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Memory expression is independent of memory labilization/reconsolidation

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ABSTRACT

There is growing evidence that certain reactivation conditions restrict the onset of both the destabilization phase and the restabilization process or reconsolidation. However, it is not yet clear how changes in memory expression during the retrieval experience can influence the emergence of the labilization/reconsolidation process. To address this issue, we used the context-signal memory model of Chasmagnathus. In this paradigm a short reminder that does not include reinforcement allows us to evaluate memory labilization and reconsolidation, whereas a short but reinforced reminder restricts the onset of such a process. The current study investigated the effects of the glutamate antagonists, APV (0.6 or 1.5 μg/g) and CNQX (1 μg/g), prior to the reminder session on both behavioral expression and the reconsolidation process. Under conditions where the reminder does not initiate the labilization/reconsolidation process, APV prevented memory expression without affecting long-term memory retention. In contrast, APV induced amnesic effects in the long-term when administered before a reminder session that triggers reconsolidation. Under the present parametric conditions, the administration of CNQX prior to the reminder that allows memory to enter reconsolidation impairs this process without disrupting memory expression. Overall, the present findings suggest that memory reactivation – but not memory expression- is necessary for labilization and reconsolidation. Retrieval and memory expression therefore appear not to be interchangeable concepts.

1. Introduction

Earlier and contemporary studies have shown that the presentation of reminder cues can make a consolidated trace re-enter a transient labile state (reconsolidation) and, consequently, become vulnerable to the same agents that block consolidation (Alberini, 2007; Dudai, 2012; Eisenberg, Kobilo, Berman, & Dudai, 2003; McKenzie & Eichenbaum, 2011; Misanin, Miller, & Lewis, 1968; Przybyslawski, Roullet, & Sara, 1999). In line with this view, the administration of glutamate receptor antagonists interferes with memory during reconsolidation, as shown in several paradigms across phyla (Izquierdo et al., 2008; Lee, Milton, & Everitt, 2006; Milton et al., 2013; Nikitin & Solntseva, 2012; Pedreira, Pérez-Cuesta, & Malldonado, 2002; Rose & Rankin, 2006; Sara & Hars, 2006; Si, Helliwell, & Maleszka, 2004). It has been argued that a functional role of reconsolidation is to allow memory updating by both linking new information to the original memory and changing the level of expression of an already consolidated memory (Coccoz, Maldonado, & Delorenzi, 2011; Dudai, 2012; Forcato, Rodriguez, & Pedreira, 2011; Frenkel, Maldonado, & Delorenzi, 2005a; Frenkel, Maldonado, & Delorenzi, 2005b; Hupbach, Gomez, Hardt, & Nadel, 2007; Lee, 2008; Milekic, Pollonini, & Alberini, 2007; Pedreira, Perez-Cuesta, Maldonado, & Pérez-cuesta, 2004; Tronson & Taylor, 2007). The re-emergence of the reconsolidation hypothesis changed the theory of fixed and unchangeable long-term memory after consolidation and led to new interpretations about certain mnesic effects and the nature of experimental amnesias (Caffaro, Suárez, Blake, & Delorenzi, 2012; Frenkel, Suarez, Maldonado, Delorenzi, & Suárez, 2010; Gold, 2006; Miller & Matzel, 2006; Nader & Wang, 2006; Sara & Hars, 2006).

Our previous studies on the modulation of memory expression (the expression of a representation in cognition or behavior, Schacter, 2007) were based on the hypothesis that, during memory consolidation and reconsolidation, neuromodulators can determine the ability of the memory to guide behavior, i.e. they can either increase or decrease its behavioral expression without

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affecting its persistence (Frenkel et al., 2005a; Frenkel et al., 2005b; Frenkel et al., 2010). To Support this hypothesis, we proposed an experimental design, based on the positive modulation of memory expression during reconsolidation. In this approach, whether an unexpressed memory is reactivated by the presentation of a reminder and becomes labile is evidenced in a subsequent test. Because "reminder effects" can be explained by processes other than memory update (Cahill, McGaugh, & Weinberger, 2001; Gold, Haycock, Marri, & McGaugh, 1973), specific controls based on the constraints of reminders for triggering reconsolidation allowed us to distinguish between reactivated but unexpressed and obliterated memories after amnesic treatments and forgetting. This approach successfully unveiled unexpressed but reactivatables memories after weak training, forgetting and treatments with either scopolamine or angiotensins antagonists (Caffaro et al., 2012; Coccoz et al., 2011; Frenkel et al., 2005a; Frenkel et al., 2005b), and was successfully applied to test the hypothesis in rodent's fear memory afterwards (Blake, Boccia, Krawczyk, Delorenzi, & Baratti, 2012). Furthermore, this strategy allowed us to show, in human declarative memory, that the period in which memory can be reactivated and become labile during reconsolidation largely exceeds the period in which that memory is expressed, proving direct evidence that conscious access to memory is not needed for reconsolidation (Coccoz, Sandoval, Stehberg, & Delorenzi, 2013).

There are several boundary condition that place constraints on the onset of the reconsolidation process, for instance, the necessity of new information at the time of memory reactivation (Dudai, 2012; Pedreira et al., 2004). Memory expression during the presentation of the reminder cues is not a condition for the onset of the labilization/reconsolidation process; unexpressed memories can be reactivated and labilized (Blake et al., 2012; Caffaro et al., 2012; Coccoz et al., 2011; Frenkel et al., 2005a; Frenkel et al., 2005b; Frenkel et al., 2010). These studies predict that there should be a dissociation between the mechanisms mediating memory reactivation (i.e. access to the memory trace) and those underlying the behavioral expression of memory (Ben Mamou, Gamache, & Nader, 2006; Caffaro et al., 2012; Coccoz et al., 2011; Milton et al., 2013; Rodriguez-Ortiz, Balderas, Garcia-DeLaTorre, & Bermudez-Rattoni, 2012; Sevenster, Beckers, & Kindt, 2012). Indeed, although the requirement of glutamate AMPA and/or NMDA receptors activation in the retrieval process is largely known (Barros, Izquierdo, Medina, & Izquierdo, 2003; Si et al., 2004; Summers, Crowe, & Ng, 2003), recent studies have shown that the blockade of AMPA receptors actually disrupts retrieval without affecting the amnesic properties of protein synthesis inhibitors after memory reactivation (Ben Mamou et al., 2006; Rodriguez-Ortiz et al., 2012). However, the question about whether a lack of memory expression after the blockade of retrieval represents a disruption to reactivation, or expression, remains open (Milton et al., 2013; Rodriguez-Ortiz et al., 2012). Reconsolidation theory states that reactivation of a memory is necessary to induce the post-reminder state of malleability (Lewis, 1979; Nader, 2003). Therefore, the aim of the present study is to test whether memory reactivation and memory expression can be affected independently by the glutamate antagonists APV and CNQX.

