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Reactivation With a Simple Exposure to the Experimental Environment is Sufficient to Induce Reconsolidation Requiring Protein Synthesis in the Hippocampal CA3 Region in Mice

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ABSTRACT: Our understanding of the memory reconsolidation process is at an earlier stage than that of consolidation. For example, it is unclear if, as for memory consolidation, reconsolidation of a memory trace necessitates protein synthesis. In fact, conflicting results appear in the literature and this discrepancy may be due to differences in the experimental reactivation procedure. Here, we addressed the question of whether protein synthesis in the CA3 hippocampal region is crucial in memory consolidation and reconsolidation of allocentric knowledge after reactivation in different experimental conditions in the Morris water maze. We showed (1) that an injection of the protein synthesis inhibitor anisomycin in the CA3 region during consolidation or after a single reactivation trial disrupted performance and (2) that protein synthesis is required even after a simple contextual reactivation without any learning trial and independently of the presence of the reinforcement. This work demonstrates that a simple exposure to the spatial environment is sufficient to reactivate the memory trace, to make it labile, and that reconsolidation of this trace requires de novo protein synthesis. © 2007 Wiley-Liss, Inc.

KEY WORDS: consolidation; reconsolidation; protein synthesis; spatial memory; hippocampus

INTRODUCTION

Cellular consolidation is the process that converts a memory trace from a labile state (short-term memory, STM) into a permanent and stable state (long-term memory, LTM). This process depends on the activation of molecular cascades requiring an activation of gene transcription (Alberini et al., 1994; Bailey et al., 1996; Freeman and Rose, 1999; Guzowski, 2002) and protein synthesis in particular (Davis and Squire, 1984; Bourtchouladze et al., 1998; Quevedo et al., 1999). However, the fact that consolidated memories are permanently stored and resistant to the degradation has been challenged. A memory model proposed by Lewis (1979) and recently developed by different authors (Przybyslawski and Sara, 1997; Sara, 2000; Nader, 2003; Alberini, 2005) suggests that the memory trace can be either in a labile active state or in an inactive stable state. Thus, during initial acquisition, but also each time, when a memory is reactivated, the memory trace returns to an active labile state and must again undergo a process of consolidation (reconsolidation) in order to be maintained in the inactive stable state (Lewis, 1979; Nader et al., 2000; Sara, 2000; Nader, 2003). The majority of studies show that, just as in initial consolidation, memory reconsolidation requires new protein synthesis (Dudai and Eisenberg, 2004 and Alberini, 2005 for review). However, some conflicting results appear in the literature showing that protein synthesis is only crucial for the first memory consolidation and not for reconsolidation (Taubenfeld et al., 2001; Hernandez et al., 2002; Bahar et al., 2004; Salinska et al., 2004). A possible explanation for these inconsistencies could be the conditions of how retrieval is initiated. Indeed, although these studies often used the same behavioral paradigm, reactivation can for instance consist of a relearning trial realized in the same conditions as acquisition (Rodriguez-Ortiz et al., 2005) or a simple presentation of the conditioned stimulus (Nader et al., 2000; Suzuki et al., 2004). In the latter case, interpretation of the results can be difficult because after retrieval, there can be competition between reconsolidation and extinction (Rodriguez-Ortiz et al., 2005).

Furthermore, the majority of the reconsolidation studies used aversive learning, such as passive avoidance or fear conditioning. These two tasks require aversive reinforcement, i.e., an electric shock, and it is difficult in this case to dissociate the brain areas involved in the stimulus association and those which are engaged in the emotional processing (Beckett et al., 1997; Richter et al., 2006). Therefore, in this present study, we investigate the role of protein synthesis during reconsolidation in a cognitive learning task in which animals do not receive electric shock. We chose the allocentric version of the Morris water maze task (MWM), in which animals have to create a viewpoint-independent representation incorporating distal cues, in order to compute the location of the platform using this cognitive map of the environment. This behavioral task has been shown to be dependent on the hippocampal formation which can be divided into three major areas: the dentate gyrus (DG), CA3, and CA1. Computational models have suggested that each hippocampal region subserves different spatial mnemonic processes. For example, the DG appears to be involved in spatial pattern separation, the CA3 in spatial and temporal working memory, spatial and temporal pattern completion, and pattern association, and the CA1 appears to be involved in temporal pattern separation and completion (Kesner et al., 2000).

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Thus, the CA3 region seems to have a strategic position in the processing of spatial information and this is confirmed by behavioral studies. These studies have shown that this CA3 region is involved in spatial working memory (Frederickson et al., 1990; Lee and Kesner, 2002, 2003) and, during memory consolidation, for nonassociative (Stupien et al., 2003) as well as associative spatial learning tasks (Zhao et al., 2000). Moreover, we previously showed that the CA3 integrity is important for spatial memory consolidation in MWM (Florian and Roullet, 2004). Thus, it has been proposed that the CA3 region plays an important role in the formation of spatial LTM. For all these reasons, in this study we focused on the role of this hippocampal region in memory consolidation and especially in memory reconsolidation. Therefore, 24 h after an initial massed acquisition in the MWM, mice underwent different types of reactivation and received an injection of anisomycin in the CA3 region. This reactivation was performed with or without a relearning trial, with or without the reinforcement, and finally with or without an exposure to the spatial environment to know which elements are necessary to initiate a phase of reconsolidation.

MATERIALS AND METHODS

Animals

A total of 123 CD1 male mice (IFFA CREDO, Lyon, France) were used in the present study. On arrival, the animals were housed in groups of five in standard breeding cages (21 × 21 × 12 cm) and placed in a rearing room at a constant temperature (22°C ± 1°C) under diurnal conditions (light–dark: 08:00–20:00), with food and water ad libitum. At the time of surgery, they were 100 days old (±10 days). They were tested during the first half of the light period (between 09:30 and 12:30). Every possible effort was made to minimize animal suffering and all procedures were in strict accordance with European Union and French national laws and regulations on the use of animals in research and NIH guidelines on animal care.

