Report

Current Biology

Memory Reactivation Enables Long-Term Prevention of Interference

Highlights

- Exposing the brain to different memories induces interference, reducing performance
- Memory reactivation prevented long-term interference even following a month
- Preventing interference specifically depended on the reactivation-induced time window

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In Brief

Exposing the brain to two different memories in a short temporal offset can induce interference, reducing performance. Herszage and Censor report that brief reactivation of a motor memory followed by brief exposure to a novel memory, specifically within a limited time window following reactivation, prevents future interference. These effects are long-term and observed even a month following reactivation.

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Report

Memory Reactivation Enables Long-Term Prevention of Interference

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SUMMARY

The ability of the human brain to successively learn or perform two competing tasks constitutes a major challenge in daily function. Indeed, exposing the brain to two different competing memories within a short temporal offset can induce interference, resulting in deteriorated performance in at least one of the learned memories [1–4]. Although previous studies have investigated online interference and its effects on performance [5–13], whether the human brain can enable long-term prevention of future interference is unknown. To address this question, we utilized the memory reactivation-reconsolidation framework [2, 12] stemming from studies at the synaptic level [14–17], according to which reactivation of a memory enables its update. In a set of experiments, using the motor sequence learning task [18] we report that a unique pairing of reactivating the original memory (right hand) in synchrony with novel memory trials (left hand) prevented future interference between the two memories. Strikingly, these effects were long-term and observed a month following reactivation. Further experiments showed that preventing future interference was not due to practice per se, but rather specifically depended on a limited time window induced by reactivation of the original memory. These results suggest a mechanism according to which memory reactivation enables long-term prevention of interference, possibly by creating an updated memory trace integrating original and novel memories during the reconsolidation time window. The opportunity to induce a long-term preventive effect on memories may enable the utilization of strategies optimizing normal human learning, as well as recovery following neurological insults.

RESULTS

One of the most common challenges of the human brain relevant for daily function is to be able to learn or perform an encoded task immediately following a competing novel task. Indeed, accumulative evidence across memory domains has shown that exposing the human brain to two different competing memories within a short temporal offset can induce interference, resulting in deteriorated performance [1–4]. Proactive interference, deteriorated expression of a memory trace caused by a preceding competing novel memory trace, drew interest across many memory domains, such as episodic memory [19], language learning [20], working memory [21, 22], and motor memory [10, 11, 23]. This large volume of studies has driven the investigation of the neural mechanism of proactive interference. For example, a study [24] looking into interference in rats found that the utilization of protein resources common to both memories leads to interference. More recent work has suggested that proactive interference is regulated by the process of neurogenesis [25].

Although a large amount of work has been dedicated to the research of interference as an online mechanism and its effects on performance [5–13], whether long-term prevention of future interference can be developed is unknown. Could updating an existing memory trace with novel information reduce interference in the future? The opportunity to induce a hardwired long-term preventive effect on memories may provide mechanistic insights into basic memory processing, as well as subsequent clinical implications.

In order to create an updated memory trace, we utilized the framework of reactivation-reconsolidation, which is known to allow modifications of consolidated memory traces [2, 12]. This framework stems from studies at the synaptic level [14–17], showing that even fully consolidated memories, presumably stabilized, can be updated if they are reactivated. The ability to update such memories is limited to a time window, after which the memory returns to a stable state through the process of reconsolidation. Moreover, following training, skill memory shows additional improvements without further practice, known as "offline gains" [1, 2, 12, 13, 26, 27].

In a set of experiments, we tested the concept of using the time frame following reactivation of an existing memory in order to achieve an updated memory trace that would prevent future interference by a novel memory. We used the motor sequence learning task [18], which has been widely used to study long-term motor sequence memory [12, 13, 18, 26, 28]. The original sequence memory was executed by the right hand and the novel memory by the left hand [18, 29–32].

