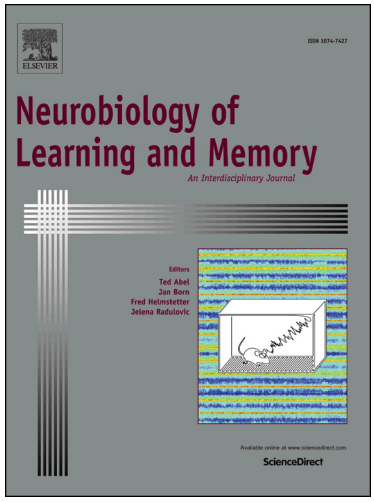

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Retrieval under stress decreases the long-term expression of a human declarative memory via reconsolidation.

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Abstract

Acute stress impairs memory retrieval of several types of memories. An increase in glucocorticoids, several minutes after stressful events, is described as essential to the impairing retrieval-effects of stressors. Moreover, memory retrieval under stress can have long-term consequences. Through what process does the reactivated memory under stress, despite the disrupting retrieval effects, modify long-term memories? The reconsolidation hypothesis proposes that a previously consolidated memory reactivated by a reminder enters a vulnerability phase (labilization) during which it is transiently sensitive to modulation, followed by a re-stabilization phase. However, previous studies show that the expression of memories during reminder sessions is not a condition to trigger the reconsolidation process since unexpressed memories can be reactivated and labilized. Here we evaluate whether it is possible to reactivate-labilize a memory under the impairing-effects of a mild stressor. We used a paradigm of human declarative memory whose reminder structure allows us to differentiate between a reactivated-labile memory state and a reactivated but non-labile state. Subjects memorized a list of five cue-syllables associated with their respective response-syllables. Four days later, results showed that the retrieval of the paired-associate memory was impaired when tested 20 min after a mild stressor (cold pressor stress (CPS)) administration, coincident with cortisol levels increase. Then, we investigated the long-term effects of CPS administration prior to the reminder session. Under conditions where the reminder initiates the reconsolidation process, CPS impaired the long-term memory expression tested 24h later. In contrast, CPS did not show effects when administered before a reminder session that does not trigger reconsolidation. Results showed that memory reactivation-labilization occurs even when retrieval was impaired. Memory reactivation under stress could hinder -via reconsolidation- the probability of the traces to be expressed in the long term.

1. Introduction

There is growing consensus that a single stressful experience modulates memory processes (Roozendaal, McEwen, and Chattarji, 2009; Sandi and Pinelo-Nava, 2007; Wolf, 2009). In fact, both human and non-human studies show that emotionally relevant events activate hormonal and brain systems that enhance the consolidation of newly acquired memories (McGaugh and Roozendaal, 2002). Thus, endogenous modulating systems provide a basis for selecting experiences for long-term storage (McGaugh, 2000). In contrast to such promoting influence during consolidation, acute stress impairs memory retrieval (Gagnon and Wagner, 2016; Roozendaal, Griffith, Buranday, de Quervain, and McGaugh, 2003). Thus, stress experience before testing impairs the retrieval of several types of memories including declarative and episodic (Roozendaal, 2002; Roozendaal and McGaugh, 2011); but see (Schwabe and Wolf, 2014). The release of glucocorticoids shortly after stress is described as a key factor of such impairing influence (Roozendaal and McGaugh, 2011). While the impairing effect on retrieval is stronger for emotionally arousing items, this effect has been also documented for neutral information (Gagnon and Wagner, 2016; Tollenaar, Elzinga, Spinhoven, and Everaerd, 2009; Wolf, Kuhlmann, Buss, Hellhammer, and Kirschbaum, 2004).

Views regarding retrieval are shifting under the light of reconsolidation findings (Dudai and Morris, 2013; Miller and Matzel, 2006; Nader and Wang, 2006; Sara and Hars, 2006). Our previous studies highlighted that retrieval and memory expression are not interchangeable concepts. Hence, memory expression during the reminder session is not a prerequisite to trigger reconsolidation since unexpressed memories can be reactivated and reconsolidated (Barreiro, Suarez, Lynch, Molina, and Delorenzi, 2013; Blake, Boccia, Krawczyk, Delorenzi, and Baratti, 2012; Caffaro, Suarez, Blake, and Delorenzi, 2012; Coccoz, Maldonado, and Delorenzi, 2011; Frenkel, Maldonado, and Delorenzi, 2005; Frenkel, Suarez, Maldonado, and Delorenzi, 2010; Maza, Locatelli, and Delorenzi, 2016a). For instance, we showed in crabs that the retrieval deficit induced by a pharmacological manipulation (administration of glutamate receptor antagonists) interferes with memory expression (Barreiro et al., 2013; Delorenzi, Maza, Suarez, Barreiro, Molina, and Stehberg, 2014). However, the memory trace retains the potentiality of being reactivated. Indeed, the information can be accessed and used for mismatch evaluation

(disparities between the retrieval conditions and the reactivated representation of the experience); the occurrence of reconsolidation depends on detecting mismatches between actual and expected experiences during the reminder session (Pedreira and Romano, 2013). Surprise, i.e. a rupture of the expectations generated by a mismatch between the retrieval conditions and the reactivated representation of the experience (Barto, Mirolli, and Baldassarre, 2013; Rescorla, 1972), is an essential boundary condition to initiate the reconsolidation process in several species (Diaz-Mataix, Ruiz Martinez, Schafe, LeDoux, and Doyere, 2013; Dudai, 2006; 2009; Fernandez, Boccia, and Pedreira, 2016b; Forcato, Argibay, Pedreira, and Maldonado, 2009; Forcato, Burgos, Argibay, Molina, Pedreira, and Maldonado, 2007; Frenkel et al., 2005; Lee and Flavell, 2014; Morris, Inglis, Ainge, Olverman, Tulloch, Dudai, and Kelly, 2006; Pedreira, Perez-Cuesta, and Maldonado, 2004; Pedreira and Romano, 2013; Sevenster, Beckers, and Kindt, 2012; 2013; 2014; Winters, Tucci, and DaCosta-Furtado, 2009). Our studies show that, although unexpressed, the memory trace becomes labile only when mismatch takes place during the reminder session (Barreiro et al., 2013; Caffaro et al., 2012; Delorenzi et al., 2014; Frenkel et al., 2005; Frenkel et al., 2010). These results suggest that there should be a dissociation between the neurobiological mechanisms mediating memory reactivation (i.e. the access to the memory trace (Lewis, 1979)) and those underlying the behavioral expression of memory (Delorenzi et al., 2014). Concordantly, other studies show this dissociation (Ben Mamou, Gamache, and Nader, 2006; Lee and Flavell, 2014; Milton, Merlo, Ratano, Gregory, Dumbreck, and Everitt, 2013; Rodriguez-Ortiz, Balderas, Garcia-Delatorre, and Bermudez-Rattoni, 2012; Santoyo-Zedillo, Rodriguez-Ortiz, Chavez-Marchetta, Bermudez-Rattoni, and Balderas, 2014a).

We recently showed that the administration of a mild stressor (cold pressor stress (CPS)) or glucose ingestion, after memory reactivation, increase long-term expression of a human declarative memory. Remarkably, these memory improvements occur only when the reminder contains the mismatch conditions necessary to trigger reconsolidation (Coccoz et al., 2011; Coccoz, Sandoval, Stehberg, and Delorenzi, 2013; Delorenzi et al., 2014). Regardless of poor memory expression at the time of memory reactivation due to forgetting (1 or 3 weeks after training), robust memory expression can be found at testing sessions if stress (1st week) or glucose administration (3th week) are concurrent with the reconsolidation phase. Thus, the behavioral expression of consolidated memories is not required for memory reactivation and reconsolidation (Barreiro et al., 2013; Ben Mamou et al., 2006; Blake et al., 2012; Delorenzi et al.,

2014; Frenkel et al., 2005; Milton et al., 2013; Rodriguez-Ortiz et al., 2012; Santoyo-Zedillo et al., 2014a; Sevenster et al., 2012).

