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Research report

Dorsolateral periaqueductal gray stimulation prior to retrieval potentiates a contextual fear memory in rats

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HIGHLIGHTS

- Dorsolateral periaqueductal gray stimulation as a negative emotional mnemonic trace.
- Negative emotional mnemonic trace potentiate contextual fear expression at recall.
- Enhancement of fear responses are long-lasting and evident after 6 days.
- Effect is contingent to the associated context.

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ABSTRACT

The association of a neutral context with an aversive stimulus, such as foot-shock, result in a contextual fear memory. A growing number of evidence have revealed that prior exposure to diverse threatening situations facilitates the encoding of fear memory during acquisition and such reports support the widespread notion that emotionally arousal results in stronger and long-lasting memories. However, few studies have investigated if a threatening experience can affect the recall and the persistence of such fear memory trace. To test the hypothesis that an emotionally negative experience could modify the retrieval of a memory and potentiate the expression of a fear memory, the present study used the chemical stimulation (microinjection of NMDA) of the dorsolateral periaqueductal gray matter (dlPAG) of rats in order to induce an aversive emotional state. Such stimulation was performed one day after a weak fear training protocol, and the fear expression was analyzed in subsequent re-exposures to the conditioned context. The results showed that the negative emotional state induced by the dlPAG stimulation enhanced the fear memory trace when this trace was reactivated one day after this aversive experience. Additionally, the potentiation of the fear response was contingent to the associated context since no potentiation was evident when NMDA-stimulated animals were subsequently placed in a non-associated context. Finally, the model suggests that the enhancement of fear responses is long-lasting since NMDA-treated animals performed a robust fear response six days after memory retrieval.

1. Introduction

Excessive and uncontrollable fear is a main feature of several anxiety-related disorders. Therefore, it seems relevant to explore organismic or environmental challenges that could lead to inappropriate fear responses during the retrieval of an already established fear memory. A growing number of evidence has revealed that prior exposure to diverse threatening situations facilitates the encoding of fear memory during acquisition [1–4]. These reports, in fact, support the widespread notion that emotionally driven experiences results in stronger and long-lasting memories [1,4–6]. Despite the abundant evidence supporting the facilitating influence of stressful experiences on subsequent memory acquisition, it remains to be established how a threatening experience affect a fear memory trace when it is induced prior to the retrieval of a previously consolidated fear memory.

The periaqueductal gray matter (PAG) is an integrative area of the neuroaxis known to elicit various overt defensive responses. It is well established that this brain structure is not only a final common pathway for forebrain or diencephalic neural sites, but also plays a major role in the fear learning process [7–9]. Specifically its dorsal portion (dPAG) is long known to be involved in the modulation

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of aversive states (for a comprehensive review, see [10]) and in addition, the dPAG plays a relevant role in sustaining olfactory fear conditioning promoted by the chemical stimulation of the dorsal premammillary nucleus of the hypothalamus [9]. Moreover, recent results from our laboratory showed that the chemical stimulation of the dorsolateral column (dlPAG) can be used as an unconditioned stimulus (US) in an olfactory fear conditioning paradigm, pointing the PAG participation in the modulation of fear associations [11]. Additionally, an electrophysiological study suggested that PAG relays aversive information to the amygdala and instruct neural plasticity for conditioned associations [12].

It has been shown that during the encoding of a CS-US association, the training intensity is one of the factors to be considered for the formation and maintenance of a fear memory. Therefore, in the present study, we used the chemical stimulation of dlPAG as a negative emotional event followed by the recall of a fear memory induced by a weak fear conditioning protocol. We hypothesized that this event, performed one day after the encoding of the CS-US association, could affect the fear memory trace during the recall of the previous established fear memory, modifying the fear expression in response to the associated context in subsequent retrieval experiences.

