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Research

Reconsolidation of declarative memory in humans

Cecilia Forcato,[1] Valeria L. Burgos,[2] Pablo F. Argibay,[2] Victor A. Molina,[3]
María E. Pedreira,[1,4] and Hector Maldonado[1,4,5]

[1]Laboratorio de Neurobiología de la Memoria, Departamento de Fisiología y Biología Molecular y Celular, IFIBYNE-CONICET,
Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, C1428EGA Buenos Aires, Argentina; [2]Unidad de Ciencias
Cognitivas, Instituto de Ciencias Básicas y Medicina Experimental, Hospital Italiano de Buenos Aires, C1181ACH Buenos Aires,
Argentina; [3]Departamento de Farmacología Facultad de Ciencias Químicas, Universidad Nacional de Córdoba,
5000 Cordoba, Argentina

The reconsolidation hypothesis states that a consolidated memory could again become unstable and susceptible to facilitation or impairment for a discrete period of time after a reminder presentation. The phenomenon has been demonstrated in very diverse species and types of memory, including the human procedural memory of a motor skill task but not the human declarative one. Here we provide evidence for both consolidation and reconsolidation in a paired-associate learning (i.e., learning an association between a cue syllable and the respective response syllable). Subjects were given two training sessions with a 24-h interval on distinct verbal material, and afterward, they received at testing two successive retrievals corresponding to the first and second learning, respectively. Two main results are noted. First, the first acquired memory was impaired when a reminder was presented 5 min before the second training (reconsolidation), and also when the second training was given 5 min instead of 24 h after the first one (consolidation). Second, the first retrieval proved to influence negatively on the later one (the retrieval-induced forgetting [RIF] effect), and we used the absence of this RIF effect as a very indicator of the target memory impairment. We consider the demonstration of reconsolidation in human declarative memory as backing the universality of this phenomenon and having potential clinical relevance. On the other hand, we discuss the possibility of using the human declarative memory as a model to address several key topics of the reconsolidation hypothesis.

The reconsolidation hypothesis states that a consolidated memory could again become susceptible to facilitation or impairment for a discrete period of time after retrieval (Nader et al. 2000a; Sara 2000a). It was initially supported by results obtained with rodents and then confirmed with animals belonging to very different species, such as the crab, chick, fish, and freshwater snail (Anokhin et al. 2002; Eisenberg et al. 2003; Pedreira and Maldonado 2003; Sangha et al. 2003). The range was extended to humans, including the procedural memory of a motor skill task (Walker et al. 2003); however, the reconsolidation hypothesis has not yet been demonstrated for the declarative memory. This demonstration would substantially back the universality of the phenomenon, but what is more, would open the possibility of discussing the reconsolidation hypothesis from the viewpoint of the declarative memory, that is, from a type of memory that is the hallmark of man (Dudai 2002). Contentious or still conjectural topics of the reconsolidation hypothesis such as its functionality (Dudai and Eisenberg 2004; Debiec et al. 2006; Sara and Hars 2006), or the reminder's requirements (Pedreira et al. 2004), would surely gain a new perspective of analysis.

In most of the precedent studies with diverse animal species, memory reconsolidation is demonstrated by the amnesic effects induced by the administration of blockers, such as the protein synthesis inhibitors or β-blockers (Przybyslawski et al. 1999; Nader et al. 2000a), or also by the learning of a new memory (Walker et al. 2003; Boccia et al. 2005), after the presentation of a reminder. The current method to demonstrate such amnesic effects is by disclosing at testing a defective retrieval of the target

memory. The common sense of William James had already pointed to the fact that the way to study memory is through its retrieval, saying that the only proof of its retention is that it can be recalled (James 1890; Sara and Hars 2006). However, this direct method of evaluation may be sometimes misleading or inapplicable, mainly when the declarative memory is involved. In fact, memories are not stored in isolation from other memories but integrated into complex associative networks (Levy and Anderson 2002; Berman et al. 2003; Debiec et al. 2006), and then the activation of related traces may interfere with the expression of the desired retrieval, as has been documented over a century of research (McGeoch 1932; Postman 1971; Anderson and Neely 1996). In other words, a faulty retrieval at testing may be due to either problems in encoding storage or simultaneous retrieval of related information (Mayes and Downes 1997), and then this lack of specificity robs this direct method of its value to disclose deficits in memory.

In the present study, we are proposing an alternative method, based on the forgetting effect that the retrieval of the target memory could have on the recall of related memories. This effect, termed retrieval-induced forgetting (RIF) (Anderson et al. 1994; MacLeod and Macrae 2001), shows that the act of remembering can temporarily block a late retrieval of other memory, or more specifically, the expression of its retrieval. For this effect to be possible, the inducing memory must be intact, and therefore, the absence of RIF might become a good indicator of a defective target memory. That is, we are proposing that memory deficits were not revealed by the flaw of its own retrieval but by the good retrieval of a related memory.

Here we demonstrate consolidation and reconsolidation in paired-associate learning (i.e., an association between a cue syllable and the respective response syllable). Subjects were given

two training sessions, with an interval of 24 h, during which they learned two distinct verbal materials. Afterward, they received at testing two successive retrievals: the first one corresponding to the first learning, and the second retrieval to the second one. The first memory was impaired when the second learning occurred immediately after the first one (consolidation) or up to 6 h after the reminder presentation (reconsolidation). In both cases, the memory impairment was disclosed not because the first retrieval was faulty but because there was no RIF (Levy and Anderson 2002).

Results

The paired-associate memory

This first section of results deals with paired-associate learning experiments (i.e., an association between a cue syllable and the respective response syllable).

An analysis of the interaction between retrievals at testing: The L2–training given 24 h after L1–training fails to impair consolidation

In the first experiment subjects were trained on day 1 with L1 and on day 2 with L2, to be finally tested on Day 3, either with L1-testing (TL1) followed by L2-testing (TL2) or the other way around (Fig. 1). The purpose of this experiment was focused on the study of possible interferences between L1 and L2 retrievals at testing session and on evaluating their use as a method to disclose deficits in the L1 memory, i.e., the memory acquired during the first training with L1 (for term definitions, see Table 1).

