# Behavioral tagging underlies memory reconsolidation

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**Memory reconsolidation occurs when a retrieving event destabilizes transiently a consolidated memory, triggering thereby a new process of restabilization that ensures memory persistence. Although this phenomenon has received wide attention, the effect of new information cooccurring with the reconsolidation process has been less explored. Here we demonstrate that a memory-retrieving event sets a neural tag, which enables the reconsolidation of memory after binding proteins provided by the original or a different contiguous experience. We characterized the specific temporal window during which this association is effective and identified the protein kinase A (PKA) and the extracellular signal-regulated kinase 1 and 2 (ERK 1/2) pathways as the mechanisms related to the setting of the reconsolidation tag and the synthesis of proteins. Our results show, therefore, that memory reconsolidation is mediated by a "behavioral tagging" process, which is common to different memory forms. They represent a significant advance in understanding the fate of memories reconsolidated while being adjacent to other events, and provide a tool for designing noninvasive strategies to attenuate (pathological/traumatic) or improve (education-related) memories.**

memory | reconsolidation | behavioral tagging

Memory, the capacity to store, remember, and retrieve events that build an individual's identity, has a fundamental role for the survival of numerous organisms. Memory consolidation is a crucial process, which consists of the stabilization of fragile memory traces into long-term memories (LTMs) (1). Memories may be subjected to a different process termed memory reconsolidation, which occurs upon exposure to retrieving events that reactivate the memory (2, 3). Such retrieval destabilizes temporarily the information previously memorized, which undergoes then a new process of consolidation susceptible to interferences and which may result in the modification of the original LTM (4, 5). Like in memory consolidation, protein-synthesis blockade after memory reactivation leads to retrograde amnesia (3), thus showing that both consolidation and reconsolidation rely on the synthesis of proteins. Yet, memory reconsolidation cannot be considered a mere recapitulation of consolidation as it is mediated by different brain structures and molecular cascades (4, 6–8).

Because one of the fundamental functions of reconsolidation is memory updating (9, 10), the role of new information contiguous to recall (11, 12) is of fundamental importance because it might boost or interfere with memory restabilization. Studies addressing the effect of adjacent, novel events on memory consolidation have led to the behavioral tagging (BT) hypothesis (13). BT is the behavioral analog of the synaptic tagging and capture hypothesis (14), formulated to explain the synaptic specificity in functional plasticity models of long-term potentiation and depression (15, 16). It postulates that two parallel and complementary mechanisms occur during LTM formation: the setting of transient learning tags in neurons, providing a potential substrate for storing recently acquired information, and the synthesis of plasticity-related proteins (PRPs), that once captured at the tagged sites allow the consolidation of memories (17, 18). This process explains, therefore, how weak learning events, capable of setting the tags but incapable of inducing long term memories, can produce stable memories if a new salient event adjacent to them provides the proteins that contribute to memory consolidation.

The validity of the BT assumptions for memory consolidation has been demonstrated in rats through the combination of a weak inhibitory avoidance (IA) training capable of inducing short- but not long-term memory, and the adjacent exploration of a novel open field (OF) capable of inducing protein synthesis in the hippocampus. Within a critical time window, the weak IA learning could use PRPs synthesized by the exploration of the novel OF, thus resulting in the consolidation of an otherwise inexistent IA-LTM (19). Similar designs have been used to show that several LTMs processed at hippocampal and cortical levels depend on BT processes (20–24). Furthermore, memory persistence and LTM of extinction have also been shown to depend on BT mechanisms (25, 26). This process can also account for the strengthening of human memories via the coupling of training with emotional related events (27), a finding that has important consequences for improving the retention of challenging contents at school (28).

In the case of memory reconsolidation, the role of novelty after memory reactivation has been less explored. Previous work showed that reactivation of an appetitive spatial memory in rats induces a reconsolidation process that can be impaired by the beta-adrenergic antagonist propranolol and that can be rescued upon impairment by an adjacent spatial novelty (29). These

## Significance

We studied how novel events contiguous to memory retrieval affect the process of memory updating termed reconsolidation. We show that memory retrieval sets a neuronal tag to which proteins provided by the novel events can bind, restabilizing thereby memory via a behavioral-tagging mechanism. Our results thus indicate that the different phases of memory stabilization (consolidation, extinction, and now reconsolidation) are mediated by behavioral tagging, which emerges as a general mechanism of long-term memory formation. They provide, in addition, a tool for designing noninvasive strategies to attenuate (pathological/traumatic) or improve (education-related) existing memories via their reactivation with novel experiences.

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Data deposition: Latencies and exploration indexes for individual animals are available at the Open Science Framework repository (https://osf.io/r6d9u/).

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results, which may be affected by early aging (30), suggested that BT underlies memory reconsolidation but despite their evident interest they did neither demonstrate the setting of a reconsolidation tag by the retrieving event nor did they characterize the temporal dynamics of protein synthesis induced by the spatial novelty, the two elements that are essential for a demonstration of a BT process.

Here we focused on the role of new information adjacent to memory reactivation to determine if a BT process underlies memory reconsolidation. We hypothesized that retrieving events set a reconsolidation tag allowing the binding of new proteins, which may be provided by contiguous novel experiences. Using different behavioral tasks, we characterized the mechanistic underpinnings of tag setting and PRP synthesis in a reconsolidation scenario, and demonstrate that some reactivating events promote reconsolidation by providing proteins that overcome the effect of a protein-synthesis blocker. Our findings thus provide a definitive demonstration of BT as an essential mechanism for memory reconsolidation.

