Published in final edited form as:

Biol Psychiatry. 2010 January 1; 67(1): 85–87. doi:10.1016/j.biopsych.2009.08.015.

D-cycloserine facilitates extinction of naloxone-induced conditioned place aversion in morphine-dependent rats

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Abstract

BACKGROUND—Cues paired with drug administration trigger relapse to drug-seeking by inducing conditioned drug craving and withdrawal. Because drug cues hinder abstinence in addicts, therapies that reduce responsiveness to drug cues might facilitate rehabilitation. Extinction is a means of reducing conditioned responses and involves exposure to the conditioned stimulus (CS) in the absence of the unconditioned stimulus (US) with which it was paired previously. We examined conditioned withdrawal extinction using naloxone-induced conditioned place aversion (CPA) in morphine-dependent rats.

METHOD—Morphine-dependent rats were trained to associate an environment with naloxone-precipitated withdrawal. Subsequently they received extinction training in which they were confined in the previously naloxone-paired environment in the absence of acute withdrawal. In some rats, the NMDA receptor partial agonist D-cycloserine (DCS) was administered prior to extinction training.

RESULTS—Morphine withdrawal-induced CPA persists in the absence of extinction training. Administration of DCS prior to extinction training facilitates extinction.

CONCLUSIONS—DCS facilitates extinction of morphine withdrawal-associated place aversion. This effect is qualitatively similar to the effect of DCS on extinction of conditioned fear, raising the possibility of common neural mechanisms. This work extends our understanding of drug cue responsivity and provides a rationale for the development of extinction-based treatments for addiction.

Introduction

Cues paired with drug administration trigger relapse to drug-seeking behavior by inducing conditioned drug craving and withdrawal (1). Because drug cues represent a barrier to long-

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Conflict of Interest Notification Statement:

The authors report no biomedical financial interests or potential conflicts of interest.

term abstinence in recovering addicts, there is interest in therapies that reduce responsiveness to them. Extinction is a means of reducing Pavlovian conditioned responses and involves exposure to the conditioned stimulus (CS) in the absence of the unconditioned stimulus (US) with which it was paired previously. In animal models including cue-induced reinstatement of drug seeking and conditioned place preference, drug craving elicited by drug-paired cues can be extinguished by presenting the cues repeatedly without drug administration. The neurobiological mechanisms involved in extinction of conditioned drug craving appear similar to those underlying extinction of conditioned fear (2–4).

In contrast, there currently is little information about extinction of conditioned withdrawal. Conditioned withdrawal can be studied using the conditioned place aversion (CPA) paradigm. In this model, rats are allowed to briefly explore a two- or three-chambered apparatus. Each rat then is confined in one of the chambers during drug withdrawal, which in opiate-dependent animals can be precipitated by an opiate receptor antagonist such as naloxone (5). When such rats are later allowed to explore the entire apparatus, they tend to avoid the previously withdrawal-paired context. Conditioned withdrawal is defined operationally as the difference in time spent in the withdrawal-paired chamber vs. the opposite chamber, which typically is paired with a neutral treatment (saline).

Here we describe experiments designed to examine extinction of CPA established by precipitated opiate withdrawal. We show that CPAs can be extinguished by confining the animals in the formerly naloxone-paired environment in the absence of withdrawal. This reduced avoidance response is due to extinction rather than forgetting, because rats that do not receive extinction training continue to exhibit persistent place aversions. We also show that the NMDA receptor partial agonist D-cycloserine (DCS) facilitates extinction of withdrawal-induced CPAs.

Methods

Experiments were conducted according to National Institutes of Health policies. Male Sprague-Dawley rats (250–275 g; Charles River Laboratories, Raleigh, NC) were trained to acquire CPA in 3-chambered place conditioning boxes (Med Associates, St. Albans, VT) with equally-sized black and white compartments separated by a smaller gray compartment (6) (Table S1 in Supplement 1). Briefly, each rat was given a pre-training (screening) test during which it freely explored the entire apparatus for 30 min. Rats with a strong initial preference (≥60% of the session time) for any chamber were not tested further. Twenty-four hrs later rats were implanted with subcutaneous pellets under isofluorane anesthesia. Some rats were made dependent on morphine by implantation of two 75-mg morphine pellets (National Institute on Drug Abuse [NIDA], Bethesda, MD) (5). These pellets slow-release morphine continuously for a period of 14 d (7). Control rats were implanted with two placebo (blank) pellets (NIDA). Three days after implantation, rats underwent conditioning in which they were confined in either the black or white compartment for 1 hr immediately following subcutaneous (SC) injection of 0.9% saline (1 ml/kg); 3 hrs later they were confined in the opposite compartment for 1 hr immediately following SC injection of naloxone (15 μg/kg; Sigma-Aldrich, St. Louis, MO). This dose elicits motivational symptoms of withdrawal without somatic symptoms (8). Compartment assignment was

counterbalanced across rats and unbiased relative to the inherent preferences determined in the pre-training test (6). Post-training tests were identical to the pre-training test (no drugs or injections).

