Neuron, Vol. 40, 695–701, November 13, 2003, Copyright ©2003 by Cell Press

A Requirement for the Immediate Early Gene *zif268* in Reconsolidation of Recognition Memory after Retrieval

Report

Bruno Bozon, Sabrina Davis, and Serge Laroche*
Laboratoire de Neurobiologie de l'Apprentissage
de la Mémoire et de la Communication
CNRS UMR 8620
Université Paris-Sud
91405 Orsay
France

Summary

Recent research has revived interest in the possibility that previously consolidated memories need to reconsolidate when recalled to return to accessible long-term memory. Evidence suggests that both consolidation and reconsolidation of certain types of memory require protein synthesis, but whether similar molecular mechanisms are involved remains unclear. Here, we explore whether *zif268*, an activity-dependent inducible immediate early gene (IEG) required for consolidation of new memories, is also recruited for reconsolidation of recognition memory following reactivation. We show that when a consolidated memory for objects is recalled, *zif268* mutant mice are impaired in further long-term but not short-term recognition memory. The impairment is specific to reactivation with the previously memorized objects in the relevant context, occurs in delayed recall, and does not recover over several days. These findings indicate that IEG-mediated transcriptional regulation in neurons is one common molecular mechanism for the storage of newly formed and reactivated recognition memories.

Introduction

When recalled, previously consolidated memories become temporarily labile and may once again require a stabilization process necessary for their further long-term storage and availability for later recall (Misanin et al., 1968; Mactutus et al., 1979; Przybyslawski and Sara, 1997; Nader et al., 2000; Sara, 2000). This process, referred to as reconsolidation (reviewed in Nader, 2003), fits into the conceptual framework proposed by Lewis (1979) which suggests that the memory trace can shift between two states: an inactive and stable (consolidated) state corresponding to stored memories, and a transiently active and fragile state following both the initial encoding of information during learning and the readout of the trace during retrieval. The implication is that when the memory is converted again into an active state following reactivation, a further storage process is required for the trace to remain in long-term memory and be available once again for recall. A central issue in the current literature is whether reconsolidation of recalled memories recruit the same mechanisms as those used during the initial consolidation process. The demonstration that well-consolidated memories, when reactivated, are vulnerable to systemic (Judge and Quartermain, 1982; Milekic and Alberini, 2002) or region-specific (Nader et al., 2000) protein synthesis inhibition, as are newly formed memories, has provided direct evidence in favor of this hypothesis and has led to a model of cellular reconsolidation that posits that intracellular events necessary for the initial consolidation of memories are re-engaged after retrieval and are required for later recall (Debiec et al., 2002; Myers and Davis, 2002). To date, however, only a few studies have identified specific mechanisms that are involved. In transgenic mice expressing a forebrain-specific CREB repressor, it was shown that CREB was required for both consolidation and reconsolidation of fear memories (Kida et al., 2002), and a recent study has shown that blockade of MAPK phosphorylation impairs both consolidation and reconsolidation of recognition memory in rats (Kelly et al., 2003). However, inactivation of C/EPBß in the hippocampus was shown to impair consolidation in an inhibitory avoidance task but not reconsolidation (Taubenfeld et al., 2001). This raises important questions as to which molecular mechanisms may or may not be required for both processes, and whether there is any form of task specificity.

The prevailing model for cellular consolidation underlying the laying down of memory suggests that synapse-to-nuclear signaling and transcriptional regulation of genes are required to maintain long-lasting synaptic modification in neural networks that are activated during learning. One critical step in this process is the activation of inducible immediate early genes (IEGs) encoding regulatory transcription factors that interact with promoter regulatory elements on a host of downstream late-response genes. *Zif268*, also known as *Krox24*, *Egr1*, or *NGFI-A*, is one such IEG encoding a zinc finger transcription factor of the *Egr* family, which has been implicated in synaptic plasticity and memory consolidation: the induction of LTP (Cole et al., 1989; Wisden et al., 1990) and certain learning experiences (Hall et al., 2001; Guzowski et al., 2001) are associated with increased expression of *zif268* in specific brain structures, and, as we have shown previously, inactivation of *zif268* in mutant mice prevents both the maintenance of synaptic plasticity measured in the dentate gyrus and the consolidation of several types of long-term memories (Jones et al., 2001). Moreover, recent studies have shown that the expression of *zif268* is increased in several corticolimbic brain structures after reactivation of consolidated fear memories (Hall et al., 2001; Thomas et al., 2002). Thus, the objective of the present study was to test the hypothesis that *zif268* would as well be required for reconsolidation. To investigate this, we used the same mouse line carrying a null mutation in the *zif268* gene (Topilko et al., 1998) as in our previous studies and examined recognition memory in an object recognition task, a behavioral paradigm widely studied in humans to probe declarative memory (Manns et al., 2003). This task is rapidly learned and does not require explicit reinforcement; it is based on the spontaneous preference of rodents for novelty and their ability to remember previously

