Neurobiology of Learning and Memory 94 (2010) 509–520

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Angiotensin modulates long-term memory expression but not long-term memory storage in the crab *Chasmagnathus*

Lia Frenkel [1], Luis Daniel Suárez [2], Héctor Maldonado [2], Alejandro Delorenzi *

Laboratorio de Neurobiología de la Memoria, Departamento de Fisiología y Biología Molecular, IFIByNE-CONICET, Pabellón II, FCEyN, Universidad de Buenos Aires, Ciudad Universitaria (C1428EHA), Argentina

ARTICLE INFO

Article history:
Received 2 February 2010
Revised 30 August 2010
Accepted 1 September 2010
Available online 7 September 2010

Keywords:
Memory
Consolidation
Reconsolidation
Expression
Angiotensin
Modulatory system
Chasmagnathus

ABSTRACT

Memory reconsolidation is a dynamic process in which a previously consolidated memory becomes labile following reactivation by a reminder. In a previous study in the crab *Chasmagnathus* memory model, we showed that a water-shortage episode, via angiotensin modulation during reconsolidation, could reveal a memory that otherwise remains unexpressed: weakly trained animals cannot reveal long-term memory (LTM) except when an episode of noticeable ethological meaning, water deprivation, is contingent upon reconsolidation. However, these results are at variance with two of our previous interpretations: weak training protocols do not build LTM and angiotensin II modulates the strength of the information storing process. A parsimonious hypothesis is that in *Chasmagnathus* angiotensins regulate LTM expression, but not LTM storage. Here, we tested three predictions of this hypothesis. First, the well-known retrograde amnesic effect of the angiotensin II antagonist saralasin is not due to interference on memory storage, but to modulation of memory expression. Second, the recovery of the LTM memory expression of the apparently amnesic retrograde effect produced by saralasin, through the water-shortage episode contingent upon reconsolidation, must be reconsolidation specific. Consequently, summation-like effects and retrieval deficits cannot explain these results because of the parametric conditions of reconsolidation. Third, weak training protocols build an unexpressed LTM that requires mRNA transcription and translation, a diagnostic characteristic of LTM. Results show that angiotensin modulates LTM expression but not LTM memory storage in the crab *Chasmagnathus*. The results lead us to suggest that, in *Chasmagnathus*, LTM expression – the process of gaining appreciable control over behavior of the reactivated trace in the retrieval session – may be considered a distinct attribute of its long-term storage. This strategy, a positive modulation during reconsolidation, is proposed to distinguish between memories that can be reactivated, labilized and are not expressed, and memories that are not stored long term, obliterated or altered in other retrieval mechanisms.

1. Introduction

Finding out what the animal learns and remembers about the experimental training session is not a simple matter. But it is certainly a crucial matter (Cahill, McGaugh, & Weinberger, 2001). A frequent finding in the long history of memory studies has been that changing experimental conditions could reveal a memory that otherwise remains unexpressed; for instance, a result that has been interpreted as additional learning, saving, retrieval failure or summation effects in experimental amnesia studies (Cahill et al., 2001; Gold, Haycock, Marri, & McGaugh, 1973; Nader & Wang, 2006; Philips, Tzvetkova, Marinesco, & Carew, 2006). The disclosure of a LTM that remains unexpressed has also been reported in the crab *Chasmagnathus granulatus* memory model.

In this associative memory animals associate the training context with a visual danger stimulus (VDS) passing overhead. After the iterative spaced presentation of the VDS, a strong freezing-to-VDS response replaces the initial escape response (Maldonado, 2002). After a strong training protocol (15 spaced trials) crabs exhibit LTM 24–96 h later. Since the memory under study arises as a consequence of an association between the context (the CS) and the VDS (the US signal) it is termed context-signal memory (CSM) (Maldonado, 2002). No LTM is revealed when weak training (six spaced trials) is presented. Consequently, this protocol was used to investigate, among other memory mechanisms, angiotensin II (ANGII) as the main neuromodulator that mediates the memory improvements triggered by an episode of water deprivation (Table 1).

* Corresponding author. Fax: +54 11 4576 3447.
E-mail addresses: frenkel@leloir.org.ar (L. Frenkel), luisdsuarez@yahoo.com (L.D. Suárez), hector@fbmc.fcen.uba.ar (H. Maldonado), delorenzi@fbmc.fcen.uba.ar (A. Delorenzi).
[1] Present address. Laboratorio de Genética del Comportamiento, Fundación Instituto Leloir-IIBBA-CONICET, Buenos Aires, Argentina.
[2] Fax: +54 11 4576 3447.

1074-7427/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.nlm.2010.09.003

Specifically, we previously showed that after a weak training protocol, which is assumed to induce only a short-term memory but not a LTM (Table 1a), CSM tested 24 h or more appears fully expressed provided an episode of water deprivation is contingent upon the process of memory reconsolidation. As well as other previous results, this reconsolidation finding has also been interpreted as a memory strengthening effect during reconsolidation in terms of the current concept that memory modulatory systems are endogenous systems that influence LTM storage processes (Cahill & McGaugh, 1996; Ferry & McGaugh, 2000; Ferry, Roozendaal, & McGaugh, 1999; Krasne, 1978; McGaugh, 2000). However, since memory reconsolidation is a process where a previously consolidated memory becomes labile following reactivation by a reminder, a LTM might be induced by weak training protocols and, therefore, the long-term expression might be the mnesic property which is modulated by endogenous angiotensins. In other words, it is plausible that the weak training protocol generates a LTM trace that would be (a) reactivated by specific context cues but is not in the process of gaining appreciable control over behavior in the retrieval session (*sensu* (Eisenberg, Kobilo, Berman, & Dudai, 2003), that is remains unexpressed and (b) displays transient sensitivity to be modulated during the labilization period of reconsolidation. In this view, ANGII could be a neuromodulator that during consolidation and reconsolidation might determine the ability of CSM to gain appreciable control over behavior, to increase or decrease its long-term expression, but not the storage over time of the experience-dependent internal representation.

The central hypothesis of the present paper, based on previous experimental findings (Table 1), is that long-term CSM comprises two essential attributes: long-term storage and expression. The ability of water shortages, via ANGII, to produce changes in CSM might be related to changes in the expression of the reactivated memory, i.e. in the process of gaining appreciable control over behavior in the retrieval session, but not to changes in the storage over time of the learned information. Here, three series of experiments were designed to test three predictions of this hypothesis.

First, the retrograde amnesic effects of the ANGII antagonist saralasin on CSM consolidation (Table 1b) is not due to a memory-storage interference, as we previously interpreted, but to the modulation of memory expression of the reactivated memory. Therefore, unlike cycloheximide, the post-training saralasin administration after strong training in *Chasmagnathus* generates an unexpressed long-term CSM, since this memory trace can be fully expressed after the retrieval session that triggers reconsolidation contingent upon an episode of water deprivation.

Second, the hypothesis predicts that post-training saralasin administration does not affect the LTM trace since it can be reactivated during the reminder session (despite it not gaining appreciable control over behavior) and positively modulated during reconsolidation by the water deprivation episode. The recovery of CSM expression must therefore be reconsolidation specific, that is, it does not occur if (a) the context of the reminder is not the training context, (b) the water deprivation episode comes 6 h after the reminder or, remarkably, (c) no reconsolidation occurs because the unconditioned stimulus is presented immediately before the reminder terminates (Carbo Tano, Molina, Maldonado, & Pedreira, 2009; Forcato, Argibay, Pedreira, & Maldonado, 2009; Frenkel, Maldonado, & Delorenzi, 2005a; Merlo, Freudenthal, Maldonado, & Romano, 2005; Morris et al., 2006; Pedreira & Maldonado, 2003; Pedreira, Perez-Cuesta, & Maldonado, 2004; Perez-Cuesta & Maldonado, 2009). Consequently, this change in LTM expression is hard to explain in terms of additional learning, saving, residual traces, retrieval deficits or summation effects.

Third, if a weak training protocol does prompt a LTM that remains unexpressed (Table 1d), this memory trace must possess the diagnostic characteristics of LTMs. Therefore, the memory whose expression is revealed after a water deprivation episode must depend on both new protein synthesis and new mRNA transcription after acquisition, a conclusive characteristic of LTM consolidation in the neurobiological field (Davis & Squire, 1984; Stough, Shobe, & Carew, 2006) (but see Gold (2008)). In addition, post-training saralasin administration should not affect the memory that can be revealed by water deprivation since the retrograde amnesic effect of the antagonist is not due to storage interference but to a change in the LTM expression.

2. Experimental procedures

2.1. Animals

Intermolt adult male crabs of the species *C. granulatus* between 2.7 and 3.0 cm across carapace were collected from the narrow coastal inlets of San Clemente del Tuyú, Argentina. In the laboratory, crabs were kept on a 12:12 h light–dark cycle, in collective tanks (20 animals each) filled up to 2 cm deep with 12‰ seawater prepared with hw-Marinex (Winex, Germany) salt, pH 7.4–7.6. The holding and experimental rooms were kept at 22–24 °C and 80 ± 10% relative humidity. Experiments were done in the daytime within the first week after the arrival of animals. Studies in

Table 1
Select results about water deprivation (w.d.) and angiotensin II (ANGII) effects on different CSM phases. Others symbols: SAR (ANGII antagonist); reac.sess. (reactivation session regarding reconsolidation studies); US (the reinforcement); CHX (cycloheximide); (+) denotes long-term memory expression; (−) denotes no long-term memory expression. (a) Carbo Tano et al. (2009), Delorenzi et al. (1995, 1996, 1997, 2000), Delorenzi and Maldonado (1999), Frenkel et al. (2002), Frenkel et al. (2005a, 2005b), Freudenthal et al. (1998), Kaczer, Pedetta, and Maldonado (2007), Locatelli, Maldonado, and Romano (2002), Maldonado (2002), Maldonado et al. (1997), Romano, Delorenzi, et al. (1996) and Romano, Locatelli, et al. (1996) memory after a WTP is expressed at least up to 4 h, whereas at 8 h memory is no longer expressed; Smal, Suárez, Delorenzi, personal communication. (b) Delorenzi et al. (1995, 1996, 1997, 2000), Delorenzi and Maldonado (1999), and Frenkel et al. (2002). (c) Frenkel et al. (2005b). (d) Frenkel et al. (2005a).