To establish the following design, we first verified that right- and left-hand performance of this task function as two interfering memories. To do so, we examined whether these two memories

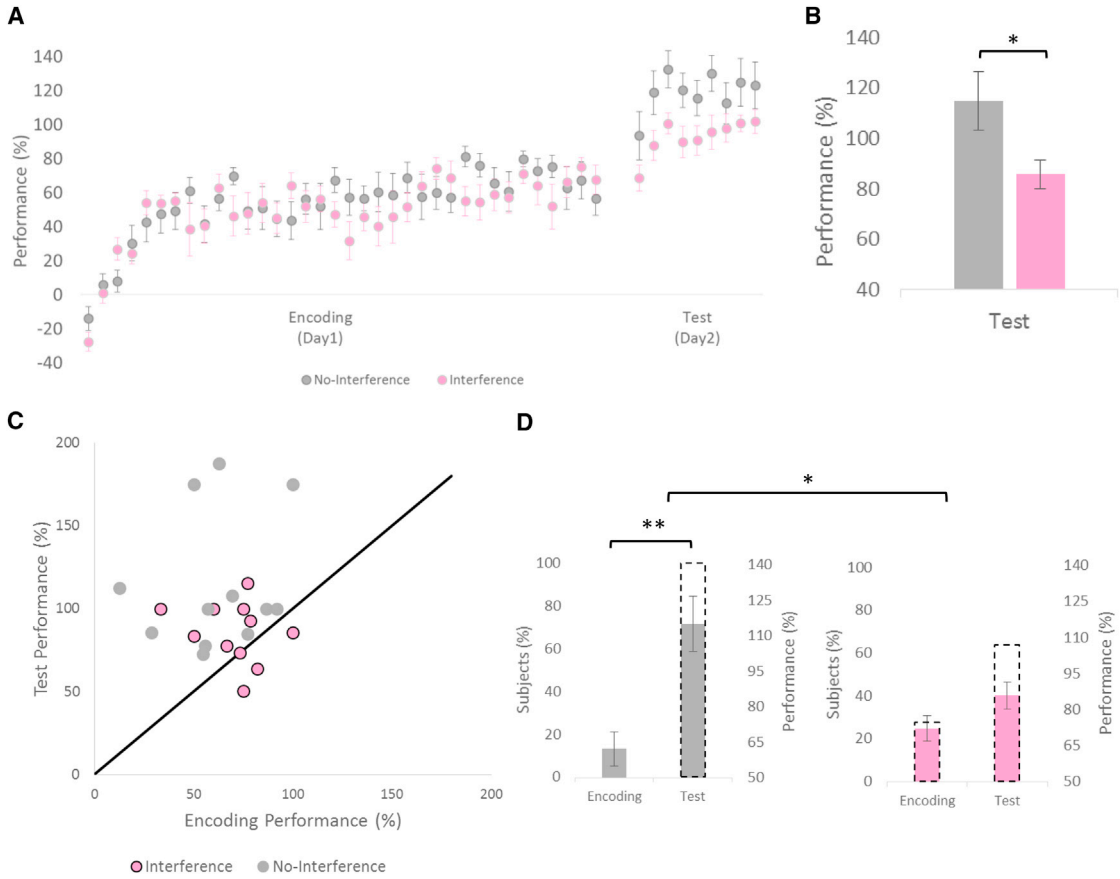

Figure 1. Interference Induced by a Left-Hand Novel Memory Decreases the Performance of the Right-Hand Original Memory

(A) Single-trial performance in experiment 1 for both no-interference (gray) and interference (pink) groups.

(B) Mean performance of the original memory (right hand) at the test session.

(C) Test versus encoding single-subject comparisons (see STAR Methods) are presented in a scatterplot along a unit slope line (y = x), where each point reflects a participant [34]. Data accumulating below the line indicate reduced performance at test, while data points above the unit line reflect subjects, who improved from the encoding session.

(D) Dashed lines reflect the percentage of participants on each side of the unit slope line in (C), and the bars reflect the mean performance values.

