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Research report

Protein synthesis inhibition in the basolateral amygdala following retrieval does not impair expression of morphine-associated conditioned place preference

A.J. Yim, C.R.G. Moraes, T.L. Ferreira, M.G.M. Oliveira*

Department of Psychobiology, Universidade Federal de São Paulo (UNIFESP), São Paulo, Brazil

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Abstract

Conditioned place preference is an animal model used to evaluate the affective properties of natural rewards and drugs of abuse. This animal model is a kind of classical conditioning that depends on learning and memory. The basolateral amygdala (BLA) plays an important role in the consolidation and extinction of memory for this task. However, there is a lack of evidence demonstrating protein synthesis dependent reconsolidation following retrieval in conditioned animals. In other words, is it possible to observe morphine-associated place preference if recall of this preference is disrupted? Accordingly, we investigated this hypothesis by BLA infusion of protein synthesis inhibitor, anisomycin, immediately after retrieval (test) in conditioned place preference paradigm. In the first experiment, the conditioned animals were exposed to the two sides of the apparatus for 15 min in a drug-free state during retrieval. In the second experiment, the animals received an injection of morphine (7.5 mg/kg, i.p.) and immediately after, they were exposed to the two sides of the apparatus for 15 min. Finally in the third experiment, after habituation and training in the conditioned place preference task, the animals received an injection of the unconditioned stimulus (morphine, i.p.; 7.5 mg/kg) followed by confinement for 10 min in the morphine-paired compartment (conditioned stimulus) during memory retrieval. For the three experiments the animals were subsequently exposed in a free-drug state to the two sides of the apparatus for the retest. Our results show that the protein synthesis inhibition in all of these experimental designs had no effect on conditioned place preference memory under conditions that would initiate reconsolidation, suggesting that if reconsolidation of a conditioned place preference task exists it is not mediated by protein synthesis in basolateral amygdala. The effect of anisomycin on consolidation of contextual fear conditioning was also investigated as a positive control to assure that the negative results were not due to methodological problems. Using the same dose of anisomycin (62.5 μg/1 μl) in morphine-associated place preference procedures, we have found that this anisomycin dose blocks the consolidation of contextual fear memory, ruling out the possibility that these negative results can be attributed to methodological problem of some sort.

Keywords: Conditioned place preference; Anisomycin; Morphine; Reconsolidation

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1. Introduction

The conditioned place preference is a very useful tool to study the reinforcing properties of natural and drug rewards in laboratory animals. This task is a kind of Pavlovian conditioning that refers to a preference for a context due to contiguous association between the environmental cues (conditioned stimulus) and natural reinforcers or drugs of abuse (unconditioned stimulus). Therefore, conditioned place preference is dependent on a special kind of associative learning in which the contextual cues become attractive and gain motivational properties that can induce drug-seeking behavior [2].

Previous studies indicate that the basolateral amygdalar (BLA) complex is involved in both acquisition and expression of learned emotional responses, like conditioned fear [23], appetitive learning and drug addiction [29]. Thus, this amygdalar complex mediates stimulus-reward learning that is required for a conditioned stimulus to gain incentive motivational and conditioned reinforcing value [8]. For instance, basolateral amygdala lesion impairs acquisition of cocaine-seeking behavior and conditioned place preference in rats [9], whereas intra-BLA infusion of the cholinergic antagonist scopolamine after training impairs

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* Corresponding author at: Department of Psychobiology, Universidade Federal de São Paulo, Rua Napoleão de Barros, 925 CEP 04024-002, São Paulo, SP, Brazil. Tel.: +55 11 5539 0155; fax: +55 11 5572 5092.

E-mail address: mgabi@psicobio.epm.br (M.G.M. Oliveira).

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consolidation of both food and amphetamine-induced conditioned place preferences [27]. Moreover, basolateral amygdala plays an important role on the extinction of approach behavior to drug-associated cues [28].

It is well established that the consolidation of new memories depend on protein synthesis to become stable and long lasting [17]. Previous studies [18,26] revealed that protein synthesis in BLA is required for consolidation of fear memories, since intra-BLA infusion of the protein synthesis inhibitor anisomycin following conditioning disrupted consolidation. Moreover, Nader et al. [21] found that long-term memories of auditory fear conditioning return to a labile state during recall, since infusion of a protein synthesis inhibitor (anisomycin) in basolateral amygdala immediately after memory reactivation produces amnesia on posterior tests. This suggests that, after retrieval, fear memories are required to undergo a process of reconsolidation in order to return to a stable form.

However, the concept that long-term memories become labile and need to undergo protein synthesis dependent reconsolidation is not a consensus in the literature, since there are conflicting data even in experiments that made use of the same tasks. For instance, whereas Debiec et al. [6] showed that intra-hippocampal infusion of the protein synthesis inhibitor anisomycin immediately after retrieval caused amnesia for contextual fear memory in later tests, Biendenkapp and Rudy [3] did not observe any loss of performance. The same contradictory results were found with inhibitory avoidance [5,19] and Morris water maze tasks [14,30].