Training (a)		Treatment on					
		Consolidation (b)		Retrieval (c)		Reconsolidation (d)	
WTP	−					reac.sess.	−
						reac.sess. plus US	−
						reac.sess. plus w.d.	+
		w.d.	+	w.d.	+	novel context plus w.d.	−
		w.d. plus SAR	−	w.d. plus SAR	−	reac.sess. plus ANGII	+
		ANGII	+	ANGII	+	reac.sess. plus US and w.d.	−
		ANGII plus SAR	−	ANGII plus SAR	−	reac.sess. plus US and ANGII	−
						reac.sess plus w.d. and CHX	−
						reac.sess. plus w.d. and SAR	−
STP	+					reac.sess.	+
		SAR	−	SAR	+	reac.sess. plus SAR	−
						reac.sess. plus US and SAR	+

Chasmagnathus have shown that 50% of animals die after 48 h of water deprivation (Santos & Costa, 1993). Each crab was used in one experiment only. Experimental procedures were in compliance with the policies on the use of Animals and Humans in Neuroscience Research. All efforts were made to minimize the number of animals used and their suffering.

2.2. The experimental device

The experimental device, the actometer (Maldonado, 2002), referred to as the training context (the CS), consisted of a bowl-shaped opaque container with a steep concave wall 12 cm high (23 cm top diameter and 9 cm floor diameter) covered to a depth of 0.5 cm with artificial sea water, where the crab was lodged before each experimental session. During each trial of 9 s, an opaque rectangular screen (25–7.5 cm), termed visual danger stimulus (VDS), was moved twice horizontally over the animal, cyclically from left to right and vice versa, at a constant speed. The VDS (the US) provoked an escape response in crabs and consequent container vibrations, converted into electrical signals through a piezoelectric transducer placed on the external wall of the container. These signals were amplified, integrated during each 9-s trial, and translated into numerical units ranging from 0 to 6000, before being processed by computer. The activity of every crab was recorded during each entire trial time. The experimental room had 40 devices, separated from each other by partitions. During the experiments, crabs were illuminated with a 5 W bulb, from above the training context. In experiment of Fig. 2A, a cylindrical (15 cm in diameter and 15 cm in height) plastic container with black and white striped walls, covered to a depth of 0.5 cm with artificial sea water, was used as a context during the reactivation session.

2.3. Escape response and freezing

The number of container vibrations during the 9 s of US presentation (a trial) depends on the magnitude of the defensive responses that each crab displays when faced with an impending threat. Two types of defensive responses are distinguished: namely, escape and freezing responses (Pereyra, Gonzalez, & Maldonado, 2000). The escape response is a directional run of the animal in an attempt to move away from the US, while the freezing response consists of a rigid motionless display in which the crab lies flat on the substratum. During repeated US presentations (training), the escape response decreases in intensity and is replaced by the progressive build-up of a strong and long-lasting freezing. No defensive responses but exploration or wandering are shown during CS exposures without US presentation. Throughout this study, data were only recorded during a trial time, i.e., during the 9-s VDS.

2.4. Strong and weak training protocols

Fifteen US, 3 min apart (strong training protocol), induce an association between the iterated US and the contextual features of the plastic container (conditioned stimulus, CS, training context). This context-signal memory (CSM) persists for at least 5 days (Maldonado, 2002; Romano et al., 2006; Tomsic, de Astrada, Sztarker, & Maldonado, 2009; Tomsic, Pedreira, Romano, Hermitte, & Maldonado, 1998). After only six training trials (weak training protocol) animals do not show long-term CSM. This protocol tests improving effects on memory (Carbo Tano et al., 2009; Delorenzi, Locatelli, Romano, Nahmod, & Maldonado, 1997; Delorenzi & Maldonado, 1999; Delorenzi et al., 1995, 1996, 2000; Frenkel, Maldonado, & Delorenzi, 2005b; Frenkel et al., 2002, 2005a; Freudenthal et al., 1998; Romano, Locatelli, Delorenzi, Pedreira, & Maldonado, 1996). Experimental protocols involve pairs of crab groups, where each pair has a trained group (TR) that receives US presentations and an untrained group (untrained group UN). Studies performed on the mechanisms underlying different memory phases have shown that CSM consolidation, extinction and reconsolidation are blocked by protein synthesis inhibitors, positively modulated by angiotensins (Table 1); selectively regulated by a muscarinic cholinergic mechanism, GABA and crucially mediated by N-methyl-D-aspartic acid (NMDA)-like glutamatergic receptors (Merlo & Romano, 2007; Pedreira, Dimant, & Maldonado, 1996; Pedreira, Dimant, Tomsic, Quesada-Allue, & Maldonado, 1995; Pedreira, Perez-Cuesta, & Maldonado, 2002; Pedreira et al., 2004; Perez-Cuesta & Maldonado, 2009; Romano, Freudenthal, Merlo, & Routtenberg, 2006; Romano, Locatelli, et al., 2006; Tomsic et al., 2009; Troncoso & Maldonado, 2002). Some neurons involved in this contextual memory have been deeply studied (Tomsic, Beron de Astrada, & Sztarker, 2003). At the molecular level, it was demonstrated the cAMP signal pathway, MAP kinases pathway and the NFκ-B transcription factor are required during CMS consolidation (Romano, Freudenthal, et al., 2006). Findings from studies carried out to investigate the mechanisms underlying reconsolidation showed a reliable CSM labilization by re-exposing the animals for 5 min to the learning context 24 h after training. This labilized memory is, for example, cycloheximide, GABA, saralasin and sulfasalazine sensitive (Carbo Tano et al., 2009; Frenkel et al., 2005a; Pedreira, Perez-Cuesta, & Maldonado, 2002; Pedreira et al., 2004; Perez-Cuesta & Maldonado, 2009; Romano, Freudenthal, et al., 2006; Romano, Locatelli, et al., 2006).

2.5. Experimental procedure and design

Experiments included two or three sessions: Training (Day 1) and testing (Day X), 24, 48 h or 5 days later. Reconsolidation experiments included a Training session (Day 1), a Reminder session (Day 2) 24 h later and a Testing session (Day 3) another 24 h later. UN or TR groups of 30–40 crabs each were set in each experiment.

2.5.1. Day 1, training session

Untrained animals (UN) were kept in the training context (CS) during the entire training session as controls, i.e., without being presented with the visual danger stimulus (VDS = US). Trained animals (TR), after being in the container for 5 min. without VDS, received 15 or six training trials, each consisting of a 9-s VDS presentation (US), separated by inter-trial intervals of 3 min. Immediately after the training session, both UN and TR crabs were moved from the training context to be housed individually in the resting containers, i.e., plastic boxes covered to a depth of 0.5 cm with brackish water and kept inside dimly lit drawers.

2.5.2. Testing session

Crabs were tested for memory expression with a US presentation in the original CS, the training context. The test trial consisted of one US in the CS. It was given on Day 2, 3 or 5.

2.5.3. Reminder session

Crabs were exposed to the training context for 5 min and then returned to their individual resting containers until the next day. This procedure turns memory into a labile state. In one experiment, a VDS was presented during the last 9 s of the reminder session. This procedure prevents memory labilization (Carbo Tano et al., 2009; Frenkel et al., 2005a; Pedreira et al., 2004; Perez-Cuesta & Maldonado, 2009).

2.6. Water deprivation

Crabs were water deprived within their individual resting containers for 2 h after context re-exposure or before the testing

session. Water deprivation for 2 h increases angiotensin II brain levels (Frenkel et al., 2002). Water deprivation occurred only in the resting containers. To test after water deprivation, actometers were filled with brackish water to a depth of 0.5 cm as in every other experimental session.

2.7. Drug administration

Crustacean saline solution (Delorenzi et al., 1996) was used as a vehicle. Fifty microliters of saline or drug solutions were given through the right side of the dorsal cephalothoraxic-abdominal membrane, by means of a syringe fitted with a sleeve to control depth of penetration to 4 mm, thus ensuring that the injected solution was released in the pericardial sac. Drugs were dissolved in physiological saline. Cycloheximide (CHX), actinomycin D (AmD) and saralasin (SAR) were purchased from Sigma. Doses were the same as those that previously showed mnesic effects (Delorenzi et al., 2000; Frenkel et al., 2002, 2005a, 2005b; Pedreira et al., 2004; Perez-Cuesta & Maldonado, 2009).

2.8. Data analysis

LTM expression was assessed by focusing data analysis on test trial scores, i.e., by estimating the difference between trained (TR) and untrained (UN) groups in response level. Rescorla (1988) convincingly argued in favor of using this sort of analysis instead of a paired training–testing comparison, stressing the need to clearly distinguish between time of input (training session) and time of assessment (testing session). This approach is amply justified in this present case since it has been demonstrated that CSM expression in crabs is independent of the escape response level at training (Maldonado, 2002). In previous experiments at our laboratory, a significant difference between the testing performances of TR- and UN-groups was invariably seen 24 h after training (UN > TR); provided that each group consisted of 25 or more crabs each and that they were given 15 or more training trials with a 3-min inter-trial interval. When animals were given six instead of 15 trials in the training session they did not show memory expression 24 h later, unless they were given a memory enhancing treatment. A TR group was said to show memory when its mean response level at the test trial was statistically lower than the respective UN-group. Accordingly, predictions were for a significant difference at testing in each UN–TR comparison, except when, for instance, memory was impaired because the treatment was amnesic. Therefore, the statistical analysis used here, in agreement with all previous studies in this memory model, was based on the UN > TR prediction and consequently data were analyzed using a priori LSD planned comparisons (orthogonal contrast analysis, three types of contrasts per experiment were used) (Howell, 1987; Rosenthal & Rosnow, 1985) following a significant main effect in a one-way ANOVA, $p < 0.05$. The type of statistical analysis used in this paper is in agreement with the extensive studies in this memory model (Carbo Tano et al., 2009; Delorenzi et al., 1995, 1996; Frenkel et al., 2005a, 2005b; Hepp, Perez-Cuesta, Maldonado, & Pedreira, 2010; Maldonado, 2002; Maldonado, Romano, & Tomsic, 1997; Pedreira et al., 2002, 2004; Perez-Cuesta & Maldonado, 2009; Romano, Lozada, & Maldonado, 1991; Tomsic et al., 1998, 2009; Troncoso & Maldonado, 2002). The experiments described in this paper used one or two untrained–trained pairs (UN–TR groups). For two pairs of groups, three types of contrasts per experiment were used: the first, between the two untrained groups of each pair; the second, between UN and TR of one pair; and the third, between UN and TR of the other pair. For one pair of groups, a *t*-test was used. Considering the expected variation of the response levels in a natural population, all the values were represented as the normalized mean and the standard error with respect to the main-control group (100%). We analysed data using STATISTICA 6.

