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The aversive properties of acute morphine dependence persist 48 h after a single exposure to morphine Evaluation by taste and place conditioning

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Abstract

The aversive properties of naloxone-precipitated morphine withdrawal from acutely administered morphine were assessed following a *single* conditioning trial using both the place conditioning and the taste conditioning paradigm. In both paradigms, the aversive properties of naloxone-precipitated morphine withdrawal were evident up to 48 h after a single injection of morphine. In neither paradigm did naloxone treatment alone produce an aversion after a single conditioning trial. These results suggest that a single morphine exposure produces long-lasting effects that persist at least 48 h beyond the agonist effects of the opiate. © 2002 Elsevier Science Inc. All rights reserved.

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1. Introduction

Morphine withdrawal typically is produced by either terminating chronic morphine exposure or by administering an opiate antagonist to chronically morphine-pretreated humans or rats (Jaffee and Martin, 1990). The latter form of withdrawal is called precipitated withdrawal. Early work suggests that naloxone-precipitated withdrawal can be produced after even a single exposure to morphine (Gellert and Sparber, 1977; Jacob and Michaud, 1974; Martin and Eades, 1964); this has been called acute physical dependence. Research with both humans and nonhumans has shown that the somatic symptoms of acute physical dependence are similar to those observed after prolonged opiate exposure (Heishman et al., 1990; June et al., 1995; Kirby and Stitzer, 1993; Martin and Eades, 1964; Wiley and Downs, 1979).

Precipitated withdrawal can be produced even when naloxone is administered up to 24 h following a single exposure to morphine (Azorlosa et al., 1994; Easterling and Holtzman, 1997; Easterling et al., 2000; Eisenberg, 1982; Gellert and Sparber, 1977; Heishman et al., 1990; June et al., 1995; Kirby and Stitzer, 1993; Schulteis et al., 1999). Human subjects remain sensitive to the withdrawal-precipitation effects of naloxone 24 h after pretreatment with a single dose of morphine, although the agonist (pupil constriction and subjective effects) activity of morphine (Heishman et al., 1990; Kirby and Stitzer, 1993) and the plasma morphine concentrations (June et al., 1995) have dissipated by that time. In rats, exposure to naloxone following a single exposure to morphine interferes with operant responding for natural rewards (Gellert and Sparber, 1977; Schulteis et al., 1999), increases the break point for intracranial self-stimulation (Easterling and Holtzman, 1997; Easterling et al., 2000), produces somatic withdrawal signs (Jacob and Michaud, 1974; Martin and Eades, 1964), and enhances corticosterone levels (Eisenberg, 1982).

Naloxone-precipitated withdrawal is measured not only by somatic symptoms of abstinence, but also by the ability of such withdrawal to serve as an aversive motivational stimulus. Precipitated withdrawal from chronically administered morphine produces both a taste avoidance (McDonald et al., 1997; Mucha, 1987, 1992) and place avoidance (Baldwin and Koob, 1993; Koob et al., 1989; Mucha, 1987; Mucha, 1991; Mucha and Iverson, 1984; Mucha et al., 1986; Parker and Joshi, 1998). Parker and Joshi (1998) report that naloxone-precipitated morphine withdrawal produced a conditioned place aversion even when morphine

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was administered 24 h prior to the naloxone injection on only two occasions. Since the aversive motivational properties were evident well after the agonist effects of morphine were dissipated, acute morphine administration appears to produce long-lasting changes at the opiate receptor.

The present study was designed to determine whether acute dependence could be produced in a single conditioning trial. Secondly, given that a *single* cycle is sufficient, how persistent is the adaptational change produced by morphine exposure across time?

2. Experiment 1

Experiment 1 examined the persistence of acute dependence produced by a single exposure to morphine using the taste avoidance paradigm. McDonald et al. (1997) reported that exposure to morphine 22 h prior to naloxone produced conditioned rejection and avoidance of a flavored solution after 2–3 conditioning trials. In Experiment 1, morphine or saline preceded exposure to saccharin solution by 22 or 46 h. Immediately following exposure to saccharin solution, the rats were injected with naloxone or saline. A two-choice (saccharin and water) preference test was used to evaluate the rats' preferences for saccharin following the conditioning trial.

