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Human reconsolidation does not always occur when a memory is retrieved: The relevance of the reminder structure

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ABSTRACT

Memory reconsolidation is defined as a process in which the retrieval of a previously consolidated memory returns to a labile state which is then subject to stabilization. The reminder is the event that begins with the presentation of the learned cue and triggers the labilization-reconsolidation process. Since the early formulation of the hypothesis, several controversial items have arisen concerning the conditions that define reconsolidation.

It is herein proposed that two diagnostic features characterize reconsolidation, namely: the labilization of the reactivated memory and the specificity of the reminder structure. To study this proposal, subjects received two different training sessions on verbal material on Day 1 and Day 2, respectively. Finally, they were tested for the first and second acquired memories on Day 3. It is demonstrated that the human declarative memory fulfills the two requirements that define the process. First, the reactivated memory is impaired by a new learning only when it is given closely after the reminder, revealing that the memory is labilized. Second, the omission of at least one of the reminder's components prevents labilization. Therefore, results show that the new learning fails to produce an amnesic effect on the target memory either when the reminder omits the learned cue or includes the beginning of the reinforcement.

1. Introduction

Perhaps the simplest way to define memory reconsolidation would be to say that it is a process in which the retrieval of a previously consolidated memory returns to a labile state that is then subject to stabilization (e.g. Morris et al., 2006). Moreover, an operative definition of memory reconsolidation generally includes the assertion that the reactivated memory can be disrupted by an interfering agent such as protein synthesis inhibitors, β-blockers or also a new learning process (Boccia, Blake, Acosta, & Baratti, 2005; Misanin, Miller, & Lewis, 1968; Nader, Schafe, & Le Doux, 2000). Taking this statement in its strict sense could lead us to conclude that whenever a memory is retrieved it becomes labile and disruptable (Nader, 2003; Sara, 2000). However, we have seen in our paradigm of learning and memory in *Chasmagnathus granulatus* that changes in some of the parametrical conditions of the reminder could prevent memory from undergoing reconsolidation (Pedreira & Maldonado, 2003; Pedreira, Perez-Cuesta, & Maldonado, 2004).

The reminder is the event that begins with the presentation of the learned cue (CS) and triggers the labilization-reconsolidation process of the memory. Based on our previous results, we consider three conditions that define the specific structure of the reminder. Firstly, the extension of the reminder has to be adequate to produce reconsolidation and not extinction (Eisenberg & Dudai, 2004; Pedreira & Maldonado, 2003). Secondly, since the previous condition implies that the animal has to compute a time interval, the reminder must have a full-stop, namely, the reminder could not produce memory labilization without CS-offset (Pedreira et al., 2004). Thirdly, the reminder does not have to include the reinforcement, therefore during the reminder exposure there is a mismatch between what the animal expects and what actually happens. This reminder condition is what we call the mismatching component (Morris et al., 2006).

In short, two diagnostic features define the reconsolidation process. Firstly, the labilization of the reactivated memory, revealed by the amnesic effect of an interfering factor presented after the reminder. Secondly, the specificity of the reminder structure, revealed by the fact that the amnesic effect of the interference is no longer observed when the reminder's parametrical conditions are changed.

The reconsolidation hypothesis has been demonstrated in very different species and types of memory, including the human procedural memory of a motor skill task (Walker, Brakefield, Hobson, & Stickgold, 2003). However, it was not until 2007 that

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the reconsolidation hypothesis was demonstrated in the human declarative memory. Two laboratories reported reconsolidation of human memories related to facts and episodes that are accessible to conscious recollection (Forcato et al., 2007; Hupbach, Gomez, Hardt, & Nadel, 2007).

In our experiment with verbal material (Forcato et al., 2007) the target memory was acquired by a first learning process (L1-training) and the interfering agent was a second learning process (L2-training). Here, we evaluate whether our experiment fulfills the two requirements that define the reconsolidation process. A series of experiments were performed with protocols similar to those used in Forcato et al. (2007). Basically, subjects were trained on two consecutive days on which they learned two distinct lists of five pairs of nonsense-syllables (L1 and L2, respectively). A group of subjects received a reminder before the L2-training on Day 2 while the other group only received the L2-training. To sum up, it was confirmed that the reminder group but not the no-reminder group showed significant deficits in L1-memory at testing on Day 3. On the other hand, it was demonstrated that the impairment of L1-memory is no longer detected when the retrieval condition of the reminder is not accomplished or when the mismatching component is excluded.

2. Materials and methods

2.1. Subjects

One hundred fifty-six healthy undergraduate and graduate students from Buenos Aires University volunteered for the study. 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 20 to 35, with a mean of 25 (51 men, 105 women). Each participant was randomly assigned to one of twelve groups.

2.2. Procedure

2.2.1. Syllable presentation

Experiments took place in a dark room and were conducted using a personal computer. Each subject was provided with earphones and seated facing a monitor placed in front of a large screen on the back wall. The experimental protocol was similar to that of Forcato et al. (2007) but differed in its use of light color, image and sound, as in the time of their presentation.