Several literature suggest that pharmacological or behavioral manipulations during reconsolidation might result in a memory interference, disturbances that affect the memory persistence itself or a failure in subsequent retrievals (Agren, Engman, Frick, Bjorkstrand, Larsson, Furmark, and Fredrikson, 2012; Schiller, Monfils, Raio, Johnson, Ledoux, and Phelps, 2010; Schwabe and Wolf, 2009; Wichert, Wolf, and Schwabe, 2011). Why does reconsolidation open an opportunity for the interference of consolidated memories? What might be the adaptive function of reconsolidation? In our view, a key function of reconsolidation is to induce a change in memory expression by the influence of a concurrent experience (Delorenzi et al., 2014; Frenkel et al., 2005). Reconsolidation is yet another example that the dynamics of the memory processes are conserved throughout evolution (Barco, Bailey, and Kandel, 2006; Dudai and Morris, 2013; Glanzman, 2010; Menzel, 1999), a feature that can be founded in the hypothesis of a common origin of the high-order memory centers in bilateral animals (Maza, Sztarker, Shkedy, Peszano, Locatelli, and Delorenzi, 2016b; Tomer, Denes, Tessmar-Raible, and Arendt, 2010; Wolff and Strausfeld, 2016). Phylogenetically distant species show a vulnerability to pharmacologic interventions during reconsolidation, from protein synthesis inhibitors to neuromodulators' agonists or antagonists, and to behavioral interventions; (Chen, Cai, Pearce, Sun, Roberts, and Glanzman, 2014; Eisenberg, Kobilo, Berman, and Dudai, 2003; Lukowiak, Fras, Smyth, Wong, and Hittel, 2007; Nader, Schafe, and Ledoux, 2000; Pedreira, 2013; Pedreira and Maldonado, 2003; Przybyslawski and Sara, 1997). Our hypothesis is that, during reconsolidation, endogenous neuromodulators can determine the ability of the memory to guide behavior by decreasing or increasing its behavioral expression, without disturbing both its persistence and its capacity to be reactivated (Caffaro et al., 2012; Delorenzi et al., 2014; Frenkel et al., 2005; Frenkel et al., 2010; Maza et al., 2016a). Accordingly, the amnesic effects found in human fear memories during reconsolidation would target the mechanisms that underlie the behavioral expression of the emotional components of fear memory, but not affect memory persistence (Agren, 2014; Kindt, Soeter, and Vervliet, 2009; Kindt and van Emmerik, 2016; Sevenster et al., 2012; Soeter and Kindt, 2010).

The working hypothesis of the present study is that, despite stress-induced retrieval deficit (by administration of CPS before testing), the potential for a memory trace to be reactivated, used

for mismatch evaluation and become labile remains unchanged (Delorenzi et al., 2014). According to other studies, the reactivation of a declarative memory after an increase in cortisol levels, due to a stressful experience or systemic administration, leads to both retrieval deficits and long-term memory attenuation (Tollenaar, Elzinga, Spinhoven, and Everaerd, 2008a; Tollenaar et al., 2009; Tollenaar, Elzinga, Spinhoven, and Everaerd, 2008b). A recent study shows similar result when fear memories are reactivated after a stressful experience (Meir Drexler and Wolf, 2016), but see (Drexler, Merz, Hamacher-Dang, Tegenthoff, and Wolf, 2015).

Here, we propose that, despite the retrieval deficit induced by CPS administration, the reactivation of this memory under stress leads to an attenuation of long-term memory expression through reconsolidation.

2. Experimental procedures

2.1. Participants

A total of 64 (36 women and 28 men) healthy undergraduate and graduate students participated as volunteers for the present study. Individuals who met any of the following criteria were excluded from participating: non-native Spanish speaking; current alcohol or substance abuse; cardiac disorders; hypertension; diabetes or treatment with psychotropic medications. All participating healthy volunteers were free of medication except for contraceptive pills (5 participants). Their ages ranged from 18 to 40, with a mean of 23.4 years old. A description separated by experimental series and groups of participants with information including age, sex, smoking status, menstrual cycle phase and use of hormonal contraception is shown at *Supplementary Material* section. Of the total, 14 subjects were excluded from the data analysis because they drank alcohol during the period of the experiment, wrote the syllables down outside the experimental room, consumed drugs, missed a step in the experimental protocol or did not meet the memory inclusion criteria by the end of the training session or coursed a stressing event during whole experiment duration. Congruent with previous studies using this memory paradigm, subjects with at least 65% correct responses in the last four training trials (13/20 correct responses) were included in the data analysis (Coccoz et al., 2011; Coccoz et al., 2013; Forcato et al., 2009; Forcato et al., 2007; Forcato, Rodriguez, and Pedreira, 2011; Forcato, Rodriguez, Pedreira, and Maldonado, 2010). All subjects were randomly assigned to groups and tested individually. In order to reduce the impact of diurnal cortisol level variations, the experiment was performed between 11:00 am and 5:00 pm (Cahill and van Stegeren, 2003). All participants were cited at experimental room at a previously accorded time, without having eaten or drunk for at least 2 hours beforehand. Before participating in the experiment, all subjects signed an informed consent, approved by the Ethic Committees of the Sociedad Argentina de Investigaciones Clínicas, and Facultad de Farmacia y Bioquímica of the Universidad de Buenos Aires.

2.2. The cold pressor stress (CPS) treatment

The procedure was the same as the one used by Cahill et al. (Cahill and van Stegeren, 2003) except that the maximum time for the CPS administration was 1 instead of 3 min, a modification required by the Ethic Committee (Coccoz et al., 2011; Coccoz et al., 2013). Briefly, subjects,

monitored by the experimenter, immersed their left arm to the elbow in ice-cold (0°-4°C) water and were told that they should keep their arms in the water for as long as possible, and that they could remove their arms whenever they liked at their discretion, and then covered by towels. In case they did not remove their arm before, participants were instructed to remove it from the water at 1 min (details in (Coccoz et al., 2011)). The mean CPS-administration time is shown at the *Result section*. As a control group, other participants were told to immerse their left arm in warm water (35-37°C) (WW).

2.2.1. Physiological and subjective measures to evaluate CPS effects

2.2.1.1. Cortisol assessment: In order to evaluate when the increase in cortisol level takes place (Experimental series 0; Figure 2), saliva samples (2ml) were obtained 5 minutes before CPS and 10, 20 and 30 minutes after CPS, and stored at -20°C prior to analyses. Cortisol levels were assayed using a commercial Elisa kit (Cortisol Saliva Elisa, DIAsource ImmunoAssays S.A., Belgium) and analyzed as concentration of cortisol (ng/ml) at basal and 10, 20 and 30 minutes post-CPS.

2.2.1.2. Blood pressure evaluation: Systolic and diastolic blood pressure was measured to asses adrenergic functioning using an automatic digital pressure monitor (*Omron Healthcare, model HEM-631int*). The cuff was placed on the wrist of the subject´s right arm (details in (Coccoz et al., 2011)) and measures were obtained before and during the CPS or WW treatment.

2.2.1.3. Subjective rating: In addition to each physiological recording, participants were asked to rate the treatment (CPS or WW) on a subjective scale: Very Unpleasant (-2), Unpleasant (-1), Indifferent (0), Pleasant (1) or Very Pleasant (2).

2.3. Experimental room

Experiments were conducted in a dim room using a personal computer. Each participant was provided with earphones and seated facing a monitor. The CPS or WW treatment was provided in a different room, adjacent to the experimental room.

2.4. The Paradigm

2.4.1. The program

In essence, participants had to learn a list of five pairs of nonsense syllables, the list was composed of five pairs of nonsense cue-response-syllables in Spanish: **ITE**-OBN, **ASP**-UOD, **FLI**-AIO, **NEB**-FOT, **DRI**-CRE (bold type: cue-syllable; regular type: response-syllable). The list was presented on the monitor screen, by a program designed using html and javascript code, so it runs locally through a common web browser. The program was a new version of the one described in Coccoz et al., 2013.

