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Polyaminergic agents modulate the reconsolidation of conditioned fear

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ABSTRACT

When consolidated memories are reactivated, they become labile and, to persist, must undergo a new stabilization process called reconsolidation. During reactivation, memory is susceptible to pharmacological interventions that may improve or impair it. Spermidine (SPD) is an endogenous polyamine that physiologically modulates the N-methyl-D-aspartate (NMDA) receptor in mammals by binding on the polyamine-binding site at the NMDA receptor. While polyamine agonists and antagonists of the polyamine binding site on the NMDA receptor respectively improve and impair early consolidation, it has not been defined whether these agents alter memory reconsolidation. Male Wistar rats were trained in a fear conditioning apparatus using a 0.4 mA footshock as unconditioned stimulus. Twenty four hours after training, animals were re-exposed to the apparatus in the absence of shock (reactivation session). Immediately after the reactivation session, SPD (1–30 mg/kg, i.p.) or the antagonist of the polyamine-binding site at the NMDA receptor, arcaine (0.1–10 mg/kg, i.p.), were injected, and the animals were tested in the same apparatus 24 h later. Freezing scores at testing were considered a measure of memory. While SPD (3 and 10 mg/kg) improved, arcaine (1 and 10 mg/kg) impaired memory reconsolidation. These drugs had no effect on memory if they were administered in the absence of reactivation, or 6 h after reactivation session. Arcaine (0.1 mg/kg, i.p.) prevented SPD (3 mg/kg)-induced improvement of memory reconsolidation. Accordingly, SPD (1 mg/kg) prevented arcaine (10 mg/kg)-induced impairment of memory reconsolidation. The amnesic effect of arcaine was not reversed by arcaine administration prior to test, ruling out state dependence in this effect. These results suggest that systemic administration of polyamine binding site ligands modulate memory reconsolidation.

1. Introduction

Memories are not formed instantaneously. After learning (acquisition), a series of molecular and cellular changes occur in different brain regions, such as hippocampus, striatum and amygdala, that lead to a progressive memory stabilization (McGaugh, 2000). This process, which initiates immediately after learning and is time-dependent, has been named "consolidation" (Dudai, 2004; McGaugh, 1966, 2000). During consolidation, memories are labile and susceptible to positive or negative modulation by different means, including pharmacological agents (McGaugh, 1966, 2000). For decades, prevailed the general belief that consolidated memories could not be modified (McGaugh, 2000). Several studies, however, have shown that reactivating or retrieving consolidated memories renders them labile again, thus requiring a new stabilization process, called reconsolidation (Dudai, 2004; Nader, Schafe, & Le Doux, 2000; Sara, 2000).

Different neurotransmitter systems have been implicated in memory reconsolidation (Nader & Hardt, 2009; Sara, 2000; Tronson & Taylor, 2007). Notwithstanding, convincing pharmacological and neurochemical evidence supports that glutamate NMDA receptors, particularly those containing a NR2B subunit, play a major role in this process (Wang, de Oliveira Alvares, & Nader, 2009). Accordingly, while NMDA receptor antagonists MK-801, AP5 and ifenprodil disrupt, the NMDA receptor agonist D-cycloserine improves fear memory reconsolidation in mice and rats (Ben Mamou, Gamache, & Nader, 2006; Lee, Milton, & Everitt, 2006; Przybyslawski & Sara, 1997; Suzuki et al., 2004). In this regard, it is worth noticing that spermidine (SPD) and spermine, endogenous polyamines that bind to and modulate NR2B subunit activity, have been implicated in memory acquisition and consolidation (Johnson, 1996; Shimada, Spangler, London, & Ingram, 1994; Williams, 1997; Williams, Romano, Dichter, & Molinoff, 1991).

Current evidence suggests that polyamines modulate consolidation by interacting with the polyamine-binding site on the NMDA receptor (Kishi, Ohno, & Watanabe, 1998; Rubin et al., 2004, 2000, 2001; Shimada et al., 1994). In line with this view, the systemic (Camera, Mello, Ceretta, & Rubin, 2007), intrahippocampal (Berlese

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et al., 2005; Gomes et al., 2010; Guerra et al., 2006; Rubin et al., 2000), and intra-amygdalar (Rubin et al., 2004, 2001) administration of SPD improves the memory of different tasks in rats. SPD-induced memory facilitation of the inhibitory avoidance task involves the sequential activation of PKC and PKA/CREB pathways in the hippocampus (Guerra et al., 2011, 2012).