2. Materials and methods

2.1. Subjects

122 Adult male Wistar rats, weighing 280–350 g obtained from the Department of Pharmacology of Federal University of Santa Catarina were used in this study. Animals were housed in polypropylene cages (50 cm × 30 cm × 15 cm) in groups of three or four, under a 12 h light/dark cycle (lights on at 7 am), in a temperature-controlled environment (23 ± 1 °C) with free access to food and water. The protocols used were approved by the Federal University of Santa Catarina, Animal Ethics Committee (23080.0055752/2006-64/UFSC) and the experiments were carried out in accordance with the Brazilian Society of Neuroscience and Behavior Guidelines for the Care and Use of Laboratory Animals.

2.2. Stereotaxic surgery

Rats were intraperitoneally anaesthetized with 1.5 ml/kg of a solution containing ketamine (10%; Cetamin®, Brazil) and xylazine (2%; Xilazin®, Brazil), associated with local anesthesia (3% lidocaine with norepinephrine 1:50,000, Dentsply, Brazil) and fixed in a stereotaxic frame (Stoelting Co., USA). A stainless steel guide cannula (13 mm length, 26G) was implanted unilaterally aimed at the caudal dlPAG according to Paxinos and Watson rat brain atlas [13] coordinates (midline = 1.9 mm; anteroposterior = 7.6 mm; dorsoventral = −2.0 mm from the skull surface at an angle of 22 °C) and fixed to the skull with a stainless steel screw and acrylic cement. A stylet introduced inside the guide cannula prevented obstruction. At the end of the surgery, subjects were injected intramuscularly with an antibiotic association of benzylpenicillin and streptomycin (1.0 ml/kg; Pentabiótico®, Brazil) to prevent possible infections. In addition, flunixin meglumine (2.5 mg/kg; Schering-Plough, Brazil), a drug with analgesic, antipyretic and anti-inflammatory properties, was administered subcutaneously. After seven days of recovery, rats were subjected to the experimental procedures.

2.3. Drugs and dlPAG infusion procedure

N-methyl-D-aspartate (NMDA; Sigma, St. Louis, MO) was dissolved in 0.1 M phosphate-buffered saline (PBS; pH 7.4) which alone served as vehicle control. The dose used was selected based on previous studies of our group, which showed that 100 pmol is a dose capable to stimulate the neurons of the dlPAG, triggering the expression of overt defensive behaviors [14] as well as olfactory fear conditioning acquisition [11]. The drug infusion (0.2 μl) of either vehicle or drug was performed at the rate of 0.6 μl/min using an infusion pump (Insight, Ribeirão Preto, SP, Brazil) with a thin dental needle (outer diameter = 0.3 mm), sized 16.2 mm, introduced through the guide cannula (13 mm), extending 3.2 mm below the cannula end, reaching the dlPAG. A polyethylene catheter (PE10; Clay Adams, USA) was interposed between the upper end of the dental needle and the microsyringe (5 μl; Hamilton), and an air bubble displaced inside the polyethylene was used to monitor the drug flow. Needle was removed 30 s after the end of drug infusion.

2.4. Apparatuses and behavioral measures

Three different chambers were used in the present study: a conditioning chamber, a propylene box and a glass box. During the experiment, the boxes were housed in a sound-attenuating room with illumination level of 80 lux. Before and after utilization, all three chambers were cleaned with a 10% ethanol solution (v/v) and dry towels.

The conditioning chamber (designated as context A, 23 cm × 20 cm × 26 cm) was constructed with stainless steel walls and a grid floor composed of 1 cm spaced stainless bars connected to a shock generator (Insight, Ribeirão Preto, SP, Brazil) that, when appropriate, delivered a 0.5 mA shock for 3 s. A propylene box (50 cm × 30 cm × 15 cm) was designated as the context B, where the animals were placed immediately after the microinjection at the dlPAG. A third context, a glass box (28 cm × 28 cm × 28 cm), was designated as context C, an environment completely different from the conditioning chamber and the context B.

Freezing duration was used as a fear response; this defensive behavior has been commonly used as a reliable index of fear and defined as the complete absence of body, head and vibrissae movement except those required for breathing [15]. The total time spent in freezing in each session was quantified in seconds and expressed as the percentage of total time.