Each trial began with the presentation of the context period which was formed by a *light* projected on the large screen, an *image* on the monitor screen; and a *sound* coming through the earphones. The three stimuli appeared together and 6 s after that the syllable period was presented. The specific context persisted during the syllable presentation (Fig. 1a) which started with the presentation of a cue-syllable on the left-hand side of the monitor screen and an empty response-box on the right. Each cue-syllable was taken at random from a list of five pairs. Subjects were given 5 s to write the corresponding response-syllable. Once that period was finished three situations were possible: first, if no syllable was written, the correct one was shown for 4 s; second, if an incorrect syllable was written, it was replaced by the correct one and it was shown for 4 s; and third, if the correct response was given, it stayed for 4 s longer. Immediately after that, another cue-syllable was shown and the process was repeated until the list was over. Altogether an actual trial lasted 51 s (6 s for context period and 45 s for syllable presentation). Throughout this paper, every time a subject faced a cue-syllable and wrote down an erroneous response or no response an error was computed.

2.2.2. The L1-training session

A trial of L1-training was composed of the context period with diverse stimuli options: the *light* could be blue or green; the *image*, three different pictures of cascades; the *sound*, three different tango melodies. Only one combination of these options (the specific context) was followed by the syllables presentation of List 1. The trial which includes the 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*. Fake trials were presented in order to enhance the level attention and to maintain the same distribution of trials as in Forcato et al. (2007). Therefore, this design allowed subjects to predict the presentation of the pair-associated task every time the specific context was completed.

The L1-training consisted of the presentation of 10 actual trials mixed with 22 fake trials (total: 32 trials), separated by a 4-s intertrial interval. In the first training trial, the L1 list was shown, and in the successive actual trials subjects were required to write down the corresponding response-syllable for each cue-syllable presented. List 1 was composed of 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. 1b, *Left panel*). Subjects that failed to obtain 65% correct responses during the last four actual trials were excluded. The training session lasted 15 min.

2.2.3. The L2-training session

A trial of L2-training was composed of the context period with diverse stimuli options: the *light* could be red or orange; the *image*, three different pictures of forests; the *sound*, three different symphonies. The context period of L2-training was radically different from that of L1-training. The L2-training consisted of the presentation of 10 actual trials mixed with 22 fake trials (total: 32 trials), separated by a 4-s intertrial interval. In the first training trial, the L2 list was shown and in the successive actual trials subjects were required to write down the corresponding response-syllable for each cue-syllable presented. List 2 was composed of 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. 1b, *Right panel*). Subjects that failed to obtain 65% correct responses during the last four actual trials were excluded. The training session lasted 15 min.

2.2.4. Testing session

The testing session consisted of the evaluation of the two memories acquired: the L1-test corresponding to the L1-training and separated by a 5-minutes interval the L2-test corresponding to the second memory acquired. Each testing (L1-test or L2-test) consisted of 4 actual trials of list L1 or list L2, respectively, mixed with 8 of the respective fake trials (total: 12 trials each). The testing session lasted 14 min (7 min for each testing).

2.2.5. Types of reminder

In order to evaluate the specificity of the reminder structure, different reminders were designed (Fig. 1c).

2.2.5.1. Cue-reminder. This trial included the specific context of L1. Immediately after the context period presentation, as expected, a cue-syllable appeared on the left-hand side of the monitor screen and the response-box on the right. However, 2 s later a notice displayed on the monitor announced that the session had to be suspended, thus not allowing the subject to write down the response-syllable (Fig. 1c, *Top diagram*).

2.2.5.2. Context-reminder. This trial included the specific context of L1 and immediately after the context period, before any syllable