Regarding appetitive tasks, Hernandez and Kelley [10] have found that the systemic administration of anisomycin did not affect the performance in a lever-pressing task, suggesting that a well learned instrumental response does not require protein synthesis dependent reconsolidation. Nevertheless, Szu-Han et al. [31] state that in an incentive learning task, in which animals learn about changes in reward value, a process of reconsolidation may undergo. Thus, they have found that the intra-amygdalar infusion of anisomycin either given after the initial devaluation or after a second devaluation session abolished the changes in the value of food reward produced by this learning, suggesting the opposite, i.e., incentive learning depends on protein synthesis within the amygdala for reconsolidation. Although the appetitive instrumental task, incentive learning and conditioned place preference are different tasks, they share one property in common, since they are reward-related tasks.

Since contextual stimulus previously associated with drug effects can elicit drug-seeking behavior [2] it would be interesting to see if it is possible to interfere with memory for this association after its reactivation. Thus the aim of the present study was to investigate whether the local infusion of anisomycin in basolateral amygdala interferes with the stability of conditioned place preference memory.

2. Materials and Methods

2.1. Subjects

Subjects were adult male Wistar rats ($n = 49$) weighting 250–350 g body weight and approximately 90 days old at the beginning of the experiments. Animals were housed in groups of two in opaque polypropylene cages (41 × 34 × 16) containing woodchip bedding in a room with controlled temperature (22 °C) and light/dark cycle (lights on 07:00–19:00). Food and water were available ad libitum. Animals were maintained in accordance with the Committee on Animal Care of the Colégio Brasileiro de Experimentação Animal (COBEA).

2.2. Drugs

Morphine chlorhydrate (Casa Granado, SP, Brazil) was dissolved in 0.9% saline solution and administered in a dose of 7.5 mg/kg. Anisomycin (Sigma, St. Louis, MO) was firstly dissolved in 3N HCl, diluted with buffer phosphate saline (PBS) and adjusted to pH 7.2 with 3N NaOH. Sterile PBS served as the vehicle control. Anisomycin was administered in a concentration of 62.5 μg/μl in a volume of 1 μl/side. Control animals received an equivalent volume of PBS (pH 7.2). The dose of anisomycin was chosen based on the work of Nader et al. [21] who have found that this dose blocks the of reconsolidation of auditory conditioned fear.

2.3. Apparatus

The place conditioning apparatus consisted of acrylic box divided into two compartments of equal size (23 × 22.5 × 22 high), one of them had grey walls with white circles fixed on them and texture floor, whereas the other one had white and grey stripes with a smooth floor. The compartments were separated by a guillotine door.

2.4. Apparatus of contextual fear conditioning

The conditioning apparatus consisted of an acrylic box, measuring 30 cm × 21 cm × 30 cm. The walls were black with some visual white patterns (two squares measuring 5.5 × 5.5 cm and three 4.0 cm × 4.0 cm squares made of white cardboard). The top was covered with transparent acrylic. The floor consisted of a metal grid (0.4 cm diameter rods placed 1.2 cm apart) connected to a shock generator and a control module (Ugo Basile model CAT7551), through which footshocks could be delivered.

2.5. Stereotaxic surgery and microinfusion

The rats were deeply anaesthetized with a combination of ketamine (90 mg/kg) and xylazine (5 mg/kg), positioned in a stereotaxic apparatus (David Kopf Instruments, Tuyunga, CA) and implanted bilaterally with stainless steel guide cannula (23 gauge) aimed at 1.0 mm above basolateral amygdala (A-P = −2.8; M-L = ± 4.7; D-V = −7.5 all relative to bregma, [22]). The cannulae was fixed with polyacrylic cement and anchored to the skull with stainless steel screws. A stylus was placed in the guide cannula to prevent clogging. Animals were allowed one week to recover from surgery before being subjected to experimental manipulations. For microinfusions, the stylus was removed from the guide cannula, the animals were gently held and a removable injector was inserted into the guide cannula, extending 1.0 mm beyond the guide tip. The injector was connected via PE 10 tubing to a 10 μl Hamilton syringe driven by a microinfusion pump (Insight, Ribeirão Preto, Brazil); and a volume of 1.0 μl was injected over 4 min (0.25 μl/min). The injector remained in the guide cannula for an additional 2 min period after infusion to minimize diffusion of the injected liquid along the injection tract.

2.6. Behavioral procedures

2.6.1. Experiment 1

Effects of BLA infusion of anisomycin immediately after reactivation of conditioned place preference memory in a subsequent retest.

This experiment consisted of four phases: habituation, conditioning, testing and retesting. This protocol was the same used by Rezayof et al. [24]. Animals were submitted to the reactivation session that occurred in the presence of conditioned stimulus only (without unconditioned stimulus) in a procedure that resembles that one used for fear conditioning experiments [6,21]. In addition, this procedure is frequently used in studies that utilize a conditioned place preference task.