3. Results

In the *Chasmagnathus* associative memory model, animals associate the training context (CS) with a visual danger stimulus (US) passing overhead. After the iterative spaced presentation of the US, a strong freezing-to-US response replaces the initial escape response. Thus, memory is revealed as a reduction in the escape response of the trained group (TR), compared with that of the paired untrained group (UN).

First, we showed (Fig. 1A), as has repeatedly been shown in this memory model (Table 1a), that while memory after a strong training protocol (STR) (15 spaced trials) is revealed in the long term (UN–STR > TR–STR; *t*-test; $t(56) = 2.68$, $p < 0.01$), animals weakly trained (WTP) (six spaced trials) do not show memory 24 h after training (UN–WTR > TR–WTR; *t*-test; $t(67) = 0.46$, $p = 0.65$). However, weak training protocols do build a LTM that remains unexpressed: CSM of weakly trained crabs can be expressed after a water deprivation episode during reconsolidation (Table 1d). Findings from previous studies investigating the mechanisms underlying reconsolidation showed a reliable CSM sensitivity (labilization) to both amnesic and improvement agents by re-exposing the animals for 5 min to the learning context (the CS), without US presentation, 24 h–72 h after training. This labile memory is sensitive to cycloheximide, angiotensin, and other pharmacological agents (Section 2.4).

Next, as has previously been demonstrated, we showed that the weak training protocol builds a memory that can be revealed by the treatment known to improve reconsolidation, a water deprivation episode, concomitantly with reconsolidation (Fig. 1A). A TR group of crabs underwent a weak *training session* while the UN-groups remained in the actometer during the same time but without stimulation with the VDS. On Day 2, in the *Reminder Session*, animals were placed in the actometers for 5 min, a procedure that turns memory into a labile state. Immediately after the *Reminder Session*, crabs were water deprived for 2 h. On Day 3, animals were placed again in the training context and memory retention was tested with a single VDS presentation. A significant difference between groups was disclosed (UN-Reconsolidation > TR-Reconsolidation; *t*-test; $t(62) = 2{,}21$, $p < 0.05$), revealing LTM in spite of the weak training session on Day 1 (Fig. 1A). As we previously demonstrated (Frenkel et al., 2005a), weakly trained crabs can reveal long-term CSM when reconsolidation, which necessarily entails that memory should be reactivated and become labile by the reminder, is contingent upon water deprivation (Table 1d).

Brain angiotensin II enhances memory during consolidation and reconsolidation, and mediates the mnesic effect of water deprivation. Concordantly, its antagonist, saralasin, administrated during these periods acts as an amnesic agent in *Chasmagnathus*. Animals do not show LTM if saralasin is injected after strong training (during consolidation) or after the presentation of a reminder (during reconsolidation) (Table 1b–d). In the next experiment we showed, as we have repeatedly demonstrated, the retrograde amnesic effects of saralasin on CSM consolidation (Table 1b).

A pair of UN–TR groups of crabs underwent a Strong Training Session. Immediately after training, animals were injected with the vehicle. On Day 2, animals were placed again in the training context and memory retention was tested with a single VDS presentation. Simultaneously, another pair of UN–TR groups underwent the same procedure except animals were injected with saralasin (0.053 ηg/animal) (Fig. 1B). Planned comparisons at testing [ANOVA: $F(3,90) = 2.73$, $p < 0.05$] disclosed significant

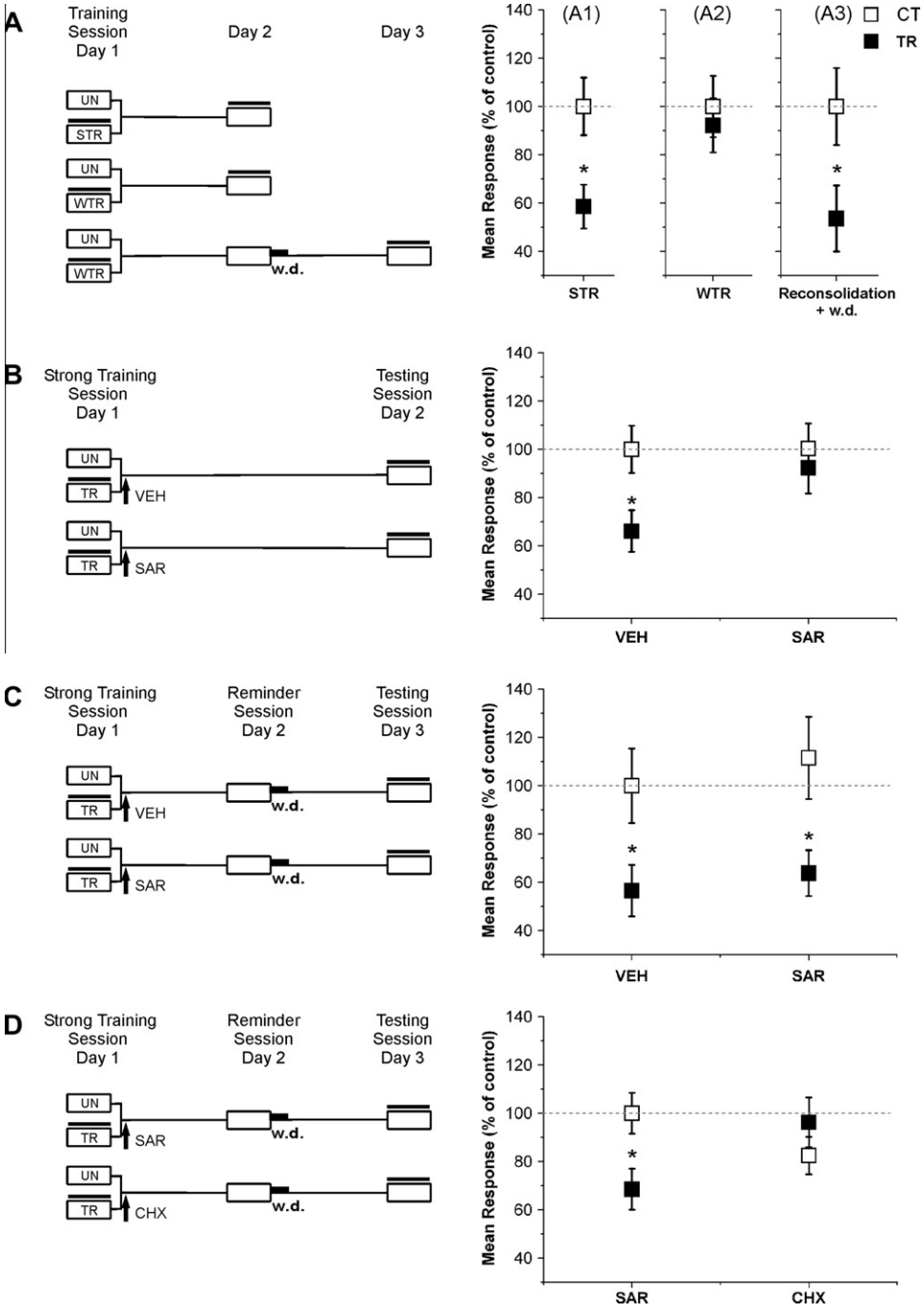

Fig. 1. Left panel: experimental designs (black bar above boxes indicate visual danger stimulus, VDS). Right panel: testing results. Graph ordinates: mean response to VDS presentation (% of control) ± SE; mean responses at test, normalized with respect to the mean response of the UN-group. A TR group is said to show memory expression at test trial when its mean response (% of its UN-group) is statistically lower than the respective UN-group. Open symbols (UN) control groups, filled symbols (TR) training groups. $^{*}p < 0.05$, $^{**}p < 0.01$. (A) Strong but not weak training generates a memory that is expressed 24 h later. (A1) Training session (Day 1): TR groups received strong (15 trials, STR pair) or weak (six trials, WTR pair) (A2) training with the visual danger stimulus, inter-trial interval: 3 min. UN-groups remained in the context for the entire session (50 or 20 min) without being exposed to the VDS. Testing session: 24 h later, groups received a VDS presentation in the training context (Day 2) ($n = 28–30$ per group). Weak training generates a memory that can be revealed by a water deprivation episode concomitantly with reconsolidation. (A3) Training session (Day 1), a TR groups of crabs underwent a weak training while the UN-groups remained in the actometer. On Day 2, in the *Reminder Session*, animals were placed in the actometers for 5 min and, immediately after this session, crabs were water deprived for 2 h (Reminder + w.d.). On Day 3, animals were tested ($n = 32$ per group). (B) Saralasin retrograde amnesic effect. Two pairs of UN–TR groups of crabs underwent a *Strong Training Session*. Immediately after training, one pair was injected with the vehicle (VEH) and the other with saralasin (0.53 ηg/animal) (SAR). Testing session 24 h latter. (C and D) The apparent amnesic effect induced by the angiotensin II antagonist administrated immediately after strong training can be recovered if animals are water deprived during reconsolidation. (C) Immediately after training all animals were injected: pair SAR received saralasin (0.053 ηg/animal) while pair VEH received the vehicle. Reminder session: 24 h later, all groups of animals were re-exposed for 5 min to the training context and water deprived (w.d.) during the next 2 h in their individual resting containers. Testing session: 24 h later, both paired-groups received a VDS presentation at test trial ($n = 25–28$ per group). (D) The same experimental design as C but the other par received cycloheximide (CHX) (0.88 μg/animal) instead vehicle ($n = 33–37$ per group).

differences between groups injected with the vehicle (UN–VEH > TR–VEH, $p < 0.05$), revealing LTM. As expected, saralasin induced an amnesic effect during consolidation. There were no significant differences between groups injected with saralasin (SAR–CHX ≈ SAR–CHX, $p = 0.57$), nor between untrained groups (UN–VEH ≈ UN–SAR, $p = 0.97$).

