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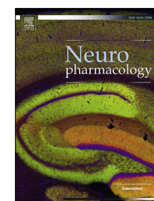

Stress-induced resistance to the fear memory labilization/reconsolidation process. Involvement of the basolateral amygdala complex

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Consolidated memories can enter into a labile state after reactivation followed by a restabilization process defined as reconsolidation. This process can be interfered with Midazolam (MDZ), a positive allosteric modulator of the GABA-A receptor. The present study has evaluated the influence of prior stress on MDZ's interfering effect. We also assessed the influence of both systemic and intra-basolateral amygdala (BLA) infusion of D-cycloserine (DCS), a partial agonist of the NMDA receptors, on the MDZ effect in previously stressed rats. Furthermore, we analyzed the effect of stress on the expression of Zif-268 and the GluN2B sites, two molecular markers of the labilization/reconsolidation process, following reactivation. The results revealed that prior stress resulted into a memory trace that was insensitive to the MDZ impairing effect. Both systemic and intra-BLA DCS administration previous to reactivation restored MDZ's disruptive effect on memory reconsolidation in stressed animals. Further, reactivation enhanced Zif-268 expression in the BLA in control unstressed rats, whereas no elevation was observed in stressed animals. In agreement with the behavioral findings, DCS restored the increased level of Zif-268 expression in the BLA in stressed animals. Moreover, memory reactivation in unstressed animals elevated GluN2B expression in the BLA, thus suggesting that this effect is involved in memory destabilization, whereas stressed animals did not reveal any changes. These findings are consistent with resistance to the MDZ effect in these rats, indicating that stress exposure prevents the onset of destabilization following reactivation.

In summary, prior stress limited both the occurrence of the reactivation-induced destabilization and restabilization.

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1. Introduction

Accumulating evidence has suggested that consolidated memory can become temporarily labile upon retrieval (Alberini and Ledoux, 2013; Nader and Einarsson, 2010; Nader and Hardt, 2009). In order for the memory to persist, a restabilization process termed reconsolidation is required, which is dependent on a new protein synthesis (Duvarci and Nader, 2004; Milekic and Alberini, 2002; Nader et al., 2000; Pedreira et al., 2002). Reactivation-induced destabilization renders memories sensitive to a pharmacological intervention, including benzodiazepines (BDZ), within a limited time window (Bustos et al., 2006). In addition, this transient plasticity enables dynamic modifications of the consolidated trace. For instance, under certain experimental conditions, novel information can be updated into the original trace and its strength can be significantly modified (Lee, 2008, 2010).

Reconsolidation is not, however, a ubiquitous process, with there being certain conditions under which reconsolidation either does not appear to occur or is highly limited. These boundary conditions place constraints on the emergence of both retrieval-induced lability and the restabilization process. For example, older (Bustos et al., 2009; Inda et al., 2011) and stronger (Suzuki et al., 2004; Wang et al., 2009) memories, as well as those reactivated for a short period (Alberini, 2005; Bustos et al., 2009) are less susceptible to engage the labilization/reconsolidation process (Tronson and Taylor, 2007).

Recent findings from our laboratory suggest that the emotional

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state generated by a stressful experience at the moment of fear memory encoding can restrict the destabilization and the engagement of reconsolidation following a later retrieval session of a 7-day fear memory (Bustos et al., 2010). Prior to reactivation, activating NMDA receptors (NMDAR) by systemic D-cycloserine (DCS), a partial NMDA agonist, promotes the destabilization of resistant memories such as those formed in previously stressed animals (Bustos et al., 2010). However, there are still no comprehensive descriptions of the behavioral conditions or the underlying mechanisms involved in stress-induced resistant fear memory.

For the memory trace to be reconsolidated, it must first enter into destabilization following reactivation (Lee, 2008). This requires GluN2B-NMDAR activation within the amygdala basolateral complex (BLA). Hence, blockage of this particular NMDA subtype receptor in the BLA limits memory destabilization and prevents reconsolidation (Ben Mamou et al., 2006; Milton et al., 2013), with the latter involving the molecular mechanisms necessary to stabilize the fragile trace. In fact, fear memory reconsolidation requires specific molecular processes such as Zif-268 activation in the BLA and in the dorsal hippocampus (Besnard et al., 2013; Diaz-Mataix et al., 2013; Hall et al., 2001; Lee et al., 2004; Lee and Hynds, 2013; Veyrac et al., 2014). Thus, disrupting Zif-268 expression following memory reactivation prevents the return to a stable state, and consequently, results in a retention deficit (Lee et al., 2004; Maddox et al., 2011). Based on the above findings, we hypothesize that prior stress should limit the activation of the underlying mechanism of the labilization/reconsolidation process in the BLA following fear memory reactivation. Hence, in the present study we evaluated the influence of prior stress exposure on both the expression of GluN2B sites and Zif-268 in the BLA induced by fear memory recall, with the aim of providing new insights into de neural mechanisms associated with stress-induced resistant fear memory.

Several authors have proposed reconsolidation as a therapeutic target for maladaptive traumatic memories associated with anxiety disorders (Finnie and Nader, 2012; Schwabe et al., 2014). Consequently, it seems relevant to evaluate pharmacological agents able to facilitate the labilization/reconsolidation process in fear memory traces resistant to disruption upon reactivation. Therefore, an additional objective of this study was to assess the influence of both systemic and intra-basolateral amygdala (BLA) infusion of DCS on memory reconsolidation in resistant memories of previously stressed rats.