*p < 0.05, **p < 0.005. Error bars represent SEM. See also Figure S1.

exhibit interference when performed in temporal proximity. 23 right-handed participants practiced a motor sequence learning task requiring them to tap, using their dominant right hand, as fast and accurate as possible, a five-digit sequence (see STAR Methods). On the following day, participants in the interference group (n = 11) performed the same task using the left hand (novel memory) and then repeated the task with the right hand (original memory). Participants of the no-interference group (n = 12) performed the task using the right hand (original memory), without prior exposure to the novel memory. We hypothesized that proactive interference effects should be evident within the time period immediately following the interfering event, thus expressed in the beginning of the session, similar to previous studies ([7, 12, 13, 33]; see STAR Methods). Since motor sequence memories improve between sessions (offline gains) [1, 2, 12, 13, 26, 27], future interference should be evident as inhibited expression of these offline gains.

Baseline performance was consistent across subjects ($F_{1,21}$ = 0.83, not significant [n.s.]; see Figure S1). Results showed decreased performance for the interference group compared to the no-interference group at the test session ($F_{1,21}$ = 4.75, $p < 0.05$; see single-trial data in Figure 1A and group average in Figure 1B). In addition, while the no-interference group expressed high offline gains between sessions ($F_{1,11}$ = 17.36, $p < 0.005$; Figure 1D; for single-subject data, see Figure 1C), offline gains were suppressed in the interference group ($F_{1,10}$ = 3.31, n.s.; Figure 1D), with a significant session × group interaction ($F_{1,21}$ = 5.7, $p < 0.03$). In sum, these results show interference between the two successive novel (left hand) and original (right hand) memories.

Could this interference be prevented using memory reactivation mechanisms? To test this hypothesis, we conducted experiment 2 (n = 24), in which the same task as in experiment 1 was used. Following encoding (see STAR Methods), on the next day, the reactivation group (n = 12) performed reactivation of the original memory (right hand) interleaved with novel memory trials (left hand). The no-reactivation group (n = 12) performed a similar amount of novel memory trials, without memory reactivation.

Baseline performance was consistent across subjects ($F_{1,22}$ = 0.17, n.s.; see Figure S1). Both groups showed significant initial

