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Changing memories after reactivation: A one-time opportunity?

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ABSTRACT

After reactivation, apparently stable memories can become sensitive to modifications again, requiring another phase of stabilization, called reconsolidation. Recent evidence shows that repeated reactivations strengthen memories and that stronger memories are more resistant to alterations during reconsolidation. Therefore, we asked whether multiple reactivations make memories less vulnerable to manipulations during reconsolidation and thus serve as boundary condition on memory reconsolidation. In Experiment 1, we tested whether new learning after the reactivation of previously learned material alters the subsequent memory for the original material. Participants learned negative and neutral pictures, reactivated them 1 week later, and learned new pictures immediately thereafter. Memory for the original pictures was assessed in a recognition test 1 day later. We found that new learning after reactivation reduced the subsequent memory of the original pictures; new learning alone or reactivation alone, however, had no effect on memory. Two further experiments investigated the impact of multiple reactivations on this memory impairing effect of new learning after reactivation. These experiments showed that the influence of new learning after reactivation disappeared neither after one (Experiment 2) nor after three (Experiment 3) additional reactivations completely, indicating that even after multiple reactivations memories remain sensitive to modifications during reconsolidation. These findings may have important implications for novel treatment approaches that aim for modification of unwanted memories during reconsolidation.

1. Introduction

After initial encoding, memories are stabilized during a process of consolidation. It has long been assumed that memory consolidation occurs only once and that consolidated memories are stable and resistant to changes (Dudai, 2004; McGaugh, 2000). This classical view on memory consolidation, however, has been challenged by recent findings suggesting that memories may re-enter an unstable state after their reactivation (Dudai, 2006; Hardt, Einarsson, & Nader, 2010; Nader & Hardt, 2009), thus requiring another phase of stabilization—called reconsolidation—that is similar but not identical to the first phase of consolidation (Lee, Everitt, & Thomas, 2004). Support for the idea of a reconsolidation process after memory reactivation comes from studies showing that reactivated memories—just like new memories—need de novo protein synthesis to persist (Nader, Schafe, & LeDoux, 2000), and that pharmacological or behavioral manipulations after the reactivation of conditioned fear memories reduce subsequent fear memory (Kindt, Soeter, & Vervliet, 2009; Przybyslawski, Roullet, & Sara, 1999; Schiller et al., 2010; Soeter & Kindt, 2011).

Early reconsolidation research focused mainly on the reconsolidation of conditioned fear memories, but recent studies have shown that memory reconsolidation occurs also in episodic memory (Finn & Roediger, 2011; Forcato et al., 2007; Hupbach, Gomez, & Nadel, 2009; Hupbach, Hardt, Gomez, & Nadel, 2008; Schwabe & Wolf, 2009; Wichert, Wolf, & Schwabe, 2011). When reactivated, episodic memories can be modified and updated by the incorporation of new information into the existing memory trace (Hupbach, Gomez, Hardt, & Nadel, 2007). Accordingly, reconsolidation manipulations may provide an opportunity to alter unwanted memories and could have important implications for the treatment of anxiety disorders, such as post-traumatic stress disorder (PTSD). Indeed, there is first evidence that therapeutic interventions during the reconsolidation window reduce subsequent responding to mental imagery of the traumatic event in PTSD patients (Brunet et al., 2008). In light of these promising findings, an important question is whether memories can always be changed after reactivation or whether there are limiting factors for reconsolidation manipulations.

During the past years, several boundary conditions on memory reconsolidation have been proposed. Rodent studies showed that

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older memories are less vulnerable to post-reactivation manipulations than younger ones (Eisenberg & Dudai, 2004; Milekic & Alberini, 2002), suggesting that memory age may be a constraint on memory reconsolidation (but see Debiec, LeDoux, & Nader, 2002). Moreover, memory strength (Suzuki et al., 2004; Wang, de Oliveira Alvares, & Nader, 2009; Winters, Tucci, & DaCosta-Furtado, 2009), reminder duration (Pedreira & Maldonado, 2003), and predictability of the reactivation stimulus (Pedreira, Pérez-Cuesta, & Maldonado, 2004) have been suggested as boundary conditions on reconsolidation. Reconsolidation research in humans showed that memory updating occurs only when original and new learning take place in the same spatial context (Hupbach et al., 2008), and that the temporal sequence of presented reminders constrain the reconsolidation process (Forcato, Rodriguez, & Pedreira, 2011).

Memory reactivation is the key to memory reconsolidation; without reactivation there is no reconsolidation. However, beyond its role as a trigger of the reconsolidation process, reactivation itself has an influence on memory: repeated reactivations enhance the resilience of a memory trace to brain damage (Lehmann & McNamara, 2011), and strengthen long-term memory (Karpicke & Roediger, 2008). Even indirect memory reactivation during sleep facilitates subsequent remembering (Rasch, Büchel, Gais, & Born, 2007; Rudoy, Voss, Westerberg, & Paller, 2009). In line with these findings, it has been proposed that memory reactivation may serve to strengthen memories (Sara, 2000). If memory reactivation strengthens memory and if stronger memories are less sensitive to modifications during reconsolidation (Suzuki et al., 2004; Winters et al., 2009), it is tempting to hypothesize that repeated memory reactivations and possibly the repeated reconsolidations following the reactivations may act as a boundary condition on reconsolidation manipulations. Given that recurrent reactivations of a traumatic event are a core symptom of PTSD, multiple reactivations as a boundary condition on reconsolidation manipulations would be highly relevant in the context of novel treatment approaches that aim at the modification of trauma memory after its reactivation (Brunet et al., 2008; Schiller et al., 2010).

In the present series of experiments, we tested the hypothesis that multiple reactivations make memories less vulnerable to alterations after reactivation and thus act as a boundary condition on reconsolidation manipulations. In the first experiment, participants learned new pictures after they had reactivated (i.e., retrieved) a set of previously learned pictures (*retrieval/new learning* condition; see Fig. 1A). To control for effects of memory reactivation without subsequent new learning and the effects of new learning without prior reactivation, we included a group that reactivated the initially learned pictures without learning new pictures (*retrieval/no new learning* condition) and a group that learned new pictures without prior reactivation of the initially learned pictures (*no retrieval/new learning* condition). In addition, we included a control group that neither reactivated the original pictures nor learned new pictures (*no retrieval/no new learning* condition). This first experiment served to show that new learning after the reactivation of previously learned material may alter the subsequent memory for the original material (Hupbach et al., 2007; Schwabe & Wolf, 2009). In order to test for the influence of a single additional reactivation on the susceptibility of memories to alterations during reconsolidation, participants in the second experiment reactivated the original pictures once before the reconsolidation manipulation (i.e., new learning after memory reactivation; Fig. 1B). In a third experiment, participants reactivated the original pictures three times before the reconsolidation manipulation (Fig. 1C), in order to test for the effect of multiple reactivations.

2. Experiment 1: does new learning during reconsolidation alter subsequent memory?

The first experiment investigated memory reconsolidation in human episodic memory and asked whether learning new information after the retrieval of previously learned information alters the subsequent memory for the originally learned information in a recognition test.

2.1. Materials and methods

2.1.1. Participants and design

Fifty-six healthy students of the Ruhr-University Bochum (28 men, 28 women; age: $M = 23.32$ years, $SEM = 0.36$) participated in this experiment. They received either course credits or a moderate monetary compensation for participation. Exclusion criteria comprised current or chronic mental disorder, drug abuse, and current treatment with medication. All participants provided written informed consent for their participation. The study was approved by the local ethics committee.

We used a between-subjects design with the factors reactivation (retrieval, no retrieval) and new learning (yes, no), resulting in four experimental groups: *retrieval/new learning*, *retrieval/no new learning*, *no retrieval/new learning*, *and no retrieval/no new learning*. Seven men and seven women were randomly assigned to each of the four groups.

2.1.2. Stimulus materials

The stimulus materials included two sets of 16 pictures taken from the International Affective Picture System (IAPS; Lang, Bradley, & Cuthbert, 2008). In order to test for potential differences in the reconsolidation of neutral and emotional memories, each set comprised eight negative and eight neutral pictures. The pictures

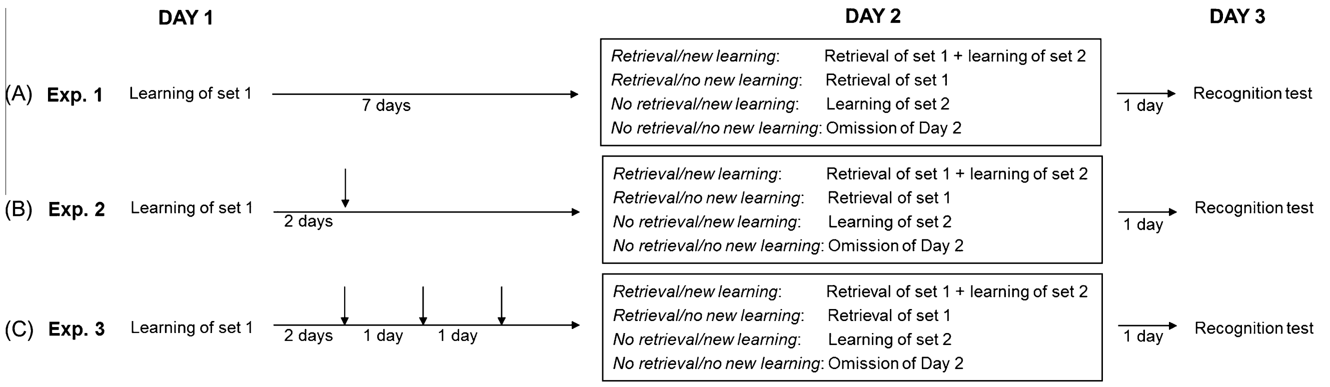

Fig. 1. Experimental design: Day 1, Day 2, and Day 3 were identical in the three experiments (for procedure see text). The number of retrievals, indicated by the vertical arrows, between Day 1 and Day 2 varied between the experiments: Exp. 1 – no additional retrieval; Exp. 2 – one additional retrieval; Exp. 3 – three additional retrievals.

of the two sets were matched with respect to emotional valence and emotional arousal, based on the IAPS standard scores for valence and arousal. To ensure that the pictures were indeed experienced as neutral and negative, participants rated each picture with respect to valence and arousal on two 0–100 scales with the endpoints being *very negative* vs. *very positive* and *not arousing* vs. *very arousing*, respectively. In retrospect, these ratings confirmed the classification of the pictures as negative and neutral: negative pictures ($M = 14.21$, $SEM = 1.18$) were rated significantly lower in valence than neutral pictures ($M = 54.22$, $SEM = 1.45$; $t\,(55) = -18.80$, $p < .001$), and were associated with significantly higher emotional arousal than neutral pictures ($M = 66.83$, $SEM = 2.29$ vs. $M = 21.06$, $SEM = 2.01$; $t\,(55) = 15.05$, $p < .001$). For recognition testing, we used an additional picture set that also consisted of eight negative and eight neutral pictures and was matched to the other two picture sets.