2.6.1.1. Habituation. On the first day, the animals were placed in one of the two compartments by chance. Half of the animals were placed in the white and grey stripes compartment, while the remaining half of the rats were placed in the grey compartment with white balls. The guillotine door was then removed and the animals were allowed to explore freely the apparatus for 15 min. The time spent by the animals in each compartment was recorded. The conditioning with morphine was always performed in the non-preferred compartment, defined as the compartment where the rat spent less than 50% of the total session time (total 900s) during the habituation phase. On the other hand, conditioning with saline was always performed in the preferred compartment, defined as the compartment where the rat spent more than 50% during the habituation phase.

2.6.1.2. Conditioning. The conditioning phase was conducted in six sessions with two sessions each day. On each day, there was a morning session (8:00–11:00) in which animals received an intraperitoneal injection of morphine chlorydrate (7.5 mg/kg) or saline and was immediately placed in the least or most preferred compartments, respectively for 50 min. After 6 h, in the evening session (14:00–17:00), the animals received another intraperitonial injection of equivalent volume of saline or morphine and were placed in the more or less preferred compartments but in such a way that each animal received the two diferent treatments on each day. Thus, each animal received three drug and three saline pairings.

2.6.1.3. Testing. The testing phase or reactivation session was carried out on day 5. The guillotine door was raised and the animals, with neither morphine nor saline injections, were placed in the apparatus with free acess to both compartments for 15 min. The time spent in each compartment was recorded by an unaware observer. Immediately after this testing, the animals received a bilateral infusion of anisomycin (62.5 μg/1 μl/side) or the same volume of PBS in basolateral amygdala and returned to their home cages.

2.6.1.4. Retesting. The retesting phase was conducted on day 6 and the procedure was identical to the testing phase.

2.6.2. Experiment 2

Effects of BLA infusion of anisomycin immediately after reactivation testing with the unconditioned stimulus (morphine) on a subsequent test.

In the object recognition task, Bozon et al. [4] found that zif 268 mutant mice (immediate early gene involved in memory reconsolidation) showed impairments after reactivation only when the object was presented in a previously associated context. Thus, it is possible that reconsolidation only occurs in the conditioning place preference when the conditioned stimulus (context) is paired at the same time with the unconditioned stimulus (morphine) during reactivation test. Thus, to test this hypothesis, the same animals of experiment 1 were used in this experiment. One week after finishing experiment 1, the animals were retested again to the conditioned place preference task. However, immediately before testing, the animals received an intra-peritoneal injection of morphine chlorydrate (7.5 mg/kg) and then were placed to explore freely the two compartments of apparatus for 15 min. Immediately after testing, half of animals that received an intra-amygdalar infusion of vehicle in experiment 1, received an intra-amygdalar infusion of anisomycin (62.5 μg/1 μl/side, $n = 7$) whereas the remaining half of rats received an intra-amygdalar infusion of vehicle ($n = 8$). The same procedure was used with the animals that received an intra-amygdalar infusion of anisomycin in the experiment 1. Thus, half of the animals that received an intra-amygdalar infusion of anisomycin in experiment 1 received an intra-amygdalar infusion of vehicle, while the remaining half received an intra-amygdalar infusion of anisomycin.

2.6.3. Experiment 3

Effects of BLA infusion of anisomycin immediately after a reactivation session with the animals confined in the morphine-paired compartment in the presence of unconditioned stimulus (morphine) on subsequent test.

As the result of the experiment 2 could be influenced by the complex history of the animals, we tested an alternative way to present the conditioned stimulus at same the time with the unconditioned stimulus during the reactivation session. Therefore, the animals received an injection of morphine immediately before being placed in morphine-paired compartment during the reactivation test.

This experiment consisted of four phases: habituation, conditioning, confinement in the morphine-paired compartment (reactivation) and testing. The experiment was conducted in six consecutive days. The habituation and conditioning phases were identical to experiment 1. One day after the conditioning phase (day 5), the animals received an injection of morphine chlorydrate (7.5 mg/kg, i.p.) and immediately after, they were confined into the morphine-paired compartment for 10 min. Shortly after this confinement, the animals received an intra-amygdalar infusion of anisomycin ($n = 9$) or PBS ($n = 9$) and returned to their home cage. The testing phase was carried out on day 6. As in the preconditioning phase, the guillotine door was raised and the animals were placed in the apparatus with free acess to both compartments for 15 min in a drug-free state. The time spent in each compartment was recorded by an unaware observer.

2.6.4. Experiment 4

Effects of BLA infusion of anisomycin immediately after training on consolidation of contextual fear conditioning.

After completion of the experiments involving conditioned place preference, we performed a contextual fear conditioning experiment in a new group of animals to assure that BLA protein synthesis is sensitive to anisomycin. We chose contextual fear conditioning as positive control because early study [18] has demonstrated that intra-BLA infusion immediately after training disrupts consolidation of this task.

