Clonidine, an α2 adrenergic receptor agonist, disrupts reconsolidation of a cocaine-paired environmental memory

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Environmental cues can elicit robust cocaine reward memories, contributing to relapse to cocaine abuse. Memories can be manipulated pharmacologically by interfering with reconsolidation after reactivation. Clonidine, an α2 noradrenergic receptor agonist, was tested for its ability to block reconsolidation of cocaine environmental-paired memory. Male Sprague-Dawley rats completed an 8-day cocaine place conditioning procedure to establish a cocaine place preference. Cocaine memory was reactivated by exposure to the cocaine-paired environment in a drug-free state, followed immediately by administration of clonidine (10 or 50 μg/kg) or vehicle. Cocaine place preference was retested 24 h and 1 week later. Clonidine significantly attenuated the previously established cocaine place preference when tested 1 or 7 days later. To investigate the generalizability of this effect to other drug classes, morphine conditioned place preference was tested. Clonidine administration after morphine memory reactivation did not significantly alter the expression of morphine place preference. These results suggest that clonidine can interfere with reconsolidation of cocaine memory and may be a useful approach to reduce relapse.

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Introduction

Exposure to environmental cues previously paired with cocaine abuse can trigger extreme levels of craving, drug seeking, and relapse (Stewart, 1992). One strategy to aid in the prevention of relapse is to dampen memories associated with prior cocaine use (Bernardi *et al.*, 2006). Recalled memories undergo a process of reconsolidation (Nader *et al.*, 2000), which can be manipulated pharmacologically, thus disrupting the memory trace (Otis and Mueller, 2011).

Norepinephrine plays an important role in memory reconsolidation, and reducing noradrenergic transmission can interfere with the reconsolidation process (Forget *et al.*, 2009; Gamache *et al.*, 2012). For example, the β-adrenergic receptor antagonist propranolol administered after memory reactivation, can inhibit reconsolidation and disrupt cocaine place preference (Fricks-Gleason and Marshall, 2008). Propranolol also blocks reconsolidation of radial arm memory (Przybyslawski *et al.*, 1999) and fear memory (Przybyslawski *et al.*, 1999; Dębiec and Ledoux, 2004). Although the efficacy of β-adrenergic receptor antagonists to attenuate both appetitive and aversive memory reconsolidation has been established, the role of α2-adrenergic receptors in reconsolidation has not been tested. Clonidine is an α2-adrenergic receptor agonist, and activation of α2 autoreceptors located on presynaptic terminals decreases norepinephrine release (Anderson and Stone, 1974). This study tested the hypothesis that clonidine would interfere with cocaine contextual memories by blocking reconsolidation. Cocaine conditioned place preference was established and the effectiveness of clonidine to attenuate cocaine place memory was analyzed. The effect of clonidine on reconsolidation of morphine contextual memory was tested to determine the generalizability of the effect across classes of drugs of abuse.

Methods

Subjects

Adult male Sprague-Dawley rats (Charles River Laboratories, Wilmington, Massachusetts, USA) were housed on a 12-h light/dark cycle in a humidity and temperature-controlled environment. Except during testing between 09: 00 and 12: 30 h, animals had free access to water and food and were housed two per cage with no enrichment objects. Experiments were completed with an approved protocol from Temple University Institutional Animal Care and Use Committee and in compliance with NIH guidelines for Care and Use of Laboratory Animals.

Conditioned place preference

An unbiased conditioned place preference design similar to our previously published methods (Shi *et al.*, 2014) was used. The conditioned place preference chamber (45 × 20 × 20 cm) contained two distinct sides separated

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by a removable partition; one side had white walls with black horizontal strips and a smooth floor, whereas the other side had white walls and black circles with a rough floor. Rats were weighed and acclimated to the testing room for 30 min before testing. Rats were administered cocaine (10 mg/kg, intraperitoneal) or vehicle and immediately confined to one of the two distinct sides in a randomized manner for 30 min. Conditioning sessions continued for 8 days in a counterbalanced method resulting in four pairing with cocaine and four pairings with vehicle. On day 9, a post-test was conducted in a drug-free state for 30 min to determine place preference. Place preference score was defined as time in drug-paired side minus time in vehicle-paired side. On day 10, rats were placed in the drug-paired side for 10 min to reactivate drug environmental-paired memories. Immediately after re-exposure, clonidine (10 or 50 μg/kg, subcutaneous) or vehicle was administered, and rats returned to home cages. Place preference was tested again 24 h (day 11) and 7 days (day 18) later. In a separate cohort of rats, cocaine place conditioning occurred using the same 8-day conditioning procedure and post-test on day 9. On day 10, animals remained in their home cages and were administered clonidine 50 μg/kg or vehicle. Preference was retested 24 h and 7 days later.