Surgery

Bilateral guide cannulae (0.56 mm in diameter) were implanted 1.2 mm above the CA3 region. The following coordinates with lambda and bregma in the same horizontal plane were used: posterior to bregma, −1.7 mm; lateral to midline, ±2.5 mm, and 1.5 mm beneath the skull surface, according to Franklin and Paxinos (1997). The subjects were then left in their home cage for a recovery period of 7–8 days.

Intracranial Injection Procedure

Anisomycin (ANI-sigma) 100 μg/μl dissolved in NaCl (pH adjusted to 7.4) or NaCl (vehicle: VEH) were bilaterally injected in a volume of 0.25 μl/side into the dorsal hippocampus. At this dose, ANI inhibits >90% of protein synthesis in the brain during the first 2 h (Flood et al., 1973; Morris et al., 2006). The injector (0.25 mm in diameter) was connected with a polyethylene tubing to a 1-μl Hamilton syringe driven by a microinjection pump at a rate of 0.1 μl/min. Mice remained in their cages during the injection period. The injection lasted 2 min 30 s for each side and the needle was left in the cannulae for an additional 60 s to allow diffusion.

Allocentric Spatial Learning in the Morris Water Maze

The water maze consisted of an ivory painted circular pool (110 cm diameter, 30 cm high) that was filled up to 15 cm from the base with water (23°C ± 1°C) and made opaque by addition of nontoxic white opacifier. A circular goal platform painted white (9 cm of diameter) was positioned in the center of one quadrant, 15 cm from the wall. A white curtain surrounded the swimming pool, delimiting the experimental environment. Several extra-maze visual cues, approximately 50–100 cm away from the pool, were attached to this curtain. Four start positions were located around the perimeter of the pool, dividing its surface into four equal quadrants. The apparatus was surmounted by a video camera connected to a video recorder and a computerized tracking system (Ethovision®, Noldus).

Massed-procedure in the spatial Morris water maze has been described previously (Florian and Roullet, 2004). Briefly, 1 week after surgery, mice were placed in the experimental room under a red heating light. From this position, the mice could not see the experimental environment directly surrounding the swimming pool. Ten minutes later, the mice were individually submitted to a single familiarization session of three trials with the platform always located in the same quadrant and protruding 0.5 cm over the surface of the water. The session started with the mouse standing on the platform for 60 s. At the beginning of each trial, the mice were released at one of the three possible starting points facing the wall, and allowed to swim freely until they reached the platform. Mice failing to find the platform within a fixed period of 60 s were gently guided by hand to the platform and a maximum escape latency of 60 s was recorded. After the animals had climbed onto the platform, they were allowed to stay on it for an additional 60 s, and subsequently replaced in the water from a different start position. The start positions were determined in a pseudorandom order, such that each was used only once in a single session.

The training phase consisted of four consecutive sessions of three trials with an intersession delay of 15–20 min during which mice were returned to their home cage. The procedure was the same as for the familiarization, except that the platform was submerged 0.5 cm beneath the surface of the water. Mice were required to navigate to the invisible platform using the spatial cues available in the experimental environment.

In all experiments, mice were tested for their LTM retention of spatial orientation by giving them a probe test consisting to a 60-s free swim trial without the platform.

In the MWM, the level of stress is dependent on the water temperature. In this study, the temperature of the water is relatively high (24–25°C) and in this condition, it was shown that animals displayed lower posttraining plasma corticosterone level than animals trained at 19°C. In this case, the MWM maze might be considered as a mildly stressful situation (Sandi et al., 1997).

Experiment 1—*Memory consolidation*: Immediately after the last training session, the mice received injections of ANI or VEH. The probe test took place 24 h after the injections.

Experiment 2—*Memory reconsolidation*: Twenty-four hours after training, a reactivation trial was performed. For the reactivation trial, after being placed for 10 min under the red heating light in the experimental room, the mouse was placed for 1 min onto the submerged platform and subjected to a single classic trial of learning. Once the animal found the platform, it stood on it for 60 s before being removed from the apparatus. Injections were performed just after the reactivation trial and the probe test took place 24 h later.

Experiment 3—*Control of specific effect of anisomycin*: Mice did not undergo reactivation and were merely left in their home cage. Injections were performed 24 h after the training sessions in the animal room and the probe test performed 24 h later.

Experiment 4—*Short-term memory post reactivation*: The procedure was the same as for Experiment 2 except that the probe test took place 1 h later.

Experiment 5—*Reactivation without a trial of relearning*: The procedure was the same as for Experiment 2 except that mice did not undergo a learning trial. Thus, the reactivation trial simply consisted of placing mice onto the submerged platform for 2 min.

Experiment 6—*Reactivation without relearning trial and without the reinforcement*: The procedure was the same as for Experiment 5 except that mice were placed on the emerged platform for 2 min. Therefore, the mice were not in contact with water (negative reinforcement) during the reactivation trial.

Experiment 7—*Reactivation without relearning trial, without reinforcement and without the exposition of experimental environment surrounding the water maze*: Twenty-four hours after the training sessions, the mice were placed for 10 min in the experimental room under a red light. The mice did not have the possibility of seeing the experimental environment surrounding the swimming pool.

For the training phase, mean escape latencies were used for each session (three trials per session).

During the probe test, the number of annulus crossings i.e., the number of times a mouse crossed an ideal circle (14 cm diameter) located around each of the four possible platform positions in the four quadrants, was analyzed. This number of annulus crossings reveals the strategy of search for the platform and the number of target annulus crossings can determinate if mice know the target location. The animal location was determined five times per second and recorded as x and y coordinates in time. Subsequently, these data were employed to calculate the swimming velocity and time spent in the periphery area (a 13-cm width band starting at the wall). These two last behavioral variables were measured to verify that anisomycin injections do not cause an undesirable motor disturbance or an abnormal behavior and thus, to verify the specificity of action of the protein synthesis inhibitor on memory.

Cannulae Placement Verification

On completion of the experiment, the mice were sacrificed; brains were removed and frozen at −20°C. Cannulae placements were determined by examination of serial coronal sections (40 μm) stained with thionine (Fig. 1). Serial sections were subjected to binocular microscopic inspection with high magnification (40×) to exclude cell layers in CA1 or CA4 regions formed mainly by infiltration of glial cells. Mice which presented injections overflow into the CA1 or CA4 regions were removed from the statistic analysis.