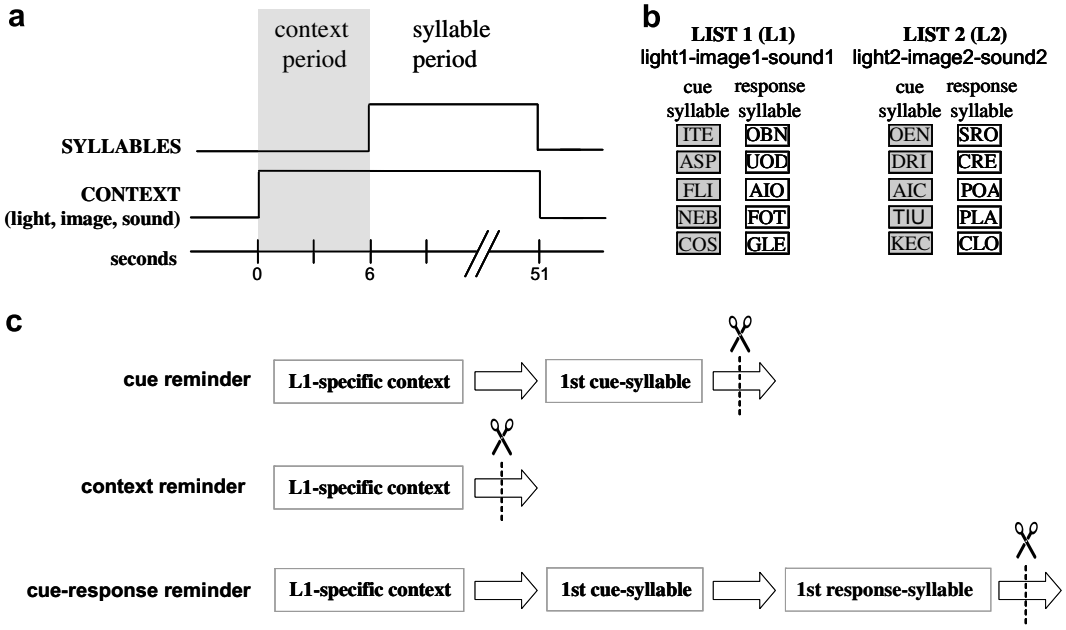


Fig. 1. Experimental protocol. The subjects were submitted to two different training sessions (L1-training and L2-training), separated by a time interval, in a 3 day experiment. (a) An actual trial was formed by the context period: a specific combination of a light (color illumination of the room), image (a picture on the monitor) and sound (music melody from earphones); and by the syllable period: 6 s after the context presentation, five pairs of cue-response-syllables were presented in random order. (b) Paired-associated memory. (*Left*) L1, the list 1 presented in the L1-training and testing. (*Right*) L2, the list 2 presented in the L2-training and testing. Both lists differ to each other in cue-response pairs as well as in color, light, image and sound that precede them. (c) Types of reminders. (*Top diagram*) The cue reminder included the specific context of L1-memory plus the presentation of one cue-syllable of L1 after which the trial was abruptly interrupted, thus not allowing the subject to write the respective response-syllable. (*Middle diagram*) The context reminder consisted of 6 s presentation of the L1-specific context, but the trial was abruptly interrupted before any syllable presentation. (*Bottom diagram*) Finally, the cue-response reminder included the L1-specific context but subjects were allowed to write the first response-syllable and after that the trial was interrupted. Scissors stand for the full-stop of each type of reminder.

presentation, a notice displayed on the monitor announced that the session had to be suspended (Fig. 1c, *Middle diagram*).

2.2.5.3. Cue-response-reminder. This trial included the specific context of L1 and immediately after the context period a cue-syllable appeared and subjects were allowed to answer with the respective response-syllable. After that, a notice displayed on the monitor announced that the session had to be suspended (Fig. 1c, *Bottom diagram*)

2.2.6. Demo

Before the training session, participants were confronted with a demo program to receive all the instructions and to understand the objective of the task. The program consisted of 4 trials, similar in structure to those of L1 or L2-training, but with another context and two different pairs of nonsense-syllables.

2.3. Method to reveal memory deficit

In our previous report (Forcato et al., 2007), an alternative method was used to evaluate the amnesic effect on the target memory. Since memories are not stored in isolation from other memories but integrated into complex associative networks (Berman, Hazvi, Stehberg, Bahar, & Dudai, 2003; Debiec & Ledoux, 2004), a faulty retrieval of the L1-memory tested before the L2-memory may be caused by either deficits in its storage or by simultaneous retrieval of related information (Mayes & Downes, 1997; McGeoch, 1932). Therefore, the current method of demonstrating amnesia by disclosing at testing the faulty retrieval of the memory was not used. Instead, our method was based on the forgetting effect that L1-memory retrieval could have on L2-memory later recall, evaluated 5 min after the L1-test, termed *retrieval-induced* forgetting (RIF effect, Anderson, Bjork, & Bjork, 1994). The results showed that L1-memory recall could temporary block the expression of L2-memory; however, for this effect to be possible, the memory first recalled must be intact. Consequently, a poor performance of L1-memory at testing may be interpreted as the loss of L1-memory and/or retrieval interference with its own expression by simultaneous recall of L2 items. On the other hand, a good performance of L2-memory at testing, that is, the absence of the RIF effect is only regarded as an expression of the L1-memory deficit. Namely, the absence of the RIF effect, rather than the failure of L1-retrieval, is specific of L1-memory impairment.

Concerning the possibility of performing inversed testing, that is, to test L2-memory 5 min before L1-memory, the results obtained in the previous work (Forcato et al., 2007) were conclusive. They showed a low number of errors for the L2-test and a high number of errors for the L1-test throughout the paper, independently of the experiment under evaluation. The good performance of the L2-test revealed the absence of simultaneous retrieval interference while the poor performance of the L1-test could be caused by impairment of L1-memory and/or the RIF effect. The lack of retrieval interference on L2-retrieval could be interpreted as a consequence of the difference in strength between L1 and L2-memories due to the fact that the former was acquired 48 h and the latter 24 h before the test session (Ebbinghaus, 1885; Wixted & Ebbesen, 1997). Therefore, in the present paper groups in which the L2-test was evaluated first were not included.