## Results

We first studied if the blockade of the reconsolidation process via the infusion of a protein synthesis inhibitor could be prevented by associating the memory reactivation session with a different behavioral task that usually induces protein synthesis in shared brain areas. Rats were habituated to an IA box for 5 min during 2 d and were then subjected to a training session performed 24 h later. In such a training, they learn not to step down on a compartment in which they receive an electric foot shock. On the fourth day, animals were exposed to a reactivation session that lasted 40 s during which they were replaced in the IA box in the absence of reinforcement and LTM was again evaluated 24 h after this session. Fig. 1*A* shows that control animals infused with a vehicle solution (Veh) in the CA1 region of the dorsal hippocampus immediately after the reactivation session exhibited IA-LTM on the next day; in contrast, reactivated animals infused with an appropriate dose of the protein-synthesis inhibitor emetine (EME; see *SI Appendix*, Fig. S1*A* for dose–response effects) were amnesic. This effect was not observed if EME was infused 24 h after training but without subjecting the animals to the reactivation session. Hence, after reactivation, the IA memory becomes labile and requires protein synthesis to persist, thus confirming the existence of a reconsolidation phase (31).

If reconsolidation occurs through a BT process, two parallel and complementary processes should be triggered when it is engaged:

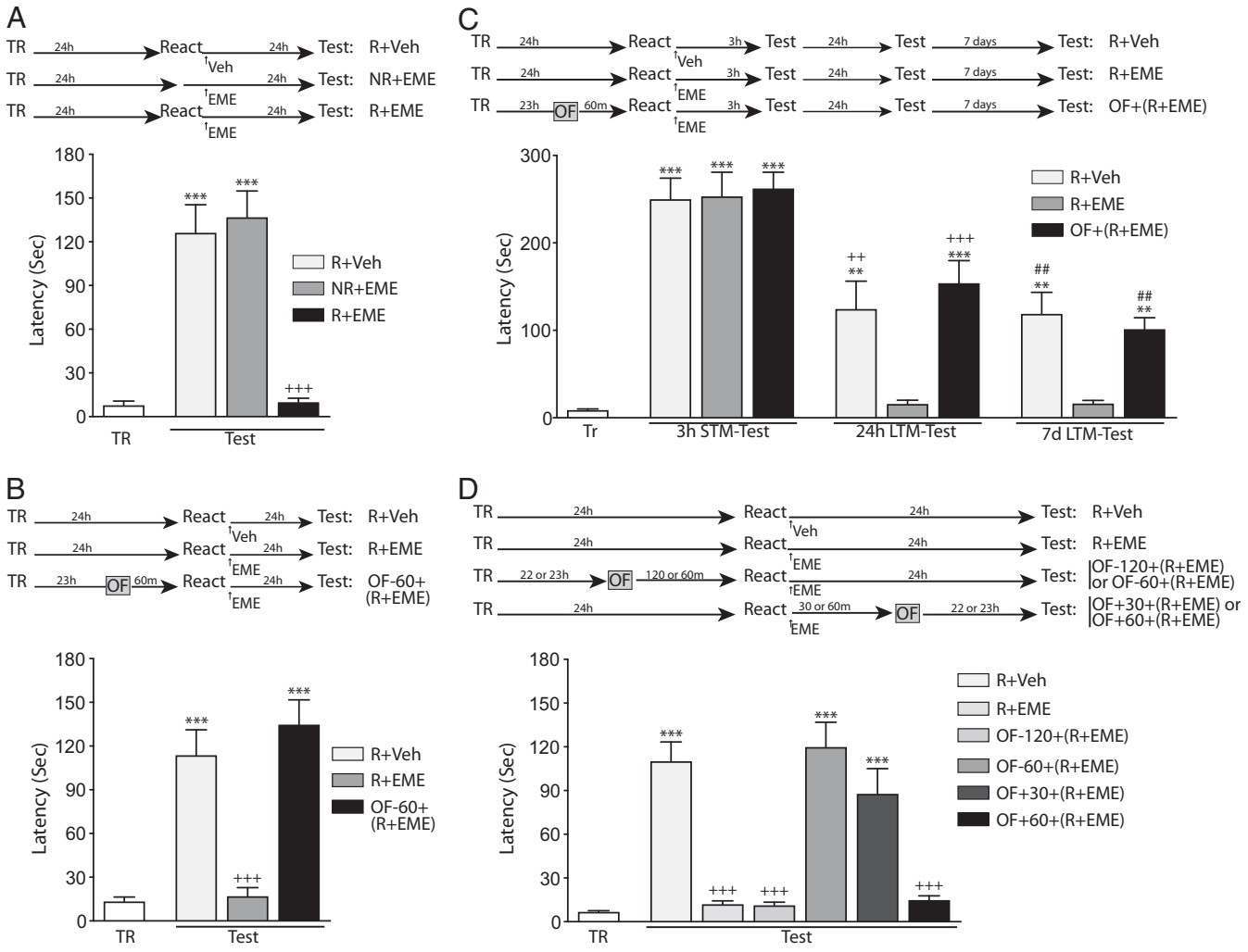


**Fig. 1.** Exploration of an OF rescues the blockade of reconsolidation induced by protein-synthesis inhibition for a persistent period of time and within a restricted time window. (*Top*) Experimental design. The figures show the latency to step-down from the platform during TR and test sessions, expressed as mean ± SEM. R: animals submitted to a reactivation session. NR: nonreactivated animals. (*A–D*) Newman–Keuls analysis after one-way ANOVA. (*A*) ***$P$ < 0.001 vs. TR; +++$P$ < 0.001 vs. R+Veh and NR+EME, $n$ = 7. (*B*) ***$P$ < 0.001 vs. TR; +++ $P$ < 0.001 vs. R+Veh and OF-60+(R+EME), $n$ = 8. (*C*) ***$P$ < 0.001 and **$P$ < 0.01 vs. TR, +++ $P$ < 0.001 and ++$P$ < 0.01 vs. R+EME (24 h LTM-Test); ##$P$ < 0.01 vs. R+EME (7 d LTM-Test); $n$ = 6–7. (*D*) ***$P$ < 0.001 vs. TR; +++$P$ < 0.001 vs. R+Veh; OF-60+(R+EME) and OF+30+(R+EME); $n$ = 8–10.