The task was carried out during two consecutive days. On the first day (training), the animals were individually placed in the black box where they remained for 5 min. The time each rat remained in freezing, defined as complete immobility and absence of vibrissae movements and sniffing, was recorded continuously minute by minute for 5 min. After this period, the rats received two footshocks (1 mA, 1 s duration) at 30 s intervals. The animals were immediately removed from the apparatus after the last footshock and received intra-BLA injection of 62.5 μg in a volume of 1 μl/side of anisomycin or saline. After this, they returned to their homecage. Contextual conditioning tests were performed on the second day, 24 h after training. The rats were placed in the same training context and no footshocks were delivered. The time in freezing was recorded continuously minute by minute for 5 min. The freezing/min ratio was taken as a measure of contextual conditioning.

2.7. Locomotor activity

Locomotor activity was measured during the pre-conditioning, testing and retesting phases. An observer measured the number of times that the animals crossed one compartment to other during 15 min.

2.8. Histology

After completion of experimental sessions, each animal was anaesthetized with chloral hydrate (1.0 ml) and received an intra-amygdalar infusion of 1.0 μl of 1% solution of methylene blue for checking the placement of cannulae. Frozen brain slices (40 μm) were analysed to verify the microinfusion sites. The sections were mounted onto slides, dried and stained with cresyl violet.

2.9. Statistical analysis

In all experiments, the conditioning scores are expressed as the total time spent in morphine-paired compartment during conditioning phase. Locomotor activity is expressed as number of crossings to one compartment to other during pre-conditioning, testing and retesting phases. Data are expressed as mean ± S.E.M. Analysis of data was performed using two-way ANOVA, with phases (habituation, testing or retesting) and treatment (anisomycin or saline) as factors. Following a significant F-value, post-hoc analysis (Newman-Keuls) were performed for assessing specific group comparisons, considering $p < 0.05$ as statistically significant. Data from contextual fear conditioning were evaluated using two-way ANOVA with group and minute as main factors. When applied, the analysis was followed by the post-hoc Newman-Keuls test.

3. Results

3.1. Histology

Fig. 1 shows the bilateral location of the injection needle tips in the basolateral amygdala and the diffusion of the ink in the experimental and control animals. Only the animals with the ink in the basolateral amygdala were included in statistical analysis.

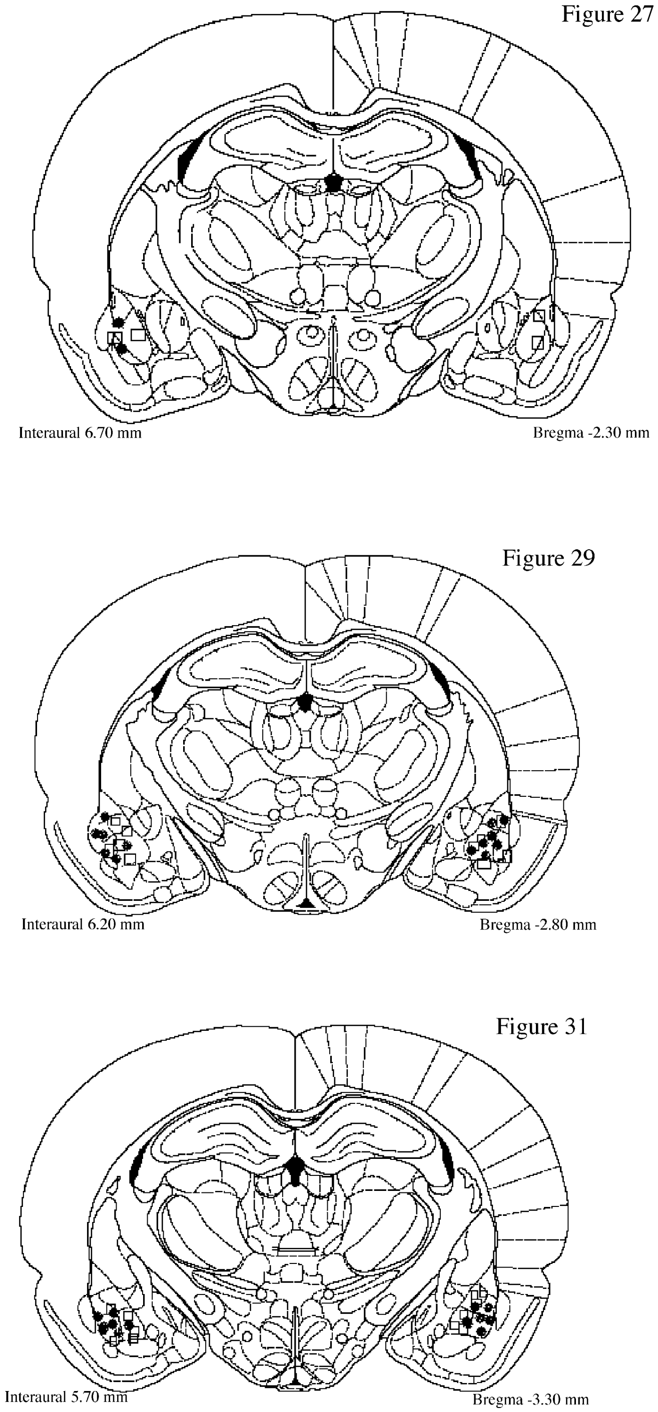

Fig. 1. Histological analysis: Location of bilateral injection needle tips in the basolateral amygdala at three different rostrocaudal planes (−2.30, −2.80 and −3.30 mm AP to bregma). Black filled circles represent anisomycin group, whereas white squares represent PBS group in experiment 1. The placements for the other experiments all showed similar distribution and therefore are not shown.