2.4. Statistics

Results are reported as mean total number of errors for the L1 or L2-test. Data from each experiment was first analyzed with a one-way analysis of variance (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 L1-test vs. the respective L1-control; and the L2-test vs. the respective L2-control.

The choice of this statistical approach was in 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 L1-memory performance at testing of a group that received both trainings with the performance of a control group that received only L1-training and, separately, by contrasting L2-memory performance at testing of a group that received both trainings with a group that received only L2-training.

3. Results

3.1. The specific parametrical conditions of the reminder structure

3.1.1. The first diagnostic feature of the memory reconsolidation: The labilization of the reactivated memory

One way to disclose the reconsolidation process is by disrupting the re-stabilization of the reactivated memory with an amnesic agent such as protein synthesis inhibitors, β-blockers or also by a new learning process. It has recently been observed in our paradigm of declarative memory in humans that the process of labilization-reconsolidation was triggered by the reminder presentation and interfered with a second learning process that impaired the reconsolidation of the target memory within a time window (Forcato et al., 2007).

The experiment included two main groups: the no-reminder group (NR) and the cue reminder group (R_C) (Fig. 2). The no-reminder group received a first training (L1-training) on Day 1 consisting of the specific context presentation followed by five pairs of nonsense-syllables (cue-syllable; response-syllable; Fig. 1b, *Left panel*) presented in a random order. For each cue-syllable, subjects had to complete the correspondent response-syllable. A second training (L2-training) with the different context and five different pairs of syllables was given (Fig. 1b, *Right panel*) on Day 2; and L1 and L2-memory were tested on Day 3, separated by a 5-minute interval. The controls were run simultaneously with the main group and were as follows: the CTL1(NR) with L1-training on Day 1, without a treatment on Day 2, and presentation of L1-test on Day 3; and the CTL2(NR), with L2-training on Day 2 and L2-test on Day 3 (Fig. 2a, *Left panel*).

The cue reminder group (R_C) was similar to the former main group, but L2-training was immediately preceded by the cue reminder. The cue reminder included the specific context of L1-memory plus the presentation of one cue-syllable of L1 after which the trial was abruptly interrupted, thus not allowing the subject to answer with the respective response-syllable (Fig. 1c, *Top diagram*). The controls were run simultaneously with the main group and were as follows: CTL1(R_C) with L1-training on Day 1, the sole presentation of the cue reminder on Day 2 and L1-test on Day 3; and the CTL2(R_C) as the CTL2(NR) (Fig. 2b, *Left panel*).

Results of the testing session of Experiment 1 on Day 3 are exhibited in Fig. 2. The analysis of mean total errors discloses a significant difference between the L1-retrieval of the no-reminder group and CTL1(NR) (Fig. 2a, *Right panel*). As we have seen in Section 2, this poor performance during the L1-retrieval could be explained in terms of an interference from retrieval of L2-memory on the expression of the L1-retrieval, 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 (*simultaneous retrieval interference*) (McGeoch, 1932). As we expected, a significant difference between L2-retrieval of the no-reminder group and CTL2(NR) was disclosed (ANOVA $F(3, 48) = 8{,}245$ $p < .001$; LSD comparisons $p_{L1} = .006$ and $p_{L2} < .001$, respectively) (Fig. 2a, *Right panel*). That is, the first retrieval negatively influences the expression of the second, which represents an instance of RIF (Anderson et al., 1994; Levy & Anderson, 2002). Since this effect depends on the integrity of the first recalled memory (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.