*Correspondence: serge.laroche@ibaic.u-psud.fr

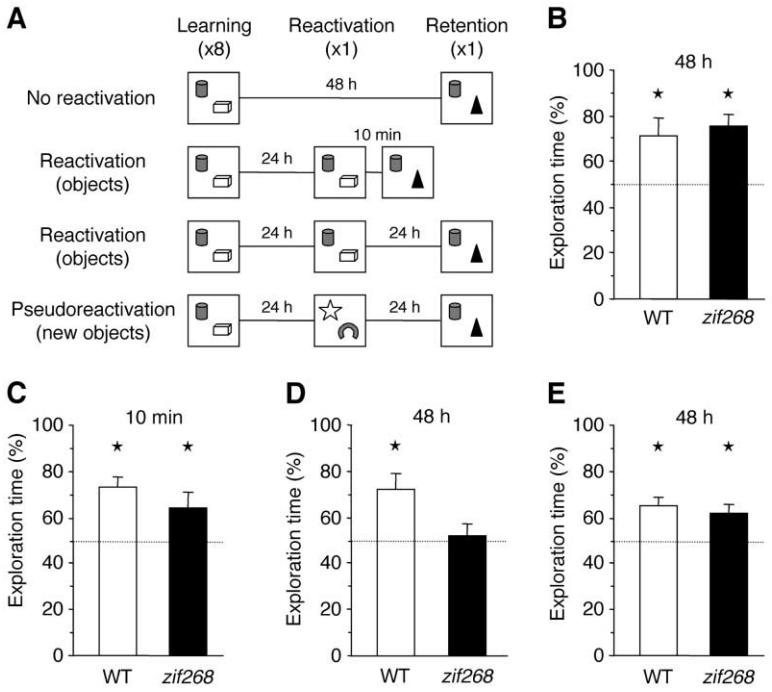

Figure 1. *Zif268* Inactivation in Mutant Mice Impairs Reconsolidation of Recognition Memory

(A) Schematic representation of the object recognition task and time structure of the protocols used in (B)–(E). *Zif268* mutant mice and wild-type (wt) littermates were exposed to two objects for eight briefly spaced sessions, and retention was tested in different conditions of reactivation or no reactivation.

(B–E) Retention performance in the different protocols is expressed as the percent time spent exploring the novel object over the total time of object exploration. (B) *Zif268* inactivation does not impair long-term recognition memory in this overtraining paradigm (wild-type, n = 6; *zif268* mutant, n = 8). (C) A brief reexposure to the familiar objects 24 hr after training does not affect postreactivation short-term memory (wild-type, n = 7; *zif268* mutant, n = 12) but (D) impairs postreactivation long-term memory in *zif268* mutant mice (wild-type, n = 15; *zif268* mutant, n = 14). (E) Recognition memory was not affected in *zif268* mutant mice by a pseudoreactivation consisting of presenting two entirely new objects on day 2 (wild-type, n = 12; *zif268* mutant, n = 17). *$p < 0.05$ (Student's t test).

encountered objects (Ennanceur and Delacour, 1988; Clark et al., 2000). In humans, monkeys, and rodents, recognition memory is affected by damage to structures of the medial temporal lobe, including the hippocampus and adjacent entorhinal and parahippocampal cortices (Wan et al., 1999; Clark et al., 2000; Manns et al., 2003).

Results and Discussion

From our previous studies, we know that *zif268* is essential for normal long-term recognition memory performance in the object recognition task (Jones et al., 2001). This itself presents as a difficulty, as determining the role of *zif268* in reconsolidation after retrieval necessitates that mice initially memorize the elements of the task as well as their wild-type littermates. However, we do know that the deficit in long-term spatial memory in at least one task, spatial learning in the water maze, can be overcome by extended and distributed training (Jones et al., 2001). Thus, we first examined whether *zif268* mutant mice could form a long-term memory for objects if given additional exposures to the objects in a distributed training paradigm. To test this, mice were placed in a small arena containing two objects (Figure 1A) that they could explore freely for four blocks of two 5 min periods (5 min ITI) instead of two, with a time interval between blocks extended to 90 min. Then, after a delay of 48 hr, a novel object replaced one, and the percentage of time spent exploring the novel compared to the familiar object was used as a measure of memory. Both *zif268* mutant mice (n = 8) and wild-type littermates (n = 6) showed preferential exploration of the novel object (Figure 1B, $p < 0.05$ in each case), thus demonstrating that they remembered the two objects they had previously experienced. If the memory of the familiar objects had faded, they would have spent an equal amount of time exploring each. ANOVA showed that there was no significant difference between groups ($F < 1$).