At the beginning, a start button should be clicked, and the program goes to a black screen for 10 seconds. During this time the subject is left alone in the room. The program continues automatically running a number of iterations that varies depending on the Day (Training day: 10 iterations, Testing day: 4 iterations). Each iteration consists of two stages: a context-stage and the syllable-stage (both described below). The list was associated with a specific context: an image on the monitor screen and a sound coming through the earphones (context-stage). During syllable-stage, every time a cue-syllable was shown a blank space appeared beside it; the cursor was posed on it, allowing the subject to enter a response using the keyboard (no interaction with the mouse was needed on this stage). After each iteration, a pause is introduced, where a silent black screen is shown. After 10 seconds, a new iteration begins. After the last iteration followed by the 10 seconds pause, a message is displayed announcing the end of the experiment.

2.4.2. Demo: before the *Training Session*, all participants were presented with a demo program explaining the instructions of the task. The program consisted of 4 trials, similar in structure to the training but with different pairs of nonsense-syllables associated with a different context.

2.4.3. Training Session (day 1): all participants underwent the same training protocol on Day 1 (details in (Coccoz et al., 2011; Forcato et al., 2007). As we commented above, each training trial was comprised of a context stage, where an image-sound combination was presented. After context stage, the series of nonsense-syllables were presented as paired-associates (the syllable stage). During the syllable stage, the background image and the sound from the context stage was preserved. In the syllable stage the five cue-syllables appeared progressively as described above, in random order, on the left-hand side of the monitor screen while an empty response-box appeared on the right-hand side. The first time that the list appeared on the computer

screen, the subject was told not to respond any syllable; after 5 s, the program shows the correct response for 4 s (in red) in order to allow the subject to memorize each response syllable associated with the matched cue syllable. In the following iterations, the subjects were given 5 s to write the corresponding response-syllable (Figure 1A). There were three situations that could occur during training: 1) if no response syllable was written down, the correct syllable was shown in red for 4 s; 2) if an incorrect response syllable was written down, it was replaced by the correct syllable and it was shown in red for 4 s; and 3) if the correct response was given, it stayed on the screen for 4 s. The complete iteration lasted 51 s: 6 s for the Context Stage plus 45 s for the syllable stage. Throughout the experiment, every time a subject faced a cue-syllable and wrote down a response, the program recorded: the exact text the subject typed (included backspaces and re-writings), the time of reaction, and the final result.

2.4.4. Testing Sessions (Day 4 or 5): the testing session consisted of the evaluation of the memory, in a random order of the 5 cue-response syllables, acquired during training. The testing session has the same structure as the training session except for the number of trials (4 instead of 10 trials) (Figure 1B). The subjects were not informed that there would be a memory test in the last session. During testing session, the participant response was recorded. In order to evaluate the main mnesic effects, only the first trial response was analyzed. The following trials of the testing session were analyzed as retraining data and used to estimate the cue-response syllable persistence.

Correct syllables responses were quantified. Three types of errors can be distinguished in this memory paradigm: Error 1) no response was written down; Error 2) the response-syllable was misspelled; or Error 3) the response-syllable was not the right one, but it belonged to the list (Figure 1F).

2.4.5. Reactivation Session (day 4): Participants were asked to perform a computer task similar to that one from the first day (Training session), but without the Demo session. The *Reactivation Session* included a reminder that reactivates and labilizes the memory (Labilizer-Reminder session; group CPS-LR) as described in (Coccoz et al., 2013; Pedreira, 2013): immediately after the training context, a cue-syllable appeared on the left-hand side of the monitor screen and the response-box. However, 2 seconds later a "System Error" notice displayed on the monitor

interrupting the session and *not allowing the subject to write down the response-syllable in the response-box* (Figure 1C). This type of reminder triggers the reconsolidation process (Coccoz et al., 2011; Forcato et al., 2009; Pedreira, 2013). As a control group regarding the specificity on reconsolidation effects, other participants passed through a No-Labilizer-Reminder session (group CPS-NLR): similar to *CPS*-LR but with the difference that the "System Error" notice was displayed on the monitor 5 s later, instead of 2 s, allowing the subject to write down the response-syllable in the response-box. This type of reminder does not trigger the reconsolidation process (Coccoz et al., 2011; Forcato et al., 2009; Pedreira, 2013)(Figure 1D).

2.5. Experimental Series

2.5.1. Series 0: Timing of Cortisol increase due to CPS

The first series of experiments intended to evaluate the timing of Cortisol increase induced by the CPS administration. Fifteen (15) participants (8 women and 7 men) were cited at experimental room between 11:00 am and 5:00 pm, and were asked to immerse their left arm into cold water (CPS) at least for 1 minute, with the possibility of removing their arm at their discretion. Blood pressures were assayed before and during the CPS treatment. Saliva samples were collected 5 minutes before CPS and 10, 20 and 30 minutes after CPS treatment, and stored at -20°C for posterior cortisol level assessment. Subjective assessments were performed as described in above. Three (3) participants were excluded from data analysis as they did not fit the inclusion criteria.

2.5.2. Series 1: The CPS effect on memory retrieval.

This series of experiments intended to evaluate whether the mild stressor CPS could have any effect on memory retrieval. In this series, twenty-five (25) subjects participated (16 women and 9 men; 5 participants were excluded from data analysis as they did not fit the memory inclusion criteria). All participants were trained at day 1 (training session); at day 4 they were asked to immerse their left arm in cold water (CPS group) or warm water (WW group) and 20 minutes later, their memory was tested (testing session *2.4.3.*) (Figure 3A). Correct syllable responses, error type, and physiological and subjective measures were assayed (2.2.1).

2.5.3. Series 2: The CPS effect on reconsolidation memory.

This series of experiments intended to evaluate whether the mild stressor CPS, 20 minutes before a reminder that labilized a memory, could have long-term effects on its testing 24 hours later. Twenty-four (24) subjects (12 women and 12 men) participated in this experimental series (six participants were excluded from data analysis as they did not fit the memory inclusion criteria). All participants were trained at day 1 (training session); at day 4 they were asked to immerse their left arm in cold water (CPS) and 20 minutes later, they were asked to perform a computer task similar to that one from the first day. Half of the participants passed through the Labilizer-Reminder session (*CPS*-LR group), and the other haft, through the No-Labilizer-Reminder session (*CPS*-NLR group). In all cases, all participants were cited at the next day (Day 5) to perform the computer task (testing session, *2.4.3.*) (Figure 4A). Correct syllable responses, error type, and physiological and subjective measures were assayed (2.2.1).

2.6. Statistics

The statistical analysis of memory performance was performed according to previous studies (Coccoz et al., 2011). Results were reported as mean and standard error of the total number of correct responses for the list. Data were analyzed using repeated measures ANOVA. The between-subjects factor was the experimental groups. The within-subjects factor was 'time of measurement': the tail end of training (Forcato et al., 2009; Forcato et al., 2010) and the testing performances of the subjects (Coccoz et al., 2011). For cortisol data were analyzed using repeated measures analysis of variance (ANOVA). For blood pressure data, a 2X2 design was employed (Schulz, Plein, Richter, Blumenthal, and Schachinger, 2011) in which the between-subjects factors were the experimental groups and the 'time of sampling´ before and during the CPS treatment measurements. *Post hoc* tests were performed using Fisher's LSD ($\alpha = 0.05$) between groups. We analyzed data using STATISTICA software (StatSoft 6.0).