To test the generalizability of the findings to another class of abused drugs, morphine (5 mg/kg, subcutaneous) place conditioning followed the same 8-day procedure, with 40 min conditioning sessions and 20 min post-test sessions. On day 9, morphine place preference was tested. On day 10, clonidine (50 μg/kg) or vehicle was administered after 10 min re-exposure to morphine-paired chamber. Place preference was retested 24 h later. In pilot experiments, the conditioning sessions were optimized for each drug to produce a significant place preference. Dose–response studies indicated that 10 mg/kg cocaine and 5 mg/kg morphine produced the greatest place preference (data not shown) and were chosen for further study.

Drugs

National Institute on Drug Abuse Drug Supply Program (Bethesda, Maryland, USA) generously supplied cocaine hydrochloride and morphine sulfate. Clonidine hydrochloride was purchased from Sigma Aldrich (St Louis, Missouri, USA). All drugs were dissolved in sterile saline and administered at 1 ml/kg body weight. Saline was used as vehicle.

Statistical analyses

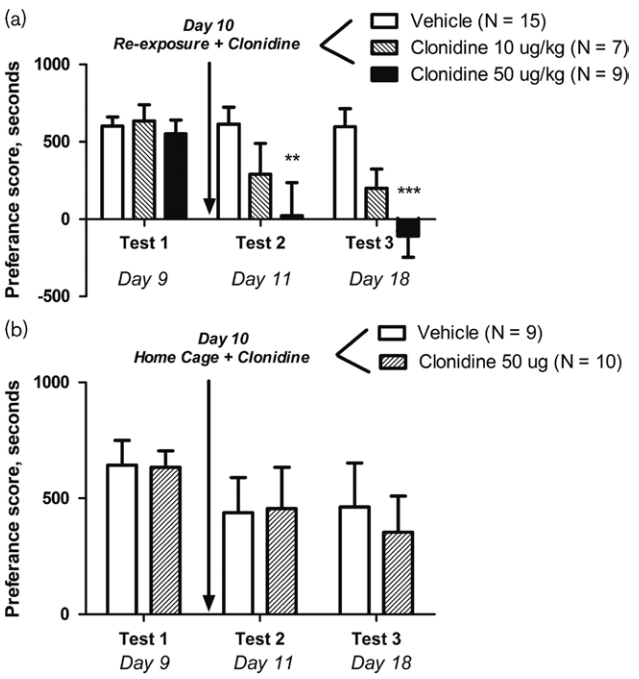
The data were analyzed with GraphPad 5, version 5.01 (GraphPad Software, La Jolla, California, USA) using a factorial repeated-measures analysis of variance (ANOVA) with between-subjects factors of day and drug (clonidine or vehicle) and Bonferroni's post-hoc analysis. The α score was set to 0.05.

Results

Clonidine administration interfered with the maintenance of cocaine conditioned place preference

Conditioned place preference was used to test the effect of clonidine on maintenance of cocaine reward memory. Cocaine place preference was established using an 8-day conditioning protocol with the preference test on day 9. Clonidine (10 or 50 μg/kg) was administered after re-exposure to the chamber previously paired with cocaine on day 10 and place preference was measured 24 h and 7 days later. Place preference scores were expressed as time in cocaine-paired side minus time in vehicle side (Fig. 1a). Using a two-way ANOVA, both main effects of day [$F(2, 56) = 8.07$, $P < 0.001$] and drug [$F(2, 56) = 7.02$, $P > 0.005$] were significant, as was the interaction [$F(4, 56) = 2.89$, $P < 0.05$]. Bonferroni's post-hoc analysis revealed that both groups showed a preference for the

Fig. 1



Effects of clonidine on previously established cocaine conditioned place preference. (a) Rats conditioned with cocaine (10 mg/kg, intraperitoneal) showed a preference to the cocaine-paired chamber when tested on day 9 (test 1). On day 10, rats were re-exposed to the cocaine-paired chamber and administered clonidine (10 or 50 μg/kg) or vehicle immediately thereafter. Place preference was retested 1 or 7 days later. Clonidine (50 μg/kg) mitigated the expression of cocaine place preference on days 11 (test 2) and 18 (test 3) as show by significantly lower preference scores versus vehicle injected rats. Two-way analysis of variance, clonidine versus vehicle on day 11, $^{**}P < 0.01$ and clonidine versus vehicle on day 18, $^{***}P > 0.001$. (b) Rats were conditioned with cocaine as above and showed a place preference to the cocaine chamber on day 9 (test 1). On day 10, clonidine (50 μg/kg) or vehicle was injected in the home cage without memory reactivation. In this case, clonidine had no effect on the established place preference when tested 1 day later (test 2). Data are expressed as mean ± SEM.