2. Methods

2.1. Animals

Male Wistar rats (280–320 g) from our breeding stock were housed in groups of 3–4 per cage with food and water ad libitum. All animals were maintained in a 12 h light/dark cycle (with light from 7:00 a.m.) at 21–22 °C, following the protocols approved by the Animal Care Committee of the Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, which is consistent with the NIH Guide for the Care and Use of Laboratory animals. The number of animals used, as well as their suffering, was minimized at the minimum possible. All experiments were conducted between 9:00 and 15:00.

2.2. Stress

Animals were stressed by immobilization in plastic restrainers under intense light for 30 min (S group), after which, the rats were returned to the colony room. This procedure was selected on the basis of previous studies at our laboratory (Bustos et al., 2010; Maldonado et al., 2014). Control animals (NS group) were transferred in their own home cages to a separate experimental room, handled for 2 min, and then returned to the colony room.

2.3. Drugs and administration

Midazolam (MDZ, Gobbi Novag S.A., Argentina) and D-cycloserine (DCS, Sigma-Aldrich, USA) were dissolved in sterile saline (SAL, 0.9%, w/v) at concentrations of 3 mg/ml and 15 mg/ml, respectively, for i.p. injection (Bustos et al., 2010). The total volume of drug used was 1.0 ml/kg, or an equivalent amount of SAL, in all cases. For intra-BLA infusion, DCS was dissolved in SAL to a final concentration of 40 μg/μl and the amount infused was 10 μg/side (Lee et al., 2009; Portero-Tresserra et al., 2013).

2.4. Contextual fear conditioning

- **Apparatus:** The conditioning chamber was constructed of gray acrylic (20 × 23 × 20 cm) with a transparent lid and was connected to a scrambled shocker (Ugo Basile Biological Research Apparatus, Italy). The grid floor consisted of 10 parallel stainless steel grid bars, each measuring 1.5 mm in diameter and spaced 1.5 cm apart (center to center). The conditioning room was illuminated by a white fluorescent tube located on the ceiling, with a ventilation fan used to provide background noise.
- **Fear Conditioning:** Rats were individually placed in the conditioning chamber after 3 min of acclimatization (pre-shock period) and 3 unsignaled scrambled footshocks (0.5 mA, 3 s duration and 30 s intershock interval) were given, with animals being kept in the chamber for an additional 50 s (post-shock period). Cannulated rats were trained with 3 footshocks of 0.65 mA in order to induce levels of conditioning similar to those exhibited by non-cannulated rats (Ortiz et al., 2015), as chronic cannulation of the BLA tends to attenuate the expression of conditioned freezing (Fendt, 2001).
- **Reactivation session:** One day after training, rats were re-exposed to the training context for 5 min without shock delivery.
- **Test sessions:** One (Test 1) and eight (Test 2) days after the reactivation session, animals were reintroduced into the training context for 10 min without shock delivery.

The freezing responses of each rat were scored during the pre-shock and post-shock periods, as well as during the reactivation and testing sessions. The total time spent freezing in each period was quantified using a stopwatch and expressed as a percentage of total time. Freezing, a commonly used index of fear in rats, was defined as the total absence of body and head movement, except for that associated with breathing (Blanchard and Blanchard, 1969).

2.5. Surgery and Intra-BLA infusion

Intra-BLA cannulae implantation, local infusion, and histological procedures were previously described by Giachero et al. (2013). The coordinates used relative to bregma were: anterior −3 mm; lateral ±5.0 mm; ventral −6.1 mm (Paxinos and Watson, 2009). Only animals with bilateral adequate injection sites were considered for statistical analysis.

A recovery period of 7 days was allowed before starting the experiments. For intra-BLA drug administration, each rat was bilaterally infused with DCS or SAL (0.25 μl/side) at a flow rate of 0.25 μl/min, with the infusion cannulae being kept in place for an additional period of 60 s in order to allow drug diffusion.

2.6. Tissue preparation and western blot

Animals were sacrificed by decapitation at the time specified in each experiment (see Experimental Design). The bilateral BLA was dissected from coronal brain slices of 2 mm using an acrylic brain matrix (Stoelting CO.) on ice, according to the BLA boundaries defined by Paxinos and Watson (2009), with BLA enriched tissue being collected using a 2 mm micro punch. These samples were homogenized by sonication in cold lysis buffer (25 mM HEPES; 0.5 M NaCl; 2 mM EDTA; 1 mM DTT; 0.1% NP40) with protease and phosphatase inhibitors (1 mM orthovanadate; 1 mM PMSF; 10 μg/ml leupeptin; 10 μg/ml aprotinin; 1 μg/ml pepstatin) before being centrifuged (20000g, 2 min). The supernatants were collected and frozen at −70 °C, and the protein yield was quantified using the Bradford assay (Biorad). On the day of the western blot experiment, the samples were combined with 1:4 sample buffer 4X (50% Glycerol; 4% SDS; 125 mM Tris; 400 mM DTT; 0.02 bromophenol blue) and boiled at 70 °C for 10 min. Then, the samples were electrophoresed and transferred onto PVDF membranes as described by Maldonado et al. (2014).