Although the involvement of NR2B-containing NMDA receptors in memory reconsolidation has been shown, no study has addressed whether polyamines, endogenous agonists of these receptors, modulate memory reconsolidation. Therefore, in the current study we investigated whether SPD and arcaine, respectively an agonist and an antagonist of the polyamine binding site on the NMDA receptors, alter fear memory reconsolidation.

2. Materials and methods

2.1. Animals

Experimentally naive male Wistar rats (260–360 g), from the animal house of the Federal University of Santa Maria were used. The animals were housed four to a cage on a 12-h day/night cycle (lights on at 7:00 a.m.) at a temperature of 21 °C with water and standard laboratory chow (Guabi, Santa Maria, Rio Grande do Sul, Brazil) *ad libitum*. All experimental procedures were conducted during the light phase of the cycle (from 11:00 a.m. to 4:00 p.m.). All experimental procedures were conducted in accordance with the policies on the use of animals and humans in neuroscience research, revised and approved by the Society for Neuroscience Research in January 1995 and with the institutional and national regulations for animal research (process 068/2011).

2.2. Drugs

Animals were injected with saline (0.9% NaCl), 1,4-diguanidinobutane sulfate (arcaine; Pfaltz & Bauer, Waterbury, CT, USA), or N-(3- aminopropyl)-1.4-butanediamine trihydrochloride (spermidine; Sigma, St. Louis, MO). All drugs solutions were prepared daily in saline and injections were performed intraperitoneally (i.p.) in a 1 ml/kg injection volume. Doses were selected based on previous studies (Camera et al., 2007) and pilot experiments.

2.3. Conditioning apparatus

Contextual fear conditioning training, reactivation and test took place in a fear conditioning chamber (30 × 25 × 25 cm), located in a well-lit room. The front wall and ceiling of the chamber were made of clear acrylic plastic, whereas the lateral and rear walls were made of opaque plastic. The floor of the chamber consisted of 32 stainless steel rods (3 mm diameter), spaced 1 cm apart and wired to a shock generator. The chamber was cleaned with 30% ethylic alcohol before and after each rat occupied it.

2.4. Behavioral procedure

2.4.1. Contextual fear conditioning

In the conditioning trial each animal was subjected to a single fear-conditioning training session, as described by Rubin et al. (2004), with some modifications. In brief, the rat was placed in the conditioning chamber (conditioned stimulus, CS) and habituated to the apparatus (CS) for 3 min. Immediately after habituation, three 1 s, 0.4 mA footshocks (unconditioned stimulus, US) were delivered. The shocks were 40 s apart. After the last CS/US pairing, rats were allowed to stay in the chamber for additional 60 s before returning to their home cages.

2.4.2. Reactivation session

Twenty four hours after the conditioning session, the rats were re-exposed to the conditioning apparatus for 3 min, but no footshocks were delivered. During this time, the rat was observed every 4 s to assess whether it was in freezing, or not, by a trained observer who was unaware of the experimental treatment conditions. Behavior was judged as freezing if there was an absence of any visible movement, except for that required for breathing. Data were converted to the percentage of samples scored as freezing.

2.4.3. Test session

Twenty-four hours after reactivation, each rat was placed back in the conditioning chamber and a 6-min test was performed. During this time, the rat was observed every 4 s to assess whether it was in freezing, or not, as described above, and data were converted to the percentage of samples scores as freezing.

2.5. Experimental groups

2.5.1. Experiment 1

This experiment was designed to investigate the effect of SPD on memory reconsolidation. Animals were trained in the fear conditioning apparatus, as described above. Twenty-four hours later, the animals were subjected to the reactivation session. Immediately after reactivation session, the animals were injected with saline or SPD (1, 3, 10 or 30 mg/kg) and, 24 h later, tested in the fear conditioning apparatus where their freezing responses were scored, as described above.

2.5.2. Experiment 2

This experiment was designed to investigate the effect of arcaine on memory reconsolidation. Animals were trained in the fear conditioning apparatus, as described above. Twenty-four after training, the animals were subjected to the reactivation session. Immediately after reactivation session, the animals were injected with saline or arcaine (0.1, 1 or 10 mg/kg) and, 24 h later, tested in the fear conditioning apparatus where their freezing responses were scored, as described above.