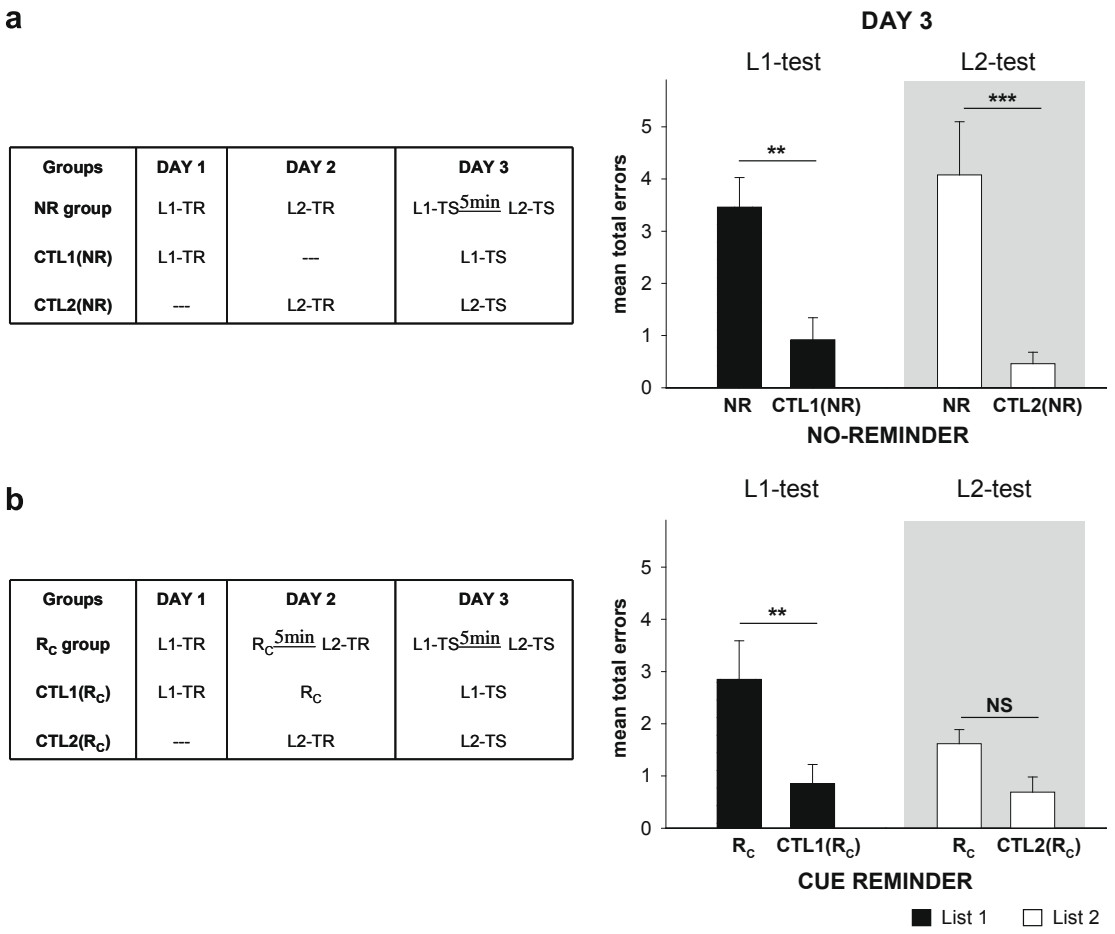
The analysis of mean total errors discloses a significant difference between the L1-retrieval of the cue reminder group (R_C) and CTL1(R_C) (Fig. 2b, *Right panel*) due to L1-memory deficit or/and simultaneous retrieval of related information. Instead, there was no significant difference between L2-retrieval of the cue reminder group and CTL2(R_C) (ANOVA $F(3, 48) = 4{,}605$ $p = .007$; LSD comparisons $p_{L1} = .003$ and $p_{L2} = .161$, respectively) indicating the absence or reduction of the RIF effect, that is, deficits in the L1-memory (Fig. 2b, *Right panel*). Therefore, during Day 2, the cue reminder induced the labilization of the consolidated L1-memory which was impaired by the immediate L2-training, corroborating the first diagnostic feature of the memory reconsolidation, i.e., the labilization of the reactivated memory.

3.1.2. The second diagnostic feature of the memory reconsolidation: The specificity of the reminder structure

The main groups had, as the cue reminder group of the first experiment had, L1-training on Day 1, a putative reminder followed by L2-training on Day 2, and a testing session on Day 3 (Fig. 3) but they differed from each other as regards the components of the putative reminder. The context reminder group (R_{CTX}), included a putative reminder where the abrupt interruption came immediately after the L1-specific context (Fig. 1c, *Middle diagram*). The controls were run simultaneously with it and were as follows: the CTL1(R_{CTX}) with L1-training on Day 1, the presentation of a context reminder on Day 2 and L1-test on Day 3; and the CTL2(R_{CTX}), with L2-training on Day 2 and L2-test on Day 3 (Fig. 3a, *Left panel*).

Finally, in the cue-response reminder group (R_{CR}) subjects were allowed to answer the first cue-syllable with the respective response-syllable before the session was interrupted (Fig. 1c, *Bottom diagram*). The controls were run simultaneously with it and were as follows: CTL1(R_{CR}) with L1-training on Day 1, the presentation of the cue-response reminder on Day 2 and L1-test on Day 3; and the CTL2(R_{CR}) as the CTL2(R_{CTX}) (Fig 3 b, *Left panel*).

Results are shown in Fig. 3. The data analysis corresponding to the context reminder group (R_{CTX}) (Fig. 3a, *Right panel*) and the cue-response reminder group (R_{CR}) (Fig. 3b, *Right panel*) discloses a significant difference between the mean total errors of L1-retrieval and those of the respective CTL1(R_{CTX}) and CTL1(R_{CR}). However, in contrast to the results showed above for the cue reminder group, both the R_{CTX} group and the R_{CR} group revealed a significant difference between the mean total errors of L2-retrieval and those of the respective CTL2(R_{CTX}) and CTL2(R_{CR}) (for R_{CTX} ANOVA $F(3,48) = 10.577$ $p < .001$; LSD comparisons $p < .001$ and $p_{L2} = .004$, respectively, and for R_{CR} ANOVA $F(3,48) = 9.900$ $p_{L1} < .001$; LSD comparisons $p_{L1} < .001$ and $p_{L2} = .038$, respectively). This picture of results indicates the presence of the RIF effect, that is, there is no deficit in L1-memory. Therefore, the context and cue-response reminders fail to induce memory labilization, leaving the first memory intact. It is important to notice that this lack of labilization is due to subtle changes in the reminder structure. In Fig. 2B we have seen that the context followed by a cue-syllable triggers the labilization-reconsolidation process of the L1-memory. However, this phenomenon is no longer observed if the cue-syllable is omitted and only the context is presented, nor if the possibility to answer with the correspondent response-syllable is added. That is to say, memory fails to be labilized when the learned cue is not presented (the context reminder group) or the mismatching component of the reminder is absent (the cue-response reminder group). These results confirm the second diagnostic feature of the memory reconsolidation, i.e., the specificity of the reminder structure.