2.1.3. Procedure

Testing took place on three experimental days: Day 1, learning; Day 2, retrieval and/or new learning; Day 3, recognition testing (see Fig. 1A).

After their arrival in the lab on Day 1, all participants saw 16 neutral and negative pictures (set 1) on a computer screen and were instructed to memorize them. While seeing a picture, participants gave valence and arousal ratings by clicking on two rating scales shown under the picture. Each picture was presented on the screen until participants finished their valence and arousal ratings. Picture presentation and rating took about 10 min. To rule out any group differences in picture encoding, participants completed a written immediate free recall test directly after the picture presentation. Participants were asked to write down all pictures they could remember, in as much detail as possible. The experimenter counted the number of remembered pictures. If participants remembered less than 13 of the 16 pictures, the procedure was repeated (without valance and arousal ratings). Participants had a maximum of two trials to reach the learning criterion of 13 remembered pictures. After these two learning trials, the learning phase was finished. Participants who did not reach the learning criterion after two learning trials followed the same procedure as all participants. There was no time limit for the free recall test; most participants finished the learning session after two learning trials and within 25 min.

The procedure on Day 2, 7 days after Day 1, depended on the experimental condition. Similar to previous studies (Hupbach et al., 2007, 2008; Wichert et al., 2011), participants in the retrieval conditions (*retrieval/new learning*, *retrieval/no new learning*) were asked to sit down quietly for 2 min and to think of the pictures that were presented on Day 1. In order to quantify memory reactivation and to control for potential group differences in reactivation, participants were then asked to verbally list the pictures they remembered. The experimenter recorded the pictures that were mentioned without giving feedback. Retrieval took place in the same spatial context as the learning session on Day 1 (Hupbach et al., 2008). Participants in the *retrieval/no new learning* group left the lab after retrieval. Participants in the *retrieval/new learning* group learned another 16 neutral and negative pictures (set 2) immediately after retrieval, following the same procedure as during initial learning on Day 1. In order to avoid any reactivation of the picture set from Day 1, participants in the *no retrieval/new learning* group learned the novel pictures without prior memory retrieval and in a different spatial context (Hupbach et al., 2008). Participants in the *no retrieval/no new learning* group completely omitted experimental Day 2.

On Day 3, 24 h after Day 2, all participants completed a recognition test on the computer. Forty-eight pictures were shown, including the pictures that were learned on Day 1, the pictures that were learned by the new learning groups on Day 2, and an additional set that has never been shown before (set 3). Pictures were presented one after another (in randomized order) and participants were asked to indicate whether they had seen the presented picture on Day 1 or not by pressing a *yes* or a *no* button on a keyboard.

Testing on the three experimental days took place between 9 am and 7 pm. For each subject, the testing sessions usually took place at the same time of the day. Groups did not differ systematically with respect to the testing time.

2.2. Results

2.2.1. Learning performance on Day 1

On Day 1, participants took on average 1.8 trials ($SEM = 0.05$) to reach the learning criterion of 13 correctly recalled pictures; the four groups (*retrieval/new learning, retrieval/no new learning, no retrieval/new learning, no retrieval/no new learning*) did not differ in the number of learning trials, $F(3,52) = 1.65$, $p = .19$, $\eta^2 = .09$ (main effect Group). At the end of Day 1, participants remembered on average 13.41 pictures ($SEM = 0.24$; Table 1) with no significant differences between groups, $F(3,52) = 0.44$, $p = .72$, $\eta^2 = .03$ (main effect Group), suggesting that there were no group differences in initial picture encoding. Overall, negative pictures were better remembered than neutral pictures, $t\,(55) = 2.45$, $p = .01$.

2.2.2. Memory retrieval and new learning on Day 2

On Day 2, participants in the two retrieval groups recalled on average 11.79 pictures ($SEM = 0.40$; Table 1). Negative pictures were more often retrieved than neutral pictures, $t\,(27) = 3.24$, $p = .003$. Unexpectedly, the *retrieval/no new learning* group recalled significantly more pictures than the *retrieval/new learning* group, $F(1,26) = 7.68$, $p = .01$, $\eta^2 = .22$ (main effect New Learning), which might point to group differences in initial memory consolidation.

Participants in the two new learning groups learned a second set of pictures either immediately after retrieval of picture set 1 or without prior memory retrieval. They took on average 1.9 trials ($SEM = 0.07$) to reach the learning criterion of 13 correctly recalled pictures from set 2 ($M = 14.19$, $SEM = 0.28$; Table 1) with no differences in their learning performance, $F(1,26) = 2.67$, $p = .10$, $\eta^2 = .11$ (main effect Reactivation). Negative pictures were again better remembered than neutral pictures, $t\,(27) = 2.78$, $p = .01$.

2.2.3. Memory performance on Day 3

2.2.3.1. Memory accuracy. Recognition accuracy (i.e., the percentage of correctly recognized pictures from set 1 [hits] minus the percentage of pictures from set 2 or set 3 that were incorrectly identified as being from set 1 [false alarms]), was analyzed by an Emotionality (negative, neutral) × Reactivation (retrieval, no retrieval) × New Learning (yes, no) ANOVA. This analysis revealed that the effect of learning new pictures depended critically on the prior retrieval of the initially learned pictures, $F(1,52) = 4.30$, $p = .04$, $\eta^2 = .08$ (Reactivation × New Learning interaction; without any three-way-interaction with Emotionality). Learning new pictures after retrieval of the original pictures reduced the subsequent memory for the original pictures (*retrieval/new learning* vs. *retrieval/no new learning*, LSD post hoc test, $p = .01$), whereas learning new pictures without prior retrieval did not alter subsequent memory for the original pictures (*no retrieval/new learning* vs. *no retrieval/no new learning*, LSD post hoc test, $p = .75$; Fig. 2A and Table 1).

Since the *retrieval/new learning* group recalled significantly fewer pictures than the *retrieval/no new learning* group during retrieval on Day 2 (see above), we performed an Emotionality (negative, neutral) × New Learning (yes, no) ANCOVA with retrieval performance on Day 2 as covariate to rule out the possibility that the

Table 1
Performance at initial learning on Day 1, retrieval and/or new learning on Day 2, and recognition testing on Day 3 for Experiment 1.

Group	Day 1	Day 2		Day 3	
	Pictures recalled after initial learning	Pictures recalled after retrieval	Pictures recalled after new learning	Recognition accuracy (%)	Hits (%)
Retrieval/new learning	13.57 (0.49)	10.79 (0.51)	14.67 (0.38)	91.79 (1.26)	92.30 (1.10)
Retrieval/ no new learning	13.50 (0.49)	12.79 (0.51)	–	96.43 (1.26)	97.70 (1.10)
No retrieval/ new learning	13.64 (0.49)	–	13.71 (0.38)	94.62 (1.26)	96.50 (1.10)
No retrieval/ no new learning	12.93 (0.49)	–	–	94.06 (1.26)	95.70 (1.10)

Data represent means and standard error of the mean (in parentheses).