3. Results

3.1. Series 0: CPS, blood pressure and cortisol increase

Since retrieval deficit occurs when cortisol levels are high, this experimental series was performed to evaluate the timing of cortisol increase induced by CPS. Fifteen (15) participants (8 women and 7 men) were cited at experimental room between 11:00 am and 5:00 pm, and were asked to immerse their left arm into cold water (CPS) at least for 1 minute, with the possibility of removing their arms at their discretion. Blood pressures were assayed before and during the CPS treatment. Saliva samples were collected 5 min before CPS treatment and 10, 20 and 30 min after CPS treatment, and stored at -20°C for posterior cortisol level assessment. Subjective assessments were performed as described in *Experimental Procedures*. Three (3) participants were excluded from data analysis as they did not fit the inclusion criteria. A description of the participants is shown in *Supplementary Material*.

Mean CPS-administration time was 45.1s (with a minimum of 24.6s to 1 min maximum). The exposure to the CPS treatment caused a significant rise in systolic and diastolic blood pressure (ANOVA: $F_{1,12}$=44.605, p= 0.000023, systolic; $F_{1,12}$=10.88, p= 0.0063, diastolic). Cortisol increase was observed at 20 min post-CPS (ANOVA: $F_{3,36}$=8.7642, p= 0.00017)(Figure 2). Subjects scaled the CPS as Unpleasant (Mean ± SEM = -1 ± 0.18).

According to previous studies, retrieval deficit occurs when cortisol levels are high (Roozendaal, 2002; Roozendaal and McGaugh, 2011; Schwabe and Wolf, 2014; Tollenaar et al., 2008a; 2009; Tollenaar et al., 2008b). Consequently, in the next experimental series, we tested whether the CPS, administered 20 min prior the testing session, could induce retrieval deficit.

3.2. Series 1: Effects of CPS into memory retrieval

Twenty five (25) subjects (16 women and 9 men) participated in this experimental series; a description of the participants is shown in *Supplementary Material*. All participants were trained at day 1. At day 4, were divided into two different experimental groups. In the CPS group, participants were asked to immerse the left arm into cold water as described before. 20 minutes later, they performed the testing session. In the WW group, participants were asked to immerse the left arm into warm water and 20 minutes later, they performed the testing session as well (Figure 3A). Blood pressures were assayed before (basal) and during treatments. Subjective assessments were performed as described former. Five (5) participants were excluded from data analysis as they did not fit the memory inclusion criteria.

3.2.1. Physiological and subjective measures.

Mean CPS-administration time was 54.97s (with a minimum of 39.7s to 1 min maximum), while mean WW-administration time was 1min. The exposure to the CPS treatment caused a significant rise in diastolic and systolic blood pressure respect to WW control: the difference between the pressure during treatment minus basal levels was 0.66 ± 2.9 mmHg in WW group and 14 ± 2.9 mmHg in CPS group for diastolic; and 1.2 ± 1.84 mmHg in WW group and 8.3 ± 1.9 mmHg in CPS group for systolic (ANOVA: $F_{4,13}$=7.0921, p= 0.00521, diastolic; p= 0.0148, systolic). As expected, all participants that were exposed to CPS rated the treatment as unpleasant (Mean ± SEM = -0.875 ± 0.3), while WW-treated subjects rated the treatment as pleasant (Mean ± SEM = 0.8 ± 0.29) (Tukey HSD test; p = 0.000114).

3.2.2. Cold pressor stress impairs memory expression at test

Repeated measures ANOVA of the *Training Tail* -the mean of correct responses for the last four trials of the training- compared to testing (Coccoz et al., 2011; Coccoz et al., 2013; Forcato et al., 2007) revealed an interaction effect between CPS and WW groups and trials ($F_{1,16}$= 6.6; p= 0.0204). In order to determine the degree of uniformity of the performances at *Training Session*, we compared the *Training Tail* (Box in Figure 3B), post hoc analyses showed no significant differences between groups (p = 0.84). A significant decrease in memory expression was observed 4 days after training in both groups (WW: p= 0.000584; CPS: p=0.000002, compared with the respective *Training Tail*). Remarkable, testing under stress induced a significant decrease in memory expression (p = 0.0029, CPS *vs.* WW group at *Testing Session)*(Figure 3B).

Error type analysis revealed significant differences between CPS and WW groups at testing for error type 1 (no completion) (ANOVA, $F_{5, 60}$ = 4.8116, p= 0.00092; Tukey HSD test: p=0.00055, mean ± SEM, WW group 1.25 ± 0.09, CPS group 3.37 ± 0.67). The other error types (2 and 3) did not show significant differences (all p > 0.8). In addition, no significant differences were observed between groups for all types of errors in the *Training Tail* (all p > 0.9).

In the next experimental series we evaluated whether, despite the impairing effect on retrieval due to the previous CPS treatment (Figure 3B), this memory can be reactivated and enter reconsolidation.

3.3. Series 2: Long-term outcomes of memory reactivation after Cold Pressor Stress

Twenty-four (24) subjects (12 women and 12 men) participated in this experimental series (six participants were excluded from data analysis as they did not fit the memory inclusion criteria). A description of the participants is shown in *Supplementary Material*.

All participants were trained at day 1. At day 4, all participants were asked to immerse the left arm into cold water (*2.5.1.*); 20 minutes later were divided into two experimental groups (Figure 4A). In the CPS-LR (Labilizer-Reminder session) group, the reminder structure that triggers reconsolidation *(2.4.5.)* was presented (Coccoz et al., 2011; Forcato et al., 2009; Pedreira, 2013). On the other hand, the CPS-NLR (No-Labilizer-Reminder session) group, the reminder that does not trigger reconsolidation *(2.4.5.)* was presented. Blood pressures were assayed before and during the CPS treatment. All participants were cited the following day (Day 5) to perform the Testing session (Figure 4A).

3.3.1. Physiological and subjective measures (Day 4)

The exposure to the CPS treatment caused a significant rise in diastolic and systolic blood pressure respect to basal levels in both groups (ANOVA: $F_{1,16}$ =0.52835, p=0.47780; Tukey HSD: p = 0.000323 (*CPS*-NLR) and p = 0.0037 (*CPS*-LR) for diastolic; p = 0.000312 (*CPS*-NLR) and p = 0.006 (*CPS*-LR) for systolic). No significant differences were observed between both *CPS*-NLR and *CPS*-LR groups (p>0.5 for diastolic; p>0.8 for systolic). Mean CPS-administration time was 56.76s (with a minimum of 41.1s to 1 min maximum) and 59.5s (with a minimum of 56s to 1 min maximum) for CPS-NLR and CPS-LR groups, respectively. As expected, all participants that were exposed to CPS rated the treatment as unpleasant (Mean ± SEM = -0.78 ± 0.22).

3.3.2. *Memory reactivation under stress impairs, via reconsolidation, long-term memory expression (Day 5)*

Repeated measures ANOVA of the *Training Tail* compared to testing revealed an interaction effect between groups and trials ($F_{1,16}$= 5.24; p = 0.036). In order to determine the degree of uniformity of the performances at *Training Session*, we compared the mean of correct responses

for the *Training Tail* (Box in Figure 4B), post hoc analyses showed no significant differences in correct responses between groups (p = 0.2). At Day 5, a significant decrease in performance was observed in both groups (*CPS*-NLR group: p = 0.000131; *CPS*-LR group: p= 0.000001, compared with the respective Training Tail). Remarkably, the mild stressor CPS treatment before the reminder session that triggers reconsolidation (Day 4) impairs the long-term memory expression at testing (p = 0.000625, *CPS*-LR *vs. CPS*-NLR)(Figure 4B). In spite of the retrieval deficit due to the previous CPS treatment (Figure 3) (*3.2.2*), the evaluation of the reminders conditions that triggers, or not, reconsolidation was possible. As a result, CPS administration before memory reactivation leaded to an attenuation of long-term memory expression that was reconsolidation-specific (Figure 4).