2.5.3. Experiment 3

To evaluate whether the systemic administration of SPD and arcaine are specific for reconsolidation of contextual fear memories two control experiments were performed. In the first, animals were trained in the fear conditioning apparatus, as described above, but were not subjected to the memory reactivation session 24 h later (“no reactivation” control). The animals were injected with saline, SPD (3 mg/kg) or arcaine (10 mg/kg) 24 h after training and, 24 h later, were tested in the fear conditioning apparatus and had their freezing responses scored, as described above. The doses of spermidine and arcaine used in this experiment were chosen based on the dose-response curve experiments (Experiments 1 and 2), which determined fully effective and non-effective doses for both compounds.

In order to confirm the specificity of enhanced or disrupted reconsolidation by SPD and arcaine, respectively, a second control experiment was performed. The animals were trained in the fear conditioning apparatus and 24 h later they were subjected to the reactivation session, as described above. Six hours after reactivation session (“delayed infusion” control), the animals were injected with saline, SPD (3 mg/kg) or arcaine (10 mg/kg). Twenty-four hours after reactivation, the animals were tested in the fear conditioning apparatus and their freezing responses were scored, as described above.

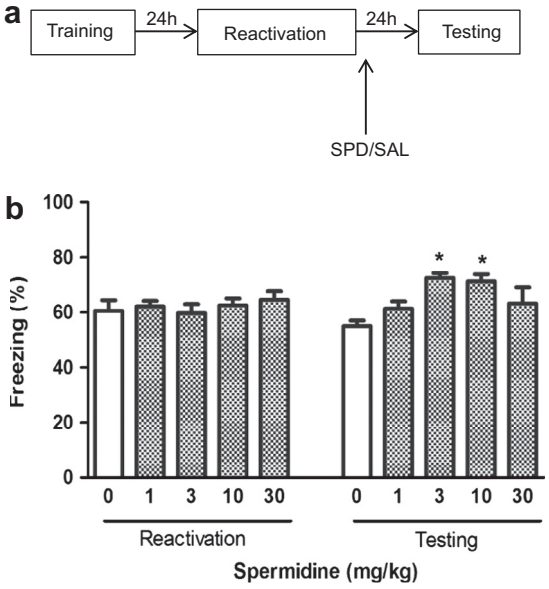

Fig. 1. Postreactivation administration of SPD improves reconsolidation of contextual fear memories. (a) Schematic of the experimental design. Rats received an i.p. injection of SPD (1–30 mg/kg) or saline (0.9% NaCl, 1 ml/kg), immediately after a reactivation session, and were tested for memory reconsolidation 1 day later. The dose of 3 and 30 mg/kg (b) was effective at enhancement memory reconsolidation. $^{*}p < 0.05$ compared with vehicle by the SNK Test. Data are the means + SEM percentage of freezing in reconsolidation testing session ($n = 9$–10 animals in each group).

2.5.4. Experiment 4

In order to investigate the involvement of polyamine-binding sites on the NMDA receptor in the effect of polyamines, we initially administered the polyaminergic agonist, SPD (at a non-effective dose, determined by the dose-effect curve shown in Fig. 1) to prevent the deleterious effect of the polyaminergic antagonist arcaine. The animals were trained in the fear conditioning apparatus and, 24 h later, they were subjected to the reactivation session, as described above. Immediately after reactivation session, the animals were injected with saline or SPD (1 mg/kg) and 15 min later they were injected with saline or arcaine (10 mg/kg) in different flanks. Twenty-four hours after reactivation, the animals were tested in the fear conditioning apparatus and their freezing responses were scored, as described above.

In order to confirm the involvement of polyamine-binding sites on the NMDA receptor in the effect of polyamines, we administered the polyaminergic antagonist arcaine (at a non-effective dose, determined by dose-effect curve shown in Fig. 2) to prevent the facilitatory effect of SPD. The animals were trained in the fear conditioning apparatus and, 24 h later, they were subjected to the reactivation session, as described above. Immediately after reactivation session, the animals were injected with saline or arcaine (0.1 mg/kg), immediately after reactivation, and saline or SPD (3 mg/kg) 15 min later, in different flanks. Twenty-four hours after reactivation, the animals were tested in the fear conditioning apparatus and their freezing responses were scored, as described above.