3.2. Behavior

3.2.1. Experiment 1

Effects of BLA infusion of anisomycin immediately after reactivation testing on a subsequent retest.

Fig. 2 shows the mean time ± S.E.M. spent in the morphine-paired compartment during the habituation, testing and retesting phases of animals that received an intra-amygdalar infusion of anisomycin ($n = 9$) or vehicle ($n = 8$) immediately after reactivation testing and retested 24 h later. A two-way ANOVA with groups (i.e., anisomycin and vehicle) and phases as factors revealed a significant difference among testing conditions ($F_{(1,30)} = 22.69$; $p < 0.001$). The Newman-Keuls post-hoc test revealed a significant difference between habituation and testing days ($p < 0.001$) and habituation and retesting days ($p < 0.001$). However, no statistical difference was found between groups ($F_{(1,15)} = 0.17$; $p = 0.68$) and no interaction was found between phases and groups ($F_{(2,30)} = 2.16$; $p = 0.13$).

Regarding locomotor activity, the mean and ± S.E.M. of the number of times that animals crossed one compartment to other for PBS group were 17.75 ± 2.6, 16.29 ± 3.0 and 13.0 ± 3.48 during habitation, testing and retesting phases, respectively, whereas the mean time and ±S.E.M. for animals that received anisomycin were 16.89 ± 1.5, 16.89 ± 1.19 and 15.11 ± 1.60 during these same phases. A two-way ANOVA with groups (PBS and anisomycin) and phases (habituation, testing and retesting) as factors revealed a significant difference among testing conditions ($F_{(2,28)} = 4.50$; $p < 0.05$). The Newman-Keuls post-hoc test revealed a significant difference between habituation and retesting days ($p = 0.02$) and testing and retesting days ($p = 0.02$). However, no significant difference was found between groups ($F_{(1,14)} = 0.39$; $p = 0.54$) and no interaction was found between groups and phases ($F_{(2,28)} = 0.51$; $p = 0.23$).

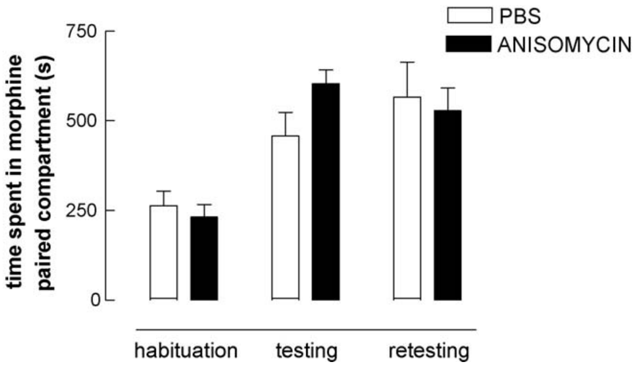

Fig. 2. The mean time ± S.E.M. spent in the morphine-paired compartment during the habituation, testing and retesting phases of animals that received an intra-amygdalar infusion of anisomicyn ($n = 9$) or vehicle ($n = 8$) immediately after reactivation testing (62.5 μg/μl/side) and retested 24 h later. The groups do not differ in any phase of the experiment (two-way ANOVA).

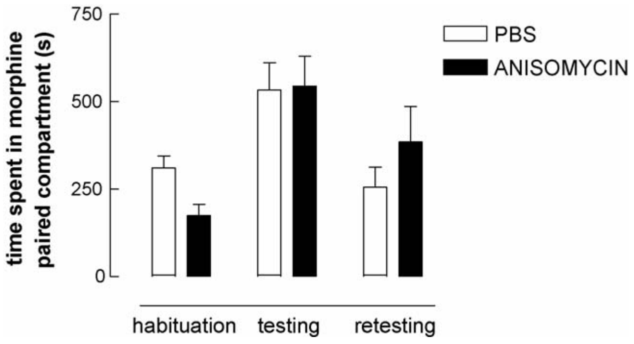

Fig. 3. The mean time ± S.E.M. spent in the morphine-paired compartment during the habituation, testing and retesting phases of animals that received an intra-peritoneal injection of morphine (7.5 mg/kg) before reactivation testing. Immediately after this testing, these animals received an intra-amygalar infusion of anisomycin (62.5 μg/μl/side, $n = 7$) or vehicle ($n = 8$) and were tested again 24 h later. The groups do not differ in any phase of the experiment (two-way ANOVA).

3.2.2. *Experiment 2*

Effects of BLA infusion of anisomycin immediately after reactivation testing with the unconditioned stimulus (morphine) on a subsequent test.