In order to appraise the persistence of the cue-response memory, retraining trials were analyzed: almost fully performance was observed in both groups already at the retraining trial 3 (trial 2; mean ± SEM: 3.25 ± 0.39 and 4.3 ± 0.35 for CPS-LR and CPS-NLR respectively)(trial 3: mean ± SEM: 4.37 ± 0.3 and 4.6 ± 0.27 for CPS-LR and CPS-NLR respectively; $F_{1, 16\ (group)}$ =1.07, p = 0.31; $F_{1, 16\ (trial)}$ =0.24, p=0.63). In addition, repeated measures ANOVA that included the four experimental groups from experimental series 1 and 2 (WW, CPS, CPS-NLR and CPS-LR) was performed to evaluate the differences between the testing results of all groups; ANOVA revealed an interaction effect between all groups and trials ($F_{3,32}$ = 4.2508; p= 0.012). No significant differences in correct responses were observed between WW (Day4) vs. *CPS*-NLR (Day5) (p=0.215) and between CPS (Day4) vs. *CPS*-LR (Day5) groups (p=0.404). Similar profile of the ones described in the post-hoc analysis performed in the two experimental series (Figures 3 and 4) was found: WW (Day4) vs. CPS (Day4), p=0.0013 and *CPS*-NLR (Day5) vs. *CPS*-LR (Day5) groups (p=0.00053).

4. Discussion

The present study found both short and long-term decreases in memory expression when memory was reactivated under stress. A key finding is that the negative modulation of memory expression induced during reconsolidation occurs even if retrieval is impaired. Despite the poor memory expression due to stressor exposure, the capacity of the memory to be reactivated, to evaluate the mismatch component of the reminder session and, then, becoming labile remains unaffected (Barreiro et al., 2013; Coccoz et al., 2013; Delorenzi et al., 2014; Frenkel et al., 2005; Rodriguez-Ortiz and Bermudez-Rattoni, 2016).

4.1 Memory reactivation under stress

The canonical view is that retrieval processes are particularly susceptible to be disrupted by acute stress, mainly explained by the induced increase in cortisol level (Buchanan, Tranel, and Adolphs, 2006; de Quervain, Roozendaal, and McGaugh, 1998; de Quervain, Roozendaal, Nitsch, McGaugh, and Hock, 2000; Lupien and Schramek, 2006; Roozendaal, 2002; Wolf et al., 2004). Although the effects of acute stressors actions on memory retrieval have predominately been described as more pronounced for emotional rather than for neutral memories, several studies have also found effects for neutral information, suggesting that pre-testing stress might preferentially affect emotional material if they are presented (Beckner, Tucker, Delville, and Mohr, 2006; Gagnon and Wagner, 2016; Luethi, Meier, and Sandi, 2008; Roozendaal, Okuda, de Quervain, and McGaugh, 2006; Sandi and Pinelo-Nava, 2007; Schwabe and Wolf, 2014; Wolf, 2009). In addition, several types of memories and retrieval tests are influenced by stressors (Gagnon and Wagner, 2016). Here, the result showed that CPS, before the cued-recall test, impairs the expression of this emotionally neutral declarative memory (Figure 3). Nonetheless, the view that stress impairs retrieval is not accurate since different effects can be obtained *via* autonomic (enhancing) and glucocorticoids (impairing) actions (Schonfeld, Ackermann, and Schwabe, 2014; Schwabe and Wolf, 2014). According with the elegant Schonfeld et al (2014) study, we paired in time the retrieval session with the expected delayed cortisol increase induced by CPS administration in order to effectively found the impairing effect during testing session (Figure 2 and 3). The short and long-term impaired effects here described are, in some way, according with other studies showing that a retrieval session under stress can impaired memory expression in a subsequent delayed cue-recall test (Meir Drexler and Wolf, 2016;

Tollenaar et al., 2008a; 2009). Among other explanations, reconsolidation is considered one of them (Gagnon and Wagner, 2016; Tollenaar et al., 2008a). Present study adds data supporting the view that reconsolidation is a key mechanism that underlie the long-term outcomes of reactivated memories under stress.

4.2. Stress and reconsolidation: positive and negative memory effects

Early and recent non-human animals studies show that both stressful experiences or glucocorticoids administration before or during reconsolidation can affect subsequent memory retention in both directions (Bustos, Giachero, Maldonado, and Molina, 2010; Cai, Blundell, Han, Greene, and Powell, 2006; Dodd and Lukowiak, 2015; Frenkel et al., 2005; Frenkel et al., 2010; Merz, Wolf, and Hennig, 2010; Tronel and Alberini, 2007). In agreement, studies in humans using diverse memory paradigms with different emotional contents show that stressors after memory reactivation, or during reconsolidation, can enhance or impair memory (Agren, 2014; Bos, Jacobs van Goethem, Beckers, and Kindt, 2014; Bos, Schuijer, Lodestijn, Beckers, and Kindt, 2014; Cheung, Garber, and Bryant, 2015; Hupbach and Dorskind, 2014; Kindt and van Emmerik, 2016; Merlo, Bekinschtein, Jonkman, and Medina, 2015; Nader, Hardt, and Lanius, 2013; Schwabe, Nader, and Pruessner, 2014; Schwabe and Wolf, 2009; 2010). Results using the present memory paradigm show that stress, glucose or a GABAergic agonist after memory reactivation improve memory (Coccoz et al., 2011; Coccoz et al., 2013; Rodriguez, Campos, Forcato, Leiguarda, Maldonado, Molina, and Pedreira, 2012). Resembling the retrieval view (Schonfeld et al., 2014; Schwabe and Wolf, 2014), present results show that CPS can exert opposite effects on reconsolidation according to administration times. We previously showed that after forgetting there would be a memory trace that would not be consciously accessed but could be reactivated and labilized by the appropriate reminder (Coccoz et al., 2013). When the CPS administration occurs after memory reactivation, the memory expression is improved in the long term (Coccoz et al., 2011). Remarkably, this effect occurs only when the CPS is given after the reminder that triggers reconsolidation. On the other hand, here we show that only when the CPS is given before the reminder that triggers reconsolidation the memory expression is impaired in the long-term (Figure 4). Consequently, the timing (before or after memory reactivation) of administration of stress protocol determines opposite short and long-term effects. There are a number of possible explanations of this difference. It is promising to

consider, among others, the possibility that the long-term improving effect occurs if the autonomic response activated by the stressor takes place shortly after the reconsolidation process is initiated. Conversely, when memory reactivation takes place 20 min post stress, the autonomic response is no longer present and the cortisol response to the stressor could be a key factor to the impairing long-term effect. This view is congruent with the description of the opposite roles of autonomic arousal and glucocorticoids in memory retrieval under stress (Schwabe and Wolf, 2014).

4.3. Memory reactivation beyond expression

The present results are in line with our view that memory expression is not required for a consolidated memory to be reactivated and then become labile by specific reminders (Delorenzi et al., 2014; Frenkel et al., 2010). In the light of this hypothesis, we showed that during reconsolidation (and consolidation) neuromodulators can determine the probability of memory to guide behavior, by either increasing or decreasing its behavioral expression, without affecting the potential of persistent memories to be reactivated and become labile in the long-term (Barreiro et al., 2013; Blake et al., 2012; Caffaro et al., 2012; Coccoz et al., 2011; Coccoz et al., 2013; Frenkel et al., 2010; Maza et al., 2016a). Concordantly, here we show that, although the very poor memory expression due to the stressor before reminder (Day 4, Figure 3), the memory of the nonsense cue-response-syllables must be reactivated. Then, the mismatch condition is evaluated (reminders that trigger or not reconsolidation), the memory trace becomes labile, and after that, the memory expression is impaired at long-term (Day 5, Figure 4). Several boundary conditions are proposed for reconsolidation: memory age, memory strength, extinction, among others (Fernandez et al., 2016b). Here, it is important to highlight that not all reactivation sessions leads memory to reconsolidation, mismatch is a boundary condition (Alberini, 2007; Diaz-Mataix et al., 2013; Dudai, 2012; Fernandez et al., 2016b; Forcato et al., 2009; Frenkel et al., 2005; Pedreira et al., 2004; Rodriguez-Ortiz and Bermudez-Rattoni, 2016; Rodriguez-Ortiz, Garcia-DeLaTorre, Benavidez, Ballesteros, and Bermudez-Rattoni, 2008; Sevenster et al., 2012; 2014). Therefore, the probability of the cue-response syllable memory to being accessed at the testing session appears not to be affected by CPS before reminder (Figure 4). Results like this are harmonious with the classical proposition that two different processes underlie retrieval: memories must first be reactivated (ecphory) and then a subsequent process

