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Naltrexone-induced conditioned place aversion following a single dose of morphine in the rat

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Abstract

Limited preclinical research has been conducted investigating the motivational or "affective" properties of withdrawal from acute opioid dependence following a single morphine exposure. Therefore, the purpose of the present study was to pharmacologically characterize the motivational properties associated with naltrexone-precipitated withdrawal after a single injection of morphine using place conditioning. Conditioned place aversion was assessed using a biased two-compartment apparatus and procedure. Adult male Sprague–Dawley rats were given 15 min free access to the entire apparatus on day one to determine initial preferences. Beginning on the second day, combinations of either saline or morphine (1.0–10 mg/kg, s.c.) followed by naltrexone (0.003–3.0 mg/kg, s.c.) 3.75 h later were administered. Rats were then immediately confined to one compartment for 30 min. The next day, rats received the alternative treatment and were confined to the opposite compartment. Twenty-four hours later animals were tested again for 15 min while they had access to the entire apparatus. Morphine followed by naltrexone conditioned significant place aversion (CPA) with just one pairing. This effect was a function of the naltrexone and morphine doses. CPA was also dependent on morphine pretreatment time, with significant aversion only occurring 4 h after morphine pretreatment. Finally continuous morphine administration followed by a single injection of naltrexone resulted in CPA. These data extend the range of behavioral effects associated with antagonist-precipitated withdrawal from acutely administered morphine and suggest that place conditioning is an effective model in assessing motivational aspects of withdrawal from acute opioid dependence in rats.

Keywords: Place conditioning; Place preference; Place avoidance; Opioids

1. Introduction

In animal subjects, the administration of a single dose of a morphine-like agonist followed by an opioid antagonist can result in a number of physiological and behavioral changes (Schulteis et al., 1997, 1999), indicative of an opiate withdrawal syndrome. These changes resemble those that appear following abstinence or opioid-antagonist administration in subjects physically dependent upon chronically-administered morphine or other mu-opioid agonists (Martin, 1983), suggesting a common mechanism. Other similarities in antagonist-precipitated changes after chronic and acute administration of morphine have been observed for a variety of dependent measures (Kosersky et al., 1974; Eisenberg, 1982; Krystal and Redmond, 1983; Ramabadran, 1983; Adams and Holtzman, 1990; Schnur, 1991; Easterling and Holtzman, 1997, 1999; Kalinichev and Holtzman, 2003; Harris and Gewirtz, 2004), further validating the model of acute opioid dependence.

An increasing number of studies have focused on elucidating the "affective/motivational" component of drug states in animals. One popular method is place conditioning (for a recent review, see Bardo and Bevins, 2000), which has the advantage over other animal models of allowing subjects to be tested in a drug-free state. Therefore, the affective component of a drug state experienced previously (i.e., pleasure or aversion) can be studied in the absence of the potentially confounding influence of the direct drug

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effects. Morphine-like opioids condition a preference for the place associated with their effects, suggesting that the effects were pleasurable or rewarding (for review see Tzschentke, 1998). On the other hand, opioid withdrawal results in place avoidance, consistent with the presumed aversive nature of the state of withdrawal. For example, in rats chronically dependent upon morphine, conditioned place aversion can be elicited by precipitating withdrawal with very low doses (0.004–0.015 mg/kg) of the antagonist naloxone (Mucha et al., 1986; Schulteis et al., 1994). This effect can be achieved with as little as one naloxone-place pairing (Mucha et al., 1986) and can persist for as long as 16 weeks (Stinus et al., 2000).

There have been a limited number of studies assessing the motivational effects associated with acutely administered morphine followed by naloxone. However, evidence suggests the effects are quite profound as well. For example, rats avoided an environment in which they received naloxone (0.5 and 1.0 mg/kg) 24 h after pretreatment with 20 mg/kg morphine (Parker and Joshi, 1998). In a more recent study (Azar et al., 2003), naloxone-precipitated withdrawal 4 h after a single injection of morphine conditioned significant place aversion after just two pairings. This effect was dose-dependent for both naloxone (0.003–16.7 mg/kg) and morphine (1.0–5.6 mg/kg). The results of these studies clearly indicate that the drug state produced by acutely administered morphine followed by naloxone is aversive.