To study the Zif-268 expression, proteins (40 μg) were separated by SDS-PAGE (10%). The resulting blots were incubated with primary antibody (1:500, Santa Cruz Biotechnology), followed by incubation with horseradish peroxidase–conjugated antibody to goat IgG, with Actin (Sigma) used as a loading control and the obtained film samples were scanned.

For GluN2B expression, 15 μg of proteins were resolved using 7.5% SDS-PAGE. Then, the resulting blots were incubated with a rabbit primary antibody (1:750, Cell Signaling Technology), followed by incubation with IRDye® 800 WC Donkey Anti Rabbit IgG, with Tubulin (Sigma) used as a loading control. The bands were visualized by scanning in an LI-COR Odyssey imager, and all images were analyzed using the Gelpro31 program.

2.7. Experimental design

Experiment 1: Animals were randomly subjected to a stress session (S group) or to just being handled (NS condition). One day later, all these animals were fear conditioned. Then, after 24 h, animals received either SAL or DCS 30 min before the reactivation session and were subsequently injected with SAL or MDZ immediately after being reactivated. The fear memory retention tests were performed one (Test 1) and eight (Test 2) days later.

Experiment 2: BLA cannulated animals were fear conditioned 24 h after a stress (S) or a handling session (NS). One day later, rats received a bilateral infusion of DCS or SAL 15 min before memory reactivation, and immediately after reactivation, animals were systemically administered with either SAL or MDZ. Then, fear memory retention tests were performed as previously described 1 or 8 days later.

Experiment 3: NS and S rats were subjected to conditioning, before being reactivated one day later (R groups) and sacrificed after a further 90 min, with the BLA being dissected for Zif-268 assessment. Non-reactivated groups (NR) were left in their home cages until being sacrificed.

Experiment 4: NS and S rats were fear conditioned and systemically administered one day later with either SAL or DCS 30 min prior to reactivation. To evaluate Zif-268 expression in the BLA, rats were sacrificed 90 min after this reactivation.

Experiment 5: NS and S rats were fear conditioned, before being reactivated (R groups) one day later and after a further 60 min were sacrificed for the assessment of GluN2B expression in the BLA. Non-reactivated groups (NR) were left in their home cages until being sacrificed.

2.8. Statistical analysis

Results are expressed as the means ± S.E.M. Data were analyzed by Student's *t*-test or ANOVAs followed by Newman-Keuls post hoc test. The significance level used for all statistical analyses was $p < 0.05$. Depending on the experiment, the factors analyzed were: Condition (S vs NS), Pre-treatment (SAL vs DCS), Reactivation (R vs NR), and Treatment (SAL vs MDZ).

3. Results

3.1. Experiment 1: systemic DCS prior to reactivation promoted the destabilization of resistant fear memories in stressed animals

In this experiment, we evaluated the influence of pre-reactivation DCS administration on MDZ's disruptive effects on 1-day fear memory in stressed rats. Fig. 1B shows that all groups exhibited similar levels of freezing during the reactivation session [$F(1, 58) = 0.002$; $p > 0.05$]. However, a decrease in freezing behavior was observed in NS groups administered with MDZ in Test 1, regardless of the pretreatment drug. In the stressed group, only DCS/MDZ-administered animals exhibited reduced levels of freezing, which were similar to those shown by NS groups treated with MDZ, with Test 2 showing similar effects to Test 1.

The ANOVA revealed a significant Condition × Pretreatment × Treatment interaction for Test 1 [$F(1, 54) = 14.08$; $p < 0.01$] and Test 2 [$F(1, 54) = 15.13$; $p < 0.01$], with post hoc comparisons indicating that the freezing levels exhibited by NS/SAL/MDZ, NS/DCS/MDZ and S/DCS/MDZ did not differ from each other, but were significantly lower than the remaining groups in both tests (Fig. 1C).

Two main results emerge from these experiments: 1) Previous stress prevented MDZ's disruptive effect on fear memory reconsolidation; 2) Systemic DCS pretreatment restored the interfering effect of MDZ on reconsolidation in stressed animals.

3.2. Experiment 2: BLA infusion of DCS promoted the destabilization of resistant fear memory in stressed animals

The aim of this experiment was to evaluate if activation of NMDARs within the BLA by DCS facilitates the onset of reactivation-induced destabilization in resistant fear memory, as shown by stressed rats. Fig. 2 shows that all groups exhibited similar levels of freezing during the reactivation session, ruling out any possible effect of stress or DCS local infusion on memory expression [$F(1, 74) = 0.59$, $p > 0.05$] (Fig. 2C). As observed in experiment 1, all NS animals treated with MDZ showed less freezing in Test 1 and Test 2 than the SAL-treated groups, whereas S animals administered with DCS/MDZ exhibited a decrease in freezing expression in both tests.

The ANOVA revealed a significant Condition × Pretreatment × Treatment interaction for Test 1 [$F(1, 70) = 8.48$, $p < 0.01$] and Test 2 [$F(1,70) = 7.88$, $p < 0.01$] with the post hoc test showing that NS/SAL/MDZ, NS/DCS/MDZ and S/DCS/MDZ did not differ from each other and were significantly lower compared to the remaining groups (Fig. 2D). In order to demonstrate that the effects of the treatments were dependent on the reactivation, groups of cannulated animals (NS and S) were fear conditioned. One day later, animals were infused either with DCS or SAL and then received MDZ after 20 min, without being reactivated (non-reactivated groups). One day later, all the animals were tested, with the results showing that all non-reactivated groups displayed comparable levels of freezing during testing (Fig. S1).