The expression of the L1–retrieval at the first phase of testing is reduced by the interference of simultaneous recruitment of items from the L2 memory and/or by a loss of the L1–memory impairment

Results shown in Figure 1 (right panels) correspond to six retrievals at the testing session of the respective groups A, B, L1-CTL, and L2-CTL, analysis of variance (ANOVA); $F_{(5,54)} = 10.546$, $P < 0.001$. If the analysis is confined to phase 1 of groups A and B, we find that the L1-retrieval at phase 1 (group A) had a number of errors significantly higher than that of the respective control L1-CTL ($P < 0.001$), whereas the performance for the L2-retrieval at phase 1 (group B) proved to be as good as that for the L2-CTL ($P = 0.546$). The poor performance during the L1-retrieval at phase 1 could be explained in terms of an interference from retrieval of L2 on the expression of the L1-retrieval (L2 → L1), namely, the retrieval of the L1-memory simultaneously recruits items from the L2-memory and these items interfere with the expression of the L1-retrieval (retrieval interference) (McGeoch 1932). On the other hand, the good performance during the

L2-retrieval at phase 1 indicates absence of interference in the opposite direction (L1 → L2). This disparity is attributed to a difference between the memory strength for L1 and that for L2, due to the fact that the former was acquired 48 h and the latter 24 h before the test session (Ebbinghaus 1885; Wixted and Ebbesen 1997). Therefore, the strongest memory interferes with the retrieval of the weaker one but not vice versa.

However, an alternative explanation of the poor performance of L1-retrieval at testing could be offered. The poor expression of the L1-retrieval at first phase of testing may be due not only to the simultaneous recruitment of the L2 memory but also (and/or) by a loss of the L1-memory, that is, by an impairment of the L1-memory consolidation because of the second training with L2.

The expression of the L2–retrieval at phase 2 of testing is negatively influenced by the previous L1–retrieval at phase 1

If the analysis of results from Experiment 1 (Fig. 1) is now confined to those of the L2-retrieval, we find a faultless expression of L2-retrieval at phase 1 (group B) but a poor one at phase 2 (group A), in comparison with L2-CTL ($P = 0.516$ and $P < 0.001$, respectively). This difference indicates that regardless of whether the retrieval of a memory is preceded by that of a related memory, the first retrieval influences negatively the expression of the second one, which represents an instance of RIF (Anderson et al. 1994; Levy and Anderson 2002). Since this effect depends on the integrity of the memory first recalled (Anderson et al. 1994), the result indicates that the L1 memory is intact, although its expression was reduced by the interference of the simultaneous retrieval of L2 memory. Therefore, we conclude that the second training given 24 h after the first one does not impair the consolidation of the L1-memory.

The absence of the RIF effect is a specific indicator of deficits in the target memory

The preceding analysis of results was based on the postulation that the L1-retrieval at the first phase of testing not only interferes with its own expression due to the simultaneous recruitment of L2 items, but it also interferes, provided that the L1-memory was intact, with the expression of the later L2-retrieval (RIF effect). In the cases of L1-memory impairment, as is expected to be found in the following series of experiments aimed at studying impairments of L1-memory consolidation and reconsolidation, a double effect should be expected: (1) a defective expression of the L1-retrieval at phase 1 and (2) a reduction or elimination of the RIF. The first effect cannot be distinguished from that of the interference due to the simultaneous recruitment of L2 items, whereas the second one specifically reveals memory impairment. That is, a poor performance for L1-retrieval at testing may be interpreted as loss of L1-memory and/or interference with its own expression by simultaneous recall of L2 items; instead, the absence of RIF is only regarded as an expression of deficits in the L1-memory. Namely, the absence of RIF, but not the failure of L1-retrieval, is specific of L1-memory impairment.

Keeping in mind the above considerations, results of the next series of experiments will be evaluated by the RIF effect from the L1-retrieval at phase 1 on the expression of L2-retrieval at phase 2. A number of errors for L2-retrieval at phase 2 (panel A) higher than that of the L2-CTL would indicate no impairment
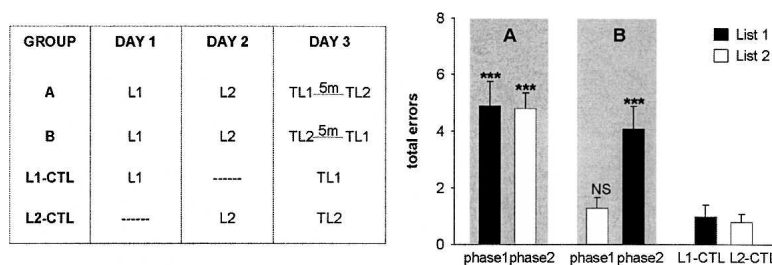

Figure 1. Analysis of interaction between retrievals at testing. (*Left*) Groups ($n = 10$): L1-CTL, L1-control; L2-CTL, L2-control; A, Group A, B, Group B; L1, L1-training; L2, L2-training; TL1, L1-testing; TL2, L2-testing; 5m, the 5-min interval between testings. Phase 1, the first list tested (TL1 or TL2); Phase 2, the second list tested (TL1 or TL2). L1-CTL and L2-CTL were evaluated on Day 3 with TL1 or TL2, respectively. Group A was evaluated with TL1 on phase 1 and TL2 on phase 2; Group B was evaluated with TL2 on phase 1 and TL1 on phase 2. (*Right*) Mean of total errors ± SEM on Day 3. Black bars, TL1 performance; white bars, TL2 performance. ***, $P < 0.001$.

Table 1. Definitions of terms

Term	Definition
Retrieval-induced forgetting (RIF)	Refers to the phenomenon in which the act of remembering some material disrupts temporally the retrieval expression of related material (Anderson et al. 1994).
Retrieval interference	Refers to the phenomenon in which the act of remembering some material recruits simultaneously items from related material producing an impairment in the expression of the desired retrieval (McGeoch 1932).
Paired-associate memory	A memory generated by a verbal learning consisting of the association between a cue syllable and a response syllable.
Prediction memory	A memory generated by the association between a specific context and the verbal learning.
Context period	Initial period of time of each trial where the context is formed. It consists of a fixed sequence of light, image, and sound.
Specific context	Combination of light, image, and sound that is always followed by the verbal learning.
Syllable period	Period of time where the nonsense syllables are presented.
Cue-response syllables	Pairs of nonsense syllables of the verbal learning.
Actual trial	It includes the specific context followed by the syllable presentation.
Fake trial	It includes a context that is never followed by the syllable presentation.
L1-training (or L1)	It consists of 10 actual trials interspersed with 22 fake trials separated by a 3-sec intertrial interval. Each actual trial includes the List 1 presented with the respective specific context.
L2-training (or L2)	It consists of 10 actual trials interspersed with 22 fake trials separated by a 3-sec intertrial interval. Each actual trial includes the List 2 presented with the respective specific context.
L1-memory	Memory acquired by the L1-training (the target memory).
L2-memory	Memory acquired by the L2-training.
Reminder	Trial that includes the specific context of L1-training plus the presentation of one cue syllable, which is abruptly interrupted.
TL1 (or L1-Testing)	Test of the L1 memory consisting of four actual trials interspersed with eight of the respective fake trials.
TL2 (or L2-Testing)	Test of the L2 memory consisting of four actual trials interspersed with eight of the respective fake trials.
Phase 1	Period of the test session where one of the two lists (L1 or L2) is first retrieved.
Phase 2	Period of the test session where one of the two lists (L1 or L2) is retrieved 5 min after phase 1.
L1-retrieval	Retrieval of the prediction and paired-associated memory corresponding to the L1 memory.
L2-retrieval	Retrieval of the prediction and paired-associated memory corresponding to the L2 memory.
Group A (A)	Experimental groups that are tested for L1 in phase 1 and for L2 in phase 2.
Group B (B)	Experimental groups that are tested for L2 in phase 1 and for L1 in phase 2.
L1-CTL (or L1-control)	Control group that is only trained and tested with L1 and TL1, respectively.
L2-CTL (or L2 control)	Control group that is only trained and tested with L2 and TL2, respectively.

of the L1-memory. In contrast, a performance for L2-retrieval at phase 2 similar to that of the L2-CTL would indicate a deficit of the L1-memory.