2.5.5. Experiment 5

This experiment was designed to investigate whether the effect of arcaine on memory reconsolidation involved state dependence. Animals were trained in the fear conditioning apparatus and, 24 h later, they were subjected to the reactivation session, as described above. Immediately after reactivation and 30 min before testing, the animals were injected with saline or arcaine (10 mg/kg). Twenty-four hours after reactivation session, the animals were tested in the fear conditioning apparatus, where their freezing responses were scored, as described above.

2.6. Statistics

The data were converted to the percentage of samples scored as freezing and analyzed by one- or two-way analysis of variance (ANOVA), depending on the experimental design. Post hoc analyses were carried out by the Student–Newman–Keuls test, when indicated. A $p < 0.05$ was considered significant.

3. Results

3.1. Postreactivation SPD improves reconsolidation of contextual fear memories biphasically

Fig. 1 shows the effect of SPD (1–30 mg/kg, i.p.) immediately post-reactivation on the reconsolidation of fear conditioning. As expected, statistical analysis (one-way ANOVA) of reactivation session freezing scores revealed no difference among groups [$F(4,45) = 0.39$, $p > 0.05$], indicating that animals' behavior was similar between groups before drug administration. Statistical analysis (one-way ANOVA) of test freezing scores revealed a significant effect of drug treatment [$F(4,44) = 4.71$, $p < 0.05$]. Post hoc analysis (SNK) revealed that while 3 and 10 mg/kg SPD increased, 1 and 30 mg/kg SPD had no effect on freezing scores at test. These results suggest that SPD facilitates memory reconsolidation in a biphasic manner.

3.2. Postreactivation administration of arcaine impairs reconsolidation of contextual fear memories in a dose-dependent manner

Fig. 2 shows the effect of arcaine (0.1–10 mg/kg, i.p.) immediately post-reactivation on fear conditioning reconsolidation. One-

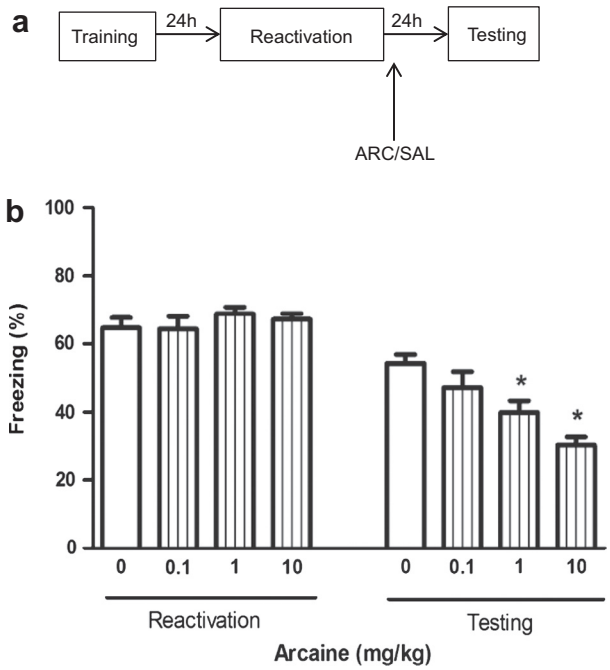

Fig. 2. Postreactivation administration of arcaine impairs the reconsolidation of contextual fear memories. (a) Schematic of the experimental design. Rats received an i.p. injection of arcaine (0.1–10 mg/kg) or saline (0.9% NaCl, 1 ml/kg), immediately after a reactivation session, and were tested for memory reconsolidation 1 day later. $^{*}p < 0.05$ compared with vehicle by the SNK Test. Data are the means + SEM percentage of freezing in reconsolidation testing session ($n = 8$ animals in each group).