In the study by Azar et al. (2003), conditioned place aversion occurred following a single exposure to 5.6 mg/kg morphine, but the effect was relatively modest. However, this pretreatment dose is lower than the dose of morphine (10 mg/kg) used in other studies of acute opioid dependence (Young, 1986; Adams and Holtzman, 1990; Easterling and Holtzman, 1997; Kalinichev and Holtzman, 2003; Harris and Gewirtz, 2004). Perhaps more robust effects would have been produced using this dose. Such evidence could provide further insight into the "affective/motivational" components associated with many of the behavioral models used to study acute opioid dependence and possibly into the underlying mechanisms mediating various aspects of drug dependence, including induction and relapse (for reviews see Koob and Le Moal, 1997; Koob, 1999). Therefore, the purpose of this study was to characterize further the motivational state associated with antagonist-precipitated withdrawal from acute opioid dependence using place conditioning following the administration of a single dose of 10 mg/kg morphine. First, we determined the minimum number of pairings of 10 mg/kg morphine followed by 0.3 mg/kg naltrexone (3.75 h later) required to condition place aversion in rats. Once we confirmed that significant conditioned place aversion could be achieved with just one pairing, our second objective was to characterize this phenomenon pharmacologically. This was accomplished by varying the doses of morphine and naltrexone and by varying the morphine pretreatment times. In addition, the place conditioning induced by a single dose of morphine and naltrexone was compared to that induced by a single naltrexone-place pairing in rats receiving a continuous infusion of morphine.

2. Methods

2.1. Animals

The subjects were adult male Sprague–Dawley rats (Charles-River Breeding Laboratories, Raleigh, NC), weighing between 250 and 370 g at the start of the study. Animals were housed 2–3 per cage and maintained in the Emory School of Medicine Division of Animal Resources Care Facility. Animals were maintained according to the "Guide for the Care and Use of Laboratory Animals" (National Academy of Sciences, 1996), and all procedures were approved by the Institutional Animal Care and Use Committee.

2.2. Apparatus

The apparatus ($40 \times 40 \times 30$ cm) was a chamber constructed of Plexiglas; it consisted of two compartments divided by a removable barrier with a doorway (14.5×13 cm), which allowed access to each compartment. One compartment was gray with a roughly textured floor, while the other was checkered with black and white squares (2 cm^2) with a smooth floor. A solid barrier without a doorway was used to confine animals to a given side of the chamber.

2.3. General procedure

On the first day of experimentation, rats were placed in the chamber and allowed to explore both compartments freely for 15 min. Locomotor activity and location were determined using AccuScan Digiscan Activity Monitors (AccuScan Instruments Inc., Columbus, OH), with the aid of the VersaMax software (Version 1.30, AccuScan Instruments Inc), and a desktop computer. The time each rat spent in a compartment was noted. The rat showed a preference for one side of the apparatus over the other. To limit variability and avoid creating the potential for a "ceiling" effect (i.e. spending a maximal amount of time in a given side), a biased procedure was used to condition aversion to the initially preferred side or to condition preference to the least preferred side. Beginning the next day, morphine (10 mg/kg) or saline was injected s.c. 3.75 h prior to conditioning; the order of the injections was randomly assigned. Then, saline or naltrexone (0.3 mg/kg) was injected and the rats were immediately confined to one compartment of the chamber for 30 min. On the next day, rats received the alternative pretreatment followed 3.75 h later by saline or naltrexone and were immediately confined to the opposite side of the chamber for 30 min. The second

solution injected (i.e., saline or naltrexone) remained constant throughout conditioning. The total number of conditioning sessions (1:1 for each treatment) depended on the experiment. Twenty four hours after the last conditioning session, animals were given free access to the entire chamber for 15 min, and the amount of time spent in each compartment was recorded.

The first experiment was designed to determine the minimum number of pairings needed to condition a place aversion. Once it was determined that place aversion could be conditioned with a single pairing of morphine→naltrexone, combinations of saline or morphine (1.0–10 mg/kg) followed by saline or naltrexone (0.003–3.0 mg/kg) were systematically varied to observe effects on conditioned place aversion. To examine effects of pretreatment time on place aversion, the amount of time between 10 mg/kg morphine and 0.3 mg/kg naltrexone was varied from 1.0 to 6.0 h. In a subsequent experiment, animals were tested weekly to determine the persistence of conditioned place aversion following one and two pairings of 10 mg/kg MOR→0.3 mg/kg NTX. Finally, conditioned place aversion following a single conditioning session was assessed in morphine-dependent rats rendered physically dependent by a continuous s.c. infusion of morphine (22.5 mg/kg/day) and then given an injection of either saline or naltrexone (0.003–0.3 mg/kg). Rats were tested for 2 weeks or until conditioned place aversion abated. The number of animals used in each experiment ranged from 6 to 10 animals except for the SAL/SAL group, which was comprised of 22 animals. A total of 221 animals were used. With the exception of the experiments assessing the persistence of conditioned place aversion, all groups were tested once.