Groups	DAY 1	DAY 2	DAY 3
NR group	L1-TR	L2-TR	L1-TS 5min L2-TS
CTL1(NR)	L1-TR	---	L1-TS
CTL2(NR)	---	L2-TR	L2-TS

Groups	DAY 1	DAY 2	DAY 3
R_C group	L1-TR	R_C 5min L2-TR	L1-TS 5min L2-TS
CTL1(R_C)	L1-TR	R_C	L1-TS
CTL2(R_C)	---	L2-TR	L2-TS

Fig. 2. The labilization of the reactivated memory. (a) (*Left*) Groups (n = 13): NR stands for the no-reminder group, CTL1(NR) for L1-control and CTL2(NR) for L2-control. L1-TR stands for L1-training, L2-TR for L2-training, L1-TS for L1-testing, L2-TS for L2-testing and 5 min for the 5 min interval between tests. (*Right*) Mean of total errors ± SEM on Day 3. Black bars stands for L1-TS performance, white bars for L2-TS performance. $^{**}p < 0.01$; $^{***}p < 0.001$. (b) (*Left*) Groups (n = 13): R_C stands for the cue reminder group, CTL1(R_C) for L1-control and CTL2(R_C) for L2-control. L1-TR, L2-TR, L1-TS, L2-TS as above. R_C stands for the cue reminder trial and 5 min for the 5 min interval between the cue reminder trial and L2-TR or between tests. (*Right*) Mean of total errors ± SEM on Day 3. $^{**}p < 0.01$; NS, $p > 0.05$.

It is important to highlight that the context reminder and the cue-response reminder also retrieved the target memory. Indeed, in the case of the context reminder the retrieval process was demonstrated in our previous work, showing that the subjects correctly predicted the appearance of the verbal task when the specific context was presented (Forcato et al., 2007). Concerning the cue-response reminder, the subjects remembered the target memory since they had answered with the correspondent response-syllable.

3.2. Training performance analysis

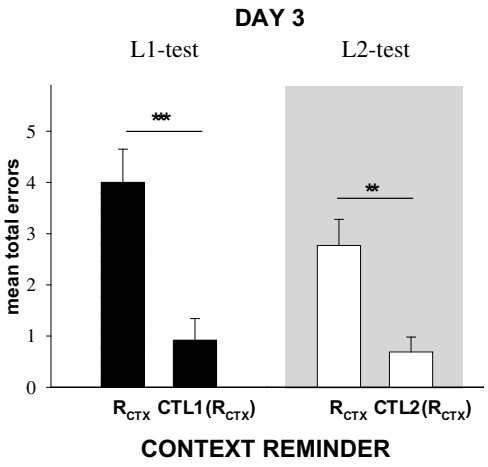
The uniformity of L1-training performance among the main groups and also the uniformity of L2-training performance was studied. The analysis of mean total errors for the last four actual trials revealed no significant difference among the groups for L1-training, $F(3,48) = .103$ $p = 0.958$ neither for L2-training, $F(3,48) = 0.084$ $p = 0.968$. Therefore, there was a noticeable uniformity in the paired-associate acquisition among experiments, confirming that the difference on L2-test performance on Day 3 was specifically due to the presence or absence of the RIF effect. On the other hand, we observed an increase in L2-learning performance compared to L1-learning performance, in the last four training-trials for all main groups. In spite of that, such differences fail to reach a significant level. For the *no-reminder group*, 87 ± 3% and 96 ± 3% of correct responses for L1 and L2, respectively, $F(1,24) = 3{,}575$ $p = .070$; for *cue reminder group*, 88 ± 3% and 95 ± 2%, $F(1,24) = 3{,}923$ $p = 0.059$; for *context reminder group*, 89 ± 3% and 94 ± 2% $F(1,24) = 2{,}069$ $p = .163$; for *cue-response reminder group*, 89 ± 3% and 94 ± 2%, $F(1,24) = 2{,}381$ $p = .135$. The increase in L2-learning performance could be explained by the familiarity with the procedural rules of the task, since subjects had learned the L1 list 24 hours before the second task.