3.1. The apparent amnesic effect induced by the angiotensin II antagonist administrated immediately after strong training can be recovered if animals are water deprived during reconsolidation

The amnesic effect of saralasin during consolidation was explained by the disruption of LTM storage in our previous studies. On the other hand, our working hypothesis predicted that the saralasin-induced amnesia would be due to the inhibition of the expression of a consolidated memory that, nevertheless, maintained its capability to be reactivated by a reminder in spite of the saralasin administration. The following two experiments were focused on this question.

In the next experiment, we used the treatment known to improve reconsolidation in order to test whether it was possible to reveal memory even after the post-training administration of saralasin (Fig. 1C). If saralasin is a true amnesic agent that impedes LTM formation, then there should be no memory to be reactivated, labilized and then susceptible to be facilitated 24 h after training, and thus animals should not show memory retention at testing on the third day.

A pair of UN–TR groups of crabs underwent a Strong Training Session; the TR group received strong training, while the UN-group remained in the actometer for the same period but without stimulation with the VDS. Immediately after training, animals were injected with saralasin (0.053 ηg/animal). On Day 2, animals were placed in the training context for 5 min (*Reminder Session*). After the *Reminder Session*, crabs were water deprived for 2 h, a treatment that improves memory expression in a subsequent test on animals weakly trained (Table 1d). On Day 3, animals were placed again in the training context and memory retention was tested with a single VDS presentation. Simultaneously, another pair of UN–TR groups underwent the same procedure, but the vehicle (VEH) was injected instead of saralasin. Planned comparisons at testing [ANOVA: $F(3,100) = 4.01$, $p < 0.01$] disclosed significant differences between groups injected with both saralasin (UN–SAR > TR–SAR, $p < 0.05$) and the vehicle (UN–VEH > TR–SVEH, $p < 0.05$), revealing LTM in spite of the saralasin administration on Day 1. There were no significant differences between untrained groups (UN–SAR ≈ UN–VEH, $p = 0.60$). That is, the apparent amnesia induced by saralasin was therefore reverted by water deprivation after a Reminder Session.

In the next experiment we tested whether saralasin-induced amnesia is similar to the amnesia induced by a protein synthesis inhibitor, which is assumed to interfere with memory consolidation and, consequently, to impede LTM formation (Fig. 1D). To this end, a pair of UN–TR groups underwent the same protocol as the SAR-pair in the previous experiment. Simultaneously, another pair of UN–TR groups underwent the same procedure, but cycloheximide (0.88 μg/animal) was injected instead of saralasin. This dose of cycloheximide inhibits protein synthesis in the crab's central nervous system and blocks LTM formation. Planned comparisons at testing [ANOVA: $F(3,136) = 2.72$, $p < 0.05$] disclosed significant differences between groups injected with saralasin (UN–SAR > TR–SAR, $p < 0.05$), revealing LTM in spite of the saralasin administration on Day 1. There were no significant differences between groups injected with cycloheximide (UN–CHX ≈ TR–CHX, $p = 0.27$), nor between untrained groups (UN–SAR ≈ UN–CHX, $p = 0.16$). The apparent amnesia induced by saralasin was therefore reverted by water deprivation after a Reminder Session. As expected, the same treatment had no effect on cycloheximide-induced amnesia due to the expected disruption of LTM formation (Alberini, 2009; Hermitte, Pedreira, Tomsic, & Maldonado, 1999; Maldonado, 2002; Pedreira & Maldonado, 2003; Pedreira et al., 1995, 1996, 2004). These findings clearly indicate the different nature of both amnesic agents.

3.2. The recovery of memory expression after the apparent amnesic effect of saralasin is reconsolidation specific

Our working hypothesis predicted that the change in CSM expression showed above was reconsolidation specific; this series of experiments addressed this question.

3.2.1. Memory enhancement induced by water deprivation after the Reminder Session depends on presentation of the reminder

In the following experiment, we tested the requirement of a reminder to reverse the saralasin-induced amnesia by water deprivation during reconsolidation (Fig. 2A). To this end, a pair of UN–TR groups underwent the same protocol as the SAR-pair in the previous experiment. Simultaneously, a second pair of UN–TR groups underwent the same procedure, with the sole exception that during the *Reminder Session* animals were not placed in the training context (CS), but in a different one. Training, saralasin injection and water deprivation were the same for both pairs of groups, and the same protocol as described in the previous experiment was applied.

At the *Testing Session*, planned comparisons [ANOVA: $F(3,106) = 4.39$, $p < 0.01$] disclosed significant differences between those groups that were re-exposed to the training context during the Reminder Session (UN-Reminder > TR-Reminder, $p < 0.01$) as expected from previous results. There were no significant differences between groups exposed to a novel context during the *Reminder Session* (UN-No Reminder ≈ UN-No Reminder, $p = 0.27$), nor between untrained groups (UN-Reminder ≈ UN-No Reminder, $p = 0.81$). This result shows that the short presentation of the training context, which triggers reconsolidation, is a crucial prerequisite for revealing memory at the *Testing Session*.

3.2.2. The contingence between water deprivation and reminder presentation is crucial to enhance memory expression after the Reminder Session

This experiment was performed to test whether memory retention showed at the Testing Session in Fig. 2A was due to the enhancing effect of water deprivation or the result of the sole presentation of the reminder. To test this possibility, memory retention was compared between water-deprived animals immediately after presentation of a reminder and water-deprived animals 6 h after the reminder presentation (Fig. 2B). At the *Testing Session*, planned comparisons [ANOVA: $F(3,84) = 2.91$, $p < 0.05$] revealed significant differences between animals that underwent water deprivation concurrent with the presentation of the reminder (UN-Concurrent > TR-Concurrent, $p < 0.05$) as expected from previous results. On the contrary, when water deprivation was delayed for 6 h, the escape response of trained animals was similar to that of untrained animals (UN-NonConcurrent ≈ TR-NonConcurrent, $p = 0.46$). There were no significant differences between control groups (UN-Concurrent ≈ UN-NonConcurrent, $p = 0.48$). This result showed that the presentation of the reminder alone was not sufficient to reverse the apparent amnesic effect induced by saralasin; in fact, the event of water deprivation must take place concurrent with the reminder presentation. In the experiment, Fig. 2A, both pairs of groups underwent the water deprivation protocol at the same time, while only the pair subjected to a reminder showed memory retention at testin. In the experiment, Fig. 2B, both groups were re-exposed to the training context and to the water deprivation protocol, differing only in the time point of the water deprivation procedure. Under these conditions, only the group in which water deprivation was concurrent with re-exposure to the training context showed memory retention at testing (Fig. 2B). Given these results, facilitation of retrieval during the Testing Session due to water deprivation 1 day before should be discarded. In fact, the last experiment showed that the group