Statistical Analysis

The SYSTAT 9.0 statistical software package was used for data analysis. The results were expressed as mean ± standard error of mean (SEM) and analyzed using one- or two-way analysis of variance (ANOVA) or a repeated measure ANOVA when appropriate. Post hoc multiple comparisons were carried out when allowed, using Tukey's honestly significant distance (HSD) test.

RESULTS

For the analysis of the training data (Fig. 2), we pooled first the entire control group (VEH, $n = 64$) and the experimental groups (ANI, $n = 59$). Concerning the latency to find the hidden platform, ANOVA for repeated measure revealed a significant session effect [F (3,357) = 64.387; $P < 0.001$] but no pretreatment effect [F (1,119) = 0.383; $P = 0.537$] and no

FIGURE 1. A representative sample of a thionine-stained brain section showing placement of the tip of cannula (black arrows).

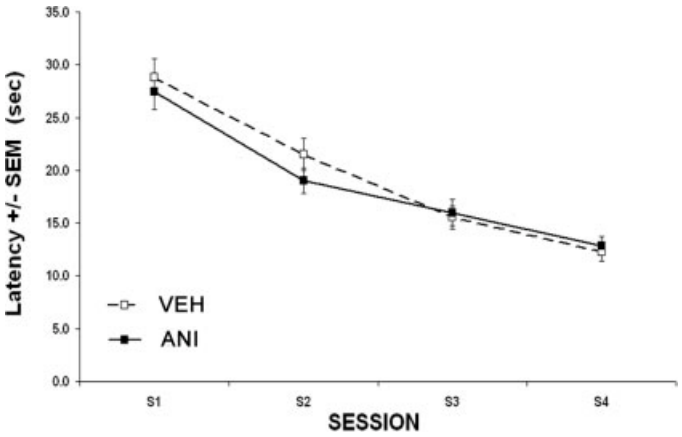

FIGURE 2. Mean latency to find the platform during the training phase of the spatial water maze task. Before treatment, the two groups of mice learned the position of the platform and displayed the same level of performance during the four learning sessions.

interaction between these two factors [F (3,357) = 0.773; P = 0.510]. These data confirmed that, in general, before treatment the two groups of mice learned the position of the platform and displayed the same level of performance during the four learning sessions. This analysis was additionally performed separately for each experiment to demonstrate the absence of acquisition differences between the two groups before treatment.

After the training phase, mice from each group were subjected to one of the seven experiments. All groups were independent i.e., each mouse was only tested once for a given experiment and a given treatment.

Experiment 1: Effect of Anisomycin Injection on the Initial Spatial Memory Consolidation

Concerning the mean latencies before escape onto the hidden platform, a repeated measure ANOVA of the entire data revealed a significant session effect [F (3,48) = 14.815; P < 0.001] but no pretreatment effect [F (1,16) = 0.282; P = 0.603] and no interaction between these two factors [F (3,48) = 0.118; P = 0.949]. These data confirmed that before treatment the two groups of mice learned the position of the platform and reached the same level of performance during the four learning sessions.

As shown in Figure 3, ANI injection just after initial acquisition impaired long-term retention during the probe test performed 24 h later. A two-way ANOVA revealed no significant effect between VEH- and ANI-injected mice [F (1,64) = 0.105; P = 0.747] on the total number of annulus crossings, but a significant quadrant effect [F (3,64) = 20.504; P < 0.001] and a significant treatment x quadrant interaction [F (3,64) = 8.921; P < 0.001] indicating that the profile of exploration of the quadrants was different in the two groups of mice. Tukey's test revealed that VEH group crossed the target annulus significantly more often than the three other annuli (P < 0.001). No such differences in annulus exploration were found for the ANI group. Moreover, VEH-injected mice crossed the target annulus more often than the ANI group (P = 0.001). Additional analyses did not reveal any significant difference between the two groups neither in swimming speed [F (1,16) = 0.129, P = 0.724] nor in the time spent in the periphery of the pool during the probe test [F (1,16) = 3.171, P = 0.094].

Experiment 2: Effect of Anisomycin Injection on Spatial Memory Reconsolidation (With One Relearning Trial)

During the training session, mice learned the position of the platform [F (3,42) = 6.287; P < 0.001] and an ANOVA revealed no significant pretreatment differences between the two groups of mice [F (1,14) = 0.371; P = 0.552] and no interaction between these two factors [F (3,42) = 0.857; P = 0.471].

Figure 4 shows the number of annulus crossings by the two groups during the probe test 24 h after a reactivation session including a relearning trial. A two-way ANOVA revealed no significant effect between VEH- and ANI-injected mice [F (1,56) = 0.836; P = 0.364], a significant quadrant effect [F (3,56) = 13.263; P < 0.001], and a treatment x quadrant interaction [F (3,56) = 5.150; P = 0.003]. The VEH group crossed the target annulus more often than the three other ones

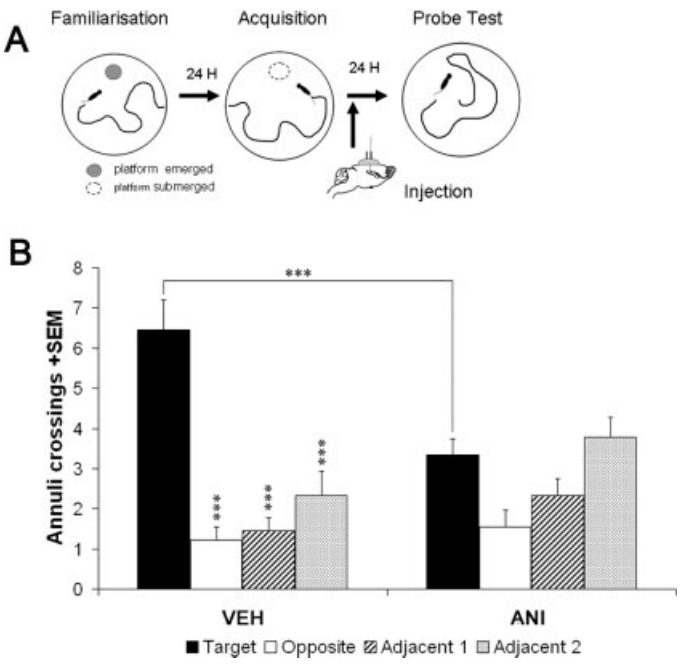

FIGURE 3. Memory consolidation. (A) Immediately after the last training session, mice received injections of ANI or NaCl. The probe test took place 24 h after the injections. (B) Number of annulus crossings during the 60-s probe trial. Anisomycin injection in the hippocampal CA3 region just after training impaired spatial memory consolidation. ***P < 0.001.