3.3. Classification and analysis of syllable errors during test session

In the above experiments, the identity of each response to each cue-syllable at testing could be established, an advantage that we did not have in the previous experiment (Forcato et al., 2007). Four types of error were distinguished. Namely: the *void error*, when no response was written down; the *whole error*, when the response-syllable was neither included in L1 nor L2; the *within-list* error, when the response-syllable was not the right one but it belonged to the same list; the *intrusive error*, when the response-syllable was one of the other list. Fig. 4 shows the mean total errors for each group. Analysis of the L1-test disclosed no significant difference between groups for any type of syllable errors (Fig. 4a) (ANOVAs $p_{void} = .151$; $p_{within\text{-}list} = .848$; $p_{whole} = .488$; $p_{intrusive} = .068$). Noticeably, considering the type of error for all groups, the void type showed the maximum concentration (53 ± 5%), whereas the number of intrusive errors scarcely reached the 6 ± 2%. A similar picture was obtained when the L2-test errors were considered (Fig. 4b). No

a

Groups	DAY 1	DAY 2	DAY 3
R_{CTX} group	L1-TR	R_{CTX} 5min L2-TR	L1-TS 5min L2-TS
CTL1(R_{CTX})	L1-TR	R_{CTX}	L1-TS
CTL2(R_{CTX})	---	L2-TR	L2-TS



b

Groups	DAY 1	DAY 2	DAY 3
R_{CR} group	L1-TR	R_{CR} 5min L2-TR	L1-TS 5min L2-TS
CTL1(R_{CR})	L1-TR	R_{CR}	L1-TS
CTL2(R_{CR})	---	L2-TR	L2-TS

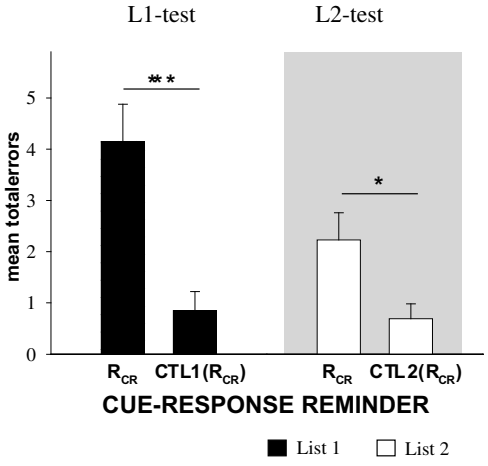


Fig. 3. The specificity of the reminder structure. (a) (*Left*) Groups (n = 13): R_{CTX} stands for the context reminder group, CTL1(R_{CTX}) for L1-control and CTL2(R_{CTX}) for L2-control. L1-TR, L2-TR, L1-TS, L2-TS as in Fig. 2. R_{CTX} stands for the context reminder and 5 min for the 5 min interval between the context reminder and L2-TR or between tests. (*Right*) Mean of total errors ± SEM on Day 3. $^{**}p < 0.01$; $^{***}p < 0.001$. (b) (*Left*) Groups (n = 13): R_{CR} stands for cue-response reminder group, CTL1(R_{CR}) for L1-control and CTL2(R_{CR}) for L2-control. L1-TR, L2-TR, L1-TS, L2-TS as in Fig. 2. R_{CR} stands for the cue-response reminder and 5 min for the 5 min interval between the cue-response reminder and L2-TR or between tests. (*Right*) Mean of total errors ± SEM on Day 3. $^{*}p < 0.05$; $^{***}p < 0.001$.

significant difference in the number of L2-test errors was disclosed between groups (ANOVAs p_{void} = .057; $p_{within\text{-}list}$ = .098; p_{whole} = .552; $p_{intrusive}$ = .303). The void error was the predominant type (67 ± 6%) and the number of intrusive errors reached the 4 ± 2%. This identification, however, does not allow us to decide *per se* whether the errors are due to retrieval interference, RIF or deficits in the tested memory.

4. Discussion

Present results indicate that our experiments on reconsolidation of human declarative memory fulfill the two diagnostic features which define the reconsolidation process: the labilization of the retrieved memory and the specificity of the reminder. Furthermore, these findings support the idea that labilization-reconsolidation does not always occur when a memory is retrieved. On the contrary, it requires a reminder with a specific structure so that omitting one of its components —such as the cue-syllable in R_{CTX} group– or omitting one of its parametrical conditions —such as the mismatching component in R_{CR} group— deactivates the reminder and prevents the labilization of the target memory.

It is worth emphasizing that in the case of the cue-response reminder group (Fig 3b, *Right panel*) the reminder fails to induce memory labilization due to the mere inclusion of one syllable-response in its structure. Such inclusion represents the beginning of reinforcement, and therefore the omission of the mismatching component. A result analogous to that of the cue-response reminder group was obtained with crabs using the model of contextual memory (Pedreira et al., 2004). It was shown that the amnesic effect produced by a high dose of cicloheximide given after the reminder termination was no longer observed when the reinforcement was presented at the last minute of the reminder, leaving the memory intact and consolidated. Results with rats in the water-maze paradigm (Morris et al., 2006) could be considered as another example of the opposite effects of a protein synthesis inhibitor (anisomicyn) depending on whether the mismatching component is included in the reminder or not. Bilateral infusion of anisomycin in the hippocampus after the reminder did not impair memory when the injection was given once the rat had found the platform. However, in the same series of experiments, rats were confronted during reactivation with the absence of the expected platform. This irreversible mismatch made the memory susceptible to anisomycin and triggered the updating of the cognitive representation of the new platform. However, in other models the labilization-reconsolidation process seems to be triggered in the presence of the reinforcement (Duvarci & Nader, 2004).