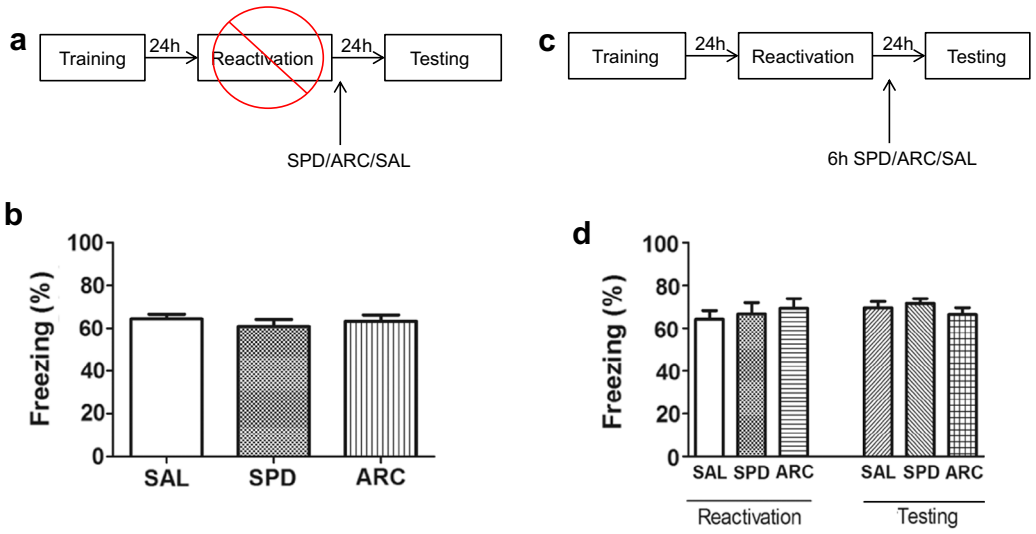

Fig. 3. SPD and arcaine are specific for reconsolidation of contextual fear memories. (a) Schematic of the experimental design in the absence of reactivation session. Rats received an i.p. injection of SPD (3 mg/kg), arcaine (ARC, 10 mg/kg) or saline (0.9% NaCl, 1 ml/kg), 24 h after training in the absence of reactivation session, and were tested for memory reconsolidation 1 day later (b). (c) Schematic of the experimental design in the delayed administration of SPD and arcaine. Rats received an i.p. injection of SPD (3 mg/kg), arcaine (ARC, 10 mg/kg) or saline (0.9% NaCl, 1 ml/kg), 6 h after reactivation session, and were tested for memory reconsolidation 1 day later (d). Data are means + SEM percentage of freezing in the testing session (n = 7–10 animals in each group).

way ANOVA of reactivation session freezing scores did not reveal significant differences among groups [$F(3,28) = 0.59$, $p > 0.05$]. Statistical analysis of test session freezing scores revealed a significant effect of pharmacological treatment [$F(3,28) = 9.15$, $p < 0.05$]. Post hoc analysis (SNK) revealed that while 1 and 10 mg/kg arcaine decreased, 0.1 mg/kg arcaine did not alter freezing scores. These results suggest that arcaine impairs memory reconsolidation in a dose-dependent manner.

3.3. SPD and arcaine specifically modulate contextual fear reconsolidation

Fig. 3 shows the effect of SPD (3 mg/kg, i.p.) and arcaine (10 mg/kg, i.p.) 24 h after training, in the absence of reactivation session and 6 h after reactivation session, on contextual fear conditioning. Statistical analysis (one-way ANOVA) revealed that SPD and arcaine did not alter contextual fear conditioning in the absence of reactivation [$F(2,18) = 0.41$, $p > 0.05$, Fig. 3b] and that the delayed injection of SPD and arcaine did not alter contextual fear conditioning [$F(2,26) = 0.84$, $p > 0.05$, Fig. 3d]. Again, as expected, statistical analysis of reactivation freezing scores revealed no differences among groups [$F(2,26) = 0.28$, $p > 0.05$, Fig. 3d].

3.4. Involvement of polyamine-binding sites at the NMDA receptor in the effect of polyamines on reconsolidation of contextual fear memories

Fig. 4b shows the effect of SPD, at a non-effective dose (1 mg/kg, immediately post-reactivation) on the impairment of reconsolidation induced by arcaine (10 mg/kg, 15 min post-reactivation). Fig. 4d shows the effect of arcaine, at a non-effective dose (0.1 mg/kg, immediately post-reactivation), on the improvement of reconsolidation of contextual fear conditioning induced by SPD (3 mg/kg, 15 min post-reactivation). Statistical analysis (one-way ANOVA) of reactivation freezing scores revealed no differences among the groups in both experiments [$F(3,16) = 1.20$, $p > 0.05$; $F(3,16) = 1.60$, $p > 0.05$, Fig. 4b and d respectively]. On the other hand, statistical analysis of test freezing scores (two-way ANOVA) presented in Fig. 4b revealed a significant pretreatment (saline or SPD) versus treatment (saline or arcaine) interaction [$F(1,16) = 57.67$, $p < 0.05$]. Post hoc analysis (SNK) revealed that SPD prevented the impairment of fear conditioning reconsolidation induced by arcaine. Statistical analysis of test freezing scores (two-way ANOVA) presented in Fig 4d revealed a significant pretreatment (saline or arcaine) versus treatment (saline or SPD) interaction [$F(1,16) = 13.50$, $p < 0.05$]. Post hoc analysis (SNK) revealed that arcaine prevented SPD-induced improvement of contextual fear conditioning reconsolidation.

