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## Delta-Opioid Receptor Antagonists Prevent Sensitization to the Conditioned Rewarding Effects of Morphine

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### Abstract

**Background**—Functional interactions between mu- and delta- opioid receptors (MOPr and DOPr, respectively) are implicated in morphine tolerance and dependence. The contribution of DOPr to the conditioned rewarding effects of morphine and the enhanced conditioned response that occurs following repeated morphine administration is unknown. This issue was addressed using the conditioned place preference procedure (CPP).

**Methods**—Rats received home cage injections of saline or morphine (5.0 mg/kg/day × 5 days) prior to conditioning. For sensitization studies, DOPr antagonists (DOPr1/2: naltrindole, DOPr2: naltriben, DOPr1: 7-benzylidenenaltrexone) were administered prior to morphine injections. Conditioning sessions (2 morphine; 2 saline) commenced 3 days later. To assess the influence of acute DOPr blockade on the conditioning of morphine reward in naïve animals, 3 morphine and 3 saline conditioning sessions were employed. Antagonists were administered prior to morphine conditioning sessions.

**Results**—Morphine was ineffective as a conditioning stimulus after 2 conditioning sessions in naïve rats. However, doses 3.0 mg/kg produced significant CPP in morphine pre-exposed rats confirming that sensitization develops to the conditioned rewarding effects of morphine. In animals that received morphine pre-exposure with naltrindole or naltriben, but not 7-benzylidenenaltrexone, sensitization was prevented. No attenuation of morphine CPP was observed in animals that received DOPr antagonists acutely, prior to conditioning sessions.

**Conclusion**—These data indicate a critical role of DOPr systems in mediating sensitization to the conditioned rewarding effects of morphine. The efficacy of naltrindole and naltriben in preventing the enhanced response to morphine suggest the specific involvement of DOPr2 in the sensitization process.

### Keywords

morphine; sensitization; conditioned place preference; delta opioid receptor; naltrindole; naltriben

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## Introduction

Exposure to stimuli associated with opiate administration elicits drug-craving and increased risk of relapse (1, 2). Accordingly, one focus of addiction research is the identification of the neural substrates mediating the conditioned reinforcing effects of drugs and pharmacological treatments that can prevent their development and/or expression.

The conditioned place preference (CPP) paradigm has been used extensively to study the contextual/cued associations that develop to drugs of abuse in experimental animals (3, 4). CPP studies have demonstrated that the rewarding effects of opiates become conditioned to stimuli associated with their administration. Several laboratories have shown that the conditioned rewarding effects of mu-opioid receptor (MOPr) agonists are enhanced in animals with a prior history of opiate exposure (5–8). Morphine doses that fail to produce CPP in naïve rats produce robust CPP in animals that received MOPr agonists repeatedly prior to the commencement of conditioning. The efficacy of morphine is also increased.

The opioid receptor types mediating the sensitized response to MOPr agonists are unclear. Repeated administration of the non-selective opioid receptor antagonist, naloxone (0.5 mg/kg) with morphine or fentanyl blocked the enhancement of CPP confirming opioid receptor mediation. However, a lower naloxone dose (0.1 mg/kg) that antagonizes the effects of MOPr-, but not delta-(DOPr) or kappa-opioid receptor (KOPr) agonists was ineffective (7). KOPr antagonism enhances sensitization to MOPr agonists (9). Therefore, attenuation of sensitization can not be attributed to KOPr blockade. These findings suggest that a DOPr-dependent mechanism may underlie sensitization to the conditioned rewarding effects of MOPr agonists.

Functional interactions of MOPr and DOPr have been described. Antisense oligonucleotides to DOPr attenuate morphine dependence (10, 11). Similarly, pharmacological antagonism or genetic deletion of DOPr reduces dependence and analgesic tolerance (12). Repeated MOPr agonist administration increases DOPr cell surface expression in brain and spinal cord (13, 14). MOPr and DOPr heterodimerization has been reported (15, 16). Evidence that the DOPr2 subtype may correspond to a MOPr-DOPr complex has been obtained (17, 18). Together these data indicate that chronic morphine exposure alters DOPr function and that aberrant DOPr activity may contribute to the behavioral dysregulation produced by repeated MOPr activation.