cocaine-paired side on day 9 and there was no significant difference between groups. When preference scores on day 9 were expressed as percent of total time on drug-paired side [(time in drug-paired side/total time) × 100], rats spent a mean of 67% (±1.3%) of the total test time on the drug-paired side. Clonidine 50 μg/kg administered after re-exposure to the cocaine-paired side on day 10 significantly decreased time spent on cocaine-paired side when tested on days 11 ($P < 0.01$) and 18 ($P < 0.001$) (Fig. 1a). Place preference scores of rats injected with clonidine 10 μg/kg were not significantly different than those injected with vehicle. This experiment revealed that clonidine (50 μg/kg) administered after memory reactivation on day 10, significantly reduced preference for cocaine-paired environment 24 h and 7 days later.

Re-exposure to cocaine-paired context is required for clonidine to attenuate an established cocaine conditioned place preference

This study determined if re-exposure to the cocaine-paired environment was necessary for clonidine to disrupt an established place preference. Rats underwent cocaine place conditioning followed by a preference test on day 9. On day 10, rats remained in their home cage and received vehicle or clonidine 50 μg/kg by the same schedule as before except without re-exposure to the cocaine-paired chamber. A factorial repeated-measures ANOVA of place preference scores on days 9, 11, and 18 (Fig. 1b) revealed no significant main effects for time [$F(2, 34) = 1.77$, P = NS], drug [$F(1, 34) = 0.05$, P = NS], or interaction [$F(2, 34) = 0.13$, P = NS]. Clonidine (50 μg/kg) administration in the absence of memory reactivation did not alter an established cocaine place preference.

Clonidine administration did not affect morphine conditioned place preference

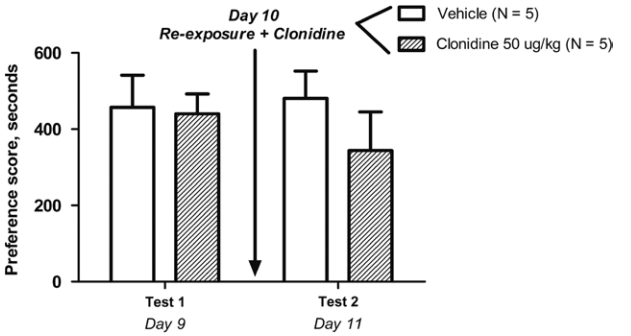
Morphine condition place preference was used to determine if the effects found with clonidine on cocaine contextual memory generalized to another drug class. Morphine (5 mg/kg) produced a place preference on day 9 (Fig. 2). Rats conditioned with morphine spent a mean of 69% (±1.9%) of time on the drug-paired side during preference testing on day 9. Rats received vehicle or clonidine (50 μg/kg) after memory reactivation on day 10 and were retested on day 11. As shown in Fig. 2, a factorial repeated-measures ANOVA revealed no significant differences between clonidine and vehicle groups for the main effects of time [$F(1, 8) = 0.24$, P = NS] and drug [$F(1, 8) = 0.82$, P = NS], or their interaction [$F(1, 8) = 0.67$, $P = 0.43$]. These results reflect no difference between the vehicle and clonidine-treated animals, indicating that clonidine did not interfere with expression of a previously established morphine place preference.

Discussion

A conditioned place procedure was used to determine if clonidine could disrupt memory reconsolidation of environmental cocaine-paired cues. Clonidine dose-dependently attenuated an established cocaine conditioned place preference. Two lines of evidence suggest that clonidine attenuated place preference by interfering with memory reconsolidation (Alberini, 2011; Alberini and LeDoux, 2013). First, clonidine effectively erased cocaine place preference only when administered after recall of the cocaine place memory which was produced by re-exposure to the cocaine-paired environment. When clonidine was administered in the home cage in the absence of re-exposure to the cocaine-paired cues, rats maintained their preference for the cocaine-paired side. Second, there was no spontaneous recovery of cocaine place preference. Even when tested 1 week after a single clonidine administration, place preference was absent while controls maintained preference. Taken together, these data indicate that clonidine can inhibit reconsolidation of cocaine associated memories.