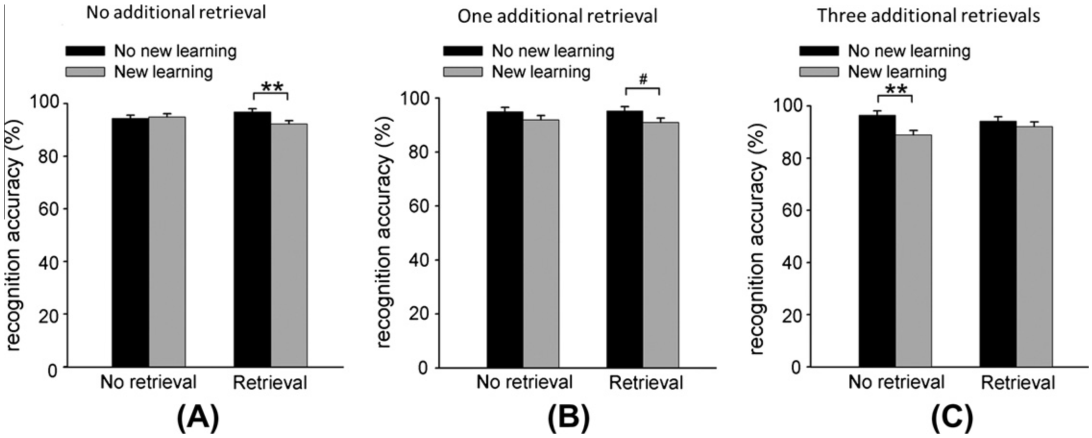

Fig. 2. Impact of new learning after retrieval (Exp. 1) when the memory was retrieved once (Exp. 2) or three times before (Exp. 3). Accuracy in the recognition test (i.e., hits minus false alarms): (A) Learning new pictures after retrieval of original pictures reduced memory performance for the original pictures. (B) The *retrieval/new learning* group tended to recognize fewer pictures correctly than the *retrieval/no new learning* group when the memory was reactivated once before. (C) The *no retrieval/new learning* group recognized significantly fewer pictures correctly than the *no retrieval/no new learning* group when the memory was reactivated three times before. LSD post hoc tests $^{**}p < .01$, $^{\#}p < .08$; data represent means ± standard errors of the mean.

group differences observed on Day 3 were owing to differences in initial memory consolidation. This analysis yielded no significant effect of retrieval performance on Day 2 on the recognition accuracy (effect retrieval performance on Day 2, $F(1,25) = 0.22$, $p = .88$, $\eta^2 = .001$), and showed that the impairing effect of new learning after retrieval on subsequent memory remained even when the retrieval performance on Day 2 was controlled for, $F(1,25) = 4.33$, $p < .05$, $\eta^2 = .15$ (main effect New Learning): the *retrieval/new learning* group ($M = 91.86$, $SEM = 1.44$) still remembered significantly less pictures than the *retrieval/no new learning* group ($M = 96.36$, $SEM = 1.44$).

2.2.3.2. Percentage of false alarms (intrusions from set 2 and intrusions from set 3). According to the concept of memory reconsolidation, one would expect the incorporation of the new information (i.e., picture set 2) into the original memory (i.e., picture set 1) after reactivation (Hupbach et al., 2007). The false alarm rate, however, was very low ($M = 1.02$, $SEM = 0.23$), with no difference between intrusions from pictures that had been presented on Day 2 (set 2; $M = 0.89$, $SEM = 0.34$) and intrusions from pictures that have not been presented before (set 3; $M = 1.01$, $SEM = 0.35$), $t(55) = -0.23$, $p = .82$. Moreover, an Emotionality (negative, neutral) × Reactivation (retrieval, no retrieval) × New Learning (yes, no) ANOVA revealed no significant influence of reactivation or new learning on intrusions from set 2 (main effect Reactivation and main effect New Learning both $Fs(1, 52) < 1.89$, $ps > .18$, $\eta^2 < .04$) or intrusions from set 3 (main effect Reactivation and main effect New Learning both $Fs(1, 52) = 2.69$, $ps = .11$, $\eta^2 = .05$).

Since pictures from set 2 were novel for the no new learning groups (*retrieval/no new learning*, *no retrieval/no new learning*) but familiar for the new learning groups (*retrieval/new learning*, *no retrieval/new learning*) in the recognition test, we compared the groups with and without new learning on Day 2 with respect to incorrectly identified pictures from set 2. Intrusions from set 2 were equally low for the groups with ($M = 1.12$, $SEM = 0.48$) and without ($M = 0.67$, $SEM = 0.48$) new learning, $F(1,54) = 0.44$, $p = .51$, $\eta^2 = .01$ (main effect New Learning), indicating that there was no difference in the incorrectly identified pictures from set 2.

2.2.3.3. Percentage of hits (correctly recognized pictures from set 1). Because the false alarm rate was very low (see above), we further analyzed the percentage of hits. An Emotionality (negative, neutral) × Reactivation (retrieval, no retrieval) × New Learning (yes, no) ANOVA on the percentage of hits revealed that the effect of learning new pictures depended critically on the prior retrieval of the initially learned pictures, $F(1,52) = 8.19$, $p = .006$, $\eta^2 = .14$ (Reactivation × New Learning interaction; without any three-way-interaction with Emotionality). Learning new pictures after retrieval of the original pictures reduced the subsequent memory for the original pictures (*retrieval/new learning* vs. *retrieval/no new learning*, LSD post hoc test, $p = .001$), whereas learning new pictures without prior retrieval did not alter subsequent memory for the original pictures (*no retrieval/new learning* vs. *no retrieval/no new learning*, LSD post hoc test, $p = .61$; Fig. 3A).

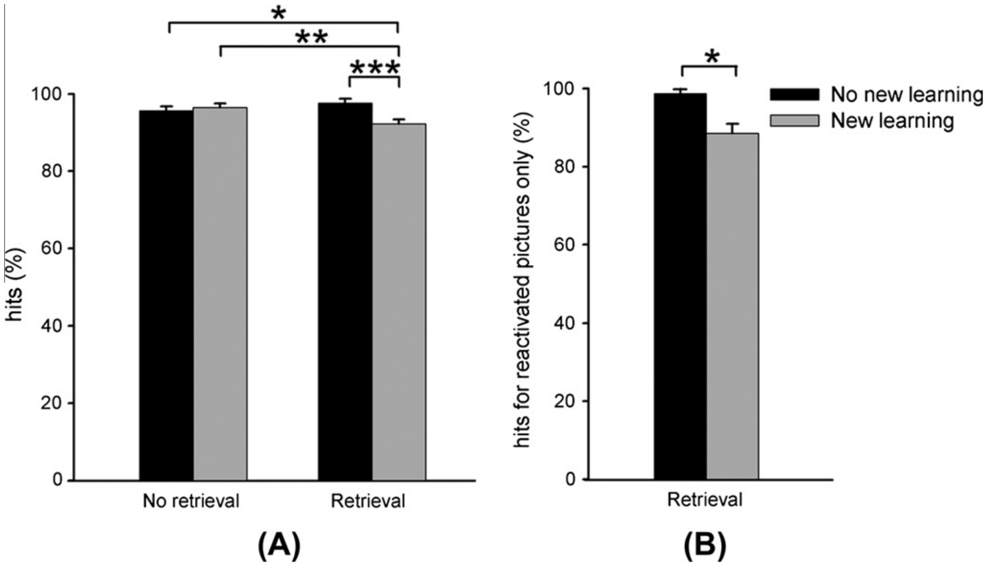

Fig. 3. Impact of new learning after memory retrieval (Exp. 1). (A) Hits (i.e., percentage of correctly recognized pictures from set 1) in the recognition test: learning new pictures after retrieval of original pictures reduced memory performance for the original pictures. (B) Hits for retrieved pictures only: learning new pictures reduced memory performance for the original pictures that were retrieved on Day 2. LSD post hoc tests $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$; data represent means ± standard errors of the mean.

2.2.3.4. Hits for retrieved pictures only. According to the concept of memory reconsolidation, only memories that are reactivated return to an unstable state in which they are modifiable (Nader & Hardt, 2009). Therefore, we analyzed those memories that were actually recalled during retrieval on Day 2. An Emotionality (negative, neutral) × New Learning (yes, no) ANOVA revealed a significant effect of new learning after retrieval, $F(1,26) = 8.61$, $p = .007$, $\eta^2 = .25$ (without an interaction with Emotionality): whereas participants who did not learn new pictures after retrieval recognized virtually all of the retrieved pictures correctly in the recognition test on Day 3, those participants who learned new pictures after retrieval showed a marked decrease in their memory for those pictures that were retrieved on Day 2 (Fig. 3B).

The percentage of hits for pictures that were not retrieved on Day 2 was significantly above chance level ($M = 77.77$, $SEM = 5.48$), $t(27) = 5.07$, $p < .001$, indicating that pictures that were not retrieved in the free recall test on Day 2 were still remembered in the recognition test on Day 3. An Emotionality (negative, neutral) × New Learning (yes, no) ANOVA on the percentage of pictures that were not retrieved revealed that new learning did not impair memory performance for pictures that were not retrieved, $F(1,26) = 0.02$, $p = .89$, $\eta^2 = .001$ (main effect New Learning): the *retrieval/new learning* group ($M = 76.96$, $SEM = 7.89$) did not differ from *retrieval/no new learning* group ($M = 78.57$, $SEM = 7.89$).

We also performed an ANOVA for recognition performance in which we included the hits for the pictures that were retrieved on Day 2 for the retrieval groups (and all pictures for the no retrieval groups). However, in this ANOVA the Reactivation × Interference interaction did not reach statistical significance ($p > .20$).

2.3. Discussion

Experiment 1. asked whether learning new material after the reactivation of initially learned material alters the subsequent memory for the original material. According to the reconsolidation view, memory reactivation through retrieval renders memories labile again and sensitive to modification through new learning (Nader & Hardt, 2009; Sara, 2000). The results of our first experiment showed that learning new pictures reduced memory performance indeed only when the memory for the previously learned pictures was reactivated before. The memory impairment in the *retrieval/new learning* group remained when we controlled for potential differences in initial consolidation. Moreover, a strong memory impairing effect of new learning was observed only for those memories that were actually retrieved before new learning; new learning did not impair memories that were not retrieved before new learning. The emotionality of the pictures did not affect this pattern of results. In sum, the findings of the first experiment suggest that new learning after the reactivation of previously learned material interferes with the reconsolidation of the original material. The key question of the present study, however, was whether multiple reactivations act as a boundary condition on memory reconsolidation and can thus reduce the influence of reconsolidation manipulations (such as new learning after reactivation) on subsequent memory. We addressed this question in the following two experiments.

3. Experiment 2: does one additional memory reactivation reduce the impairing effect of new learning during reconsolidation?

In the second experiment, we examined if one additional reactivation makes memories less sensitive to new learning after reactivation. For this purpose, participants retrieved the initially learned material once prior to the reconsolidation manipulation.

