



## Original Articles

# Cue-independent memory impairment by reactivation-coupled interference in human declarative memory



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## ABSTRACT

Memory is a dynamic process. While memory becomes increasingly resistant to interference after consolidation, a brief reactivation renders it unstable again. Previous studies have shown that interference, when applied upon reactivation, impairs the consolidated memory, presumably by disrupting the reconsolidation of the memory. However, attempts have failed in disrupting human declarative memory, raising a question about whether declarative memory becomes unstable upon reactivation. Here, we used a double-cue/one-target paradigm, which associated the same target with two different cues in initial memory formation. Only one cue/target association was later reactivated and treated with behavioral interference. Our results showed, for the first time, that reactivation-coupled interference caused cue-independent memory impairment that generalized to other cues associated with the memory. Critically, such memory impairment appeared immediately after interference, before the reconsolidation process was completed, suggesting that common manipulations of reactivation-coupled interference procedures might disrupt other processes in addition to the reconsolidation process in human declarative memory.

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## 1. Introduction

Memories are not consolidated once and forever (Nader & Hardt, 2009). Upon retrieval, a consolidated memory can re-enter a transiently labile state, which is susceptible to interference, and therefore requires a reconsolidation process to be preserved (Nader, 2003; Nader, Schafe, & LeDoux, 2000; Sara, 2000). Reactivation, which can be induced by a brief retrieval, opens a recurrent window of vulnerability for old memories to be updated or eliminated. There are three particularly relevant examples: (1) application of amnesic treatments such as protein synthesis inhibitors (Duvvarci, Nader, & LeDoux, 2005; Graeff et al., 2014; Morris et al., 2006; Nader et al., 2000; Rossato et al., 2007; Suzuki et al., 2004) and electroconvulsive shock (Kroes et al., 2014; Lewis &

Bergman, 1973; Lewis, Mahan, & Bregman, 1972; Misanin, Miller, & Lewis, 1968) shortly after reactivation damages memory retrieval; (2) extinction training on reactivated conditioning memory prevents the conditioned response from returning (Agren et al., 2012; Clem & Huganir, 2010; Liu et al., 2014; Monfils, Cowansage, Klann, & LeDoux, 2009; Schiller, Kanen, LeDoux, Monfils, & Phelps, 2013; Schiller et al., 2010; Xue et al., 2012); (3) relearning a new temporal or spatial series during reconsolidation degrades procedural memory performance (Diekelmann, Büchel, Born, & Rasch, 2011; Walker, Brakefield, Hobson, & Stickgold, 2003). In such cases, reactivation and the ensuing reconsolidation process serve as an adaptive update mechanism for consolidated memories (Besnard, Caboche, & Laroche, 2012; De Oliveira et al., 2013; Lee, 2008, 2009; Nader & Hardt, 2009; Tronson & Taylor, 2007).

Evidence for disruption of memories upon reactivation has accumulated rapidly across animal models in the last decade (Besnard et al., 2012; Nader & Einarsson, 2010; Tronson & Taylor, 2007), mostly using pharmacological compounds to block the memory's reconsolidation process. However, the invasive nature

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of pharmacological blockade renders it inappropriate for human studies (Schiller & Phelps, 2011). As a consequence, the behavioral interference procedure, a non-invasive technique, has been developed. The first attempt, introducing a new motor sequence to participants after retesting on an old motor sequence, produced severe impairment on old sequential performance (Walker et al., 2003). Likewise, extinction training during reactivation prevented the return of fear response for fear conditioning memory, as compared to the non-reactivation condition (Schiller et al., 2010; Xue et al., 2012). Similar to these studies, applications of behavioral interference to disrupt consolidated memories have been consistently established across various memory types (Besnard et al., 2012; Nader & Einarsson, 2010; Schiller & Phelps, 2011; Tronson & Taylor, 2007).

However, the effect of the behavioral interference procedure on declarative memory remains elusive. On the one hand, reactivation coupled interference manipulations elicit various forms of memory changes, ranging from weakening the reactivated memory (Chan & LaPaglia, 2013; Forcato, Argibay, Pedreira, & Maldonado, 2009; Forcato, Rodríguez, Pedreira, & Maldonado, 2010; Forcato et al., 2007), strengthening the reactivated memory (LaPaglia & Chan, 2013; Pashler, Kang, & Mozer, 2013; Potts & Shanks, 2012), or updating the memory with new information (Gershman, Schapiro, Hupbach, & Norman, 2013; Hupbach, Gomez, Hardt, & Nadel, 2007; Hupbach, Gomez, & Nadel, 2009; Hupbach, Hardt, Gomez, & Nadel, 2008; Wichert, Wolf, & Schwabe, 2013), which raises a question about whether there is a consistent role of reactivation in human declarative memory. On the other hand, the specific role of behavioral interference on reconsolidation is unknown. Pharmacological interference only shows its effects after, but not before, the reconsolidation process is completed, suggesting that this interference interrupts the reconsolidation process. In contrast, behavioral interference coupled with reactivation causes memory impairment both after and before the reconsolidation process is completed. Therefore, it is still unknown whether the unique effect of reactivation-coupled behavioral interference in the post-reconsolidation test only emerges after the reconsolidation process is completed.

Here, we investigated the effect of reactivation-coupled interference on declarative memory in humans. A double-cue/one-target paradigm was employed in which two different cues were paired with one target in initial memory formation, but only one cue/target association was later reactivated and treated with behavioral interference. Results showed that reactivation rendered declarative memory unstable, which was manifest by cue-independent memory impairment that was not restricted to the directly interfered cue/target association. Furthermore, this effect was found immediately after interference, before the completion of reconsolidation, suggesting that common manipulations of behavioral interference coupled with reactivation do not necessarily disrupt the reconsolidation process for human declarative memory.

## 2. Experiment 1: impairment of human declarative memory by reactivation-coupled interference

### 2.1. Method

#### 2.1.1. Participants and materials

Forty-four participants (aged 17–31 years, 29 females) were recruited from Peking University, Beijing, China. They were all native Chinese speakers with normal reading and comprehension ability. The memory task was composed of 152 frequency-balanced two-character Chinese words; these words were randomly selected from a large Chinese word database.

#### 2.1.2. Procedure

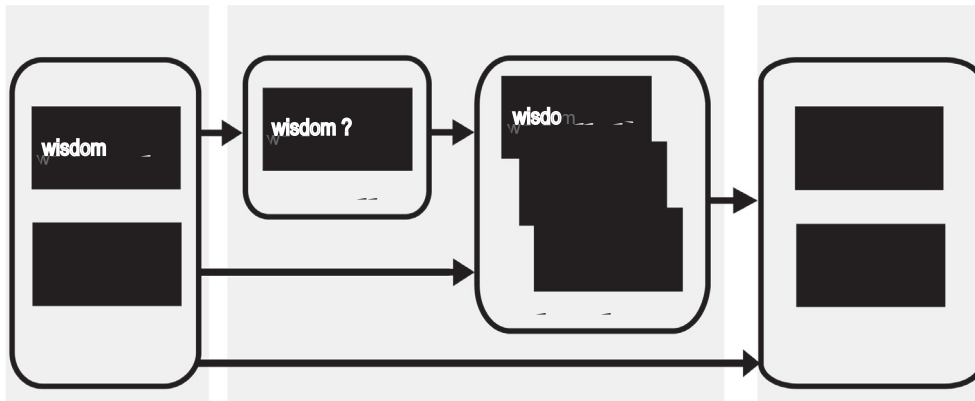
Association learning was given on Day 1, in which 48 Chinese word pairs (e.g., *wisdom-plane*) were displayed on the computer screen sequentially, each for 3 s. Each target word was paired with two different cue words (e.g. *wisdom-plane* and *gardener-plane*), thus participants learned two series, composed of 24 word pairs each, in the form of A-X and B-X. After initial learning, participants completed a self-test with corrective feedback. Afterwards, they completed a 5 min arithmetic task as a distraction, and then took a test without feedback. The self-test phase repeated until participants reached 100% accuracy.