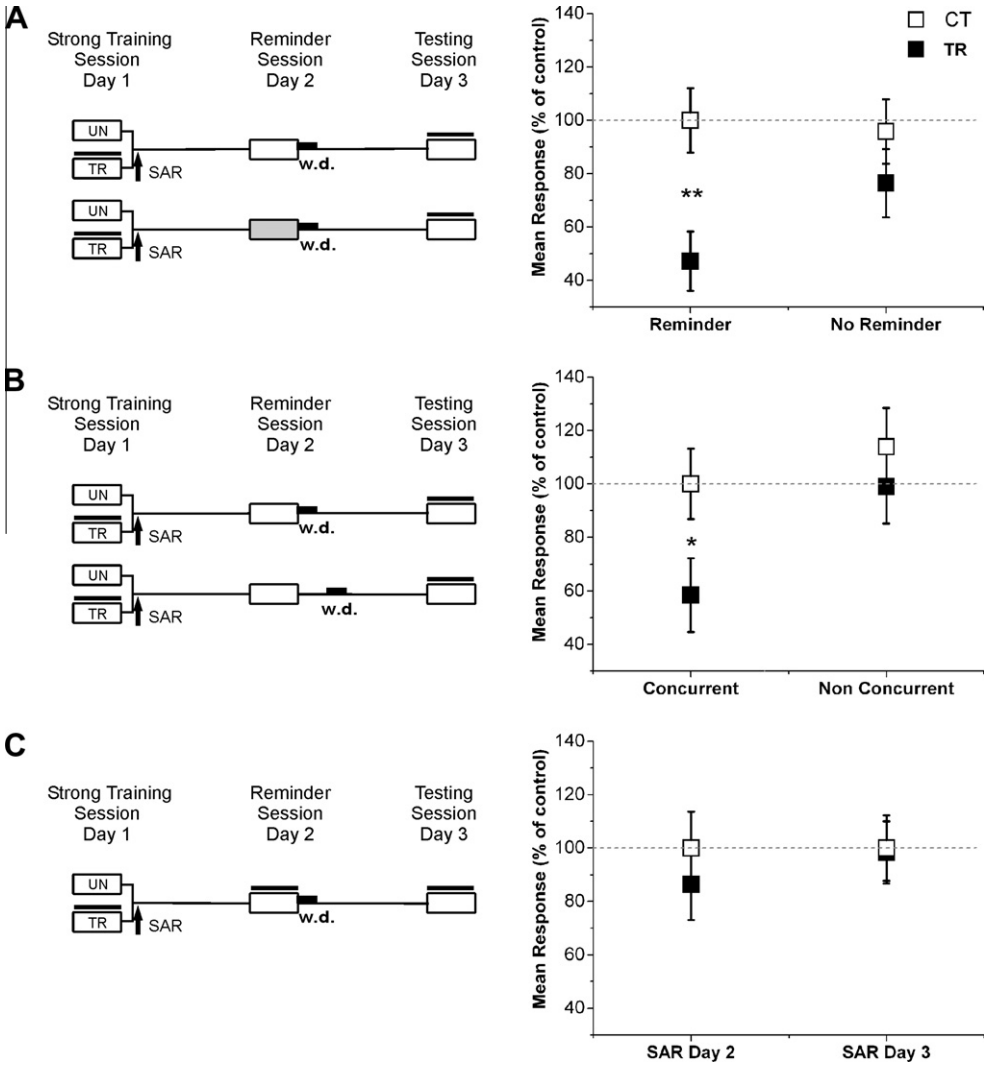

Fig. 2. The recovery of memory expression after the apparent amnesic effect of saralasin is reconsolidation specific. Left panel: experimental designs; right panel: testing results. All symbols as Fig. 1. (A) Memory enhancement induced by water deprivation after *Reminder Session* depends on presentation of the reminder. Training session: TR groups received strong training (15 trials). UN-groups remained in the context for the entire session without being exposed to the VDS. Immediately after training all animals were injected with saralasin (0.053 ηg/animal). Reminder Session: 24 h later, a pair of UN–TR groups of animals were re-exposed to the training context for 5 min (Reminder pair), while the other pair was exposed during the same time to a different context (No Reminder pair). Both pairs of groups were water deprived (w.d.) for the next 2 h in their individual resting containers. Testing session: 24 h later, both paired-groups received a VDS presentation in the training context (n = 24–32 per group). (B) The contingence between water deprivation and the reminder presentation is crucial to enhance memory expression after the *Reminder Session*. Training session: TR groups received strong training (15 trials). UN-groups remained in the context for the entire session without being exposed to the VDS. Immediately after training all animals were injected with saralasin (0.053 ηg/animal). Reminder Session: 24 h later, animals were re-exposed to the training context for 5 min. A pair of UN–TR groups of animals were immediately water deprived (w.d.) for the next 2 h in their individual resting containers (Concurrent pair), while the other pair started the water deprivation protocol (2 h of w.d.) 6 hs after the reminder presentation (NonConcurrent pair). Testing session: 24 h later (n = 20–24 per group). (C) The change in LTM expression in strongly trained, saralasin-treated crabs is reconsolidation specific. Training session: TR group received strong training (15 trials) with the VDS. The UN-group remained in the context for the entire session without being exposed to the VDS. Immediately after training, both groups received a saralasin (0.053 ng/animal) injection. First testing session: 24 h after training, both groups received a VDS presentation at the test trial. Immediately after this test, all animals were water deprived for 2 h in their individual containers. Second testing session: 48 h after training, both groups received a VDS presentation at the test trial (n = 35–38 per group).

that was water deprived at a time closer to the test did not show CSM at testing.

3.2.3. The change in LTM expression in strongly trained, saralasin-treated crabs is reconsolidation specific

The last experiment of this series showed that the presentation of the CS (a reminder) before water deprivation was not sufficient to revert the amnesic effect of saralasin, but it was necessary to present the reminder under conditions that trigger reconsolidation (Fig. 2C). The reminder was not a sufficient condition to induce reconsolidation. During the *Reminder Session* a mismatch needs to take place between what is expected in a given situation and what actually occurs in several memory models (Dudai, 2006, 2009; Forcato, Rodriguez, Pedreira, & Maldonado, 2009; Forcato, Argibay, et al., 2009; Frenkel et al., 2005a; Morris et al., 2006; Nader, Schafe, & Ledoux, 2000; Pedreira et al., 2004; Perez-Cuesta & Maldonado, 2009). Specifically, in the *Chasmagnathus* memory model, reconsolidation does not take place after a reinforced presentation of the training context (i.e. if a single VDS (the US) is presented during re-exposure) (Frenkel et al., 2005a; Pedreira et al., 2004; Perez-Cuesta & Maldonado, 2009). Based on this evidence, we tested whether water deprivation after a reminder can induce memory expression at the *Testing Session* in conditions in which the reminder cannot trigger reconsolidation. A pair of UN–TR groups underwent a *Strong Training Session* and were injected with saralasin as previously described. On Day 2, in the *Reminder*

Session, animals were placed in the actometers for 5 min and a single US was presented. Immediately after this reinforced reminder presentation, animals were water deprived for 2 h. On Day 3 animals were tested for memory expression. Statistical analysis (*t*-test) revealed no significant differences neither between groups on Day 2 during US presentation (UN–SAR ≈ TR–SAR; *t*-test; $t(72) = 0.089$, $p = 0.93$), as expected after a post-training saralasin injection, nor at the *Testing Session* on Day 3 (UN–SAR ≈ TR–SAR; *t*-test; $t(72) = 0.710$, $p = 0.48$). Thus, the reinforced reminder impeded the improving effect of water deprivation that modifies memory expression, even when this reminder included more training cues than those presented during the *Reminder Session* that actually trigger the reconsolidation process (i.e. the US). The main characteristic of the reminder presented here was that it did not trigger reconsolidation of memory after its reactivation.

Briefly, the results showed that memory expression after the negative modulation of saralasin during consolidation can be facilitated by water deprivation concurrent with the presentation of a reminder (the CS) that induces reactivation and labilization of memory, but not by a reminder that does not induce labilization of the reactivated memory. Consequently, the increase in CSM expression showed in the previous experiments is hard to explain in terms of the recovery of amnesia as a consequence of additional learning, saving, residual traces, retrieval deficits or summation effects (e.g. (Gold et al., 1973; Haycock, Gold, Macri, & McGaugh, 1973; Miller & Matzel, 2006; Nader & Wang, 2006)).

3.3. Weak training protocols build a LTM which remains unexpressed and which depends on both new protein synthesis and new mRNA transcription after acquisition

Weak training protocols do build a LTM that can be reactivated during the *Reminder Session* but stay unexpressed (Table 1c and d, Fig. 1A); however, it is possible that this protocol generates an intermediate-term memory that does not possess the diagnostic characteristics of LTMs. As strongly trained animals fail to recover memory expression when treated with cycloheximide after training (Fig. 1D), it is not plausible that weakly trained animals can recover memory expression by the reconsolidation and water-deprivation contingency procedure if they are treated with cycloheximide after training. However, a CSM of weakly trained crabs can be revealed after a water deprivation episode (Table 1c). In the following three experiments, we tested whether the weak training protocol builds a memory, whose expression is revealed after water deprivation, that depends on protein synthesis after acquisition, a definite characteristic of LTM consolidation.

The next experiment was aimed to explore whether weak training builds a LTM that depends on transcription of new proteins. First, a pair of UN–TR groups was trained using a weak training protocol, and then injected with cycloheximide (0.88 μg/animal). Simultaneously, another pair of UN–TR groups was also weakly trained, but injected with saline solution. Both pairs of groups were tested on Day 2, after being water deprived for 2 h in order to allow the expression of memory at the Testing Session (Fig. 3A). Planned comparisons [ANOVA: $F(3,135) = 2.77$, $p < 0.05$] disclosed a significant difference in response between vehicle-injected groups (UN–VEH > TR–VEH, $p < 0.01$), but not between cycloheximide-injected groups (UN–CHX ≈ TR–CHX, $p = 0.68$). There were no unspecific effects of the drug on the behavior of control animals (UN–VEH ≈ UN–CHX, $p = 0.98$).

In the following experiment, animals were weakly trained and injected with the transcription inhibitor actinomycin D (0.62 μg/animal). A vehicle control pair of UN–TR groups was performed simultaneously, and both pairs of groups were tested 48 h later, after 2 h of water deprivation to allow memory expression (Fig. 3B). Analysis of response scores at the Testing Session [ANOVA: $F(3,149) = 3.00$, $p < 0.05$] revealed CSM for animals injected with the vehicle (UN–VEH > TR–VEH, $p < 0.05$), while there were no significant differences between actinomycin D-injected groups (UN–AMD ≈ TR–AMD, $p = 0.51$) nor between untrained groups (UN–VEH ≈ UN–AMD, $p = 0.89$).

Finally, we tested whether the weak training protocol builds a memory that cannot be blocked by saralasin administration after acquisition. Animals were weakly trained and injected with the angiotensin II antagonist saralasin (0.053ηg/animal). A vehicle control pair of UN–TR groups was performed simultaneously, and both pairs of groups were tested 48 h later, after 2 h of water deprivation to allow memory expression (Fig. 3C). Analysis of response scores at the Testing Session [ANOVA: $F(3,147) = 5132$, $p < 0.01$] revealed CSM for animals injected with both the vehicle (UN–VEH > TR–VEH, $p < 0.01$) and saralasin-injected groups (UN–SAR > TR–SAR, $p < 0.05$). No differences were found between untrained groups (UN–VEH ≈ UN–SAR, $p = 0.84$).

In conclusion, weak training generates a LTM that depends on transcription and translation, but it will not be expressed unless animals are water deprived before testing. However, this training generated a memory whose formation could not be blocked by post-training administration of the angiotensin II antagonist.

4. Discussion

The key finding of this study is that the sensitivity of CSM to be modulated by endogenous angiotensin II during consolidation is an attribute regarding its long-term expression, i.e. the process of gaining appreciable control over behavior in the retrieval session, but not regarding both the storage over time and the capability to be reactivated by specific context cues. Crabs, strongly trained and saralasin-treated during consolidation, construct a LTM that is not expressed but which can be reactivated during the reminder session and expressed long term through an enhancing treatment during reconsolidation. Either strongly or weakly trained, saralasin-treated animals do build a potentially reactivated but unexpressed LTM, comparable in nature to the unexpressed LTM generated by weak training protocols. A reminder is capable of reactivating and making this unexpressed long-term CSM labile, which both depends on mRNA transcription and translation and persists at least 24–48 h after training. However, both its storage over time and its capability to be reactivated in the long term are independent of the neuromodulator angiotensin II.