2.4. Induction of dependence by continuous morphine infusion

This procedure has been described previously (Kalinichev and Holtzman, 2003). In short, rats were anesthetized with halothane and then shaved from the back of the head just behind the ears to below the scapulae. The area was then cleaned with 70% ethanol. A 1.5-cm incision was made between the scapulae. A small cavity was made using blunt-ended scissors and a model 2ML1 (7-day) Alzet osmotic pump (Durect Corporation, Cupertino, CA) was inserted. The wound was then closed with MikRon Autoclip wound clips (Beckton Dickinson, Sparks, MD) and treated with nitrofurazone soluble powder (Fermenta Animal Health Co., Kansas City, MO) to minimize the chances of infection. The rats were given 3 days to recover from surgery. Drug concentrations were based on the infusion rate of the pump and on the weight of the animal. The daily dose of morphine (22.5 mg/kg) is higher than doses shown to reliably produce tolerance to and physical dependence upon morphine in the rat (Easterling and Holtzman, 1997; Kalinichev and Holtzman, 2003).

2.5. Drugs

Morphine sulfate (Penick Co., Nutley, NJ), naltrexone hydrochloride (Sigma-Aldrich Co., St. Louis, MO), levorphanol tartrate (Roche Laboratories, Nutley, NJ), heroin hydrochloride, and etorphine hydrochloride (National Institute on Drug Abuse, Bethesda, MD) were prepared in 0.9% saline. Buprenorphine hydrochloride (National Institute on Drug Abuse, Bethesda, MD) was dissolved in de-ionized water. All drugs were injected s.c. in a volume of 1.0 mL/kg of body weight. All drug doses are expressed as the free base.

2.6. Data analysis

Data with two or more groups were analyzed using a one-factor (dose or pretreatment time) or two-factor (agonist pretreatment × naltrexone dose) analysis of variance (ANOVA). For experiments assessing the persistence (in weeks) of conditioned place avoidance, data were analyzed using a repeated one-factor ANOVA. All post hoc comparisons were made using Dunnett's test (treatments vs. control). Individual treatment groups were also analyzed using a one sample *t*-test (mean vs. 0). p-values < 0.05 were accepted as statistically significant.

3. Results

3.1. Place conditioning following saline and morphine pretreatment

Rats receiving only saline (i.e. saline→saline) for both conditioning days spent significantly more time on the initially-non-preferred side of the apparatus [Fig. 1; $t(21) = -2.52$, $p < 0.05$]. However, the pre-conditioning and post-conditioning difference in the time spent on the initially non-preferred side was small, averaging 48.0±20.0 s. Rats treated with saline→naltrexone (0.3 or 3.0 mg/kg) on the preferred side versus saline→saline on the non-preferred side also tended to spend more time on the initially non-preferred side, but owing to group variability the change in time spent on that side did not differ significantly from that of either the saline→saline group or from 0 (Fig. 1).

Four-hour pretreatment with 10 but not 1.0 or 3.0 mg/kg morphine followed 3.75 h later by saline conditioned place preference to the initially-preferred side with only a single pairing [Fig. 2A–C; $F(3,43) = 3.86$, $p < 0.05$]; rats that received 10 mg/kg morphine→saline spent significantly more time on the preferred side of the place conditioning apparatus than did those receiving saline→saline (Fig. 2C). However, this effect did not occur following 2 h pretreatment with 10 mg/kg morphine→saline as rats spent an average of 42.4±53.0 s on the initially preferred side (Fig. 2C). In contrast, place aversion was conditioned with a