Forty-eight hours later, on Day 3, interference training was conducted only on A-X pairs; accordingly, cue A was referred to as the trained cue and cue B as the independent cue. A-X pairs were divided into three subsets: one subset received reactivation-coupled interference (R-interference), one subset received interference without reactivation (NR-interference), and the remaining subset served as the baseline control. Memory reactivation was conducted using an old/new recognition test, during which one cue A word for R-interference or one lure word was shown alone on the screen for 2 s, and participants judged whether they had seen it in the previous learning session. The old/new recognition test included eight lure words that had not been learned before. Immediately after judgment, the cue A words and the lure words underwent interference manipulation. Interference was conducted by pairing each cue with a series of three new words appearing on the right side successively for three times, each for 3 s, and participants were asked to memorize these new associations. For NR-Interference condition, interference was given directly without reactivation. Interference training repeated three times. The order for R-interference and NR-interference was counterbalanced within participants. The three subsets of A-X pairs were counterbalanced between participants for different conditions.

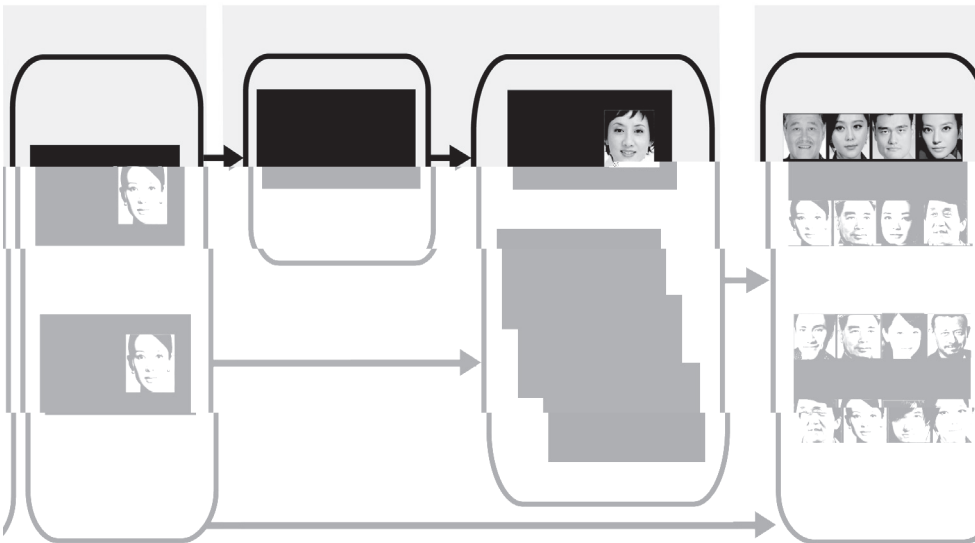
A recall test was given 24 h later (Day 4) in which all the 48 cue words were presented consecutively on the screen. Participants typed the corresponding target words originally paired in the learning session into the computer. The order of testing for the 6 groups was randomized within participants.

### 2.2. Results and discussion

Memory impairment was calculated by subtracting the recall accuracies in the control conditions from those in the corresponding experimental conditions (Fig. 1A). Because no significant difference was found in memory performance between the control condition of trained-cue retrieval and that of independent-cue retrieval in the ensuing experiments ( $p > 0.05$ ), the recall accuracies in both control conditions were averaged and then used as a baseline for subtraction in the following analysis. Memory impairment was examined by a 2 (cue type: trained cue vs. independent cue)  $\times$  2 (interference type: reactivation-coupled interference (R-interference) vs. non-reactivation-coupled interference (NR-interference)) repeated measures ANOVA. Results (Fig. 2B) showed that, neither the main effect of cue type ( $F(1,43) = 3.27$ ,  $p > 0.05$ ,  $MSE = 0.03$ ,  $\eta_p^2 = 0.07$ ) nor that of interference type ( $F(1,43) = 0.53$ ,  $p > 0.05$ ,  $MSE = 0.03$ ,  $\eta_p^2 = 0.01$ ) were significant, but their interaction effect was significant ( $F(1,43) = 4.43$ ,  $p < 0.05$ ,  $MSE = 0.02$ ,  $\eta_p^2 = 0.09$ ). Investigations on the simple effects showed that, contrary to the usual view (Chan & LaPaglia, 2013), R-interference did not result in more severe memory impairments than NR-interference ( $t(43) = 0.75$ ,  $p > 0.05$ ) for trained-cue retrieval. In contrast, more memory impairment of the target words was found in the R-interference condition compared to the NR-interference condition for independent-cue retrieval ( $t(43) = -2.24$ ,  $p < 0.05$ ), indicating that memory impairment by R-interference was not restricted to the cues directly interfered.



B



These findings demonstrate that declarative memory becomes susceptible to interference upon reactivation. However, the effect is not manifest by more impairment in the R-interference condition than in the NR-interference condition. Instead, our results suggest that R-interference of one cue/target association also disrupts retrieval of the target by other cues, resulting in a cue-independent memory impairment. In contrast, NR-interference only blocks the retrieval of the specific cue/target association, which is in line with previous evidence (Anderson & Green, 2001).

### 3. Experiment 2: the immediate effect of reactivation-coupled interference on declarative memory before the completion of reconsolidation

Memory reconsolidation is a time-dependent process, which begins soon after reactivation and lasts at least an hour (Nader & Einarsson, 2010; Schiller & Phelps, 2011; Tronson & Taylor, 2007). In animal studies, pharmacological interference impairs reactivated memory after, but not before, the reconsolidation process is completed, suggesting that this interference disrupts the reconsolidation process (Nader et al., 2000). Likewise, unique memory impairment is caused by reactivation-coupled behavioral interference (R-interference) but not by behavioral interference without reactivation (NR-interference) after the reconsolidation process is completed (Monfils et al., 2009). However, in the immediate test, both R-interference and NR-interference cause memory impairment (e.g. Agren et al., 2012; Liu et al., 2014; Schiller et al., 2010). In such cases, it is possible that the lack of a difference in the immediate test is due to the low sensitivity of the traditional single-cue/one-target procedure. The double-cue paradigm might serve as a more sensitive tool, as it tests the memory change through both the trained cue and the independent cue. Next, we test the memory performance before reconsolidation is completed using the double-cue paradigm.

