



Short communication

Systemic or intra-prelimbic cortex infusion of prazosin impairs fear memory reconsolidation

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HIGHLIGHTS

- ▶ Post-retrieval infusion of prazosin impairs subsequent retrieval of fear memory.
- ▶ Impairment in fear memory induced by prazosin is dependent of memory reactivation.
- ▶ Impairment in fear memory induced by prazosin does not spontaneously recover with time.
- ▶ Intra-prelimbic cortex infusion of prazosin impairs fear memory reconsolidation.

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ABSTRACT

The alpha-1 adrenergic antagonist prazosin has been used to alleviate the symptoms of PTSD, but the mechanism remains unclear. One possibility is that prazosin may disrupt fear memory reconsolidation, leading to attenuation of fear responses. To test this hypothesis, we administered a single systemic injection of prazosin during the reconsolidation of olfactory fear conditioning in rats. We found that a post-retrieval injection of prazosin disrupted subsequent retrieval of fear. Similarly, intra-prelimbic cortex infusion of prazosin during the reconsolidation period also disrupted subsequent retrieval of fear. These findings suggest that fear memory undergoes reconsolidation through activation of alpha-1 adrenergic receptors in the prelimbic cortex.

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Fear learning is an essential ability for survival in a threatening environment. However, dysregulation of fear circuits may cause the persistence of fear responses resulting in excessive fear and anxiety [1]. Recent research has focused in developing strategies for suppressing these maladaptive fear responses [2]. Currently, the extinction based therapy is considered one of the most common treatments for anxiety disorders such as post-traumatic stress disorder (PTSD) [3,4]. However, it has the inconvenient that fear memories may reappear with the passage of time or may reinstate toward stressful situations [5]. Pioneer studies have demonstrated that during a short period after fear retrieval, previously consolidated fear memories can become labile and subjective to pharmacological interference, particularly by drugs that inhibit the molecular mechanism involved in memory formation [6–8]. This disruptive process initiated after memory retrieval is referred to as “reconsolidation blockade”, and has been suggested

as a novel therapeutic strategy to reduce fear in PTSD patients with the promise of avoiding the reappearance of fear [9,10].

The noradrenergic system has long been involved in the modulation of emotional memories [see 11 for a review]. Several clinical studies in humans have demonstrated the efficacy of alpha-1 adrenergic antagonist prazosin in alleviating the symptoms of PTSD [12–15], but the mechanism involved in the attenuation of fear responses remains to be elucidated. One possible explanation could be that prazosin facilitates the mechanisms of fear extinction. However, contrary to this assumption, previous studies in rodents have demonstrated that the blockade of alpha-1 adrenergic receptor with prazosin disrupts the mechanisms of fear extinction [16,17]. Another possibility is that prazosin treatment would disrupt the reconsolidation of fear memories, leading to a reduction in PTSD symptoms, but no study has addressed the role of alpha-1 adrenergic receptors in fear memory reconsolidation.

Since olfaction is the most important sensory system in rodents [18], fear conditioning toward olfactory stimuli results in robust and long-lasting fear responses [19,20]. In the present study, we took advantage of the olfactory fear conditioning paradigm in rats to investigate the role of alpha-1 adrenergic receptors in fear memory reconsolidation. We then focused in the medial prefrontal cortex (mPFC) as a potential substrate mediating the effects of

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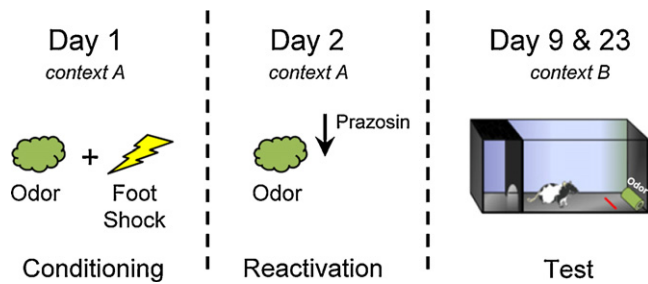


Fig. 1. Schematic representation of the olfactory fear conditioning paradigm. On day 1, rats received 5 pairings of amyl acetate odor and electrical footshock during a 4 min session in context A. On day 2 (reactivation session), animals were returned to the same conditioning chamber in the presence of amyl acetate for 2 min (without footshock) and were systemically or intra-mPFC injected with vehicle or prazosin immediately after being removed from the chamber. Additional control groups were fear conditioned as previously mentioned and received vehicle or prazosin injections in the home cage during day 2 (not reactivated). The retention of olfactory fear conditioning was assessed in a different context (context B) one week later (test) or three weeks (spontaneous recovery) after reactivation, in which a cloth impregnated with the conditioned odor amyl acetate was used as an odor source. We measured the percentage of time rats spent hiding in a different compartment away from the conditioned odor cloth (compartment on the left) and the percentage of time rats spent close to the odor cloth (drawing of cloth on right with red line indicating 7 cm from odor cloth).

prazosin, since this brain region has been shown to be essential for the encoding of olfactory-based emotional learning [21,22].

