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Research report

Impairment of contextual conditioned fear extinction after microinjection of alpha-1-adrenergic blocker prazosin into the medial prefrontal cortex

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ABSTRACT

Long-lasting memories of aversive or stressful events have been associated with the noradrenergic system activation. Alpha-1-adrenergic antagonist prazosin has successfully been used in the last years to treat anxiety disorders related to aversive memories recurrence in humans. Contextual conditioned fear extinction paradigm in rats has been used to better understand the mechanisms involved in the attenuation of defensive behaviour after a traumatic situation. Here we investigated the effects of systemic administration of prazosin in the fear extinction processes. Rats were previously paired in a contextual fear conditioning box (1 footshock, 1 mA, 2 s duration), further returning to the same box during three consecutive days receiving an intraperitoneal injection of vehicle or prazosin 30 min before (acquisition of extinction; 0.1 or 0.5 mg/kg) or immediately after (consolidation of extinction, 0.5 or 1.5 mg/kg) each extinction session (10 min). On the last day, all animals were re-exposed undrugged to the apparatus. Since the medial prefrontal cortex (mPFC) has been described as a key structure in the modulation of conditioned fear extinction, the effects of intra-mPFC microinjection (0.2 μl per side) of vehicle (PBS) or prazosin (0.75 or 2.5 nmol) in the acquisition of fear extinction (10 min before extinction session 1) were further evaluated. Subjects were drug-free re-exposed to the same box in the next day (extinction session 2). The percentage of freezing time was used as the memory retention parameter. The results showed that either systemic or intra-mPFC-alpha-1-adrenergic blockade increased the freezing time in the last extinction sessions, suggesting impairment of the extinction of contextual conditioned fear in rats.

1. Introduction

Post-traumatic stress disorder (PTSD) is a psychological condition costing billions of dollars for taxpayers in the United States each year [76]. This anxiety disorder associated with previous exposure to different traumas such as wars, catastrophes, sexual abuse and terrorism [28], extraordinary stress experiences may result in persistent aversive memories, which can reverberate for decades [26].

Therapies to reduce the PTSD-signs have been based on the attempt to extinguish aversive memories, abolishing all the present symptoms [69]. Most of the knowledge about the mechanisms involved in fear extinction comes from classical-fear-conditioning studies, initially proposed by Pavlov [51] and widely used in laboratory animals for research groups worldwide [17,20,58]. In this paradigm, a neutral stimulus (usually a sound, an odor, a light or the environment) is paired with an aversive unconditioned stimulus (US; footshock in the paws) able to induce defensive responses. After effective pairings, the neutral stimulus acquires aversive properties and becomes a conditioned stimulus (CS) which alone elicits the same responses observed when the US was present. Repeated exposure to the CS in the absence of the US results in reduced occurrence of the conditioned response, which over time begins to fade in a phenomenon known as extinction [47,82]. This reduction in defensive behaviour toward the repeated conditioned stimulus presentation in rodents has been described as an analogue of many cognitive behaviour therapies widely used to treat anxiety disorders in humans [14,24,40]. Thus, associating pharmacotherapy with extinction intervention could be a promising therapeutic to PTSD patients [41].

It has been suggested that memories of stressful events – as occurs in both PTSD for humans or fear conditioning for rodents – are stored more lively and permanently than neutral or trivial ones, and can persist after a single experience [2,4,31]. Several studies have suggested that this influence in the acquisition/consolidation of intense emotional memories is mediated by the cathecolamine norepinephrine in humans [10,23,73] and rodents [1,22,43]. In fact, clinical studies indicate that patients with PTSD have tonically

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elevated central nervous system concentration of norepinephrine, a critical effector of the mammalian stress response (for review see [78]). In addition, an increased activity of the locus coeruleus, the main noradrenergic nucleus of the brain, has been observed in several different species after fearful or high-stress situations [9]. Consequently, adrenergic agents that inhibit norepinephrine, such as the alpha-1-adrenergic antagonist prazosin, have been used in the last few years to treat anxiety disorders that involve aversive memory recurrence, such as PTSD [7,21,42,59,80]. The efficacy of prazosin in reducing trauma-related nightmares and sleep disturbances associated with PTSD, along with the favorable adverse-effect profile and the low cost, has made this drug a promising agent for the treatment of this anxiety disorder [79]. At this point, evaluating the effects of prazosin in the contextual fear conditioning extinction in rodents can supply insights for understanding the neurobiological, neuroanatomical and pharmacological mechanisms underlying the therapeutic efficacy of this compound in humans.

In the present study, we start investigating the effects of alpha-1-adrenergic blockade in the acquisition or consolidation of contextual conditioned fear extinction by systemically injecting different doses of prazosin 30 min before, or immediately after, each extinction session. A cardiovascular assay protocol was used to confirm that prazosin doses based on literature were in fact effective in antagonizing the alpha-1-adrenergic receptor, and to eliminate potential cardiovascular interferences over basal heart rate and blood pressure measurement occasioned by acute or repeated prazosin administration. Finally, we investigated the medial prefrontal cortex (mPFC) as a feasible neuroanatomical substrate mediating the alpha-1-adrenergic modulation of contextual fear extinction, since this region has been widely described as a critical structure in memory circuits for fear extinction [29,33,53].

2. Materials and methods

2.1. Subjects

Male Long-Evans Hooded rats ($n = 105$) weighing 300–360 g, aged 12–16 weeks at the time of experiments were obtained from the Universidade Federal de Santa Catarina. They were housed in polypropylene cages ($50 \times 30 \times 15$ cm) in groups of three or four and maintained in a 12 h dark/light cycle (lights on in 7:00 a.m.), and in a controlled temperature ($23 \pm 1\ ^\circ C$) room with free access to water and food. All procedures were approved by the Animal Ethics Committee (23080.006118/2004-36/UFSC) and were carried out in accordance with Brazilian Society of Neuroscience and Behavior Guidelines for Care and Use of laboratory animals.

2.2. Drugs

Prazosin hydrochloride (Sigma–Aldrich, USA) was dissolved (0.1, 0.5 and 1.5 mg/ml) in distilled water – which, alone, served as a vehicle control – and administered via intraperitoneal (IP) in an injection volume of 1 ml/kg, 30 min before (acquisition of extinction) or immediately after (consolidation of extinction) extinction sessions 1, 2 and 3.

The systemic doses of prazosin (0.1, 0.5 and 1.5 mg/kg) were selected based on previous studies [67,74]. In cardiovascular assays, the doses of prazosin used were chosen according to the following assumptions: (1) in acute injection the same effective dose found in the acquisition of extinction (0.5 mg/kg) was used; and (2) in repeated injections, the higher dose used in the consolidation of extinction (1.5 mg/kg) was employed. The systemic doses of phenylephrine (Sigma–Aldrich, USA; 0.6, 2 or 6 μg/kg) were chosen based on pilot studies.