the setting of a reconsolidation learning tag and the synthesis of PRPs. Moreover, if the setting of the tag is independent of protein synthesis, then the amnesia induced by EME infusion after memory reactivation could be prevented by providing the required proteins through a different associated task, performed when EME is not present. To test this hypothesis, we repeated the previous experiment but we allowed rats to explore a novel OF 60 min before exposing them to the IA-memory reactivation session in the presence of EME. Fig. 1*B* shows that exposure to the novel OF prevented the amnestic effect of EME infusion, thus confirming that the coupling of novel events capable of providing PRPs during the reconsolidation phase has a facilitating effect.

We next evaluated whether the rescuing effect is specifically related to the protein-synthesis process. In other words, we studied if such a rescuing is possible in the case of the postretrieval LTM (PR-LTM) that depends on protein synthesis, but not in the case of the postretrieval short-term memory (PR-STM) that is independent of protein synthesis (31–33). Thus, we repeated the previous experiment but testing the EME-infused animals 3 h, 24 h, and 7 d after memory reactivation (Fig. 1*C*). Infusing EME after the reactivation session did not affect IA memory 3 h later but it induced a long-term amnesia 24 h and 7 d after the reactivation session. When the animals were allowed to explore the novel OF 60 min before the reactivation session, and EME was afterward infused, the PR-STM was again not affected. However, in the case of the 24-h memory, this treatment rescued the PR-LTM, which would be otherwise impaired in the absence of the OF experience (Fig. 1*C*). This rescuing effect persisted at least for 7 d.

As the lifetime of the tag is limited and the newly synthetized proteins are subjected to time-dependent degradation, the rescuing effect of the novel experience should be temporally restricted. To test this hypothesis, we studied the time course of the memory-rescue efficiency of the novel OF by exposing different groups of animals to a novel OF at different times before or after the reactivation of the IA memory in the presence of EME. We found that OF exploration rescued memory reconsolidation when it was performed 1 h before or 30 min after the reactivation session, but not at farther time points (Fig. 1*D*), thus defining the temporal window that is necessary for the PRPs and the tag to coexist in order to promote memory reconsolidation.

To confirm that the novel OF experience provided newly synthesized proteins to the tag established upon memory reactivation, we allowed animals to explore a novel OF 60 min before a memory reactivation session in the presence of EME, but this time, they were also infused with EME or Veh immediately after the OF session. Fig. 2 shows that in the Veh group the reconsolidation process was successfully achieved, thus showing again that PRPs produced during the OF session allow overcoming the effect of EME delivered upon memory reactivation. Yet, animals infused with EME both immediately after the OF exploration and upon memory reactivation had no memory, thus confirming that the PRPs rescuing reconsolidation under EME were synthesized upon the exploration of the novel arena.

As the existence of BT implies the setting of a tag triggered by memory reactivation, impairing this process could also lead to a blockade of memory reconsolidation and to LTM amnesia. In the absence of such a tag, an associated task providing new proteins should be ineffective to rescue the impaired memory. To test this hypothesis, we inhibited protein kinase A (PKA) and the extracellular signal-regulated kinase 1 and 2 (ERK1/2) pathways, which mediate the reconsolidation process of different memories and are involved in BT during memory consolidation (34, 35). To this end, we infused either Veh, Rp-cAMP (PKA inhibitor), or U0126 (MEK inhibitor) upon memory reactivation, at doses which proved to be effective in similar contexts (35–39). We determined if this interference was rescued by the previous exploration of a novel OF. Fig. 3*A* shows that the infusion of Rp-cAMP immediately after the reactivation session impaired memory reconsolidation in a way that

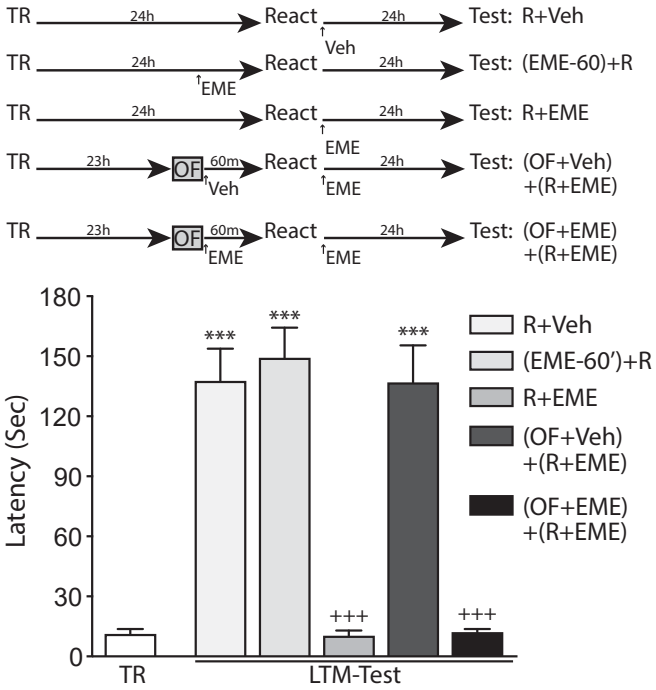


**Fig. 2.** Exploration of an OF rescues IA-memory reconsolidation through a protein-dependent mechanism. (*Top*) Experimental design. The figures show the latency to step-down from the platform during training (TR) and test sessions, expressed as mean ± SEM. R: animals submitted to a reactivation session. ***$P < 0.001$ vs. TR; +++$P < 0.001$ vs. R+Veh, (EME-60′)+R and (OF+Veh)+(R+EME). Newman–Keuls analysis after one-way ANOVA, $n = 6$–8.