Fig. 3 shows the mean time ± S.E.M. spent in the morphine-paired compartment during habituation, testing and retesting phases of animals that received an intra-peritoneal injection of morphine (7.5 mg/kg) before reactivation testing and that immediately after this testing received an intra-amygalar infusion of anisomycin (62.5 μg/μl/side, $n = 7$) or vehicle ($n = 8$) and were tested again 24 h later. A two-way ANOVA revealed a significant difference among testing conditions ($F_{(2,26)} = 14.53$, $P < 0.001$). The Newman-Keuls post-hoc test revealed significant differences between habituation and testing days ($p < 0.001$) and between testing and retesting days ($p < 0.01$). No statistical difference was found between groups ($F_{(1,16)} < 0.001$; $p = 0.98$) and no significant interaction was found between groups and phases ($F_{(2,26)} = 2.69$; $P = 0.08$). The Newman-Keuls post-hoc test revealed significant differences between pre-conditioning day and testing day ($p < 0.001$) and between testing and retesting day ($p < 0.01$).

3.2.3. *Experiment 3*

Effects of BLA infusion of anisomycin immediately after a reactivation session with the animals confined in the morphine-paired compartment in the presence of unconditioned stimulus (morphine) on subsequent test.

Fig. 4 shows the mean time spent ± S.E.M. in the morphine-paired compartment during habituation and testing phases for morphine-treated rats (7.5 mg/kg) that received an intra-amygdalar injection of anisomycin ($n = 9$) or PBS ($n = 9$) immediately following the reactivation session.

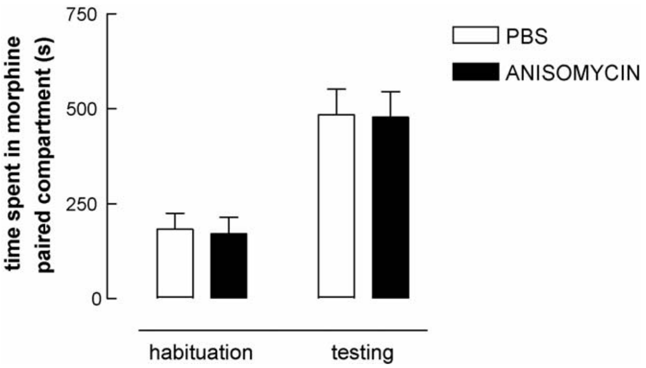

Fig. 4. The mean time ± S.E.M. spent in the morphine-paired compartment during the habituation and testing conditions of animals that received an intra-peritoneal injection of morphine (7.5 mg/kg) before being confined for 10 min in the morphine-paired compartment. Immediately after confining, the animals received an intra-amygdalar infusion of anisomicyn (62.5 μg/μl/side, $n = 9$) or PBS ($n = 9$) and were tested 24 h later. The groups do not differ in any phase of the experiment (two-way ANOVA).

A two-way ANOVA revealed statistical differences between phases (habituation and testing, ($F_{(1,16)} = 33.01$; $p < 0.001$). The Newman-Keuls post-hoc test revealed a significant difference between habituation and testing days ($p = 0.001$). However, no statistical difference was found between groups ($F_{(1,16)} = 0.02$; $p = 0.87$) and no interaction between group and testing conditions ($F_{(1,16)} = 0.96$) (Fig. 4).

Regarding locomotor activity, the mean and ±S.E.M. of number of times that animals crossed one compartment to other for PBS group were 15.56 ± 2.1 and 22.44 ± 1.37 during habitation and testing phases, respectively, whereas the mean time and ±S.E.M. for animals that received anisomycin were 14.33 ± 2.3 and 18.00 ± 2.5 during habituation and testing phases, respectively. A two-way ANOVA revealed statistical differences between phases ($F_{(1,16)} = 4.58$; $p = 0.04$) The Newman-Keuls post-hoc test showed a significant difference between habituation and testing day ($p = 0.048$) but there is no statistical difference between groups ($F_{(1,16)} = 2{,}54$; $p = 0.13$) and no interaction between group and phases ($F_{(1,16)} = 0.42$; $p = 0.52$).

3.2.4. *Experiment 4*

Effects of BLA infusion of anisomycin immediately after training on consolidation of contextual fear conditioning.

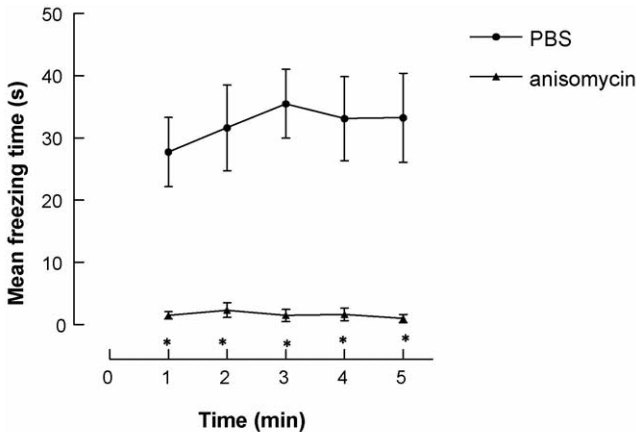

Fig. 5. The effects of post-training intra-BLA anisomycin administration on freezing response of rats in contextual fear conditioning. Mean freezing time ± S.E.M. of anisomycin ($n = 6$) and vehicle ($n = 8$) on test day measured during 5 min.