To sum up, it is assumed that a mismatch between what was expected and what actually occurred could result from a failed prediction. A wide range of memory flaws could account for such failure, ranging from outdated to faulty or incomplete information.

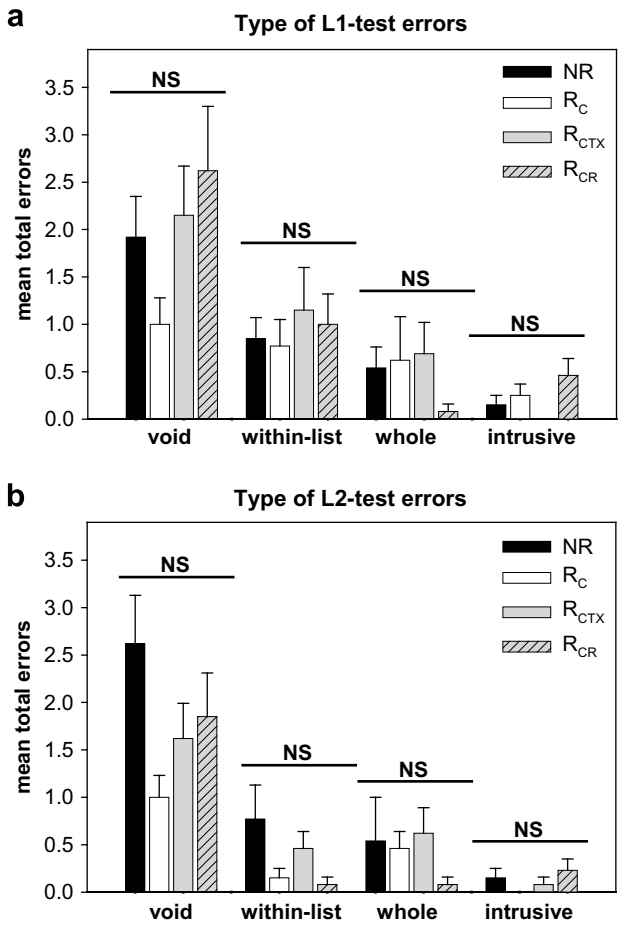


Fig. 4. Type of syllable errors. (a) Type of L1-test errors. Groups (n = 13): NR stands for the no-reminder group (black bars), R_C for the cue reminder group (white bars), R_{CTX} for the context reminder group (Grey bars) and R_{CR} for the cue-response reminder group (stripped bars). NS, $p > 0.05$. Mean total errors ± SEM on Day 3. (b) Type of L2-test errors. Groups (n = 13): symbols as above. Mean total errors ± SEM on Day 3.

Therefore it seems reasonable to suppose, in agreement with several authors (Nader et al., 2000; Sara, 2000), that labilization-reconsolidation plays a repairing role by enabling the system to integrate new information in the background of the past (Pedreira et al., 2004).

The analysis of the error types deserves special consideration, in particular the case of the intrusive error. If it were proven that this type of error is significantly more frequent in the L1-test of the cue reminder group than in that of the other three groups, such an outcome could be taken as indicative that items of L2 are incorporated in the labilized L1-memory. The incorporation of new items into the original information was actually reported in a previous work on reconsolidation of episodic memories in humans (Hupbach et al., 2007). These experiments are similar to ours but with lists of objects instead of lists of syllable pairs. However, no comparable result was found in our experiments. On the contrary, here the overwhelming majority of errors were of the void type and only an insignificant number of intrusions was detected in the four main groups (Fig. 4a). This disparity of results could be explained by a noticeable difference between the experimental designs. In our protocol the contexts of L1- and L2-learnings were sharply different from each other (Fig. 1b) thus ensuring a maximum reduction of the possibility of source confusion during retrieval (Lindsay, Allen, Chan, & Dahl, 2004). Instead, in Hupbach et al. (2007) the lists had the same context for both learnings in the reminder group, which partially explains the high number of intrusion errors in terms of source confusion (Lindsay et al. 2004). On the contrary, in the no-reminder group the context for both learnings was different, hindering the possibility of source confusion.

Therefore, we consider that the possible role of memory reconsolidation is to update the original information, but a different experimental design would be necessary in order to test this hypothesis.

The demonstration that also in the human declarative reconsolidation the reminder requires a specific structure, including the relevant mismatching component, support the possible universality of the reminder conditions. This view 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). On the other hand, we confirm the promising use of the human model, emphasized in our previous work (Forcato et al., 2007), concerning the study of contentious or still conjectural issues about reconsolidation.

Author contributions

Maldonado H. and Pedreira M.E. contributed equally to this work.

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