3.1. Materials and methods

3.1.1. Participants

Fifty-six healthy students of the Ruhr-University Bochum (28 men, 28 women; age: $M = 23.73$ years, $SEM = 0.33$) participated in this experiment. None of the participants had already participated in Experiment 1. Participants received either course credits or a moderate monetary compensation for participation. Exclusion criteria comprised current or chronic mental disorder, drug abuse, and current treatment with medication. All participants provided written informed consent for their participation. This study was approved by a local ethics committee. Seven men and seven women were randomly assigned to each of the four experimental groups (*retrieval/new learning, retrieval/no new learning, no retrieval/new learning, and no retrieval/no new learning*).

3.1.2. Stimulus materials and procedure

Materials were the same as in Experiment 1. As in the previous experiment, participants' ratings indicated that negative pictures (valence: $M = 12.57$, $SEM = 1.15$; arousal: $M = 69.60$, $SEM = 2.24$; $t(55) = -24.32$, $p < .001$) were experienced as significantly more negative and more arousing than neutral pictures (valence: $M = 55.54$, $SEM = 1.08$; arousal: $M = 14.72$, $SEM = 1.31$; $t(55) = 24.59$, $p < .001$).

Day 1, Day 2, and Day 3 were identical to the experimental days in Experiment 1. However, in order to assess the influence of one additional reactivation on the susceptibility of memories to reconsolidation manipulations, all participants retrieved the original memories once between experimental Days 1 and 2 (see Fig. 1B), following the same procedure as described for retrieval on Day 2 (see above). To ensure sufficient consolidation of the initially learned material, the additional retrieval took place at least two (and at max. four) days after Day 1 (i.e., the additional retrieval session could occur between 5 and 3 days before experimental Day 2). The timing of the last additional retrieval relative to Day 1 and Day 2 was counterbalanced across groups.

3.2. Results

3.2.1. Learning performance on Day 1

Participants took on average 1.77 trials ($SEM = 0.06$) to reach the learning criterion (13 correctly recalled pictures) and the four groups did not differ in the number of learning trials, $F(3,52) = 1.44$, $p = .24$, $\eta^2 = .08$ (main effect Group). At the end of Day 1, participants remembered on average 13.12 pictures ($SEM = 0.26$; Table 2) with no significant differences between groups, $F(3,52) = 0.97$, $p = .41$, $\eta^2 = .05$ (main effect Group). Negative pictures were better remembered than neutral pictures, $t(55) = 3.85$, $p < .001$.

3.2.2. Additional memory retrieval

Participants recalled on average 12.09 pictures ($SEM = 0.25$; Table 2) during the additional retrieval between experimental Days 1 and 2, with no differences between the four groups, $F(3,52) = 0.37$, $p = .78$, $\eta^2 = .02$ (main effect Group). Negative pictures were more often retrieved than neutral pictures, $t(55) = 3.95$, $p < .001$.

3.2.3. Memory retrieval and new learning on Day 2

On Day 2, the retrieval groups (*retrieval/new learning, retrieval/no new learning*) recalled on average 12.29 pictures ($SEM = 0.35$; Table 2) without any differences in their retrieval performance, $F(1,26) = 0.65$, $p = .43$, $\eta^2 = .02$ (main effect New Learning).

The new learning groups (*retrieval/new learning, no retrieval/new learning*) took on average 1.75 trials ($SEM = 0.08$) to reach the learning criterion for the second picture set ($M = 13.57$, $SEM = 0.32$; Table 2) and did not differ in their learning performance, $F(1,26) = 0.05$, $p = .83$, $\eta^2 = .002$. Again, negative pictures were better remembered than neutral ones, $t(27) = 4.33$, $p < .001$.

3.2.4. Memory performance on Day 3

3.2.4.1. Memory accuracy. An Emotionality (negative, neutral) × Reactivation (retrieval, no retrieval) × New Learning (yes, no) ANOVA on the recognition accuracy showed no significant effect of new learning after reactivation (Reactivation × New Learning interaction, $F(1,52) = 0.17$, $p = .68$, $\eta^2 = .003$). However, it revealed a significant main effect of new learning, $F(1,52) = 5.07$, $p < .03$, $\eta^2 = .09$: the new learning groups (*retrieval/new learning,* no *retrieval/new learning)* recognized significantly fewer pictures than the no new learning groups (*retrieval/no new learning, no retrieval/no new learning*; Table 2).

3.2.4.2. Percentage of false alarms (intrusions from set 2 and intrusions from set 3). Same as in Experiment 1, the false alarms rate was very low ($M = 1.20$, $SEM = 0.29$), with no difference between intrusions from pictures that have been presented before (set 2; $M = 1.48$, $SEM = 0.47$) and intrusions from pictures that have not been presented before (set 3; $M = 0.69$, $SEM = 0.34$), $t(55) = 1.41$, $p = .17$. Intrusions from set 2 were equally frequent for the groups with ($M = 1.47$, $SEM = 0.57$) and without new learning ($M = 1.34$, $SEM = 0.57$), $F(1,54) = 0.03$, $p = .87$, $\eta^2 < .001$ (main effect New Learning). Moreover, an Emotionality (negative, neutral) × Reactivation (retrieval, no retrieval) × New Learning (yes, no) ANOVA revealed no significant no influence of reactivation or new learning on intrusions from set 2 (main effect Reactivation and main effect New Learning both $Fs(1,52) < 0.03$, $ps > .87$, $\eta^2 < .001$) or intrusions from set 3 (main effect Reactivation and main effect New Learning both $Fs(1,52) < 1.55$, $ps > .22$, $\eta^2 < .03$).

3.2.4.3. Percentage of hits (correctly recognized pictures from set 1). An Emotionality (negative, neutral) × Reactivation (retrieval, no retrieval) × New Learning (yes, no) ANOVA on the percentage of hits showed no significant effect of new learning after reactivation (Reactivation × New Learning interaction, $F(1,52) = 0.99$, $p = .33$, $\eta^2 = .02$). It rather revealed a significant main effect of new learning, $F(1,52) = 5.79$, $p < .02$, $\eta^2 = .10$ (main effect New Learning): the new learning groups recognized significantly fewer pictures from set 1 than the no new learning groups ($M = 93.13$, $SEM = 1.02$ vs. $M = 96.61$, $SEM = 1.02$; Table 2).

3.2.4.4. Hits for retrieved pictures only. An Emotionality (negative, neutral) × New Learning (yes, no) ANOVA for only those pictures that were recalled during retrieval on Day 2 revealed that new learning after retrieval decreased memory performance for the retrieved pictures, $F(1,26) = 3.83$, $p = .06$, $\eta^2 = .13$ (Fig. 4B).

Same as in Experiment 1, the percentage of hits for pictures that were not retrieved on Day 2 was significantly above chance level ($M = 72.47$ $SEM = 6.10$), $t(27) = 3.68$, $p = .001$. An Emotionality (negative, neutral) × New Learning (yes, no) ANOVA revealed that new learning did not impair memory performance for pictures that were not retrieved on Day 2, $F(1,26) = 1.33$, $p = .26$, $\eta^2 = .05$ (main effect New Learning): the *retrieval/new learning* group ($M = 65.47$, $SEM = 8.58$) did not differ from *retrieval/no new learning* group ($M = 79.46$, $SEM = 8.58$).

We also performed an ANOVA for recognition performance in which we included the hits for the pictures that were retrieved on Day 2 for the retrieval groups (and all pictures for the no retrieval groups). In this ANOVA the Reactivation × Interference interaction did also not reach statistical significance ($p > .60$).

3.3. Discussion

Since it has been proposed that memory reactivation may serve to strengthen an existing memory trace (Wang et al., 2009) and that stronger memories are less vulnerable to alterations during reconsolidation (Milekic & Alberini, 2002), Experiment 2 asked if one additional reactivation prior to the reconsolidation manipulation reduces the impairing effect of new learning after reactivation. In this experiment, the critical Reactivation × New Learning interaction was not significant which might lead to the conclusion that the impairing effect of new learning after reactivation disappeared when the initially learned material was reactivated before the reconsolidation manipulation. However, the pattern of results displayed in Fig. 4A questions this interpretation, showing that memory impairment was most pronounced in the *retrieval/new learning*

Table 2
Performance at initial learning on Day 1, the additional memory retrieval, retrieval and/or new learning on Day 2, and recognition testing on Day 3 for Experiment 2.

Group	Day 1	Additional retrieval	Day 2		Day 3	
	Pictures recalled after initial learning	Pictures recalled after retrieval	Pictures recalled after retrieval	Pictures recalled after new learning	Recognition accuracy (%)	Hits (%)
Retrieval/ new learning	13.14 (0.53)	12.52 (0.52)	12.57 (0.50)	13.64 (0.45)	90.93 (1.61)	92.20 (1.40)
Retrieval/ no new learning	13.21 (0.53)	11.86 (0.52)	12.00 (0.50)	–	95.22 (1.61)	97.10 (1.40)
No retrieval/ new learning	13.69 (0.53)	12.14 (0.52)	–	13.50 (0.45)	91.91 (1.61)	94.10 (1.40)
No retrieval/ no new learning	12.43 (0.53)	11.86 (0.52)	–	–	94.87 (1.61)	96.10 (1.40)

Data represent means and standard error of the mean (in parentheses).

group. Moreover, in line with the reconsolidation account, memory performance was reduced for those pictures that were actually retrieved on Day 2 (Fig. 4B) but not for pictures that were not retrieved on Day 2, suggesting that the impairing effects of new learning after reactivation were still present after one additional memory reactivation.