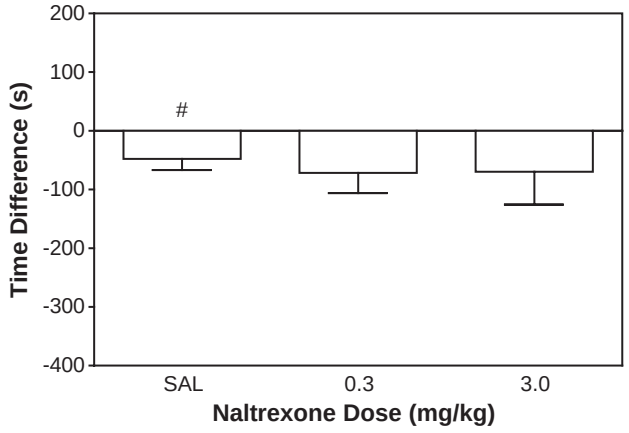

Fig. 1. Saline→naltrexone does not condition place aversion compared to saline→saline. Following a 15 min pretest to determine initial preference, rats were conditioned over two days using saline pretreatment followed by saline (SAL) and naltrexone (NTX; 0.3 or 3.0 mg/kg). Testing (15 min) occurred 24 h after conditioning. The results are expressed as the difference between the time spent on the initially preferred side on the test day and the amount of time spent on the initially preferred side prior to conditioning. Values are the mean±S.E.M. (SAL $n=22$; Other groups $n=6$ and 9). #Significantly different from 0, one sample t-test, $p<0.05$.

single drug-compartment pairing when morphine administration was followed by an injection of naltrexone. This effect was morphine-dose-dependent [$F(2,58)=6.74$, $p<0.05$]. Place aversion did not develop following pretreatment with 1.0 mg/kg morphine and 0.03–3.0 mg/kg naltrexone (Fig. 2A). However, rats spent significantly more time in the initially non-preferred side of the apparatus after pretreatment with 3.0 mg/kg morphine and 0.3 mg/kg naltrexone [$t(7)=-2.36$, $p<0.05$], and tended to spend more time on that side after pretreatment with 3.0 mg/kg morphine and 0.03 or 3.0 mg/kg naltrexone (Fig. 2B). Pretreatment with 10 mg/kg morphine followed by naltrexone resulted in a biphasic dose–response curve (Fig. 2C), which was dependent upon the dose of naltrexone [$F(6,68)=3.84$, $p<0.05$]. This dose of morphine and the lowest dose of naltrexone (0.003 mg/kg) did not condition aversion. However, rats spent significantly more time in the initially-non-preferred side of the apparatus after receiving 10 mg/kg morphine and 0.03 mg/kg [$t(9)=-2.45$, $p<0.05$], 0.1 mg/kg [$t(7)=-3.83$, $p<0.05$], or 0.3 mg/kg [$t(6)=-20.0$, $p<0.05$] naltrexone, and tended to spend more time there after 1.0 and 3.0 mg/kg. The magnitude of the place aversion conditioned by 10 mg/kg morphine and naltrexone was consistently greater than that conditioned by 3.0 mg/kg morphine and the corresponding doses of naltrexone (Fig. 2B and C).

3.2. Temporal dependency and persistence of conditioned place aversion

To assess the dependency of conditioned place aversion on the interval between the injection of morphine (10 mg/kg) and naltrexone (0.3 mg/kg), morphine pretreatment was systematically varied from 1 to 6 h before the conditioning trial. There was a significant effect of morphine pretreatment time on conditioned place aversion [Fig. 3; $F(4,45)=6.87$, $p<0.05$]. One- or two-hour pretreatment with morphine followed by naltrexone did not condition a place aversion; 4-h pretreatment with morphine did, but the