Xilazine (5.0 mg/kg IP, Anasedan® 2%, Vetbrands, Brazil) and ketamine (75 mg/kg IP, Francotar® 10%, Virbac, Brazil) were used as anesthetics to the stereotaxic surgery and cardiovascular analysis. Heparin 30 IU [diluted in a saline sterile buffered solution with: (mM) NaCl 137, KCl 2.7, KH_2PO_4 1.5, $NaHPO_4$ 8.1; pH = 7.4] was used as anticoagulant for cardiovascular assays.

For intra-mPFC microinjection, prazosin (Sigma–Aldrich, USA) was freshly dissolved in phosphate-buffered saline (PBS), which alone served as a vehicle control. A correction in the pH (7.4) and a moderate sonic agitation process allowed a satisfactory homogeneity. Since prazosin in higher concentrations precipitate after some minutes, it was necessary to agitate it promptly before injections. Bilateral infusion of prazosin (0.75 or 2.5 nmol per side) or PBS was performed 10 min before testing in a volume of 0.2 μl per side, based on previous report [74].

2.3. Cardiovascular assay

Subjects were anesthetized with xilazine and ketamine, and 30 UI of heparin was administered through a needle inserted in the left femoral vein to prevent clot formation. A polyethylene catheter (PE-10), previously implanted into the right carotid artery, was connected to a pressure transducer coupled to acquisition hardware and software (PowerLab 8/30 running Chart 5.4; AD Instruments), enabling the heart rate and mean arterial pressure valuation.

2.4. Contextual conditioned fear chamber

Contextual fear conditioning was performed in a chamber ($35 \times 20 \times 30$ cm) constructed with side walls of aluminum and a front of clear plexiglas. The grid floor of the apparatus, shaped with bars of stainless steel (9 mm spaced), was connected to a shock generator (Insight, Ribeirão Preto, SP, Brazil) from which footshock was programmed and delivered. Conditioning occurred in a sound-attenuating room with an illumination level of 100 lux. The chamber was cleaned with 10% alcohol–water solution after each rat exposure and dried thoroughly. A video camera system, located 50 cm to the front wall of the apparatus and joined to a DVD recorder, enabled a trained observer to score the time percentage of freezing behaviour (characterized as the complete absence of movement except those for respiration), which was used as a memory retrieval index.

2.5. Surgery

Rats were anaesthetized with xilazine (5.0 mg/kg) and ketamine (75 mg/kg) via IP and positioned in a stereotaxic frame. A longitudinal incision was made on the scalp and the bone was then exposed. Two stainless screws were fixed on the skull using a screwdriver. Two stainless steel guide cannulas (diameter = 0.7 mm; length = 11 mm) were stereotaxically implanted with the cannula tips aimed to mPFC [coordinates: anteroposterior, +3.0 mm from bregma; laterolateral, ±0.6 mm from midline; dorsoventral, −1.8 mm from skull surface [52]. The screws and the cannulas were anchored to the skull with dental cement. Rats were allowed 7 days to recover from surgery.

2.6. Intracerebral injection

Prazosin or PBS microinjections were bilaterally infused 10 min before acquisition of extinction. Two stainless steel needles (14 mm) were connected by polyethylene (PE10) tubing to a Hamilton microsyringe (5 μl), and inserted in the guide cannulas while the rat was kindly restrained. An infusion pump (Insight, B12000 model) was used to assure the bilateral microinjections over a 20-s time period (0.6 μl/min). The needles were kept within the cannulas for an additional 20 s after drug infusion, to maximize the diffusion and to prevent backflow of drug into the cannulas. All drug solutions were freshly prepared immediately before each experiment.

2.7. Procedures

2.7.1. Cardiovascular measurement

Anaesthetized rats were properly connected to cardiovascular transducers and an acute intraperitoneal injection of saline ($N = 4$) or prazosin 0.5 mg/kg ($N = 4$) was performed to evaluate possible baseline cardiovascular function effects. This dose was selected because it was the effective dose injected before the acquisition of extinction. Changes in heart rate and mean arterial pressure were registered and compared with baseline values. This analysis was important to exclude interferences in locomotor activity resulting from hypotension, during the extinction sessions. Increasing doses of the alpha-1-adrenergic agonist, phenylephrine (0.6, 2 or 6 μg/kg) 30 min after prazosin 0.5 mg/kg, was performed to confirm the alpha-1-adrenergic receptor blockade.

The 1.5 mg/kg prazosin dose ($N = 5$) was infused in three consecutive days and in the fourth day, simulating the drug-free session in behavioural experiments, the possible residual presence of the drug was evaluated by injecting crescent doses of phenylephrine. The highest dose of 1.5 mg/kg was selected, in this case, to simulate the treatment regimen adopted during the extinction process, excluding a possible carryover prazosin effect in the drug-free session.

2.7.2. Evaluation of systemic blockade of alpha-1-adrenergic receptor on the acquisition and consolidation of contextual conditioned fear extinction

Rats were allocated in an adjacent room 30 min before conditioning. They were transferred to the experiment room and allowed to explore the conditioning chamber for 1 min. After this time, subjects received one footshock (1 mA, 2 s) and were kept in the apparatus for an additional 1 min. In the next day (extinction session 1), rats were replaced in the conditioning chamber and were randomly divided to receive prazosin or the vehicle 30 min before (acquisition of extinction) or immediately after (consolidation of extinction) this session. Rats received the same treatment and returned to the apparatus during two more consecutive days (extinction sessions 2 and 3). On the fourth day (extinction session 4), subjects were replaced undrugged in the conditioning box to evaluate the behavioural responses

in the absence of possible drug effects. All extinction sessions lasted 10 min during which the time percentage of freezing behaviour was registered and compared between them. Extinction index was represented by the percentage of freezing reduction from the first to the last extinction session, and was calculated by the following expression: the total time of freezing behaviour in the first extinction session, minus the total time of freezing on the last extinction session, divided by the total time of freezing in the first extinction session.

2.7.3. Changes in the acquisition of contextual conditioned fear extinction after alpha-1-adrenergic blockade within the mPFC

Rats were context-paired as previously described in the systemic experiments. On the next day (extinction session 1), subjects were randomly divided to receive a bilateral microinjection of prazosin (0.75 or 2.5 nmol/side) or PBS within the mPFC 10 min before returning to the conditioning chamber. They were drug-free re-exposed to the apparatus after 24 h (extinction session 2) and the time percentage of freezing behaviour was registered again. Both extinction sessions lasted 10 min.

After experiments, rats were deeply anesthetized with chloral hydrate (150 mg/kg) and microinjected, with the same needle previously used, with Evan Blue dye (0.2 μl, 3%) to stain the microinjection site placement. They were perfused transcardially with saline (NaCl 0.9%) and paraformaldehyde (10%) during 10 min each. Brains were removed from the skulls and stored in little vials filled up with 4% paraformaldehyde solution (wt/vol) after decapitation. At least 24 h before sectioning, brains were transferred to a 30% sucrose solution (wt/vol) for cryoprotection. A cryostat (Leica CM1850) was used to cut brains into coronal slices (50 μm). The sections were placed on gelatin-coated slides and the microinjection sites delimited by Evans Blue dye were examined in an optical microscopy. Only animals with dye microinjections bilaterally located in the mPFC (infralimbic and ventral border of prelimbic cortex) were included in the statistical analysis.