Our working hypothesis is that NMDA and AMPA antagonists can induce a retrieval deficit that is due to a disruption of memory expression, although the capability of memory to be reactivated and become labile remains unaffected. We predict that, although the glutamate receptors antagonist attenuates memory expression, the antagonist will have amnesic effects as a result of a disruption of reconsolidation. This result should prove that even in the absence of expression, memory was reactivated and labilized. However, several of the amnesic effects after memory reactivation can be described as independent of reconsolidation (Cahill et al., 2001; Gisquet-Verrier & Riccio, 2012; Gold, 2006). If this long-term amnesic effect of glutamate antagonists depends on memory reactivation-labilization of the non-expressible memory, then it should be demonstrated that the amnesic effect is due to the reconsolidation process. This issue can be addressed in the context-signal memory of *Chasmagnathus* since it was demonstrated that memory labilization and reconsolidation are not triggered by the reminder presentation *per se*, but when the reminder fulfils certain parametric conditions. In particular, a reminder of short duration that does not include reinforcement is able to trigger labilization and reconsolidation, whereas a short but reinforced reminder is not able to trigger such a process (Frenkel et al., 2005a; Frenkel et al., 2005b; Pedreira et al., 2004; Romano et al., 2006). Therefore, if memory can be interfered with during reconsolidation, regardless of its expression being attenuated, the long-term amnesic effect should not occur when reinforcement is presented contingent on the reminder. In addition, several other conditions have been proposed that constrain reconsolidation, including the effects of this process need time to develop (Frenkel et al., 2005a; Frenkel et al., 2005b; Nader & Hardt, 2009). Thus, the amnesic effects on the unexpressed, but reactivated, memory will be specifically disclosed in the long, but not short, term.

Our results support the perspective that the reactivation-labilization and expression mechanisms can be dissociated.

2. Experimental procedures

2.1. Animals

Intermolt adult male crabs of the species *Neohelice granulata (Chasmagnathus granulatus)* between 2.7 and 3.0 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. 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, consisted of a bowl-shaped opaque container, illuminated by a bottom light (5 W bulb), 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 seawater, where the crab was lodged before each experimental session. Eighteen seconds before each trial the bottom light went off and the top light (5 W bulb) was turned on. Then, a 9 s trial was given, in which 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. During this 9 s trial, the top light was kept on. The VDS provoked an escape response in crabs and consequent container vibrations, which were converted into electrical signals through a piezoelectric transducer placed on the external wall of the container. These signals were amplified and integrated during each 9-s trial, and translated into numerical units ranging from 0 to 16,000, 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. In experiment

of Fig. 2B, 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 seawater, was used as a different training context during the Reminder Session on the second day. Between Training, Reminder and Testing Sessions, crabs stayed in resting containers (plastic cylindrical containers, 15 cm in diameter and 15 cm in height) covered to a depth of 0.5 cm with brackish water and kept inside dimly lit drawers.

2.3. Escape response and freezing

The intensity of container vibrations during a VDS 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, González Portino, & Maldonado, 2000). The escape response is a directional run of the animal in an attempt to move away from the VDS, while the freezing response consists of a rigid motionless display in which the crab lies flat on the substratum. During repeated VDS presentations (training), the escape response decreases in intensity and is progressively replaced by a freezing response. Throughout this study, data were recorded during a trial time, i.e., during the 9-s VDS presentation.

2.4. Training protocol

A training protocol consists of fifteen trials (9 s, two VDS presentations), with 3 min intertrial intervals (total training duration; 42 min), after a 10 min adaptation period in which animals do not receive VDS. Since the memory under study arises as a consequence of an association between the context and the VDS, it was termed context-signal memory. Context-signal memory expression is revealed at testing session as a significant decrease in the activity of the animals when the VDS is presented. This decrease in activity is due to an increase in the number of animals displaying the freezing response. Here, we used a variation of the training protocol. In this case, 18 s before each trial the bottom light went off and the top light came on. Next, during the 9 s trial, the VDS was presented with the top lights on. This training protocol also induces an association between the iterated VDS presentation and the contextual features of the training context, where a robust freezing is also acquired throughout the 15-trial training and a clear retention is found when tested with the VDS presentation short term (2 and 4 h) and long term (1–4 days) (Fustiñana, Carbó Tano, Romano, & Pedreira, 2013; Maldonado, 2002; Pereyra et al., 2000; Tomsic, Pedreira, Romano, Hermitte, & Maldonado, 1998).

2.5. Experimental procedure and design

Experiments included three sessions: Training Session (Day 1), Reminder Session (Day 2) 24 h later and Testing Session (Day 3) another 24 h later. The experimental protocols involve pairs of crab groups, where each pair consists of a trained group (TR) and an untrained group (UN). Untrained animals remained in the actometer during the entire Training Session, but they were not presented with the VDS. For the untrained groups, response was recorded in intervals of 9-s coincident with VDS presentation in the respective trained group. In the Reminder Session and Testing Session, untrained groups were treated as trained groups. Each group has between 25 and 40 crabs.