A vast number of studies have shown that a treatment or condition can act to reveal a memory that otherwise remains unexpressed. For instance, it was widely demonstrated that the phenomenon of latent memory is a common feature of learning and memory across the animal kingdom, as well as in many studies which showed recovery of retrograde amnesias (Cahill et al., 2001; Gold et al., 1973; Haycock et al., 1973; Nader & Wang, 2006; Parvez, Stewart, Sangha, & Lukowiak, 2005; Philips et al., 2006; Rescorla, 1988). Classical studies demonstrated that poor memory shown in animals, produced either by weak training or by strong training followed by amnesic treatments, was improved by a non-contingent unconditioned stimulus presentation. It is also possible that the memory deficit caused by cycloheximide was larger and hence needed a stronger protocol to overcome the deficit (Gold, McDonald, & McGaugh, 1974). Or that the recovery of memory expression of saralasin groups was due to additional learning built on the residual memory trace, because water deprivation acts as an unconditioned stimulus and is associated with the context (Squire, 2006). The recovery of amnesia involving reminders has also been performed to demonstrate that performance is recovered (see special section The Neurobiology of Amnesia in Learn Mem, vol. 13, issue 5, 2006). Taken together, the diversity of approaches that

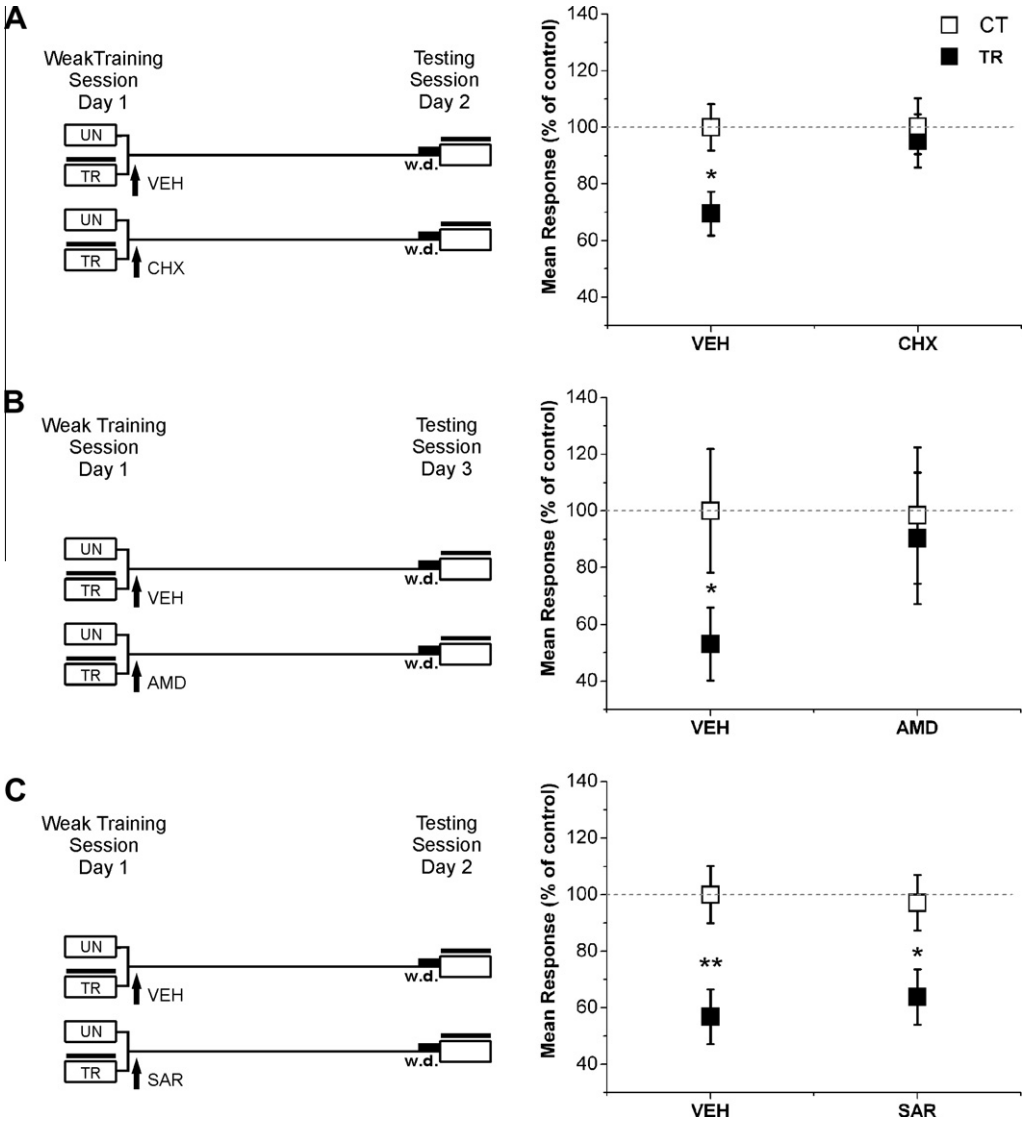

Fig. 3. Weak training protocols build a LTM that remains unexpressed and depends on both new protein synthesis and new mRNA transcription after acquisition. Left panel: experimental designs; right panel: testing results. All symbols as Fig. 1. (A) Weak training protocol builds a memory that depends on mRNA translation. Training session (Day 1): TR groups received weak training (six trials). UN-groups remained in the context for the entire session without being exposed to the VDS. Immediately after training, the pair VEH received a vehicle injection while the pair CHX received cycloheximide (0.88 μg/g crab). Testing session (Day 2): 24 h later, both paired-groups were water deprived for 2 h in their individual containers before testing (n = 34–35 per group). (B) A weak training protocol builds a memory that depends on mRNA transcription. Training session: TR groups received weak training (six trials). UN-groups remained in the context. Immediately after training, the pair VEH received a vehicle injection while the pair AmD received actinomycin D (0.62 μg/g crab). Testing session: 48 h later, both paired-groups were water deprived for 2 h in their individual containers before testing (n = 38–39 per group). (C) A weak training protocol builds a memory whose formation cannot be blocked by post-training administration of the angiotensin II antagonist. Training session: TR groups received weak training (six trials. UN-groups remained in the context. Immediately after training, the pair VEH received a vehicle injection while the pair SAR received saralasin (0.053 ηg/animal). Testing session: 48 h later, both paired-groups were water deprived for 2 h in their individual containers before testing (n = 37–38 per group).

were able to recover memory from experimental amnesias might be considered as new learning added onto a residual memory trace (Squire, 2006). However, several features in the present approach are different from and contrast with the possible interpretations presented before, because the improving mnesic effects specifically belong to the reconsolidation process. Neither retrieval nor training–testing conditions were changed. Results show that saralasin is not a true amnesic agent like cycloheximide, which interferes with the cascade of cellular and molecular events related to LTM storage (Figs. 1C, 2 and 3D). The lack of memory expression is not the result of the absence of storage over time, because CSM can be specifically reactivated and become labile under the specific reminder conditions that trigger reconsolidation. In this reminder, a mismatch between what is expected and what actually occurs is crucial for the occurrence of the labile phase (Frenkel et al., 2005a; Morris et al., 2006; Pedreira et al., 2004; Perez-Cuesta & Maldonado, 2009). Summation and adding-like effects predict that the US presentation during the reminder session must also result in a change in LTM expression when water deprivation is contingent upon the *Reactivation Session*. For instance, the change in memory expression of saralasin groups in Figs. 1 and 2 may be due to additional learning built on the residual memory trace in the sense that water deprivation acts as an unconditioned stimulus and is associated with the context (Gold et al., 1973; Squire, 2006). However, the US presentation within the reminder session canceled the reconsolidation process and, consequently, no change in memory expression is disclosed despite the water deprivation episode after CS–US presentation. The reminder session is necessary because its

absence does not increase long-term CSM expression, despite the water deprivation treatment. As observed in other models, a reminder session that triggers reconsolidation *per se*, for instance in the absence of water deprivation, results in no change in LTM (Alberini, 2007; Carbo Tano et al., 2009; Dudai, 2006, 2009; Frenkel et al., 2005a; Morris et al., 2006; Pedreira et al., 2004; Sara, 2000a, 2000b). Retrieval deficit can also explain some recoveries after amnesic procedures (Miller & Matzel, 2006) (see special section The Neurobiology of Amnesia in Learn Mem, vol. 13, issue 5, 2006). All in all, the present findings are also extremely hard to explain in terms of reminder deficits. Despite the saralasin post-training administration, the long-term CSM is clearly revealed by means of a specific reminder structure, through the reactivation and labilization of the unexpressed memory, which is a necessary condition for the occurrence of reconsolidation. No memory reactivation was shown after cycloheximide administration. The lack of CSM expression is better explained by the fact that the saralasin retrograde amnesic effect is due to an inhibition of memory's long-term expression, i.e. of the process of gaining appreciable control over behavior in the retrieval session of the reactivated memory trace.

The present results necessarily lead us to a different interpretation of both the long-term nature of the mnesic angiotensin actions and the long-term existence of the CSM generated by the weak training protocol (Table 1). Weak training protocols were assumed not to build long-term CSM (Carbo Tano et al., 2009; Delorenzi & Maldonado, 1999; Delorenzi et al., 1995, 1996, 1997, 2000; Frenkel et al., 2002, 2005a, 2005b; Freudenthal et al., 1998; Maldonado, 2002; Maldonado et al., 1997; Romano, Delorenzi, Pedreira, Tomsic, & Maldonado, 1996; Romano, Locatelli, et al., 1996; Romano, Freudenthal, et al., 2006; Romano, Locatelli, et al., 2006). However, the present results show that strongly trained, saralasin-treated animals do build an unexpressed LTM of a similar nature to those build by weak training. It is viable that weak training yields a CSM that survives days after consolidation, without expression, until an episode makes it possible for the animal to gain control over its behavior. Weakly trained crabs can reveal long-term CSM when reconsolidation, which necessarily entails that memory should be reactivated and become labile by the reminder, is contingent upon water deprivation (Table 1d). Like the saralasin retrograde effects showed here, we have demonstrated that this mnesic effect on LTM expression is specific upon the reconsolidation process, too (Table 1d) (Frenkel et al., 2005a). Therefore, weak training protocols do build a LTM trace which can be reactivated, but it does not gain appreciable control over behavior, i.e. remains unexpressed. However, this memory could be the result of an intermediate-term memory that is independent of new protein synthesis or of new mRNA transcription after acquisition, a definite characteristic of LTM. Due to the fact that strongly trained crabs failed to recover CSM expression when treated with cycloheximide after training (Fig. 1C), it is not plausible to test whether weakly trained animals recover memory expression by the reconsolidation and water-deprivation contingency procedure. Nevertheless, in order to reactivate a potential memory trace independent of the synthesis of new proteins, we attempted to recover memory expression during retrieval of weakly trained animals treated with cycloheximide or actinomycin after training. Weakly trained crabs failed to recover expression, although the test trial was preceded by water deprivation, if they were treated with cycloheximide or actinomycin D immediately after training (Fig. 3A and B). Remarkably, weakly trained, saralasin-treated animals do build a potentially reactivated but unexpressed LTM (Fig. 3C). A LTM without expression could be expressed later, but in every case the consolidated memory, as a result of either saralasin post-training treatment or weak training, shared the diagnostic feature of LTM (Alberini, 2009; Davis & Squire, 1984; Stough et al., 2006) (but see (Gold, 2008)). Here, the LTM is therefore revealed through the memory reactivation–labilization via the specific reminder structure that triggers the reconsolidation process.