An involvement of DOPr in morphine tolerance and dependence has been demonstrated (10, 11, 12). The role of DOPr in sensitization to the rewarding effects of morphine is unknown. An unbiased CPP procedure was used to address this issue. Morphine conditioning was assessed in naïve rats and those that received a sensitizing morphine treatment alone or with a DOPr antagonist prior to conditioning. Because pharmacological data indicate DOPr subtypes (DOPr1; DOPr2), the DOPr 1/2 antagonist, naltrindole (19), the DOPr2 antagonist, naltriben, (20) and the DOPr1 antagonist, 7-benzylidenenaltrexone (BNTX; 21) were evaluated.

## Materials and Methods

### Animals

Male Sprague-Dawley rats (300–350 g; Charles River) were housed in a temperature controlled colony room and maintained on a 12 h:12 h light:dark cycle with food and water available ad-libitum. Facilities were accredited by AALAC and experiments were approved by the NIDA Institutional Care and Use Committee.

## Drug Treatments

For sensitization studies, rats received home-cage injections of saline or morphine (5.0 mg/kg/day  $\times$  5 days; s.c). Fifteen min prior to injections they received an s.c injection of saline, naltindole, naltriben or BNTX. Conditioning commenced 3 days later. Experiments were conducted in squads of twenty animals. Separate squads of animals were run for each antagonist treatment. Each squad consisted of control and experimental animals. Controls animals received vehicle prior to repeated morphine injections. Experimental animals received vehicle or one of the assigned antagonist doses prior to morphine injection. Morphine-evoked conditioning was then assessed. The influence of prior, repeated administration of antagonist, alone, on morphine conditioning was assessed in additional squads of animals. Conditioning commenced 3 days later.

To assess the effects of antagonist treatment on the establishment of morphine CPP, additional rats received injections of antagonist or vehicle 15 min prior to morphine conditioning sessions.

## CPP Procedure

Conditioning was conducted in Plexiglas boxes (7, 22) under dim illumination (8.5–10 lux). Under these conditions, rats exhibit no preference for either of the place cues. Two conditionings sessions (50 min)/day were conducted with at least 6 hrs separating each. Rats were injected with saline and confined to one compartment. Following morphine administration (3.0; 5.0 mg/kg; s.c) they were confined to the other compartment. For sensitization studies, four conditioning sessions (2 morphine; 2 saline) were conducted since 2 drug pairings is insufficient for the development of CPP in animals that are opiate naïve prior to the commencement of conditioning (7, 22). To assess the effects of acute DOPr antagonism on the establishment of CPP, six conditioning sessions (3 morphine; 3 saline) were conducted since three drug pairings produces robust CPP in opiate naive animals (7, 22; 23). Treatment compartment and the presentation order of morphine and saline were counterbalanced. Conditioning was assessed 1 day after the last conditioning session. Uninjected rats were allowed access to both compartments for 900 sec. Time spent in each was assessed by analysis of the video-recorded test session. Data analysis was conducted by a blinded observer. The rat's location was determined by the position of the front paws.

## Statistical Analysis

Conditioning scores represent time spent in the morphine-paired place minus that spent in the saline-paired place (mean  $\pm$  SEM). The Wilcoxon test was used to determine whether individual doses produced significant CPP. A two-factor (pretreatment  $\times$  morphine dose) ANOVA or when appropriate, a single-factor ANOVA or the Student t-test was used to determine the effects of treatments upon conditioning. Post-hoc analysis was conducted using the Dunnet's test.

## Drugs

Drugs, generously provided by NIDA Drug Supply (Bethesda, MD, USA), were prepared in saline.

## Results

Rats conditioned with saline exhibited no preference for either of the place cues. The mean time spent in the black and white compartments, respectively, was: 330  $\pm$  17 vs. 350  $\pm$  22 sec (4 sessions) and 385  $\pm$  51 vs. 329  $\pm$  38 sec (6 sessions). There was no difference between groups in time spent in the two compartments (4 sessions:  $t=0.8$ ,  $df=7$ ;  $p=0.5$ ; 6 sessions:  $t=0.6$ ,  $df=7$ ;  $p=0.6$ ) confirming that the conditioning procedure was unbiased.

As previously reported (7, 8, 22), morphine failed to induce CPP in control animals after 2 drug conditioning sessions (Fig 1). In animals previously exposed to morphine, doses of 3.0 and 5.0 mg/kg produced significant CPP. ANOVA revealed a significant effect of pretreatment [ $F(1,51) = 4.6$ ;  $p = 0.04$ ] but no dose [ $F(2,51) = 1.3$ ;  $p = 0.3$ ] or interaction [ $F(2,46) = 0.8$ ;  $p = 0.5$ ] effects. The CPP observed in animals with a prior morphine history is evidential of the development of sensitization.