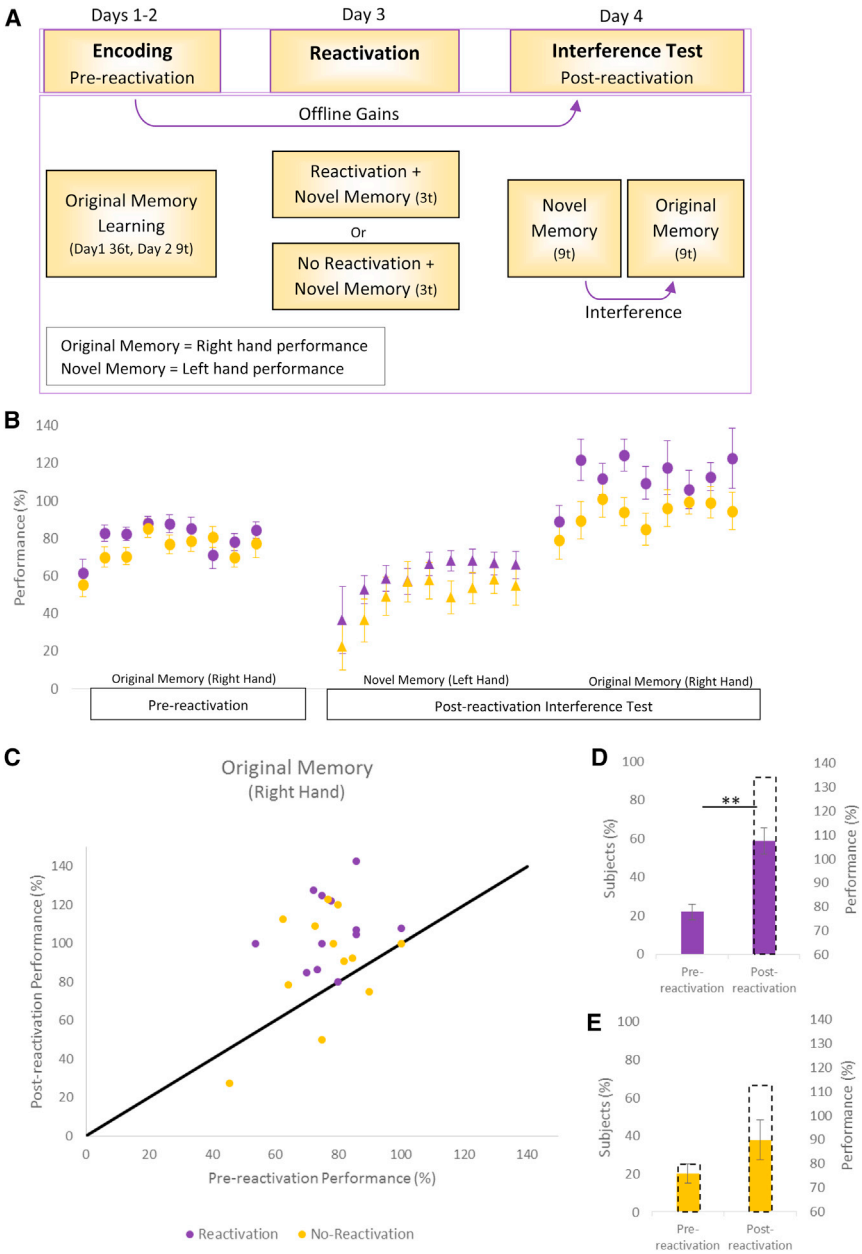

Figure 2. Interference Is Prevented Following Memory Reactivation

(A) General design of experiment 2. Following encoding (days 1–2), participants performed a session (day 3) containing practice of a novel memory, with (reactivation group) or without (no-reactivation group) reactivation of the original memory. On the following day (day 4), a post-reactivation test was conducted, testing both memories. Since offline performance gains are usually expressed as improvements of memories, interference caused by the novel memory should disrupt the expression of the offline gains of the original memory. #t indicates the number of trials.

(B) Single-trial performance in experiment 2 for both reactivation (purple) and no-reactivation (yellow) groups. Original memory (right hand) is depicted as circles and novel memory (left hand) as triangles.

(C) Post-reactivation versus pre-reactivation single-subject comparisons (see STAR Methods) are presented in a scatterplot along a unit slope line (y = x), where each point reflects a participant [34]. Data accumulating below the line indicate reduced performance post-reactivation, thus no expression of offline gains in performance, while data points above the unit line reflect subjects who showed expression of offline gains.

(D and E) Dashed lines reflect the percentage of participants on each side of the unit slope line in (B), and the bars reflect the mean performance values. A one-way ANOVA with a session factor tested the expression of offline gains in each group. Interference in expression of offline gains of the original memory was prevented for participants in the reactivation group, showing highly efficient pre-post reactivation gains.

*p < 0.05, **p < 0.001. Error bars represent SEM. See also Figure S1.

offline gains ($F_{1,11}$ = 22.76, p < 0.005 in the reactivation group; $F_{1,11}$ = 19.54, p < 0.005 in the no-reactivation group), indicating successful consolidation of the encoded memory [1, 2, 12, 13, 26, 27], and subsequently the performance was consistent at the end of encoding sessions (end of day 2, $F_{1,22}$ = 0.25, n.s.). Repeated-measures ANOVA tested for offline gains in original memory (right hand) performance at the interference test session. The results, supported by single-trial (Figure 2B) and single-subject (Figure 2C) data, show that interference in expression of offline gains of the original memory was prevented for participants in the reactivation group, showing highly efficient pre-post reactivation gains (reactivation group $F_{1,11}$ = 26.37, p < 0.001, Figure 2D; no-reactivation group $F_{1,11}$ = 3.53, n.s., Figure 2E; no group × session interaction, $F_{1,22}$ = 2.91, n.s.).