#### 3.1. Experiment 2.1

##### 3.1.1. Method

**3.1.1.1. Participants and materials.** Forty-two participants (aged 18–27 years, 28 females) were recruited from Peking University, Beijing, China. They were all native Chinese speakers with normal reading and comprehension abilities. The materials were identical to those used in Exp. 1.

**3.1.1.2. Procedure.** We used the procedure from Exp. 1 with the exceptions that (1) the retention interval between the learning and the interference training session was 24 h; (2) the interference training and the test session were conducted on the same day with a 5 min interval, within which participants completed over 20 arithmetic questions as a distraction (Fig. 3A).

##### 3.1.2. Results and discussion

Similar results (Fig. 3B) were found as those in Exp. 1. The interaction effect of cue type and interference type was significant ( $F(1,41) = 8.40$ ,  $p < 0.01$ ,  $MSE = 0.02$ ,  $\eta_p^2 = 0.17$ ). Follow-up tests showed that, in comparison with NR-interference, R-interference caused less memory impairment ( $t(41) = 2.25$ ,  $p < 0.05$ ) for trained-cue retrieval and more memory impairment for independent-cue retrieval ( $t(41) = -2.18$ ,  $p < 0.05$ ). Therefore, reactivation-coupled interference caused cue-independent forgetting even in the immediate test, not requiring the reconsolidation process to be completed.

#### 3.2. Experiment 2.2a and 2.2b

To further test whether the immediate effect of interference after reactivation is independent of test types and materials, we repeated Exp. 2.1 with another test type, recognition tests. This allowed us to examine whether the cue-independent memory impairment by R-interference was test-independent.

##### 3.2.1. Method

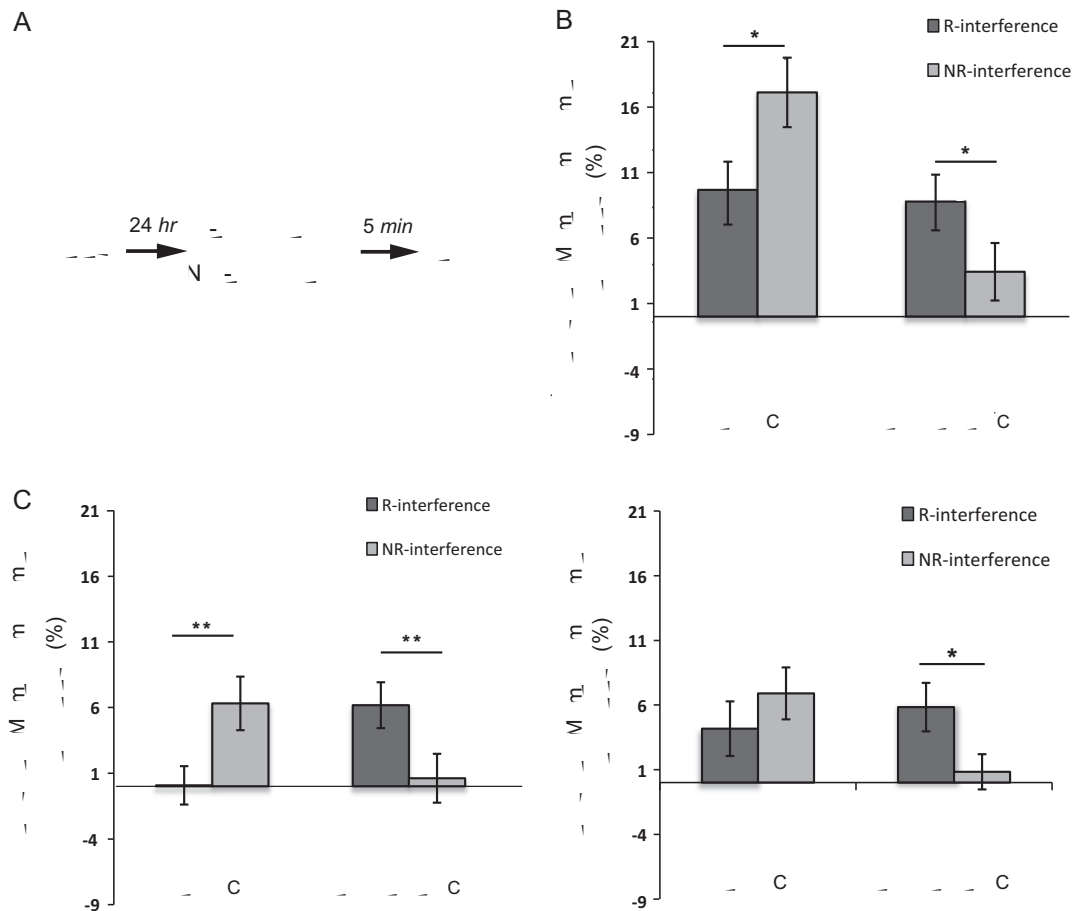
**3.2.1.1. Participants and materials.** Forty-eight (aged 17–28 years, 31 females) and forty-four (aged 18–25 years, 25 females) students from Peking University, Beijing, China, attended Exp. 2.2a and Exp. 2.2b respectively. They were native Chinese speakers with normal reading and comprehension ability. Words used in the experiments were all frequency-balanced two-character Chinese words. Pictures of faces of well-known celebrities were used. The pictures were black and white and were set to a standard size with the same pixel quality.

**3.2.1.2. Procedure of Exp. 2.2a.** We used the procedure from Exp. 2.1, with the following exceptions. On Day 1, participants learned 90 word-face pairs (45 A-X and 45 B-X pairs). Each pair was presented for 3 s and repeated for four runs. Twenty-four hours later, on Day 2, interference training was conducted on two A-X subsets in four runs. The training procedure was similar to that of Exp. 1 with three exceptions: (1) each cue was paired with a new picture of a face as the interfering target; (2) the new cue-target pairs repeated once in each run; (3) no lure words were included. Memory for all the originally learned pairs were tested by a recognition test, in which every cue was displayed at the center of the screen and participants were asked to choose the correct target face out of the 8 faces. To avoid participants making judgments based on familiarity with the target, the 7 alternative choices were randomly selected from the other target items learned on Day 1.

**3.2.1.3. Procedure of Exp. 2.2b.** The experimental procedure of Exp. 2.2b was the same as that of Exp. 2.2a, with the exception that Exp. 2.2b employed the Exp. 1 interference training procedure, in which each cue was paired with 3 substitute words successively, and the interference was repeated 3 times in each run for 3 runs.