Fig. 2 shows conditioning produced by morphine (5.0 mg/kg) in rats that had received the repeated morphine pretreatment regimen with the DOPr1/2 antagonist, naltrindole. The naltrindole doses employed were ineffective in producing a conditioned response in naïve animals (24). ANOVA revealed a significant effect of naltrindole [ $F(4,38) = 6.2$ ;  $p = 0.001$ ]. Morphine produced CPP in animals that had received once daily injections of morphine with saline but was ineffective in animals that received the morphine pretreatment with 0.03 or 0.1 mg/kg naltrindole.

To determine whether prior repeated administration of naltrindole, alone, prevents acquisition of morphine CPP in naïve animals, additional rats received home cage injections of naltrindole (0; 0.1 mg/kg/day  $\times$  5 days). Conditioning sessions (3 morphine; 3 saline) commenced 3 days later. As previously reported (22, 23), morphine produced significant CPP in naïve animals when the number of environmental/drug pairings was increased (Fig 3a). CPP was also observed in animals that received naltrindole, repeatedly, prior to the commencement of conditioning. There was no difference between groups in this effect ( $t = 0.4$ ;  $df = 1,14$ ;  $p = 0.5$ ). Similarly, naltrindole administration prior to each morphine conditioning session did not affect morphine CPP [Fig. 3b:  $F(2,25) = 1.3$ ;  $p = 0.3$ ].

Fig. 4 shows morphine conditioning (2 sessions) in animals that received the five day morphine pretreatment with the DOPr2 antagonist, naltriben. In animals that had received naltriben with morphine for five days, the conditioned response to morphine response was attenuated [ $F(3,43) = 4.7$ ;  $p = 0.007$ ]. Thus, morphine failed to produce significant CPP in animals that had received 0.01–0.3 mg/kg naltriben with morphine. Furthermore, following the 0.1 mg/kg naltriben + morphine treatment, morphine CPP was significantly reduced relative to vehicle + morphine treated animals. Repeated naltriben administration (0.03 mg/kg/day  $\times$  5 days), alone, prior to the commencement of conditioning sessions (3 morphine; 3 saline) did not alter morphine CPP (Fig 5a). The magnitude of CPP did not differ between the control and naltriben pretreatment groups ( $t = 0.5$ ;  $df = 1,17$ ;  $p = 0.6$ ). Similarly, naltrindole administration prior to each of morphine conditioning sessions did not attenuate morphine CPP (Fig. 5b:  $t = 1.2$ ;  $df = 1,18$ ;  $p = 0.3$ ).

The DOPr1 antagonist, BNTX (0.03–0.3mg/kg), was ineffective in attenuating the enhancement of morphine conditioning produced by prior morphine administration (Fig 6a) [ $F(3,36) = 0.3$ ;  $p = 0.8$ ]. Prior repeated administration of BNTZ alone (Fig 6b) or its administration prior to morphine conditioning sessions were also without effect (6c).

## DISCUSSION

These studies confirm that the conditioned rewarding effects of morphine are enhanced in animals with a prior history of morphine administration. In animals that received morphine repeatedly with a DOPr1/2 or a selective DOPr2 antagonist, no enhancement of the conditioned response was seen. Selective DOPR1 antagonism was without effect.

We previously showed that six conditioning sessions (3 morphine; 3 saline) is necessary for the conditioning of morphine reward in rats that are opiate naïve prior to place conditioning. In animals with a prior history of MOPr agonist administration, the potency and efficacy of morphine in producing CPP is increased (7, 22). In the present study, morphine doses which

were ineffective as conditioning stimuli after 2 environmental pairings produced robust CPP in animals that had received context independent injections of morphine prior to the commencement of conditioning. These findings confirm that sensitization develops to the conditioned rewarding effects of morphine. Previous time course studies revealed that a period of abstinence is necessary for expression of the enhanced conditioned response (7). Thus, it can not be attributed to an increase in the total dose of morphine administered. Rather, neuroadaptations occurring in response to repeated morphine administration underlie the development of sensitization.

In animals that received naltrindole with the morphine pretreatment regimen, the enhancement of CPP was prevented. The effective doses of naltrindole are DOPr selective (25, 26) suggesting that the abolition of sensitization results from specific DOPr blockade. The abolition of sensitization can not be attributed to malaise or any conditioned effects of the antagonist since the naltrindole doses employed lack aversive effects (7) and naltrindole administered alone for 5 days prior to conditioning was without effect.