2.5.1. Day 1, Training session

TR group: This group underwent a training protocol. First, each crab was placed in an individual training context for 10 min, without being stimulated with VDS. After this adaptation period, animals received 15 training trials (Section 2.4.), separated by inter-trial intervals of 3 min. Finally, animals were immediately placed in individual resting containers until the following day. UN group: Each crab of this untrained group was placed in an individual training context and remained there for the same time and conditions as for the trained animals (Section 2.2.), but without receiving the VDS. Then, animals were immediately placed in individual resting containers until the following day.

2.5.2. Day 2 – Reminder session

Three situations may arise in this session according to the design of the experiment in question (Maldonado, 2002; Pedreira et al., 2004): (a) Crabs were re-exposed to the training context for 5 min, in which the bottom light went off and a top light came on for the last 27 s without the VDS presentation. Then, animals were returned to their respective resting containers until the following day. This procedure reactivates and turns memory into a labile state, initiating the reconsolidation process. (b) Crabs were re-exposed to the training context for 5 min, in which the bottom light went off and a top light came on for the last 27 s, with the VDS being presented for the last 9 s. Animals were then returned to their respective resting containers until the following day. This procedure prevents memory labilization, and reconsolidation is not initiated. (c) Crabs were exposed to a cylindrical and striped context and the VDS was not presented. This context is perceived as a novel context, and thus it does not trigger memory labilization and reconsolidation. After the presentation of each reminder, crabs were returned to their resting containers.

2.5.3. Day 3 – Testing session

On the third day, crabs were re-exposed to the training context for 10 min and at the end memory expression was tested with a single VDS presentation as in Section 2.2.

2.6. Drug administration

Ten minutes prior to the reminder session the following treatments were given: Crustacean physiological saline solution (SAL) (Delorenzi et al., 1996); vehicle (2% dimethylsulfoxide in saline); the glutamate NMDA receptor antagonist, DL-2-Amino-5-phosphonopentanoic acid (APV) (0.6 or 1.5 μg/g); or the glutamate AMPA/kainate receptor antagonist 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX) (1 μg/g). The injections were given through the right side of the dorsal cephalothoraxic-abdominal membrane, by means of a syringe fitted with a sleeve to control the depth of penetration to 4 mm, thus ensuring that the injected solution was released in the pericardial sac (Maldonado, 2002). CNQX was diluted in vehicle while APV was diluted in SAL. All drugs were purchased from Sigma Chemical Co. Saint Louis, Missouri, USA.

2.7. Memory retention criterion and data analysis

LTM expression was assessed on Day 3 by focusing data analysis on test trial scores, i.e., by estimating the difference between the response level of the trained group (TR) and that of the respective untrained group (UN). 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 memory expression in crabs is independent of the escape response level at training (Maldonado, 2002; Romano et al., 2006; Tomsic et al., 2009). Data were analyzed using analysis of variance (ANOVA) and a priori planned comparisons. The parameter under measure (container vibrations as a result of

complex motor activities) reflects qualitatively different responses (as, for example, freezing and escape), and therefore the variance of this parameter is different when different responses prevail. As the variance of activity scores increases with the mean, thus violating the homogeneity of variance assumption of ANOVA, the data were log2 transformed. All experiments described in this paper used one or two untrained–trained pairs (UN–TR). Three types of contrast per experiment were used for two pairs: 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. 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. When only one pair of groups was compared, a *t*-test was used. Because the values resulting from the 9 s vibration integration, measured by four microphones, are expressed in arbitrary units, we use the transformed values (log2 of response), which have a normal distribution, to express the activity of groups of animals as median and standard errors. We analyzed data using STATISTICA (StatSoft 6.0).

3. Results

3.1. APV impairs memory expression

In the Chasmagnathus associative memory model, animals associate the training context with the VDS passing overhead. After the iterative presentation of the VDS, a strong freezing-to-VDS 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 (UN) group; (UN > TR).

In the first experiment, we assessed whether the administration of the NMDA antagonist APV, before the testing session, disrupts memory expression (Fig. 1).

On Day 1, two pairs of UN–TR groups of crabs underwent the Training Session, then the animals were moved to the resting containers until the next day. On Day 2 a pair of UN–TR groups of crabs was administered with physiological saline solution before the testing session (SAL-TR and SAL-UN groups) and simultaneously another pair of UN–TR groups of crabs was administered with 1.5 μg/g of APV (APV-TR and APV-UN groups), all animals underwent the Testing Session and then were returned to their resting containers. Finally, on Day 3, all animals underwent a second Testing Session (Fig. 1). It is important to note that in this experiment crabs were re-exposed during the testing sessions to the training context for 5 min, with the VDS being presented for the last 9 s. As explained in methods, this procedure prevents the onset of memory labilization, and reconsolidation (see Fig. 1) but the behavior can be fully expressed. Results at training are shown in Fig. 1; no differences were disclosed between APV and SAL groups during Training Session (Day 1). ANOVA (repeated measures), performed on the scores of each trial, disclosed no significant group differences [*F*group (1,63) = 1.55; $p = 0.218$] and, as expected, a significant trial effect [*F*trial(1,14) = 25.25; $p < 0.001$].

Planned comparisons on activity scores on Day 2 [ANOVA:$F(3,127) = 5.01$, $p < 0.01$] and Day 3 [ANOVA: $F(3,104) = 5.98$, $p < 0.001$] disclosed significant differences in escape response between Pair SAL groups: on Day 2 (SAL-UN > SAL-TR, $^{**}p < 0.01$) and on Day 3 (SAL-UN > SAL-TR, $^{*}p < 0.05$); that is, a lower escape response was evident in the SAL-TR group revealing memory expression at both Testing Sessions (Day 2 and 3). However, in spite of the fact that there were no significant differences between APV groups on the first testing session (Day 2) (APV-UN ≈ APV-TR, $p = 0.12$) there was a significant difference on the second testing session (Day 3) (APV-UN > APV-TR, $^{*}p < 0.05$). That is, APV administration (1.5 μg/g) impairs memory expression when it is administered prior to testing (Day 2). However, during the second test APV treated crabs displayed a lower escape response revealing memory expression (Day 3). There were no differences between untrained groups on either Day 2 or Day 3 (SAL-UN ≈ APV-UN, $p = 0.85$, Day 2; $p = 0.72$ Day 3).