##### 3.2.2. Results and discussion

Similar results were found for the two experiments (Fig. 3C and D). Significant interaction effects (Exp. 2.2a,  $F(1,47) = 27.17$ ,  $p < 0.001$ ,  $MSE = 0.01$ ,  $\eta_p^2 = 0.37$ ; Exp. 2.2b,  $F(1,43) = 7.02$ ,  $p < 0.05$ ,  $MSE = 0.01$ ,  $\eta_p^2 = 0.14$ ) but no main effects ( $p > 0.05$ ) were found for the two factors, cue type and interference type. For the trained-cue retrieval, R-interference resulted in comparable (Exp. 2.2b,  $t(43) = 1.21$ ,  $p > 0.05$ ) or even less (Exp. 2.2a,  $t(47) = 2.79$ ,  $p < 0.01$ ) memory impairment than NR-interference. The reverse was true for independent-cue retrieval, in which R-interference consistently caused more memory impairment than NR-interference (Exp. 2.2a,  $t(47) = -3.15$ ,  $p < 0.01$ ; Exp. 2.2b,  $t(43) = -2.42$ ,  $p < 0.05$ ). These results further confirmed that R-interference did not cause more severe memory impairment than NR-interference for the directly interfered declarative memory, but caused memory impairment that generalized to independent cues. More importantly, here we established that memory impairment occurred immediately after behavioral interference, contradicting the time-restricted effect in previous studies (Schiller & Phelps, 2011; Schiller et al., 2010). Given that the unique memory impairment of R-interference was found before reconsolidation process completed, this suggests that interference with behavioral means may disrupt some other processes in addition to the reconsolidation process of declarative memory.



**Fig. 3.** (A) Timeline for Exp. 2.1, Exp. 2.2a, and Exp. 2.2b. Percentages of memory impaired under different conditions relative to the baseline condition are shown in (B) (Exp. 2.1), (C) (Exp. 2.2a), and (D) (Exp. 2.2b). Generalized memory impairment under R-interference was found immediately after interference training in (B)–(D). For independent-cue retrieval, R-interference led to significantly greater memory impairment than NR-interference in all the figures. \*  $p < 0.05$ , \*\*\*  $p < 0.001$  (two-tailed  $t$  test); error bars, SEM.

#### 4. Experiment 3a and 3b: cue-independent memory impairment not caused by memory reactivation

Two control experiments were further conducted to exclude the possibility that the manipulation of reactivation itself accounts for the memory impairment under independent-cue retrieval. Cues that underwent R-interference in previous experiments now underwent reactivation without interference. Two experiments were conducted (Fig. 4A and B): Exp. 3a used word-word pairs with a post-reconsolidation test; Exp. 3b used word-face pairs with an immediate test.

##### 4.1. Method

###### 4.1.1. Participants and materials

Forty-two (aged 17–24 years, 28 females) and fifty-two (aged 17–30 years, 35 females) participants from Peking University, Beijing, China, attended Exp. 3a and Exp. 3b respectively. They were all native Chinese speakers with normal reading and comprehension abilities. The critical task included frequency-balanced two-character Chinese words and pictures of faces of well-known celebrities.

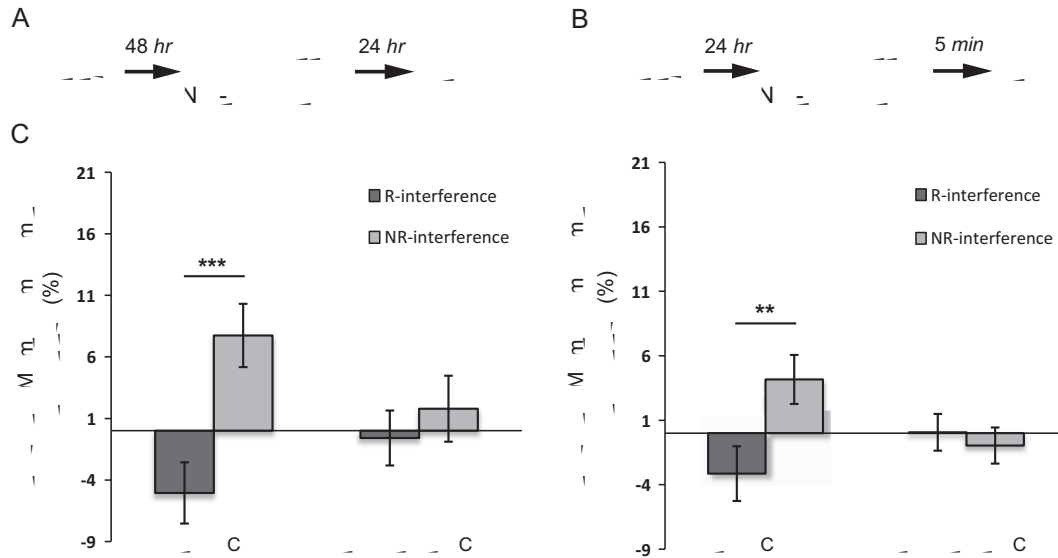
###### 4.1.2. Procedure

The procedures of Exp. 3a and Exp. 3b were similar to that of Exp. 1 and Exp. 2.2a respectively, with the exception that no interference was given after the 2 s reactivation judgment, which meant

that the trained cues were either only reactivated (Reactivation instead of R-interference) or directly interfered (NR-interference) during interference training session.

##### 4.2. Results and discussion

In both experiments, reactivation caused less memory impairment than interference (Exp. 3a,  $F(1,41) = 13.44$ ,  $p < 0.01$ ,  $MSE = 0.02$ ,  $\eta_p^2 = 0.25$ ; Exp. 3b,  $F(1,51) = 5.37$ ,  $p < 0.05$ ,  $MSE = 0.01$ ,  $\eta_p^2 = 0.10$ ). A significant interaction effect was found in both experiments (Exp. 3a, Fig. 4C:  $F(1,41) = 5.80$ ,  $p < 0.05$ ,  $MSE = 0.02$ ,  $\eta_p^2 = 0.12$ ; Exp. 3b, Fig. 4D:  $F(1,51) = 7.43$ ,  $p < 0.01$ ,  $MSE = 0.01$ ,  $\eta_p^2 = 0.13$ ). As expected, there was no difference in memory impairment caused by reactivation and that caused by NR-interference for independent-cue retrieval (Exp. 3a,  $t(41) = 0.85$ ,  $p > 0.05$ ; Exp. 3b,  $t(51) = -0.79$ ,  $p > 0.05$ ). Furthermore, there was less memory impairment under reactivation than under NR-interference for trained-cue retrieval (Exp. 3a,  $t(41) = 4.03$ ,  $p < 0.001$ ; Exp. 3b,  $t(51) = 3.13$ ,  $p < 0.01$ ). When compared with the control condition, reactivation alone did not lead to any memory impairment on the corresponding cue/target association (Exp. 3b,  $t(51) = 1.48$ ,  $p > 0.05$ ), and sometimes reactivation even improved memory performance (as in Exp. 3a,  $t(41) = 2.04$ ,  $p < 0.05$ ). Therefore, the memory impairment in neither the trained- nor the independent-cue retrieval condition could be attributed to reactivation alone.



**Fig. 4.** (A) Timeline for Exp. 3a. (B) Timeline for Exp. 3b. Reactivation represents only reactivation without further interference, while NR-interference represents direct interference without reactivation. Percentage of memory impaired under different conditions relative to the baseline condition was illustrated in (C) (Exp. 3a) and (D) (Exp. 3b). Both (C) and (D) showed that there was no difference in memory impairment caused by Reactivation and NR-interference in independent- or trained-cue retrieval. \*\* $p < 0.01$ , \*\*\* $p < 0.001$  (two-tailed  $t$  test); error bars, SEM.

## 5. Experiment 4: absence of cue-independent memory impairment by interference on unconsolidated memories

Next we examined whether the cue-independent memory impairment found in our study only exists for consolidated memory, as suggested by previous studies (Agren et al., 2012; Schiller et al., 2010, 2013; Xue et al., 2012).