It might well be that the single reactivation before the reconsolidation manipulation was not sufficient to ‘shield’ the memory against modifications during reconsolidation. Therefore, we performed a third experiment in which we assessed the effect of multiple reactivations on memory performance after reconsolidation manipulations.

4. Experiment 3: do multiple memory reactivations reduce the impairing effect of new learning during reconsolidation?

The third experiment examined if multiple reactivations make memories less sensitive to reconsolidation manipulations. To this end, participants retrieved the original memories three times prior to the standard reconsolidation manipulation on Day 2.

4.1. Materials and methods

4.1.1. Participants

Fifty-six healthy students of the Ruhr-University Bochum (28 men, 28 women; age: $M = 23.39$ years, $SEM = 0.31$) participated in this experiment. None of the participants had participated in Experiment 1 or 2. Participants received either course credits or a moderate monetary compensation for participation. Exclusion criteria comprised current or chronic mental disorder, drug abuse, and current treatment with medication. All participants provided written informed consent for their participation. This study was approved by the local ethics committee. Again, seven men and seven women were randomly assigned to each of the four experimental groups (*retrieval/new learning, retrieval/no new learning, no retrieval/new learning, and no retrieval/no new learning*). One participant of the *retrieval/new learning* group had to be excluded because he did not comply with the instructions.

4.1.2. Stimulus materials and procedure

The stimulus materials were exactly the same as in Experiment 1 and Experiment 2. Participants’ ratings verified again that negative pictures were experienced as negative ($M = 16.51$, $SEM = 1.28$) and neutral pictures as neutral ($M = 57.25$, $SEM = 1.40$; $t(55) = -20.28$, $p < .001$), and that negative pictures were significantly more arousing than neutral pictures ($M = 58.24$, $SEM = 2.67$ vs. $M = 11.73$, $SEM = 1.44$; $t(55) = 15.52$, $p < .001$).

The procedure on experimental Days 1–3 was exactly the same as in Experiments 1 and 2: participants learned the first picture set on Day 1, retrieved it and learned the new picture set on Day 2 (depending on the experimental group), and completed a recognition test on Day 3.

In order to assess the impact of multiple reactivations on the effect of new learning after reactivation, participants had three additional retrievals (on separate days) of picture set 1 between Day 1 and Day 2 (see Fig. 1C). To ensure sufficient consolidation of the learned material, the first additional retrieval took place at least two (and at max. four) days after Day 1 (i.e., the last additional retrieval session could occur between 1 and 3 days before experimental Day 2). The second and the third retrieval took place on the 2 days following the first retrieval. The procedure for these additional retrievals was the same as described for the retrieval on Day 2. The timing of the last additional retrieval relative to Day 1 and Day 2 was counterbalanced across groups.

4.2. Results

4.2.1. Learning performance on Day 1

Participants took on average 1.7 trials ($SEM = 0.06$) to reach the learning criterion (13 correctly recalled pictures) and the four groups did not differ in the number of learning trials, $F(3,52) = 0.50$, $p = .50$, $\eta^2 = .04$ (main effect Group). At the end of Day 1, participants remembered on average 13.3 pictures ($SEM = 0.27$; Table 3) with no significant differences between groups, $F(3,52) = 1.55$, $p = .21$, $\eta^2 = .08$ (main effect Group), indicating that groups did not differ in initial memory encoding. Negative pictures were better remembered than neutral pictures, $t(55) = 3.69$, $p < .001$.

4.2.2. Additional memory retrievals

During the three additional retrievals prior to the reconsolidation manipulation on experimental Day 2, participants recalled on average between 12 and 13 pictures without any differences between groups (first retrieval: $M = 12.32$, $SEM = .25$; second retrieval: $M = 12.91$, $SEM = .2$; third retrieval: $M = 13.07$, $SEM = .24$; $Fs(3, 52) < 0.64$, $ps > .43$, $\eta^2 < .03$ [main effect Group]; Table 3). Negative pictures were better recalled than neutral pictures, $ts(55) > 2.53$, $ps < .01$. At the third retrieval, participants remembered significantly more pictures than at the first retrieval, $F(1,52) = 32.37$, $p < .001$, $\eta^2 = .38$, indicating that additional memory retrievals indeed enhanced memory.

4.2.3. Memory retrieval and new learning on Day 2

On Day 2, 1 week after initial learning, the retrieval groups (*retrieval/new learning, retrieval/no new learning*) recalled on average 12.87 pictures ($SEM = 0.35$; Table 3) and did not differ in their retrieval performance, $F(1,26) = 0.71$, $p = .41$, $\eta^2 = .03$ (main effect New Learning).

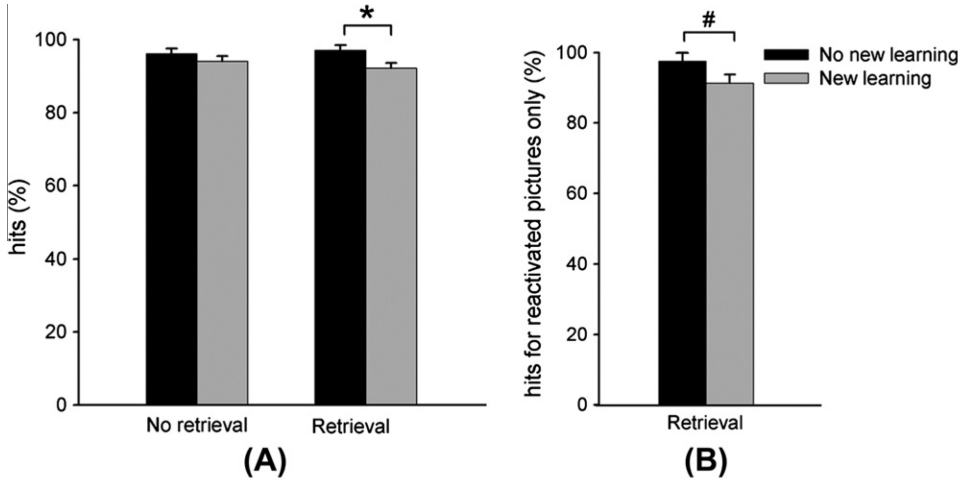

Fig. 4. Impact of new learning after retrieval when the memory was retrieved once before (Exp. 2). (A) Hits (i.e., percentage of correctly recognized pictures from set 1) in the recognition test: the *retrieval/new learning* group recognized significantly fewer pictures than the *retrieval/no new learning* group. (B) Hits for retrieved pictures only: learning new pictures reduced memory performance for the original pictures that were retrieved on Day 2. LSD post hoc tests $^{*}p < .05$, $^{\#}p < .08$; data represent means ± standard errors of the mean.

The new learning groups (*retrieval/new learning, no retrieval/new learning*) took on average 1.7 trials (*SEM* = 0.09) to reach the learning criterion for the second picture set (*M* = 13.54, *SEM* = 0.31; Table 3) and did not differ in their learning performance, $F(1,26) = 0.12$, $p = .74$, $\eta^2 = .004$ (main effect Reactivation). Negative pictures were again better remembered than neutral pictures, $t(27) = 2.70$, $p = .01$.

4.2.4. Memory performance on Day 3

4.2.4.1. Memory accuracy. An Emotionality (negative, neutral) × Reactivation (retrieval, no retrieval) × New Learning (yes, no) ANOVA on the recognition accuracy showed no interaction effect between reactivation and new learning, $F(1,51) = 2.48$, $p = .12$, $\eta^2 = .05$, and no main effect of reactivation, $F(1,51) = 0.07$, $p = .80$, $\eta^2 = .001$. New learning, however, reduced recognition accuracy significantly, $F(1,51) = 7.64$, $p = .008$, $\eta^2 = .13$: whereas participants who did not learn new pictures recognized virtually all of the retrieved pictures correctly (*M* = 95.30, *SEM* = 1.2), those participants who learned new pictures showed a marked decrease in their recognition accuracy (*M* = 90.52, *SEM* = 1.2; Table 3). Moreover, whereas there was no difference between the retrieval groups (*retrieval/new learning* vs. *retrieval/no new learning*, LSD post hoc test, $p = .41$), the *no retrieval/new learning* group recognized fewer pictures than the *no retrieval/no new learning* group (LSD post hoc test, $p = .003$; Fig. 2C).

4.2.4.2. Percentage of false alarms (intrusions from set 2 and intrusions from set 3). Same as in Experiments 1 and 2, the false alarms rate was very low (*M* = 1.40, *SEM* = 0.36), with no difference between intrusions from pictures that have been presented before (set 2; *M* = 0.80, *SEM* = 0.33) and intrusions from pictures that have not been presented before (set 3; *M* = 1.68, *SEM* = 0.49), $t(54) = -1.73$, $p = .09$. Intrusions from set 2 were equally frequent for the groups with (*M* = 0.23, *SEM* = 0.45) and without new learning (*M* = 1.33, *SEM* = 0.44), $F(1,53) = 2.99$, $p = .09$, $\eta^2 = .05$. Moreover, an Emotionality (negative, neutral) × Reactivation (retrieval, no retrieval) × New Learning (yes, no) ANOVA revealed no significant influence of reactivation or new learning on intrusions from set 2 (main effect Reactivation and main effect New Learning both $Fs(1,51) < 2.99$, $ps > .10$, $\eta^2 < .05$) or intrusions from set 3 (main effect Reactivation and main effect New Learning both $Fs(1,51) < 0.72$, $ps > .40$, $\eta^2 < .01$).