These findings showed that APV prevents memory expression, but it does not produce amnesia because this experimental procedure does not initiate the labilization/reconsolidation process.

3.2. Memory reactivation is independent of memory expression

In this next experiment, we assessed if the memory reconsolidation process takes place when memory expression is disrupted by APV. To achieve this goal, we evaluated whether the administration of APV, prior to the Reminder Session that triggers the reconsolidation process, disrupts memory retention.

If APV (1.5 μg/g) interferes with memory expression, but not with labilization, then the memory trace would be reactivated, labilized and then susceptible to be disrupted by the NMDA antagonist (Pedreira et al., 2002). In such a case, animals should not show memory retention at testing on the third day. In contrast, if the interference of memory expression by APV also prevents labilization, animals should show memory retention at testing on the

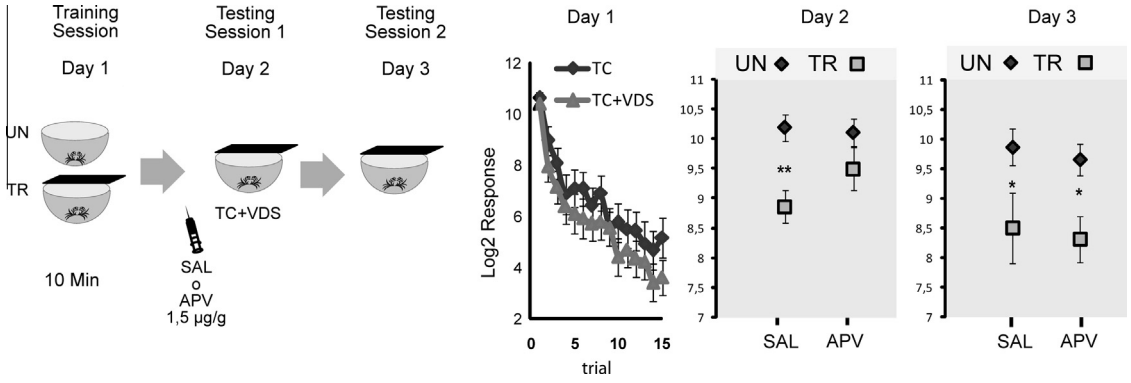

Fig. 1. *Pre-testing administration of APV impairs memory expression.* Left panel: experimental designs (black bar above boxes indicates visual danger stimulus, VDS). Right panel: *Reminder* and *Testing Session* results. Graphs ordinates: log2 trial scores during VDS presentation (means ± SE); filled symbols (♦): UT, untrained groups; open symbols (■): TR, trained groups. On Day 1 TR groups received a 15-spaced trial training, while UT groups remained in the actometer for the same time without stimulation., Day 2, before *Testing* pair SAL groups were injected with saline solution and pair APV groups were administrated with APV (1.5 μg/g). memory was tested with a single VDS presentation. Then all animals were returned to their resting containers. Day 3, memory was tested with a single VDS presentation. For all groups, $N = 32$.

third day. The following experiment focused on this question (Fig. 2).

On Day 1, two pairs of UN–TR groups of crabs underwent the Training Session, then animals were moved to the resting containers until the next day. On Day 2 all crabs were administered with APV (1.5 μg/g) before the reminder session. A pair of UN–TR groups were re-exposed to the original training context (TC) for 5 min (this procedure reactivates and initiates memory labilization during reconsolidation), then were returned to their resting containers, (UN–TC and TR–TC groups). Simultaneously, another pair of UN–TR groups was re-exposed to the original training context for 5 min and at the end received a single VDS presentation (UN – TC + VDS and TR – TC + VDS), as explained above this procedure does not trigger the reconsolidation process. Then, animals were returned to their resting containers. On Day 3, all animals were tested.

Results at training are shown in Fig. 2; no differences were disclosed between training groups. ANOVA (repeated measures) performed on the scores of each trial, disclosed no significant group differences [Fgroup (1,61) = 0.04; $p = 0.95$] and, as expected, a significant trial effect [Ftrial(1,14) = 27.17; $p < 0.001$].

On Day 2, as in the first experiment, the groups injected with APV pre testing did not show memory expression: there were no significant differences between control and training groups that received the VDS (TR – TC + VDS and UN – TC + VDS, $t(62) = 1.37$, $p = 0.17$).

On day 3 planned comparisons on activity scores, [ANOVA: $F(3,124) = 6.68$, $p < 0.05$ showed differences between TR – TC + VDS and UN – TC + VDS ($p < 0.01$)], but not between untrained groups (UN – TC ≈ UN – TC + VDS, $p = 0.74$). Interestingly, there was no difference between TR–TC and UN–TC groups ($p = 0.26$) (Fig. 2), indicating no memory retention in the TR–TC group. That is, APV administered prior to a reminder procedure that allows memory to enter reconsolidation can impair this process even when the NMDA-antagonist disrupts memory expression. In those animals in which memory was no labilized due to the parametric conditions of the reminder (there was not a mismatch between the animal's prediction and what actually occurred), APV did not affect memory reconsolidation and therefore memory retention was disclosed on the third day.

We replicated the previous experiment with a lower dose of APV (0.6 μg/g) and we obtained the same results: On day 1, no differences were disclosed between training groups. ANOVA (repeated measures) performed on the scores of each trial, disclosed no significant group differences [Fgroup (1,61) = 0.34; $p = 0.56$] and, as expected, a significant trial effect [Ftrial(1,14) = 27.55; $p < 0.001$]. On Day 2 there were no significant differences between control and training groups that received the VDS (9.61 ± 0.37; 10.06 ± 0.26 TR – TC + VDS and UN – TC + VDS respectively, $t(62) = 0.98$, $p = 0.33$). On day 3, planned comparisons on activity scores, [ANOVA: $F(3,121) = 4.97$, $p < 0.01$] showed differences between groups that received the VDS (8.58 ± 0.67; 10.44 ± 0.22 TR – TC + VDS and UN – TC + VDS respectively, $p < 0.01$) but, there was not memory retention for the groups exposed to the original training context without the VDS (9.99 ± 0.38; 10.30 ± 0.34 TR–TC and UN–TC, $p = 0.36$). There were no differences between untrained groups (UN – TC ≈ UN – TC + VDS, $p = 0.93$).