(conversion) will determine whether they can or cannot be behaviorally expressed (Tulving, 1983). Expression is not a necessary condition either to reactivate long-term memories or to use the reactivated information to evaluate the mismatch conditions. Results showed here add new evidence supporting the view that the mechanisms mediating memory reconsolidation and the mechanisms that underlie the behavioral expression of memory are different (Barreiro et al., 2013; Ben Mamou et al., 2006; Caffaro et al., 2012; Coccoz et al., 2013; Finn, Roediger, and Rosenzweig, 2012; Frenkel et al., 2005; Frenkel et al., 2010; Lee and Flavell, 2014; Merlo et al., 2015; Milton et al., 2013; Rodriguez-Ortiz et al., 2012; Rodriguez-Ortiz and Bermudez-Rattoni, 2016; Sevenster et al., 2012). Similar to the enhancing effects on memory reconsolidation, the impairing effects in the behavioral expression of long-term memories induced during reconsolidation might be due to, for instance, changes in decision-making processes or the modulation of putative retrieval-links that are critical for long-term memory expression (Brembs, 2011; Delorenzi et al., 2014; Dudai and Eisenberg, 2004; Menzel, 2012; Shadlen and Kiani, 2013). Consequently, reconsolidation might reflect a series of processes that allows memory updating by increasing or decreasing the hierarchy of memories that potentially control behavior. Here, results show that at retraining, Day 5 (*3.3.2*), both experimental groups presented almost fully performance suggesting that, although unexpressed in the long-term, the cue-response memory persists also in the CPS-LR (Labilizer-Reminder session) group. Accordingly, other studies show that during reconsolidation it is possible to affect the mechanisms that underlie the behavioral expression of the emotional components of fear memories without necessarily affecting memory persistence (Kindt et al., 2009; Sevenster et al., 2012; 2013; Soeter and Kindt, 2010).

4.4. A look at retrieval through the glasses of reconsolidation

Unlike consolidation and reconsolidation, the neurobiological research to the domain of the rich theoretical concepts of the retrieval process have been more limited (Barros, Izquierdo, Medina, and Izquierdo, 2003; Dudai, 2002; Summers, Crowe, and Ng, 2003; Sweatt, 2007). From the very beginnings of the rebirth of reconsolidation, and as a result of their pioneering studies, Sara and colleagues pointed out that the reconsolidation hypothesis will lead to new looks at the retrieval process (Przybyslawski, Roullet, and Sara, 1999; Przybyslawski and Sara, 1997; Sara, 2000). In neurobiological accounts, retrieval can be considered as reactivation of inactive memory traces

that guide behavior (Roediger, Dudai, and Fitzpatrick, 2007), a concept traceable to the view that memory only lends itself to study through its retrieval ("The only proof of there being retention is that recall actually takes place", William James warning (1872), from (Sara, 2000)). The experimental design exemplified in the present study perhaps might invite to look again that William James paradigmatic advice. Our studies regarding the action of neuromodulators during both memory consolidation and reconsolidation have show that unexpressed memories can be reactivated and become labile, stressing that retrieval and memory expression are not interchangeable concepts (Delorenzi et al., 2014). In the experimental design what is evaluated at testing sessions is whether unexpressed memories have been previously reactivated and become labile by a reminder that trigger reconsolidation. Although unexpressed, the corroboration that a consolidated memory is retrieved might be that the trace has been reactivated and the information learned used to evaluate mismatch conditions during reminder sessions (Caffaro et al., 2012; Coccoz et al., 2011; Frenkel et al., 2005; Frenkel et al., 2010; Rodriguez-Ortiz et al., 2012; Rodriguez-Ortiz and Bermudez-Rattoni, 2016; Santoyo-Zedillo, Rodriguez-Ortiz, Chavez-Marchetta, Bermudez-Rattoni, and Balderas, 2014b). Neural correlates of memory have usually been explored considering that memory retrieval and memory expression are interchangeable concepts. However, we find in the crab *Neohelice* changes in neural activity induced by training that correlates with memory persistence but not with the probability of this memory to be expressed in the long term (Maza et al., 2016a). The experimental design here discussed can add another view to explain, for example, findings showing that memories may persist covertly after its apparent elimination by some amnesic treatments (Delorenzi et al., 2014; Gisquet-Verrier and Riccio, 2012; Gold, 2006; Nader and Wang, 2006; Ryan, Roy, Pignatelli, Arons, and Tonegawa, 2015).

The terms active, reactive and expression might be constructive for descriptions of the processes that retrieve consolidated memory traces.

4.5. Limitations

The present experimental series were designed in order to evaluate whether an unexpressed memory can be reactivated and enter reconsolidation. The procedure used to interfere memory expression in the reminder sessions was mild stressor CPS; the same used in other studies (e.g. (Cahill and van Stegeren, 2003)) except that in our studies the maximum time for the CPS administration was 1 instead of 3 min (2.2) (Coccoz et al., 2011; Coccoz et al., 2013). Since

retrieval impairments were previously described when cortisol level is high, the Series 0 (Figure 2) evaluates the timing of cortisol increase induced by CPS when the maximum time of exposure was 1 min; limitations were the absent of a warm-water control and that cortisol was only measured during this experimental series. Nonetheless, when an unstressed group was used as control, behavioral results were in agreement with a number of previous studies (Series 1, Figure 3)(Gagnon and Wagner, 2016). In addition, experimental series 2 was performed in order to evaluate whether the reactivate trace could be used to evaluate the mismatch condition during the reminder session even when stressor administration before reactivation impairs memory expression. Thus, all participants were treated with CPS and then were separated into two experimental groups according to the type of the reminder that include, or not, mismatch conditions (Fernandez, Bavassi, Forcato, and Pedreira, 2016a). A limitation of the design might be the absent of a stress-free control. Nonetheless, we decide to use as control subjects that performed identical procedures as the experimental group but the reminder that does not trigger reconsolidation was included. Indeed, the long-term effect of reactivate memory under stress was no disclosed in this group (*3.3.)*. Although it would be interesting to analyze differences between women and men, the experimental design of the present study does not allow this examination.

4.6. General conclusions:

Overall, present and previous studies show that - depending on time of administration - a mild stressor can have either enhancing or impairing effect on emotionally-neutral human memory throughout its action on reconsolidation process. Stress impairs retrieval by disrupting memory expression. However, memory expression is not required for memory reactivation-labilization. Stress leaves its footprint in the reactivated memory by changing -via reconsolidation- the memory "strength", the probability of the traces to be expressed in the long term.