With this protocol, we were able to assess the effect of retrieval interposed between training and retention. In the first experiment (Figure 1A), *zif268* mutant mice and wild-type littermates were allowed to explore two objects on day 1 as before and were then briefly reexposed to the same two objects for a single 5 min reactivation session on day 2. During this phase, the two groups showed equal exploration of the two familiar objects, with no difference between groups (all p values > 0.05, data not shown). Postreactivation long-term memory (PR-LTM, Nader et al., 2000) was then tested 24 hr later (day 3) in a single 5 min session in which a novel object replaced one of the familiar objects (Figure 1A). Wild-type mice (n = 15) explored the novel object significantly more than the familiar object (Figure 1D; $p < 0.001$), demonstrating a similar recognition performance to that when no reactivation was interposed. In contrast, *zif268* mutant mice (n = 14) showed equal exploration of the two objects (Figure 1D), with a level of exploration of the novel object not significantly different from chance ($p > 0.05$). ANOVA confirmed a significant difference between groups ($F_{1,27} = 7.14$; $p < 0.05$) and between the performance of *zif268* mutant mice with and without the reactivation session ($F_{1,20} = 10.13$; $p < 0.01$). Importantly, when postreactivation short-term memory (PR-STM) was tested 10 min after the reactivation session, both wild-type (n = 7) and *zif268* mutant mice (n = 12) showed preferential exploration of the novel object ($p < 0.05$ in each case) that was significantly greater than chance level (Figure 1C), with no difference between groups ($F_{1,17} = 2.86$; $p > 0.05$). These results showing impaired PR-LTM but intact PR-STM rule out the possibility that the deficit 24 hr after reactivation

is due to nonspecific effects, such as impaired motor activity or the spontaneous tendency of mice to explore novelty. Thus, *zif268* mutant mice are able to form a long-term recognition memory in conditions of extended exposure to the objects, and *zif268* inactivation does not affect retrieval. A consolidated and stable recognition memory, however, can again become labile after brief reactivation, and *zif268* mutant mice cannot in this case reconsolidate the memory for objects.

To determine whether the impairment in recognition memory after retrieval is specific to reactivation of the target memory, two entirely novel objects were presented on day 2 (pseudoreactivation, Figure 1A) instead of the two objects experienced on day 1, and a retention test was conducted on day 3, as before, by presenting one object from day 1 and a novel object. On day 3 (Figure 1E), both wild-type ($n = 12$) and *zif268* mutant mice ($n = 17$) showed preferential exploration of the novel object ($p < 0.05$ in each case) that was significantly greater than chance, with no difference between groups ($F < 1$). Compared with the memory deficit observed in *zif268* mutant mice when the two relevant "target" objects are present during reactivation, these results suggest that neither handling nor other nonspecific effects are responsible for the observed deficit. Thus, overall, the findings indicate that *zif268*-dependent impairment in recognition memory after retrieval requires the target memory to be actively reactivated.

Contextual cues associated with the training experience can act as reminders to promote retrieval (Spear 1973; Deweer et al., 1980; Gisquet-Verrier et al., 1989; Sara, 2000), suggesting that the context in which learning has taken place is an important attribute of the memorized episode and can potentially activate target memory traces. Two other series of experiments were conducted to explore the relative contribution of the target objects and of the context to the reactivation process (Figure 2). First, to test whether a *zif268*-dependent reconsolidation process occurs in object recognition memory if contextual information is provided alone, we used the same protocol as before, with the exception that reactivation consisted of exposing the mice to the training context alone, without the objects (Figure 2A). Twenty-four hours after contextual reactivation, both wild-type ($n = 10$) and *zif268* mutant mice ($n = 6$) showed preferential exploration of the novel object (Figure 2B) that was significantly greater than chance level ($p < 0.05$); and there was no group difference ($F < 1$). The absence of effect with the context alone suggests that the context is not an effective cue for reactivating the target memory in this paradigm. This may be due to the relatively low salience of contextual cues in a task that does not make use of explicit reinforcement. Moreover, the mice are well familiarized with the context, and it is thus likely that its value as a cue associated with the set of objects is devaluated; its potential for promoting retrieval of the target memory being lost. In the second experiment, we presented the two previously experienced objects during reactivation on day 2 but in an entirely different context (changing the size and shape of the test arena and the experimental testing room). When the retention test was conducted on day 3 back in the original training context, neither the wild-type ($n = 12$) nor the *zif268* mutant mice ($n = 14$) were impaired