4.2.4.3. Percentage of hits (correctly recognized pictures from set 1). An Emotionality (negative, neutral) × Reactivation (retrieval, no retrieval) × New Learning (yes, no) ANOVA on the percentage of hits showed no interaction effect between reactivation and new learning, $F(1,51) = 1.31$, $p = .26$, $\eta^2 = .03$, and no main effect of reactivation, $F(1,51) = 0.39$, $p = .54$, $\eta^2 = .008$. New learning, however, reduced recognition performance significantly, $F(1,51) = 15.23$, $p < .001$, $\eta^2 = .23$ (main effect New Learning): the new learning groups recognized fewer pictures correctly than the no new learning groups (*no retrieval/new learning* vs. *no retrieval/no new learning*, LSD post hoc test, $p = .001$; *retrieval/new learning* vs. *retrieval/no new learning*, LSD post hoc test, $p = .06$; Fig. 5A and Table 3).

4.2.4.4. Hits for retrieved pictures only. Analyzing the impact of new learning on those pictures that were actually retrieved on Day 2, an Emotionality (negative, neutral) × New Learning (yes, no) ANOVA revealed that the *retrieval/new learning* group tended to recognize fewer of the retrieved pictures than the *retrieval/no new learning* group, $F(1,25) = 2.52$, $p = .08$, $\eta^2 = .09$ (Fig. 5B).

Same as in Experiment 1 and Experiment 2, the percentage of hits for pictures that were not retrieved on Day 2 was significantly above chance level (*M* = 74.26, *SEM* = 5.25), $t(27) = 4.62$, $p < .001$. An Emotionality (negative, neutral) × New Learning (yes, no) ANOVA revealed that new learning did not impair memory performance for pictures that were not retrieved on Day 2, $F(1,26) = 2.38$, $p = .14$, $\eta^2 = .08$ (main effect New Learning): the *retrieval/new learning* group (*M* = 66.37, *SEM* = 7.24) did not differ from the *retrieval/no new learning* group (*M* = 82.14, *SEM* = 7.24).

We also performed an ANOVA for recognition performance in which we included the hits for the pictures that were retrieved on Day 2 for the retrieval groups (and all pictures for the no retrieval groups). In this ANOVA the Reactivation × Interference interaction did also not reach statistical significance ($p > .60$).

4.3. Discussion

Our second experiment suggested that the impairing effect of new learning during reconsolidation was not abolished after one additional reactivation. Because it remained possible that a single reactivation before the reconsolidation manipulation was not sufficient to reduce the impairing influence of new learning after reactivation, our third experiment asked whether multiple reactivations make memories less susceptible to reconsolidation manipulations. The results were similar to those of the Experiment 2. The lack of a significant Reactivation × New Learning interaction suggests that the obtained effect of new learning was not reactivation-dependent. However, the finding that memory was reduced in both new learning groups (*retrieval/new learning*, *no retrieval/new learning*; Fig. 5A) might be taken as evidence for an impairing effect of new learning after reactivation: since participants in the *no retrieval/new learning* group had three reactivations before new learning on Day 2, they might have expected another reactivation session and might thus have reactivated the initially learned pictures without any instructions to do so. Another finding speaking in favor of an impairing effect of new learning during reconsolidation is that memory performance was reduced for those pictures that were actually retrieved on Day 2 (Fig. 5B) but not for pictures that were not retrieved on Day 2.

In addition, our third experiment provided direct evidence for the idea that repeated reactivation strengthens memory (Karpicke & Roediger, 2008): participants recalled more pictures at the third retrieval than at the first, although they did not see the pictures again in between.

5. Comparison across experiments

Because the stimulus materials and the experimental procedures on Day 1, Day 2, and Day 3 were exactly the same in the three experiments, we compared the percentage of hits across experiments by means of a three-way ANOVA with the factors Reactivation (retrieval, no retrieval), New Learning (yes, no), and Number of Additional Reactivations (none, one, three). This analysis yielded a significant three-way interaction, $F(2,155) = 3.92$, $p = .02$, $\eta^2 = .05$, indicating that the effect of new learning after memory reactivation depended on the number of memory reactivations: the effect of new learning was reactivation-dependent in the first experiment, when the participants did not retrieve the learned material between Day 1 and Day 2, but not in Experiments 2 and 3, when participants retrieved the learned material at least once before the experimental manipulation on Day 2 (results described above). A direct comparison of the three *retrieval/new learning* groups across experiments, however, showed that their memory performance did not change significantly with additional reactivations, $F(2,38) = 0.20$, $p = .82$, $\eta^2 = .01$ (main effect Number of Additional Reactivations).

As the data suggest an increasing influence of new learning (without prior reactivation) with increasing number of additional reactivations, we directly compared memory performance for the three *no retrieval/new learning* groups across the three experiments. An Emotionality (negative, neutral) × Number of Additional Reactivations (none, one, three) ANOVA revealed that the percentage of hits indeed decreased with additional reactivations in the *no retrieval/no new learning* groups, $F(2,39) = 3.61$, $p = .04$, $\eta^2 = .16$ (main effect Number of Additional Reactivations; no additional reactivation: $M = 96.50$, $SEM = 1.50$; one additional reactivation: $M = 94.10$, $SEM = 1.50$; three additional reactivations: $M = 90.70$, $SEM = 1.50$; LSD post hoc test, three additional reactivations vs. no additional reactivation $p = .01$; other $ps > .13$). Moreover, a Group (no retrieval/new learning, no retrieval/no new learning) × Number of Additional Reactivations (none, one, three) ANOVA on the percentage of hits revealed a strong increasing influence of new learning with additional reactivations, $F(2,78) = 5.64$, $p = .005$, $\eta^2 = .16$ (Group × Number of Additional Reactivations interaction): whereas memory performance in the *no retrieval/no new learning* group stayed constant with additional reactivations (no additional reactivation: $M = 95.70$, $SEM = 1.20$; one additional reactivation: $M = 96.10$, $SEM = 1,20$; three additional reactivations:

Table 3
Performance at initial learning on Day 1, the three additional memory retrievals, retrieval and/or new learning on Day 2, and recognition testing on Day 3 for Experiment 3.

Group	Day 1	1st Additional retrieval	2nd Additional retrieval	3rd Additional retrieval	Day 2		Day 3	
	Pictures recalled after initial learning	Pictures recalled after retrieval	Pictures recalled after retrieval	Pictures recalled after retrieval	Pictures recalled after retrieval	Pictures recalled after new learning	Recognition accuracy (%)	Hits (%)
Retrieval/new learning	12.36 (0.53)	11.64 (0.50)	12.36 (0.50)	12.50 (0.49)	12.57 (0.50)	13.43 (0.45)	92.10 (1.78)	93.30 (1.50)
Retrieval/no new learning	13.55 (0.53)	12.36 (0.50)	13.00 (0.50)	13.36 (0.49)	13.17 (0.50)	–	94.16 (1.71)	97.20 (1.40)
No retrieval/new learning	13.57 (0.53)	12.50 (0.50)	13.14 (0.50)	13.26 (0.49)	–	13.64 (0.45)	88.93 (1.71)	90.70 (1.40)
No retrieval/no new learning	13.83 (0.53)	12.79 (0.50)	13.14 (0.50)	13.14 (0.49)	–	–	96.43 (1.71)	98.00 (1.40)

Data represent means and standard error of the mean (in parentheses).

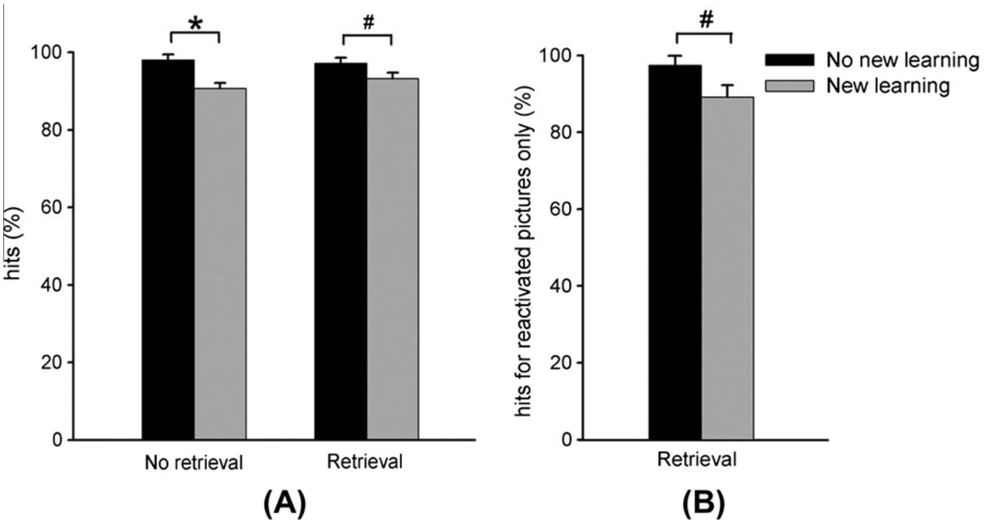

Fig. 5. Impact of new learning after retrieval when the memory was retrieved three times before (Exp. 3). (A) Hits (i.e., percentage of correctly recognized pictures from set 1) in the recognition test: the *no retrieval/new learning* group recognized significantly fewer pictures than the *no retrieval/no new learning* group; the *retrieval/new learning* group tended to recognize fewer pictures than the *retrieval/no new learning* group. (B) Hits for retrieved pictures only: learning new pictures reduced memory performance for the original pictures that were retrieved on Day 2. LSD post hoc tests $^{***}p < .001$, $^{\#}p < .08$; data represent means ± standard errors of the mean.

$M = 98.00$, $SEM = 1.20$), memory performance in the *no retrieval/new learning* group decreased with increasing number of additional reactivations (no additional reactivation: $M = 96.50$, $SEM = 1.20$; one additional reactivation: $M = 94.10$, $SEM = 1{,}20$; three additional reactivations: $M = 90.70$, $SEM = 1.20$; Figs. 3A, 4A, and 5A).