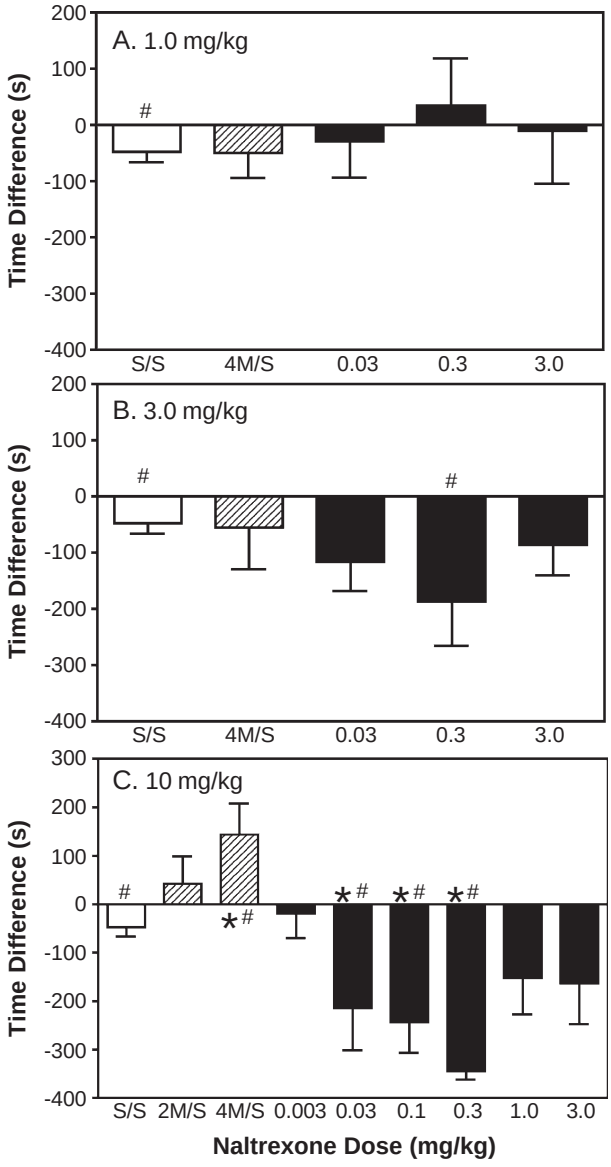

Fig. 2. Place conditioning is a function of morphine and naltrexone dose. Following a 15 min pretest to determine initial preference, rats were conditioned over 2 days using 1.0 (A), 3.0 (B), or 10 mg/kg morphine (C) pretreatment followed 1.75 h (10 mg/kg morphine→saline only; 2M/S) or 3.75 h by saline (4M/S group) or naltrexone (0.003–3.0 mg/kg). Testing (15 min) occurred 24 h after conditioning. The results are expressed as the difference between the time spent on the initially preferred side on the test day and the amount of time spent on the initially preferred side prior to conditioning. The saline→saline group (S/S) from Fig. 1 is reproduced in each panel. Values are the mean±S.E.M. (S/S $n=22$; Other groups $n=6-10$). #Significantly different from 0, one sample t-test, $p<0.05$. *Significantly different from S/S group, one-factor ANOVA, Dunnett's post hoc, $p<0.05$.

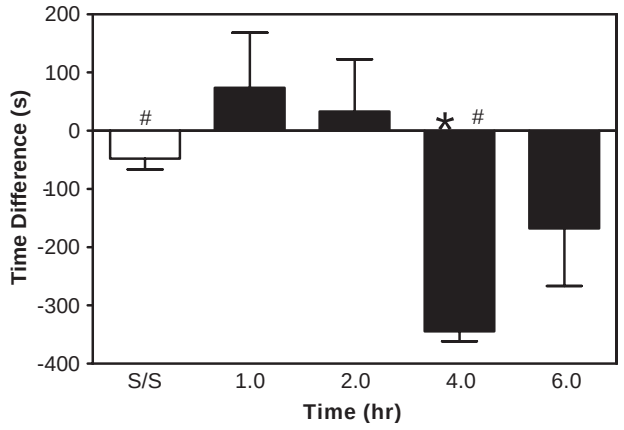

Fig. 3. Conditioned place aversion is a function of morphine pretreatment time. The effects of naltrexone (0.3 mg/kg, s.c.) following morphine (10 mg/kg, s.c.) given 1.0–6.0 h prior conditioning were assessed. Saline→saline (S/S) and morphine→naltrexone at 4 h are reproduced from Figs. 1 and 2, respectively. Values are the mean±S.E.M. (S/S $n=22$; Other groups $n=6–7$). Other details are as in Fig. 2. #Significantly different from 0, one sample t-test, $p<0.05$. *Significantly different from S/S group, one-factor ANOVA, Dunnett's post hoc, $p<0.05$.