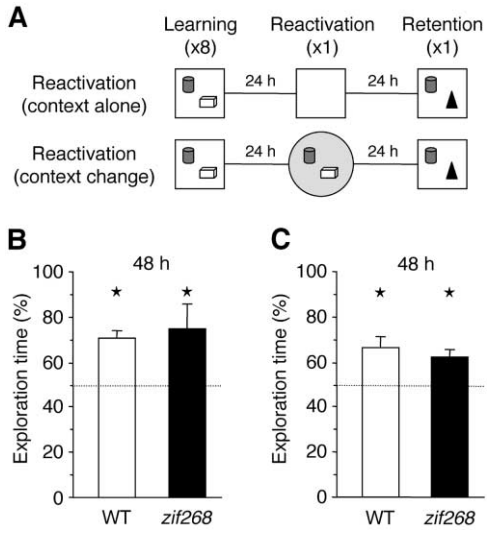

Figure 2. The Effect of Contextual Information on Reconsolidation

(A) Schematic representation of the time structure of the protocols used in (B)–(C). (B) Recognition memory was not affected in *zif268* mutant mice by reexposure to the context alone without the objects (wild-type, $n = 10$; *zif268* mutant, $n = 6$) or (C) by presenting the objects in an entirely different context (wild-type, $n = 12$; *zif268* mutant, $n = 14$). *$p < 0.05$ (Student's t test).

(Figure 2C), both showing preferential exploration of the novel object ($p < 0.05$) with no significant difference between groups ($F < 1$).

In all, these experiments define some of the elements that seem to be necessary for *zif268*-dependent reconsolidation of recognition memory. Clearly, contextual information alone is not sufficient, and the target items (the previously experienced objects) are required. However, the impairment after retrieval is only observed when the target objects are presented in the relevant context. This may suggest that the whole memory representation includes contextual attributes of the learning episode and that the full reactivation of the consolidated memory in this task requires a match between the target items and the context within which they occurred. A similar effect has been observed using electroconvulsive shock after reactivation of fear memory (DeVietti and Holliday, 1972). Alternatively, it remains entirely possible that during the reactivation test in a different context a distinct new memory trace was formed which did not imply reactivation and restorage of the former memory (see Nader, 2003). Whether the objects alone could be sufficient to reactivate the memory fully cannot be firmly established at this point, as it is impracticable to expose the animals to the objects without contextual information being either relevant or irrelevant.

To examine temporal constraints on the requirement for *zif268* in reconsolidation, we tested recognition memory 5 days after learning and increased either the time between training and reactivation or between reactivation and test (Figure 3A). In the first experiment, mice were trained as before, and memory was tested 5 days later. Both wild-type ($n = 6$) and mutant mice ($n = 6$) showed significantly greater exploration of the novel object on day 5 (Figure 3B; $p < 0.05$ in each case), with

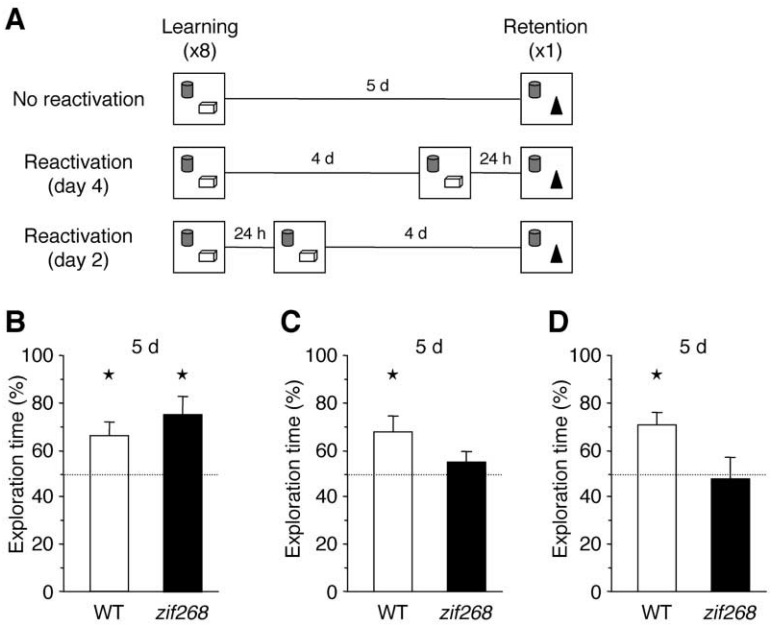

Figure 3. The Impairment of Recognition Memory after Reactivation in *zif268* Mutant Mice Is Long Lasting and Not Temporally Graded

(A) Schematic representation of the time structure of the protocols used in (B)–(D). (B) Retention performance on day 5 shows that both wild-type (wt, n = 6) and *zif268* mutant mice (n = 6) can form a long-term recognition memory in the overtraining paradigm. (C) Reactivation of the memory by brief reexposure to the objects on day 4, 1 day before test (wild-type, n = 11; *zif268* mutant, n = 12), or (D) 24 hr after training, 4 days before test (wild-type, n = 8; *zif268* mutant, n = 8), similarly impairs postreactivation long-term recognition memory in *zif268* mutant mice. *$p < 0.05$ (Student's t test).