could not be prevented by the prior OF exploration (60 min before), suggesting that PKA is involved in the setting of the tag. The infusion of U0126 also impaired memory reconsolidation, but in this case, IA-LTM could be rescued by the prior OF exploration (Fig. 3*B*). This result indicates that the ERK 1/2 pathway does not participate in the tag-setting process. Moreover, the infusion of EME after the exploration of the novel arena completely blocked the memory rescue (Fig. 3*C*) when reactivation was followed by U0126 infusion. Overall, these results suggest that while the PKA pathway participates in the tag setting, the ERK 1/2 pathway is involved in the synthesis of PRPs required for the reconsolidation.

We next asked if the tagging and capture process underlying IA reconsolidation also operates in the reconsolidation of a different kind of memory. We thus repeated the previous experiments using the spatial version of the object recognition task (SOR). In this task, an animal reveals its learning of the spatial configuration of two identical objects when it exhibits higher exploration of the object that was displaced to a novel location in a test (20, 40–42). The time exploring both objects allows calculating a discrimination index (DI). The test also forms a hippocampus-dependent memory but, contrary to the IA task that induces an aversive memory, it induces a nonaversive spatial memory.

First, we observed that animals injected with Veh after a reactivation session exhibited significant LTM in a test with a displaced object (Fig. 4*A*). *SI Appendix*, Figs. S2–S4 show that in all cases, a consolidated memory of the content learned during the training session was present in the reactivation session, with equivalent discrimination indexes between the different experimental groups within each experiment. Infusing EME at an appropriate dose (*SI Appendix*, Fig. S1*B* for dose–response effects) immediately after a 2-min SOR reactivation session performed 24 h after training induced LTM amnesia 24 h later, confirming the necessity of protein synthesis for the reconsolidation process triggered by the

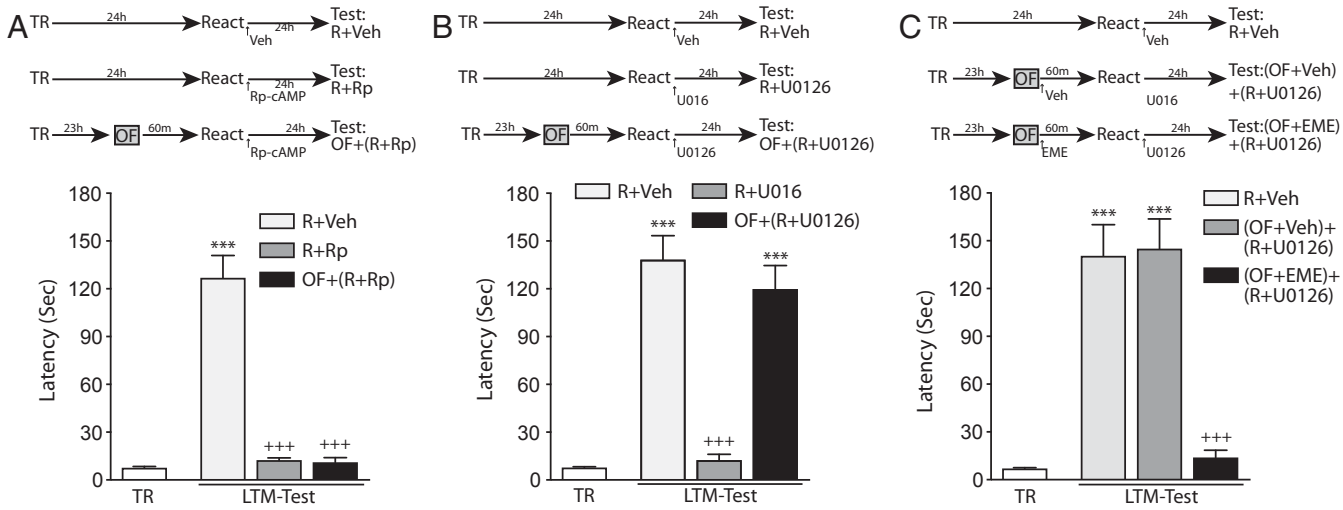


**Fig. 3.** PKA and ERK 1/2 are required for different processes during IA-memory reconsolidation. (*Top*) Experimental design. The figures show the latency to step-down from the platform during TR and test sessions, expressed as mean ± SEM. R: animals submitted to a reactivation session. (*A–C*) Newman–Keuls analysis after one-way ANOVA. (*A*) ***$P < 0.001$ vs. TR; +++$P < 0.001$ vs. R+Veh, $n = 10–12$. (*B*) ***$P < 0.001$ vs. TR; +++$P < 0.001$ vs. R+Veh and OF+(R+U0126), $n = 11–13$. (*C*) ***$P < 0.001$ vs. TR; +++$P < 0.001$ vs. R+Veh and (OF+Veh)+(R+U0126), $n = 6$.

reactivation session (Fig. 4*A*). Again, this impairment of SOR reconsolidation by EME was prevented by OF exploration occurring 60 min before memory reactivation (Fig. 4*B*). Furthermore, the amnesia induced by EME infusion after reactivation and its prevention by the exploration of the novel arena were specific for the PR-LTM (24 h), as no effect was observed for the postretrieval STM (3 h). This result confirms the specificity of the phenomenon for the long-term protein synthesis-dependent reconsolidation (Fig. 4*C*). Finally, we observed that the rescuing effect of the novel OF occurred when exploration took place between 120 min before and 60 min after the reactivation session (Fig. 4*D*).