In summary, the two previous experiments showed that APV induces amnesic effects when administered previous to a reminder session that triggers reconsolidation. Another condition of the reconsolidation process is that the effects on such process will be disclosed in the long but not in the short-term (Dudai & Eisenberg, 2004; Frenkel et al., 2005a; Frenkel et al., 2005b; Gisquet-Verrier & Riccio, 2012; Pedreira et al., 2004). Therefore, the following series of experiments tested whether the amnesic effect of APV previous to the reminder is disclosed in the long -but not in the short- term.

On Day 1, a pair of UN–TR groups of crabs underwent the Training Session, then animals were moved to the resting containers until the next day. On Day 2 all crabs were administered with SAL and were re-exposed to the original training context (TC) for 5 min, then were returned to their resting containers (SAL-TR and SAL-UN groups). Four hours after the reminder session all animals were tested. On Day 3, all animals underwent a second testing session. At both Testing Session there were significant differences between groups (SAL-TR and SAL-UN, $t(54) = 3.31$, $p < 0.01$, Day 2; $t(54) = 4.23$, $p < 0.01$, Day 3) showing memory at both testing sessions Fig. 3A. Fig. 3B shows the short and long term testing sessions in groups of animals that were exposed to the TC or a novel context on Day 2, after APV administration. On Day 1 two pairs of UN–TR groups of crabs underwent the Training Session; next, animals were moved to the resting containers until the next day. On Day 2 all crabs were administered with APV (0.6 μg/g), a pair of UN–TR animals were re-exposed to the original TC for 5 min (this procedure reactivates and initiates memory labilization during reconsolidation), then were returned to their resting containers (TR–TC

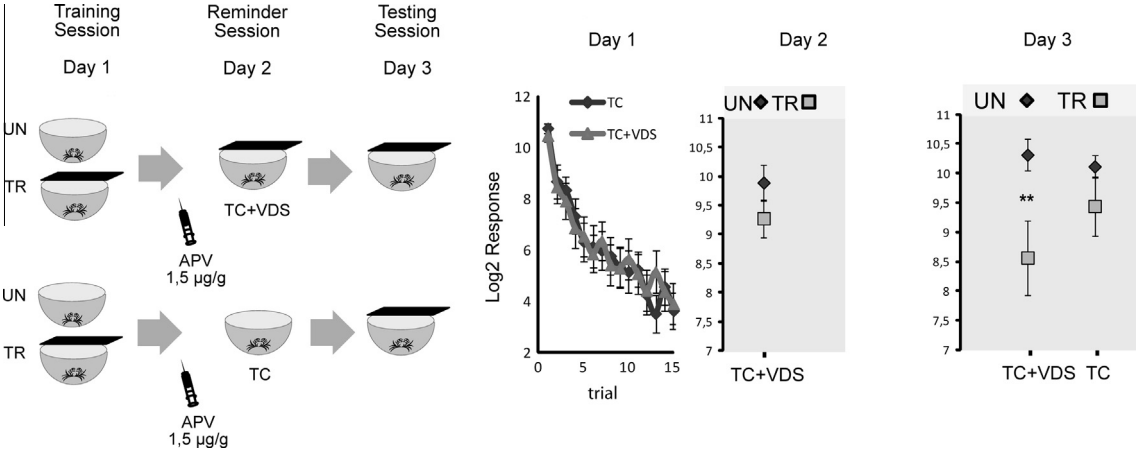

Fig. 2. *Memory reactivation is independent of memory expression.* Day 1, Training Session. Day 2, all animals were injected with APV (1.5 μg/g). Pair TC groups were re-exposed to the training context for 5 min (this procedure reactivates and initiates memory labilization during reconsolidation) and Pair TC + VDS groups were re-exposed to the training context for 5 min and at the end received a single VDS presentation (This reminder does not trigger the reconsolidation process). Day 3 (*Testing Session*), memory was tested with a single VDS presentation. TR–TC, $N = 32$; UN–TC, $N = 32$; TR – TC + VDS, $N = 30$; UN – TC + VDS, $N = 32$. Symbols as in Fig. 1.