2.1. Method

2.1.1. Subjects

The subjects were 49 male Sprague–Dawley rats weighing between 190 and 260 g on the conditioning day. They were maintained in individual stainless steel cages with food and water ad lib except as specified. The room was illuminated on a 12:12 light/dark schedule with the lights on at 8:00 a.m. and all procedures occurred in the light phase. Upon arrival in the laboratory, the rats were handled daily for one week prior to the conditioning trials. All procedures were approved by the Institutional Animal Care Committee according to the guidelines of the Canadian Council for Animal Care.

2.1.2. Drugs

Morphine sulphate was obtained from the British Drug House (BDH), Toronto, ONT, Canada, and mixed with physiological saline to produce a solution of 20 mg/ml (w/v). Naloxone HCl was obtained from Dupont Wilmington, DE, and mixed with physiological saline to produce a solution of 1 mg/ml (w/v). All injections were administered subcutaneously at a volume of 1 ml/kg.

2.1.3. Procedure

Table 1 presents the design of Experiment 1. There were a total of six groups, which differed on the basis of the preconditioning treatment (saline [S], morphine — 22 h [M22] before naloxone, and morphine — 46 h [M46] before conditioning) and the conditioning treatment (naloxone [N] or saline [S]). The groups were as follows: SS ($n=9$), SN ($n=7$), MS22 ($n=9$), MS46 ($n=8$), MN22 ($n=9$), MN46 ($n=8$).

Table 1
Design of taste conditioning trial in Experiment 1

Groups	Day 3 pretreatment	Day 4 pretreatment	Day 5 conditioning
MN22 ($n=9$)	Saline	Morphine	Sac → naloxone
MS22 ($n=9$)	Saline	Morphine	Sac → saline
MN46 ($n=8$)	Morphine	Saline	Sac → naloxone
MS46 ($n=8$)	Morphine	Saline	Sac → saline
SN ($n=7$)	Saline	Saline	Sac → naloxone
SS ($n=9$)	Saline	Saline	Sac → saline

Following a one-week adaptation period to the laboratory, the experimental procedures began. The rats were deprived of water for 24 h and on each of Days 1–4 were trained to consume their daily water supply in 30 min from a graduated tube attached to their home cage. The groups did not differ in their water intake on Day 4 (mean = 19.5 ml). On Days 3 and 4, 2 h after the water drinking session, all rats were injected with the appropriate solution as indicated in Table 1. On Day 3, rats in groups MN46 and MS46 were injected with morphine and rats in groups MN22 and MS22 were injected with saline. On Day 4, rats in groups MN22 and MS22 were injected with morphine and rats in group MN46 and MS46 were injected with saline. Rats in groups SN and SS were never injected with morphine, but received saline injections on both days.

On the conditioning trial (Day 5), the rats were presented with a graduated tube containing 0.1% saccharin solution for 30 min. Immediately following the removal of the tube, they were injected with either naloxone (groups MN46, MN22, and SN) or saline (groups MS46, MS22 and SS) solution. Four hours after the conditioning trial, the rats were placed on ad lib water for 3 days to replenish their thirst.

The testing trial occurred on Day 10. The rats were again placed on a drinking schedule of 30 min per day for 2 days prior to the test trial. On the test trial, they were presented with two graduated tubes, one containing 0.1% saccharin solution and the other containing tap water for 240 min and the amounts consumed at 30, 120, and 240 min were measured. The intake measures were converted to saccharin preference ratios (milliliter saccharin consumed/total saccharin + water consumed).

2.2. Results

Rats pretreated with morphine 22 and 46 h prior to a single conditioning trial with naloxone displayed taste avoidance, although naloxone alone did not produce taste avoidance following a single conditioning trial. Fig. 1 presents the mean ± S.E.M. saccharin preference ratio across 240 min of testing for each of the groups in Experiment 1. A