3.5. Effect of arcaine on reconsolidation was not caused by state dependence

Fig. 5 shows the effect of arcaine immediately post-reactivation and 30 min before testing on contextual fear conditioning scores at test. Again, statistical analysis of reactivation freezing scores (one-way ANOVA) revealed no differences among groups [$F(3,16) = 0.23$, $p > 0.05$]. On the other hand, statistical analysis of freezing scores at test (two-way ANOVA) revealed only a significant effect of post-reactivation pharmacological treatment [$F(1,16) = 0.46$, $p > 0.05$]. Post hoc analysis (SNK) confirmed that administration of arcaine immediately after reactivation impaired reconsolidation regardless whether arcaine was injected again before test or not. These results rule out state dependence as a possible explanation for the currently reported arcaine-induced impairment of reconsolidation.

4. Discussion

The current study showed that while the polyamine SPD improves, arcaine, an antagonist of the polyamine binding site at the NMDA receptor, impairs the reconsolidation of contextual fear conditioning.

In a general sense, the current finding that arcaine disrupts the reconsolidation of the memory of contextual fear conditioning task corroborates those studies that have shown that NMDA receptor antagonists impair memory reconsolidation (Charlier & Tirelli, 2011; Lee et al., 2006; Pedreira, Perez-Cuesta, & Maldonado, 2002). Early studies by Przybyslawski and Sara (1997) have shown that blocking NMDA receptor with MK-801 up to 1 h after the reactivation session impairs memory reconsolidation, when memory is assessed 24 h or 48 h later, suggesting that NMDA modulators have

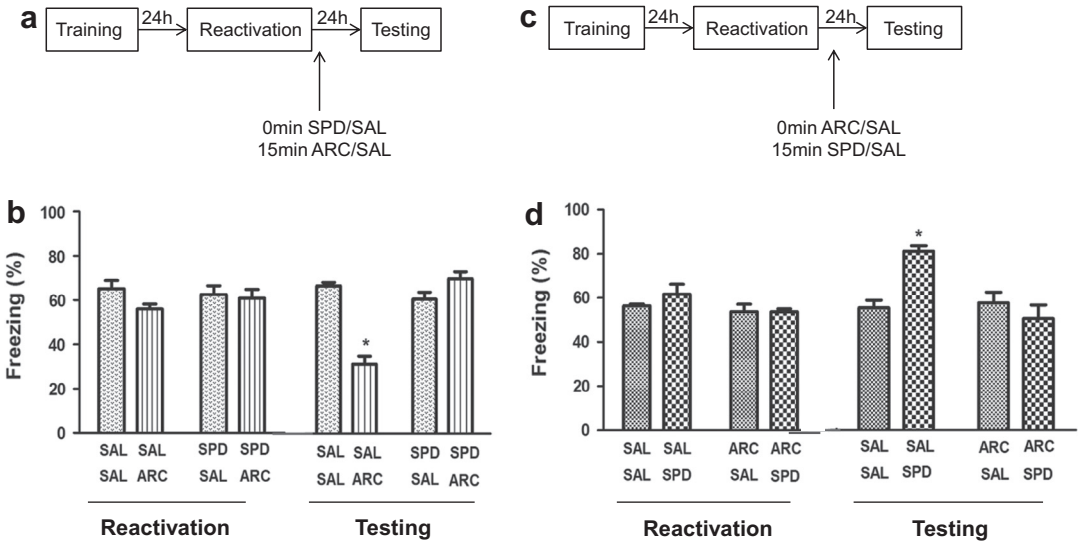

Fig. 4. Involvement of polyamine-binding sites on the NMDA receptor in the effect of polyamines on reconsolidation. (a) Schematic of the experimental design to prevent the amnesic effect of arcaine by SPD. Rats received an i.p. injection of SPD (1 mg/kg) or saline (0.9% NaCl, 1 ml/kg) immediately and saline (0.9% NaCl, 1 ml/kg) or arcaine (ARC, 10 mg/kg) 15 min after the reactivation session, and were tested for memory reconsolidation 1 day later (b). (c) Schematic of the experimental design to prevent the facilitatory effect of SPD by arcaine. Rats received an i.p. injection of arcaine (ARC, 0.1 mg/kg) or saline (0.9% NaCl, 1 ml/kg) immediately and SPD (3 mg/kg, i.p.) or saline (0.9% NaCl, 1 ml/kg) 15 min after the reactivation session, and were tested for memory reconsolidation 1 day later (d). $^{*}p < 0.05$ compared with vehicle by the SNK. Data are means + SEM percentage of freezing in the testing session ($n = 5$ animals in each group).