The L2–training given 5 min after L1–training impairs consolidation of L1–memory

The purpose of the next experiment was to explore whether the consolidation of the target memory (the L1-memory) could be affected by giving the L2-training on Day 1 immediately after the L1-training. Therefore, the protocol of this experiment (Fig. 2) was similar to above except for the interval between trainings, which in this case was only 5 min. Results of the testing session on Day 3 are exhibited in Figure 2 (right panels) (ANOVA, $F_{(5,54)} = 11.5$, $P < 0.001$). The analysis of data corresponding to group A discloses a significant difference between mean errors of L1-retrieval at phase 1 and that of the L1-CTL ($P < 0.002$) but not between L2-retrieval at phase 2 and the L2-CTL ($P = 0.323$). This picture of results is indicative of an absent or insufficient RIF effect as a consequence of deficits in the L1-memory. On the other hand, the mean errors of L2-retrieval at phase 1 (group B) was similar to that of L2-CTL ($P = 0.710$), a result that was expected since the impairment of L1-memory rules out the possibility of an interference from the L1-retrieval (ANOVA, $F_{(5,54)} = 5.091$, $P < 0.001$).

In summary, the L2-training given 5 min after the L1-training, impairs the consolidation of the memory previously acquired. The deficit of L1-memory consolidation is specifically disclosed by the absence or reduction of the RIF effect.

Study of the effect of L2–training given at diverse intervals after reminder on reconsolidation of L1–memory

The following experiments were aimed at testing the reconsolidation phenomenon (Fig. 3). In Experiment 1, subjects were trained with L1 on Day 1 and with L2 on Day 2, 5 min after the reminder presentation. The reminder trial included the specific context of L1-training plus the presentation of one cue syllable, which was abruptly interrupted after 2 sec of exposure, thus not allowing the subject to write down the response syllable. Results of test session on Day 3 (ANOVA, $F_{(5,54)} = 10.546$, $P < 0.001$) were closely similar to those obtained above as to memory consolidation. The analysis of data of group A reveals a significant difference between mean errors of L1-retrieval at phase 1 and that of the L1-CTL ($P < 0.001$) but not between L2-retrieval at phase 2 and the L2-CTL ($P = 0.255$). This picture of results is indicative of an absent or insufficient RIF effect as a consequence of deficits in L1-memory. On the other hand, results in group B repeat those obtained throughout this paper, namely, the mean errors of L2-retrieval at phase 2 were as that of the L2-CTL ($P = 0.568$) and the performance of L1-retrieval at phase 2 was higher than that of the L1-CTL ($P < 0.001$).

The remaining two experiments of this series were carried out with the goal of pointing out the time window of the L1-memory reconsolidation. The results obtained with L2-training given 6 h after reminder (Fig. 3, Experiment 2) (ANOVA, $F_{(5,54)} = 13.038$, $P < 0.001$) showed a significance difference ($P < 0.002$) for L1-retrieval at phase 1 vs. L1-CTL, and no difference ($P = 0.214$) for L2-retrieval at phase 2 vs. L2-CTL. These findings were similar to those obtained with L2-training tested 5 min after reminder (Fig. 3, Experiment 1), disclosing no RIF effect and then a weakened L1-memory. Instead, results with the L2-training given 10 h after reminder (Fig. 3, Experiment 3), showed a significant difference for L1-retrieval at phase 1 vs. L1-CTL ($P < 0.001$), and also for L2-retrieval at phase 2 vs. L2-CTL ($P < 0.004$), indicating an RIF effect and L1-memory intact.

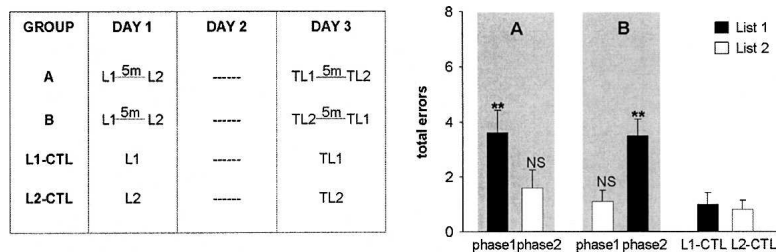Therefore, the L2-training given 5 min or 6 h after present-

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Figure 2. Effect of an interfering training on memory consolidation. (*Left*) Groups (*n* = 10): controls, A, B, L1, L2, TL1, TL2, Phase 1, and Phase 2, as in Figure 1; 5m, the 5 min between trainings or between testings. (*Right*) Mean of total errors ± SEM on Day 3. **, $P < 0.01$.

ing the reminder impairs the memory of L1 acquired 24 h before. On the contrary, the same training given 10 h after the reminder leaves the first memory intact, suggesting a period of reconsolidation longer than 6 h and shorter than 10. This period appears longer than that generally estimated for consolidation but the comparison is not possible here since we have not investigated the temporal window for consolidation. The deficit of L1-memory in the two former experiments is specifically disclosed by the absence or reduction of the RIF effect.

Figure 3. Effect of an interfering training on memory reconsolidation. (Experiment 1) (*Left*) Groups (*n* = 10): controls, A, B, L1, L2, TL1, TL2, Phase 1, and Phase 2, as in Figure 1; R, the reminder trial; 5m, the 5 min between the reminder trial and L2, or between testings. (*Right*) Mean of total errors ± SEM on Day 3. ***, $P < 0.001$. (Experiment 2) (*Left*) Groups (*n* = 10): controls, A, B, L1, L2, TL1, TL2, Phase 1, and Phase 2, as in Figure 1; 6h, the 6 h between the reminder trial and the L2 training; 5m, the 5 min between testings; R, as in Experiment 1. (*Right*) Mean of total errors ± SEM on Day 3. **, $P < 0.01$. (Experiment 3) (*Left*) Groups (*n* = 10): controls, A, B, L1, L2, TL1, TL2, Phase 1, and Phase 2, as in Figure 1; 10h, the 10 h between the reminder trial and the L2 training; 5m and R, as in Experiment 1. (*Right*) Mean of total errors ± SEM on Day 3. **, $P < 0.01$; ***, $P < 0.001$.