The intra-BLA anisomycin animals were impaired in the consolidation of contextual fear conditioning (Fig. 5). There was a significant group effect [$F_{(1,12)} = 22.80$; $P < 0.001$]. Post-hoc (Newman-Keuls $P < 0.01$) test showed that intra-BLA anisomycin animals showed less freezing time than the control animals in test day. The main effects minute [$F_{(4,48)} = 0.71$; $P > 0.1$] and interaction (group × minute) [$F_{(4,48)} = 0.63$; $P > 0.1$] were not significant.

4. Discussion

The present results suggest that conditioned place preference task does not undergo protein synthesis dependent reconsolidation after retrieval in BLA. It should also be considered that intra-amygdalar infusion of anisomycin did not interfere with locomotor activity, which may influence the conditioned place preference response. The protocol used in experiment 1 was the same used by Rezayof et al. [24]. In this experiment, animals were submitted to the test only in the presence of conditioned stimulus in a procedure that resembles that one used for fear conditioning experiments. In addition, this procedure is the most frequently used in studies that utilize conditioned place preference task. The importance of the unconditioned presentation together with conditioned stimuli was also tested since Bozon et al. [4] have shown that during recall of an object recognition memory, neither contextual information nor the exposure to previously presented objects in a different context is sufficient in itself to destabilize the memory. Hence, using mutant mice to zif 268, a gene necessary to reconsolidation of a recognition object task, Bozon et al. [4] observed that the memory becomes unstable only when the objects are recalled in the previously associated context. In experiment 2, using the same animals tested for the place preference task in the first experiment, we tested these animals in the presence of the unconditioned stimulus (morphine). Immediately after testing, the animals received an intra-BLA infusion of anisomycin and retested 24 later. Protein synthesis inhibition did not impair performance of the animals in the retest day. The absence of effect of anisomycin in the experiment 2 could be due to the complex history of the animals. For instance, the first test without morphine could interfere with the performance in the following tests. In order to further verify the hypothesis that the presence of unconditioned together with the conditioned stimuli is important for the reconsolidation phenomena, we performed the experiment 3 in naïve animals. In this experiment, the animals were confined in the paired compartment and were presented with the unconditioned stimulus (morphine) for 10 min during the reactivation of conditioned place preference memory followed by infusion intra-amygdalar of anisomycin. Again, anisomycin did not interfere with the performance of the animals in a subsequent test, suggesting that this task did not undergo protein synthesis dependent processes in the basolateral amygdala after retrieval.

Several reports have indicated that inhibition of protein synthesis in basolateral amygdala with anisomycin impairs consolidation of both contextual and auditory fear conditioning [18,26]. To ascertain that the lack of a positive result in conditioned place preference task is not due to a methodological problem of some sort, we performed an experiment with contextual fear conditioning which aimed to replicate the findings of a previous study performed by Maren et al. [18]. These authors have shown that anisomycin is able to disrupt the consolidation of contextual fear conditioning when it is infused in the basolateral amygdala after training. Similarly, infusing the same dose of anisomycin used in the conditioning place preference task (62.5 μg/μl/side) into the basolateral amygdala immediately after training, we also obtained an impairment of consolidation of contextual fear conditioning. This result shows that protein synthesis in basolateral amygdala was sensitive to anisomycin in our experimental procedure and excludes the possibility of anisomycin is not blocking protein synthesis in this cerebral structure.

This present result is not the first one to report a failure to find a reconsolidation effect. Previous studies have not found reconsolidation in different tasks including inhibitory avoidance [5] and contextual fear conditioning [3,15]. In place preference conditioning, as in contextual fear conditioning, context is paired with a stimulus that has motivational value. It is possible that the number of pairings is a critical factor related to the conflicting results. Suzuki et al. [30] showed that in anisomycin blocked reconsolidation for contextual fear conditioning when only one footshock was delivered, but it did not block reconsolidation when three footshocks were delivered. They also demonstrated that longer reexposures to the training context triggered reconsolidation of stronger memories. Taken together, these results suggest that memory reconsolidation is dependent on the strength of memory and the number of pairings of conditioned and unconditioned stimuli. Since conditioned place preference requires several pairings between conditioned and unconditioned stimuli, it is possible that this task is more resistant to the effects of protein synthesis inhibition during retrieval. However, it should be mentioned that, in the same work, Suzuki et al. [30] found that Morris water maze, a task that requires several trials for training, anisomycin blocked reconsolidation. In an inhibitory avoidance task, Milekic and Alberini [19] showed that the requirement for protein synthesis of a reactivated memory is evident only when memory is recent. Thus, they found that systemically administered anisomycin-induced impairment is dependent upon the interval between training and testing session. When the interval between training and testing sessions was shorter (2 and 7 days), animals that received anisomycin immediately following reactivation testing showed impairment in their performance in a subsequent retest. However, when the interval between training and testing sessions was longer (14 and 28 days), animals that received anisomycin immediately after recall did not showed amnesia for this task in a later retest. A possible explanation, according to Kandel, [13] is that consolidation of new memories depends on protein synthesis to form new synapses. These new synapses are weaker and susceptible to protein synthesis inhibition. Consequently, the reactivation of new memories could potentially destabilize a large amount of these new synapses, causing memory impairment. Conversely, as the time goes by, the synaptic connections become more stable and less vulnerable to the effects of protein synthesis inhibition after reactivation. Since conditioned place preference requires several pairings between the conditioned stimulus and unconditioned stimulus during training, it is possible that memory for this task may undergo several rounds of consolidation followed by reconsolidation during the first few pairings. However, in the testing phase, this memory was stronger and insensitive to the effects of protein synthesis inhibition upon retrieval.