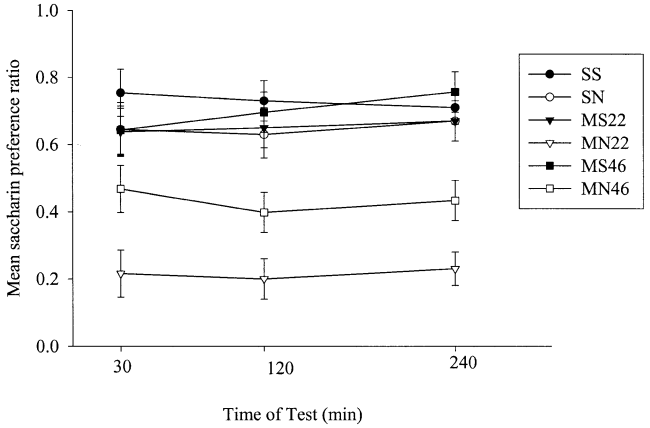

Fig. 1. Mean ± S.E.M. saccharin preference ratios (ml saccharin consumed/ml saccharin + ml water consumed) over a 240-min drinking period for the various groups in Experiment 1.

$3 \times 2 \times 3$ mixed-factors ANOVA for the between-groups factors of pretreatment condition and conditioning treatment and the within-groups factor of Time of test revealed significant main effects of pretreatment, $F(2,43) = 10.03$; $P < .001$, and conditioning treatment, $F(1,43) = 30.8$; $P < .001$. The only interaction that was statistically significant was the pretreatment by conditioning treatment, $F(2,43) = 4.9$; $P < .025$. Subsequent analysis of simple main effects revealed a significant pretreatment condition effect for the naloxone-conditioned groups, $F(2,43) = 54.8$; $P < .001$, but not for the saline-conditioned groups, $F(2,43) < 1$. LSD pairwise comparison tests revealed that among the naloxone-conditioned groups, Group MN22 had a lower saccharin preference ratio than Group MN46 ($P < .01$) and Group MN46 had a lower saccharin preference ratio than Group SN ($P < .01$). Additionally, simple main effects for the conditioning treatment effect revealed that Groups MN22 and MN46 had lower saccharin preference ratios than Groups MS22 and MS46, respectively, F's$(1,43) > 63.3$; P's $< .001$; however, Group SN did not differ significantly from Group SS, $F(1, 43) = 0.3$.

2.3. Discussion

The agonist effects of acute morphine dissipate well within 12 h of morphine exposure (Heishman et al., 1990; June et al., 1995; Kirby and Stitzer, 1993), yet precipitated morphine withdrawal can be induced up to 46 h following a *single* exposure to morphine. The strength of the taste aversion degraded as the interval between morphine pretreatment and naloxone treatment increased, but it was still present 46 h following the morphine pretreatment. Naloxone alone, however, did not produce one-trial conditioned taste avoidance. These results suggest that the persistent adaptational changes which occur following a single exposure to morphine (Gellert and Sparber, 1977; Jacob and Michaud, 1974; Martin and Eades, 1964; Schulteis et al., 1999) last for up to 46 h in rats.

3. Experiment 2

Place aversion conditioning is a sensitive measure of the aversive properties of opiate withdrawal (Koob et al., 1989; Mucha, 1987; Mucha, 1991; Mucha and Iverson, 1984; Mucha et al., 1986). The procedure consists of exposing rats to one distinctive chamber while in a saline state and another chamber while in a naloxone-precipitated morphine withdrawal state. When subsequently tested in a drug-free choice test between the chambers, rats spend less time in the chamber associated with precipitated withdrawal than in the chamber associated with saline (Koob et al., 1989; Mucha and Iverson, 1984; Parker and Joshi, 1998). More recently, Parker and Joshi (1998) reported that the conditioned place aversion produced by naloxone after two conditioning trials was enhanced by pretreatment with morphine 24 h prior to the conditioning trials. Experiment 2 evaluated the persistence of the aversive properties of acute morphine withdrawal using the place conditioning paradigm. Rats received a single conditioning trial cycle during which injections of morphine or saline were followed by naloxone or saline, 24 or 48 h later.

3.1. Method

3.1.1. Subjects

The subjects were 96 experimentally naïve male Sprague–Dawley rats (Charles River Labs, St. Constant, Quebec), weighing 250–300 g on the first conditioning trial. The rats were housed in pairs in clear plastic cages in a room illuminated on a 12-h light/12-h dark cycle with the lights on at 8:00 am. All procedures were conducted during the light phase of the cycle. Food and water were available ad lib.