The impairing effect of the L2–training on the target memory reconsolidation lasts at least for 48 h

In order to test the disruptive effect on memory reconsolidation, 48 h after the L2-training, the following two experiments were performed (Fig. 4). The protocol of Experiment 1 with groups A, L1-CTL, and L2-CTL, ANOVA, $F_{(3,36)} = 10.355$, $P < 0.001$, was similar to the one shown above in Figure 1; however, the test session was carried o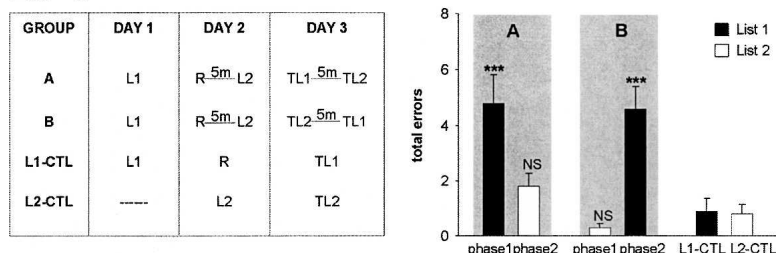ut 72 h rather than 48 h after L1-training. Results of group A (Fig. 4, Experiment 1) again show a RIF effect since the number of errors for L1-retrieval at phase 1 and L2-retrieval at phase 2 were higher than those of their respective control groups, L1-CTL ($P < 0.001$) and L2-CTL ($P < 0.002$). Thus, the RIF is disclosed at a test session carried out 72 h after the first training which denotes an enduring L1-memory.

The protocol of Experiment 2 (Fig. 4) includes group A, L1-CTL, and L2-CTL and a reminder placed 5 min before the L2-training. The pattern of results, (ANOVA, $F_{(3,36)} = 3.869$, $P < 0.017$) proved to be closely similar to that of Figure 3, Experiment 1, since the mean error of L2-retrieval at phase 2 was similar to that of the L2CTL ($P = 0.153$), whereas that of L1-retrieval at phase 1 resulted higher than the mean error of the respective L1-CTL ($P < 0.001$). Therefore, the absence of RIF is shown not only at the test session given at 48 h, but also 72 h after the first training. This lasting absence of RIF effect discloses an enduring deficiency in the L1-memory, namely, the demonstration that the disrupting effect on the memory reconsolidation persists no only for 24 h, but for 48 h after post-reminder L2-training.
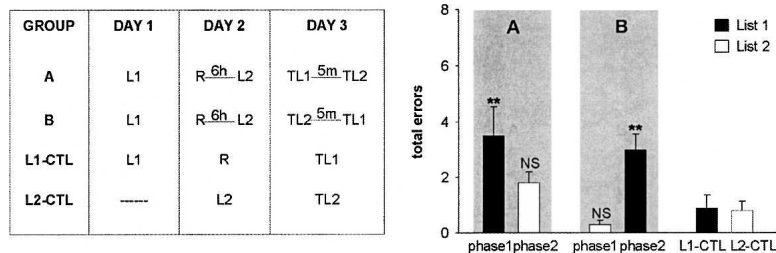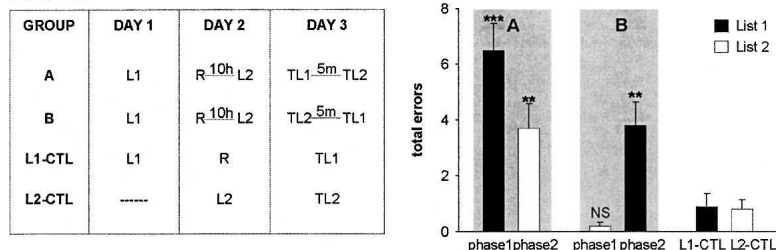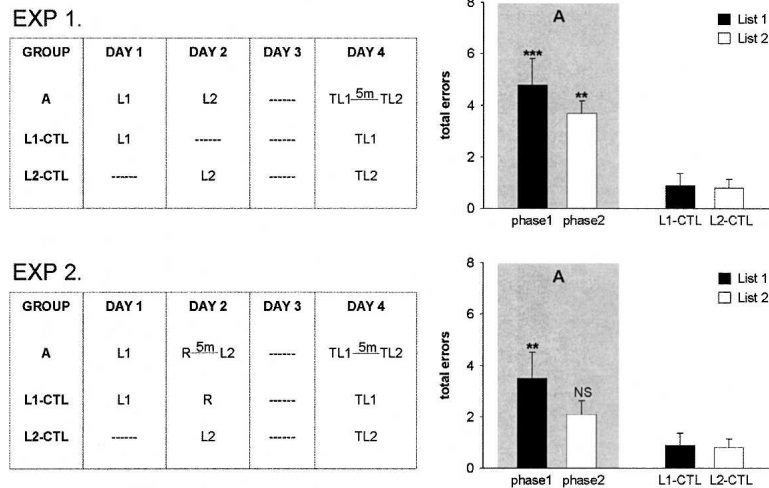
The impact of the context period on the paired–associate learning and the uniformity of trainings

The control groups, L1-CTL and L2-CTL, evidenced very good retention of the paired-associate memory up to at least 4 d after the verbal material was acquired. The range of mean errors per retrieval was between 0.8 and 1.0 out of 20. We attempt here to determine to what extent the inclusion of a period of context formation before syllables presentation could have improved the performance of the subjects. A first group of 10 subjects (the context group) was given 10 L1-actual trials, each with 20 sec of context period (red light + New York City + jazz) followed by the syllables period, and separated by a 3-sec intertrial interval. A second group of 10 subjects (the no-context group) started the syllables period 20 sec after the beginning of the trial but with no context formation; therefore, they were illuminated only from the keyboard lamp during the entire trial. Although both groups were given

Figure 4. The effect of an interfering training on memory reconsolidation lasts at least 48 h. (Experiment 1) (*Left*) Groups (*n* = 10): controls A, L1, L2, TL1, TL2, Phase 1, and Phase 2, as in Figure 1; 5m, the 5 min between testings. (*Right*) Mean of total errors ± SEM on Day 4. **, *P* < 0.01; ***, *P* < 0.001. (Experiment 2) (*Left*) Groups (*n* = 10): controls A, L1, L2, TL1, TL2, Phase 1, and Phase 2, as in Figure 1; 5m, the 5 min between the reminder trial and L2-training, or for the 5 min between testings; R, as in Figure 3. (*Right*) Mean of total errors ± SEM on Day 4. **, *P* < 0.01.

the same paired-associate training (i.e., the same pairs of cue syllable and response syllable), there was a significant difference in the number of response errors at L1-testing between both groups (ANOVA, $F_{(1,18)}$ = 6.015, *P* = 0.027) (Fig. 5A).