2.5. Experimental procedures

All experiments were carried out during the diurnal phase, between 1:00 and 5:00 pm. Each experiment was recorded by a video camera while a monitor and a DVD-recording system were installed in an adjacent room.

2.5.1. Weak contextual fear conditioning

In order to establish the experimental conditions for the emergence of a weak fear memory, animals were subjected to diverse training protocols, varying the number of footschocks applied. The training consisted in placing each rat in the chamber (context A) and allowing a 1 min acclimation period (pre-shock period); after this period, rats received one, three or five foot-shocks (0.5 mA, 3 s duration at an inter-shock interval 30 s; unconditioned stimuli). Animals remained in the chamber for an additional minute (post-shock period). Rats assigned to control group (0 foot-shock) were placed in conditioning chamber for 3 min without receiving any unconditioned stimuli. All groups remained a total of 3 min, except the group that received 5 shocks which remained 4 min in the conditioning chamber (context A). At the end of this period, rats were removed and subsequently placed in their home cages.

2.5.2. dlPAG chemical stimulation with NMDA infusion

Rats were randomly assigned to receive either the infusion of NMDA 100 pmol or PBS (vehicle) into the dlPAG. Immediately after the drug infusion, subjects were placed in context B for 10 min, after this period animals were returned to their home cages.

2.5.3. Test sessions

Depending on the experimental design, test sessions (test 1, 2 or 3) were performed either in context A or context C, 24 h, 48 h or 6 days after the dlPAG infusion. The test was performed by placing each rat in the selected context for 3 min, without receiving foot-shock, and the time spent in freezing was scored and expressed as percentage of total time (3 min).

2.6. Experimental design

2.6.1. Experiment 1

This experiment was designed to establish a sub-threshold fear conditioning protocol that would enable a further increased fear response, when later exposed to the CS (context A). For this purpose, one, three or five foot-shocks were associated with a neutral context based on previous preliminary studies. Rats were submitted to the weak contextual fear conditioning (day 1) and the fear expression was evaluated in context A, on day 2 (test 1) and also on day 3 (test 2), when they were returned to the same context. Conditional emotional responses were assessed as previously described.

2.6.2. Experiment 2

This experiment was designed to evaluate whether the chemical stimulation of the dlPAG, a midbrain structure known to signalize danger, affects the fear memory trace during recall and during a subsequent test session. On day 1, rats were subjected to the weak contextual fear conditioning protocol (context A) receiving 0, 1 or 3 foot-shocks; on day 2, rats were either infused intra-dlPAG with NMDA or vehicle (context B) and on day 3 rats were re-exposed to the context A for test 1. Six days later (day 9), an additional testing session was performed (test 2) in context A to measure fear conditioned responses and 24 h later, animals were again tested for their freezing response (test 3) in a novel context (context C).

2.6.3. Experiment 3

The third experiment was designed to evaluate if the re-exposure to context A was a pre-requisite to observe the potentiated fear response in NMDA-treated rats that were previously conditioned. Therefore, in the test 1 (on day 3), rats were randomly assigned to two experimental groups depending on the context (re-exposure to context A or exposure to context C). This session last 3 min and the total time spent in freezing was scored. An additional test (test 2) was performed 24 h later by placing all rats (both groups) in context A, for 3 min and time spent in freezing scored.