In Experiment 1, rats were implanted with morphine pellets or blank pellets. Extinction training began 24 hrs after the post-training test and consisted of 30 min confinements in the previously saline- and naloxone-paired boxes. On each confinement day the rats were exposed to each chamber once, with 3 hrs between exposures; the order of exposure was counterbalanced across rats and reversed relative to the preceding session. Saline was administered immediately prior to each confinement. Three confinement days were conducted at 48 hr intervals, and 24 hrs after each confinement day was a 30 min post-training (extinction) test. In Experiment 2, all rats were implanted with morphine pellets and given a single post-training test either 1 d or 7 d after training. In Experiment 3, all rats were implanted with morphine pellets and exposed to an abbreviated extinction training and test protocol consisting of one confinement day and one extinction test. D-cycloserine (15 mg/kg; Sigma-Aldrich) was administered IP immediately prior to confinement in the previously naloxone-paired box, whereas saline was administered IP immediately prior to confinement in the opposite box.

Data are expressed as change scores (seconds in the naloxone-paired box minus seconds in the saline-paired box) for a post-training or extinction test minus the aversion score for the same animal in the pre-training test. Because rats that did not develop a CPA cannot (by definition) undergo CPA extinction, rats not exhibiting a strong aversion (≤-300 sec in the post-training test) were excluded from the parts of Experiments 1 and 3 where extinction was quantified. Data were analyzed with t-tests or two-way ANOVA (Treatment x Test Day) with Test Day as a repeated measure, followed by simple main effects tests or post hoc Scheffé comparisons.

Results

In Experiment 1, rats with morphine pellets (n=20) expressed strong CPAs in the post-training test, whereas rats with blank pellets (n=20) did not ($t(38)=3.76$; $p<0.01$) (Fig. 1A). Fourteen rats in the morphine group met the criterion for inclusion in the extinction study (Fig. 1B). Changes in time spent in withdrawal-associated environments depended on main effects of Treatment ($F(1,32)=12.23$, $p<0.01$) and Test Day ($F(1,32)=5.28$, $p<0.01$). Simple main effects tests indicated that morphine-dependent rats spent less time in the naloxone-associated environment than did controls during the post-training test ($F(1,32)=21.83$, $p<0.01$), extinction test 1 ($F(1,32)=8.93$, $p<0.01$), and extinction test 2 ($F(1,32)=4.73$, $p<0.05$). Group differences were not significant by extinction test 3.

In Experiment 2, rats were tested for the first time either 1 d (n=19) or 7 d (n=17) after training (corresponding to the post-training test and third extinction test time points in Experiment 1). The CPAs in the 1 d and 7 d test groups did not differ (Fig. 1C), indicating no spontaneous decrease in CPA over this period of time.

In Experiment 3, 31 morphine pellet-implanted rats met the criterion for inclusion in the extinction study. The change in time spent in the withdrawal-associated environment depended on an interaction of Treatment and Test Day ($F(1,29)=4.74$, $p<0.05$) (Fig. 2). DCS prior to confinement in the previously naloxone-paired box facilitated CPA extinction: there was a significant reduction in the CPA between the post-training test and the first extinction test in the group treated with DCS (n=16) (Scheffé test, $p<0.01$), whereas there was no difference after saline treatment (n=15).

Discussion

Naloxone-induced CPA in morphine-dependent rats is diminished following exposure to the previously naloxone-paired environment in the absence of acute withdrawal. CPA persists for at least 7 days unless the rats receive additional exposures to the environment without withdrawal, indicating that the effect is due to extinction and not forgetting. Administration of DCS immediately prior to extinction training dramatically increases the rate of extinction of the CPA. Our data suggest that extinction of conditioned drug withdrawal involves mechanisms similar to those involved in other types of extinction (e.g., extinction of conditioned fear) (9). Common approaches might treat a wide spectrum of disorders in which conditioning contributes to maladaptive behavior.

Avoidance responses are difficult to extinguish unless exposure to the CS is forced (10). To ensure that the rats would be exposed to the aversion-paired CS, we conducted extinction training by confining them in the contexts and testing them in separate sessions rather than simply testing them repeatedly. This approach guarantees equivalent CS exposure in all rats and enables control over extinction rates through titration of the number and duration of exposures.

DCS facilitates extinction of conditioned fear (11,12), cocaine conditioned place preference (13,14), and ethanol seeking maintained by response-contingent delivery of ethanol-paired cues (15). DCS also enhances cue exposure therapy in patients with anxiety-related disorders (16–18). CPA extinction thus seems similar to other types of extinction in terms of sensitivity to DCS. The putative mechanism of DCS is enhanced NMDA receptor function, which facilitates new learning (19). Our findings highlight the possibility that a variety of experience-dependent maladaptive behaviors involve common neural substrates, and may provide an explanation for co-morbidity of psychiatric conditions such as stress/anxiety disorders and addiction (20).