Moreover, we try to explore the degree of uniformity of the performances at paired-associate trainings throughout the experiments of this study. For this purpose, we compare the mean errors for the last four actual trials of the training tail (Fig. 5B) corresponding to the control groups of the seven experiments (L1-CTL and L2-CTL). An ANOVA of these values across experiments and type of training (L1, L2), indicated that there was no experiment or type of training effect or interaction (*P* = 0.961, 0766, and 0.998, respectively). That is, there was a noticeable uniformity in the acquisition course of the paired-associate memory through experiments and type of training.

The prediction memory

Over training, subjects learn to identify the specific context, namely, the only light-image-sound sequence that is followed by the syllables display. The level of the prediction memory was assessed by the number of proper responses in pressing the expectancy keys (YES or NO) in both actual and fake trials. We compare the learning curves of prediction with those of learning to respond to a cue syllable. In Figure 5B, we show this contrast for the L1-training of the experiment shown above in Figure 1. The successive regression analysis (Lozada et al. 1990) revealed different values for both asymptote and slope for either the prediction or the paired-associate learning (asymptotic trial: 8 and 21, respectively; slope value: −12.867 and −4.898, respectively). This result is consequently shown for all the 38 training curves of the paper.

The mean of accumulative errors corresponding to either the training tail or the retrieval (four actual trials + eight fake trials) is practically equal to zero for the prediction memory throughout this paper. In short, subjects learn quickly to distinguish the specific context and predict the immediate appearance of the syllables, as well as to retrieve practically without errors the response syllables, even in those experiments that included memory impairments.

Discussion

The main finding of this study is the demonstration, in humans, that previously consolidated declarative memory returns to a labile state and becomes subject to stabilization again. This process of labilization-reconsolidation is triggered by the presentation of a reminder and characterized by the possibility that a second training can impair the declarative memory within a time window.

The demonstration backs the universality of the reconsolidation phenomenon, which is consistent with the idea that general principles of memory organization, as well as basic components of the mechanisms serving memory, would be used across evolution by phylogenetically very disparate animals (Carew 2000; Pedreira and Maldonado 2003). Moreover, the conclusion that a consolidated declarative memory can be actually disrupted after a reminder has potential clinical relevance as has been suggested several times (Bustos et al. 2006; Debiec et al. 2006; Foa 2006).

In order to analyze how the reminder triggers reconsolidation, it seems pertinent to deal separately with the two mnemic processes that are involved in the present study: the paired-associate memory, and the one we term the prediction memory. The reminder of the former memory consisted of the presentation of the L1 context (red light + New York City + jazz) and immediately then, one cue syllable on the left side of the monitor's screen and the response box on the right. However, 2 sec later the trial was abruptly interrupted. All the contextual parameters and the cue syllable vanished and only the notice on the monitor's screen announcing the unexpected end of the trial remained. Thus, the reminder included also in this case what we consider the double requirement for producing the memory labilization and its following reconsolidation; namely, the initial retrieval of the target memory by presenting the cue syllable and the termination of the reminder without the appearance of the correct response syllable (Pedreira et al. 2004). The memory retrieved by the cue syllable implies, as every memory, an expectation (Dudai 2002), specifically, the subject here expects the reinforcement of the correct response syllable. Therefore, the reminder closure marks the irreversible mismatch between what was expected and what actually occurred. In this framework, we discuss the possible functionality of the reconsolidation process as an answer to such "frustration." A wide range of memory flaws could account for a failed prediction, ranging from outdated to faulty or incomplete information. Therefore, it seems reasonable to suppose (Nader et al. 2000b; Sara 2000b) that labilization-reconsolidation plays a repair role by enabling the system to integrate new information on the background of the past. This memory updating mechanism would not entail an obligatory phase of every retrieved memory but a mechanism of exception, triggered only if the presentation of the unreinforced reminder was terminated and if its duration was sufficiently short (Pedreira et al. 2004). The demonstration that the process of memory reconsolidation also occurs with declarative memory in humans offers the remarkable opportunity of testing straightforwardly the role of this process and its functional value. Experiments may be performed aimed at testing that the reminder necessarily includes an episode of frustration, and also, that the updating of
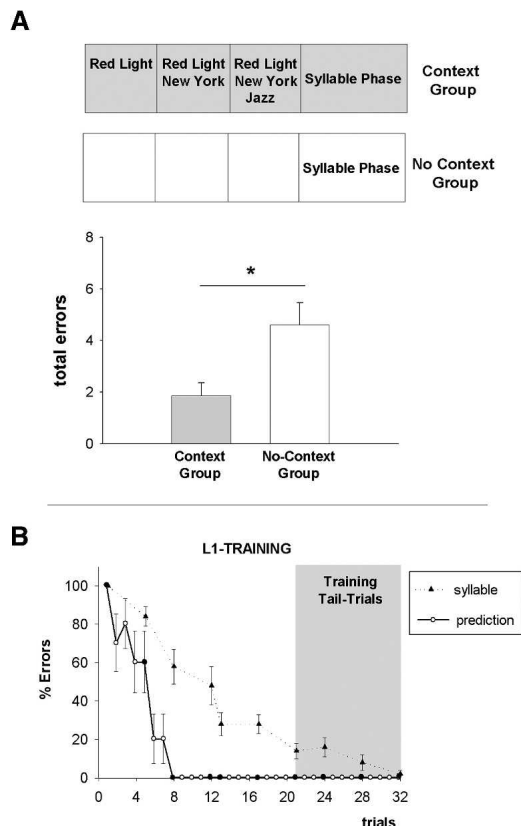
Figure 5. (*A*) Impact of the context phase on the paired-associated learning. (*Upper* panel) Diagram of the training session. The context group received the list of syllables after the presentation of the specific context (5-sec red light, 5-sec red light plus New York City image, and 10 sec of light, image, and jazz music). The No-Context Group, was trained in the same basic context (same dark room, personal computer, earphones) but before the syllable presentation received a 20-sec interval without any light, image, or sound stimulation. (*Lower* panel) Groups ($n = 10$). Gray bar, the Context Group; white bar, the No-Context Group. Mean of total errors $\pm$ SEM on testing, *, $P < 0.05$. (*B*) Training syllable and prediction. Mean percentage errors $\pm$ SEM per trial during training. Dotted line, syllable errors; solid line, prediction errors; black circles, actual trials; white circles, fake trials. The gray rectangle corresponds to the training tail trials (last four syllable training trails and last 12 prediction training trails). The training sessions (L1-training or L2-training) comprised 10 actual trials interspersed in 22 fake trials (total: 32 trials).

information occurs during the reconsolidation period but not when the memory is stable again. However, although results of the present experiments as well as others performed with other animals (Pedreira et al. 2002; Debiec and LeDoux 2004; Suzuki et al. 2004) suggest that reconsolidation is engaged when unexpected events occur, it is pertinent to note that demonstrations of reconsolidation have also been shown using the identical stimuli as was employed during training (Duvarci and Nader 2004; Eisenberg and Dudai 2004).