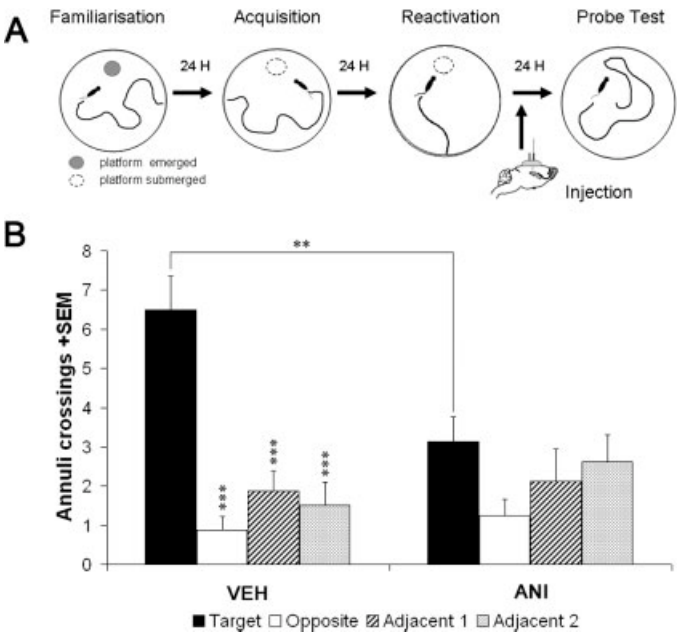

FIGURE 4. Memory reconsolidation. (A) Twenty-four hours after training, a reactivation trial was performed with one relearning trial. The drug infusions occurred immediately after reactivation. The probe test took place 24 h after the injections. (B) Number of annulus crossings during the 60-s probe trial. Anisomycin caused a clear disruption of spatial memory reconsolidation. **$P < 0.01$; ***$P < 0.001$.

($P < 0.001$) and there was no significant difference in the exploration of the four quadrants for the ANI group. Moreover, the VEH group crossed the target annulus more often than the ANI group ($P = 0.008$). Additional analyses did not reveal any significant difference between VEH- and ANI-injected mice in swimming speed [F (1,14) = 0.179, $P = 0.679$] or on the time spent in the periphery of the pool during the probe test [F (1,14) = 0.072, $P = 0.792$].

Experiment 3: Control of the Specific Effect of Anisomycin (Injection Without Reactivation)

During the training session, mice learned the position of the platform [F (3,48) = 14.474; $P < 0.001$] and an ANOVA revealed no significant pretreatment differences between the two groups [F (1,16) = 0.006; $P = 0.939$] and no interaction between these two factors [F (3,48) = 1.920; $P = 0.159$].

VEH- and ANI-injected mice crossed the annulus located in the target quadrant significantly more often than the remaining three annuli (Fig. 5). The two-way ANOVA revealed a significant quadrant effect [F (3,64) = 25.126; $P < 0.001$], no treatment effect [F (1,64) = 0.034; $P = 0.854$], and no interaction between these two factors [F (3,64) = 0.781; $P = 0.509$]. Moreover, no significant effects were found between the two groups in swimming speed [F (1,16) = 1.074, $P = 0.315$] or on the time spent in the periphery of the pool during the probe test [F (1,16) = 0.192, $P = 0.667$].

Experiment 4: Effect of Post Reactivation Anisomycin Injection on Short-Term Memory

During the training session, mice learned the position of the platform [F (3,48) = 7.219; $P < 0.001$] and ANOVA revealed no significant pretreatment differences between the two groups [F (1,16) = 0.938; $P = 0.347$] and no interaction between these two factors [F (3,48) = 0.259; $P = 0.854$].

VEH- and ANI-injected mice crossed the annulus located in the target quadrant significantly more often than the remaining three annuli (Fig. 6). The two-way ANOVA revealed a significant quadrant effect [F (3,64) = 30.468; $P < 0.001$] and no treatment effect [F (1,64) = 0.160; $P = 0.690$], and no interaction between these two factors [F (3,64) = 0.451; $P = 0.718$]. Additional analyses did not reveal any significant differences between the two groups in swimming speed [F (1,16) = 1.099, $P = 0.310$] or on the time spent in the periphery of the pool during the probe test [F (1,16) = 1.672, $P = 0.214$].

Together, the results of the first four experiments indicate that injection of anisomycin impaired initial spatial consolidation and produced amnesia for already consolidated spatial memory when this was reactivated before anisomycin injection. Moreover, anisomycin injection had no effect if it was performed without a reactivation trial and this treatment had no effect if the probe test was performed 1 h after the reactivation. These results indicate that the observed effect in Experiments 1 and 2 was not due to nonspecific effects of the drug.