Regarding reward-related tasks, the few existing results are also contradictory. Firstly, Hernandez et al. [11] demonstrated that an instrumental task requires protein synthesis in nucleus accumbens core only during the early stages of learning, since animals that received an anisomycin infusion in this nucleus immediately following the first five trials did not learn this task. However, if the task has been well learned, anisomycin had no effect on performance. Furthermore, the same authors [10] also found that systemic administration of anisomycin after retrieval impaired performance but attributed this loss to a conditioning of taste aversion for sugar induced by anisomycin. These authors suggest that well learned instrumental learning does not undergo protein synthesis dependent reconsolidation. However, Lee et al. [16] have shown that memories related to cocaine-seeking behavior undergo reconsolidation. Thus, when animals trained to associate cocaine self-administration with a 20 s light conditioned stimulus (CS) reactivate this memory, it becomes labile and sensitive to intra-BLA infusion of anisomycin in such a way that CS loses its reinforcement properties and does not support a new instrumental learning. In addition, they suggest that amygdalar zif 268 is required to reconsolidation of this task, since intra-BLA infusion 90 min prior to reactivation session of zif 268 antisense oligodeoxynucleotides disrupts a well learned CS-cocaine memory. Hernandez et al. [10] and Lee et al. [16] have reached opposite interpretation about the ability of a well learned response undergo protein synthesis dependent reconsolidation. One main problem in comparing very different tasks relies on the difficult in establishing a fair basis for comparison; on other word, the term “well learned” may not reflect a similar amount of learning in one task and another. Perhaps, one should concentrate rather on the different kind of learning tasks used by these authors. While Hernandez et al. [10] used an instrumental task Lee et al. [16] based their interpretation on a secondary reinforced procedure.

Another line of reasoning assumes that regions other than basolateral amygdala are involved in the reconsolidation of a conditioned place preference task such as the nucleus accumbens, the hippocampus or the medial pre-frontal cortex. These structures were found to be also involved with associative learning processes contributing to drug-seeking behavior [25]. Bahar et al. [1] showed that amygdalar circuits required for either consolidation or extinction of taste aversion are not required for reconsolidation of this task. However, Eisenberg et al. [7] have found that the microinfusion of anisomycin in the taste cortex immediately after retrieval of conditioned taste aversion test results in apparent amnesia for this memory, suggesting that anisomycin blocked reconsolidation in this cortical area. Recently, Valjent et al. [32] reported that systemic administration of anisomycin or ERK inhibition after a CPP test can disrupt performance in a posterior test. These data suggest that protein synthesis dependent reconsolidation occurs in a CPP test but our data show that this phenomenon does not seems to occur in amygdala. One likely candidate for mediate reconsolidation is the nucleus accumbens core in which Miller and Marshal [20] injected an inhibitor of ERK kinase after CPP test and observed impairment in a subsequent test. Therefore, although reconsolidation was not observed in amygdala, it was observed in other structure of the brain.

Alternatively, it is possible that the involvement of the BLA in this task is not related to protein synthesis. The positive control has shown that the right site for conditioned fear was hit. Nonetheless, it remains unclear if the same region that is important for conditioned fear is also important for CPP. Previous work [12,27] suggests that this is the case since post-training drug administration in BLA amygdale such as scopolamine or buvocaine impairs consolidation of CPP. Also pretraining lesion impairs acquisition of this task [9].

In summary, the results shown here suggest that memory for morphine-associated place preference does not undergo protein synthesis dependent reconsolidation in BLA nucleus differently of what happens on fear conditioning [21]. Our results does not give support to the idea that reconsolidation could be used as a new paradigmatic concept that could be used in the management of drug addiction. However, the effects of anisomycin administration on memory reactivation are yet poorly understood in the context of the reward property of drugs of abuse. More studies will be necessary in order to establish the tasks and specific procedures that can sustain reconsolidation in the different forms of appetitive learning.

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