The reconsolidation of the paired-associate memory was interrupted by the learning of other verbal material (Müller and Pilzecker 1900). The use of a training session as an amnesic agent, instead of some invasive agents such as chemical blockers or physical interferences, has the advantage of being free of undesirable metabolic side effects or physical perturbations. Moreover, although both trainings were given to acquire different verbal materials, they share several parameters of the experimental context as well as the pattern of syllables presentation, thus involving clearly related memories. This gave the possibility of

disclosing retrieval interferences during testing and allowed to use the effect of the RIF as a measure of a deficit in the target memory. The RIF effect was detected in all the experiments of this study except in those that included memory impairments. The memory impairment is not disclosed by failures of its own retrieval but the faultlessness in the retrieval of a related memory. Thus, the absence or defect of RIF, from L1-retrieval on the later L2-retrieval, proved to be an indicator of an impairment of the paired-associate memory. A study that takes into account the retrieval interaction and evaluates through them the deficits in memory storage, seems to be more in keeping with the view that memories are not stored in isolation of other memories but integrated into complex networks (Levy and Anderson 2002; Berman et al. 2003; Debiec et al. 2006), a proposal especially valid in the case of the human declarative memory. Thus, the RIF effect has been used throughout this paper for testing long-term memory and post-reactivation long-term memory, but if it is used at a very short time after learning it will allow us to study intriguing questions of the reconsolidation theory as to how long the memory remains labile but intact after reactivation (Pedreira et al. 2004), and also to test the specificity of the RIF effect (Nader et al. 2000a).

In contrast with what has just been shown, the association between the specific context and the following display of the cue syllables (the prediction memory) was never disrupted by the second training. The prediction memory level was assessed by the number of incorrect responses in pressing the expectancy keys in all of the trials (actual and fake trials). The learning curves fell abruptly, and subjects showed no mistakes after seven to eight trials. At test sessions, all the subjects demonstrated near 100% of correct responses, and no statistical differences were disclosed for any comparison between groups. Thus, the blockade of the consolidation (or reconsolidation) by a second training is found for paired-associate memory but not for the prediction one. This contrast could be explained by the difference in number of trials between prediction and paired-associate learning (10 and 32 trials, respectively), or by the difference in complexity between them, which makes one memory weaker than the other. Alternatively, another account may be offered, at least concerning reconsolidation, based on the hypothesis that for this to take place, the predicted reinforcement should not be delivered during the entire reminder presentation (Pedreira et al. 2004). According to the experimental protocol, however, the reminder trial ends after a 2-sec exposure of the cue syllable. Thus, for the paired-associate memory, the promised reinforcement failed to occur, while for the prediction memory, it was fulfilled. Therefore, the same reminder fails to produce reconsolidation if the recalled memory includes promises that are fulfilled, and triggers reconsolidation if it includes predictions that fail.

At this juncture, we would like to note two new perspectives opened by the present results. First, it is of great value from a clinical point of view, that a traumatic or pathogenic memory could be reactivated by selective reminders and disrupted not only by a pharmacological agent but also by other learning (Bustos et al. 2006; Debiec et al. 2006; Foa 2006). Second, the possibility is very promising for future reconsolidation studies of using the human declarative memory as a model to address contentious or still conjectural topics about reconsolidation, such as its functionality (Frenkel et al. 2005; Debiec et al. 2006) or its role in updating information (Sara and Hars 2006). It is pertinent to add that in a recent work, published when the present manuscript was under revision, it was reported that human episodic memories can undergo reconsolidation (Hupbach et al. 2007); however, the omission of the RIF analysis deprives this work of relevant information that would allow discarding alternative explanations for the reminder effect.

Materials and Methods

Subjects

Two-hundred-eighty healthy undergraduate and graduate students from Buenos Aires University volunteered for the study (60% women, 40% men). Before their participation in the experiment, subjects signed an informed consent approved by the Comité independiente de Ética para Protocolos de Investigación (CEPI) Hospital Italiano de Buenos Aires. Their ages ranged from 18 to 35 yr, with a mean of 25. The participants were randomly assigned to one of 40 groups.

Procedure

Context formation and syllables presentation

Experiments took place in a dark room and were conducted using a personal computer. Each subject was provided with earphones, and seated in front of a monitor placed ahead of a large screen on the back wall (Fig. 6A). Each training trial included a first period during which the context was formed (the context period), followed by a second one during which a series of nonsense syllables were presented as paired associates (the syllable period). The context period comprised a fix sequence of three accumulative steps: a first step of light alone projected on the large screen for 5 sec; a second step of light plus image for 5 sec, with the addition of an image displayed on the monitor's screen; and a third step of light plus image plus sound for 10 sec, with the addition of a sound coming through the earphones. The specific context persisted during the syllable presentation (Fig. 6B). The syllable period that follows the former, started with the presen-

tation of a cue syllable on the left side of the monitor's screen and an empty response box on the right. Each cue syllable was taken at random and successively from a list of five. Subjects were required to write down the corresponding response syllable before 5 sec. After finishing that period three situations were possible: first, if no syllable was written, the correct one was shown for 4 sec; second, if an incorrect syllable was written, it was replaced by the correct one and it was shown for 4 sec; and third, if the correct response was given, it stayed for a further 4 sec. Immediately after, another cue syllable was shown and the process was repeated until the list was over. All together, an actual trial lasted 65 sec (20 sec for the context period and 45 sec for the syllable presentation).

The L1–training session

A trial of the List 1 (L1)-training had the three steps of the context formation, each with two possible options: the light could be red or blue; the image, a picture of Ravello or one of New York City; the sound, a jazz song or an Italian one. Only one combination of these options (the specific context) was followed by the syllables presentation of List 1. The trial that includes specific context followed by the syllables presentation is termed the actual trial, while the others with only context (i.e., without syllables presentation) are called the fake trials. In L1-training, the specific context of the actual trial was: red light + picture of New York City + jazz melody.

The L1-training consisted of 10 actual trials interspersed with 22 fake trials (total: 32 trials), separated by a 3-sec intertrial interval. List 1 was constituted by five pairs of nonsense cue-response syllables in rioplatense Spanish: **ITE**-OBN, **ASP**-UOD, **FLI**-AIO, **NEB**-FOT, **COS**-GLE (bold type: cue syllable; regular type: response syllable) (Fig. 6C). Subjects that failed to reach 70% of correct responses during the last four actual trials were excluded.