In light of these results, the stability of an interference-prevention effect remained unclear. Would prevention of interference be evidently stable after a long period of time, indicating a long-term plasticity mechanism? To determine whether interference prevention was a stable, long-term phenomenon, participants (n = 23) attended a session after a period of at least 1 month from reactivation, in which interference was tested (see STAR Methods).

A one-way ANOVA with a group factor compared the long-term original memory performance, at least 1 month following reactivation (46.5 ± 15.1 days following a reactivation condition; 41.3 ± 13.5 days following a no-reactivation condition; values indicating mean ± SD). Results indicated that long-term interference was reduced for participants in the reactivation group, showing higher performance of the original memory compared to the no-reactivation group ($F_{1,21}$ = 5.93, p < 0.03; Figure 3B). This demonstrates that memory reactivation, even if performed 1 month prior to the interference test, enables long-term prevention of interference.

Could it be that interference is prevented by practice per se, and not due to reactivation-reconsolidation mechanisms? To

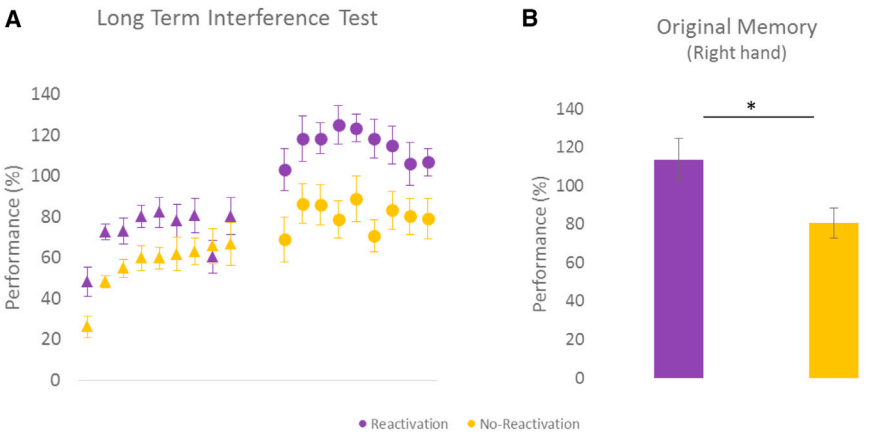

Figure 3. Long-Term Reduction of Interference

(A) Performance in each trial in the long-term interference test session. Original memory (right hand) is depicted as circles and novel memory (left hand) as triangles.

(B) Mean performance of the original memory (right hand) at the long-term interference test in experiment 3.

*p < 0.05. Error bars represent SEM.

address this question, we performed experiment 4, in which experimental conditions did not differ in practice itself but differed in whether it was conducted within the limited reconsolidation time window. Based on previous studies, which found that the reconsolidation time window lasts for up to 6 hr from reactivation [2, 7], participants (n = 10) performed the left-hand trial after either 10 min (within the reactivation-induced reconsolidation time window of susceptibility) or 6 hr (outside the reactivation-induced time window) following the right-hand reactivation trial. Importantly, this design was within subject, hence participants performed the experiment within a 10-min window on 1 week and a 6-hr window on the other, in a counter-balanced manner (see STAR Methods). The results, supported by single-trial data (Figure 4A), showed that future, post-reactivation interference was reduced when subjects performed the novel memory trial 10 min after reactivating the original memory ($t(9) = 2.05$, $p < 0.05$; Figure 4B). These results indicate that practice per se is not sufficient to create an updated memory trace preventing interference, but rather that the timing of the exposure to the novel memory is crucial and dependent on the reconsolidation time window.