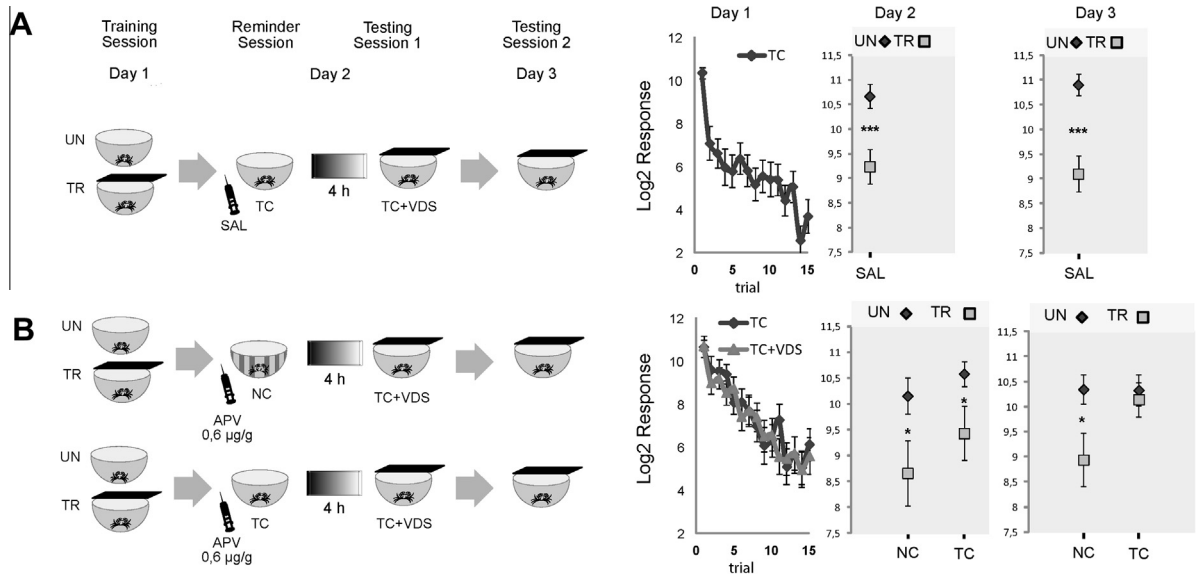

Fig. 3. *APV administrated pre reminder effects are disclosed in the long -but not in the short-term.* (A) *Saline treatment.* Day 1, Training Session. Day 2, all animals were injected with SAL before the reminder session and were re-exposed to the training context for 5 min, 4 h after the reminder session all animals underwent a testing session. Day 3 (Testing Session), memory was tested with a single VDS presentation. For all groups, $N = 28$. (B) *APV treatment.* Day 1, Training Session. Day 2, all animals were injected with APV (0.6 μg/g) before the reminder session. Pair TC groups were re-exposed to the training context for 5 min (this procedure reactivates and initiates memory labilization during reconsolidation) and Pair NC groups were re-exposed to a novel context for 5 min (this reminder does not trigger the reconsolidation process) 4 h after the reminder session all animals underwent a testing session. Day 3 (Testing Session), memory was tested with a single VDS presentation. TR–TC, $N = 32$; UN–TC, $N = 31$; TR–NC, $N = 32$; UN–NC, $N = 32$. Symbols as in Fig. 1.

and UN–TC groups). Simultaneously, another pair of UN–TR groups of crabs was exposed to a novel context (NC) for 5 min and then were was returned to their resting containers (TR–NC and UN–NC groups). Four hours after the reminder session all animals were tested. On Day 3, all animals underwent an additional testing session.

No differences were disclosed between training groups. ANOVA (repeated measures) performed on the scores of each trial, disclosed no significant group differences [Fgroup $(1,64) = 0.07$; $p = 0.80$] and, as expected, a significant trial effect [Ftrial$(1,14) = 20.81$; $p < 0.001$]. At the short-term Testing Session, planned comparisons on activity scores [ANOVA: $F(3,123) = 3.46$, $p < 0.01$] showed memory retention in animals exposed to either the novel or the trained context (UT–NC > TR–NC, $p < 0.05$; UN–TC > TR–TC, $p < 0.05$). However, at the long-term Testing Session planned comparisons [ANOVA: $F(3,123) = 3.14$, $p < 0.05$] showed memory retention for animals exposed to the novel context (UT–NC > TR–NC, $p < 0.05$) but, there was no memory retention in the animals re-exposed to the original training context on Day 2 (UN–TC > TR–TC, $p < 0.05$). These findings showed that the amnesic effects due to APV are reconsolidation specific because they are disclosed in the long but not in the short-term. There were no difference between untrained groups on Testing Session (short-term: SCP–UN–TC ≈ SCP–UN–NC, $p = 0.98$; long-term: SCP–UN–TC ≈ SCP–UN–NC, $p = 0.52$).

3.3. The AMPA antagonist CNQX impairs memory reconsolidation but does not impair memory expression

In the next experiment, we assessed whether the administration of the AMPA antagonist CNQX, before the testing session, disrupts memory expression (Fig. 1).

Fig. 4A displays the effect of the vehicle treatment. On Day 1, a pairs of UN–TR groups of crabs underwent the Training Session, and then the animals were moved to the resting containers until the next day. On Day 2 crabs were administered with vehicle before the Testing Session (VEH-TR and VEH-UN groups). Finally, on Day 3, all animals underwent a second Testing Session). Results at training are shown in Fig. 4A. At both Testing Session there were significant differences between groups (VEH-TR and VEH-UN, $t(68) = 2.52$, $p < 0.05$, Day 2; $t(68) = 2.05$, $p < 0.01$, Day 3) indicating memory retention at both testing sessions. Fig. 4B shows the effect of CNQX treatment. On Day 1, two pairs of UN–TR groups of crabs underwent the Training Session, then animals were moved to the resting containers until the next day. On Day 2 all crabs were administered with CNQX (1 μg/g) before the reminder session. A pair of UN–TR groups was re-exposed to the original training context (TC) for 5 min (this procedure reactivates and initiates memory labilization during reconsolidation), then the animals were returned to their resting containers, (TR–TC and UN–TC groups). Simultaneously, another pair of UN–TR groups was re-exposed to the original training context (TC) for 5 min and at the end received a single VDS presentation (Training context + VDS, TC + VDS), which is the procedure that does not trigger the reconsolidation process. Then, animals were returned to their resting containers. On Day 3, all animals were tested (TR – TC + VDS and UN – TC + VDS groups).

Results at training are shown in Fig. 4B; no differences were disclosed between training groups. ANOVA (repeated measures) performed on the scores of each trial, disclosed no significant group differences [Fgroup $(1,64) = 1.25$; $p = 0.27$] and, as expected, a significant trial effect [Ftrial$(1,14) = 19.20$; $p < 0.001$].