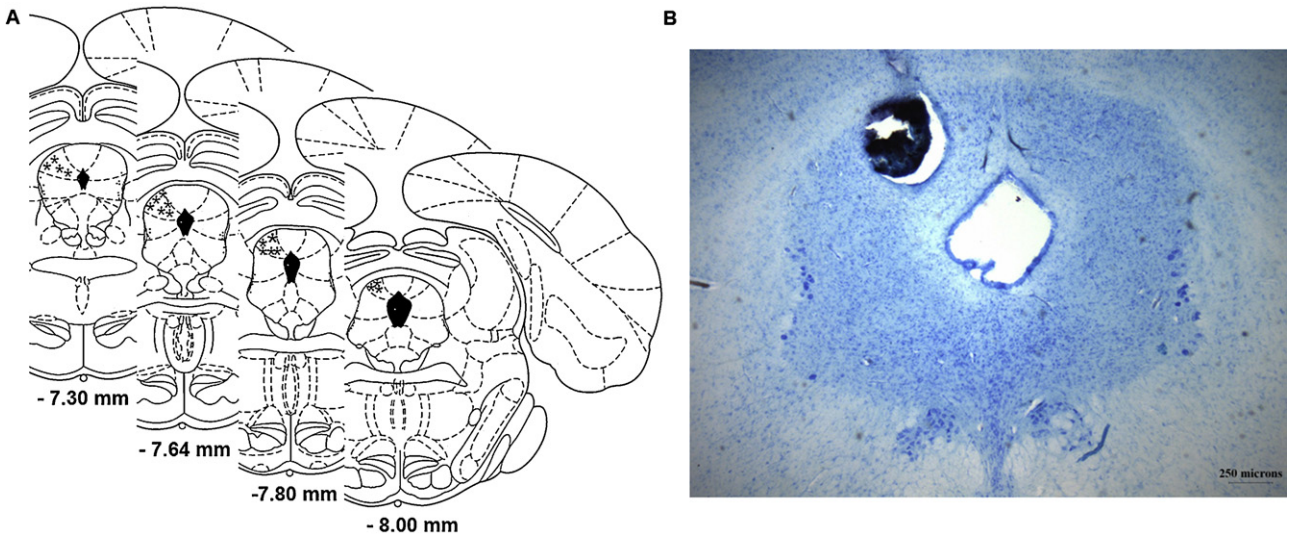

Fig. 1. Microinjection sites. (A) Injection sites in coronal midbrain sections illustrated in diagrams according to the atlas of Paxinos and Watson [13]. (B) Photomicrography representing an infusion site marked by Evans-Blue dye within the dlPAG.

2.6.4. Experiment 4

The fourth experiment was designed to evaluate whether the chemical stimulation of the dlPAG, affects the fear memory trace when the re-exposure to context A was performed 48 h after the NMDA infusion. On day 1, rats were subjected to the contextual fear conditioning protocol receiving 3 foot-shocks; on day 2, rats were infused with intra-dlPAG with either NMDA or vehicle and on day 4 subjects were re-exposed to the conditioning context for the test session.

2.7. Perfusion and histology

After the behavioral tests, rats were deeply anesthetized using chloral hydrate 15% (2.3 mg/kg, intraperitoneally) and transcardially perfused with 0.9% NaCl followed by a 10% formaldehyde solution. A 0.2 μl injection of Evans Blue was then applied through the guide cannula to mark the exact location of the treatment microinjection. The brains were then removed and immersed in a 10% formaldehyde solution and after 24 h transferred to a 30% sucrose solution for 24 h. Slices (50 μm thick) were obtained in a cryostat (CM1850; Leica, Germany) in the transverse plane and mounted on glass microscope slides. The sections were stained with Giemsa to anatomically localize the Evans Blue marks. The location was mostly between −7.6 and −8.0 mm posterior from bregma according to rat brain atlas [13] (Fig. 1).

2.8. Statistical analysis

Results were expressed by mean + SEM. Data from experiments 1, 2 and 3 were analyzed by repeated measures analysis of variance (ANOVA). Following significant ANOVA results ($p < 0.05$), *post hoc* comparisons using Newman–Keuls test were performed. Data from experiment 4 was analyzed by student "*t*" test for independent groups.

3. Results

3.1. Experiment I. Weak contextual fear conditioning

Repeated measures ANOVA performed with data from Experiment 1 depicted in Fig. 2, showed a significant trial effect [$F(1,30) = 37.61$; $p = 0.00001$], a significant effect of number of foot-shocks [$F(3,30) = 3.5737$; $p = 0.02540$] and an effect of interaction (session *versus* number of shocks) [$F(3,30) = 4.0116$; $p = 0.01632$]. The *post hoc* analysis revealed that rats which received 5 foot-shocks during acquisition, exhibited a significant ($p < 0.05$) increase in the % of freezing during test 1 (re-exposure, day 2) when compared to 0 and 1 foot-shock groups. Freezing time for rats within the 3 foot-shock group were not different from 0, 1 or 5 foot-shock groups. In addition, although no difference was found among groups during test 2, rats that received 3 or 5 foot-shocks showed significantly more freezing during test 1 than test 2.