In Experiment 2, we showed that a consolidated spatial memory can again become labile after reactivation. In the

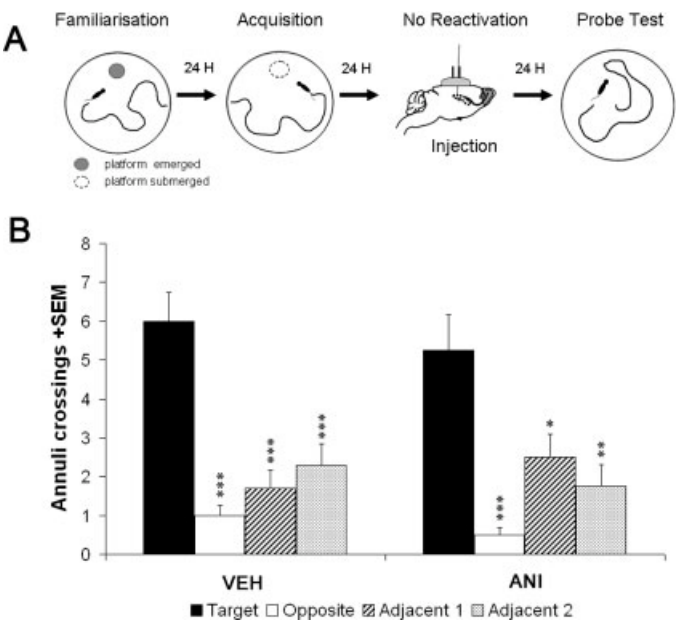

FIGURE 5. Control of specific effect of anisomycin. (A) Mice did not undergo reactivation and were merely left in their home cage. Injections were performed 24 h after the training sessions in the animal room and the probe test realized 24 h later. (B) Histograms represent the number of annulus crossings during the 60-s probe trial. Anisomycin injection had no effect if it is performed without a reactivation trial. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

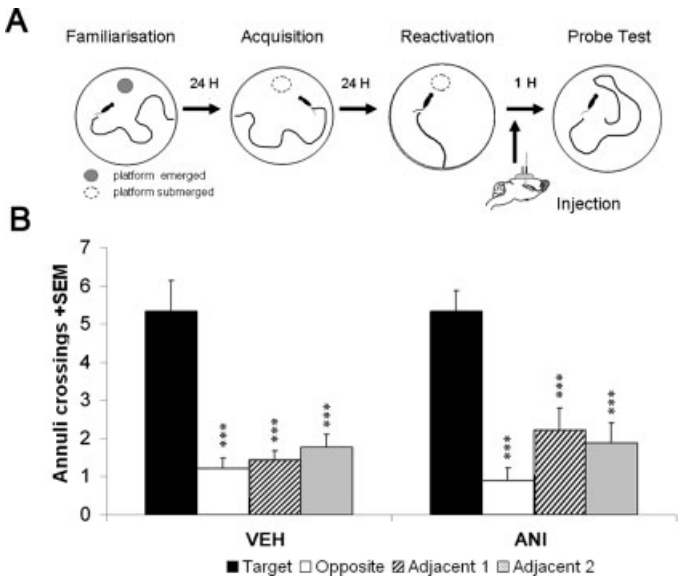

FIGURE 6. Short-term memory postreactivation. (A) Twenty-four hours after training, a reactivation trial was performed with one trial of relearning. The drug infusions occurred immediately after reactivation. The probe test took place 1 h after the injections. (B) Number of annulus crossings during the 60-s probe trial. Anisomycin injection had no effect in short term memory post reactivation. ***$P < 0.001$.

remaining experiments, we attempted to determine which elements were necessary during retrieval to induce a reactivation of the memory trace and to require new protein synthesis. We sought to establish whether a learning trial, the presence of reinforcement, and the presentation of the experimental environment are crucial to induce a reconsolidation phase.

Experiment 5: Reactivation Without Relearning Trial

During the training session, mice learned the position of the platform [F (3,51) = 7.826; $P < 0.001$] and an ANOVA revealed no significant pretreatment differences between the two groups [F (1,17) = 0.165; $P = 0.690$] and no interaction between these two factors [F (3,51) = 0.373; $P = 0.773$].

Figure 7 shows the effects of immediately postreactivation ANI and VEH injections on the number of annulus crossings during the probe test. ANOVA revealed a treatment effect [F (1, 68) = 4.130; $P = 0.046$], a significant quadrant effect [F (3, 68) = 18.861, $P < 0.001$] and more important, a treatment x quadrant interaction [F (3, 68) = 5.537, $P = 0.002$]. Subsequent comparison indicated a significant difference between the target annulus and the other three annuli for VEH mice ($P < 0.001$) but not for the ANI-injected mice. Moreover, these VEH mice crossed the target annulus more often than the ANI group ($P = 0.001$). Additional analyses did not reveal a significant difference between VEH- and ANI-injected mice in swimming speed [F (1,16) = 1.287, $P = 0.273$]. However, a marginal treatment effect was detected on the time spent in the periphery of the pool during the probe test [F (1,16) = 4.516, $P = 0.051$], but this difference was only due to one mouse in the ANI group which passed the majority of the probe test along the walls. Thus, without a relearning trial, anisomycin injection provoked the same performance deficit as observed in Experiment 2 in which a relearning trial was administered.

Experiment 6: Reactivation Without Relearning Trial and Without the Presence of the Reinforcement

During the training session, mice learned the position of the platform [F (3,45) = 7.711; $P < 0.001$] and an ANOVA revealed no significant pretreatment differences between the two groups [F (1,15) = 0.006; $P = 0.941$] and no interaction between these two factors [F (3,45) = 0.137; $P = 0.938$].

The two-factor ANOVA indicated no treatment effect [F (1,60) = 0.872, $P = 0.354$], but a significant quadrant effect [F (3,60) = 25.830, $P < 0.001$] and a treatment x quadrant interaction [F (3,60) = 9.066, $P < 0.001$]. For the VEH group (Fig. 8), Tukey's test revealed a significant difference between the target annulus and the three other annuli ($P < 0.001$) and these mice crossed the target annulus more often than the ANI group. For the ANI-injected mice, no significant difference was found between the four annuli. Therefore, even if the mice were not in contact with water during the reactivation trial, a protein synthesis dependent reconsolidation process