2.8. Statistical analysis

Student's *t*-test for dependent samples was used to compare mean arterial pressure increases following phenylephrine, before and after prazosin acute treatment. Student's *t*-test for independent samples was used to detect cardiovascular measurement differences between the groups treated with saline or prazosin during three consecutive days. ANOVA repeated measures followed by Duncan's test was used to compare the effects of repeated systemic or intra-mPFC administration of prazosin or the vehicle/PBS on the mean time percentage of freezing behaviour during extinction sessions. One-way ANOVA followed by Duncan's test was used to evaluate differences in the extinction index between the groups. The level of statistical significance adopted was $p < .05$. All analyses were performed using the Statistica software package (Version 8.0, StatSoft®, Tulsa, USA).

3. Results

3.1. Analysis of cardiovascular parameters after acute or repeated administration of prazosin

As illustrated in Fig. 1A, administration of prazosin, 0.5 mg/kg, thirty min before was able to prevent ($p < .05$) increases in MAP induced by crescent doses of phenylephrine (0.6, 2, and 6 μg/kg), with no changes in basal HR and MAP (Fig. 1A, inset). These findings indicated that doses and the injection interval regimen were consistent with behavioural studies. In order to test for drug carryover effect, three repeated days of prazosin treatment (1.5 mg/kg) did not alter cardiovascular parameters nor prevent vasoconstriction induced by phelylephrine administration in the fourth day, discarding residual prazosin presence during last extinction session (Fig. 1B).

3.2. Effects of systemic prazosin treatment in the acquisition and consolidation of contextual conditioned fear extinction

ANOVA repeated measures (treatment vs. extinction session) revealed a significant effect for the factor treatment [$F_{(2,2)} = 3.88$, $p < .05$] and extinction session [$F_{(3,66)} = 52.0$, $p < .00001$] in the parameter time percentage of freezing behaviour in rats systemically injected with vehicle, prazosin 0.1 mg/kg or prazosin 0.5 mg/kg during three repeated days, 30 min before the acquisition of contextual conditioned fear extinction. Subsequent analysis using Duncan's post hoc test revealed a significant ($p < .05$) reduction in the percentage of freezing time from the first to the second extinction session in the vehicle-group, but not in the prazosin-treated groups (Fig. 2A). In addition, an increased percentage ($p < .05$) of freezing time behaviour during the third extinction session was observed in the group administered with prazosin (0.5 mg/kg) when compared to vehicle-control group in the same session (Fig. 2A). In fact, ANOVA followed by Duncan's post hoc test revealed a significant reduction [$F_{(2,22)} = 3.0$; $p < .05$] in the extinction index parameter in this group when compared to vehicle-control group (Fig. 2B).

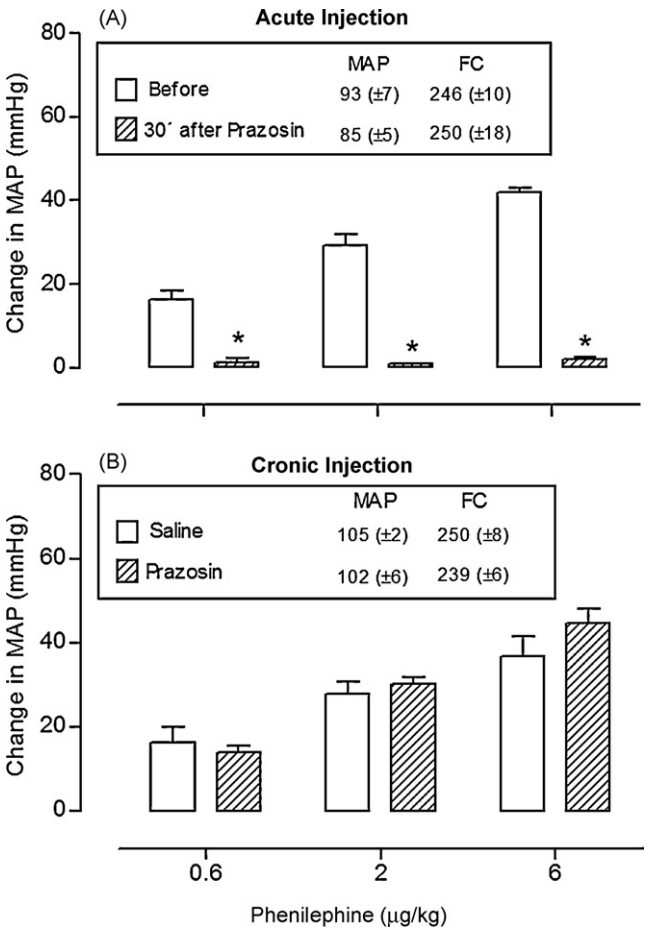

Fig. 1. Mean arterial pressure (MAP) dose–response curve of phenylephrine (0.6, 2, 6 μg/kg, IV, 30 min after) in anaesthetized rats pre-treated with prazosin in acute (A; 0.5 mg/kg, IP, 30 min before) or repeated (B; 1.5 mg/kg, three consecutive days) regimen. Inset represents MAP and heart rate basal values (mean ± SEM) before and after prazosin pre-treatment. Data are expressed as mean + SEM. Student's *t*-test. ($N = 4$–5 per group). $^*p < .05$ compared to respective group before treatment.

ANOVA performed on data obtained from the group receiving systemic prazosin treatment immediately after the extinction sessions showed a significant effect only for the factor extinction session [$F_{(3,90)} = 47.08$, $p < .00001$]. Duncan's post hoc test revealed a decrease in the percentage of freezing time behaviour from the first to the third or fourth extinction sessions in all groups independently of the treatment (Fig. 3A). No statistical differences were detected in the extinction index parameter among the distinct groups (Fig. 3B).