On Day 2, there was a significant difference between groups that received the VDS (TR – TC + VDS and UT – TC + VDS, $t(62) = 2.42$, $p < 0.05$). On day 3 planned comparisons on activity scores, [ANOVA: $F(3,119) = 3.51$, $p < 0.05$], as we expected, showed differences between TR – TC + VDS and UT – TC + VDS ($p < 0.01$) indicating that CNQX does not affect memory retention when administered prior to a reminder that does not allow the emergence of reconsolidation. In contrast, there was no difference between TR–TC and UT–TC groups, indicating that CNQX blocked memory retention ($p = 0.45$) (Fig. 4B). There were no differences between untrained groups (UT – TC ≈ UT – TC + VDS, $p = 0.81$) on Day 3. In conclusion, the CNQX administered before the reminder

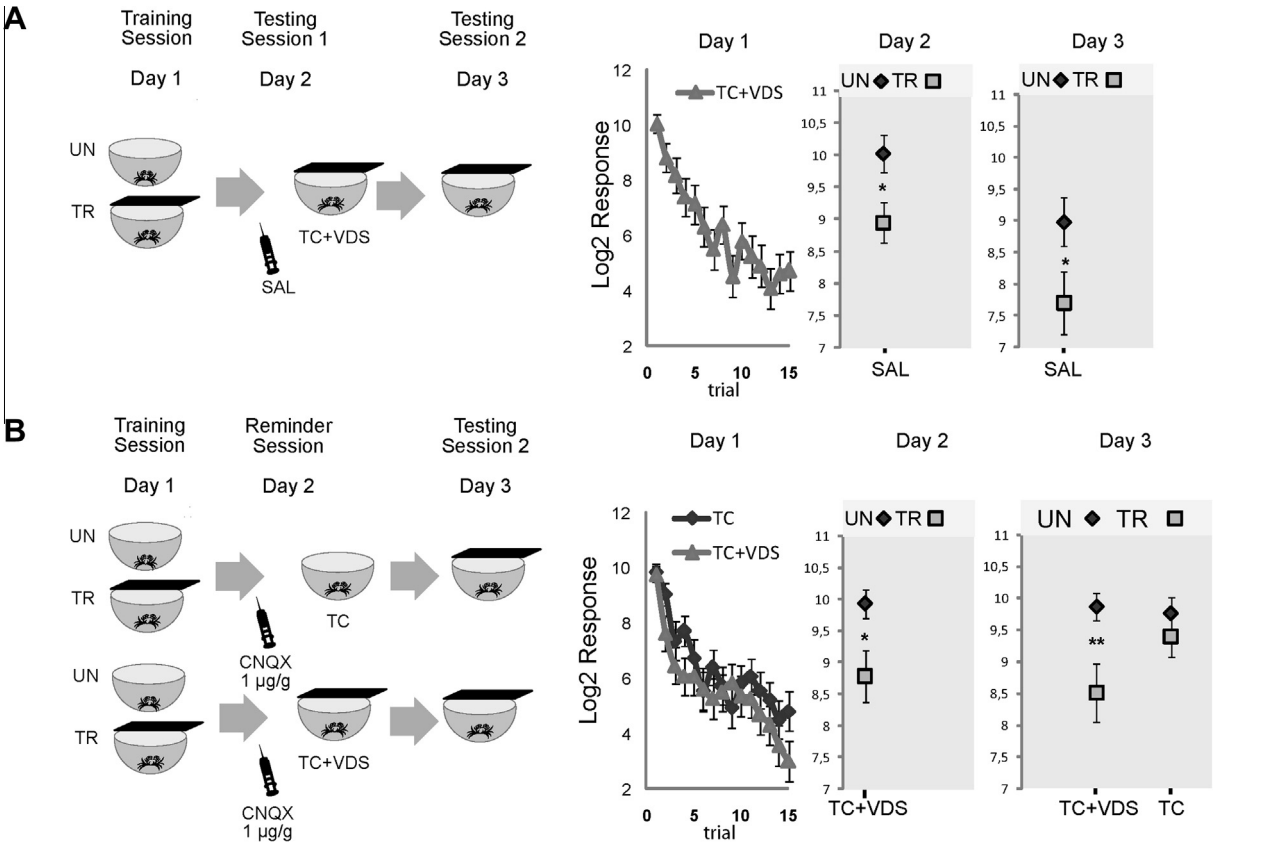

Fig. 4. *CNQX impairs memory reconsolidation but does not impair memory expression.* (A) *Vehicle treatment*. Day 1, Training Session. Day 2, all animals were injected with VEH before the reminder session and were re-exposed to the training context for 5 min. Day 3 (Testing Session), memory was tested with a single VDS presentation. VEH-TR, $N = 35$; VEH-UN, $N = 35$ (B) *CNQX treatment*. Day 1, two pairs of UN–TR groups of crabs underwent the Training Session. Day 2, all animals were injected with CNQX (1 µg/g) before the reminder presentation. Pair TC groups were re-exposed to the training context for 5 min (this procedure reactivates and initiates memory labilization during reconsolidation) and Pair TC + VDS groups were re-exposed to the training context for 5 min and at the end received a single VDS presentation (this reminder does not trigger the reconsolidation process). Day 3 (Testing Session), memory was tested with a single VDS presentation. TR–TC; UN–TC; TR – NC + VDS, $N = 31$; UN – TC + VDS, $N = 30$. Symbols as in Fig. 1.

that allows memory to enter reconsolidation impairs this process without disrupting memory expression under the present parametric conditions.

4. Discussion

The key finding of this study is that the disruption of retrieval induced by APV (1.5 µg/g and 0.6 µg/g) resulted from the disruption of behavioral expression of the accessed memory trace.

Even though the administration of CNQX prior to the reminder did not impair memory expression, it was effective in disrupting memory reconsolidation. This can be inferred because animals treated before a reminder that triggers reconsolidation were amnesic on the following day, during testing (Fig. 4). The same effect of CNQX on memory reconsolidation was also observed in *Caenorhabditis elegans* (Rose & Rankin, 2006). However, in this study we used one dose of CNQX and the injection was systemic. Regarding this point, we cannot conclude that AMPA receptors are not implicated in memory expression. Further research is required to definitively conclude that CNQX does not influence the behavioral performance during reactivation. For instance, it has been found that AMPA receptors antagonist can impair memory expression when locally administered in the basolateral amygdala (Ben Mamou et al., 2006; Milton et al., 2013; Rodriguez-Ortiz et al., 2012).