All in all, the current results strongly suggest that in *Chasmagnathus* angiotensin is a neuromodulator that during consolidation and reconsolidation determines the ability of the CSM to guide behavior, to increase or decrease its long-term expression, but not its long-term storage. This view is in contrast with our previous interpretation of the mnesic actions of angiotensins on CSM because it contained the concept that memory modulatory systems are endogenous systems that influence memory storage processes (Cahill & McGaugh, 1996; Ferry et al., 1999; Krasne, 1978; McGaugh, 2000). Thus, endogenous modulating systems seem to provide the basis for selecting experiences for long-term storage. Typically, weak training protocols show evident short-term, but not LTM expression and are generally designed to reveal memory-enhancing effects for post-training treatments. However, in *Chasmagnathus* a long-term CSM without expression can subsist, be reactivated and become labile. Consequently, CSM expression – the potentiality of the memory trace to gain appreciable control over behavior at testing sessions – may be considered as a distinct attribute of the storage over time of its internal representation. It has previously been proposed that memory traces must be reactivated before they can be expressed (Tulving, 1983). The memory improvements shown here might reinforce items critical for the retrieval process of long-term expression rather than storage, a hypothesized function of the reconsolidation process (Dudai & Eisenberg, 2004). We propose that the endogenous angiotensinergic system in *Chasmagnathus* memory model provides a basis for understanding how previous experiences, among those stored long term, will be expressed in the long term. Given that these findings are limited to both this neuromodulator and this memory model, the extent to which the weak training protocols can be a useful tool for dissecting cellular and molecular mechanisms underlying encoding, processing and storing memory from those cellular and molecular mechanisms responsible for expressing it remains to be determined.

Studies on reconsolidation have been essential to propose the endogenous angiotensinergic system as a modulator of long-term expression, but not of long-term retention of the memory trace. Although reconsolidation processes have often been unveiled through their vulnerability to amnesic agents (Alberini, 2007; Dudai, 2009; Eisenberg et al., 2003; Forcato, Argibay, et al., 2009; Morris et al., 2006; Nader & Hardt, 2009; Nader et al., 2000; Pedreira et al., 2004; Sara & Hars, 2006), reconsolidation might also provide an opportunity for memory improvement. Enhancing agents contingent upon reconsolidation have been shown (Frenkel et al., 2005a; Sara, 2000b; Tronson & Taylor, 2007). Importantly, real-life events such as additional learning and water-shortage induced memory improvement on a labilized memory during the reconsolidation process (Frenkel et al., 2005a; Lee, 2008). Overall, the present results support our initial proposition that memory reconsolidation is a process modulated by concurrent experiences, where neuromodulators – triggered by real-life events – could improve and, consequently, modulate the expression of previously consolidated memories (Frenkel et al., 2005a). Here, we show that during reconsolidation it is possible to change the long-term expression of a memory and thus disclose unexpressed memories.

4.1. The positive modulation of memory expression during reconsolidation to distinguish between memories that are not expressed and those that are not stored

The approach currently presented, i.e. a positive modulation of memory expression during reconsolidation, could be useful for distinguishing between reactivated but unexpressed and obliterated

memories (Cahill et al., 2001; de Hoz, Martin, & Morris, 2004; Hardt, Wang, & Nader, 2009; Nader & Wang, 2006; Rescorla, 1988; Squire, 2006; Tulving, 1983) (see special section The Neurobiology of Amnesia in Learn Mem, vol. 13, issue 5, 2006). The positive modification in memory expression after the reminder presentation shown here (and Table 1d) is specifically attributable to the reconsolidation process, but hard to explain as the result of, for example, adding or summation effects, partial retraining or retrieval deficits. What is evaluated at the test on Day 3 in the *Testing Session* is not whether memory survived or not, but whether it had been reactivated and become labile on Day 2 in the *Reactivation Session*, by the presentation of a reminder under conditions that trigger the reconsolidation process, previous to water deprivation treatment. This approach would equip, for instance, the retrieval and storage views of amnesias with a new prediction in the case of positive results (Hardt et al., 2009; Nader & Wang, 2006). The amnesic effects of a certain treatment, here saralasin in *Chasmagnathus*, cannot be explained as retrieval or storage deficit, but as a decrease in LTM expression. In the case of negative results, like in the cycloheximide groups of Figs. 1D and 3A, it is not possible to dissect whether the interference affected the consolidation process (the classical view) or obstructed the retrieval process (an alternative one, e.g.(Miller & Matzel, 2006)). We propose the use of this strategy, i.e. the positive modulation of memory expression during reconsolidation to distinguish between memories that can be reactivated, labilized and are not expressed, and memories that are not stored long term, obliterated or altered in other retrieval mechanisms.

Acknowledgments

This work was supported by Universidad de Buenos Aires (X426), CONICET (PIP 5466) and Fundación Ciencias Exactas y Naturales. The authors thank: J. Aggio, M. Berón de Astrada, F. Locatelli, E. Merlo, M.E. Pedreira, V. Molina, A. Romano and J. Stehberg for helpful comments about the manuscript and A. Vidal and B. Dimant for technical support.

References

Alberini, C. M. (2007). Reconsolidation: The samsara of memory consolidation. *Debates in Neuroscience, 1*, 17–24.
Alberini, C. M. (2009). Transcription factors in long-term memory and synaptic plasticity. *Physiological Reviews, 89*, 121–145.
Cahill, L., & McGaugh, J. L. (1996). Modulation of memory storage. *Current Opinion in Neurobiology, 6*, 237–242.
Cahill, L., McGaugh, J. L., & Weinberger, N. M. (2001). The neurobiology of learning and memory: Some reminders to remember. *Trends in Neurosciences, 24*, 578–581.
Carbo Tano, M., Molina, V. A., Maldonado, H., & Pedreira, M. E. (2009). Memory consolidation and reconsolidation in an invertebrate model: The role of the GABAergic system. *Neuroscience, 158*, 387–401.
Davis, H. P., & Squire, L. R. (1984). Protein synthesis and memory: A review. *Psychological Bulletin, 96*, 518–559.
de Hoz, L., Martin, S. J., & Morris, R. G. (2004). Forgetting, reminding, and remembering: The retrieval of lost spatial memory. *PLoS Biology, 2*, E225.
Delorenzi, A., Dimant, B., Frenkel, L., Nahmod, V. E., Nassel, D. R., & Maldonado, H. (2000). High environmental salinity induces memory enhancement and increases levels of brain angiotensin-like peptides in the crab *Chasmagnathus granulatus*. *Journal of Experimental Biology, 203*, 3369–3379.
Delorenzi, A., Locatelli, F., Romano, A., Nahmod, V., & Maldonado, H. (1997). Angiotensin II (3–8) induces long-term memory improvement in the crab *Chasmagnathus*. *Neuroscience Letters, 226*, 143–146.
Delorenzi, A., & Maldonado, H. (1999). Memory enhancement by the angiotensinergic system in the crab *Chasmagnathus* is mediated by endogenous angiotensin II. *Neuroscience Letters, 266*, 1–4.
Delorenzi, A., Pedreira, M. E., Romano, A., Garcia, S. I., Pirola, C. J., Nahmod, V. E., et al. (1996). Angiotensin II enhances long-term memory in the crab *Chasmagnathus*. *Brain Research Bulletin, 41*, 211–220.
Delorenzi, A., Pedreira, M. E., Romano, A., Pirola, C. J., Nahmod, V. E., & Maldonado, H. (1995). Acute administration of angiotensin II improves long-term habituation in the crab *Chasmagnathus*. *Neuroscience Letters, 196*, 193–196.
Dudai, Y. (2006). Reconsolidation: The advantage of being refocused. *Current Opinion in Neurobiology, 16*, 174–178.
Dudai, Y. (2009). Predicting not to predict too much: How the cellular machinery of memory anticipates the uncertain future. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 364*, 1255–1262.
Dudai, Y., & Eisenberg, M. (2004). Rites of passage of the engram: Reconsolidation and the lingering consolidation hypothesis. *Neuron, 44*, 93–100.
Eisenberg, M., Kobilo, T., Berman, D. E., & Dudai, Y. (2003). Stability of retrieved memory: Inverse correlation with trace dominance. *Science, 301*, 1102–1104.
Ferry, B., & McGaugh, J. L. (2000). Role of amygdala norepinephrine in mediating stress hormone regulation of memory storage. *Acta Pharmacologica Sinica, 21*, 481–493.
Ferry, B., Roozendaal, B., & McGaugh, J. L. (1999). Role of norepinephrine in mediating stress hormone regulation of long-term memory storage: A critical involvement of the amygdala. *Biological Psychiatry, 46*, 1140–1152.
Forcato, C., Argibay, P. F., Pedreira, M. E., & Maldonado, H. (2009). Human reconsolidation does not always occur when a memory is retrieved: The relevance of the reminder structure. *Neurobiology of Learning and Memory, 91*, 50–57.
Forcato, C., Rodriguez, M. L., Pedreira, M. E., & Maldonado, H. (2009). Reconsolidation in humans opens up declarative memory to the entrance of new information. *Neurobiology of Learning and Memory*.
Frenkel, L., Freudenthal, R., Romano, A., Nahmod, V. E., Maldonado, H., & Delorenzi, A. (2002). Angiotensin II and the transcription factor Rel/NF-kappaB link environmental water shortage with memory improvement. *Neuroscience, 115*, 1079–1087.
Frenkel, L., Maldonado, H., & Delorenzi, A. (2005a). Memory strengthening by a real-life episode during reconsolidation: An outcome of water deprivation via brain angiotensin II. *European Journal of Neuroscience, 22*, 1757–1766.
Frenkel, L., Maldonado, H., & Delorenzi, A. (2005b). Retrieval improvement is induced by water shortage through angiotensin II. *Neurobiology of Learning and Memory, 83*, 173–177.
Freudenthal, R., Locatelli, F., Hermitte, G., Maldonado, H., LaFourcade, C., Delorenzi, A., et al. (1998). Kappa-B like DNA-binding activity is enhanced after spaced training that induces long-term memory in the crab *Chasmagnathus*. *Neuroscience Letters, 242*, 143–146.
Gold, P. E. (2008). Protein synthesis inhibition and memory: Formation vs amnesia. *Neurobiology of Learning and Memory, 89*, 201–211.
Gold, P. E., Haycock, J. W., Marri, J., & McGaugh, J. L. (1973). Retrograde amnesia and the "reminder effect": An alternative interpretation. *Science, 180*, 1199–1201.
Gold, P. E., McDonald, R., & McGaugh, J. L. (1974). Direct cortical stimulation: A further study of treatment intensity effect on retrograde amnesia gradients. *Behavioral Biology, 10*, 485–490.
Hardt, O., Wang, S. H., & Nader, K. (2009). Storage or retrieval deficit: The yin and yang of amnesia. *Learning and Memory, 16*, 224–230.
Haycock, J. W., Gold, P. E., Macri, J., & McGaugh, J. L. (1973). Noncontingent footshock attenuation of retrograde amnesia: A generalization effect. *Physiology and Behavior, 11*, 99–102.
Hepp, Y., Perez-Cuesta, L. M., Maldonado, H., & Pedreira, M. E. (2010). Extinction memory in the crab *Chasmagnathus*: Recovery protocols and effects of multi-trial extinction training. *Animal Cognition, 13*, 391–403.
Hermitte, G., Pedreira, M. E., Tomsic, D., & Maldonado, H. (1999). Context shift and protein synthesis inhibition disrupt long-term habituation after spaced, but not massed, training in the crab *Chasmagnathus*. *Neurobiology of Learning and Memory, 71*, 34–49.
Howell, D. C. (1987). *Statistical methods for psychology*. Boston: Duxbury.
Kaczer, L., Pedetta, S., & Maldonado, H. (2007). Aggressiveness and memory: Subordinate crabs present higher memory ability than dominants after an agonistic experience. *Neurobiology of Learning and Memory, 87*, 140–148.
Krasne, F. B. (1978). Extrinsic control of intrinsic neuronal plasticity: An hypothesis from work on simple systems. *Brain Research, 140*, 197–216.
Lee, J. L. (2008). Memory reconsolidation mediates the strengthening of memories by additional learning. *Nature Neuroscience, 11*, 1264–1266.
Locatelli, F., Maldonado, H., & Romano, A. (2002). Two critical periods for cAMP-dependent protein kinase activity during long-term memory consolidation in the crab *Chasmagnathus*. *Neurobiology of Learning and Memory, 77*, 234–249.
Maldonado, H. (2002). Crustacean as model to investigate memory illustrated by extensive behavioral and physiological studies in *Chasmagnathus*. In K. Wiese (Ed.), *The crustacean nervous system* (pp. 314–327). Berlin: Springer.
Maldonado, H., Romano, A., & Tomsic, D. (1997). Long-term habituation (LTH) in the crab *Chasmagnathus*: A model for behavioral and mechanistic studies of memory. *Brazilian Journal of Medical and Biological Research, 30*, 813–826.
McGaugh, J. L. (2000). Memory – A century of consolidation. *Science, 287*, 248–251.
Merlo, E., Freudenthal, R., Maldonado, H., & Romano, A. (2005). Activation of the transcription factor NF-kappaB by retrieval is required for long-term memory reconsolidation. *Learning and Memory, 12*, 23–29.
Merlo, E., & Romano, A. (2007). Long-term memory consolidation depends on proteasome activity in the crab *Chasmagnathus*. *Neuroscience, 147*, 46–52.
Miller, R. R., & Matzel, L. D. (2006). Retrieval failure versus memory loss in experimental amnesia: Definitions and processes. *Learning and Memory, 13*, 491–497.
Morris, R. G., Inglis, J., Ainge, J. A., Olverman, H. J., Tulloch, J., Dudai, Y., et al. (2006). Memory reconsolidation: Sensitivity of spatial memory to inhibition of protein synthesis in dorsal hippocampus during encoding and retrieval. *Neuron, 50*, 479–489.
Nader, K., & Hardt, O. (2009). A single standard for memory: The case for reconsolidation. *Nature Reviews Neuroscience, 10*, 224–234.