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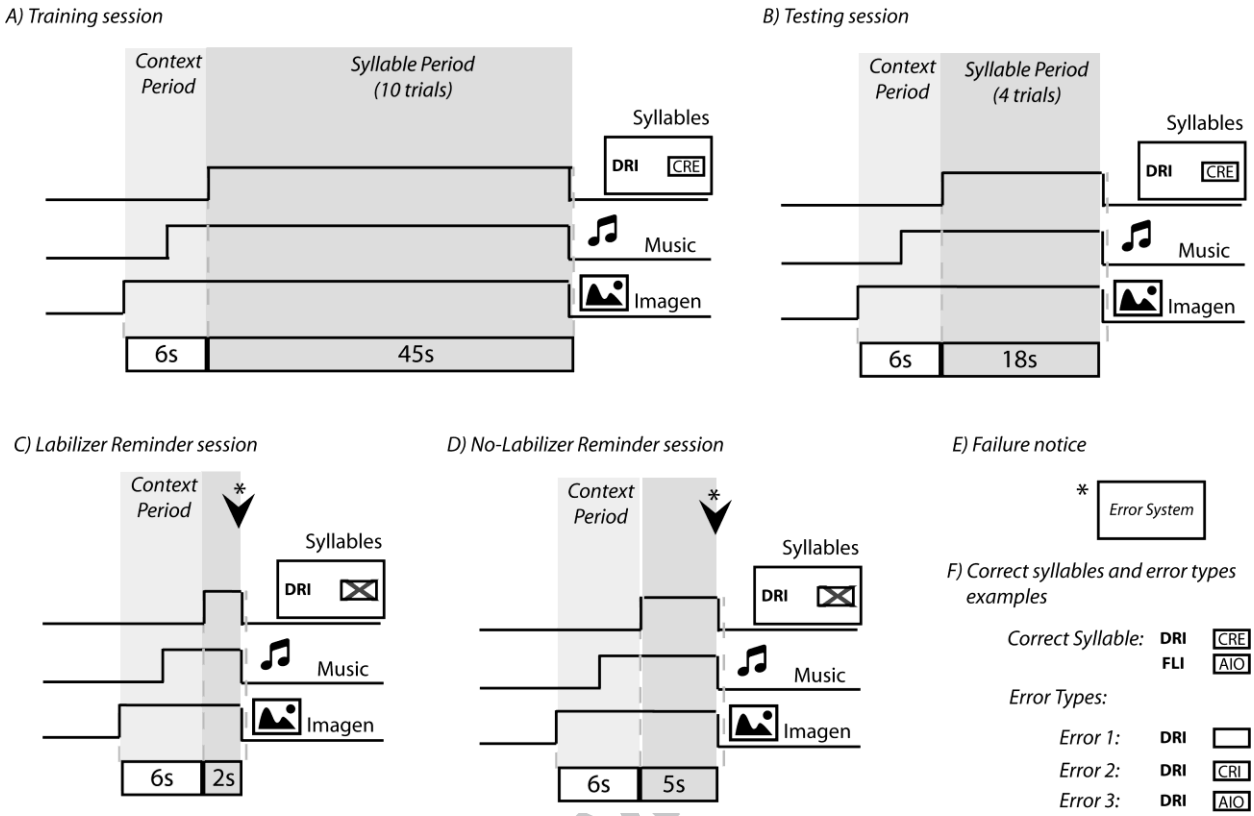
Figure Legends

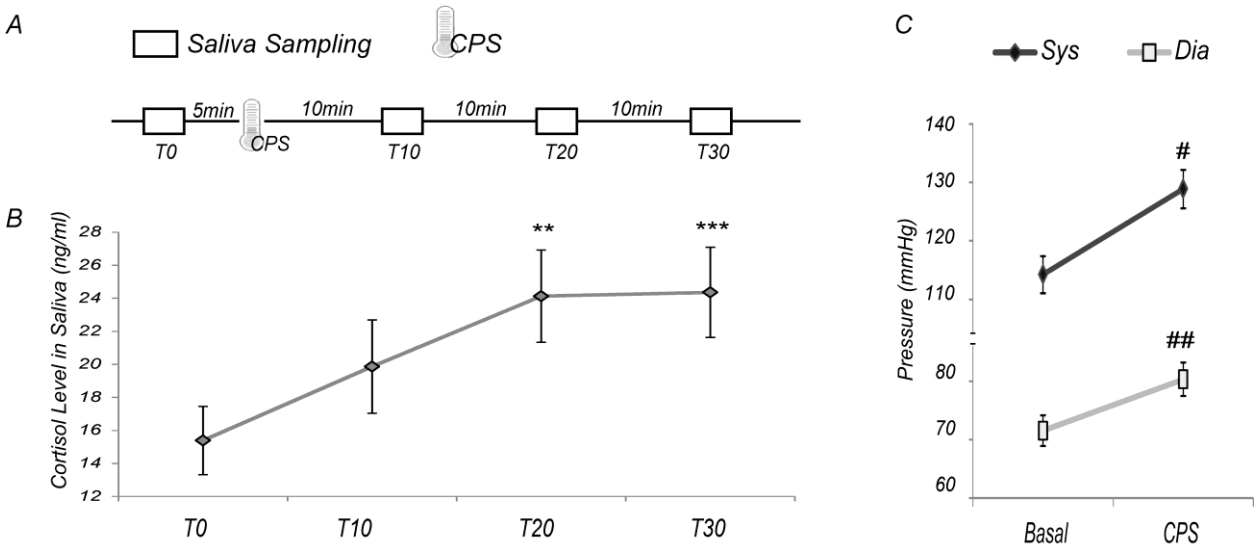
Figure 1. Experimental design for the Training Session (A), the Testing Session (B), Labilizer-Reminder-LR session, (C) and the No-Labilizer-Reminder-NLR session (D). Failure notice in the LR and NLR (E). Correct Syllables response and error types 1, 2 and 3 examples (F).

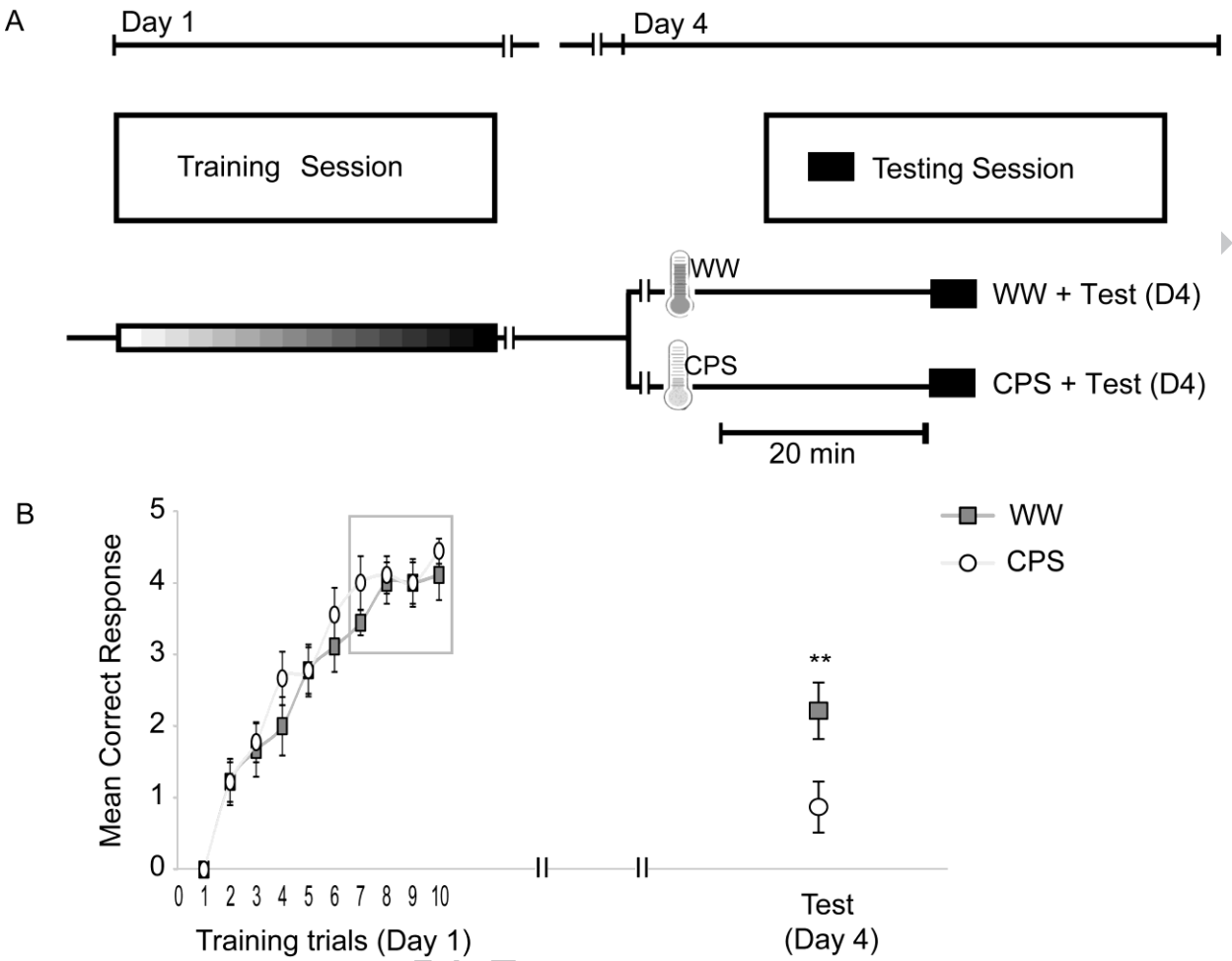
Figure 2. A. Experimental design for saliva sampling before (T0-Basal) and 10 (T10), 20 (T20) and 30 minutes (T30) after cold pressor stress (CPS); B. Cortisol levels (ng/ml) in saliva at basal and different times post-CPS samples; C. Systolic and diastolic blood pressure (mmHg) before (basal) and during CPS. Mean ± SEM. ** p=0.00078 and ***p=0.0006, both compared with basal level; #p=0.00016 and ##p=0.0085, both compared with basal pressure.