### 5.1. Method

#### 5.1.1. Participants and materials

Forty-two participants (aged 17–29 years, 22 females) were recruited from Peking University, Beijing, China. They were all native Chinese speakers with normal reading and comprehension abilities. The critical task included frequency-balanced two-character Chinese words and pictures of faces of well-known celebrities.

#### 5.1.2. Procedure

We developed a modified procedure of Exp. 2.1, except that all interference trainings were given immediately after memory was formed (Fig. 5A).

### 5.2. Results and discussion

As shown in Fig. 5C, no interaction effect was found for cue type and interference type ( $F(1,41) = 1.01$ ,  $p > 0.05$ ,  $MSE = 0.03$ ,  $\eta_p^2 = 0.02$ ). Moreover, when compared with the control condition, R-interference did not cause worse memory performance in independent-cue retrieval ( $t(41) = -0.06$ ,  $p > 0.05$ ). Therefore, the manipulation of behavioral interference was only effective after a period of initial memory consolidation.

## 6. Experiment 5: Cue-independent memory impairment not caused by reactivation of all the cue-target associations

Given that consolidation is needed for the cue-independent forgetting effect to take place, this suggests that the two cues and their target form a composite memory that can be influenced

together during interference training. There are two possible explanations for the cue-independent forgetting effect. First, due to innate associations (i.e. A-B or A-X-B), the independent cue/target association (i.e. B-X) was reactivated upon reactivation, thus both the cue/target associations (i.e. A-X and B-X) became unstable and were disrupted by the ensuing interference information (Liu et al., 2014). Alternatively, reactivation of the target memory may only render the directly reactivated association unstable; the reason why other cues also failed to retrieve the target memory was because the unstable memory was already updated by interference information. In this way, memory for the target item itself was updated.

We developed a modified procedure of Exp. 2.2a, with participants learning associations in the form of A-M (i.e., trained-cue group in this experiment) and M-X (i.e., independent-cue group in this experiment), to test the two alternatives. If the first explanation is correct, by directly pairing the two cues together for learning (i.e. A-M), it would be more likely for participants to think of the other cue (i.e. M) when one cue (i.e. A) was reactivated. Thus, the ensuing interference training should also disrupt the M-X memory and cause more memory impairment for M-X than NR-interference. In contrast, if reactivation is only on A-M, M-X will not be influenced.

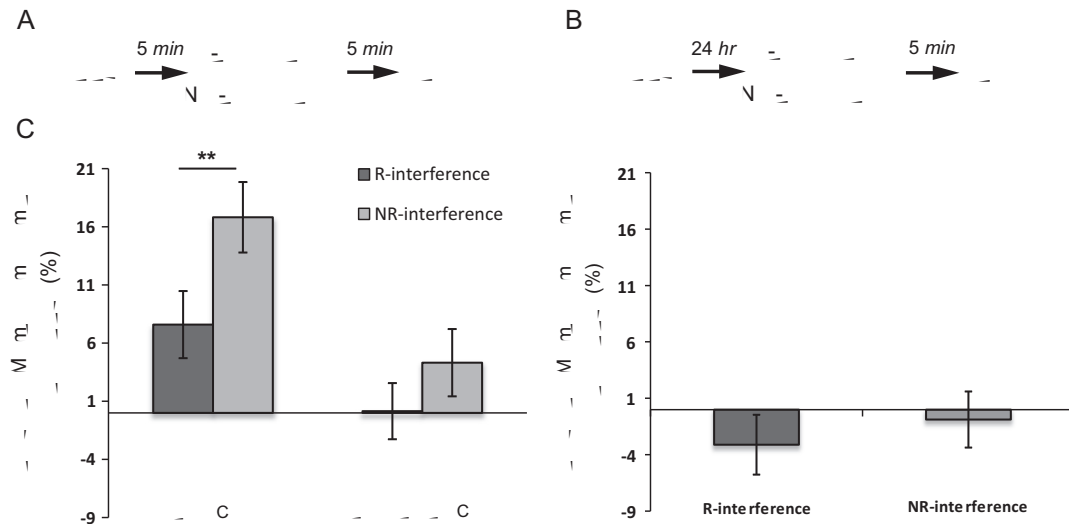
### 6.1. Method

#### 6.1.1. Participants and materials

Forty-two participants (aged 18–28 years, 30 females) were recruited from Peking University, Beijing, China. They were all native Chinese speakers with normal reading and comprehension abilities. The critical task included frequency-balanced two-character Chinese words and pictures of faces of well-known celebrities.

#### 6.1.2. Procedure

The procedure of Exp. 5 (Fig. 5B) was similar to that of Exp. 2.2a with the following changes. On Day 1, participants learned 45 word-word pairs (in the form of A-M) and 45 word-face pairs (in the form of M-X), instead of the 90 word-face pairs (in the form



**Fig. 5.** (A) Timeline for Exp. 4. (B) Timeline for Exp. 5. (C) Percentage of memory impaired under different conditions relative to the baseline condition in Exp. 4. R-interference did not cause more memory impairment in independent-cue retrieval, when memory was not consolidated. The memory impairment pattern was not affected by NR-interference. (D) Percentage of memory impaired under different conditions in Exp. 5. Reactivation-coupled interference of cue A did not cause generalized memory impairment of the whole memory sets (A-M-X). \*\*  $p < 0.01$  (two-tailed  $t$  test); error bars, SEM.

of A-X and B-X). On Day 2, A-M pairs underwent R-interference, NR-interference, or no training. Interference was done by pairing one new face picture to cue A. The final test was a 1 out of 8 choices recognition test, conducted only on M-X pairs.

## 6.2. Results and discussion

Results (Fig. 5D) showed no significant difference in memory impairment on M-X between R-interference and NR-interference ( $t(41) = 0.82$ ,  $p > 0.05$ ), supporting the second explanation. This result is consistent with previous findings on conditioning memory, when pharmacological blockade of a secondary conditioned memory under reactivation (CS2-CS1) failed to disrupt the primary conditioning response (CS1-US) (Debiec, Doyere, Nader, & LeDoux, 2006). Therefore, this result suggests that reactivation might produces a content-limited change, which would not be easily generalized to other memory associations.

## 7. Experiment 6: impairing strong memory through reactivation-coupled interference on related weak memory

So far, we have demonstrated that reactivation-coupled interference causes memory impairment that is independent of the directly interfered associations. This gives us a chance to test whether we can disrupt a strong memory by exerting reactivation-coupled interference on a related weak memory. In daily life, we might recall certain memories from seeing or hearing certain things, and sometimes these memories cause distress and pain. Therefore, it would be beneficial to find an indirect way to disrupt the recall of an unwanted memory.

### 7.1. Method

#### 7.1.1. Participants and materials

Thirty-six participants (aged 19–26 years, 20 females) were recruited from Peking University, Beijing, China. They were all native Chinese speakers with normal reading and comprehension ability. Frequency-balanced two-character Chinese words were used.