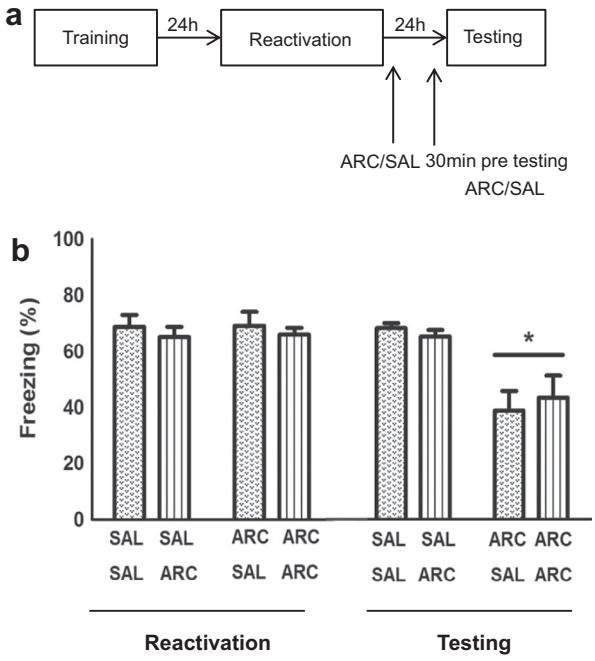

Fig. 5. Effect of arcaine on reconsolidation was not caused by state dependence. (a) Schematic of the experimental design. Rats received an i.p. injection of arcaine (ARC, 10 mg/kg) or saline (0.9% NaCl, 1 ml/kg) immediately after reactivation and 30 min before testing, and were tested for memory reconsolidation. $^{*}p < 0.05$ compared with vehicle by the SNK Test. Data are the means + SEM percentage of freezing in the testing session ($n = 5$ animals in each group).

a limited time window to interfere with memory reconsolidation. More recently, additional evidence has been gathered indicating that NR2B-containing NMDA receptors are critical for reconsolidation. Mamou et al. (2006), have shown that NR2B subunits are necessary for transforming stable fear conditioning memories into labile ones during reactivation in the basolateral amygdala (BLA), and Wang et al. (2009) have shown a relationship between NR2B expression and the ability of an auditory fear memory to undergo reconsolidation in the BLA. Interestingly, the deleterious effect of arcaine on memory reconsolidation was fully prevented by a non-effective dose of SPD. This is in full agreement with the view that arcaine and SPD compete for the same binding site at the NMDA receptor (Reynolds, 1990). Notwithstanding, arcaine inhibits [3H]MK801 binding in the absence of added polyamines, implying a constitutive role of ligand occupation of the polyamine site for NMDA receptor function (Reynolds, 1990). Therefore, since arcaine may alter NMDA receptor function in the absence of polyamines, one must be cautious while presuming a physiological role for endogenous polyamines on memory based only on data obtained with arcaine.

In the current study we also showed that SPD improves memory reconsolidation. These results are also in agreement with those from Lee et al. (2006) and Yamada, Zushida, Wada, and Sekiguchi (2009), who have shown that enhancing NMDA receptor-mediated glutamatergic transmission with D-cycloserine (DCS), a NMDA receptor partial agonist, facilitates the reconsolidation of fear conditioning memory. The currently described biphasic effect of SPD on memory reconsolidation is also in agreement with the view that polyamines modulate the NMDA receptor biphasically (Rock & Macdonald, 1995; Williams, 1997; Williams et al., 1991). Accordingly, polyamines, at low micromolar concentrations, enhance [3H]MK-801 and [3H]TCP binding to the NMDA receptor channel, whereas higher concentrations of polyamines do not alter the binding of these ligands, resulting in a biphasic concentration dose–response curve (Ransom & Stec, 1988; Sacaan & Johnson, 1990; Williams, 1997). Accordingly, low concentrations of polyamines enhance NMDA-evoked currents, whereas higher concentrations of polyamines produce less enhancement of, or inhibit, NMDA receptor currents (McGurk, Bennett, & Zukin, 1990; Rock & Macdonald, 1995; Sprosen & Woodruff, 1990; Williams, Dawson, Romano, Dichter, & Molinoff, 1990). Moreover, these results agree with previous studies that have shown that intrahippocampal or intra-amygdala administration of SPD improves the memory of the inhibitory avoidance task (Berlese et al., 2005; Rubin et al., 2000) and fear conditioning (Rubin et al., 2004) in a biphasic manner.