These findings revealed that intra-BLA DCS infusion restored the susceptibility of fear memory reconsolidation to MDZ disruptive effects.

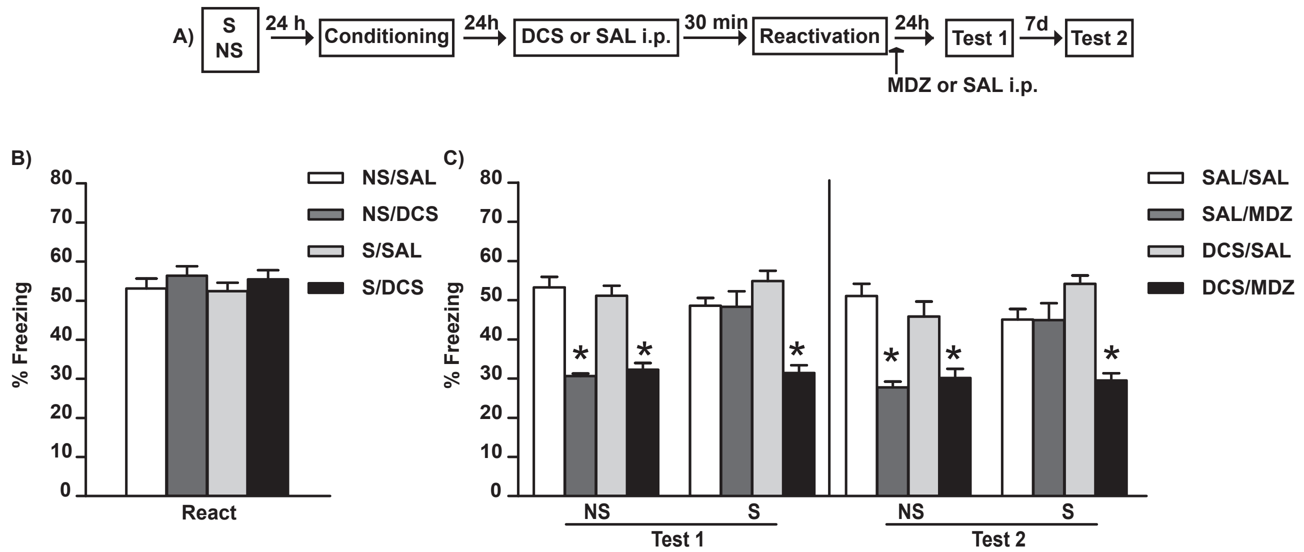

Fig. 1. Systemic DCS prior to reactivation promotes the destabilization of resistant fear memories in stressed animals. A) Schematic representation of the experimental design. B) No differences between groups were observed in freezing during the reactivation session. NS/SAL (n = 15), NS/DCS (n = 14), S/SAL (n = 17), S/DCS (n = 16). C) Systemic administration of DCS before reactivation facilitated the disruptive effect of systemic MDZ on memory reconsolidation in stressed animals. NS/SAL/SAL (n = 7), NS/SAL/MDZ (n = 8), NS/DCS/SAL (n = 7), NS/DCS/MDZ (n = 7), S/SAL/SAL (n = 8), S/SAL/MDZ (n = 9), S/DCS/SAL (n = 7), S/DCS/MDZ (n = 9). Data are expressed as the mean ± SEM of the freezing percentage. (*) significantly different compared with the remaining groups ($p < 0.01$).