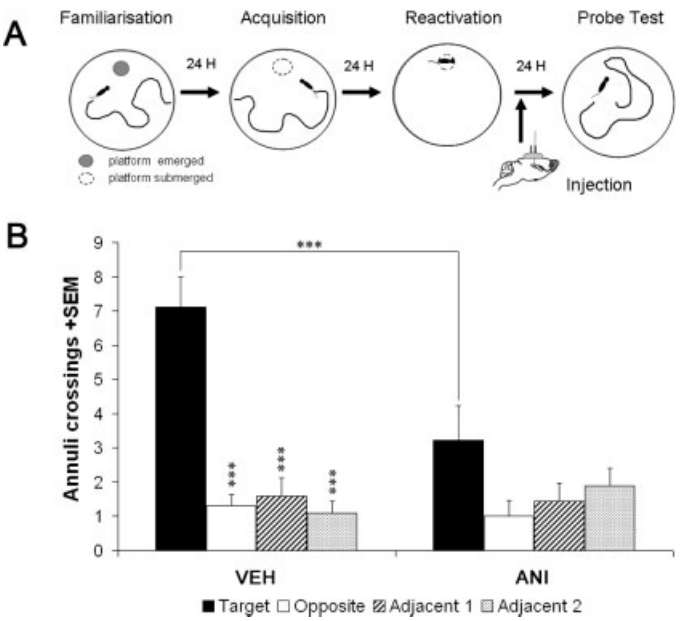

FIGURE 7. Reactivation without a trial of relearning. (A) Twenty-four hours after training, a reactivation was performed but mice did not undergo a learning trial. Thus, the reactivation trial simply consisted of placing mice onto the submerged platform for 2 min. The probe test took place 24 h after the injections. (B) Number of annulus crossings during the 60-s probe trial. Without this additional learning trial during the reactivation phase, anisomycin injection always impaired spatial memory reconsolidation. ***$P < 0.001$.

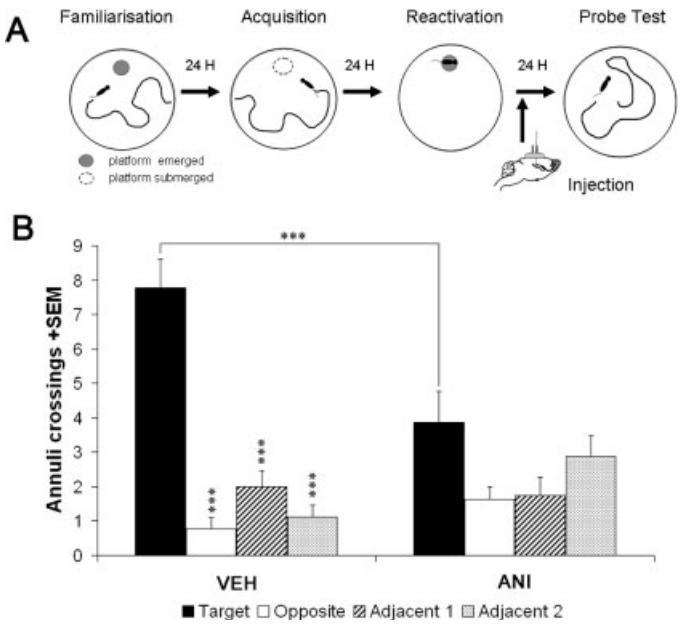

FIGURE 8. Reactivation without relearning trial and without the reinforcement. (A) To understand the importance of the presence/absence of reinforcement during reactivation, mice were only placed in an emerged platform during the reactivation phase, without the contact of the water. The probe test took place 24 h after the injections. (B) Number of annulus crossings during the 60-s probe trial. Without the contact of water (negative reinforcement) during the reactivation phase, anisomycin injection always impaired spatial memory reconsolidation. ***$P < 0.001$.

was nevertheless triggered by this reactivation. Additional analyses did not reveal any significant difference between the two groups in swimming speed [F (1,15) = 0.037, P = 0.849] or on the time spent in the periphery of the pool during the probe test [F (1,15) = 1.189, P = 0.293].

Experiment 7: Reactivation Without Relearning Trial, Without the Presence of Reinforcement and Without Exposure to the Experimental Environment Surrounding the Water Maze

During the training session, mice learned the position of the platform [F (3,45) = 16.037; $P < 0.001$] and an ANOVA revealed no significant pretreatment differences between the two groups [F (1,15) = 2.857; P = 0.112] and no interaction between these two factors [F (3,45) = 0.249; P = 0.258].

Figure 9 shows the number of annulus crossings in the four quadrants during probe test, for ANI- and VEH-injected mice. VEH as well as ANI-treated mice crossed the target quadrant more often than the remaining three quadrants. The two factors ANOVA revealed no treatment effect [F (1,60) = 0.156; P = 0.694], a significant quadrant effect [F (3,60) = 36.940; $P < 0.001$], but no interaction between the two factors [F (3,60) = 0,424; P = 0.737]. Moreover, no significant effects were detected between the two groups in swimming speed [F (1,15) = 1.032, P = 0.326] or on the time spent in the periphery of the pool during the probe test [F (1,15) = 0.001, P = 0.973].

Thus, a phase of memory reconsolidation is not initiated if the mice are only placed in the experimental room without exposure to the experimental environment.

DISCUSSION

This work aimed to test the role of protein synthesis in the hippocampal CA3 region during consolidation and reconsolidation in mice. We used massed-training in the allocentric version of the MWM, administered in a single 90-min session, which allowed us to study posttraining events occurring during initial memory consolidation or during reconsolidation (Florian and Roullet, 2004). In all experiments, animals were able to locate the hidden platform during training and VEH-injected mice searched for it in the correct quadrant during the probe test performed either 24 h after the training sessions or 24 h after reactivation.