no difference between groups ($F_{1,10} = 1.1$; $p > 0.05$), thus reinforcing the fact that *zif268* mutant mice can form a stable, well-consolidated memory for objects in our overtraining paradigm. In contrast, when memory was recalled by a brief exposure to the objects on day 4, performance of the *zif268* mutant mice 24 hr later ($n = 12$; $p > 0.05$) but not of the wild-type mice ($n = 11$; $p < 0.05$) fell to chance—the mutant mice spending an equal time exploring the two objects (Figure 3C). Between-groups comparisons revealed that performance of the wild-type mice with and without reactivation did not differ ($F < 1$), while there was a significant difference in the performance of *zif268* mutant mice ($F_{1,16} = 6.50$; $p < 0.05$). Although only a limited time scale could be explored in this paradigm, the results indicate that there is not a rapid temporal gradient of susceptibility to *zif268* inactivation of a reactivated recognition memory, suggesting that both recent and relatively remote recognition memories undergo *zif268*-dependent reconsolidation when recalled. Other studies have explored the vulnerability of more remote memories, using protein synthesis inhibitors. Nader et al. (2000) found that recalled fear memories are still sensitive to injection of a protein synthesis inhibitor in the amygdala 2 weeks after training, and Debiec et al. (2002), using intrahippocampal injections, reported a similar effect on a reactivated contextual fear memory 45 days after training. Although memories for emotionally neutral stimuli such as that for objects is less resistant to forgetting than aversive, emotionally arousing memories (Cahill and McGaugh, 1998), and therefore the delay we used between learning and reactivation was shorter than examined in these studies, both results suggest that memory may not become immune to reconsolidation rapidly. However, Milekic and Alberini (2002) found that the requirement for protein synthesis of a reactivated memory in an inhibitory avoidance task fades progressively within a 2 week period, when the recency of the memory decreases. Thus, it remains a possibility that a slower process of consolidation may with time render reconsolidation of recalled memories unnecessary. Task specificity, strength of initial learning, and the number of times a memory is reactivated explicitly (cued recall) or implicitly (rehearsal) and reconsolidated may be important variables to explore to provide further understanding of the processes and mechanisms of reconsolidation.

Concerns have been raised in a few instances of reconsolidation studies that a recovery of memory may occur when an "amnestic" treatment is given after retrieval. For example, using a protein synthesis inhibitor in an aversive avoidance task, Judge and Quartermain (1982) observed that the amnesia produced by anisomycin after retrieval but not after initial learning had dissipated in 3–4 days, suggesting that consolidation and reconsolidation may not be qualitatively similar (see also Mactutus et al., 1979, using hypothermia). Thus, we also tested recognition memory performance on day 5 when memory was reactivated 1 day after training (Figure 3A). The results showed that *zif268* mutant mice had no recognition memory on day 5, 4 days after the recall test (Figure 3D). Here again, wild-type mice showed preferential exploration of the novel object on day 5 ($n = 8$; $p < 0.01$) whereas *zif268* mutant mice did not ($n = 8$; $p > 0.05$), and the two groups differed significantly ($F_{1,14} = 4.99$; $p < 0.05$). In addition, while performance of the wild-type mice with and without reactivation did not differ ($F < 1$), that of the mutant mice was significantly different in the two conditions ($F_{1,12} = 5.16$; $p < 0.05$). These results indicate that the disruptive effect of *zif268* inactivation on reconsolidation is enduring for at least several days, and it seems unlikely that in this case there would be spontaneous recovery of the memory.

Overall, the present findings are consistent with the view that reactivation of a consolidated memory for objects presumably returns it into an active state that needs reconsolidation for further storage, and they reveal that the IEG *zif268* is required for reconsolidation

of recognition memory after retrieval. Two other studies have shown that reactivation of a consolidated memory is associated with an increase in the expression of *zif268* in several corticolimbic structures (Hall et al., 2001; Thomas et al., 2002). Thus, our results showing that *zif268* is required for the expression of long-term but not short-term recognition memory after retrieval support the notion that, after retrieval, activation of *zif268* is critical for the trace to return to long-term memory and be available for later recall.