Male Long-Evans rats were fear conditioned in a conditioning chamber (context A) saturated with amyl acetate odor receiving 5 electrical footshock (day 1). The next day, subjects were returned to the same chamber in the presence of the conditioned odor for a 2 min reactivation session, which was immediately followed by a systemic or intra-mPFC injection of vehicle or prazosin. The retention of olfactory fear conditioning was assessed one week later in an odor box (context B), where rats were initially habituated in the absence of the conditioned odor. On the next day (test) and two weeks later (spontaneous recovery test), a cloth impregnated with the conditioned odor was used as an odor source in one side of the chamber. A one week period between reactivation and test was kept to ensure that prazosin or any active metabolites were completely cleared out during test (Fig. 1; see supplemental material for more details).

The percentage of time spent freezing was used as a memory retention parameter during the reactivation session on day 2. The following behavioral responses were measured during the exposure to the context B: the percentage of hide time (time hiding in a different compartment away from the conditioned odor cloth) and the percentage of approach time (time when rats are within 7 cm of the odor cloth). The freezing response during conditioned odor presentation in context B was not significantly high (less than 10% of the time) compared to other studies using freezing as a measure of fear. Therefore, freezing behavior was not considered in the context B. In fact, previous studies in rats have demonstrated that freezing is elicited only in situations where a flight route is not available or when rats are not able to maintain a defensive distance from the threatening stimulus [23].

In the first set of experiments, rats were randomly divided to receive a single systemic injection of vehicle (Veh, $n = 10$) or prazosin (Pz, 1.5 mg/Kg; $n = 10$) immediately after the reactivation session. Two additional groups that were not submitted to the reactivation session received vehicle ($n = 8$) or prazosin (1.5 mg/kg; $n = 8$) and remained in the home cage. A student's *t*-test for independent samples did not reveal differences in freezing time during the reactivation session between groups (Veh: 54%; Pz: 51%, $t_{20} = 0.24$; $p = 0.81$), suggesting that both groups acquired the same levels of fear conditioning. A one-way ANOVA performed