We further showed that the OF exploration rescued memory reconsolidation due to its capacity to induce protein synthesis in the hippocampus, as the infusion of EME immediately after the exploration impaired rescuing (Fig. 5). We also observed that OF exploration was unable to prevent amnesia induced by the infusion of Rp-cAMP in the dorsal hippocampus immediately after the reactivation session (Fig. 6*A*). This suggests again that the PKA pathway is involved in a fundamental process that, via the setting of the reconsolidation tag, allows the restabilization of the mnemonic trace. On the other hand, the amnesia induced by U0126 could be prevented by the previous exploration of the novel OF (Fig. 6*B*), and this prevention was in turn impaired by the infusion of EME immediately after the novel experience (Fig. 6*C*), suggesting that the ERK pathway is involved in the synthesis of PRPs required for SOR memory reconsolidation.

## Discussion

Our results demonstrate conclusively that memory reconsolidation occurs through a tagging and capture process, which underlies the stabilization of memories resulting from different hippocampus-dependent learning tasks such as IA and SOR. In both experimental contexts, we demonstrated that infusing the protein synthesis inhibitor EME upon memory reactivation impairs the reconsolidation process, leading to long-term amnesia. This amnesia could be overcome if animals explored a novel arena within a critical time window around memory reactivation. The rescuing effect of novelty was dependent on its capacity to induce the synthesis of PRPs in the hippocampus, and on their subsequent capture by a tag set upon the reactivation session. It was specific for the PR-LTM and persistent in time. Moreover, while the PKA pathway was required for the process of tag setting, the ERK 1/2 pathway was specifically activated to engage the synthesis of PRPs required for the reconsolidation.

Overall, the present results provide a mechanistic analysis that was absent in prior works indicating that BT could mediate memory reconsolidation in rodents (29, 30). In these works, which focused on both appetitive spatial learning and contextual fear conditioning, a novel spatial experience (placing rats in a novel box) contiguous to memory reactivation, improved the persistence of the memory subjected to reconsolidation by the reactivating event (29, 30). Furthermore, when the process of reconsolidation was impaired by the delivery of the beta-adrenergic antagonist propranolol, the introduction of spatial novelty reversed the impairment (29). These findings are relevant, as they are consistent with the participation of a BT process in memory reconsolidation, but they lack the demonstration that the reactivating event sets a reconsolidation tag and that the contiguous novel experience provides PRPs that once captured by the tag lead to longer memory persistence. These two phenomena require specific analyses as they have separate time courses, one which refers to the temporal duration of the tag and the other to the temporal duration of PRPs availability after synthesis. As these analyses were absent in prior works, their conclusions were carefully formulated with respect to the possibility that BT underlies the process of memory reconsolidation (29). In contrast, our work characterized the temporal dynamics and the molecular nature of both phenomena, providing thereby the concrete demonstration of their participation in a reconsolidation scenario.

Different works reported that the BT process underlies LTM consolidation in the case of aversive, appetitive, and spatial memories (13). This conclusion applies also to memory persistence (26) and to the long-term extinction of aversive memories (25). Our work shows that besides these scenarios, BT also participates in memory reconsolidation, a fact that highlights its generality as a fundamental mechanism underlying the formation of lasting memories.

Inhibiting the PKA and the ERK1/2 pathways upon memory reactivation in the IA or SOR tasks revealed the different implication of these pathways in the process of memory reconsolidation. Infusing the PKA inhibitor induced a retrograde amnesia that could not be prevented by the exploration of a novel arena. In this case, even when proteins were provided by the exploration of the OF, memory was not reconsolidated, confirming the importance of