3.3. Effects of intra-mPFC microinjection of prazosin in the acquisition of contextual conditioned fear extinction

Overall ANOVA comparing the effects of intra-mPFC microinjection of PBS, prazosin 0.75 or prazosin 2.5 nmol/side, 10 min before extinction session 1, showed a significant effect for the factor extinction session [$F_{(1,28)} = 18.60$, $p < .0001$] and for the interaction between treatment vs. extinction session [$F_{(2,28)} = 6.64$, $p < .01$]. Post

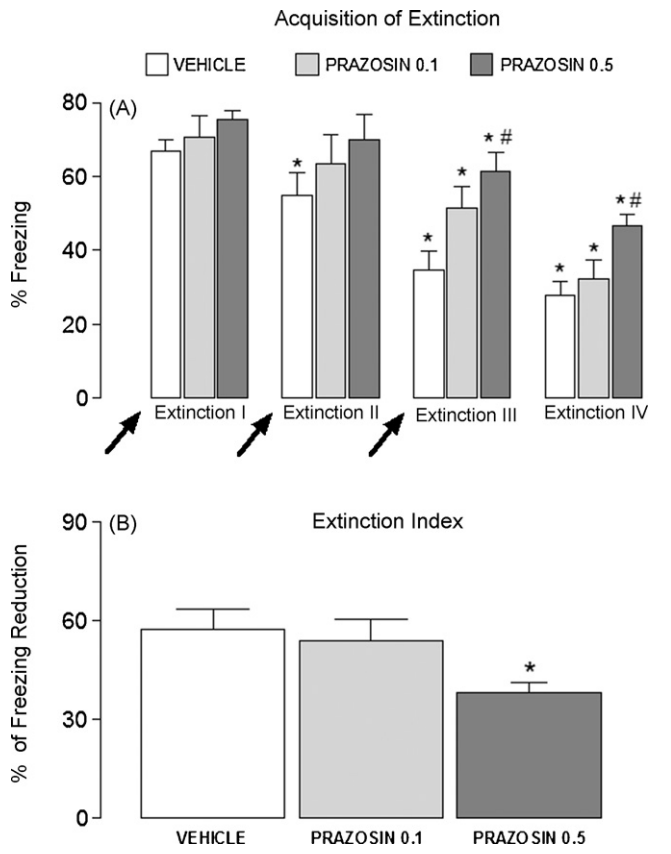

Fig. 2. (A) Effects of repeated injection of prazosin (0.1 or 0.5 mg/kg, IP) or vehicle during acquisition of extinction (30 min before extinction sessions 1, 2 and 3) on the percentage of freezing behaviour in the extinction sessions. $^{*}p < .05$ compared to respective group in the extinction session 1; $^{\#}p < .05$ compared to vehicle-control group in the same extinction session. (B) Extinction index represented by the percentage of reduction in the freezing behaviour time from the first to the last extinction session. $^{*}p < .05$ compared to vehicle-control group. ANOVA followed by Duncan's post hoc test. Data are expressed as mean + SEM ($N = 7$–9 per group). Arrows indicate the moment of prazosin injection.

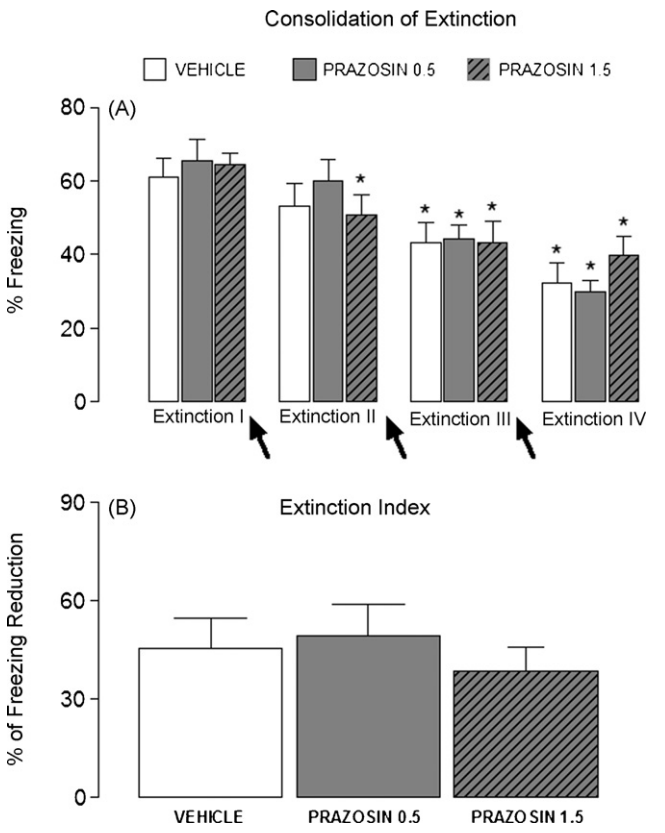

Fig. 3. Percentage of freezing time behaviour after repeated injection of prazosin (0.5 or 1.5 mg/kg, IP) or vehicle during consolidation of extinction (immediately after extinction sessions 1, 2 and 3). (B) Extinction index represented by the percentage of reduction in the freezing behaviour time from the first to the last extinction session. $^{*}p < .05$ compared to respective group in the extinction session 1. ANOVA followed by Duncan's post hoc test. Data are expressed in mean + SEM ($N = 9$–12 per group). Arrows indicate the moment of prazosin injection.

hoc comparisons revealed a significant ($p < .05$) decrease on the percentage of freezing behaviour from the first to the second extinction session in the PBS-control group, indicating that two extinction sessions of 10 min (24 h intercalated) were enough to initiate contextual fear conditioning extinction (Fig. 4A). This reduction in freezing behaviour was not observed when the subjects were microinjected with prazosin 0.75 nmol/side before extinction session 1 (Fig. 4A). In fact, ANOVA followed by Duncan's post hoc test revealed a significant reduction ($F_{(2,28)} = 6.29$; $p < .01$) in the extinction index in this group when compared to PBS-control group (Fig. 4B).

4. Discussion

The present study demonstrated that systemic infusions of the selective alpha-1-adrenergic antagonist, prazosin, before (but not immediately after) extinction sessions were able to impair the extinction of contextual conditioned fear in rats. This disruption in the acquisition of fear extinction was also observed when subjects were microinjected with prazosin directly into the mPFC, a neuroanatomical site significantly related to extinction mechanisms (for review see [57]).

Clinical evidences in humans have shown the effectiveness of prazosin in treating and managing the symptoms of PTSD [21,60,79–81]. These beneficial effects of prazosin seems to be related with its ability in inhibiting adrenergic activity, since symptoms of this disorder have been associated with an increased central nervous system noradrenergic activity [15]. Based on this information, the present study was undertaken with the hypothesis that administering prazosin prior to consecutive re-exposure to the aversive context should decrease defensive behaviour and enhance acquisition of contextual conditioned fear extinction in rats.

The criterion used in the present study to determine the occurrence of extinction was the significant reduction in the freezing response in a determined session when compared to the first extinction session according to previous studies in the literature [6,49]. Based on this criterion, pilot studies performed in our laboratory have shown that three extinction sessions, of 10 min each, were sufficient to reduce the occurrence of freezing in rats. Therefore, an additional day (fourth extinction session) was performed to evaluate the recall of extinction in the absence of drug effects.

Surprisingly, present findings were contrary to our expectation. Repeated systemic injections of prazosin (0.5 mg/kg) increased the percentage of freezing behaviour along the extinction sessions and reduced the extinction index when compared to vehicle-control group. It means that while the percentage of freezing behaviour was significantly reduced from extinction session 1 to extinction session 4 in both vehicle-control group and prazosin 0.1 mg/kg, no changes were visualized on this parameter in the group treated with prazosin 0.5 mg/kg, indicating impairment in the acquisition of fear extinction.