3.1.2. Apparatus

The place conditioning apparatus consisted of two wooden chambers ($35 \times 25 \times 30$ cm) which were painted flat black. During conditioning, the chambers were separated by a wooden divider. Each chamber of the conditioning apparatus differed on the basis of floor cues. The floor of one chamber consisted of sandpaper strips, which were 5 cm wide and separated from the next strip by 5 cm. The other chamber had wire mesh flooring (1×1 cm). When assessed by group means, rats display equal preference for the two floors (Parker, 1995). The divider was removed during testing and the amount of time the rats explored each chamber of the conditioning apparatus was monitored by a video-tracking apparatus from a video camera mounted to the ceiling (Videomex-V, Columbus Instruments, Columbus, OH).

3.1.3. Procedure

The rats arrived in the laboratory 6 days prior to the onset of the experiment and were handled on each of these days. The experiment consisted of a single conditioning cycle and a place preference test. The design of the place conditioning

Table 2
Design of place conditioning cycle of Experiment 2

Groups	Day 1: nontreatment trial	Day 2 pretreatment	Day 3 pretreatment	Day 4: treatment trial
MN24 ($n=16$)	Saline → mesh or sand	Saline	Morphine	Naloxone → sand or mesh
MS24 ($n=16$)	Saline → mesh or sand	Saline	Morphine	Saline → sand or mesh
MN48 ($n=16$)	Saline → mesh or sand	Morphine	Saline	Naloxone → sand or mesh
MS48 ($n=16$)	Saline → mesh or sand	Morphine	Saline	Saline → sand or mesh
SN ($n=16$)	Saline → mesh or sand	Saline	Saline	Naloxone → sand or mesh
SS ($n=16$)	Saline → mesh or sand	Saline	Saline	Saline → sand or mesh

trial is presented in Table 2. Rats were randomly assigned to each of the groups ($n=16$). During the conditioning cycle, Day 1 was designated as the nontreatment trial and Day 4 was designated as the treatment trial. On Day 1, all rats were injected with saline and 5 min later were placed in either the chamber with the mesh floor or the chamber with the sandpaper floor (counterbalanced among the groups) for 30 min. On Day 4, the treatment trial, rats were injected with either naloxone (1 mg/kg sc) or saline and 5 min later were placed in the chamber with the floor opposite to the nontreatment floor (sandpaper or mesh) for 30 min. After each trial, the chambers were cleaned with soapy water and thoroughly dried.

On Days 2 and 3 of the conditioning cycle, all rats received a total of two pretreatment injections [morphine (20 mg/kg sc) or saline], one each at 24 and 48 h prior to the treatment trial on Day 4. The solution injected varied at each interval among the groups. The rats assigned to Groups MN48 and MS48 received morphine on Day 2 and saline on Day 3. The rats assigned to Groups MN24 and MS24 received morphine on Day 3 and saline on Day 2. The rats assigned to Groups SN and SS received saline on both Days 2 and 3.

The conditioned place preference test was conducted 48 h after the treatment trial. The divider was removed from the conditioning apparatus and drug-free rats were individually placed in the center of the place preference apparatus facing the wall perpendicular to the chambers. In doing so, each rat was presented with the opportunity to explore both chambers for 15 min. The video-tracking apparatus automatically recorded the amount of time in seconds that each rat spent in each of the two chambers.

3.2. Results

A single exposure to morphine 24 or 48 h prior to naloxone produced a place aversion. Fig. 2 presents the mean seconds on the treatment-paired floor minus the nontreatment-paired floor for the various groups in Experiment 2. The difference scores were entered into a 2×3 between-