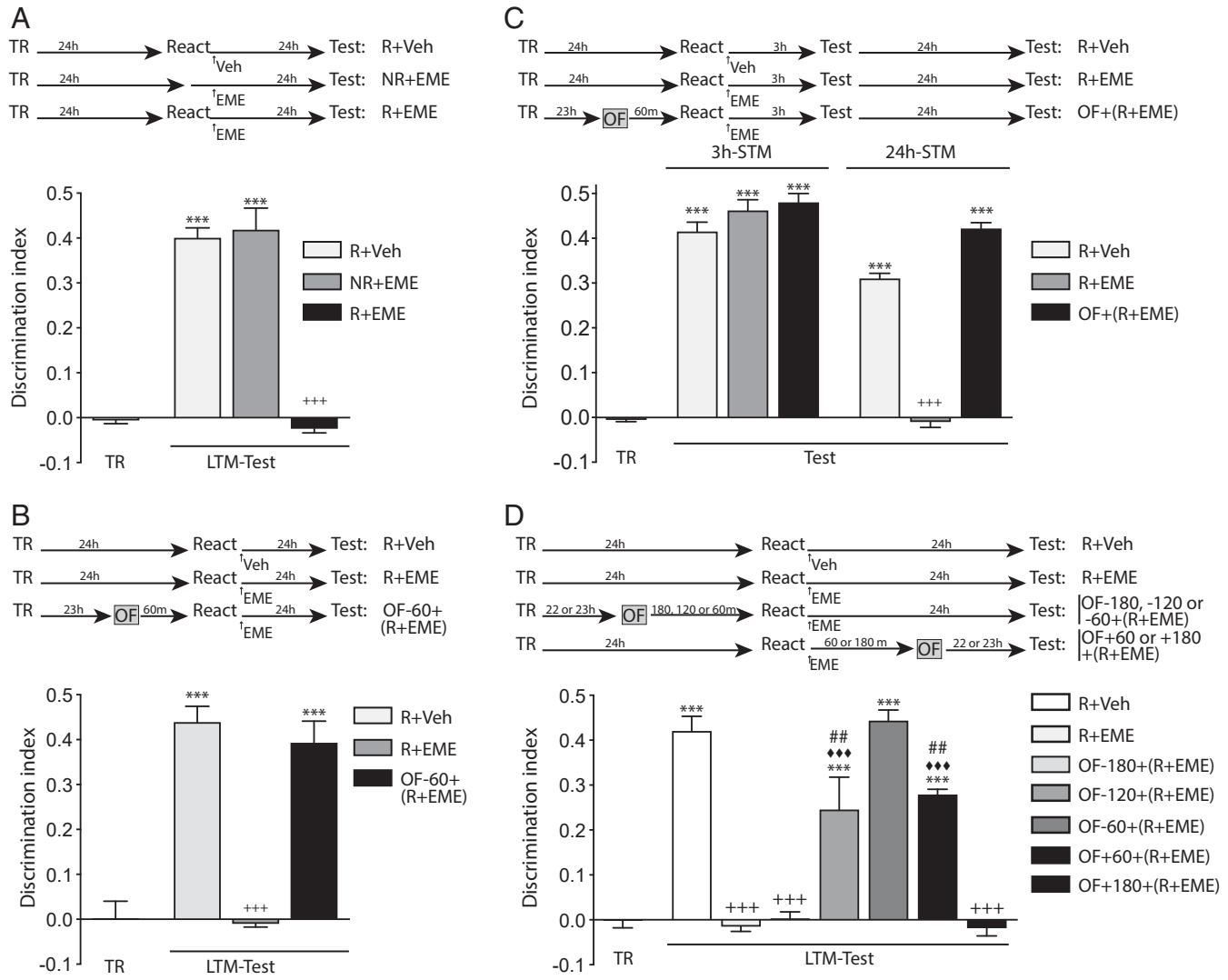


**Fig. 4.** Exploration of an OF rescues the blockade of reconsolidation induced by EME for a persistent period of time and within a restricted time window. (*Top*) Experimental design. The figures show the discrimination index between the object moved to the novel position and the nonmoved object, expressed as mean ± SEM, during TR and test sessions. R: animals submitted to a reactivation session. NR: nonreactivated animals. (*A–D*) Newman–Keuls analysis after one-way ANOVA. (*A*) ***$P < 0.001$ vs. TR; +++$P < 0.001$ vs. R+Veh and NR+EME $n = 6–7$. (*B*) ***$P < 0.001$ vs. TR; +++$P < 0.001$ vs. R+Veh and OF+(R+EME), $n = 7–8$. (*C*) ***$P < 0.001$ vs. TR, +++$P < 0.001$ vs. R+Veh and OF-60+(R+EME) in 24 h test, $n = 6$. (*D*) ***$P < 0.001$ vs. TR; +++$P < 0.001$ vs. R+Veh, OF-120+(R+EME), OF-60+(R+EME) and OF+60+(R+EME); ♦♦♦$P < 0.001$ vs. R+Veh; ## $P < 0.01$ vs. OF-60+(R+EME), $n = 6–9$.

the PKA pathway for tag setting. In the absence of the tag, PRPs could not be captured to restabilize the mnemonic trace. As affecting the tag results in the impossibility of capturing PRPs from any source, we cannot exclude that the PKA pathway also plays a role in the synthesis of PRPs during memory reconsolidation. In contrast, the participation of ERK1/2 pathway in memory reconsolidation was better delimited. Inhibition of this pathway after memory reactivation induced long-term retrograde amnesia that could be overcome through exploration of a novel OF, i.e., through protein synthesis. This result indicates that ERK1/2 pathway is required for the PRPs synthesis process rather than for setting the reconsolidation tag. It also shows that if there was an effect of the inhibitor U0126 on the learning tag, it was not relevant to impair its function.

A tagging and capture mechanism relies on the coexistence of the tag and the newly synthesized proteins in order for the capture process to occur (17, 19). Both the duration of the tag and the protein synthesis/degradation dynamics define the time window during which cooccurring events can contribute PRPs and determine the fate of the memory. Our data unveil that a putative reconsolidation tag lasts around 30–45 min for the IA memory and around 1–2 h for the SOR memory, showing that despite their transient nature, the duration of the tags depends on which memory is being reconsolidated. These time windows explain why, in most studies, memory reconsolidation is impaired by the action of protein synthesis inhibitors and other substances provided immediately after memory reactivation, but not 4 or 6 h later (3, 31, 43, 44). They account for the different timescales of interference observed in the reconsolidation of distinct memories, and define a clear interval around a memory reactivation session during which events and interventions could be used to modify an established memory trace.