3.2. Experiment II. Intra-dlPAG infusion with NMDA facilitated the freezing responses in trained rats re-exposed to the associated context

Data analysis showed a significant effect of the number of foot-shocks [$F(2,40) = 9.93$, $p = 0.0031$], treatment [$F(1,40) = 4.45$, $p = 0.0411$] and trial [$F(2,80) = 9.52$, $p = 0.0002$] (Fig. 3). In addition, an interaction between number of foot-shocks *versus* treatment [$F(2,40) = 7.26$, $p = 0.002$], number of foot-shocks *versus* trial [$F(4,80) = 9.87$, $p = 0.0001$], treatment *versus* trial [$F(2,80) = 9.25$, $p = 0.0001$] and an interaction among number of foot-shocks *versus* treatment effect *versus* trial [$F(4,80) = 5.97$, $p = 0.0002$] was found. The *post hoc* test revealed a significant ($p < 0.05$) increase in the % of time freezing in the 3 foot-shocks-NMDA group ($N = 11$) as compared to the 3 foot-shocks-PBS group ($N = 6$) and to all remaining groups during the re-exposure to context A. Similarly, *post hoc* comparisons during test 1, also performed in the context A, performed 6 days after the re-exposure, showed a significant ($p < 0.05$) increase in the freezing time in the 3 foot-shocks-NMDA injected group as compared to the 3 foot-shocks-PBS group and to all other groups. In constrast, no significant difference was found among the groups, when rats were tested in context C (test 2). Furthermore, the *post*

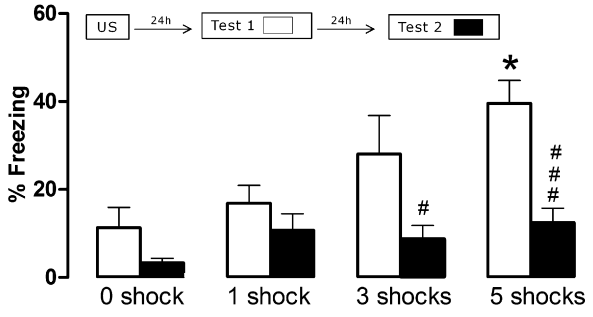

Fig. 2. Freezing levels expressed by rats conditioned with 0 ($n = 8$), 1 ($n = 8$), 3 ($n = 9$) or 5 ($n = 9$) foot-shocks during Test 1 (■) or Test 2 (□) sessions. Data are expressed as mean + epm. $^{*}p < 0.05$ as compared to 0 and 1 shock group during Test 1 (repeated measures ANOVA followed by Newman Keuls *post hoc* test); $^{\#}p < 0.05$ and $^{\#\#\#}p < 0.0005$ when compared to previous test 1.