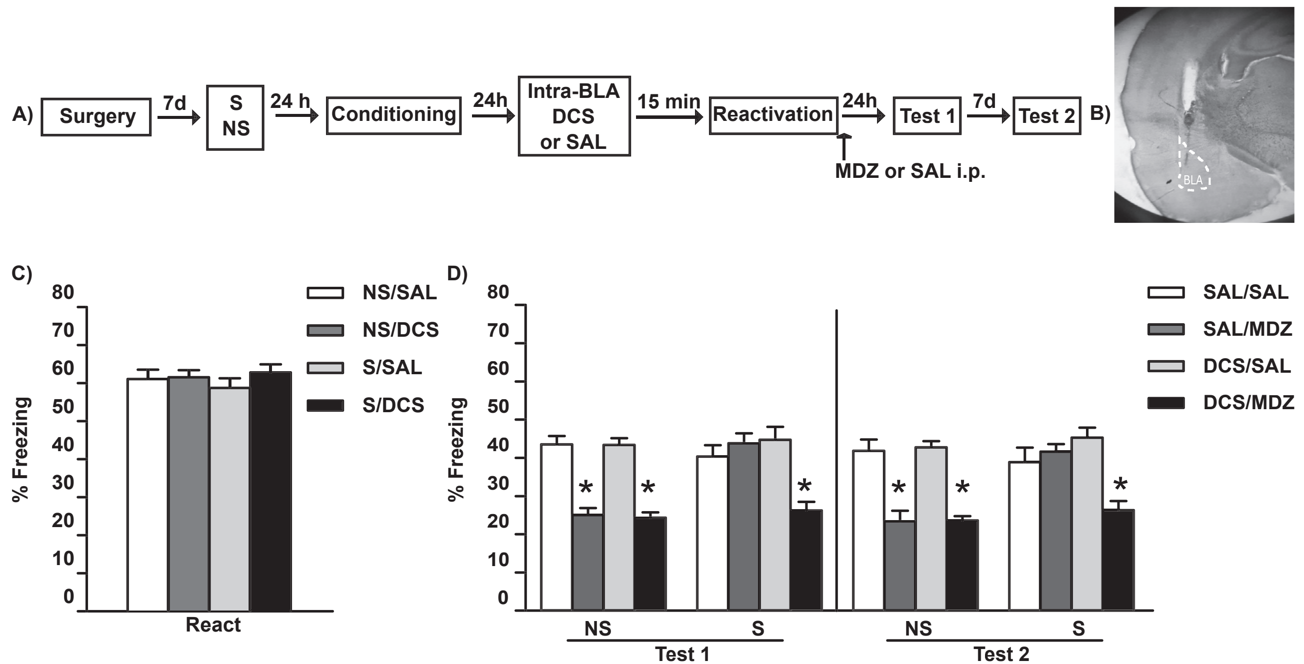

Fig. 2. BLA infusion of DCS promotes the destabilization of resistant fear memory in stressed animals. A) Schematic representation of the experimental design. B) Representative image of the infusion site in the BLA. C) No differences between groups were observed in freezing during the reactivation session. NS/SAL (n = 22), NS/DCS (n = 19), S/SAL (n = 19), S/DCS (n = 23). D) Intra-BLA administration of DCS before reactivation facilitated the disruptive effect of systemic MDZ on memory reconsolidation in stressed animals. NS/SAL/SAL (n = 11), NS/SAL/MDZ (n = 11), NS/DCS/SAL (n = 10), NS/DCS/MDZ (n = 9), S/SAL/SAL (n = 9), S/SAL/MDZ (n = 10), S/DCS/SAL (n = 12), S/DCS/MDZ (n = 11). Data are expressed as the mean ± SEM of the freezing percentage. (*) significantly different compared with the remaining groups ($p < 0.01$).

3.3. Experiment 3: previous stress exposure prevented the retrieval-induced increase of Zif-268 expression in the BLA

The goal of this experiment was to evaluate the Zif-268 expression in the BLA after memory reactivation in NS and S animals. As can be observed in Fig. 3B, both the NS and S reactivated groups displayed similar fear levels during reactivation [$t = -0.26$; $p > 0.05$]. However, an increase in Zif-268 expression was only observed in the NS/R group. The ANOVA revealed a significant Condition × Reactivation interaction [$F(1, 26) = 10.72$, $p < 0.01$], with the post hoc test showing that Zif-268 expression in NS/R group was significantly higher compared with the remaining groups (Fig. 3C). These data suggest that previous stress exposure prevented the reactivation-induced increase of Zif-268 expression in the BLA.