Although prazosin treatment (0.5 mg/kg) before extinction sessions was effective in disrupting the fear extinction process, equal dose of this same compound infused after each extinction session

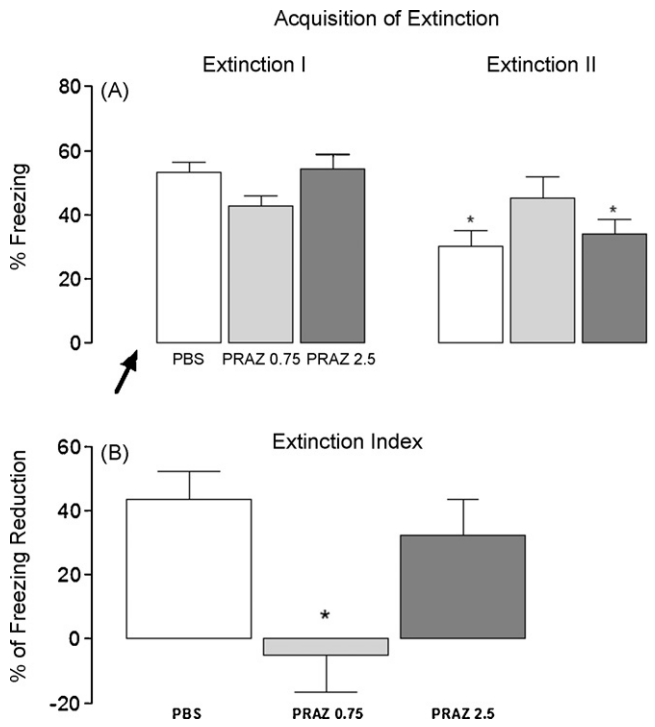

Fig. 4. (A) Percentage of freezing time behaviour after intra-mPFC microinjection of prazosin (0.75 or 2.5 nmol/side) or PBS during acquisition of extinction (10 min before extinction session 1). *$p < .05$ compared to respective group in the extinction session 1; (B) extinction index represented by the percentage of reduction in the freezing behaviour time from the first to the last extinction session. *$p < .05$ compared to PBS-control group (ANOVA followed by Duncan's post hoc test). Data are expressed in mean + SEM ($N = 9$–12 per group). Arrow indicates the moment of drugs infusion.

(consolidation of extinction) failed to interfere with this process. Previous studies have been shown that process involving consolidation mechanisms exhibit a critical time window in which different neurotransmitter and brain structures are required [38,70]. Thus, in the present study, we tested a higher dose of prazosin (1.5 mg/kg IP), which could extend the time for action of this antagonist, favoring the occurrence of carryover effects. However, compared to control group, no effects on freezing behaviour were observed again.

Since systemic injections of prazosin during acquisition of extinction were performed before each extinction session, the increased percentage of freezing visualized in the group treated with prazosin 0.5 mg/kg could suggest both interferences in the spontaneous locomotor activity or anxioselective effects of this compound. However, previous studies involving a behavioural pattern monitor for locomotion evaluation [74] or other distinct models of anxiety [13,16,45] demonstrated that doses of prazosin similar with those used in the present study neither altered the locomotor activity nor the defensive response in rats. Moreover, the increased freezing behaviour visualized in the prazosin-treated group was also observed during the extinction session 4, when rats were drug-free re-exposed to the apparatus. This suggests that, rather than locomotor deficits or anxioselective effects, the alpha-1-adrenergic receptors blockade seems to be involved with cognitive mechanisms responsible by the persistence of fear responses.

Considering that the alpha-1-adrenergic receptors blockade can still induce changes in the cardiovascular pattern, we investigated the effects of acute administration of prazosin in the basal values of HR and MAP. Using anaesthetized rats we demonstrated that prazosin injection (0.5 mg/kg IP; 30 min before) was able to block the MAP increase induced by crescent doses of phenylephrine. This finding suggest that the dose and the interval of injection adopted during the acquisition of extinction were effective in antagonize the alpha-1-adrenergic receptor, without affecting basal cardiovascular values. This blockade of vasoconstrictor effects of phenylephrine was not visualized when subjects were repeatedly treated with prazosin (1.5 mg/kg IP) during three consecutive days and drug-free tested in the fourth day, which excludes any possibility of residual drug presence in significant quantities during the last extinction session.

Although this is an exclusive study evaluating the participation of alpha-1-adrenergic receptors in fear extinction, other studies have shown an essential role for these receptors in different tests involving mnemonic processes [23,56,71]. Riekkinen et al. [67] described impairment in the acquisition of spatial memory in rats pre-treated with prazosin. On the other hand, Manion et al. [36] reported that administration of prazosin before exposure to stressful events was able to blockade the subsequent exaggeration of acoustic startle response in rats. Both findings indicate that alpha-1-adrenergic receptors are essential to mechanisms of learning. In this case, since fear extinction has been suggested as a new inhibitory learning [8], these mentioned studies could support the idea that systemic alpha-1-adrenergic blockade impairs fear extinction mechanisms.

In a subsequent study, the mPFC as a potential neural system mediating the effects of alpha-1-adrenergic receptors in fear extinction, was evaluated. The mPFC, as previously mentioned, has been widely described as a fundamental structure in fear extinction mechanisms [77]. This structure receives substantial afferent connections of noradrenergic fibers from locus coeruleus, the main noradrenergic nucleus of the brain [68], and presents a substantial concentration of alpha-1-adrenergic receptors [19,54]. Thus, intra-mPFC microinjection of alpha-1-adrenergic antagonist prazosin was used to evaluate the involvement of these receptors in the acquisition of fear extinction.

Previous studies carried out in our laboratory have shown that while rats systemically treated with vehicle need approximately three extinction sessions to extinguish, PBS-control group, previously submitted to stereotaxic surgery procedures, spent only two extinction sessions of 10 min to exhibit a significant decrease in the time percentage of freezing. In fact, this finding was confirmed in the present study where PBS-control group, and also the group treated with prazosin 2.5 nmol/side, demonstrated a significant reduction in the percentage of freezing behaviour from the first to the second extinction session. In line with systemic treatment, local microinjection of prazosin (0.75 nmol/side) into the mPFC reduced the extinction index parameter when compared to control-subjects microinjected with PBS, indicating a disruption in the acquisition of fear extinction.

There are some evidences showing that the mPFC modulates the cardiovascular components of the defensive behaviour in a contextual fear paradigm [64,66,84]. While the chemical stimulation of the mPFC was able to elicit a MAP and HR increase [83]; the pharmacological temporary inactivation of this structure reduced these parameters during a contextual retrieval session [66]. These changes in the cardiovascular parameters after mPFC-excitotoxic lesion [25] or mPFC-alpha-1-adrenegic blockade [65] were not observed in non-stressed rats, reinforcing the idea that this structure has no tonic influence on cardiovascular activity. For this reason, and also because intra-mPFC infusion of prazosin did not interfere with freezing behaviour in the first extinction session, changes in the cardiovascular parameters after the alpha-1-adrenergic blockade within the mPFC were not assessed in the present study.