To evaluate the prediction memory (the association between the specific context and the syllable presentation), subjects were instructed to press the YES or NO button (the expectancy keys) on the keyboard 3 sec after the last step of the light–image–sound sequence has started. The subject has to press YES if he thinks that the syllable presentation is to come about, NO in the opposite case.

The L2–training session

A trial of the L2-training had the three steps of the context formation, each with two possible options: the light could be green or yellow; the image, a picture of a forest or one of Waikiki beach; the sound, a symphony or a blues song. In the L2-training, the specific context of the actual trial was: green light + picture of the forest + symphony.

The L2-training consisted of 10 actual trials interspersed with 22 fake trials (total: 32 trials), separated by a 3 sec intertrial interval. List 2 was constituted by five pairs of nonsense cue-response syllables in rioplatense Spanish: **OEN**-SRO, **DRI**-CRE, **AIC**-POA, **TIU**-PLA, **KEC**-CLO (bold type: cue syllable; normal type, response syllable) (Fig. 6C). Subjects that failed to reach 70% of correct responses during the last four actual trials were excluded.

To evaluate the prediction memory, subjects were instructed to press the YES or NO button, as above.
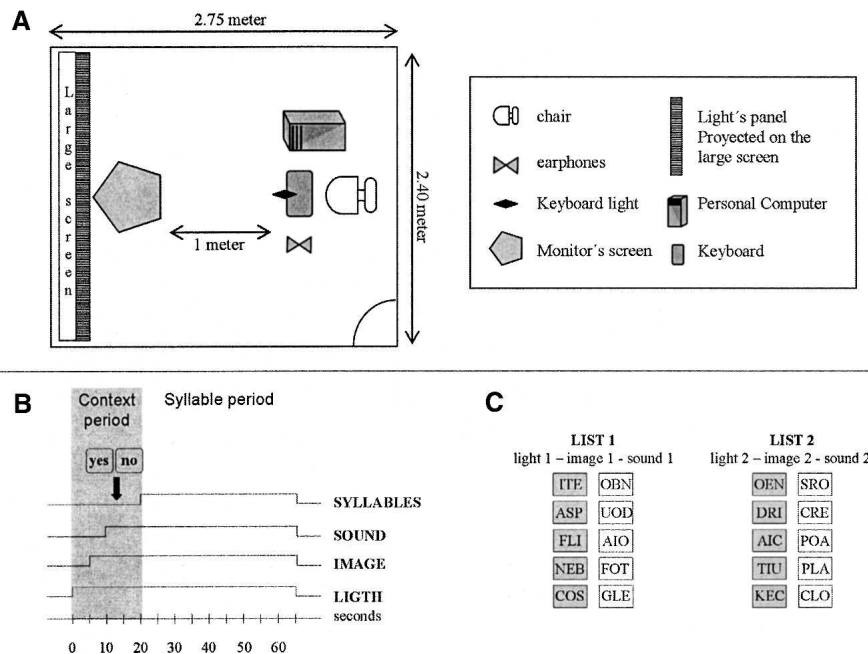

Figure 6. Experimental protocol. Subjects were submitted to two different training sessions (L1-training and L2-training), separated by a time interval, in a 3-d experiment. During each training trial, the subject was seated in front of a monitor placed ahead of a large screen. (*A*) Diagram of the experimental room. Symbols are explained in the *right* panel. (*B*) An actual trial included a sequence of three accumulative steps termed the specific context: first step, light (color illumination of the large screen); second step, image (a picture on the monitor); third step, sound (music melody from earphones). Ten seconds after presentation of the sound, five pairs of cue-response syllables were presented successively and at random order (each pair consisted of two squares superimposed on the monitor's image: the *left* square was the cue box, which includes a nonsense syllable of three letters, and the *right* one, the empty response box, was where the subject was required to write down the corresponding syllable). Expectancy keys: Participants were told that as soon as the sound presentation was started they should press one of the two keys. "Yes," I expect the cue-response display comes: "No," I don't. (*C*) Lists: L1 and L2 differed each other in cue-response pairs as well as in color, light, image, and sound that precede them or those of the respective fake trials.

Testing

The testing session consisted of two retrievals of verbal material presented in alternative order: TL1 corresponding to the first training (L1) followed by TL2 corresponding to the second training (L2) (groups A) or TL2 followed by TL1 (groups B). Each retrieval (TL1 or TL2) consisted of four actual trials of list L1 or list L2, respectively, interspersed with eight of the respective fake trials (total: 12 trials each). Thus, each retrieval had a distribution in number and types of trials similar to the last 12 trials of the respective training (termed the training tail). Subjects had to press the key YES or NO (once the last step of the context period was started).

Reminder

The reminder trial included the specific context of L1. Immediately after completing it, and as expected, a cue syllable appeared on the left side of the monitor's image and the response box on the right. However, 2 sec later a notice displayed on the monitor announced that the session has to being suspended, thus not allowing the subject to write down the response syllable in the box.

Demo

Before the training session, participants were confronted with a demo program to receive all the instructions and to understand the goal of the task. The program consisted of four trials, similar in structure to but with a context and two pairs of nonsense syllables different from those of L1- or L2-training.

Statistics

Results are reported as mean number of errors for each retrieval during test session. Data from each experiment were first analyzed with a one-way ANOVA with a number of levels equal to the number of retrievals. It was followed by a priori planned comparisons (FISHER, $\alpha = 0.05$) between the number of errors of L1-retrieval at phase 1 (or at phase2) of testing vs. the respective control L1CTL; and L2-retrieval at phase 2 (or phase 1) of testing vs. the respective control L2-CTL.

The choice of this statistical approach was on line with the purpose of evaluating the mutual influence at testing between the retrievals of two different verbal materials acquired under different training conditions. Consequently, the effect was properly revealed by contrasting the performance for L1-retrieval of a group that received both trainings with the performance of a control group that received only L1, and separately, by contrasting the performance for L2-retrieval of a group that received both trainings with a group that received only L2.

For training curves the analysis was conducted by using the method of successive regression (Lozada et al. 1990).

Acknowledgments

References

Anderson, M.C. and Neely, J.H. 1996. Interference and inhibition in memory retrieval. In *Memory. Handbook of perception and cognition* (eds. E.L. Bjork and R.A. Bjork), 2nd ed., pp. 237–313. Academic Press, New York.

Anderson, M.C., Bjork, R.A., and Bjork, E.L. 1994. Remembering can cause forgetting: Retrieval dynamics in long-term memory. *J. Exp. Psychol. Learn. Mem. Cogn.* **20**: 1063–1087.