The requirement for *zif268* in the consolidation of recognition memory is supported by our previous findings in the same behavioral paradigm (Jones et al., 2001). From this we can infer that the *zif268* gene is necessary for both the formation of recognition memory and the restabilization of a previously consolidated recognition memory after recall. The two behavioral situations are not entirely equivalent, however, and it is still uncertain whether memory reactivation induces a *zif268*-dependent process similar to that required for consolidation and whether *zif268* would be required for reconsolidation in the standard conditions of learning without overtraining. A direct test of this hypothesis would require conditions where *zif268* could be inactivated after reactivation only. Notably, in this experiment we used a behavioral procedure to bypass the requirement for *zif268* during the initial consolidation. The implication is that in this particular situation the initial memory formed in the absence of a functional *zif268* gene. Why then would this memory require *zif268* for restabilization after recall? Our interpretation is that the molecular mechanisms that allow information to enter an accessible long-term memory store or to reenter into a similar state after recall recruit *zif268* function in the normal conditions of learning and recall. With extended training in the overtraining paradigm, the behavioral manipulation would allow a compensatory mechanism to be recruited during learning, but the routinely used *zif268*-dependent mechanism would be initiated by the brief reactivation and fail in the *zif268* mutant mice. In this view, a signaling cascade leading to activation of *zif268* and presumably to other IEGs would be initiated and required for both consolidation and reconsolidation, and a compensatory mechanism would be set into motion in the specific behavioral conditions of overtraining used here but not after retrieval because of the brief reexposure, simply as we know it does not after learning in the standard condition with brief exposures (Jones et al., 2001). Perhaps a behaviorally induced compensatory mechanism could overcome the reconsolidation deficit as well if there was overexposure to the objects during retrieval, but this cannot be tested because it would be equivalent to relearning. This compensatory mechanism is to date unknown. One could speculate that another member of the *Egr* family of transcription factors might be recruited. Molecular considerations indeed suggest a potential for functional compensation as there is a high homology in the encoded zinc finger proteins that is likely to result in the activation of the same set of downstream effector genes characterized by the consensus *Egr* binding motif on their promoter regions (Swirnoff and Milbrandt, 1995), thus providing a simple way for full neuronal and functional compensation. *Krox20* (*Egr2*) is an intuitively likely candidate. As *zif268*, *Krox20* is rapidly regulated in models of synaptic plasticity such as long-term potentiation (LTP) and, interestingly, the upregulation of Krox20 protein outlasts that of zif268 and requires stronger cell stimulation (Williams et al., 1995), which at a conceptual level may equate to the overtraining protocol. Thus, a testable hypothesis is that *Krox20* may be instrumental in compensating for the lack of *zif268* during learning in conditions of overtraining, leading to the same *Egr*-dependent genomic response of the activated neurons, but this mechanism would not be able to come into play in reconsolidation because a brief reexposure to the objects would not be sufficient to engage *Krox20*.

The consolidation of recognition memory has been shown to be impaired by hippocampal-restricted inhibition of CREB/ATF transcription factors (Pittenger et al., 2002), by inhibition of MAPK (Kelly et al., 2003), and by *zif268* inactivation (Jones et al., 2001), and improved by genetic inhibition of calcineurin or of protein phophatase 1 (Malleret et al., 2001; Genoux et al., 2002). Recently, Kida et al. (2002) reported that CREB function is required both for consolidation and reconsolidation of fear memory, and in the same line we reported that pharmacological blockade of MAPK phosphorylation impairs both consolidation and reconsolidation of recognition memory in rats (Kelly et al., 2003). The general question thus arose as to whether transcriptional mechanisms are also activated and required for reconsolidation after recall. This first report on the effect of inactivation of an IEG provides strong support for the requirement of at least in part similar transcriptional mechanisms in both consolidation and reconsolidation of recognition memory. As MAPK and CREB are components of an upstream cascade controlling plasticity-dependent transcriptional regulation of *zif268* (Davis et al., 2000), the available information to date suggests that a similar signaling cascade is implicated in both processes. Given that *zif268* is likely to control the expression of a host of late-response, effector genes regulated via the *Egr*-response element, it is conceivable that for a large part similar transcriptional mechanisms mediate both consolidation and reconsolidation of recognition memory and that these mechanisms are necessary, whether after learning or recall, for storage and later availability of long-term recognition memory.

Experimental Procedures

Animals

Zif268 mutant mice were generated using 129/SV ES cells injected into C57BL/6J blastocytes and backcrossed onto a C57BL/6J background (Topilko et al., 1998). The targeted inactivation of the *zif268* gene involved an insertion of a *lacZ-neo* cassette between the promoter and coding sequence. There was an additional frameshift mutation at the level of an *N*del restriction site that corresponds with the beginning of the DNA binding domain. As described in Jones et al. (2001), previous histochemical, physiological, and behavioral screening has shown that gross brain anatomy, basal synaptic transmission in the hippocampus, cell excitability, and general behavior and motor activity are normal in *zif268* mutant mice. In situ hybridization studies also confirmed the complete absence of *zif268* in the mutant mice, while both constitutive and LTP-inducible expression of the *LacZ* gene in the *zif268* mutant was comparable to that of the *zif268* gene in wild-type mice, suggesting that signaling events upstream of *zif268* transcription were not affected. Wild-type and *zif268* mutant mice (4–9 months) were housed in a temperature- and light-controlled mouse colony room (12 hr light/dark cycle), in

groups of 4 or 5, and had ad libitum access to food and water. All experiments were conducted in accordance with the recommendations of the EU directive (86/609/EEC) and the French National Committee (87/848).