Previous studies have demonstrated that the mPFC projects heavily to the amygdala [37,50], a complex structure that plays an important role in the acquisition and expression of conditioned fear

associations (for review see [34]). This mPFC-neural-pathway targets significantly the intercalated cells of the amygdala, a nucleus of GABAergic neurons located between the central and basolateral portions of the amygdala, which are responsible for feed-forward inhibition of central nuclei output neurons [5,35]. Therefore, the impairment of contextual conditioned fear extinction after the mPFC-alpha-1-adrenergic receptor antagonism observed in the current study may be attributed to a blockade of the mPFC-activation of intercalated cells over the central amygdala, resulting in an exacerbated expression of conditioned fear during the following extinction session. In addition, descending projections from the mPFC to the hippocampus and the mesencephalic periaqueductal gray matter have also been revealed [85]. Since these neural sites are related to the contextual/environmental information of the aversive event [3] and the physiological and behavioural aspects of the conditioned defensive response [86], mPFC-interference over these regions could also be involved in the modulation of contextual conditioned fear extinction [30,39].

The absence of extinction process seen in the group microinjected with the higher dose of prazosin (2.5 nmol/side) into the mPFC may be attributed to the lack of selectivity for this compound when administered in elevated doses. In fact, Regan et al. [62] described that high doses of prazosin can block alpha-2-adrenergic receptors. These receptors, present in the presynaptic terminals, when activated, promote a decrease in the norepinephrine release in the synaptic cleft [32]. Thus, the blockade of alpha-2-adrenergic receptors could induce an increase in noradrenergic neurotransmission, facilitating extinction processes through the activation of vmPFC-beta-1-adrenergic receptors [12,44]. In fact, previous studies have indicated a facilitation (Do-Monte and Carobrez, unpublished results) or impairment [46] in the mechanisms of extinction, respectively after the microinjection of the agonist beta-adrenergic isoproterenol or the beta-blocker propranolol directly into the vmPFC.

Exposure therapy is a set of techniques, based on principles of conditioning and learning, which help patients to confront their feared objects, situation, memories or images in safe circumstances [11]. The repeated exposure to the traumatic memory utilized for PTSD treatment, facilitates extinction of feared emotional response to this memory. Given its well-documented clinical efficacy, this cognitive behavioural therapy approach has been considered the first-line of treatment for PTSD [24,48,61,72]. Despite the abundance of evidence pointing to the efficacy of exposure therapy, PTSD remains a difficult disorder to treat and identifying alternative treatment options is imperative. For this reason, studies are currently evaluating the use of pharmacological intervention to enhance or accelerate the cognitive process involved in this therapy [18,63].

Clinical reports in humans and experimental studies in laboratory animals provide support for the hypothesis that drugs that inhibit the mnemonic processes may impair fear extinction [27,46] while cognitive enhancers can facilitate this mechanism [55,63,87]. Accordingly, the present findings demonstrated that repeated systemic treatment or intra-mPFC infusion of prazosin, which disrupt memory formation in different animal models [67,75,88], also impaired the mechanisms of fear extinction. Considering that prazosin has been widely prescribed for reducing nightmares and sleep disturbance in PTSD patients, a potential detrimental effect of this drug in reducing the beneficial effect of exposure therapy must be further examined.

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References

[1] Adamec R, Muir C, Grimes M, Pearcey K. Involvement of noradrenergic and corticoid receptors in the consolidation of the lasting anxiogenic effects of predator stress. Behav Brain Res 2007;179:192–207.
[2] Adamec RE, Shallow T. Lasting effects on rodent anxiety of a single exposure to a cat. Physiol Behav 1993;54:101–9.
[3] Anagnostaras SG, Gale GD, Fanselow MS. Hippocampus and contextual fear conditioning: recent controversies and advances. Hippocampus 2001;11:8–17.
[4] Anderson AK, Yamaguchi Y, Grabski W, Lacka D. Emotional memories are not all created equal: evidence for selective memory enhancement. Learn Mem 2006;13:711–8.
[5] Berretta S, Pantazopoulos H, Caldera M, Pantazopoulos P, Pare D. Infralimbic cortex activation increases c-Fos expression in intercalated neurons of the amygdala. Neuroscience 2005;132:943–53.
[6] Bitencourt RM, Pamplona FA, Takahashi RN. Facilitation of contextual fear memory extinction and anti-anxiogenic effects of AM404 and cannabidiol in conditioned rats. Eur Neuropsychopharmacol 2008;18:849–59.
[7] Boehnlein JK, Kinzie JD. Pharmacologic reduction of CNS noradrenergic activity in PTSD: the case for clonidine and prazosin. J Psychiatr Pract 2007;13:72–8.
[8] Bouton ME, Westbrook RF, Corcoran KA, Maren S. Contextual and temporal modulation of extinction: behavioral and biological mechanisms. Biol Psychiatry 2006;60:352–60.
[9] Bremner JD, Krystal JH, Southwick SM, Charney DS. Noradrenergic mechanisms in stress and anxiety. I. Preclinical studies. Synapse 1996;23:28–38.
[10] Cahill L, Prins B, Weber M, McGaugh JL. Beta-adrenergic activation and memory for emotional events. Nature 1994;371:702–4.
[11] Cahill SP, Foa EB, Hembree EA, Marshall RD, Nacash N. Dissemination of exposure therapy in the treatment of posttraumatic stress disorder. J Trauma Stress 2006;19:597–610.
[12] Cain CK, Blouin AM, Barad M. Adrenergic transmission facilitates extinction of conditional fear in mice. Learn Mem 2004;11:179–87.
[13] Cecchi M, Khoshbouei H, Morilak DA. Modulatory effects of norepinephrine, acting on alpha 1 receptors in the central nucleus of the amygdala, on behavioral and neuroendocrine responses to acute immobilization stress. Neuropharmacology 2002;43:1139–47.
[14] Cloitre M. Effective psychotherapies for posttraumatic stress disorder: a review and critique. CNS Spectr 2009;14:32–43.
[15] Cukor J, Spitalnick J, Difede J, Rizzo A, Rothbaum BO. Emerging treatments for PTSD. Clin Psychol Rev 2009;29:715–26.
[16] Davis M, Kehne JH, Commissaris RL. Antagonism of apomorphine-enhanced startle by alpha 1-adrenergic antagonists. Eur J Pharmacol 1985;108:233–41.
[17] Davis M, Myers KM, Chhatwal J, Ressler KJ. Pharmacological treatments that facilitate extinction of fear: relevance to psychotherapy. NeuroRx 2006;3:82–96.
[18] Davis M, Ressler K, Rothbaum BO, Richardson R. Effects of D-cycloserine on extinction: translation from preclinical to clinical work. Biol Psychiatry 2006;60:369–75.
[19] Day HE, Campeau S, Watson Jr SJ, Akil H. Distribution of alpha 1a-, alpha 1b- and alpha 1d-adrenergic receptor mRNA in the rat brain and spinal cord. J Chem Neuroanat 1997;13:115–39.
[20] Delgado MR, Nearing KI, Ledoux JE, Phelps EA. Neural circuitry underlying the regulation of conditioned fear and its relation to extinction. Neuron 2008;59:829–38.
[21] Dierks MR, Jordan JK, Sheehan AH. Prazosin treatment of nightmares related to posttraumatic stress disorder. Ann Pharmacother 2007;41:1013–7.
[22] Do Monte FH, Canteras NS, Fernandes D, Assreuy J, Carobrez AP. New perspectives on beta-adrenergic mediation of innate and learned fear responses to predator odor. J Neurosci 2008;28:13296–302.
[23] Ferry B, Roozendaal B, McGaugh JL. Involvement of alpha1-adrenoceptors in the basolateral amygdala in modulation of memory storage. Eur J Pharmacol 1999;372:9–16.
[24] Foa EB. Psychosocial therapy for posttraumatic stress disorder. J Clin Psychiatry 2006;67(Suppl. 2):40–5.
[25] Frysztak RJ, Neafsey EJ. The effect of medial frontal cortex lesions on cardiovascular conditioned emotional responses in the rat. Brain Res 1994;643:181–93.
[26] Garakani A, Mathew SJ, Charney DS. Neurobiology of anxiety disorders and implications for treatment. Mt Sinai J Med 2006;73:941–9.
[27] Hart G, Harris JA, Westbrook RF. Systemic or intra-amygdala injection of a benzodiazepine (midazolam) impairs extinction but spares re-extinction of conditioned fear responses. Learn Mem 2009;16:53–61.
[28] Heim C, Nemeroff CB. Neurobiology of posttraumatic stress disorder. CNS Spectr 2009;14:13–24.
[29] Hugues S, Garcia R. Reorganization of learning-associated prefrontal synaptic plasticity between the recall of recent and remote fear extinction memory. Learn Mem 2007;14:520–4.
[30] Ji J, Maren S. Hippocampal involvement in contextual modulation of fear extinction. Hippocampus 2007;17:749–58.