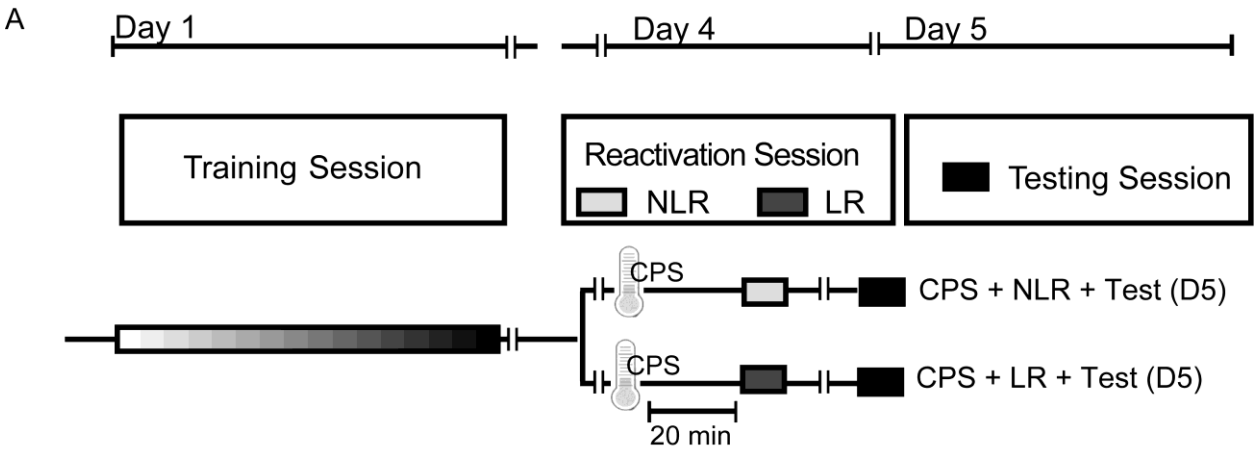
Figure 3. A. Experimental design for Series 1 experimental groups: at day 1, Training Session was performed; at day 4, participants immersed their left arm in warm water (WW) or cold water (CPS) for at least 1 min and 20 minutes later, Testing Session was performed; B. Mean Correct Responses during the 10 trials of the Training Session and the first trial of the Testing Session for both WW and CPS groups. Grey box represents the Training Tail. Mean of correct responses ± SEM. ** p= 0.0029.

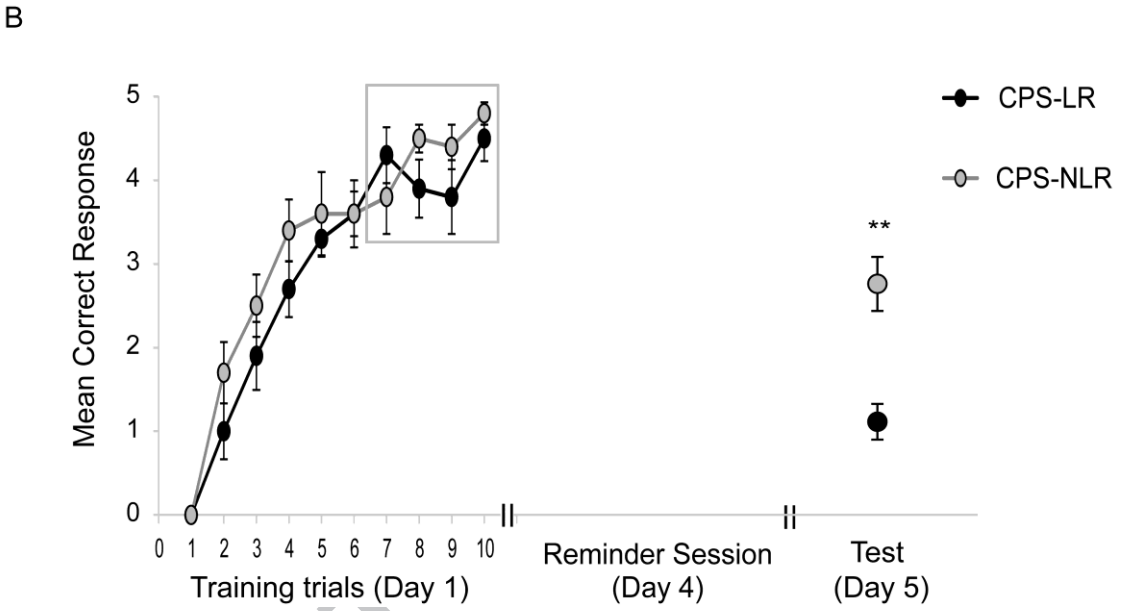
Figure 4. A. Experimental design for Series 2 experimental groups: at day 1, Training Session was performed; at day 4, all participants immersed their left arm in cold water (CPS) for at least 1 min and 20 min later, participants were asked to perform a computed task similar to that one from day 1 (without Demo session) but in this case, 2s post cue-syllable appearance, a failure notice displayed not allowing the subject to write down the response-syllable in the response-box (Labilizer-Reminder session; CPS-LR group) or the failure notice disrupts 5s post cue-syllable appearance, allowing the subject to write down the response-syllable in the response-box (No-Labilizer-Reminder session; CPS-NLR). Testing Session was performed at Day 5. B. Mean Correct Responses during the 10 trials of the Training Session and the first trial of the Testing Session for both CPS-NLR and CPS-LR groups. Grey box represents the Training Tail. Mean of correct responses ± SEM. ** p= 0.000625.











Highlights

Retrieval under stress decreases the long-term expression of a human declarative memory via reconsolidation. *Pablo Nicolás Fernández Larrosa, Alejandro Ojea, Ignacio Ojea, Victor Alejandro Molina, María Aurelia Zorrilla-Zubilete and Alejandro Delorenzi.*

- The canonical view is that stress disrupts memory retrieval.
- Reconsolidation studies reshape several memory concepts, including retrieval.
- Present results show that a mild stressor disrupts memory expression.
- However, the memory trace retains the potentiality of being reactivated.
- The reactivated, but unexpressed, information is used to initiate reconsolidation.

The Long-Term Outcome Of Reactivate Memory Under Stress Is Reconsolidation-Specific.

The cannonical view postulates that stress impairs memory retrieval. However, the unexpressed memory trace could be reactivated. Then, the mismatch condition at the reminder session is indeed evaluated and reconsolidation is (A), or not (B), initiated. The negative modulation in the long-term memory expression only occurs if reconsolidation was initiated.