### 7.1.2. Procedure

A similar procedure as Exp. 2.1 was used with the following exceptions. First, participants learned 108 word pairs (54 A-X and 54 B-X pairs), with A-X pairs learned six times and B-X pairs learned three times. Therefore, strong memory was formed for A-X pairs. Second, during the training phase, one A-X subset (strong memory) received direct interference without reactivation. Another A-X subset was interfered with indirectly, in which the corresponding B-X pairs (weak memory) were reactivated and then treated with interference. Interference in both groups was done by sequentially pairing two new words with the cue. Recognition tests were given on all the originally learned pairs.

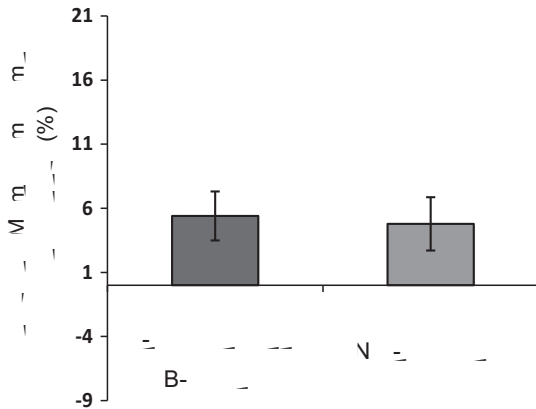
### 7.2. Results and discussion

We aimed to compare the effect of direct interference with that of indirect R-Interference on memory disruption. Data analysis was conducted on the strong memory (i.e. A-X) group (Fig. 6). Results showed that although R-interference on B-X pairs failed to cause more severe memory impairment than direct NR-interference on A-X pairs ( $t(35) = 0.26$ ,  $p = 0.80$ ), it caused significant memory impairment in A-X pairs, as compared with the control group ( $t(35) = -2.83$ ,  $p < 0.01$ ). This result suggests that we could take advantage of the cue-independent characteristics of reactivation-coupled interference, by using this interference to disrupt a strong memory with a related weak memory.

## 8. Discussion

Our work supports the idea that reactivation renders human declarative memory unstable. Results from five experiments (Exps. 1, 2.1, 2.2a, 2.2b, and 6) demonstrate that (1) reactivation renders consolidated memory unstable, and (2) behavioral interference during reactivation causes memory impairment that generalizes to retrievals of other cues related to the interfered target memory. This cue-independent character of memory impairment by reactivation-coupled interference establishes the feasibility to disrupt an unwanted memory indirectly.

There have been controversies over whether reactivation renders human declarative memory unstable (Schiller & Phelps, 2011), given that reactivation-coupled interference often failed to



**Fig. 6.** Percentage of memory impaired under different conditions relative to the baseline condition in Exp. 6. R-Interference on weak memory (B-X pairs) caused memory impairment in related strong memory (A-X pairs). The effect was comparable to direct interference on A-X pairs. \**p*

cause memory impairment (e.g. [Hupbach et al., 2007, 2008, 2009](#); [Gershman et al., 2013](#); [Wichert et al., 2013](#)). Similarly, no memory impairment by R-interference was found in the trained-cue condition in Exp. 2.2a. We believe that one reason for these null findings is that reactivation manipulation enhances the directly reactivated declarative memory to some extent ([Kroes et al., 2014](#); [LaPaglia & Chan, 2013](#); [Pashler et al., 2013](#); [Potts & Shanks, 2012](#)), as has been shown in previous studies ([Chan, McDermott, & Roediger, 2006](#); [Roediger & Karpicke, 2006](#); [Karpicke & Roediger, 2008](#)). Alternatively, reactivation may work to reinstate context information and to bind the reactivated memory to the context ([Sederberg, Gershman, Polyn, & Norman, 2011](#)), providing more clues for memory to be recollected upon testing. In both cases, the facilitation of memory recollection by reactivation (as in Exp. 3a) may counterbalance the impairment by later interference. Therefore, memory changes can vary depending on the characteristics of the original memory and the information participants encounter upon reactivation. By exploiting an independent retrieval cue in our study, we get eliminate the confounding effect of reactivation and consistently establish memory impairment by reactivation-coupled interference.

The results also showed that reactivation-coupled interference caused memory impairment that generalized to other retrieving cues. This was revealed by the double-cue/one-target paradigm, which has been used in conditioning memory studies ([Liu et al., 2014](#); [Schiller et al., 2010](#); [Tronel, Milekic, & Alberini, 2005](#)). [Schiller et al. \(2010\)](#) separately paired two different conditioned stimuli (CS) with the same electric shock (US), and found that the reactivation and extinction of one CS did not affect the conditioned response to the other CS. Though their result is different from ours, it does not rule out the possibility that cue-independent memory disruption exists in other types of memory because memory for electric shock might be so general that the unconditioned shocks paired with two conditioned cues were two different sets of memories. This possibility has been suggested by [Debiec, Diaz-Mataix, Bush, Doyere, and LeDoux \(2013\)](#), who found that when two different CSs were paired with one US as a compound, disruption of one CS-US association was generalized to the other one. Similar effects have also been established when reactivation of US in addition to extinction training for one CS-US association causes a generalized forgetting effect ([Liu et al., 2014](#)). Together with our findings in declarative memory, these findings suggest that the cue-independent impairment may be

common in reactivation-coupled behavioral interference for different memory forms.

Furthermore, we established that the memory impairment by reactivation-coupled interference appeared immediately after behavioral interference. The time criteria of reconsolidation disruption are well established in both animal and human studies, in which disruption of reconsolidation by pharmacological or electroconvulsive means causes memory impairment after, but not before, the reconsolidation process completes ([Kroes et al., 2014](#); [Nader et al., 2000](#)). The same conclusions have been drawn in behavioral interference studies, as R-interference leads to greater memory impairment than NR-interference in the post-reconsolidation test but not the immediate test (e.g. [Agren et al., 2012](#); [Liu et al., 2014](#); [Schiller et al., 2010, 2013](#); [Xue et al., 2012](#)). However, our findings suggest that the lack of an immediate effect from previous studies is due to the low sensitivity of the traditional single-cue/one-target paradigm. Additional research is needed to test whether this immediate effect exists in other memory types. In addition, given that memory deficits appeared even before the reconsolidation time window closed, it suggests that processes other than reconsolidation were affected ([Nader et al., 2000](#); [Schiller & Phelps, 2011](#); [Tronson & Taylor, 2007](#)). However, to prove this possibility, further evidence is needed to show that the immediate effect still persists when the reconsolidation process is blocked. If the immediate effect persists without a period of reconsolidation, then it further points out the necessity to test whether the established effect of reconsolidation disruption in the post-reconsolidation test comes solely from reconsolidation disruption.

The mechanism underlying the cue-independent forgetting effect is unknown. One possibility is that, upon reactivation, only the target memory is reactivated and is then updated by ensuing interfering information. The target memory thus becomes unavailable to both trained and independent retrieval cues. The cue-independent forgetting effect has also been established in retrieval-induced forgetting ([Anderson, 2003](#); [Anderson, Bjork, & Bjork, 1994](#); [Anderson & Spellman, 1995](#)), where inhibitory control on target memory renders it unavailable to related retrieving cues ([Anderson & Green, 2001](#); [Anderson & Spellman, 1995](#); [Wang, Cao, Zhu, Cai, & Wu, 2015](#)). It is thus possible that inhibitory control operates in reactivation-coupled interference. Participants may have the urge to think of the originally learned memory upon reactivation, which can be so strong as to interfere with the ensuing interference learning process. To overcome this, participants could be suppressing, via inhibitory control, the original memory during the repeated learning of interference information. However, inhibitory control explanation does not address the null effect for unconsolidated memory. Therefore, more evidence is needed for interpreting the effect of reactivation-coupled interference on declarative memory.