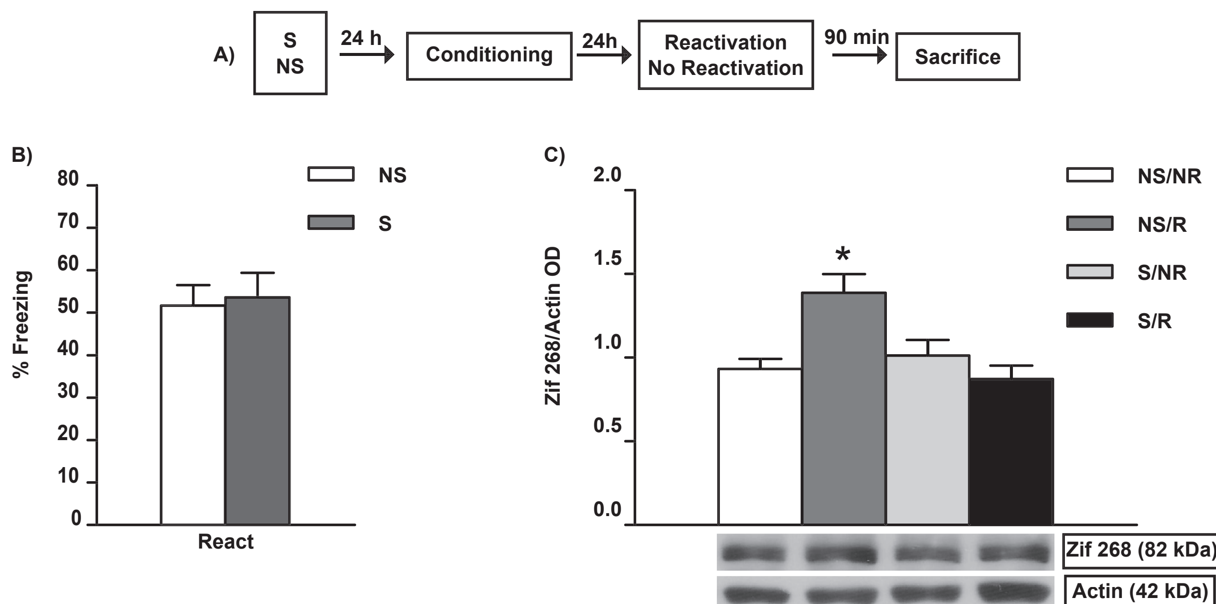

Fig. 3. Previous stress exposure prevents the retrieval-induced increase of zif-268 expression in the BLA. A) Schematic representation of the experimental design. B) Both reactivated groups exhibited similar levels of the freezing during reactivation session. NS/R (n = 8), S/R (n = 7). C) The increase in Zif-268 expression was prevented in stressed animals subjected to the reactivation procedure. NS/NR (n = 7), NS/R (n = 8), S/NR (n = 8), S/R (n = 7). Data are expressed as the mean ± SEM of the freezing percentage or the relative optical density of Zif-268/Actin. (*) significantly different compared with the remaining groups ($p < 0.01$).

3.4. Experiment 4: DCS administration prior to reactivation facilitated the increase of Zif-268 expression in the BLA following reactivation in stressed animals

In this experiment, we evaluated the influence of systemic DCS administered prior to reactivation on Zif-268 expression in the BLA of the NS and S groups. As illustrated in Fig. 4B, all groups revealed similar freezing levels during the reactivation session [$F(1, 19) = 3.83$, $p > 0.05$]. The western blot analysis revealed a decreased Zif-268 expression in the S/SAL group, with ANOVA showing a significant Condition × Treatment interaction [$F(1, 19) = 5.53$, $p < 0.05$]. In addition, the significantly lower Zif-268 level in the S/SAL group than the remaining groups was confirmed by the post hoc test (Fig. 4C).

These findings indicate that DCS facilitated the reactivation-induced increase of Zif-268 expression in the BLA of stressed rats.

3.5. Experiment 5: prior stress exposure prevented reactivation-induced enhancement in GluN2B subunit expression in the BLA

The aim of this experiment was to examine the expression of the GluN2B subunit in the BLA after reactivation in NS and S animals. As can be seen in Fig. 5B, both the NS and S reactivated groups displayed similar fear responses [$t = 1.05$; $p > 0.05$], but an increase in GluN2B expression was observed in NS/R animals. The ANOVA revealed a significant Condition × Reactivation interaction [$F(1, 27) = 6.74$, $p < 0.05$], with the Post hoc test confirming that GluN2B expression in the NS/R group was significantly higher than in the remaining groups (Fig. 5C). In summary, these experimental findings showed that the reactivation trial enhanced GluN2B expression in the BLA in control unstressed rats whereas no elevation was observed in stressed rats.

4. Discussion

It has been previously reported (Bustos et al., 2006, 2009) that MDZ, a fast-acting positive modulator of the GABA-A receptor, disrupts fear memory reconsolidation in control unstressed animals. However, in the present study, prior exposure to a single restraint episode led to a memory trace that became insensitive to the reconsolidation-impairing effect of MDZ when reactivation took place one day after training. Moreover, previous data have shown that stress-induced resistance after reactivation is also noticeable even when using a different protocol (Bustos et al., 2010). Here, prior stress exposure did not modify fear expression during pre or postshock periods (Fig. S2). All the above evidence confirms that stress prior to memory encoding results in the resistance of the memory trace to the MDZ-disruptive effect on fear memory reconsolidation.

It has been suggested that the reconsolidation process is composed of two distinctive and mechanistically different phases, namely, a reactivation-induced destabilization and a subsequent restabilization process (Lee, 2008). Therefore, we wondered if these phases could be restricted in memories formed under stress. Moreover, it was previously shown that stress-induced resistance to MDZ is only detectable when stressed animals are exposed to the conditioned environment (Bustos et al., 2010), thus indicating that exposure to the associated context is a requirement for resistance to take place.

It is also known that DCS, when acting at the strychnine-insensitive glycine-recognition site of the NMDA receptor complex, enhances NMDA receptor mediated glutamatergic transmission (Rouaud and Billard, 2003). Related to this, activation of NMDA sites before reactivation is a necessary requirement for the onset of the labilization/reconsolidation process after retrieval (Tronson and Taylor, 2007). Hence, the stimulation of these receptors, for instance by DCS, should restore the vulnerability to MDZ's disruptive action after retrieval in resistant memories of stressed animals. In agreement, the findings of the present study revealed that both systemic and intra-BLA DCS prior to reactivation restored the interfering effect of MDZ on memory reconsolidation in stressed rats. Furthermore, consistent with prior findings (Bustos et al., 2010; Ortiz et al., 2015), DCS administration did not affect freezing during reactivation in unstressed or stressed animals, thus