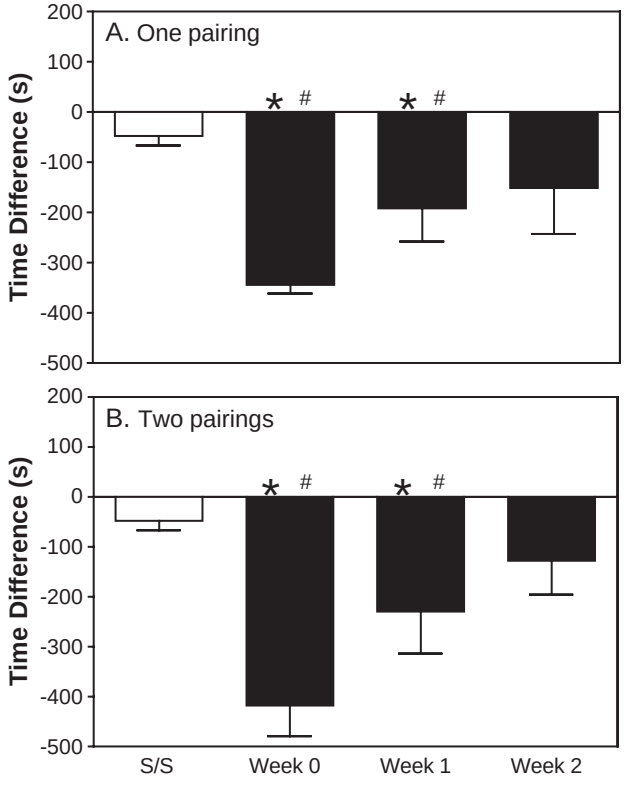

Fig. 4. Conditioned place aversion is reversible. The effects of naltrexone (0.3 mg/kg, SC) following morphine (10 mg/kg, s.c.; 3.75 h pretreatment) following one (A) or two (B) pairing sessions were assessed. Values are the mean±S.E.M. (SAL/SAL $n=22$; Other groups $n=7–8$). Saline→saline (S/S) and morphine→naltrexone (top panel; one pairing, week 0) are reproduced from Figs. 1 and 2, respectively. Other details are as in Fig. 2. #Significantly different from 0, one sample t-test, $p<0.05$. *Significantly different from S/S group, repeated one-factor ANOVA, Dunnett's post hoc, $p<0.05$.

effect was smaller and no longer statistically significant when pretreatment was extended to 6 h.

To assess the persistence of conditioned place aversion following a single pairing of 10 mg/kg morphine followed 3.75 h later by 0.3 mg/kg naltrexone, subjects were tested weekly until the effect was gone. There was a significant effect of time on conditioned place aversion [$F(3,41)=7.15$, $p<0.05$]: rats spent significantly more time in the initially-

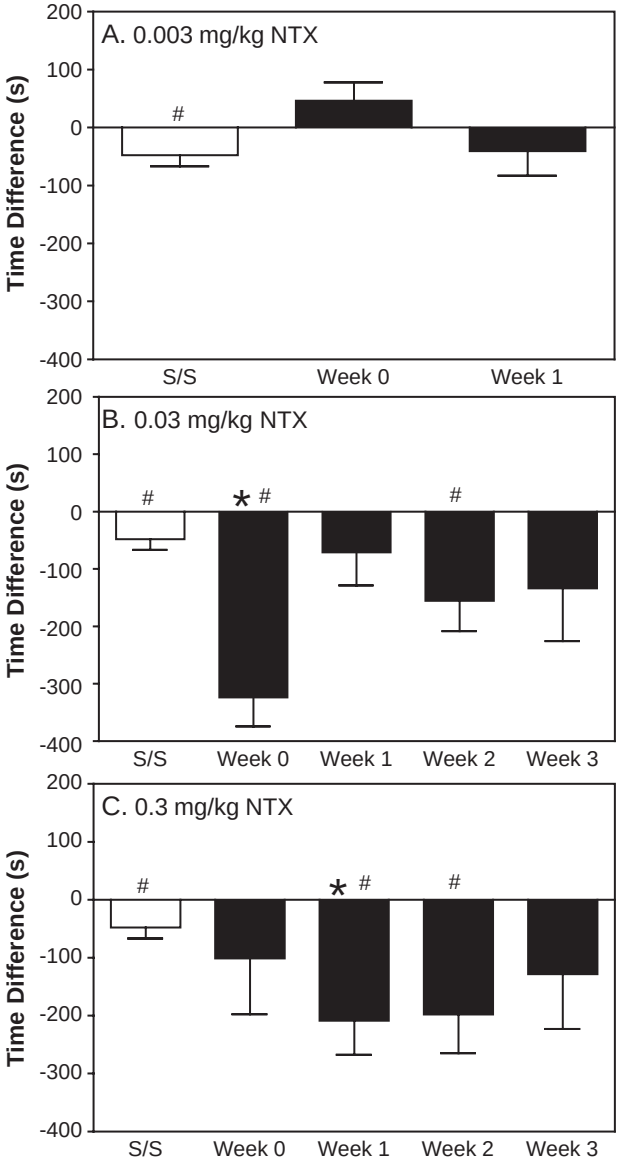

Fig. 5. Following a continuous morphine infusion, conditioned place aversion is reversible and a function of naltrexone dose after a single pairing. The effects of conditioning with saline or naltrexone (0.003–0.3 mg/kg) following opioid dependence induced by continuous morphine infusion (22.5 mg/kg/day) were assessed. Testing occurred 24 h after conditioning (week 0) and was repeated weekly for up to 3 weeks. The saline→saline group (S/S) from Fig. 1 is reproduced in each panel. Values are the mean±S.E.M. (S/S $n=22$; Other groups $n=6–7$). Other details are as in Fig. 2. #Significantly different from 0, one sample t-test, $p<0.05$. *Significantly different from S/S group, repeated one-factor ANOVA, Dunnett's post hoc, $p<0.05$.

non-preferred side of the apparatus for up to 1 week following conditioning (Fig. 4A). A similar effect was observed when two pairings of 10 mg/kg morphine followed by 0.3 mg/kg naltrexone were used [Fig. 4B; $F(3,38)=11.70$, $p<0.05$]. The effect following two pairings appeared to be stronger in the first post-conditioning test (week 0) as compared to one pairing, but there was no difference between one and two pairings after that (i.e. at 1 and 2 weeks post-conditioning).