In light of the results of experiment 4, indicating that prevention of interference is not dependent on practice per se but specifically on the reactivation-induced time window, we conducted a further experiment testing whether the same structure of trials would prevent interference if conducted following memory encoding (instead of following memory reactivation). Therefore, experiment 5 tested whether a similar intervention as in the reactivation procedure of experiment 4, but rather performed immediately following memory encoding, would improve performance at the interference test session compared to controls who encoded the memory without this intervention (see STAR Methods). Performance (see Figure S2) indicated that the same structure of trials does not prevent interference if it is not conducted following reactivation of the consolidated memory, further suggesting that preventing future interference is dependent on reactivation mechanisms.

DISCUSSION

The findings indicate that reactivating an existing memory in synchrony with novel memory trials induces a preventive effect, reducing future interference between the two memories. This preventive effect is long-term, implying the existence of a hard-wired neural mechanism underlying an updated memory trace, possibly integrating original and novel memories. Such long-term neural mechanisms may either impact memory storage capacity or enable reduction of induced retrieval deficits [35], both crucially affecting functional performance.

Figure 4. Preventing Future Interference Depends on the Reconsolidation Time Window of the Original Memory

(A) Original memory (right hand) performance at each trial in experiment 4.

(B) Future, post-reactivation interference (original right-hand performance) was reduced if the novel memory was introduced 10 min following reactivation of the original memory. Each line represents a subject; black line reflects mean values.

*p < 0.05. Error bars indicate SEM.

The results of experiment 1 isolated the effects of proactive interference, directly showing that if the novel memory is performed immediately before the original memory at test session, it induces proactive interference. Importantly, experiment 4 shows that preventing interference was not due to practice per se, but rather specifically depended on a limited time window of susceptibility for update induced by memory reactivation and thus was not evident when the novel memory was introduced 6 hr following memory reactivation (outside the reactivation-induced time window). This limited time window for memory update, evident in reconsolidation studies ranging from synaptic [14–17] to systems levels [2, 12, 33], suggests that prevention of future interference may be explained by reactivation-reconsolidation mechanisms. Furthermore, experiment 5 showed that the exact same structure and timing of trials as in the reactivation procedure of experiment 4 does not prevent interference if not conducted following reactivation of a consolidated memory. This further asserts that preventing future interference is dependent on reactivation mechanisms and cannot be attributed to practice order and structure (e.g., interleaved or blocked practice [36–38]), which consistently across all sets of experiments prevented interference only in reactivation conditions. It is important to note that the reactivation-reconsolidation mechanism, consistent with previous reconsolidation studies [2, 7, 13], includes only a brief 30-s exposure to the task rather than overtraining [39]. Furthermore, our experiments show that resistance to interference effects were long-term and observed a month following reactivation.

It remains to be determined whether such mechanisms may operate similarly in additional memory domains, or whether interference between two different memory types [8] may be similarly prevented. Of note, recent evidence has cast doubt regarding the ability of post-reactivation interference to degrade the consolidated memory [40]. The heart of this critique relates to a study [2] in which the behavioral effect relied on an end-point measure (accuracy) different from the commonly used end-point measure [1, 7, 13], which combines speed and accuracy, as done here. Thus, the end-point measure used in our study combining both speed and accuracy has proven to be highly replicable across multiple labs and studies, showing that memory reactivation paired with different types of interference can interfere with subsequent memory gains and with the corresponding neural signatures (including both [40] and [2] studies, as well as [7, 13, 41]).

Here, we extend this framework to show that memory reactivation enables long-term prevention of interference. The fact that prevention of interference sustained for a long period after reactivation suggests that the neural plasticity supporting this effect created a long-term change in the networks underlying motor sequence memory. This account is in line with recent evidence, based on non-invasive magnetic stimulation used as a virtual lesion protocol [12, 13, 41], pointing to a mechanism whereby following motor sequence memory reactivation, interactions between primary motor cortex (M1) and existing cortical and sub-cortical memory traces mediate memory update, if followed by reconsolidation. According to this view, such extended inter-regional neural interactions are recruited following memory reactivation to enable its update, which in turn contributes to the interference prevention in a later session.