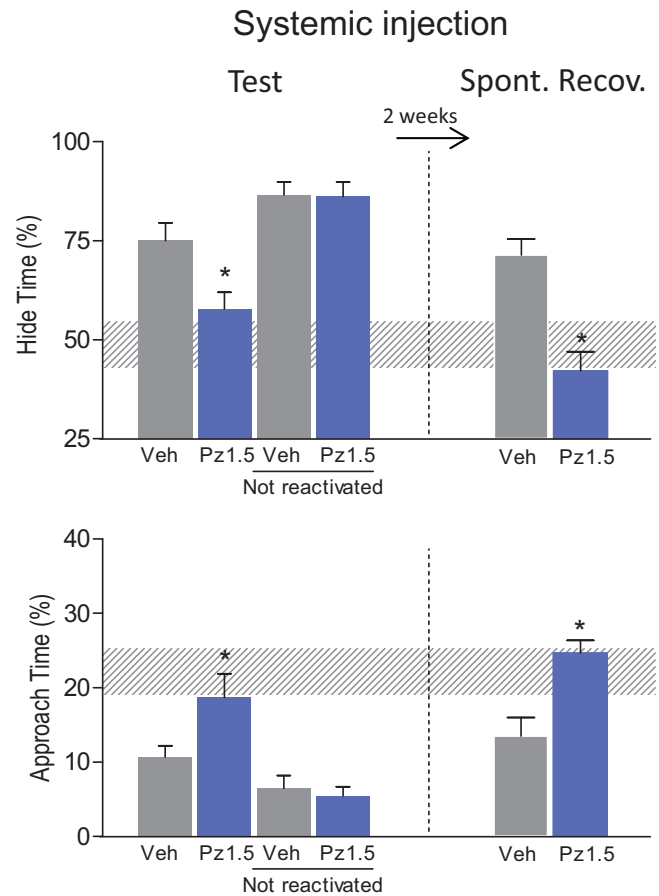


Fig. 2. Systemic post-retrieval infusion of prazosin disrupts fear memory reconsolidation. A single injection of prazosin (1.5 mg/Kg) immediately after reactivation session impaired subsequent retrieval of olfactory fear memory, as indicated by a significant reduction in the percentage of hide time and an increase in the percentage of approach time. This effect was dependent of fear reactivation, since prazosin treated rats that did not receive fear reactivation exhibited levels of fear similar to vehicle group. When re-tested in context B two weeks later, prazosin treated rats still showed impairment in fear retrieval, indicating that fear did not spontaneously recover three weeks after disrupting reconsolidation. Hatched horizontal bars represent the mean and the confidence limits ($\pm 95\%$) for all the subjects during the familiarization session in context B. * $p < 0.05$ compared to vehicle group. One way ANOVA followed by Tukey's test. Data are expressed as mean \pm SEM ($n = 8-10$ per group).

during a familiarization session in context B did not reveal statistical differences between the groups in the parameters hide time ($F_{(3,36)} = 0.6$; $p = 0.56$) and approach time ($F_{(3,36)} = 1.39$; $p = 0.26$). Thus, data obtained during the familiarization session for all groups were merged and expressed as the mean and the confidence limits ($\pm 95\%$) for all the subjects (see Fig. 2, hatched horizontal bars). In general, rats spent $\sim 50\%$ of the time in the hide compartment and $\sim 50\%$ of the time in the opened area, indicating that they did not generalize the olfactory conditioned fear when exposed to the novel context.

However, during the odor test, an ANOVA overall comparison revealed a significant treatment effect between the groups for the parameters hide time ($F_{(3,32)} = 11.23$; $p = 0.00003$) and approach time ($F_{(3,32)} = 9.59$; $p = 0.0001$). Further analysis using Tukey's test revealed a significant ($p < 0.05$) effect in the reactivated group treated with prazosin when compared to the vehicle group (see Fig. 2). Prazosin-treated rats showed a decreased time in the hide box and an increased time spent near the conditioned odor source, suggesting that post-reactivation blockade of alpha-1 adrenergic receptors impaired the retrieval of olfactory fear conditioning one week later. Contrary, non-reactivated rats treated with the same

dose of prazosin did not show impairment in the retention of olfactory fear when compared to non-reactivated control group (all p 's > 0.05), demonstrating that the disruptive effect of prazosin depends on prior memory reactivation.

Evidences from literature have shown that memories may follow two distinct processes after being triggered by memory retrieval: extinction or reconsolidation. Differences between these processes seem to be dependent on the retrieval time: while short retrieval sessions lead to reconsolidation, long retrieval sessions lead to extinction mechanisms [24–26]. Although previous studies have demonstrated that a short reactivation session promotes fear reconsolidation instead of fear extinction, it is possible that, under our experimental conditions, prazosin is reducing fear retrieval by facilitating the mechanisms of fear extinction. It has been shown that spontaneous recovery of fear occurs over the course of few weeks after fear memory extinction [27]. Therefore, to investigate if fear memory would reappear with the passage of time, rats were re-tested in context B two weeks later.

Our results showed that prazosin pretreatment reduced the hide time ($t_{20} = 4.6$; $p = 0.005$) and increased the approach time ($t_{20} = -3.61$; $p = 0.003$) during the spontaneous recovery test performed two weeks later, when compared to vehicle control group (Fig. 2). These findings suggest that prazosin impairs the reconsolidation of olfactory fear, since fear memory fail to re-emerge three weeks following treatment. Another explanation could be that prazosin-treated rats spent more time around the odor source during the first test, which would result in some extinction of the shock-odor association and consequent reduction of fear during the following test. However, it is little likely since previous findings from our laboratory showed that rats restricted close to the conditioned odor needed more than three extinction sessions of ten minutes to extinguish fear (unpublished data).

The present findings agree with a previous study in which blockade of alpha-1 adrenergic receptors during the post-retrieval period disrupted memory reconsolidation in a paradigm involving drug-associated cues in rats [28]. In a recent study, Olson et al. (2011) showed that prazosin reduced the expression of fear potentiated startle, aggression and social interaction in mice previously exposed to a traumatic experience of stress [29]. Our results, however, provide the first evidence that post-retrieval blockade of alpha-1 adrenergic receptors may result in disruption of the fear memory reconsolidation.