3.3. Continuous morphine administration

The motivational aspects of naltrexone-precipitated withdrawal during a continuous infusion of morphine (22.5 mg/kg/day) were assessed for up to 3 weeks after place conditioning. As shown in Fig. 5, place conditioning was dependent on the dose of naltrexone [$F(3,35)=9.98$, $p<0.05$] and the interval between conditioning and testing [0.003 mg/kg naltrexone: $F(1,21)=42.89$; 0.03 mg/kg naltrexone: $F(3,63)=33.29$; and 0.3 mg/kg naltrexone: $F(3,63)=31.18$, respectively, $p<0.05$]. The lowest dose of naltrexone (0.003 mg/kg) administered during the infusion of morphine did not result in conditioned place aversion in either the test the day after the conditioning trials or the test a week later (Fig. 5A). A 10-fold higher dose of naltrexone (0.03 mg/kg) produced a significant place aversion 0 and 2 weeks after the conditioning trials [$t(5)=-6.44$ and $t(6)=-2.92$, $p<0.05$, respectively] and a nonsignificant trend towards place aversion 1 and 3 weeks post-conditioning (Fig. 5B). Conditioning with 0.3 mg/kg naltrexone resulted in significant place aversion 1 and 2 weeks later [$t(5)=-3.53$ and $t(5)=-2.96$, $p<0.05$, respectively] and nonsignificant trends towards place aversion immediately after conditioning and 3 weeks later (Fig. 5C). The amount of time spent in the non-preferred side of the apparatus immediately after conditioning with 0.03 mg/kg naltrexone (week 0) and 1 week after conditioning with 0.3 mg/kg naltrexone were significantly greater than that seen with acutely administered saline→saline [Fig. 5B and C; $t(5,21)=5.14$ and $t(5,21)=2.59$, $p<0.05$, respectively].

4. Discussion

The present study demonstrates that significant place aversion can be conditioned after just one pairing of acutely administered morphine followed 3.75 h later by naltrexone. This phenomenon required the presence of both morphine and naltrexone and was a function of morphine dose, naltrexone dose, and the interval between the two drugs. Our findings are consistent with those from other place conditioning studies reporting the negative motivational effects of withdrawal from acutely administered opioids (Parker and Joshi, 1998; Azar et al., 2003). Our data are also consistent with studies demonstrating that rats made physically dependent upon chronically administered morphine demonstrate significant place avoidance following a single conditioning session with naloxone (Mucha et al., 1986; Mucha, 1987; Kosten, 1994; Shippenberg et al., 2000). Taken together, these data reaffirm and extend the parallels between acute opioid and chronic opioid dependence.

Our findings demonstrate that the procedure, despite being biased, can still reveal place preference. In our hands, after a 3.75-h pretreatment, rats spent significantly more time in the chamber paired with 10 mg/kg morphine followed by saline as compared to the chamber paired with saline followed by saline. This finding is similar to other studies, where a single intravenous bolus of morphine given concurrently during conditioning elicited place preference (Bardo and Neisewander, 1986; Bardo et al., 2003). It is also consistent with the place conditioning literature in general, which suggests that morphine-like drugs are rewarding (for review see Tzschentke, 1998). However, 2 h pretreatment with morphine failed to condition place preference. This finding is somewhat "counter-intuitive" given the results following 3.75 h morphine pretreatment. Additionally, a number of studies report place preference with morphine post-injection intervals less than 4 h prior to conditioning, albeit following multiple conditioning trials (Mucha and Iversen, 1984; Shippenberg et al., 1996; Olmstead and Franklin, 1997; Parker and McDonald, 2000). Pharmacokinetics is an unlikely explanation as peak plasma levels of morphine (SC) are reached well within 2 h and have declined significantly by 4 h (Barjavel et al., 1995). Perhaps the rewarding properties associated with morphine are the result of cellular events occurring well "downstream" of the initial drug–receptor interactions and require a minimum amount of time for development. Such a notion would be consistent with drug discrimination studies assessing the effects of acute opioid dependence, where development of discriminative cues required a minimum amount of time following morphine administration (Easterling and Holtzman, 1999; White and Holtzman, 2003).

