the formation of an association between new (S2) and reactivated information (memory of S1-US), which makes this paradigm proper for investigating the mechanisms involved in linking new and reactivated information. As described above, we know that C/EBPβ is essential in the hippocampus for consolidation but not reconsolidation of IA memory. Conversely, C/EBPβ in the amygdala has the opposite role: it is necessary for IA reconsolidation but not consolidation. We therefore designed a second-order conditioning IA paradigm and tested whether the formation of the association between the new information S2 and the reactivated memory induced by S1-US is sensitive to the disruption of the C/EBPβ in the amygdala (reconsolidation mechanisms), hippocampus (consolidation mechanism) or both [92]. If, as hypothesized, reconsolidation mediates the formation of an association between new and reactivated information, then C/EBPβ knock-down in amygdala should disrupt the formation of this association.

Rats were trained in an IA shuttle box (context A) in which a light was switched on while the animal was in the safe part of the context. Subsequently, the light was used to reactivate this memory in a different context (context B = S2). As previously found with other second-order fear-conditioning tasks [121–124], reactivation of IA memory in a

new context resulted in the formation of an association that linked S2 with the recalled fear originally induced by S1-US. In fact, at subsequent tests in context B, the animals that were shocked only in context A, and underwent light exposure in context B, learned to fear context B. When protein synthesis was disrupted throughout the brain by systemic injection of protein synthesis inhibitors after reactivation by the light in context B, both the new fear of context B and the original memory for context A were disrupted. This indicated that both the new and original, reactivated memories were labile. However, this requirement for protein synthesis was the sum of two mechanistically distinct processes. The inhibition of reconsolidation obtained by blocking a molecular mechanism selectively engaged in this process, namely expression of C/EBPβ in the amygdala, only affected the original and reactivated memory but left the new association intact. Conversely, formation of the new association was selectively disrupted by blocking the consolidation requirement, that is, hippocampal C/EBPβ expression, which, on the other hand, left the original memory intact. These results indicated that the formation of the fear memory for context B, which represents the link between the new and the reactivated information, is mediated by molecular mechanisms similar to those underlying initial consolidation of a new memory. In other words, although the original and reactivated memory undergoes reconsolidation, this process occurs independently of the formation of the new association S2-fear [92].

Although it is not clear whether these experiments with second-order conditioning completely address the role of possible new encoding hypothesized to occur at each reactivation [2, 125, 126], it is reasonable to conclude that these or similar processes are engaged in memory updating. Thus, it is possible that the process of memory updating, or at least the formation of an association that links new and reactivated information, occurs without destabilizing the original memory trace and independently of the reconsolidation of the retrieved memory.

Another function believed to be mediated by the process of reconsolidation is to strengthen the memory or increase its duration [127]. Currently, there no formal demonstrations of this function, however, we believe that this question will be addressed in the near future.

Summary and concluding remarks

In summary, the evidence provided by the studies carried out thus far in the reconsolidation field leads to the following conclusions:

1) Memory consolidation and reconsolidation engage neurocircuitry that is only partly similar. Regions involved in consolidation are not always involved in reconsolidation.
2) Memory consolidation and reconsolidation are processed by molecular mechanisms that are mostly shared. However, like neurocircuitry, some mechanisms involved in consolidation do not participate in reconsolidation.
3) Memory reconsolidation shows an age-restricted requirement for protein synthesis, the length of which seems to relate to the strength of both training and reactivation.
4) The labile phase of memory reconsolidation does not participate in the formation of new associations that link novel information with a reactivated memory. On the contrary, linking new and reactivated information is similar to the formation of a new memory.

These conclusions are in agreement with the working model described in Alberini [72], in which we proposed that the process of reconsolidation represents a phase of the extended consolidation process that occurs over a relatively long period of time and perhaps may reflect the temporal extension of RA. This process is therefore not restricted to the initial protein synthesis-dependent phase induced by learning. According to this model, consolidation may include a number of subsequent reactivation events whose function is to further strengthen and/or prolong memory retention.

Finally, although numerous studies have addressed a number of relevant questions about memory reconsolidation, a great deal remains to be discovered, particularly regarding the important consequences of these studies for clinical applications. The possibility of disrupting or weakening the strength of established memories predicts the possibility of therapeutic intervention over memories linked to pathological states, including post-traumatic stress disorder, phobias, obsessive-compulsive disorders, depression and addiction.

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