In the first experiment, we showed that anisomycin injection into the hippocampal CA3 region immediately after training impaired memory consolidation. Indeed, contrary to control

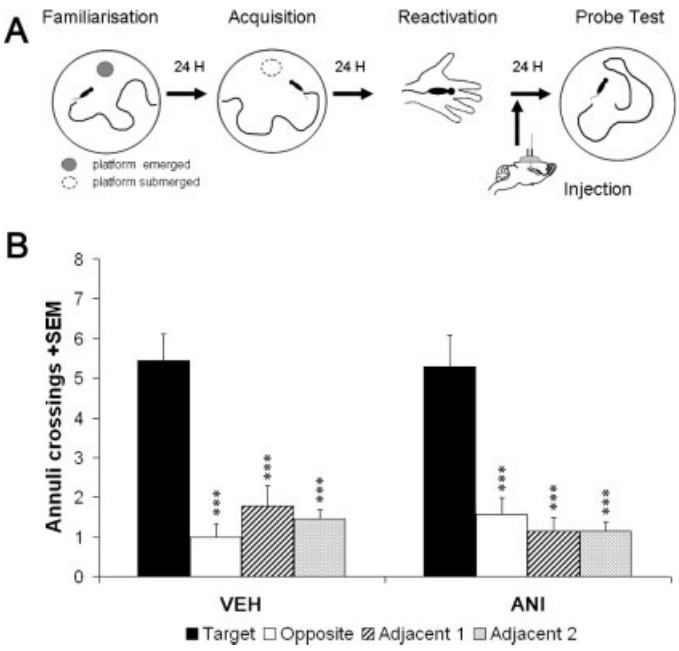

FIGURE 9. Reactivation without relearning trial, without reinforcement and without the exposition of experimental environment surrounding the water maze. (A) Twenty-four hours after the training sessions, mice were placed for 10 min in the experimental room under a red light. Mice were not able to see the experimental environment surrounding the swimming pool. (B) Number of annulus crossings during the 60-s probe trial. If animals were not exposed to the experimental environment, anisomycin injection had no effect. Thus, manipulating animals in the experimental room and placing them under a red heating light (as during acquisition) is not sufficient to reactivate the memory trace. ***$P <$ 0.001.

mice, the experimental group was not able to find the platform location during the probe trial when protein synthesis was blocked. Therefore, protein synthesis is necessary in this hippocampal region for encoding recent experiences in LTM as already shown for other structures and for different memory tasks (Davis and Squire, 1984; Bourtchouladze et al., 1998; Quevedo et al., 1999). In the second experiment, we found that mice treated with anisomycin after reactivation by a relearning trial, displayed the same deficit as in Experiment 1 when the injection was performed during the initial consolidation phase. Thus, a consolidated and stable spatial memory can become labile again after reactivation and new protein synthesis is then required to maintain memories that have been reactivated. Importantly, we have demonstrated that anisomycin causes an impairment in LTM, but not in STM when infused immediately postreactivation and that anisomycin injection had no effect if performed without a reactivation trial. Thus, the impairments observed in the first two experiments cannot be attributed to nonspecific drug effects as neurotoxicity or general impairment.

Many studies have shown the importance of protein synthesis during initial consolidation and during reconsolidation. However, most of these studies used systemic or icv injections (cf Alberini, 2005, for details). These types of injection prevent the identification of the structures involved in these processes and in particular whether these structures are the same in both consolidation and reconsolidation. On the other hand, when protein synthesis blockers are directly injected into a particular structure, the results are sometimes unmatched. For instance, anisomycin injections in the hippocampus blocks initial consolidation without affecting reconsolidation in the inhibitory avoidance task (Taubenfeld et al., 2001; Vianna et al., 2001) and in instrumental learning if injections were performed in the nucleus accumbens (Hernandez et al., 2002). By contrast, protein synthesis is required in the amygdala for both the consolidation and reconsolidation of auditory fear conditioning (Nader et al., 2000), in hippocampus for the contextual fear conditioning (Debiec et al., 2002), in the ventromedial prefrontal cortex (vmPFC) for object recognition task (Akirav and Maroun, 2006) and, in our experiment, in the CA3 hippocampal region for allocentric spatial learning in the MWM. Although these data looks apparently conflicting, such differences can easily be explained by differences in the behavioral task used and/or in the conditions of memory reactivation. Indeed, as suggested by Alberini (2005), some structures could be common to both processes and others specialized either for consolidation or reconsolidation. Moreover, it is interesting to ascertain that reconsolidation seems to be a general mechanism as it appears in different kinds of learning. Indeed, reconsolidation has been demonstrated after reactivation in tasks such as passive avoidance (Taubenfeld et al., 2001; Power et al., 2006) or fear conditioning paradigms (Nader et al., 2000; Suzuki et al., 2004), two associative and aversive tasks. The reconsolidation process was also described after reactivation in the object recognition task, a nonassociative and nonaversive paradigm (Bozon et al., 2003; Akirav and Maroun, 2006). The present study uses an allocentric version of the MWM, an associative learning task. This paradigm uses water as negative reinforcement which is clearly less aversive than the electric shock used in fear conditioning or in passive avoidance tasks, especially in our condition of water temperature (24–25°C).

In three other experiments, after extensive learning in the MWM, the reactivation phase consisted of one or several probe tests without a platform (Lattal et al., 2004; Suzuki et al., 2004, Rossato et al., 2006). In this case, the interpretation of such results can be difficult because after retrieval, competition between reconsolidation and extinction, two mechanisms requiring protein synthesis, could occur (Einsenberg et al., 2003; Pedreira and Maldonado, 2003). In a recent study, Morris et al. (2006) showed that after reactivation in the MWM, memory was sensitive to anisomycin only in a delayed matching-to-place task procedure and not in a reference memory task procedure. The latter was very similar to that used in our experiment, i.e., the platform was always in the same place during training, but nevertheless we obtained conflicting results. Various factors can explain this apparent inconsistency. For example, the number of trials before reactivation and injection was doubled in the Morris et al.'s study and the delay between the two sessions of learning also differed because one experiment used a massed procedure and the other a distributed procedure. Thus, in the Morris et al. study, rat performance reached asymptotic level over several days which was not the case in the present study. Altogether, these results show that the mechanisms supporting reconsolidation of spatial memory in the hippocampus may differ depending on experimental conditions, notably on learning conditions and possibly on the level of acquisition performances before reactivation.