Nader, K., Schafe, G. E., & Ledoux, J. E. (2000). The labile nature of consolidation theory. *Nature Reviews Neuroscience, 1*, 216–219.

Nader, K., & Wang, S. H. (2006). Fading in. *Learning and Memory, 13*, 530–535.

Parvez, K., Stewart, O., Sangha, S., & Lukowiak, K. (2005). Boosting intermediate-term into long-term memory. *Journal of Experimental Biology, 208*, 1525–1536.

Pedreira, M. E., Dimant, B., & Maldonado, H. (1996). Inhibitors of protein and RNA synthesis block context memory and long-term habituation in the crab *Chasmagnathus*. *Pharmacology, Biochemistry and Behavior, 54*, 611–617.

Pedreira, M. E., Dimant, B., Tomsic, D., Quesada-Allue, L. A., & Maldonado, H. (1995). Cycloheximide inhibits context memory and long-term habituation in the crab *Chasmagnathus*. *Pharmacology, Biochemistry and Behavior, 52*, 385–395.

Pedreira, M. E., & Maldonado, H. (2003). Protein synthesis subserves reconsolidation or extinction depending on reminder duration. *Neuron, 9*(38), 863–869.

Pedreira, M. E., Perez-Cuesta, L. M., & Maldonado, H. (2002). Reactivation and reconsolidation of long-term memory in the crab *Chasmagnathus*: Protein synthesis requirement and mediation by NMDA-type glutamatergic receptors. *Journal of Neuroscience, 22*, 8305–8311.

Pedreira, M. E., Perez-Cuesta, L. M., & Maldonado, H. (2004). Mismatch between what is expected and what actually occurs triggers memory reconsolidation or extinction. *Learning and Memory, 11*, 579–585.

Pereyra, P., Gonzalez, P. E., & Maldonado, H. (2000). Long-lasting and context-specific freezing preference is acquired after spaced repeated presentations of a danger stimulus in the crab *Chasmagnathus*. *Neurobiology of Learning and Memory, 74*, 119–134.

Perez-Cuesta, L. M., & Maldonado, H. (2009). Memory reconsolidation and extinction in the crab: Mutual exclusion or coexistence? *Learning and Memory, 16*, 714–721.

Philips, G. T., Tzvetkova, E. I., Marinesco, S., & Carew, T. J. (2006). Latent memory for sensitization in Aplysia. *Learning and Memory, 13*, 224–229.

Rescorla, R. A. (1988). Behavioral studies of Pavlovian conditioning. *Annual Review of Neuroscience, 11*, 329–352.

Romano, A., Delorenzi, A., Pedreira, M. E., Tomsic, D., & Maldonado, H. (1996). Acute administration of a permeant analog of cAMP and a phosphodiesterase inhibitor improve long-term habituation in the crab *Chasmagnathus*. *Behavioural Brain Research, 75*, 119–125.

Romano, A., Freudenthal, R., Merlo, E., & Routtenberg, A. (2006). Evolutionarily-conserved role of the NF-kappaB transcription factor in neural plasticity and memory. *European Journal of Neuroscience, 24*, 1507–1516.

Romano, A., Locatelli, F., Delorenzi, A., Pedreira, M. E., & Maldonado, H. (1996). Effects of activation and inhibition of cAMP-dependent protein kinase on long-term habituation in the crab *Chasmagnathus*. *Brain Research, 735*, 131–140.

Romano, A., Locatelli, F., Freudenthal, R., Merlo, E., Feld, M., Ariel, P., et al. (2006). Lessons from a crab: Molecular mechanisms in different memory phases of *Chasmagnathus*. *Biological Bulletin, 210*, 280–288.

Romano, A., Lozada, M., & Maldonado, H. (1991). Nonhabituation processes affect stimulus specificity of response habituation in the crab *Chasmagnathus granulatus*. *Behavioral Neuroscience, 105*, 542–552.

Rosenthal, R., & Rosnow, R. L. (1985). *Contrasts analysis focused comparisons in the analysis of variance*. Cambridge, UK: Cambridge UP.

Santos, M. C. F., & Costa, V. I. (1993). The short-term respiratory responses on three crabs exposed to water–air media. *Comparative Biochemistry and Physiology, 104A*, 785–791.

Sara, S. J. (2000a). Retrieval and reconsolidation: Toward a neurobiology of remembering. *Learning and Memory, 7*, 73–84.

Sara, S. J. (2000b). Strengthening the shaky trace through retrieval. *Nature Reviews Neuroscience, 1*, 212–213.

Sara, S. J., & Hars, B. (2006). In memory of consolidation. *Learning and Memory, 13*, 515–521.

Squire, L. R. (2006). Lost forever or temporarily misplaced? The long debate about the nature of memory impairment. *Learning and Memory, 13*, 522–529.

Stough, S., Shobe, J. L., & Carew, T. J. (2006). Intermediate-term processes in memory formation. *Current Opinion in Neurobiology, 16*, 672–678.

Tomsic, D., Beron de Astrada, M., & Sztarker, J. (2003). Identification of individual neurons reflecting short- and long-term visual memory in an arthropodo. *Journal of Neuroscience, 23*, 8539–8546.

Tomsic, D., de Astrada, M. B., Sztarker, J., & Maldonado, H. (2009). Behavioral and neuronal attributes of short- and long-term habituation in the crab *Chasmagnathus*. *Neurobiology of Learning and Memory, 92*, 176–182.

Tomsic, D., Pedreira, M. E., Romano, A., Hermitte, G., & Maldonado, H. (1998). Context-US association as a determinant of long-term habituation in the crab *Chasmagnathus*. *Animal Learning and Behavior, 26*, 196–209.

Troncoso, J., & Maldonado, H. (2002). Two related forms of memory in the crab *Chasmagnathus* are differentially affected by NMDA receptor antagonists. *Pharmacology, Biochemistry and Behavior, 72*, 251–265.

Tronson, N. C., & Taylor, J. R. (2007). Molecular mechanisms of memory reconsolidation. *Nature Reviews Neuroscience, 8*, 262–275.

Tulving, E. (1983). Ecphoric processes in episodic memory. *Philosophical Transactions of the Royal Society of London. Series B, 302*, 361–370.