Furthermore, the comparison across experiments suggests that additional memory reactivations enhanced memory. An analysis of the percentage of hits for the *no retrieval/no new learning* groups revealed that participants who reactivated the previously learned pictures three times prior to the reconsolidation manipulation on Day 2 (Experiment 3) recognized significantly more pictures correctly on Day 3 than participants who did not reactivate the learned pictures prior to the reconsolidation manipulation on Day 2 (Experiment 1), $F(1,26) = 5.17$, $p = .03$, $\eta^2 = .17$ (main effect Number of Additional Reactivations; three additional reactivations: $M = 98.00$, $SEM = 0.07$, no additional reactivation: $M = 95.70$, $SEM = 0.07$). Moreover, three prior memory reactivations significantly enhanced retrieval performance on Day 2, $F(1,54) = 4.11$, $p = .05$, $\eta^2 = .07$ (main effect Number of Additional Reactivations; three additional reactivations: $M = 12.87$, $SEM = 0.38$, no additional reactivation: $M = 11.79$, $SEM = 0.38$). These findings corroborate the idea that multiple reactivations strengthen memory (Karpicke & Roediger, 2008). Since repeated reactivations are assumed to enhance memory, we also compared the *retrieval/no new learning* group with the *no retrieval/no new learning* group across experiments. An ANOVA with the factors Emotionality (negative, neutral), Group (no retrieval/no new learning, retrieval/no new learning), and Number of Additional Reactivations (none, one, three) on the percentage of hits, however, revealed no significant effects of Reactivation, Number of Additional Reactivations, or their interaction, $Fs(2, 78) < 1.87$, $ps > .16$, $\eta s^2 < .05$, indicating no significant differences between these two groups with increasing number of reactivations.

6. General discussion

Over the past decade, evidence has been accumulated that memories—when reactivated during retrieval—undergo a process of reconsolidation during which they are modifiable (Brunet et al., 2008; Forcato et al., 2007; Hupbach et al., 2007, 2008; Kindt et al., 2009; Nader et al., 2000; Schiller et al., 2010; Schwabe, Nader, Wolf, Beaudry, & Pruessner, 2012; Schwabe & Wolf, 2009). It has been suggested that reconsolidation's fundamental role is to allow old memories to be updated each time they are reactivated (Hupbach et al., 2007). However, memory reconsolidation does not seem to occur under all conditions. Several boundary conditions have been proposed to constrain memory reconsolidation (e.g., Eisenberg & Dudai, 2004; Forcato, Rodriguez, Pedreira, & Maldonado, 2010; Hupbach et al., 2008; Pedreira et al., 2004; Suzuki et al., 2004). In the present series of experiments, we investigated whether multiple reactivations make memories less susceptible to the effects of new learning after reactivation and thus act as a boundary condition on memory reconsolidation. Our findings indicate that even three additional reactivations are not sufficient to abolish the impairing effect of new learning during reconsolidation completely, suggesting that multiple reactivations are no boundary condition on memory reconsolidation.

Our first experiment showed that new learning can influence the reconsolidation of episodic memories, irrespective of the emotionality of the memories. Learning new pictures impaired the subsequent memory for previously learned pictures only when these memories were reactivated before new learning. This finding is in line with previous studies showing that new learning after the reactivation of previously learned material alters the original memory (Finn & Roediger, 2011; Forcato et al., 2007; Hupbach et al., 2008; Schwabe & Wolf, 2009; Wichert et al., 2011). Importantly, this effect is clearly distinct from a classical retroactive interference effect. In the classical retroactive interference paradigm, learning of the interfering stimulus-pair usually takes place right after learning the original stimulus pair, (i.e., the critical reactivation component is missing in these studies; Hupbach et al., 2007). Moreover, the present experiment included a control group that learned new pictures without prior memory reactivation, and in contrast to the *retrieval/new learning* group, this group showed no memory impairment, thus speaking against a retroactive interference effect and in favor of our reconsolidation interpretation.

Does this impairing effect of new learning during reconsolidation disappear after multiple memory reactivations? It has been assumed that the reactivation of a memory trace gives rise to an expanded and thus strengthened memory trace (Nadel & Moscovitch, 1997), and recent evidence indeed indicates that memory retrieval strengthens memory (Karpicke & Roediger, 2008). Because memory strength may decrease the susceptibility of memories to modifications during reconsolidation (Wang et al., 2009; Winters et al., 2009), we hypothesized that multiple reactivations would result in strengthened memories that are less vulnerable to reconsolidation manipulations. We found that additional reactivations enhanced retrieval performance, indicating that repeated reactivations indeed strengthened participants' memory for the initially learned pictures. However, these additional reactivations did not abolish the impairing effect of new learning after reactivation: relative to the group that did not learn new pictures after reactivation (*retrieval/no new learning* group), the *retrieval/new learning* group was impaired after one and even after three additional reactivations. Moreover, the effect of new learning after reactivation was still observed for those pictures that were actually retrieved during reactivation, giving further evidence for the impairing effects of new learning during reconsolidation when the original memory was reactivated additional times before.

The critical Reactivation × New Learning interaction, however, disappeared both after one additional reactivation (Experiment 2) and after three additional reactivations (Experiment 3) which might be taken as evidence against impairing effects of new learning during reconsolidation. Our pattern of results suggests that the absence of the interaction effect in Experiments 2 and 3 is due to the fact that the group that learned new pictures without prior reactivation of the initially learned pictures (*no retrieval/new learning* group) showed—same as the *retrieval/new learning* group—impaired memory performance. One likely explanation for this finding takes into account that participants who had already completed one or three reactivation sessions before experimental Day 2 may have expected another reactivation session for experimental Day 2 and (implicitly) reactivated the memory for the initially learned pictures, even without the instruction to do so. Spontaneous memory reactivations caused by participants' expectations can hardly be ruled out in experiments in which participants retrieve memories repeatedly; and at the end of the experiment, several participants indicated indeed that they expected another retrieval session on Day 2. It seems justified to assume that participants' expectations for another reactivation session would be higher after three than after one (additional) reactivation. In line with this prediction, the *no retrieval/new learning* group showed impaired memory after three additional reactivations when the expectation for another reactivation was high, but not yet after one additional reactivation when the expectation for another reactivation was rather low. If participants in the *no retrieval/new learning* group reactivated the original material on Day 2 implicitly, the absence of a Reactivation × New Learning interaction does not argue against an impairing effect of new learning after reactivation. Instead, the impairment seen in this group provides even further evidence that multiple reactivations did not prevent memory impairments by new learning during reconsolidation.

Another explanation for the finding that the critical Reactivation × New Learning interaction disappeared both after one as well as after three additional reactivations is that three prior reactivations enhanced memory performance on Day 2 but that the final memory performance of the *retrieval/no new learning* group did not differ from the *no retrieval/no new learning* group across experiments, indicating that memory retrieval on Day 2 had no additive effect when the memory has been retrieved additional times before. This explanation fits with the finding that memory performance (i.e., correctly identified pictures from Day 1) was rather high already after at least one reactivation (no additional reactivation: *retrieval/no new learning* group 97.70%; one additional reactivation: *no retrieval/no new learning* group 96.10%; three additional reactivations: *no retrieval/no new learning* group 98.00%), not leaving room for further memory enhancing effects of reactivation on Day 2. Although reactivation alone did not have additive memory enhancing effects in Experiments 2 and 3, this does not rule out impairing effects of new learning after reactivation: as described above, the impairing influence of new learning after (implicit) reactivation was still present when the memory was additionally reactivated once or three times before.

We aimed to avoid unintended reactivations of the initially learned pictures (Hupbach et al., 2008) in two ways. First, new learning without retrieval took place in a different spatial context, whereas new learning after retrieval took place in the same spatial context as in the learning session on Day 1. However, this might have had the consequence that the recognition test on Day 3 was a source discrimination test for those participants who learned new pictures. The decision whether a picture has been shown on Day 1 or not was solely based on temporal information in the *retrieval/new learning* group, but on additional spatial information in the *no retrieval/new learning* group. According to the source monitoring framework (Johnson, Hashtroudi, & Lindsay, 1993) and based on earlier work on memory reconsolidation (Hupbach et al., 2007, 2009), one would thus expect to see higher false alarm rates in the *retrieval/new learning* group in the final memory test. In our study, however, false alarms were virtually absent in both new learning groups. Instead, new learning after reactivation reduced the correct identification of initially learned pictures (described below).

Second, we decided against a short-term memory test for the pictures from Day 1 after reactivation and/or new learning on Day 2. The reconsolidation account holds that the reconsolidation process needs time to be accomplished and that the effects of new learning after reactivation are seen in long-term but not in short-term memory (Nader et al., 2000). In episodic memory, however, a short-term memory test for the initially learned pictures is rather problematic because both a free recall test and a recognition test would represent a reactivation for all experimental groups. Moreover, in a recognition test, new pictures are presented which would be a reconsolidation manipulation on its own. Thus, a short-term memory test on Day 2 would have most likely interfered with the intended reconsolidation manipulations.