It is noteworthy that in the majority of studies comparing consolidation and reconsolidation, the retrieval condition is different from the acquisition condition. In fact, during the consolidation phase the reinforcement is present, while it is often absent in the reconsolidation phase. For example, in a fear conditioning task, the reconsolidation phase consists of a simple presentation of the conditioned stimulus, (Nader et al., 2000; Suzuki et al., 2004) while the conditioned and unconditioned stimuli are present in the consolidation paradigm. In the present study, the reactivation session included one learning trial (Experiment 2), in which mice were placed in the same conditions for both consolidation and reconsolidation. Even so, it is difficult to know whether a protein-synthesis dependent reconsolidation process was triggered after all types of memory reactivation or only when a relearning trial occurred. For this reason, we added the Experiment 5 without this supplementary learning trial and under these conditions, anisomycin injection always impaired recall performances. Thus, memory reconsolidation is triggered even if the reactivation phase does not comprise of a relearning trial. In this experiment, mice were in contact with the reinforcement (i.e., the water) and, as seen previously in numerous studies, reinforcement is absent during the reactivation phase. Therefore, in Experiment 6, to test the importance of the reinforcement during reactivation, mice were placed onto an emerged platform during the reactivation phase, without contact with water. Our results showed that mice

treated with a protein synthesis inhibitor displayed a deficit during the probe test. Thus, a phase of reconsolidation was initiated after a reactivation independently of the absence or presence of the negative reinforcement. However, it is still difficult to compare results obtained in our experimental procedures with those classically used without reinforcement such as fear conditioning.

The last experiment showed that simply manipulating animals in the experimental room and placing them under a red heating light, as it was usually done right before starting the acquisition, is not sufficient to initiate a phase of reconsolidation or, at least, to make it sensitive to interference. Thus, if animals were not exposed to the experimental environment, anisomycin injection had no effect. Biendenkapp and Rudy (2004) reported similar results in a context-preexposure facilitation effect paradigm when a protein-synthesis inhibitor was injected into the entire dorsal hippocampus. In this experiment, rats were transported in a particular bucket before context exposure which may have resulted in the bucket acting as a retrieval cue, sufficient to activate the context representation. The authors found that hippocampal injection of anisomycin after transporting rats in the bucket, and without reexposing them to the context, had no effect on performance. Thus, in accordance with our data, this study demonstrated that without exposure to the experimental environment, reconsolidation is not initiated in the hippocampus. However, in a second set of experiments, also focalised in the dorsal hippocampus, Biendenkapp and Rudy (2004) demonstrated that a single reexposure to the context does not lead to reconsolidation either. In contrast, Debiec et al. (2002) demonstrated that a reconsolidation process was initiated in this structure after exposure to the context in a fear conditioning task. Moreover, we found in our study that placing the mouse onto the platform inside the experimental environment leads to reconsolidation in the hippocampus. Thus, the role of this structure in context-dependent reconsolidation does not seem so simple and results seem contradictory, as evident in reconsolidation studies after spatial training (as mentioned earlier). Nevertheless, Biendenkapp and Rudy (2004) proposed an interesting hypothesis that could explain this apparent discrepancy. They described context as a place in which events occur, and it may be that the necessary condition for retrieval to destabilize the memory is that it must contain an event. Thus, this hypothesis could explain why the authors did not observe memory destabilization in their experiment, because when rats were placed back into the context, no particular event occurred. On the contrary, in the contextual fear conditioning task used by Debiec et al., an event (electric shock) occurred in a specific context. In the same way, in our study based on the MWM, the fact that mice have to escape from water could constitute the event that leads to memory's destabilization.

In addition to task and protocol differences between studies about reconsolidation processes taking place in the hippocampus, the fact that the functional heterogeneity of this structure is never taken into account could constitute another source of discrepancy. Indeed, injections were usually performed into the entire dorsal hippocampus (Debiec et al., 2002; Biendenkapp and Rudy, 2004; Morris et al., 2006) and consequently, it is not possible to find out the exact function of the different hippocampal subregions in the reconsolidation process. In our study, injections were performed only in the CA3 region and our result showed that this particular region is implicated during both initial consolidation and reconsolidation. In a previous study, we used diethyldithiocarbamate (DDC) which chelates most of the heavy metals in the brain to inactivate the CA3 region. A focal injection of this chelator after a spatial acquisition phase in the same procedure used in this experiment, led to a deficit of spatial memory consolidation processes (Florian and Roullet, 2004). In addition, blocking CREB transcription factor during initial consolidation in the CA3 region (Florian et al., 2006b) caused a strong spatial LTM deficit, while injection of a PSA-NCAM mimetic peptide in this subfield increased the long-term spatial memory performance (Florian et al., 2006a). The early phase of memory consolidation is often associated with structural remodelling in both the dendritic and axonal synaptic fields in the hippocampus (Murphy and Regan, 1998), particularly in the CA3 region (Stewart et al., 2005). Moreover, different computational theories consider the CA3 region as an autoassociating-recurrent network during the acquisition phase (Bennett et al., 1994; Rolls and Treves, 1994; Wallenstein and Hasselmo, 1997; Wiebe et al., 1997). In fact, inputs arriving via the DG or/and perforant path afferents are thought to produce a pattern of CA3 ensemble output that reflects the pattern of inputs received. Therefore, during consolidation, synapses are modified to reinforce this ensemble pattern by strengthening connections between coactive neurons within the ensemble (McNaughton and Morris, 1987; Treves and Rolls, 1992, 1994). The CA3 output pattern in turn activates CA1 neurons, thus producing a new pattern that serves as the output of the hippocampal circuit (Nakasawa et al., 2002). In our experiment, blocking protein synthesis in the CA3 region during initial memory consolidation, prevents synapses from being modified and strengthened to reinforce the ensemble pattern in CA3. In addition, in this case, no output pattern would be observed in CA1. In the experimental reconsolidation procedure, the initial consolidation was normal and in this case, it could be suggested that the CA1 output pattern of activation is also normal. Our result show that allocentric knowledge undergoes a protein-synthesis dependent reconsolidation process in CA3 after reactivation suggesting that reconsolidation also involves synaptic plasticity in this hippocampal region. These results may also suggest that a direct activation of the CA1 neurons via the perforant path during the probe test seems to be insufficient for the animals to retrieve spatial information. However, it would be very interesting to replicate this experiment with focal injections in the CA1 region to understand the respective role of these hippocampal structures in the treatment of spatial information after different kinds of reactivation.

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