Significant place aversion could be conditioned after naltrexone and pretreatment with 3.0 and 10 mg/kg morphine but not 1.0 mg/kg morphine, clearly demonstrating a dose-dependent effect of morphine. Significant place avoidance was also biphasic as 10 mg/kg morphine combined with doses of naltrexone less than 0.03 or higher than 0.3 mg/kg failed to condition a significant place aversion. A similar trend was seen with 3.0 mg/kg morphine pretreatment. However, conditioned place aversion following opioid antagonist-precipitated withdrawal typically follows a monophasic or "all or none" response pattern with increasing antagonist dose (Mucha et al., 1986; Mucha, 1987; Parker and Joshi, 1998; Stinus et al., 2000; Parker et al., 2002; Azar et al., 2003). In the current study, naltrexone was paired with morphine in one compartment and with saline in the other compartment. Naltrexone and naloxone alone can condition place aversion (Tzschentke, 1998) and as suggested in Fig. 1. Perhaps the combination of morphine and 0.3 mg/kg naltrexone results in a maximally aversive

withdrawal state (i.e. one that is not increased by a higher dose of naltrexone). If so, the combination of saline pretreatment and doses of naltrexone higher than 0.3 mg/kg probably produce a mildly aversive state, reducing the aversion difference between the two conditioning trials. Similar biphasic curves occurred with rats trained to discriminate 10 mg/kg morphine followed by 0.3 mg/kg naltrexone from saline followed by 0.3 mg/kg naltrexone (Holtzman, 2003).

Place avoidance was dependent upon morphine pretreatment time as it could be conditioned 4 h after morphine pretreatment but not earlier or later. This suggests that acute opioid dependence in the place conditioning paradigm requires a minimum time to develop and dissipates relatively quickly. These findings are consistent with those of drug discrimination studies (Easterling and Holtzman, 1999; White and Holtzman, 2003) and other studies of acute opioid dependence in animals and humans (Young, 1986; Heishman et al., 1989; Wang et al., 1994; June et al., 1995; White and Holtzman, 2001; Kalinichev and Holtzman, 2003; Harris and Gewirtz, 2004). However, there have been place conditioning studies where rats spent significantly less time in an environment paired with 1.0 mg/kg naloxone 24 and 48 h after single injections of 20 mg/kg morphine (Parker and Joshi, 1998; Parker et al., 2002). Perhaps the use of such a high dose of morphine and multiple (two) pairings resulted in conditions comparable to those associated with chronic opioid dependence, which elicits very long lasting place avoidance (Stinus et al., 2000).

Following both acute and continuous morphine administration, a single pairing of naltrexone-precipitated withdrawal conditioned significant place aversion, further extending the similarities between acute and chronic opioid dependence. The conditioned aversion was transient, lasting no more than 2 weeks, and the naltrexone dose–response relationship was more or less the same regardless of the duration of agonist administration—once, twice, or continuous. In another study, two pairings of 5.6 mg/kg morphine followed by naloxone produced a greater than 100-fold decrease in the ED_{50} of naloxone compared to that of a single pairing, and that ED_{50} was comparable to the ED_{50} of naloxone during chronically administered morphine (Schulteis et al., 1994; Azar et al., 2003). To the best of our knowledge, no one has determined the persistence of conditioned place aversion in chronically morphine-dependent rats following just one pairing with an antagonist. However, naloxone-precipitated withdrawal from chronically administered morphine conditioned long-lasting (16 weeks) place aversion with just 3 pairings (Stinus et al., 2000). It is not clear what procedural aspects of our study account for the failure to see changes in the persistence of conditioned place aversion and sensitivity to naltrexone as a function of the amount of exposure to morphine. One possibility is that because our animals were tested repeatedly, the conditioned aversion might have extinguished upon multiple re-exposures to the test chamber. Perhaps the aversion would have persisted longer had we tested a different group at each of the several time points.

In conclusion, these results demonstrate a number of pharmacological similarities between place aversion conditioned by antagonist-precipitated withdrawal from acute morphine dependence and other models of acute opioid dependence, including drug discrimination. Therefore, place conditioning can provide another methodological approach for probing the mechanisms that underlie acute opioid dependence, an approach that does not require the extensive training of animals.

Acknowledgments

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