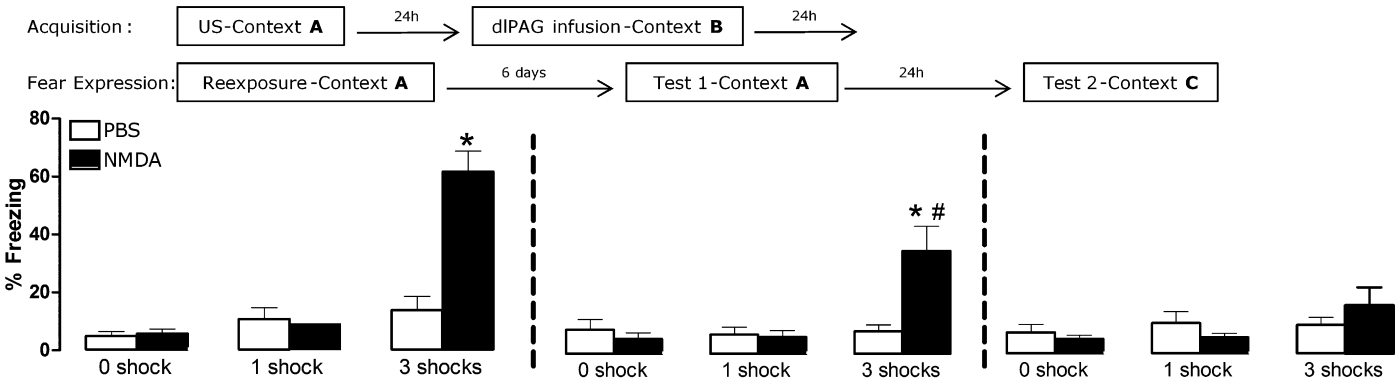

Fig. 3. Intra-dlPAG infusion with NMDA enhanced contextual fear conditioning responses during the re-exposure (left panel) and test 1 (middle panel) sessions in the same context (A) in the group that received 3 foot-shocks. No effect was detected during test 2 performed in a different context (C, right panel). $^{*}p < 0.05$ as compared to the respective PBS group. $^{\#}p < 0.01$ compared to 3 foot-shock NMDA infused rats, during test 1. ANOVA followed by Newman Keuls *post hoc* test (0 shock: PBS $n = 8$, NMDA $n = 7$; 1 shock: PBS $n = 7$, NMDA $n = 7$; 3 shocks: PBS $n = 6$, NMDA $n = 11$).

hoc test also revealed a significant ($p < 0.05$) decrease in the % freezing time in the 3 foot-shocks-NMDA group during test exposure in context A as compared to its own performance during the first retrieval session (re-exposure to context A).

In summary, the current results showed that dlPAG NMDA activation enhanced the fear response when subjects were exposed to the associated context. This effect was long lasting since, although reduced, it was still evident 6 days after the re-exposure to context A. In contrast, no sign of fear was evident when animals were subjected to a novel context, indicating that such treatment (NMDA) did not result in an unspecific fear response.

3.3. Experiment III. The enhancement of fear memory induced by intra-dlPAG NMDA infusion is context dependent

Results from experiment 2 showed that prior NMDA activation in dlPAG enhanced both the expression and the persistence of the fear memory trace. The present experiment was conducted to evaluate if the re-exposure to the associated context was a prerequisite to observe such enhancement. Fig. 4 shows data from two different groups, *i.e.*, freezing levels during test 1 in context A or C, and test 2, in context A. ANOVA revealed a significant treatment effect [$F(1,31) = 31.56$, $p = 0.001$], a significant context effect [$F(1,31) = 46.43$, $p = 0.001$], an interaction between treatment *versus* context effect [$F(1,31) = 27.45$, $p = 0.001$] and a context *versus* session interaction [$F(1,31) = 9.00$, $p = 0.005$]. Newman Keuls *post hoc* test revealed that rats injected with NMDA into dlPAG on day 2 and re-exposed to context A on day 3 (NMDA-re-exposed to A, $N = 9$) displayed a significant increase ($p < 0.005$) of the freezing behavior when compared to the PBS-re-exposed to A rats ($N = 10$) and to the exposed to C-NMDA treated rats ($N = 10$) during test 1 and test 2. Collectively, these data demonstrate that the reactivation of the fear memory trace in the associated context is a prerequisite to observe the enhancement induced by dlPAG activation since subjects placed in a novel context (non-reactivation procedure) following NMDA infusion displayed a fear level similar to that observed in PBS treated rats.