[31] Kazui H, Mori E, Hashimoto M, Hirono N. Enhancement of declarative memory by emotional arousal and visual memory function in Alzheimer's disease. J Neuropsychiatry Clin Neurosci 2003;15:221–6.
[32] Langer SZ, Briley MS, Raisman R. Regulation of neurotransmission through presynaptic receptors and other mechanisms: possible clinical relevance and therapeutic potential. Adv Biochem Psychopharmacol 1980;21:203–12.
[33] Laurent V, Westbrook RF. Inactivation of the infralimbic but not the prelimbic cortex impairs consolidation and retrieval of fear extinction. Learn Mem 2009;16:520–9.
[34] LeDoux JE. Emotion circuits in the brain. Annu Rev Neurosci 2000;23:155–84.
[35] Likhtik E, Popa D, Apergis-Schoute J, Fidacaro GA, Pare D. Amygdala intercalated neurons are required for expression of fear extinction. Nature 2008;454:642–5.
[36] Manion ST, Gamble EH, Li H. Prazosin administered prior to inescapable stressor blocks subsequent exaggeration of acoustic startle response in rats. Pharmacol Biochem Behav 2007;86:559–65.
[37] McDonald AJ, Mascagni F, Guo L. Projections of the medial and lateral prefrontal cortices to the amygdala: a Phaseolus vulgaris leucoagglutinin study in the rat. Neuroscience 1996;71:55–75.
[38] McGaugh JL. Memory—a century of consolidation. Science 2000;287:248–51.
[39] McNally GP, Pigg M, Weidemann G. Opioid receptors in the midbrain periaqueductal gray regulate extinction of pavlovian fear conditioning. J Neurosci 2004;24:6912–9.
[40] McNally RJ. Mechanisms of exposure therapy: how neuroscience can improve psychological treatments for anxiety disorders. Clin Psychol Rev 2007;27:750–9.
[41] Milad MR, Rauch SL, Pitman RK, Quirk GJ. Fear extinction in rats: implications for human brain imaging and anxiety disorders. Biol Psychol 2006;73:61–71.
[42] Miller LJ. Prazosin for the treatment of posttraumatic stress disorder sleep disturbances. Pharmacotherapy 2008;28:656–66.
[43] Miranda MA, Ferry B, Ferreira G. Basolateral amygdala noradrenergic activity is involved in the acquisition of conditioned odor aversion in the rat. Neurobiol Learn Mem 2007;88:260–3.
[44] Morris RW, Bouton ME. The effect of yohimbine on the extinction of conditioned fear: a role for context. Behav Neurosci 2007;121:501–14.
[45] Motta V, Maisonnette S, Morato S, Castrechini P, Brandao ML. Effects of blockade of 5-HT2 receptors and activation of 5-HT1A receptors on the exploratory activity of rats in the elevated plus-maze. Psychopharmacology (Berl) 1992;107:135–9.
[46] Mueller D, Porter JT, Quirk GJ. Noradrenergic signaling in infralimbic cortex increases cell excitability and strengthens memory for fear extinction. J Neurosci 2008;28:369–75.
[47] Myers KM, Davis M. Mechanisms of fear extinction. Mol Psychiatry 2007;12:120–50.
[48] Nacasch N, Foa EB, Fostick L, Polliack M, Dinstein Y, Tzur D, et al. Prolonged exposure therapy for chronic combat-related PTSD: a case report of five veterans. CNS Spectr 2007;12:690–5.
[49] Pamplona FA, Prediger RD, Pandolfo P, Takahashi RN. The cannabinoid receptor agonist WIN 55,212-2 facilitates the extinction of contextual fear memory and spatial memory in rats. Psychopharmacology (Berl) 2006;188:641–9.
[50] Pare D, Quirk GJ, Ledoux JE. New vistas on amygdala networks in conditioned fear. J Neurophysiol 2004;92:1–9.
[51] Pavlov IP. Conditioned reflex. London: Oxford University Press; 1927.
[52] Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 3rd ed. Sidney: Academic; 1997.
[53] Peters J, Kalivas PW, Quirk GJ. Extinction circuits for fear and addiction overlap in prefrontal cortex. Learn Mem 2009;16:279–88.
[54] Pieribone VA, Nicholas AP, Dagerlind A, Hokfelt T. Distribution of alpha 1 adrenoceptors in rat brain revealed by in situ hybridization experiments utilizing subtype-specific probes. J Neurosci 1994;14:4252–68.
[55] Powers MB, Smits JA, Otto MW, Sanders C, Emmelkamp PM. Facilitation of fear extinction in phobic participants with a novel cognitive enhancer: a randomized placebo controlled trial of yohimbine augmentation. J Anxiety Disord 2009;23:350–6.
[56] Puumala T, Greijus S, Narinen K, Haapalinna A, Riekkinen Sr P, Sirvio J. Stimulation of alpha-1 adrenergic receptors facilitates spatial learning in rats. Eur Neuropsychopharmacol 1998;8:17–26.
[57] Quirk GJ, Garcia R, Gonzalez-Lima F. Prefrontal mechanisms in extinction of conditioned fear. Biol Psychiatry 2006;60:337–43.
[58] Quirk GJ, Mueller D. Neural mechanisms of extinction learning and retrieval. Neuropsychopharmacology 2008;33:56–72.
[59] Raskind MA, Peskind ER, Hoff DJ, Hart KL, Holmes HA, Warren D, et al. A parallel group placebo controlled study of prazosin for trauma nightmares and sleep disturbance in combat veterans with post-traumatic stress disorder. Biol Psychiatry 2007;61:928–34.
[60] Raskind MA, Peskind ER, Kanter ED, Petrie EC, Radant A, Thompson CE, et al. Reduction of nightmares and other PTSD symptoms in combat veterans by prazosin: a placebo-controlled study. Am J Psychiatry 2003;160:371–3.