The possibility of attenuating established memories as a result of impairing reconsolidation opens perspectives for treating pathologies related to traumatic memories, phobias, and addictions (10). Indeed, this strategy proved to be efficient in certain cases (45–49). Our work allows improving this efficiency as it provides an innovative perspective to define the pharmacological or

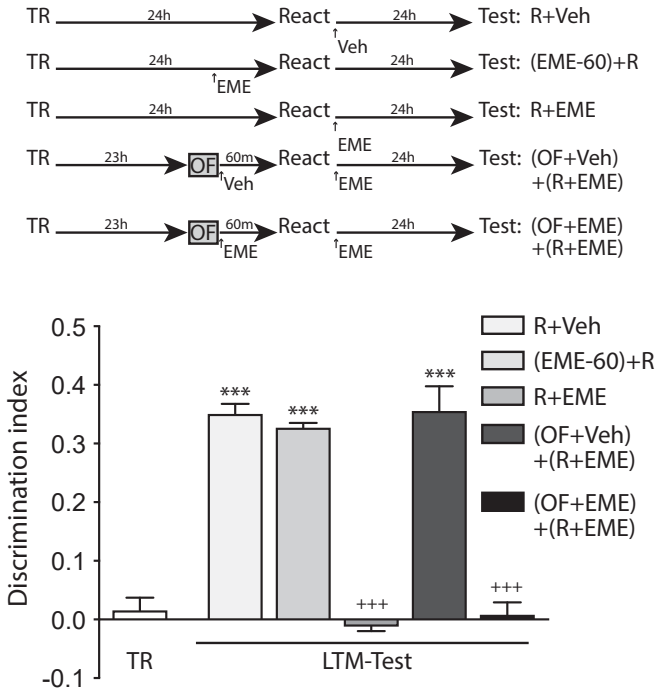


**Fig. 5.** Exploration of an OF rescues SOR-memory reconsolidation through a protein dependent mechanism. (*Top*) Experimental design. The figures show the discrimination index between the object moved to the novel position and the nonmoved object, expressed as mean ± SEM, during TR and test sessions. R: animals submitted to a reactivation session. ***$P < 0.001$ vs. TR; +++$P < 0.001$ vs. R+Veh, (EME-60′)+R and (OF+Veh)+(R+EME). Newman–Keuls analysis after one-way ANOVA, $n = 6$–8.

behavioral manipulations that may affect memory reconsolidation, and the timescales at which different events can affect it. We posit that traumatic memories could be effectively blocked if their reactivation would be associated with a novel experience impairing the reconsolidation tag or the protein synthesis process, or competing for the proteins required for reconsolidation. In a different scenario, our findings could help developing efficient strategies to improve retention of valuable memories such as those acquired in an educational context. Providing further PRPs during the reconsolidation phase could enhance PRP capture by reconsolidation tags and thus enhance the original and the updated memory traces. In conclusion, the characterization of behavioral tagging as a fundamental mechanism underlying memory reconsolidation opens new research avenues for designing effective therapies in translational research or to develop newstrategies to improve retention in educational contexts.

## Materials and Methods

**Subjects.** The study was conducted using male Wistar rats (weight: 280–300 g at the moment of the experiment) from the animal core facility of the Faculty of Exact and Natural Sciences of the University of Buenos Aires (UBA). Rats were housed in groups of three per cage, containing water and food *ad libitum*, at a constant room temperature of 22 °C and under a 12-h light/dark cycle (lights on: 6 AM). The experiments were performed during the light phase.

The experimental procedures used in this study were approved by the Institutional committee for the use and care of laboratory animals of the Faculty of Medicine of the UBA, and respected the guidelines of the National Institute of Health Guide for the Care and Use of Laboratory Animals.

**Drugs.** Emetine, U0126, and Rp-cAMP were purchased from Sigma. Emetine (50 μg per side diluted in saline), Rp-cAMP (0.5 μg per side diluted in saline), or U0126 (0.4 μg per side diluted in 10% dimethyl sulfoxide in saline) were infused in volume of 0.8 μL per side to specifically inhibit protein synthesis, PKA or MEK protein kinase activities, respectively.

The drugs and doses used in this study were chosen based on their demonstrated target specificity (50–52) and clear effects on memory (19, 35–39, 53, 54). In addition, the dose of EME used in our behavioral experiments (50 μg) was obtained from a dose–response curve (with doses of 0.5; 5.0, and 50 μg per side), which showed its effectiveness to impair memory reconsolidation in both tasks (*SI Appendix*, Fig. S1)

**Surgical and Drug-Infusion Procedures.** The procedures for implanting the cannulas, infusing drugs, and examining the localization of cannulas histologically were performed as described in a previous work (19). Antero-Posterior (AP), Ventral (V), and Lateral (L) coordinates were established using the Bregma as the reference point. Briefly, guide cannulas were stereotaxically implanted 1 mm above the pyramidal cell layer of the CA1 region of the dorsal hippocampus (AP −3.9 mm, V 3.0 mm, L ±3.0 mm) in rats that were deeply anesthetized. The coordinates of the rat brain atlas by