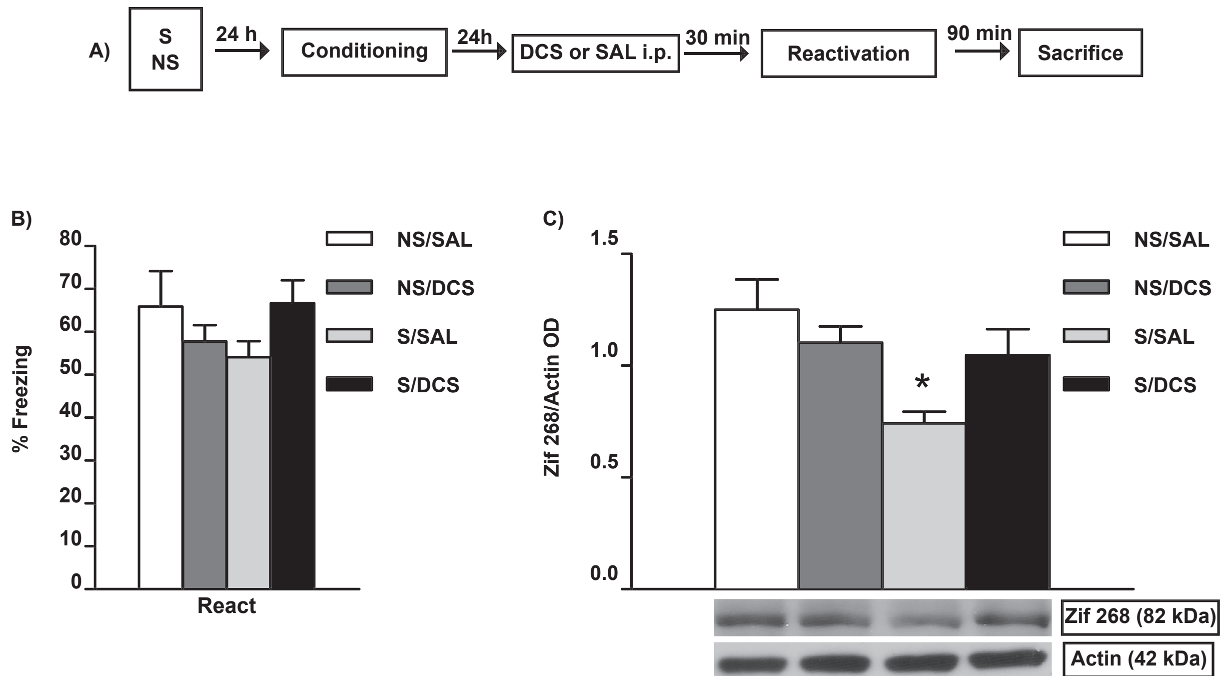

Fig. 4. DCS administration prior to reactivation facilitates the increase of zif-268 expression in the BLA following reactivation in stressed animals. A) Schematic representation of the experimental design. B) No differences between groups were observed in freezing during the reactivation session. NS/SAL (n = 5), NS/DCS (n = 6), S/SAL (n = 6), S/DCS (n = 6). C) Systemic administration of DCS before reactivation restored the increase in Zif-268 expression in stressed animals subjected to reactivation. NS/SAL (n = 5), NS/DCS (n = 6), S/SAL (n = 6), S/DCS (n = 6). Data are expressed as the mean ± SEM of the freezing percentage or the relative optical density of Zif-268/Actin. (*) significantly different compared with the remaining groups ($p < 0.05$).

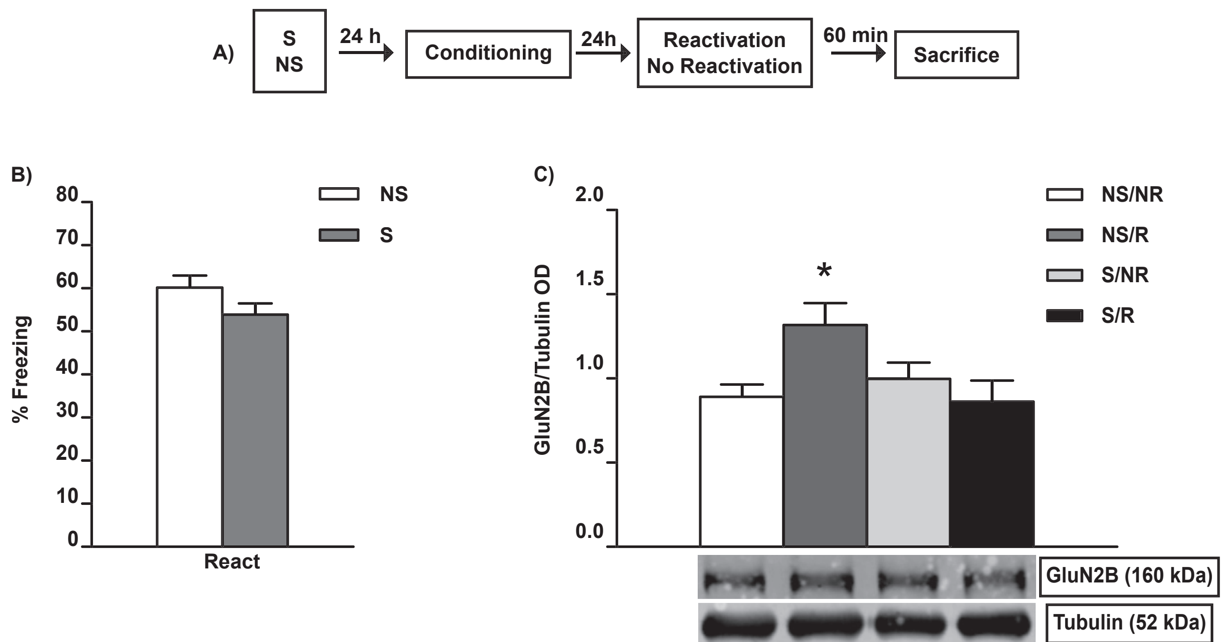

Fig. 5. Prior stress exposure prevents reactivation-induced enhancement in GluN2B subunit expression in the BLA. A) Schematic representation of the experimental design. B) Similar levels of freezing were observed in both groups during the reactivation session. NS/R (n = 8), S/R (n = 7). C) The enhancement in GluN2B expression was prevented in stressed animals subjected to the reactivation procedure. NS/NR (n = 8), NS/R (n = 8), S/NR (n = 8), S/R (n = 7). Data are expressed as the mean ± SEM of the freezing percentage or the relative optical density of GluN2B/Tubulin. (*) significantly different compared with the remaining groups ($p < 0.05$).

implying that this drug had no influence on the retrieval process or the expression of freezing behavior.