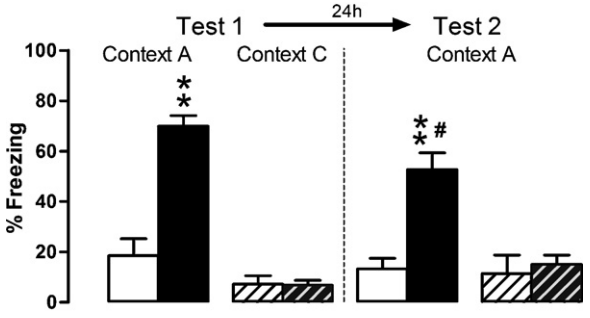

Fig. 4. Re-exposure to the conditioning environment is necessary to promote the enhancement of fear responses to the associated context. Freezing levels during test 1 (left panel) and test 2 (right panel) are shown. Drugs were microinjected one day before test 1; white bars = PBS ($n = 10$), black bars = NMDA ($n = 9$), stripped bars = rats placed in context C (n PBS = 6; n NMDA = 10) during test 1. $^{*}p < 0.001$ as compared to PBS group; $^{\#}p < 0.05$ as compared to respective group during test 1. Repeated measures ANOVA followed by Newman Keuls *post hoc* test.

3.4. Experiment IV. The enhancement of fear memory is dependent of the time elapsed between the intra-dlPAG NMDA infusion and memory reactivation

Data depicted in Fig. 5A represent the % of time spent freezing during test 1 (re-exposure to context A), following 48 h of the intra-dlPAG-NMDA infusion. Student's "*t*" test performed with data depicted in Fig. 5A, failed to show a significant treatment effect regarding PBS or NMDA. In Fig. 5B, a frequency distribution chart for subjects receiving NMDA 48 h before was presented. Freezing data collected from subjects receiving NMDA 48 h before were found distributed in a more scattered and less consistent pattern.

4. Discussion

It is well established that the dlPAG stimulation promotes a negative emotional state in rodents and in humans (for review see [10]). The physiological and behavioral responses induced by dlPAG stimulation were correlated with the fearful sensations and imminent death feelings experienced by panic attack patients [16–18]. The

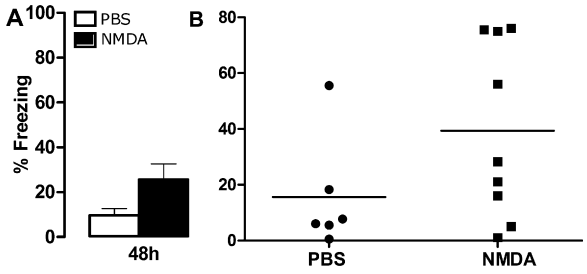

Fig. 5. (A) Forty-eight hours re-exposure session from dlPAG-NMDA treatment failed to show enhanced fear response (PBS group: $N = 6$; NMDA group: $N = 9$). (B) Frequency distribution chart representing freezing behavior during re-exposure for subjects receiving PBS or NMDA 48 h before.

activation of this midbrain area occurs in rats exposed to its natural predator [19], therefore it seems possible to suggest that dlPAG activation is involved in the mechanism of long-lasting anxiety effects observed in animal models of PTSD that uses predatory threat [20]. Additionally, the chemical stimulation of this brain area with NMDA is able to attribute an aversive relevance to a neutral stimulus [11], supporting the view that this manipulation promotes a negative emotional state which is biologically relevant. In the present study, all animals injected with NMDA 100 pmol into the dlPAG showed defensive behaviors such as running, galloping, which were restricted to the first min after the NMDA infusion and freezing, a typical response observed as a consequence of this midbrain area stimulation [21].

The weak training protocol was designed to allow low levels of freezing expression during later re-exposures to the conditioned context. As depicted in Fig. 2, rats receiving 0, 1 or 3 shocks showed lower levels of freezing as compared to those receiving 5 foot-shocks, allowing to detect further elevation of fear. Nevertheless, although rats receiving 5 foot-shocks remained a total of 4 min in the conditioning chamber during the training procedure, it seems highly probable that the improvement of fear acquisition is associated to the number of shocks applied rather than to a brief increase (1 min) in the time spent in the conditoning chamber.