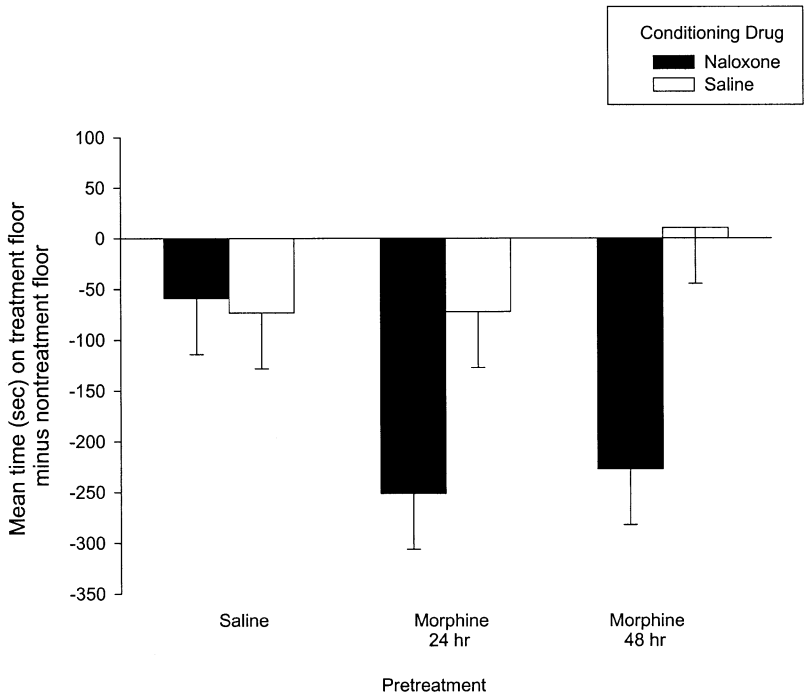

Fig. 2. Mean ± S.E.M. seconds on the treatment-paired floor minus the nontreatment-paired floor for the groups conditioned with naloxone and pretreated with morphine (24 or 48 h) or saline in Experiment 2.

groups ANOVA, which revealed a significant effect of conditioning drug, $F(1,90) = 7.0$; $P < .01$, and a Conditioning drug × Pretreatment interaction, $F(2,90) = 3.0$; $P < .05$. Subsequent analysis of the simple main effects revealed that rats treated with naloxone at 24 h, $F(1,30) = 7.0$; $P < .01$ and at 48 h, $F(1,30) = 9.3$; $P < .01$, following morphine pretreatment spent significantly less time in the treatment-paired chamber than those treated with saline. However, naloxone treatment in the absence of morphine pretreatment did not produce a place aversion in a single conditioning trial, $F(1,30) = 0.03$; $P = .86$.

3.3. Discussion

Naloxone-precipitated morphine withdrawal produced a place aversion, even when the interval between morphine and naloxone was 48 h. The place aversion did not degrade as the interval between morphine and naloxone increased, which suggests that the biological changes produced by a single exposure to morphine persist even beyond 48 h.

4. General discussion

Naloxone-precipitated morphine withdrawal produced both taste avoidance and place avoidance following a *single* conditioning trial. In both paradigms, the aversive properties of naloxone-precipitated morphine withdrawal were evident 46–48 h after a single injection of morphine (at a high dose of 20 mg/kg sc). In neither paradigm did naloxone treatment produce an aversion following a single conditioning trial. In Experiment 2, when a contextual cue served as the conditioned stimulus, the aversive effect of naloxone-precipitated withdrawal did not significantly degrade over the 48-h period; however, in Experiment 1, a stronger taste aversion was produced when withdrawal was precipitated 22 h following morphine than 46 h following morphine. Although the reason for this difference is unknown, it probably reflects a difference in the sensitivity of the two paradigms. Since smaller rats were used in Experiment 1 than in Experiment 2, it is also possible that the size of the animal affected the pharmacodynamics of the drug.

Although other investigators have documented occurrences of acute morphine dependence following a single exposure to morphine (Easterling and Holtzman, 1997; Easterling et al., 2000; Heishman et al., 1990; Martin and Eades, 1964; Schulteis et al., 1999), the present findings are the first to demonstrate that the motivationally aversive properties of acute opiate withdrawal are sufficient to produce both taste and place avoidance even 46–48 h after a single morphine exposure. These results are consistent with the research of Gellert and Sparber (1977) who reported that in the rat, naloxone administration 48 h after a single morphine exposure produced a behavioral index of naloxone-precipitated morphine withdrawal and naloxone was not aversive in morphine-naïve rats. Furthermore, Eisenberg (1982) reported that naloxone administration 24 h after a single morphine exposure induced a biochemical index (increased plasma corticosterone level) of precipitated morphine withdrawal in the rat. The increase of plasma corticosterone level induced by naloxone administration 24 h after morphine exposure was of significantly greater magnitude than that produced by naloxone administration 24 h after saline administration. The results of the present study indicate that these prolonged receptor changes following a single exposure to morphine may also be measured by the aversive motivational effects of naloxone-precipitated withdrawal. The present study provides simple and sensitive behavioral measures, which can be used to evaluate the persistence of acute morphine dependence.

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