Anokhin, K.V., Tiunova, A.A., and Rose, S.P. 2002. Reminder effects—Reconsolidation or retrieval deficit? Pharmacological dissection with protein synthesis inhibitors following reminder for a passive-avoidance task in young chicks. *Eur. J. Neurosci.* **15**: 1759–1765.

Berman, D.E., Hazvi, S., Stehberg, J., Bahar, A., and Dudai, Y. 2003. Conflicting processes in the extinction of conditioned taste aversion: Behavioral and molecular aspects of latency, apparent stagnation, and spontaneous recovery. *Learn. Mem.* **10**: 16–25.

Boccia, M.M., Blake, M.G., Acosta, G.B., and Baratti, C.M. 2005. Memory consolidation and reconsolidation of an inhibitory avoidance task in mice: Effect of a new different learning task. *Neuroscience* **135**: 19–29.

Bustos, S.G., Maldonado, H., and Molina, V.A. 2006. Midazolam disrupts fear memory reconsolidation. *Neuroscience* **139**: 831–842.

Carew, T.J. 2000. *Behavioral neurobiology. The cellular organization of natural behaviour.* Sinauer, Sunderland, MA.

Debiec, J. and LeDoux, J.E. 2004. Disruption of reconsolidation but not consolidation of auditory fear conditioning by noradrenergic blockade in the amigdala. *Neuroscience* **129**: 267–272.

Debiec, J., Doyere, V., Nader, K., and Ledoux, J.E. 2006. Directly reactivated, but not indirectly reactivated, memories undergo reconsolidation in the amygdala. *Proc. Natl. Acad. Sci.* **103**: 3428–3433.

Dudai, Y. 2002. *Memory from A to Z. Keywords, concepts and beyond.* Oxford University Press, Oxford, UK.

Dudai, Y. and Eisenberg, M. 2004. Rites of passage of the engram: Reconsolidation and the lingering consolidation hypothesis. *Neuron* **44**: 93–100.

Duvarci, S. and Nader, K. 2004. Characterization of fear memory reconsolidation. *J. Neurosci.* **24**: 9269–9275.

Ebbinghaus, D. 1885. *Über das Gedächtnis*. Dunker & Humbolt, Leipzig, Germany.

Eisenberg, M. and Dudai, Y. 2004. Reconsolidation of fresh, remote, and extinguished fear memory in medaka: Old fears don't die. *Eur. J. Neurosci.* **20**: 3397–3403.

Eisenberg, M., Kobilo, T., Berman, D.E., and Dudai, Y. 2003. Stability of retrieved memory: Inverse correlation with trace dominance. *Science* **301**: 1102–1104.

Foa, E.B. 2006. Social anxiety disorder treatments: Psychosocial therapies. *J. Clin. Psychiatry* **67 (Suppl 12)**: 27–30.

Frenkel, L., Maldonado, H., and Delorenzi, A. 2005. Memory strengthening by a real-life episode during reconsolidation: An outcome of water deprivation via brain angiotensin II. *Eur. J. Neurosci.* **22**: 1757–1766.

Hupbach, A., Gomez, R., Hardt, O., and Nadel, L. 2007. Reconsolidation of episodic memories: A subtle reminder triggers integration of new information. *Learn. Mem.* (in press).

James, W. 1890. *The principles of psychology*. Holt, New York.

Levy, B.J. and Anderson, M.C. 2002. Inhibitory processes and the control of memory retrieval. *Trends Cogn. Sci.* **6**: 299–305.

Lozada, M., Romano, A., and Maldonado, H. 1990. Long-term habituation to a danger stimulus in the crab *Chasmagnathus granulatus*. *Physiol. Behav.* **47**: 35–41.

MacLeod, M.D. and Macrae, C.N. 2001. Gone but not forgotten: The transient nature of retrieval-induced forgetting. *Psychol. Sci.* **12**: 148–152.

Mayes, A. and Downes, J. 1997. Theories of organic amnesia. *Memory* **5**: 1–2.

McGeoch, J.A. 1932. Forgetting and the law of disuse. *Psychol. Rev.* **39**: 352–370.

Müller, G.E. and Pilzecker, A. 1900. Experimentelle beitrage zur lerhe Vom gedächtnis. *Z. Psychol.* **1**: 1–300.

Nader, K., Schafe, G.E., and Le Doux, J.E. 2000a. Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. *Nature* **406**: 722–726.

Nader, K., Schafe, G.E., and Le Doux, J.E. 2000b. The labile nature of consolidation theory. *Nat. Rev. Neurosci.* **1**: 216–219.

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

Pedreira, M.E., Perez-Cuesta, L.M., and Maldonado, H. 2002. Reconsolidation of long-term memory in Crac Chasmagnathus protein synthesis requirement and mediation by NMDA-type glutamatergic receptors. *J. Neurosci.* **22**: 8805–8811.

Pedreira, M.E., Perez-Cuesta, L.M., and Maldonado, H. 2004. Mismatch between what is expected and what actually occurs triggers memory reconsolidation or extinction. *Learn. Mem.* **11**: 579–585.

Postman, L. 1971. Transfer, interference and forgetting. In *Woodworth and Schlosberg's: Experimental psychology* (eds. J.W. Kling and L.A. Riggs), 3d ed., pp. 1019–1132. Holt, Rinehart & Winston, New York.

Przybyslawski, J., Roullet, P., and Sara, S.J. 1999. Attenuation of

emotional and nonemotional memories after their reactivation: Role of beta adrenergic receptors. *J. Neurosci.* **19:** 6623–6628.

Sangha, S., Scheibenstock, A., and Lukowiak, K. 2003. Reconsolidation of a long-term memory in Lymnaea requires new protein and RNA synthesis and the soma of right pedal dorsal 1. *J. Neurosci.* **23:** 8034–8040.

Sara, S.J. 2000a. Retrieval and reconsolidation: Toward a neurobiology of remembering. *Learn. Mem.* **7:** 73–84.

Sara, S.J. 2000b. Strengthening the shaky trace through retrieval. *Nat. Rev. Neurosci.* **1:** 212–213.

Sara, S.J. and Hars, B. 2006. In memory of consolidation. *Learn. Mem.* **13:** 515–521.

Suzuki, A., Josselyn, S.A., Frankland, P.W., Masushige, S., Silvia, A.J., and Kida, S. 2004. Memory reconsolidation and extinction have distinct temporal and biochemical signatures. *J. Neurosci.* **24:** 4787–4795.

Walker, M.P., Brakefield, T., Hobson, J.A., and Stickgold, R. 2003. Dissociable stages of human memory consolidation and reconsolidation. *Nature* **425:** 616–620.

Wixted, J.T. and Ebbesen, E.B. 1997. Genuine power curves in forgetting: A quantitative analysis of individual subject forgetting functions. *Mem. Cognit.* **25:** 731–739.