Research on extinction of drug-paired cues has focused on conditioned drug craving, even though conditioned withdrawal contributes to relapse (1). Considering interest in the use of extinction protocols in clinical settings to reduce responsiveness to drug cues in recovering addicts, it is important to understand the behavioral features and neurobiological mechanisms of reward-like and aversive-like conditioned responses that are established by drugs of abuse.

Since DCS is known to be safe in humans (16), our data provide a rationale for studies in addicts. Regardless of whether DCS is useful for diminishing relapse, a more thorough

understanding of the mechanisms of extinction of conditioned withdrawal might facilitate the development of improved treatments for addictive disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the National Institute on Drug Abuse (DA012736 to W.A.C.).

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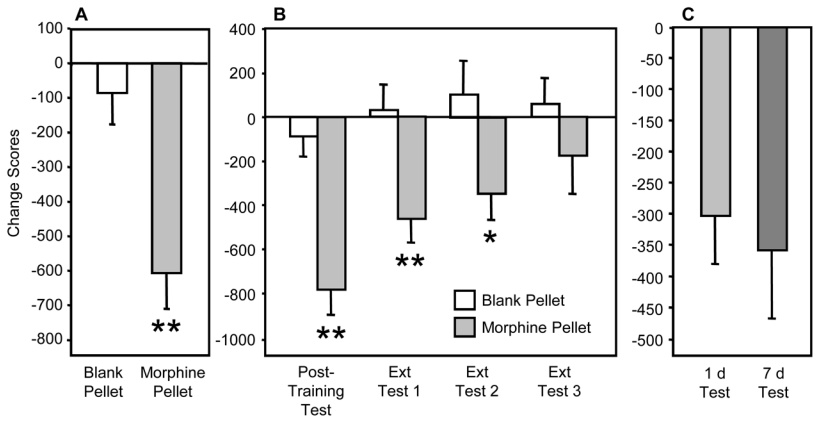

Figure 1.

Extinction of naloxone-induced conditioned place aversion in morphine-dependent rats. **A.** Rats implanted with morphine pellets exhibit conditioned place aversion (CPA) to an environment previously paired with a low dose of naloxone (15 μg/kg), whereas animals implanted with blank (placebo) pellets do not. **B.** The magnitude of CPA in morphine-dependent rats declines following repeated confinements in the previously naloxone-paired environment in the absence of acute withdrawal**. C.** Morphine-dependent rats not exposed to the previously naloxone-paired environment in the absence of withdrawal exhibit persistent CPA. *$p < .05$, ** indicates $p < .01$ vs. blank pellet, Scheffé test.

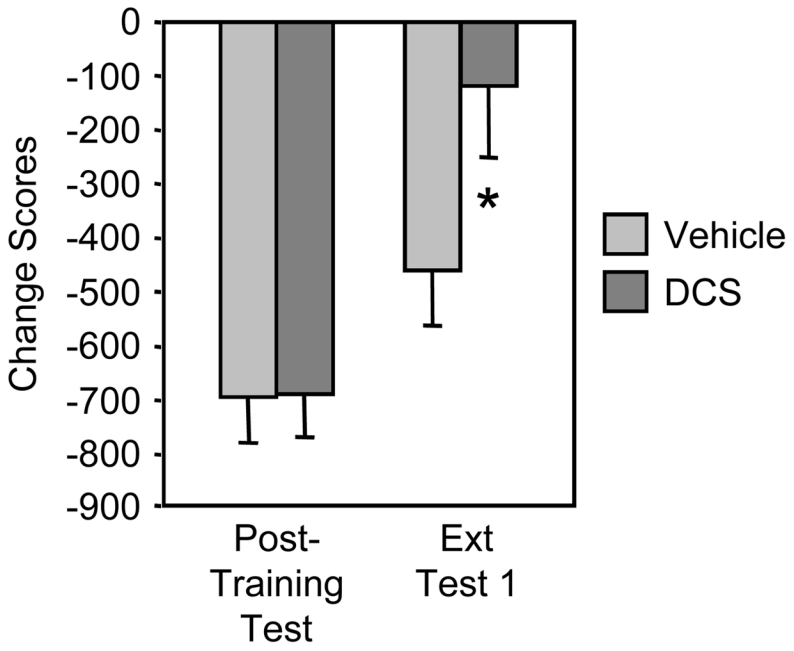

Figure 2.
D-cycloserine administered prior to confinement in the previously naloxone-paired box facilitates CPA extinction. *p<.05 vs. vehicle, Scheffé test.