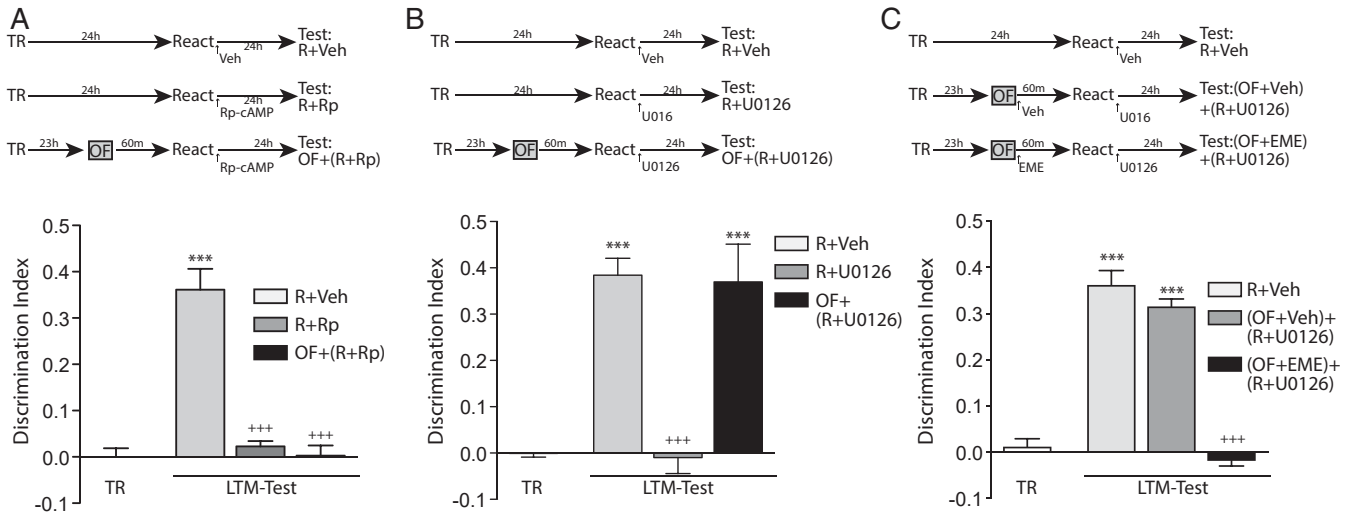


**Fig. 6.** PKA and ERK 1/2 are required for different processes during SOR-memory reconsolidation. (*Top*) Experimental design. The figures show the discrimination index between the object moved to the novel position and the nonmoved object, expressed as mean ± SEM, during TR and test sessions. R: animals submitted to a reactivation session. (*A*–*C*) Newman–Keuls analysis after one-way ANOVA. (*A*) ***$P < 0.001$ vs. TR; +++$P < 0.001$ vs. R+Veh, $n = 9$–14. (*B*) ***$P < 0.001$ vs. TR; +++$P < 0.001$ vs. R+Veh and OF-60+(R+U0126), $n = 7$. (*C*) ***$P < 0.001$ vs. TR; +++$P < 0.001$ vs. R+Veh and (OF+Veh)+(R+U0126), $n = 6$.

Paxinos and Watson (55) were used as a reference. After a resting and recovery period of at least 1 wk, procedural manipulations were performed. Drugs were infused using a 30-gauge infusion needle, which was fitted into the guide cannula. The tip of the infusion needle protruded 1.0 mm beyond the guide cannula. The data included in the analyses corresponded to animals with correct cannula implants (>95% of the rats).

**Behavioral Setups, Training, and Testing Procedures.** To avoid unnecessary emotional stress, all rats were handled daily for 3 min during 3 d before any behavioral procedure. Animals were then randomly assigned to each experimental group/condition. Experiments were performed by more than one experimenter (generally two or three) given the complexity of the experimental schedules that went over more than 1 d. Each experimenter was blind with respect to the treatment (behavioral or drug infusion) assigned to a given animal.

The OF apparatuses were previously described (19). A novel environment exploration consisted of a 5-min OF session. For the IA, we used a step-down inhibitory avoidance system manufactured by Med Associates Inc. The training protocol is an adaptation of that described previously by Radiske et al. (31) showing the existence of reconsolidation in the IA task. Rats were subjected to two habituation sessions, a training session, a reactivation session, and a test session, each of them spaced by 24 h. During the habituation session animals were placed in the platform and let to explore freely the avoidance box for 5 min. During day three, in the training session, rats were placed in the platform and immediately after stepping down with their four paws, they received an electric foot shock (0.5 mA during 3 s). After the end of the shock, rats were removed from the training box and returned to their home cages. On day four, in the reactivation session, animals were placed in the platform and taken from it after 40 s. On day five, a full test session was performed placing the animals in the platform and measuring the latency to step down up to a maximum of 400 s. In this task, the increase in the latency to step down from training to test is indicative of memory. A greater latency is indicative of a better memory. As training performance was always equivalent among the different experimental groups, the training (TR) group data resulted from a random pool of animals matching the same number of animals in the larger experimental group. In this way, an artificial increase in the global degrees of freedom was avoided.

The arena used for the SOR task was an acrylic box (60 cm width × 40 cm length × 50 cm height) presenting different visual clues (black and white patterns). During the first 2 d animals were habituated to the context letting them freely explore the arena without objects for 30 min each a day. On day three, when the training phase started, two identical objects were placed in two adjacent corners of the arena, distant 7 cm from the lateral walls. During this phase, animals could explore the arena during 8 min. We measured the time dedicated to explore each object. On the next day, memory was reactivated by switching one of the objects to a new position and allowing the rats to explore for 2 min. Finally, during the test session, the object moved for the reactivation session was moved again to a new position and animals were left to explore one more time during 2 min. The exploration time was recorded for each object and expressed as a relative discrimination index: (time in novel–time in old position)/(time in novel + time in old position). Positive values of this index reflect the presence of memory. Values around zero or negative values reflect the absence of memory.

To reduce the use of animals, rats were used twice, once in the IA task and once in the SOR task. Performance in these tasks is not affected by their sequential ordering (42) The rats could rest between 1 and 2 wk between the two tasks.

**Data Analysis.** One-way ANOVA followed by post hoc Newman–Keuls tests for multiple comparisons were used to perform between-group comparisons by means of GraphPad Prism 8 (GraphPad Software Inc.). Full statistics, F values, and degrees of freedom for each figure panel are provided in *SI Appendix*.

**Data Availability.** Latencies and discrimination indexes for individual animals are available at the Open Science Framework repository (https://osf.io/r6d9u/).

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