It should be noted that present APV results contrast the ones of Ben Mamou et al. (2006). On their study APV did not prevent memory expression or reconsolidation when infused in amygdala prior to retrieval, but protected memory from anisomycin-induced impairments. Additionally, they found that CNQX impaired memory expression but not reconsolidation when infused prior to retrieval. In this sense, several studies reports that retrieval requires intact glutamate receptors in several brain areas. However, AMPA and NMDA receptor antagonist can affect the process depending on the route of administration. For instance, in rats AMPA receptors are necessary for retrieval of fear memories in CA1 and the basolateral amygdala and NMDA receptors are necessary in all the neocortical structures. (Barros et al., 2000; Ben Mamou et al., 2006; Izquierdo et al., 2005; Milton et al., 2013; Rodriguez-Ortiz et al., 2012). In this study we employed a different memory model and different doses than Ben Mamou et al. (2006), which were administered systemically. All these factors appear to be the main source of differences between both studies.

A vast number of studies have shown that several agents or conditions can induce retrieval deficits (Barros et al., 2003; Si et al., 2004; Summers et al., 2003). Indeed, a dissociation of the requirement for different NDMA receptors for memory destabilization and restabilization has been shown (Milton et al., 2013). However, two key issues remain open. First, memory reactivation *per se* can be a sufficient factor to explain the amnesic actions of APV after memory retrieval, making these post-retrieval consequences independent of the reconsolidation process (Cahill et al., 2001; Gisquet-Verrier & Riccio, 2012). Second, some experimental approaches were unable to discern whether the glutamate antagonist administration disrupted the retrieval of memory, or

whether retrieved memory could not be expressed (Milton et al., 2013; Rodriguez-Ortiz et al., 2012).

The classic controls for memory reconsolidation (Nader, Schafe, & Ledoux, 2000) are to prove the necessity of the reminder, and that the post-retrieval treatment is effective only if it is performed during a temporal window after memory reactivation (Fig. 3). The results of this study show that the long-term amnesic effect after memory reactivation depends on the type of reminder used (Figs. 1 and 2). Previous and present results in this memory model show that the reminder structure required to trigger reconsolidation is a non-reinforcement condition. The non-reinforcement requirement has led to the mismatch hypothesis being proposed (Pedreira et al., 2004; Perez-Cuesta & Maldonado, 2009): memory becomes labile only when there is a mismatch between the animal's prediction and what actually occurs. This proposal has been confirmed in several memory models as a memory updating hypothesis (Coccoz et al., 2011; Dudai, 2009; Díaz-Mataix, Ruiz Martinez, Schafe, LeDoux, & Doyère, 2013; Forcato, Argibay, Pedreira, & Maldonado, 2009; Forcato, Rodriguez, Pedreira, & Maldonado, 2010; Rodriguez-Ortiz, De, Gutierrez, & Bermudez-Rattoni, 2005; Rodriguez-Ortiz, Garcia-DeLaTorre, Benavidez, Ballesteros, & Bermudez-Rattoni, 2008). Reconsolidation theory proposes that a labile state of the memory arises as result of memory reactivation (Lewis, 1979; Nader, 2003). Evidence has shown that not every time a memory trace is reactivated, is it labilized (Squire, Slater, & Chace, 1976); thus leading to the idea of boundary conditions for reconsolidation emergence (Nader, 2003). The mismatch hypothesis predicts that, even when retrieval is not sufficient for destabilization of the memory, it is necessary: prediction of the US occurrence is necessary for the frustration of expectations. A few studies have shown that in the absence of expression memory can enter a malleable state only when predictions are not accomplished (Coccoz et al., 2011; Díaz-Mataix et al., 2013; Forcato et al., 2011; Frenkel et al., 2005a; Frenkel et al., 2005b; Morris et al., 2006; Pedreira et al., 2004; Sevenster et al., 2012). However, a recent study by Milton et al. (2013) has proposed that memory retrieval can occur in parallel with memory destabilization and restabilization. In this study, using a similar approach to that of Ben Mamou et al. (2006) and the present study, Milton et al. proposed that destabilization, restabilization and retrieval of memory could be dissociated, and then the retrieval of the consolidated memory should not be necessary for its reconsolidation. This conclusion seems to contradict studies supporting the mismatch hypothesis but, as they noticed, "behavioral procedures used to induce memory destabilization often induce memory retrieval as well". A key feature of the paradigm used in the present study is that expression can be blocked for a memory that does not undergo reconsolidation (this memory is not labilized after reactivation). We show that even when memory expression and reconsolidation can be dissociated, prediction on the occurrence of the US and evaluation of whether this prediction was accomplished, or not, seems to be a prerequisite for labilization when memory is not expressed. In their study, Milton et al. used the behavioral expression of memory as a measure of memory reactivation; however, expression is just one of the possible fates of a reactivated memory (Fig. 5).

Present results confirm that labilization and expression mechanisms are independent processes in invertebrates too. Retrieval and memory expression therefore appear not to be interchangeable concepts.

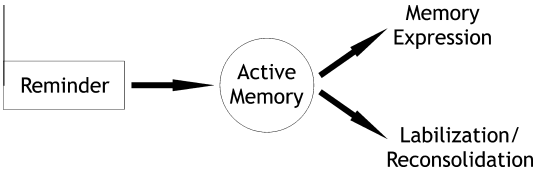

Fig. 5. *Memory Retrieval.* The perception of physical stimuli similar to those perceived during training ("Reminder") triggers a particular brain state depending on how memory was consolidated and on how it is retrieved ("Active Trace"). From this brain state, different (and independent) situations can arise: on the one hand, this active trace can lead to changes in behavior ("Memory Expression"); on the other hand, only when new information is available, the active trace can become labile and undergo a restabilization process ("Labilization/Reconsolidation"). In the *Chasmagnathus'* Context-Signal Memory model, and with the doses used here, APV has shown to be effective in blocking both memory expression and memory reconsolidation, while it did not impair labilization; CNQX blocked reconsolidation, but it did not block labilization or the behavioral expression of memory.

Acknowledgments

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