Previous studies on the reconsolidation of episodic memories reported that new learning after reactivation leads to the incorporation of new information into the old memory: when asked to recall the initially learned material, participants incorrectly reported some of the material that was learned after memory reactivation (Forcato et al., 2010; Hupbach et al., 2007, 2009). In the present experiments, such intrusions were virtually absent; false alarm rates were very low. Instead, we observed impaired memory for the initially learned material in participants who learned new information after retrieval of the older memory, which is in line with the results of other studies (Schwabe & Wolf, 2009; Strange, Kroes, Fan, & Dolan, 2010). Methodological differences between studies might explain these differences. For example, in the present study, we assessed memory in a recognition test, whereas other studies used cued or free recall tests (Forcato et al., 2007, 2010; Hupbach et al., 2007, 2008). Different memory tests are associated with different memory demands and different memory performance (Adlam, Malloy, Mishkin, & Vargha-Khadem, 2009; Cabeza et al., 1997). Furthermore, whereas the time intervals between initial learning and reactivation/new learning on the one hand, and reactivation/new learning and memory testing on the other hand was the same in earlier studies (Hupbach et al., 2007, 2008), these intervals differed in the present study. Since temporal information might be a cue to differentiate between two sets of learned material (Hupbach et al., 2009; Sederberg, Gershman, Polyn, & Norman, 2011), the differences in temporal context might also explain the observed differences in memory performance.

Reconsolidation manipulations may provide an opportunity to change unwanted memories in psychiatric disorders such as PTSD (Brunet et al., 2008) which is characterized by frequent and recurrent reactivations of a traumatic event. If these repeated reactivations of the traumatic event render participants' memories resistant to manipulations during reconsolidation, this would have major consequences for the chances to use a reconsolidation-based treatment approach in PTSD. The findings of the present study indicate that modifying effects of new learning after reactivation endure three prior reactivations, suggesting that a moderate number of reactivations may not impair reconsolidation-based treatment. However, patients with PTSD suffer from frequent trauma memory reactivations and we cannot rule out that these memories become stronger and ultimately resistant to modifications during reconsolidation after numerous reactivations.

Taken together, our study investigated whether multiple memory reactivations preserve a memory from being affected by modification during reconsolidation. Our findings show that neither one nor three additional reactivations abolish the impairing effects of new learning after reactivation completely. Accordingly, the opportunity for memory changes during reconsolidation seems to endure at least a moderate number of reactivations, suggesting that multiple reactivations do not necessarily serve as boundary condition on reconsolidation in episodic memory. Whether more frequently reactivated memories become immune to modifications during reconsolidation remains to be investigated in future research.

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References

Adlam, A.-L. R., Malloy, M., Mishkin, M., & Vargha-Khadem, F. (2009). Dissociation between recognition and recall in developmental amnesia. *Neuropsychologia, 47*, 2207–2210.

Brunet, A., Orr, S. P., Tremblay, J., Robertson, K., Nader, K., & Pitman, R. K. (2008). Effect of post-retrieval propranolol on psychophysiologic responding during subsequent script-driven traumatic imagery in post-traumatic stress disorder. *Journal of Psychiatric Research, 42*, 503–506.

Cabeza, R., Kapur, S., Craik, F. I. M., McIntosh, A. R., Houle, S., & Tulving, E. (1997). Functional neuroanatomy of recall and recognition: A PET study of episodic memory. *Journal of Cognitive Neuroscience, 9*, 254–265.

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

Dudai, Y. (2004). The neurobiology of consolidation, or, how stable is the engram? *Annual Review of Psychology, 55*, 51–86.

Dudai, Y. (2006). Reconsolidation: The advantage of being refocused. *Current Opinion in Neurobiology, 16*, 174–178.

Eisenberg, M., & Dudai, Y. (2004). Reconsolidation of fresh, remote, and extinguished fear memory in madeka: Old fears don't die. *European Journal of Neuroscience, 20*, 3397–3403.

Finn, B., & Roediger, H. L. (2011). Enhancing retention through reconsolidation: Negative emotional arousal following retrieval enhances later recall. *Psychological Science, 22*, 781–786.

Forcato, C., Burgos, V. L., Argibay, P. F., Molina, V. A., Pedreira, M. E., & Maldonado, H. (2007). Reconsolidation of declarative memory in humans. *Learning & Memory, 14*, 295–303.

Forcato, C., Rodriguez, M. L. C., & Pedreira, M. E. (2011). Repeated labilization–reconsolidation processes strengthen declarative memory in humans. *PLoS ONE, 6*, e23305.

Forcato, C., Rodriguez, M. L. C., Pedreira, M. E., & Maldonado, H. (2010). Reconsolidation in humans opens up declarative memory to the entrance of new information. *Neurobiology of Learning and Memory, 93*, 77–84.

Hardt, O., Einarsson, E. Ö., & Nader, K. (2010). A bridge over troubled water: Reconsolidation as a link between cognitive and neuroscientific memory research traditions. *Annual Review of Psychology, 61*, 141–167.

Hupbach, A., Gomez, R., Hardt, O., & Nadel, L. (2007). Reconsolidation of episodic memories: A subtle reminder triggers integration of new information. *Learning & Memory, 14*, 47–53.

Hupbach, A., Gomez, R., & Nadel, L. (2009). Episodic memory reconsolidation: Updating or source confusion? *Memory, 15*, 502–510.

Hupbach, A., Hardt, O., Gomez, R., & Nadel, L. (2008). The dynamics of memory: Context-dependent updating. *Learning & Memory, 15*, 574–579.

Johnson, M. K., Hashtroudi, S., & Lindsay, D. S. (1993). Source monitoring. *Psychological Bulletin, 114*, 3–28.

Karpicke, J. D., & Roediger, H. K. (2008). The critical importance of retrieval for learning. *Science, 319*, 966–968.

Kindt, M., Soeter, M., & Vervliet, B. (2009). Beyond extinction: Erasing human fear responses and preventing the return of fear. *Nature Neuroscience, 12*, 256–258.

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (2008). *International affective picture system (IAPS): Affective ratings of pictures and instruction manual. Technical report A-8*. Gainesville, FL: University of Florida.

Lee, J. L. C., Everitt, B. J., & Thomas, K. L. (2004). Independent cellular processes for hippocampal memory consolidation and reconsolidation. *Science, 304*, 839–843.

Lehmann, H., & McNamara, K. C. (2011). Repeatedly reactivated memories become more resistant to hippocampal damage. *Learning & Memory, 18*, 132–135.

McGaugh, J. L. (2000). Memory – A century of consolidation. *Science, 287*, 248–251.

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

Nadel, L., & Moscovitch, M. (1997). Memory consolidation, retrograde amnesia and the hippocampal complex. *Current Opinion in Neurobiology, 7*, 217–227.

Nader, K., & Hardt, O. (2009). A single standard for memory: The case for reconsolidation. *Nature Reviews Neuroscience, 10*, 224–234.

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

Pedreira, M. E., & Maldonado, H. (2003). Protein synthesis subserves reconsolidation or extinction depending on reminder duration. *Neuron, 38*, 863–869.

Pedreira, M. E., Pérez-Cuesta, L. M., & Maldonado, H. (2004). Mismatch between what is expected and what actually occurs triggers memory reconsolidation or extinction. *Learning & Memory, 11*, 579–585.

Przybyslawski, J., Roullet, P., & Sara, S. J. (1999). Attenuation of emotional and nonemotional memories after their reactivation: Role of β adrenergic receptors. *Journal of Neuroscience, 19*, 6623–6628.

Rasch, B., Büchel, C., Gais, S., & Born, J. (2007). Odor cues during slow-wave sleep prompt declarative memory consolidation. *Science, 315*, 1426–1429.

Rudoy, J. D., Voss, J. L., Westerberg, C. E., & Paller, K. A. (2009). Strengthening individual memories by reactivating them during sleep. *Science, 326*, 1079.

Sara, S. J. (2000). Retrieval and reconsolidation: Toward a neurobiology of remembering. *Learning & Memory, 7*, 73–84.

Schiller, D., Monfils, M.-H., Raio, C. M., Johnson, D. C., LeDoux, J. E., & Phelps, E. A. (2010). Preventing the return of fear in humans using reconsolidation update mechanisms. *Nature, 463*, 49–53.

Schwabe, L., Nader, K., Wolf, O. T., Beaudry, T., & Pruessner, J. C. (2012). Neural signature of reconsolidation impairments by propranolol in humans. *Biological Psychiatry, 71*, 380–386.

Schwabe, L., & Wolf, O. T. (2009). New episodic learning interferes with the reconsolidation of autobiographical memories. *PLoS ONE, 4*, e7519.

Sederberg, P. B., Gershman, S. J., Polyn, S. M., & Norman, K. A. (2011). Human memory reconsolidation can be explained using the temporal context model. *Psychonomic Bulletin & Review, 18*, 455–468.

Soeter, M., & Kindt, M. (2011). Disrupting reconsolidation: Pharmacological and behavioral manipulations. *Learning & Memory, 18*, 357–366.

Strange, B. A., Kroes, M. C. W., Fan, J. E., & Dolan, R. J. (2010). Emotion causes targeted forgetting of established memories. *Frontiers in Behavioral Neuroscience, 4*, 175.

Suzuki, A., Josselyn, S. A., Frankland, P. W., Masushige, S., Silva, A. J., & Kida, S. (2004). Memory reconsolidation and extinction have distinct temporal and biochemical signatures. *The Journal of Neuroscience, 24*, 4787–4795.

Wang, S.-H., de Oliveira Alvares, L., & Nader, K. (2009). Cellular and systems mechanisms of memory strength as a constraint on auditory fear reconsolidation. *Nature Neuroscience, 12*, 905–913.

Wichert, S., Wolf, O. T., & Schwabe, L. (2011). Reactivation, interference, and reconsolidation: Are recent and remote memories likewise susceptible? *Behavioral Neuroscience, 125*, 699–704.

Winters, B., Tucci, M., & DaCosta-Furtado, M. (2009). Older and stronger object memories are selectively destabilized by reactivation in the presence of new information. *Learning & Memory, 16*, 545–553.