Behavioral Analysis

Mice were tested in a square open field (58 cm in length, 34 cm in height) located in a room with somber lighting and constant background noise. In the object recognition protocol, two different objects were placed in the open field during the sample phase and, after a delay, one object was changed for a novel object, and mice were tested for their memory of the original two objects by measuring the amount of time spent exploring the novel object compared to the familiar object. The objects either consisted of wooden pieces of various forms or were constructed from assembling interlocking block pieces. They were cleansed thoroughly between trials to ensure the absence of olfactory cues. The mice were first habituated to the open field in the absence of objects, for 20 min a day for 2 days. Then, on day 1 of each experiment (sample phase), both wild-type and *zif268* mutant mice were given four blocks of two 5 min trials of exploration of two objects with a within-block intertrial interval of 5 min and a 90 min interval between blocks. In the standard conditions, retention was tested either 48 hr (first experiment) or 5 days later (second experiment) by placing the mice back in the open field for a 5 min session and by randomly exchanging one of the familiar objects for a novel object. Measurement of the time spent exploring each object was recorded, and exploration of the novel object was expressed as a percentage of the total time of object exploration in seconds. The criteria for exploration were based strictly on active exploration, where mice had both forelimbs within a circle of 15 cm around an object, head oriented toward it or touching it with their noses. The reactivation test interposed between the sample phase and the retention test (Figure 1A) consisted in placing the mice back in the open field for a single 5 min session of exploration of the same objects as those in the sample phase in order to reactivate the memory trace. In experiment one, reactivation was provided 24 hr after learning (day 2) and retention was tested either 10 min or 24 hr later (day 3). Other experimental conditions for the reactivation session on day 2 included exposing the mice to two entirely novel objects, to the training context alone in the absence of the objects, or to the familiar objects in a different context. In experiment two, to determine whether there are any temporal limitations on reconsolidation of recognition memory, mice were given the same sample phase protocol for learning and were tested 5 days later. The reactivation test (as above) was provided either on day 1 or 4 after learning, and retention was tested on day 5 in both cases. Analysis of variance (ANOVA) and Student's t test were used to analyze the data.

Acknowledgments

This research was funded by CNRS PICS program and by a HFSP grant (RGP0040/2002-C) (to S.L.). B.B. was supported by a doctorate fellowship from the Fondation pour la Recherche Médicale. We are grateful to Pascale Veyrac for rearing the mice.

Received: January 4, 2003
Revised: September 19, 2003
Accepted: September 25, 2003
Published: November 12, 2003

References

Cahill, L., and McGaugh, J.L. (1998). Mechanisms of emotional arousal and lasting declarative memory. Trends Neurosci. *21*, 294–299.

Clark, R.E., Zola, S.M., and Squire, L.R. (2000). Impaired recognition memory in rats after damage to the hippocampus. J. Neurosci. *20*, 8853–8860.

Cole, A.J., Saffen, D.W., Baraban, J.M., and Worley, P.F. (1989). Rapid increase of an immediate early gene messenger RNA in hippocampal neurons by synaptic NMDA receptor activation. Nature *340*, 474–476.

Davis, S., Vanhoutte, P., Pages, C., Caboche, J., and Laroche, S. (2000). The MAPK/ERK cascade targets both elk-1 and cAMP response element-binding protein to control long-term potentiation-dependent gene expression in the dentate gyrus in vivo. J. Neurosci. *20*, 4563–4572.

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

DeVietti, T.L., and Holliday, J.H. (1972). Retrograde amnesia produced by electroconvulsive shock after reactivation of a consolidated memory trace: a replication. Psychon. Sci. *29*, 137–138.

Deweer, B., Sara, S.J., and Hars, B. (1980). Contextual cues and memory retrieval in rats: Alleviation of forgetting by a pretest exposure to background stimuli. Anim. Learn. Behav. *8*, 265–272.

Ennanceur, A., and Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats: 1. Behavioural data. Behav. Brain Res. *100*, 85–92.

Genoux, D., Haditsch, U., Knobloch, M., Michalon, A., Storm, D., and Mansuy, I.M. (2002). Protein phosphatase 1 is a molecular constraint on learning and memory. Nature *418*, 970–975.

Gisquet-Verrier, P., Dekeyne, A., and Alexinsky, T. (1989). Differential effects of several retrieval cues over time: Evidence for time-dependent reorganization of memory. Anim. Learn. Behav. *17*, 394–408.

Guzowski, J.F., Setlow, B., Wagner, E.K., and McGaugh, L. (2001). Experience-dependent gene expression in the rat hippocampus after spatial learning: a comparison of the immediate-early genes *Arc*, *c-fos*, and *zif268*. J. Neurosci. *21*, 5089–5098.

Hall, J., Thomas, K.L., and Everitt, B.J. (2001). Cellular imaging of *zif268* expression in the hippocampus and amygdala during contextual and cued fear memory retrieval: selective activation of hippocampal CA1 neurons during recall of contextual memories. J. Neurosci. *2*, 2186–2193.