[61] Rauch SA, Grunfeld TE, Yadin E, Cahill SP, Hembree E, Foa EB. Changes in reported physical health symptoms and social function with prolonged exposure therapy for chronic posttraumatic stress disorder. Depress Anxiety 2009;26:732–8.
[62] Regan JW, Kobilka TS, Yang-Feng TL, Caron MG, Lefkowitz RJ, Kobilka BK. Cloning and expression of a human kidney cDNA for an alpha 2-adrenergic receptor subtype. Proc Natl Acad Sci USA 1988;85:6301–5.
[63] Ressler KJ, Rothbaum BO, Tannenbaum L, Anderson P, Graap K, Zimand E, et al. Cognitive enhancers as adjuncts to psychotherapy: use of D-cycloserine in phobic individuals to facilitate extinction of fear. Arch Gen Psychiatry 2004;61:1136–44.
[64] Resstel LB, Correa FM. Involvement of the medial prefrontal cortex in central cardiovascular modulation in the rat. Auton Neurosci 2006;126/127:130–8.
[65] Resstel LB, Fernandes KB, Correa FM. alpha-Adrenergic and muscarinic cholinergic receptors are not involved in the modulation of the parasympathetic baroreflex by the medial prefrontal cortex in rats. Life Sci 2005;77:1441–51.
[66] Resstel LB, Joca SR, Guimaraes FG, Correa FM. Involvement of medial prefrontal cortex neurons in behavioral and cardiovascular responses to contextual fear conditioning. Neuroscience 2006;143:377–85.
[67] Riekkinen M, Stefanski R, Kuitunen J, Riekkinen Jr P. Effects of combined block of alpha 1-adrenoceptors and NMDA receptors on spatial and passive avoidance behavior in rats. Eur J Pharmacol 1996;300:9–16.
[68] Robbins TW. Cortical noradrenaline, attention and arousal. Psychol Med 1984;14:13–21.
[69] Rothbaum BO, Davis M. Applying learning principles to the treatment of posttrauma reactions. Ann N Y Acad Sci 2003;1008:112–21.
[70] Sara SJ, Roullet P, Przybyslawski J. Consolidation of memory for odor-reward association: beta-adrenergic receptor involvement in the late phase. Learn Mem 1999;6:88–96.
[71] Scheiderer CL, Dobrunz LE, McMahon LL. Novel form of long-term synaptic depression in rat hippocampus induced by activation of alpha 1 adrenergic receptors. J Neurophysiol 2004;91:1071–7.
[72] Schnurr PP, Friedman MJ, Engel CC, Foa EB, Shea MT, Chow BK, et al. Cognitive behavioral therapy for posttraumatic stress disorder in women: a randomized controlled trial. JAMA 2007;297:820–30.
[73] Segal SK, Cahill L. Endogenous noradrenergic activation and memory for emotional material in men and women. Psychoneuroendocrinology 2009;34:1263–71.
[74] Selken J, Nichols DE. Alpha1-adrenergic receptors mediate the locomotor response to systemic administration of (+/−)-3,4-methylenedioxymethamphetamine (MDMA) in rats. Pharmacol Biochem Behav 2007;86:622–30.
[75] Sirvio J, MacDonald E. Central alpha1-adrenoceptors: their role in the modulation of attention and memory formation. Pharmacol Ther 1999;83:49–65.
[76] Site. National Institute of Mental Health. www.nimh.nih.gov.
[77] Sotres-Bayon F, Cain CK, LeDoux JE. Brain mechanisms of fear extinction: historical perspectives on the contribution of prefrontal cortex. Biol Psychiatry 2006;60:329–36.
[78] Strawn JR, Geracioti Jr TD. Noradrenergic dysfunction and the psychopharmacology of posttraumatic stress disorder. Depress Anxiety 2008;25:260–71.
[79] Taylor FB, Martin P, Thompson C, Williams J, Mellman TA, Gross C, et al. Prazosin effects on objective sleep measures and clinical symptoms in civilian trauma posttraumatic stress disorder: a placebo-controlled study. Biol Psychiatry 2008;63:629–32.
[80] Taylor HR, Freeman MK, Cates ME. Prazosin for treatment of nightmares related to posttraumatic stress disorder. Am J Health Syst Pharm 2008;65:716–22.
[81] Thompson CE, Taylor FB, McFall ME, Barnes RF, Raskind MA. Nonnightmare distressed awakenings in veterans with posttraumatic stress disorder: response to prazosin. J Trauma Stress 2008;21:417–20.
[82] Tronson NC, Taylor JR. Molecular mechanisms of memory reconsolidation. Nat Rev Neurosci 2007;8:262–75.
[83] Verberne AJ. Medullary sympathoexcitatory neurons are inhibited by activation of the medial prefrontal cortex in the rat. Am J Physiol 1996;270:R713–9.
[84] Verberne AJ, Owens NC. Cortical modulation of the cardiovascular system. Prog Neurobiol 1998;54:149–68.
[85] Vertes RP. Differential projections of the infralimbic and prelimbic cortex in the rat. Synapse 2004;51:32–58.
[86] Vianna DM, Landeira-Fernandez J, Brandao ML. Dorsolateral and ventral regions of the periaqueductal gray matter are involved in distinct types of fear. Neurosci Biobehav Rev 2001;25:711–9.
[87] Wrubel KM, Barrett D, Shumake J, Johnson SE, Gonzalez-Lima F. Methylene blue facilitates the extinction of fear in an animal model of susceptibility to learned helplessness. Neurobiol Learn Mem 2007;87:209–17.
[88] Zarrindast MR, Hadi M, Homayoun H, Postami P, Shafaghi B, Khavandgar S. The role of alpha-adrenoceptors in the amnestic effect of intracerebroventricular dexamethasone. Pharmacol Res 2002;46:339–44.