These results may have important implications for normal daily function and learning, often negatively affected by interference. In addition, the opportunity to induce long-term prevention of interference is also relevant for development of strategies to improve function in neurological conditions, such as enhancing motor neurorehabilitation following stroke or reducing forgetting in conditions involving memory impairments.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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  - Data Analysis
  - Statistical Analysis

SUPPLEMENTAL INFORMATION

Supplemental Information includes two figures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2017.04.025.

AUTHOR CONTRIBUTIONS

J.H. and N.C. designed the study. J.H. performed the research. J.H. and N.C. analyzed the data and wrote the manuscript.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and Algorithms		
MATLAB	MathWorks	https://www.mathworks.com/
SPSS Statistics 24	IBM	https://www.ibm.com/analytics/us/en/technology/spss/

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests should be directed to and will be fulfilled by the Lead Contact, Nitzan Censor (censornitzan@post.tau.ac.il).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

81 naive healthy subjects, ages 18-40 years (56 female, average age 24.4 years, SD = 2.8 years) gave their written informed consent to participate in the project, which was approved by the Tel Aviv University Institutional Ethics Committee. All procedures were in accordance with approved guidelines. All participants were right handed, were not musicians or video gamers [42], and reported at least 6 hr of sleep the night before each experimental session. Two participants were excluded after the first session due to failure to complete the task, and two due to extreme variations between the initial encoding sessions (influence tests: DFFITS = 70.82 and DFFITS = 35.39 [43]).

METHOD DETAILS

Stimuli and Task

Participants practiced the motor skill learning task [18] in which they were asked to tap as fast and as accurate as they can, a 5 digit sequence (4-1-3-2-4). Tapping was performed using either a keyboard or a 4-key response box (Cedrus, Lumina, Model LU440), placed in front of the subjects during the experiment. During each trial, the sequence was presented constantly on a computer screen. The same sequence was used in all experiments and sessions (but see experiment 4). The novel memory refers to the same sequence as in the original (right-hand) memory, transformed in a movement-based manner to be executed with the left hand [27, 32]. Throughout each trial, each key press produced a dot displayed at the top portion of the screen, with the dots accumulating from left to right as the trial progressed. Every trial was followed by a break of the same duration.

Experimental Design

Experiment 1 tested whether two motor memories (right- and left-hand performance) can induce interference between them. Hence, subjects learned the task with 36 trials of 10 s each interleaved with 10 s breaks, tapping with their right hand for a total duration of 12 min. On the following day, we tested the original (right hand) memory. Participants of the Interference group performed 9 trials using the left hand (novel memory), and after a 10 min break performed 9 additional trials using the right hand (the original memory practice on the previous day). On the same day, participants of the No-Interference group performed only the original memory (right hand) trials.

To test if memory reactivation could indeed prevent interference, subjects in experiment 2 learned the task for 12 min, as in experiment 1. On the next day, the pre-reactivation day, they completed a short practice with the right hand for 9 trials of 10 s each [7]. On the following day, the reactivation day, participants in the Reactivation group performed one trial of the original memory (right hand), and after a 5 min break performed tapping of the right (original memory, 3 trials) and the left (novel memory, 3 trials) hand in an interleaved manner. Participants in the No-Reactivation group performed on that day 3 novel-memory trials (left hand), similar to the Reactivation group, but without memory reactivation. On the final day, the interference test day, all participants performed 9 trials using the left hand, and after a 10 min break performed 9 additional trials using the right hand.

Experiment 3 was conducted to test whether the prevention of interference was a long-term effect. 23 participants from experiment 2 returned for another test session consisting of 9 trials with the left hand, followed by a 10 min break and 9 trials using the right hand. Participants arrived to this long-term test-session after a period of 45 days in average (SD = 14.4 days) from the first session.