In conclusion, the present study demonstrates a behavioral interference procedure to elicit generalized memory impairment of consolidated declarative memory. It raises the possibility of using a neutral cue to eliminate an unwanted or strong memory associated with various cues. Because experiences in daily life are linked with multiple contextual cues, reactivation-coupled interference offers a new approach to the treatment of post-traumatic stress disorder.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.cognition.2016.06.015>.

## References

- Agren, T., Engman, J., Frick, A., Bjorkstrand, J., Larsson, E. M., Furmark, T., & Fredrikson, M. (2012). Disruption of reconsolidation erases a fear memory trace in the human amygdala. *Science*, 337, 1550–1552. <http://dx.doi.org/10.1126/science.1223006>.
- Anderson, M. (2003). Rethinking interference theory: Executive control and the mechanisms of forgetting. *Journal of Memory and Language*, 49(4), 415–445. <http://dx.doi.org/10.1016/j.jml.2003.08.006>.
- Anderson, M., Bjork, R. A., & Bjork, E. L. (1994). Remembering can cause forgetting: Retrieval dynamics in long-term memory. *Journal of Experimental Psychology: Learning, Memory, and Cognition*, 20, 1063–1087.
- Anderson, M. C., & Green, C. (2001). Suppressing unwanted memories by executive control. *Nature*, 410(6826), 366–369. <http://dx.doi.org/10.1038/35066572>.
- Anderson, M., & Spellman, B. A. (1995). On the status of inhibitory mechanisms in cognition: Memory retrieval as a model case. *Psychological Review*, 102, 68–100.
- Besnard, A., Caboche, J., & Laroche, S. (2012). Reconsolidation of memory: A decade of debate. *Progress in Neurobiology*, 99, 61–80. <http://dx.doi.org/10.1016/j.pneurobio.2012.07.002>.
- Chan, J. C., & LaPaglia, J. A. (2013). Impairing existing declarative memory in humans by disrupting reconsolidation. *Proceedings of the National Academy of Sciences of the United States of America*, 110, 9309–9313. <http://dx.doi.org/10.1073/pnas.1218472110>.
- Chan, J. C., McDermott, K. B., & Roediger, H. L. III. (2006). Retrieval-induced facilitation: Initially nontested material can benefit from prior testing of related material. *Journal of Experimental Psychology: General*, 135, 553–571. <http://dx.doi.org/10.1037/0096-3445.135.4.553>.
- Clem, R. L., & Huganir, R. L. (2010). Calcium-permeable AMPA receptor dynamics mediate fear memory erasure. *Science*, 330, 1108–1112. <http://dx.doi.org/10.1126/science.1195298>.
- De Oliveira Alves, L., Crestani, A. P., Cassini, L. F., Haubrich, J., Santana, F., & Quillfeldt, J. A. (2013). Reactivation enables memory updating, precision-keeping and strengthening: Exploring the possible biological roles of reconsolidation. *Neuroscience*, 244, 42–48. <http://dx.doi.org/10.1016/j.neuroscience.2013.04.005>.
- Debiec, J., Diaz-Mataix, L., Bush, D. E. A., Doyere, V., & LeDoux, J. E. (2013). The selectivity of aversive memory reconsolidation and extinction processes depends on the initial encoding of the Pavlovian association. *Learning & Memory*, 20, 695–699. <http://dx.doi.org/10.1101/lm.031609.113>.
- Debiec, J., Doyere, V., Nader, K., & LeDoux, J. E. (2006). Directly reactivated, but not indirectly reactivated, memories undergo reconsolidation in the amygdala. *Proceedings of the National Academy of Sciences of the United States of America*, 103(9), 3428–3433. <http://dx.doi.org/10.1073/pnas.0507168103>.
- Diekelmann, S., Büchel, C., Born, J., & Rasch, B. (2011). Labile or stable: Opposing consequences for memory when reactivated during waking and sleep. *Nature Neuroscience*, 14(3), 381–386. <http://dx.doi.org/10.1038/nn.2744>.
- Duvarci, S., Nader, K., & LeDoux, J. E. (2005). Activation of extracellular signal-regulated kinase–mitogen-activated protein kinase cascade in the amygdala is required for memory reconsolidation of auditory fear conditioning. *European Journal of Neuroscience*, 21, 283–289. <http://dx.doi.org/10.1111/j.1460-9568.2004.03824.x>.
- Forcato, C., Argibay, P. F., Pedreira, M. E., & Maldonado, H. (2009). Human reconsolidation does not always occur when a memory is retrieved: The relevance of the reminder structure. *Neurobiology of Learning and Memory*, 91(1), 50–57. <http://dx.doi.org/10.1016/j.nlm.2008.09.011>.
- 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(4), 295–303. <http://dx.doi.org/10.1101/lm.486107>.
- Forcato, C., Rodríguez, 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(1), 77–84. <http://dx.doi.org/10.1016/j.nlm.2009.08.006>.
- Gershman, S. J., Schapiro, A. C., Hupbach, A., & Norman, K. A. (2013). Neural context reinstatement predicts memory misattribution. *Journal of Neuroscience*, 33(20), 8590–8595. <http://dx.doi.org/10.1523/jneurosci.0096-13.2013>.
- Graeff, J., Joseph, N. F., Hom, M. E., Samiei, A., Meng, J., Seo, J., ... Tsai, L.-H. (2014). Epigenetic priming of memory updating during reconsolidation to attenuate remote fear memories. *Cell*, 156(1–2), 261–276. <http://dx.doi.org/10.1016/j.cell.2013.12.020>.
- Hupbach, A., Gomez, R., Hardt, O., & Nadel, L. (2007). Reconsolidation of episodic memories: A subtle reminder triggers integration of new information. *Learning & Memory*, 14(1–2), 47–53. <http://dx.doi.org/10.1101/lm.365707>.
- Hupbach, A., Gomez, R., & Nadel, L. (2009). Episodic memory reconsolidation: Updating or source confusion? *Memory*, 17(5), 502–510. <http://dx.doi.org/10.1080/09658210902882399>.
- Hupbach, A., Hardt, O., Gomez, R., & Nadel, L. (2008). The dynamics of memory: Context-dependent updating. *Learning & Memory*, 15(8), 574–579. <http://dx.doi.org/10.1101/lm.1022308>.
- Karpicke, J. D., & Roediger, H. L. III. (2008). The critical importance of retrieval for learning. *Science*, 319(5865), 966–968. <http://dx.doi.org/10.1126/science.1152408>.
- Kroes, M. C., Tendolkar, I., van Wingen, G. A., van Waarde, J. A., Strange, B. A., & Fernandez, G. (2014). An electroconvulsive therapy procedure impairs reconsolidation of episodic memories in humans. *Nature Neuroscience*, 17, 204–206. <http://dx.doi.org/10.1038/nn.3609>.