The effect of DCS intra-BLA suggested a crucial role of NMDA sites within the BLA in the emergence of the labilization/reconsolidation process of a contextual fear memory. Moreover, previous investigations have reported a similar notion for drug-related memories (Milton et al., 2008), thereby supporting the widespread view that BLA is a primary locus in mediating memory formation and reconsolidation (Baldi and Bucherelli, 2015; LeDoux, 2007; Milton et al., 2008; Pape and Pare, 2010).

With regard to the transcription factor Zif-268, its strong involvement in memory reconsolidation has been widely reported (Veyrac et al., 2014). Related to this, and consistent with other studies (Hall et al., 2001; Maddox et al., 2011), our findings showed that the retrieval of a contextual fear memory under conditions that led to reconsolidation resulted in a clear up-regulation of Zif-268 expression in BLA. However, there was no increase observed in stressed animals subjected to the retrieval trial, which may indicate prior stress exposure impeded the reconsolidation process following reactivation. In agreement with our behavioral findings, DCS restored the elevation of Zif-268 expression within the BLA in stressed animals, which suggests that reconsolidation took place due to DCS pretreatment in stressed rats.

The expression of Zif-268 has also been functionally associated with NMDA sites in the amygdala (Lee et al., 2009; Milton et al., 2008). Given that reconsolidation and reactivation-induced Zif-268 elevation are both dependent on NMDA activation, it seems likely that the DCS restoring influence on the impairing effect of stress on reconsolidation involves the stimulation of NMDA sites from the BLA. Additionally, it has been proposed that Zif-268 within the amygdala participates in the DCS-induced facilitation of other cognitive processes, such as extinction memory (Wu et al., 2015). The fact that DCS restores both the ability of MDZ to interfere with fear reconsolidation and also the usual reactivation-induced elevation of Zif-268 expression in stressed rats suggests that at reactivation, prior stress affects NMDA signaling mechanisms within the BLA.

It is important to emphasize that NMDA receptors containing GluN2B subunits in BLA are necessary for memory destabilization. In fact, this particular NMDA subtype is critically involved in the protein degradation by the ubiquitin/proteasome required for memory destabilization after reactivation (Jarome and Helmstetter, 2013; Jarome et al., 2011; Lee et al., 2008). Moreover, inhibitors of proteasome activity block reactivation-induced destabilization and the enhancing effects of DCS on NMDA receptor-mediated synaptic transmission (Mao et al., 2008), suggesting that DCS effects are partly associated with the activity of the ubiquitin/proteasome system.

Consistent with the fact that GluN2B subunits are required for memory destabilization, intra-BLA administration of a selective antagonist of this NMDA subtype prevents the instability induced by fear memory reactivation (Ben Mamou et al., 2006; Milton et al., 2013). Furthermore, it has been suggested that the GluN2B mechanism can be down-regulated under conditions that limit the emergence of the reconsolidation process (Wang et al., 2009). Therefore, it seems likely that the activation of these sites would facilitate reactivation-induced destabilization. Our current findings show that a brief reactivation elevated GluN2B expression in the BLA thus suggesting that this mechanism was implicated in promoting memory destabilization in unstressed rats, whereas, this modification was not detected in stressed animals. In this latter case, this could indicate the non-occurrence of the reactivation-dependent destabilization process in stressed rats. In agreement, fear behavior remained unchanged in the stressed rats that had been administered with MDZ following reactivation, while it was reduced in control unstressed rats under the same drug.

5. Conclusions

The present findings confirm that prior stress exposure affects the occurrence of fear memory reconsolidation, with this process consisting of a reactivation-dependent destabilization and a subsequent restabilization phase (Lee, 2008). Stress limits destabilization because it attenuates the MDZ disruptive effect and prevents elevation of GluN2B expression in the BLA. Moreover, this environmental challenge restricts the elevation of Zif-268 in the BLA, a transcription factor crucially involved in the restabilization phase. Hence, highly arousing experiences are determinant for the subsequent emergence of reconsolidation of the memory trace following reactivation. Finally, if the labilization/reconsolidation process is an expression of the dynamic nature of memory, as has been proposed (Nader, 2015), a previous history of stress would limit the flexibility of the memory trace following retrieval.

An understanding of how stress affects the labilization/reconsolidation process is crucial, since targeting reconsolidation of traumatic memories has been suggested as a potential treatment for post-traumatic stress disorder (Besnard et al., 2012; Parsons and Ressler, 2013; Taylor and Torregrossa, 2015).

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Conflicts of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.neuropharm.2016.06.033.

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