The present results showed that the aversive event induced by dlPAG stimulation enhances the fear memory trace when there is a recall experience following the dlPAG stimulation. This enhancement is contingent to the associated context since no potentiation was evident when NMDA stimulated animals were subsequently placed in a non-associated context. Moreover, this NMDA-induced promoting influence persists for at least 6 days after the recall experience (test 1), indicating that such activation not only promotes fear expression but also strengths the fear memory trace. It is also noteworthy to indicate that animals exposed to three shocks without dlPAG microinjection displayed a moderate freezing during test 1 and did not maintain such freezing response during the subsequent test 2. A possible explanation for this effect might be due to the fact that a weak memory trace, as that induced by the current learning protocol, when retrieved by re-exposure to the associated environment, is susceptible to an extinction process that might develop in response to a successive exposure to the context. In addition, there were no signs of fear generalization since animals exposed to the NMDA infusion and later on subjected to a different context showed no freezing increase in a novel context, neither when exposed on day 3 (Fig. 4, experiment III) nor on day 10 (Fig. 3, experiment II), that is, before or after memory retrieval. The freezing enhancement observed in both experiments seems to be context-specific. Moreover, the effect on freezing response is likely to occur within a limited time window, since subjects re-exposed to the conditioning context 48 h after dlPAG stimulation did not show such an enhancement (Fig. 5).

There are numerous evidence in the literature regarding the promoting influence of threatening experiences on fear memory formation when the traumatic event has occurred prior to fear acquisition [1–4], however, relatively few studies have addressed the question regarding the strength of a memory trace when the retrieval of such trace occurs under an aversive internal state as that presumably induced following the stimulation of a well-known brain defensive area.

The stimulation of dlPAG neurons through the activation of glutamatergic receptors by injecting a NMDA receptor agonist is known to induce defensive behaviors such as freezing and escape, as well as, avoidance to the open arms in the elevated plus-maze paradigm [14]. Previous studies using electrophysiology showed that PAG is activated by limbic structures and suggested that PAG can also activate the limbic system [22]. The chemical stimulation of dlPAG increases c-Fos expression in brain areas involved in the promotion of fear learning such as the amygdala, the hypothalamus and other columns of the PAG, indicating a modulatory role of dlPAG in those brain structures activities [23]. This dlPAG influence on telencephalic structures required for the learning process was recently suggested by our group using a paradigm of olfactory fear conditioning, which used the dlPAG chemical stimulation (NMDA microinjection) as a US [11]. In another study, it was also demonstrated that the fear conditioning induced by noradrenergic activation of the dorsal premammillary nucleus of hypothalamus (PMd) also depends on dlPAG activity [9]. Moreover, Johansen and colleagues [12] studied the influence of PAG activity on amygdalar nuclei in an auditory fear conditioning paradigm and suggested that the PAG activity instructs neural plasticity in amygdala for fear association, further supporting the view that PAG stimulation modulates other brain areas involved in fear learning. Thus, it seems possible to suggest that the dlPAG activation *via* the stimulation of NMDA receptors promotes a hyperexcitability in those areas required for learning and retrieval, strengthening the memory trace after recall.

Apparently, the finding that dlPAG stimulation, which presumably results in an aversive internal state, promotes fear memory is contradictory to the transient impairing effect of stress on retrieval in aversive motivated and spatial tasks [24,25]. It should be noted, however, that stress-induced interference on memory retrieval is temporary and dependent on stress-induced secretion of glucocorticoid [5]. In our case, the retrieval session was performed one day after the dlPAG stimulation when glucocorticoid should have returned to basal levels.

In conclusion, the present results suggest that the negative emotional state induced by the dlPAG stimulation interacts with the retrieval of a memory trace. It is possible to speculate that this emotionally arousal is added to the original trace modifying its emotional relevance and enhancing fear responses in later exposures to the associated context. Further experiments are necessary to investigate the molecular mechanisms involved in such phenomena. The present data contributes to the study of the interaction between aversive events separated in time, since it shows that a negative experience biologically relevant can produce an emotional state which interact with the retrieval of a previously consolidated memory, resulting in a more robust fear memory trace.

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