- LaPaglia, J. A., & Chan, J. C. K. (2013). Testing increases suggestibility for narrative-based misinformation but reduces suggestibility for question-based misinformation. *Behavioral Sciences & The Law*, 31(5), 593–606. <http://dx.doi.org/10.1002/bsl.2090>.
- Lee, J. L. (2008). Memory reconsolidation mediates the strengthening of memories by additional learning. *Nature Neuroscience*, 11, 1264–1266. <http://dx.doi.org/10.1038/nn.2205>.
- Lee, J. L. (2009). Reconsolidation: Maintaining memory relevance. *Trends in Neurosciences*, 32, 413–420. <http://dx.doi.org/10.1016/j.tins.2009.05.002>.
- Lewis, D. J., & Bergman, N. J. (1973). Source of cues for cue-dependent amnesia in rats. *Journal of Comparative and Physiological Psychology*, 85, 421–426. <http://dx.doi.org/10.1037/h0035020>.
- Lewis, D. J., Mahan, J. J., & Bregman, N. J. (1972). Cue-dependent amnesia in rats. *Journal of Comparative and Physiological Psychology*, 81, 243–247. <http://dx.doi.org/10.1037/h0033524>.
- Liu, J., Zhao, L., Xue, Y., Shi, J., Suo, L., Luo, Y., ... Lu, L. (2014). An unconditioned stimulus retrieval extinction procedure to prevent the return of fear memory. *Biological Psychiatry*, 76(11), 895–901. <http://dx.doi.org/10.1016/j.biopsych.2014.03.027>.
- Misanin, J. R., Miller, R. R., & Lewis, D. J. (1968). Retrograde amnesia produced by electroconvulsive shock after reactivation of consolidated memory trace. *Science*, 160, 554–555. <http://dx.doi.org/10.1126/science.160.3827.554>.
- Monfils, M. H., Cowansage, K. K., Klann, E., & LeDoux, J. E. (2009). Extinction-reconsolidation boundaries: Key to persistent attenuation of fear memories. *Science*, 324, 951–955. <http://dx.doi.org/10.1126/science.1167975>.
- Morris, R. G., Inglis, J., Ainge, J. A., Olverman, H. J., Tulloch, J., Dudai, Y., & Kelly, P. A. (2006). Memory reconsolidation: Sensitivity of spatial memory to inhibition of protein synthesis in dorsal hippocampus during encoding and retrieval. *Neuron*, 50, 479–489. <http://dx.doi.org/10.1016/j.neuron.2006.04.012>.
- Nader, K. (2003). Memory traces unbound. *Trends in Neurosciences*, 26, 65–72. [http://dx.doi.org/10.1016/s0166-2236\(02\)00042-5](http://dx.doi.org/10.1016/s0166-2236(02)00042-5).
- Nader, K., & Einarsson, E. O. (2010). Memory reconsolidation: An update. *Annals of the New York Academy of Sciences*, 1191, 27–41. <http://dx.doi.org/10.1111/j.1749-6632.2010.05443.x>.
- Nader, K., & Hardt, O. (2009). A single standard for memory: The case for reconsolidation. *Nature Reviews Neuroscience*, 10(3), 224–234. <http://dx.doi.org/10.1038/nrn2590>.
- 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. <http://dx.doi.org/10.1038/35021052>.
- Pashler, H., Kang, S. H. K., & Mozer, M. C. (2013). Reviewing erroneous information facilitates memory updating. *Cognition*, 128(3), 424–430. <http://dx.doi.org/10.1016/j.cognition.2013.05.002>.
- Potts, R., & Shanks, D. R. (2012). Can testing immunize memories against interference? *Journal of Experimental Psychology: Learning, Memory, and Cognition*, 38, 1780–1785. <http://dx.doi.org/10.1037/a0028218>.
- Roediger, H. L., III, & Karpicke, J. D. (2006). Test-enhanced learning: Taking memory tests improves long-term retention. *Psychological Science*, 17(3), 249–255. <http://dx.doi.org/10.1111/j.1467-9280.2006.01693.x>.
- Rossato, J. I., Bevilacqua, L. R., Myskiw, J. C., Medina, J. H., Izquierdo, I., & Cammarota, M. (2007). On the role of hippocampal protein synthesis in the consolidation and reconsolidation of object recognition memory. *Learning & Memory*, 14, 36–46. <http://dx.doi.org/10.1101/lm.422607>.
- Sara, S. J. (2000). Retrieval and reconsolidation: Toward a neurobiology of remembering. *Learning & Memory*, 7, 73–84. <http://dx.doi.org/10.1101/lm.7.2.73>.
- 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. <http://dx.doi.org/10.1038/nature08637>.
- Schiller, D., Kanen, J. W., LeDoux, J. E., Monfils, M. H., & Phelps, E. A. (2013). Extinction during reconsolidation of threat memory diminishes prefrontal cortex involvement. *Proceedings of the National Academy of Sciences of the United States of America*, 110, 20040–20045. <http://dx.doi.org/10.1073/pnas.1320322110>.
- Schiller, D., & Phelps, E. A. (2011). Does reconsolidation occur in humans? *Frontiers in Behavioral Neuroscience*, 5, 24. <http://dx.doi.org/10.3389/fnbeh.2011.00024>.
- 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(3), 455–468. <http://dx.doi.org/10.3758/s13423-011-0086-9>.
- 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. *Journal of Neuroscience*, 24, 4787–4795. <http://dx.doi.org/10.1523/JNEUROSCI.5491-03.2004>.
- Tronel, S., Milekic, M. H., & Alberini, C. M. (2005). Linking new information to a reactivated memory requires consolidation and not reconsolidation mechanisms. *PLoS Biology*, 3(9), 1630–1638. <http://dx.doi.org/10.1371/journal.pbio.0030293>.
- Tronson, N. C., & Taylor, J. R. (2007). Molecular mechanisms of memory reconsolidation. *Nature Reviews Neuroscience*, 8, 262–275. <http://dx.doi.org/10.1038/nrn2090>.

- Walker, M. P., Brakefield, T., Hobson, J. A., & Stickgold, R. (2003). Dissociable stages of human memory consolidation and reconsolidation. *Nature*, *425*, 616–620.
- Wang, Y., Cao, Z., Zhu, Z., Cai, H., & Wu, Y. (2015). Cue-independent forgetting by intentional suppression – Evidence for inhibition as the mechanism of intentional forgetting. *Cognition*, *143*, 31–35. <http://dx.doi.org/10.1016/j.cognition.2015.05.025>.
- Wichert, S., Wolf, O. T., & Schwabe, L. (2013). Updating of episodic memories depends on the strength of new learning after memory reactivation. *Behavioral Neuroscience*, *127*(3), 331–338. <http://dx.doi.org/10.1037/a0032028>.
- Xue, Y. X., Luo, Y. X., Wu, P., Shi, H. S., Xue, L. F., Chen, C., ... Lu, L. (2012). A memory retrieval-extinction procedure to prevent drug craving and relapse. *Science*, *336*, 241–245. <http://dx.doi.org/10.1126/science.1215070>.