Activation of alpha-1 adrenergic receptors triggers an intracellular cascade that results in increased levels of the enzyme protein kinase C (PKC). It has been recently shown that many PKC substrates are involved in the biochemical pathways that are critical for memory reconsolidation [30]. Therefore, reduction in neuronal PKC levels after prazosin could explain the disruption of fear memory reconsolidation observed in the present study.

Among brain regions implicated in the control of aversive emotional states, the mPFC, more specifically the prelimbic (PL) subregion, has been shown to be an essential neural site for the acquisition of olfactory fear conditioning [31]. Previous neuroanatomical studies have demonstrated that PL is strongly interconnected with the basolateral nucleus of the amygdala [32,33], another key structure in the aversive association that occurs between footshock and olfactory stimulus [34]. In addition, the mPFC, including PL, has a substantial concentration of alpha-1 adrenergic receptors [35], which makes this region a potential neural substrate mediating the disruptive effects of prazosin on fear reconsolidation.

We started investigating this possibility by injecting vehicle PBS ($n = 8$), prazosin 0.3 μg ($n = 6$) or prazosin 0.6 μg ($n = 6$) directly into PL immediately after the reactivation session. A one-way ANOVA comparing the percentage of time freezing during the reactivation session did not reveal significant differences between groups (Veh:

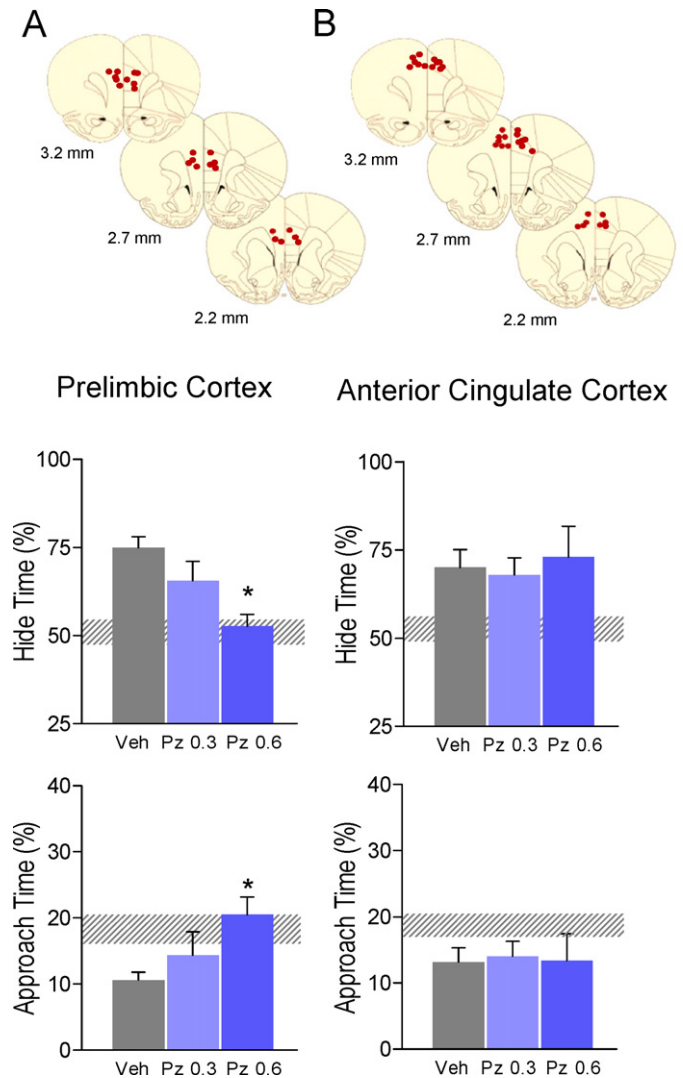


Fig. 3. Intra-prelimbic cortex infusion of prazosin disrupts fear memory reconsolidation. Post-retrieval infusion of prazosin (0.6 $\mu\text{g}/\text{side}$) into the prelimbic cortex (A), but not into the anterior cingulate cortex (B), impaired subsequent retrieval of olfactory fear memory, as indicated by a significant reduction in the percentage of hide time and an increase in the percentage of approach time. Hatched horizontal bars represent the mean and the confidence limits ($\pm 95\%$) for all the subjects during the familiarization session in context B. * $p < 0.05$ compared to vehicle group. One way ANOVA followed by Tukey's test. Data are expressed as mean \pm SEM ($n = 6$ –12 per group).