Jones, M.W., Errington, M.L., French, P.J., Fine, A., Bliss, T.V.P., Garel, S., Charnay, P., Bozon, B., Laroche, S., and Davis, S. (2001). A requirement for the immediate early gene Zif268 in the expression of late LTP and the consolidation of long-term memories. Nat. Neurosci. *4*, 289–296.

Judge, M.E., and Quartermain, D. (1982). Characteristics of retrograde amnesia following reactivation of memory in mice. Physiol. Behav. *28*, 585–590.

Kida, S., Josselyn, S.A., de Ortiz, S.P., Kogan, J.H., Chevere, I., Masushige, S., and Silva, A.J. (2002). CREB is required for the stability of new and reactivated fear memories. Nat. Neurosci. *5*, 348–355.

Kelly, A., Laroche, S., and Davis, S. (2003). Activation of mitogen-activated protein kinase/extracellular signal-regulated kinase in hippocampal circuitry is required for consolidation and reconsolidation of recognition memory. J. Neurosci. *12*, 5354–5360.

Lewis, D.J. (1979). Psychobiology of active and inactive memory. Psychol. Bull. *86*, 1054–1083.

Mactutus, C.F., Riccio, D.C., and Ferek, J.M. (1979). Retrograde amnesia for old (reactivated) memory: some anomalous characteristics. Science *204*, 1319–1320.

Malleret, G., Haditsch, U., Genoux, D., Jones, M.W., Bliss, T.V.P., Vanhoose, A.M., Weitlauf, C., Kandel, E.R., Winder, D.G., and Mansuy, I.M. (2001). Inducible and reversible enhancement of learning, memory, and long-term potentiation by genetic inhibition of calcineurin. Cell *104*, 675–686.

Manns, J.R., Hopkins, R.O., Reed, J.M., Kitchener, E.G., and Squire, L.R. (2003). Recognition memory and the human hippocampus. Neuron *37*, 171–180.

Milekic, M.H., and Alberini, C.M. (2002). Temporally graded requirement for protein synthesis following memory reactivation. Neuron *36*, 521–525.

Misanin, J.R., Miller, R.R., and Lewis, D.J. (1968). Retrograde amnesia produced by electroconvulsive shock after reactivation of a consolidated memory trace. Science *160*, 554–555.

Myers, K.M., and Davis, M. (2002). System-level reconsolidation: reengagement of the hippocampus with memory reactivation. Neuron *36*, 340–343.

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

Nader, K., Schafe, G.E., and Le Doux, J.E. (2000). Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. Nature *406*, 722–726.

Pittenger, C., Huang, Y.Y., Paletzki, R.F., Bourtchouladze, R., Scanlin, H., Vronskaya, S., and Kandel, E.R. (2002). Reversible inhibition of CREB/ATF transcription factors in region CA1 of the dorsal hippocampus disrupts hippocampus-dependent spatial memory. Neuron *34*, 447–462.

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

Sara, S.J. (2000). Retrieval and reconsolidation: towards a neurobiology of remembering. Learn. Mem. *7*, 73–84.

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

Swirnoff, A.H., and Milbrandt, J. (1995). DNA-binding specificity of NGFI-A and related zinc finger transcription factors. Mol. Cell. Biol. *15*, 2275–2287.

Taubenfeld, S.M., Milekic, M.H., Monti, B., and Alberini, C.M. (2001). The consolidation of new but not reactivated memory requires hippocampal C/EBPß. Nat. Neurosci. *4*, 813–818.

Thomas, K.L., Hall, J., and Everitt, B.J. (2002). Cellular imaging with *zif268* expression in the rat nucleus accumbens and the frontal cortex further dissociated the neural pathways activated following the retrieval of contextual and cued memory. Eur. J. Neurosci. *16*, 1789–1796.

Topilko, P., Schneider-Maunoury, S., Levi, G., Trembleau, A., Gourdji, D., Driancourt, M.A., Rao, C.V., and Charnay, P. (1998). Multiple pituitary and ovarian defects in Krox-24 (NGFI-A, Egr-1)-targeted mice. Mol. Endocrinol. *12*, 107–122.

Wan, H., Aggleton, J.P., and Brown, M.W. (1999). Different contributions of the hippocampus and perirhinal cortex to recognition memory. J. Neurosci. *19*, 1142–1148.

Williams, J., Dragunow, M., Lawlor, P.A., Mason, S.E., Abraham, W.C., Leah, J., Bravo, R., Demmer, J., and Tate, W.P. (1995). Krox20 may play a key role in the stabilisation of long-term potentiation. Brain Res. Mol. Brain Res. *28*, 87–93.

Wisden, W., Errington, M.L., Williams, S., Dunnett, S.B., Waters, C., Hitchcock, D., Evan, D., Bliss, T.V.P., and Hunt, S.P. (1990). Differential expression of immediate early gene in the hippocampus and spinal cord. Neuron *4*, 603–614.