Clonidine, through its actions on the α2-adrenergic autoreceptor, dampens noradrenergic neurotransmission by reducing norepinephrine release. The finding that clonidine can interfere with a cocaine memory trace is in agreement with prior studies using the β-adrenergic receptor antagonist propranolol. Propranolol has been shown to be an amnestic agent under several conditions, and can disrupt the reconsolidation of cocaine contextual memories when administered after memory reactivation (Bernardi *et al.*, 2006; Fricks-Gleason and Marshall, 2008; Otis *et al.*, 2013). Further, the α1-adrenergic antagonist, prazosin, also abolishes an established cocaine place preference (Bernardi *et al.*, 2009), supporting the importance of noradrenergic neurotransmission in the maintenance of cocaine contextual memories.

Fig. 2



Effects of clonidine on previously established morphine conditioned place preference. (a) Rats conditioned with morphine (5 mg/kg, subcutaneous) showed a preference to the morphine-paired chamber when tested on day 9 (test 1). On day 10, rats were re-exposed to the morphine-paired chamber and clonidine (50 μg/kg) or vehicle was administered immediately thereafter. Place preference was retested 24 h later. Clonidine did not significantly alter an established morphine conditioned place preference when tested on day 11 (test 2). Data are expressed as mean ± SEM and were analyzed with a two-way analysis of variance ($P > 0.05$).

A second aim of the study was to determine if clonidine could interfere with reconsolidation of place preference produced by another drug of abuse. Results demonstrated that maintenance of morphine place preference was unaffected by clonidine administration under similar procedures and dose of clonidine that disrupted cocaine memory reconsolidation. Previous work found that morphine place preference could be attenuated by a protein synthesis inhibitor (Milekic *et al.*, 2006; Robinson and Franklin, 2007) but only when administered after re-experience of both the drug and environmental context (Milekic *et al.*, 2006). Milekic *et al.* (2006) conclude that an established morphine place preference does not become labile after contextual recall but requires the concomitant re-experience of both context and drug. It is possible that clonidine did not attenuate morphine place preference in this study because it was not administered after contextual and concomitant drug re-exposure. However, a study by Robinson and Franklin (2010) reports disruption of an established morphine place preference by propranolol administration post contextual re-exposure. They further demonstrate that propranolol is effective in disrupting morphine place preference in rats that had undergone four morphine conditioning sessions but not eight sessions. They conclude that the strength of the morphine contextual memory (weak in the case of four pairings versus strong in the case of eight pairings) impacts the ability of propranolol to interfere with reconsolidation of a morphine place preference (Robinson and Franklin, 2010). The ineffectiveness of clonidine to disrupt a morphine place preference in this study suggests a potential difference in the role of norepinephrine in maintenance of cocaine versus morphine contextual memories, a difference in the reactivation process for cocaine versus morphine memories, or a difference in the strength of the memories. Additional studies are needed to elucidate the mechanism underlying the differences between maintenance of cocaine and morphine contextual memories and the effect of clonidine thereon. It is of interest that a preliminary human laboratory study reports lower cue-induced craving in persons with opioid use disorder who are maintained on a combination of naltrexone and lofexidine, another α2-adrenergic receptor agonist, versus naltrexone alone (Sinha *et al.*, 2007).

Previous animal and human studies have shown another type of memory, fear memory, can be impaired by inhibiting noradrenergic neurotransmission. For example, using fear conditioning in rats, blocking α2-receptor, α1-receptor, or β-adrenergic receptor immediately after fear memory reactivation significantly attenuates conditioned fear responses indicating successful attenuation of fear memory (Debiec and LeDoux, 2006; Gamache *et al.*, 2012; do Monte *et al.*, 2013; Gazarini *et al.*, 2013). In people with PTSD, propranolol administered after recall of the trauma significantly reduces physiological responses such as elevated heart rate and skin conductance 1 week later after recounting the traumatic memory (Brunet *et al.*, 2008). However, clonidine is not effective in blocking the development or expression of fear memory in a study of predator odor stress in rats (Zoladz *et al.*, 2013) further supporting the contention that clonidine specifically interferes with the reconsolidation process. Taken together, these findings indicate that blocking the noradrenergic system during reconsolidation of fear memory can destabilize the memory and erase the mnemonic trace.

Overall, our findings demonstrate that clonidine, an α2-adrenergic receptor agonist, successfully attenuated reconsolidation of cocaine environmental-paired memory when administered following memory reactivation. Without memory reactivation, cocaine place preference was not impaired by clonidine. These effects were not generalizable to morphine place preference as clonidine did not impair reconsolidation of morphine environmental-paired cues under conditions that effectively erased cocaine place preference. Future studies are needed to determine if clonidine can interfere with cue memories associated with cocaine self-administration behaviors. Effectively mitigating both substance abuse and fear environmental-cue memory reconsolidation, clonidine is a prime therapeutic candidate for further study.

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Conflicts of interest

There are no conflicts of interest.

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