63%; Pz 0.3 = 74%; Pz 0.6 = 72%, $F_{(2,17)} = 1.8$; $p = 0.18$), suggesting that all groups acquired the same levels of fear conditioning. Likewise, no statistical differences were observed between the groups in the parameters hide time ($F_{(2,17)} = 0.3$; $p = 0.70$) and approach time ($F_{(2,17)} = 0.6$; $p = 0.55$) during the familiarization in the context B. Similar to the previous experiment, group data obtained during the familiarization day was merged and expressed as the mean and the confidence limits ($\pm 95\%$) for all the subjects (see Fig. 3A, hatched horizontal bars).

During the odor test, an ANOVA overall comparison showed a significant treatment effect between the groups for the parameters hide time ($F_{(2,17)} = 7.8$; $p = 0.002$) and approach time ($F_{(2,17)} = 4.39$; $p = 0.02$). Further analysis using Tukey's test revealed a significant ($p < 0.05$) effect in the group treated with prazosin 0.6 μg in the above parameters when compared to the vehicle control group (see Fig. 3A). Rats treated with an intra-PL infusion of prazosin 0.6 μg showed a decreased time in the hide box and an increased time spent near the conditioned odor source, indicating that

post-reactivation blockade of PL- α -1 adrenergic receptors impaired the retrieval of olfactory fear conditioning one week later. These data suggest that fear memory undergoes reconsolidation through activation of α -1 adrenergic receptors in PL, and that prazosin impairs this process.

Drug backflow along the cannula track can occur during intracerebral infusion resulting in non-specific targeting effects. We therefore investigated if the anterior cingulate cortex (ACC), another subregion of the mPFC located immediately above PL, could also be mediating the disruptive effects of prazosin on fear memory. To test this, another group of rats was microinjected with either vehicle PBS ($n=12$), prazosin 0.3 μ g ($n=10$) or prazosin 0.6 μ g ($n=7$) within the ACC immediately after the reactivation session. A one-way ANOVA did not reveal significant differences between the groups in the percentage of time freezing during the reactivation session (Veh: 52%; Pz 0.3: 63%; Pz 0.6: 60%, $F_{(2,26)}=0.48$; $p=0.62$). In addition, no statistical differences were observed between the groups in the parameters hide time ($F_{(2,26)}=1.05$; $p=0.36$) and approach time ($F_{(2,26)}=0.39$; $p=0.67$) during the familiarization session in context B. Thus, group data obtained during familiarization day was again merged and expressed as the mean and the confidence limits ($\pm 95\%$) for all the subjects (see Fig. 3B, hatched horizontal bars).

Contrary to intra-PL infusion of prazosin (0.6 μ g), intra-ACC infusion of prazosin at both doses (0.3 μ g or 0.6 μ g) did not affect the hide time ($F_{(2,26)}=0.15$; $p=0.85$) and the approach time ($F_{(2,26)}=0.03$; $p=0.97$) during the odor test, when compared to vehicle control group (Fig. 3B). One could argue that the lack of effect in fear reconsolidation after intra-ACC infusion of prazosin is due to a fewer number of α -1 adrenergic receptors in this area. However, the concentration of α -1 adrenergic receptors in the ACC is comparable to those described in PL [35]. A recent study showed that infusion of protein synthesis inhibitor anisomycin into the ACC blocked fear reconsolidation [36]. Differences between our study and Einarsson and Nader (2012) may be attributed to differences between the conditioned stimuli, since we used an olfactory cue, and they used a contextual cue. Nevertheless, both studies suggest that fear reconsolidation occurs in the mPFC, although the specific subregion that is recruited may depend on the conditioned stimuli.

In conclusion, our findings suggest that impairment in fear memory reconsolidation induced by blockade of α -1 adrenergic receptors is specific to PL. This is the first study showing that the PL subregion of the mPFC is part of the neural system modulating fear memory reconsolidation. Taken together, the present results support the idea that the beneficial effects of prazosin treatment in PTSD patients may be due to impairment in fear reconsolidation, rather than facilitation in fear extinction. This speculation is also sustained by previous studies in rodents showing that prazosin treatment impairs the mechanisms of fear extinction [16,17]. In this way, a precise control in the session duration during cognitive behavioral therapy seems to be critical to determine the fear memory progression in patients with anxiety disorders receiving prazosin treatment.

Conflict of interest

The authors declare no conflict of interest.

Contributions

F.H.M., R.R.S. and T.T.W. designed and conducted all experiments. F.H.M. wrote the manuscript. A.P.C. supervised the project. All authors edited and accepted the final version of the manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bbr.2013.01.031>.

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