One must also outline that the facilitatory role of SPD was fully prevented by the administration of a non-effective dose of arcaine,

providing pharmacological evidence that SPD effects involve the polyamine binding site at the NMDA receptor.

Since we have previously shown that intrahippocampal SPD facilitates the extinction of fear conditioning (Gomes et al., 2010), some important methodological differences between the experimental protocols used to investigate extinction and reconsolidation must be emphasized. Reconsolidation protocols demand a brief exposure of the animal to the conditioning context (Sara, 2000). Such a short exposure strengths the association between context and shock, as context brings about the vivid recall of the traumatic shock experience. In this case, the duration of exposure is not enough for the animal realize that the conditioned stimulus (CS) does not predict the unconditioned stimulus (US) anymore. Accordingly, longer periods of re-exposure to the training context allow such a dissociation between CS and US. The dissociation between context and shock implies a new learning (Ji & Maren, 2007; Myers & Davis, 2002; Szapiro, Vianna, McGaugh, Medina, & Izquierdo, 2003) and, consequently, the formation of a different memory (engram). In this context, it is reasonable that SPD facilitates extinction of fear conditioning (Gomes et al., 2010), because it depends on the activation of NMDA receptors (Myers & Davis, 2002), particularly those containing the NR2B subunit (Sotres-Bayon, Diaz-Mataix, Bush, & LeDoux, 2009). Therefore, one might argue that our results (this study and Gomes et al., 2010) constitute pharmacological evidence supporting the involvement of the NMDA receptor in fear conditioning reconsolidation and extinction, as proposed by different authors (Bustos, Giachero, Maldonado, & Molina, 2010; Lee et al., 2006; Sotres-Bayon, Bush, & LeDoux, 2007; Suzuki et al., 2004; Weber, Hart, & Richardson, 2007; Yamada et al., 2009).

It is also interesting that the facilitatory effect of SPD on memory reconsolidation did not occur when this polyamine was administered 6 h after the reactivation session. This time window for the effect of SPD on reconsolidation is similar to that determined for SPD on early consolidation of inhibitory avoidance (Berlese et al., 2005). Nader et al. (2000) have shown that reconsolidation is sensitive to protein synthesis inhibition also at a restricted time window (less than 6 h), suggesting that both consolidation and reconsolidation have time windows within which protein synthesis is required. Notwithstanding, it is not known whether the facilitatory effects of SPD on memory depend on protein synthesis, and which cerebral structures are affected by SPD and arcaine, as well. In this context, micrroinfusion studies may provide experimental evidence to answer these questions.

At last, since previous studies have shown that arcaine causes state-dependent learning (Ceretta, Camera, Mello, & Rubin, 2008; Mariani et al., 2011) one might question whether the effect of arcaine on memory reconsolidation is a form of state dependence, i.e., that retrieval is dependent on a physiological/pharmacological state present during acquisition (Izquierdo & Dias, 1983a,b,c, 1985; Overton, 1964; Shulz, Sosnik, Ego, Haidarliu, & Ahissar, 2000). However, the deleterious effect of arcaine on memory reactivation was not reversed by the administration of arcaine before testing (Fig. 5), ruling out state-dependent learning in the currently reported effects of arcaine.

In summary, this study showed that while the systemic injection of SPD immediately after reactivation improves, the injection of arcaine impairs memory reconsolidation. We also showed that systemic injection of arcaine prevents the improvement of memory reconsolidation induced by SPD and that the injection of SPD prevents arcaine-induced impairment of memory reconsolidation. These findings suggest that ligands of the polyamine binding site at the NMDA receptor modulate memory reconsolidation. Moreover, arcaine-induced impairment of reconsolidation is not related to state dependence. These results indicate a role for polyamines on memory reconsolidation.

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