In experiment 4, designed to test whether interference is prevented by practice per se or due to reactivation-reconsolidation mechanisms, 10 participants completed three weekly sessions: encoding, reactivation and interference-test. In the encoding session, on the first day participants conducted 12 trials of 30 s each, in which they practiced the same 5 digit sequence as in experiments 1-3 using the right hand. In the reactivation session, participants completed a single reactivation trial for 30 s with their right hand, and

after a 10 min or 6 hr break, completed a single trial using the left hand. To reduce variability, this experiment had a within-subjects design across two subsequent weeks. Since previous studies showed no transfer of learning between two different sequences in the motor sequence learning task [2, 18], participants performed the same procedure on both weeks, changing the tapped sequence (either 4-1-3-2-4 or 2-3-1-4-2) [2]. Participants who completed the task with a 10 min break on the first week, completed a 6 hr break on the second week, and vice versa. On test day, the third day, participants completed 3 trials (30 s each) with the left hand, followed by a 10 min break and 3 trials using the right hand. The order of conditions (length of breaks on the reactivation day) and sequences were counter-balanced across participants.

To further validate the specificity of these findings to reactivation-based mechanisms, we conducted experiment 5. This experiment tested whether the same structure and timing of trials as in experiment 4, would prevent interference if conducted following encoding of the original memory (i.e., dissociated from reactivation, which can only be induced after memories have been fully consolidated). As in experiment 4, on the first day participants here performed 12 trials of 30 s each, in which they practiced the same 5 digit sequence as in all other experiments, using the right hand. Immediately following this session, participants of the Experimental Group (n = 9) performed two additional trials: a single right-hand trial, and after a 10 min break, a single trial using the left hand (similar to the reactivation session in experiment 4). Meanwhile, participants in the control group (n = 11) similarly encoded the original memory, without the additional left and right hand trials. On the next day, participants performed a standard test session, consisting of 3 trials (30 s each) performed with the left hand, followed by a 10 min break and 3 trials performed with the right hand.

QUANTIFICATION AND STATISTICAL ANALYSIS

Data Analysis

The most common motor sequence learning end-point measure was used, measuring the number of correct sequences completed on each trial. As in procedural tasks the within-session improvements tend to be higher than the between-sessions improvements (i.e the ''offline gains''), the common method of analysis is to compare the performance at the end of a session with the performance at the beginning of the next session, when examining the between-session gains [2, 7, 12, 13, 33, 44, 45]. Thus, performance was measured as the average of the first three trials of each session, with learning measured as the difference between performance at the end of encoding session (three last trials) and the first three trials of test session on the following day. For consistency between the experiments, in experiments 4-5 one 30 s trial (instead of three 10 s trials) was taken for similar analysis. Consistent with previous studies [12], to exclude effects of fatigue we excluded the last trial in cases in which there was a sudden drop in that trial of 25% or more in performance and a 3-times-or-more increase in tapping errors performed.

Performance was normalized by Individual Learning Centiles (ILC) dividing each participant's learning curve by 100, that is, the difference between the maximal number of correct sequences per trial tapped on encoding days (first two days in experiment 2-3, and first day in experiments 1,4-5) and the initial correct number of sequences (when trials of 10 s were performed, as in experiment 1-3, the average of 3 first trials was used. In experiments 4-5, which included trials of 30 s, the single first trial was used):

$$ILC = \frac{\max(learning\ curve) - mean(trials_{1,2,3})}{100}$$

Then, performance on each trial(i) was calculated as:

$$Performance_i = \frac{CorrectSequences_i - mean(trials_{1,2,3})}{ILC}$$

Statistical Analysis

A one-way ANOVA with a group factor was used to test for between groups differences in performance (Figures 1B, 3B, and S2). Repeated-measures ANOVA was conducted in experiments 1-3 to evaluate interference or offline gains between sessions, by comparing normalized performance at pre- and post-reactivation sessions for each group (Figures 1D, 2D, and 2E). To test differences in baseline performance Repeated-measures ANOVA was performed on all trials of the first encoding sessions, with group as between subjects factor (Figures S1 and S2). In addition, in experiment 4, a one-tailed paired t test was conducted to test the hypothesis that performance improves when the novel memory is presented within the reconsolidation window (Figure 4). Standard error-rates of 0.05 were used.