Naltrindole produced biphasic effects. Although an explanation for the decreased potency of higher doses is lacking, naltrindole binds with low affinity to MOPr and KOPr (27) and exerts DOPr agonist effects at high doses (28). Non-opioid receptor mediated effects of high naltrindole concentrations have been reported (29). Therefore, the diminished response to higher doses likely reflects loss of receptor selectivity.

DOPr subtypes have been proposed on the basis of differential antagonism of agonist induced antinociception, binding assays and tolerance studies (30). Recent data suggest that the cloned DOPr corresponds to DOPr2 whereas hetero-oligomerization of DOPr and KOPr gives rise to DOPr1 (31–33). BNTX is considered a selective DOPr1 antagonist whereas naltriben is DOPr2 selective (20, 21).

BNTX failed to modify the development of sensitization. The dose range employed was that which antagonized the behavioral effects of DOPr1 agonists (34, 35). Therefore, the lack of effect of BNTX is consistent with the hypothesis that sensitization to the conditioned rewarding effects of morphine occurs via a DOPr1-independent mechanism. The DOPr2 antagonist, naltriben, produced a dose-related attenuation of sensitization. The differential effects of naltrindole and naltriben as compared to BNTX suggest a critical role of DOPr2 in mediating the sensitized conditioned response to morphine that develops as a consequence of repeated morphine administration.

Given the influence of naltrindole and naltriben on sensitization, we examined whether these antagonists can attenuate the establishment of morphine CPP in opiate naïve animals. Morphine produced significant CPP in naïve animals when the number of environmental-drug pairings was increased from 2 to 3. CPP was also observed in rats that received antagonists repeatedly, prior to conditioning commencement. Thus, in the absence of concurrent MOPr activation, repeated DOPr blockade does not attenuate the conditioned rewarding effects of morphine.

CPP was observed when naltrindole or naltriben were administered acutely, prior to each of the three morphine conditioning sessions. These data suggest that although DOPr2 receptor activation is necessary for sensitization, it does not contribute to the conditioning of morphine reward in previously opiate naïve rats. Consistent with these findings, the DOPr antagonist, DN-20, does not attenuate CPP produced by a MOPr agonist in ICR mice (37). Similarly the locomotor activating and dopamine releasing effects produced by acute morphine administration are unaltered in DOPr knock out mice (36). Interestingly, DOPr antagonists were previously shown to attenuate morphine-induced CPP in ddY mice (38) and pharmacological inactivation or DOPr gene deletion attenuates conditioning in C57bl/6

mice (39). These data suggest that the role of DOPr in mediating the acute conditioned rewarding effects of MOPr agonists differ depending on the species and strain tested and highlight the need for caution in extrapolation of data to the human condition.

The mechanism by which DOPr2 blockade prevents the development of sensitization is unknown. Repeated MOPr activation increases DOPr function and cell surface expression (13, 14, 40). Whether repeated morphine administration produces similar effects in brain regions important for the motivational effects of opiates is unknown. However, DOPr recruitment by repeated morphine may be one mechanism by which DOPr2 antagonism attenuates sensitization.

MOPr and DOPr heterodimerization has been reported (15, 41, 42) in heterologous systems. Using bivalent ligands composed of a MOPr agonist pharmacophore linked to a DOPr antagonist pharmacophore through variable-length spacers, Daniels *et al* (43) obtained evidence that a physical interaction between DOPr and MOPr modulates MOPr-mediated tolerance and dependence. In contrast to monovalent MOPr agonists, bivalent ligands composed of a MOPr agonist and a DOPr antagonist did not produce CPP in ICR mice (37). Such findings indicate that heteromeric MOPr and DOPr contribute to the rewarding effects of MOPr agonists in this strain.

In conclusion, these data provide the first demonstration that DOPr2 activation is required for the development of sensitization to the conditioned rewarding effects of morphine. DOPr antagonists prevent the development of tolerance to the antinociceptive effects of MOPr agonists (10–12, 44). Given the postulated roles of sensitization and conditioning in addiction, these findings suggest that drugs which function as MOPr agonists/ DOPr2 antagonists may be effective analgesics with reduced tolerance and addiction liability. Studies, however, are needed to determine whether DOPr/MOPr interactions result from activation of opioid receptors located on separate neurons or at the intracellular level, via receptor cross-talk, and whether these receptor interactions are observed in human subjects.

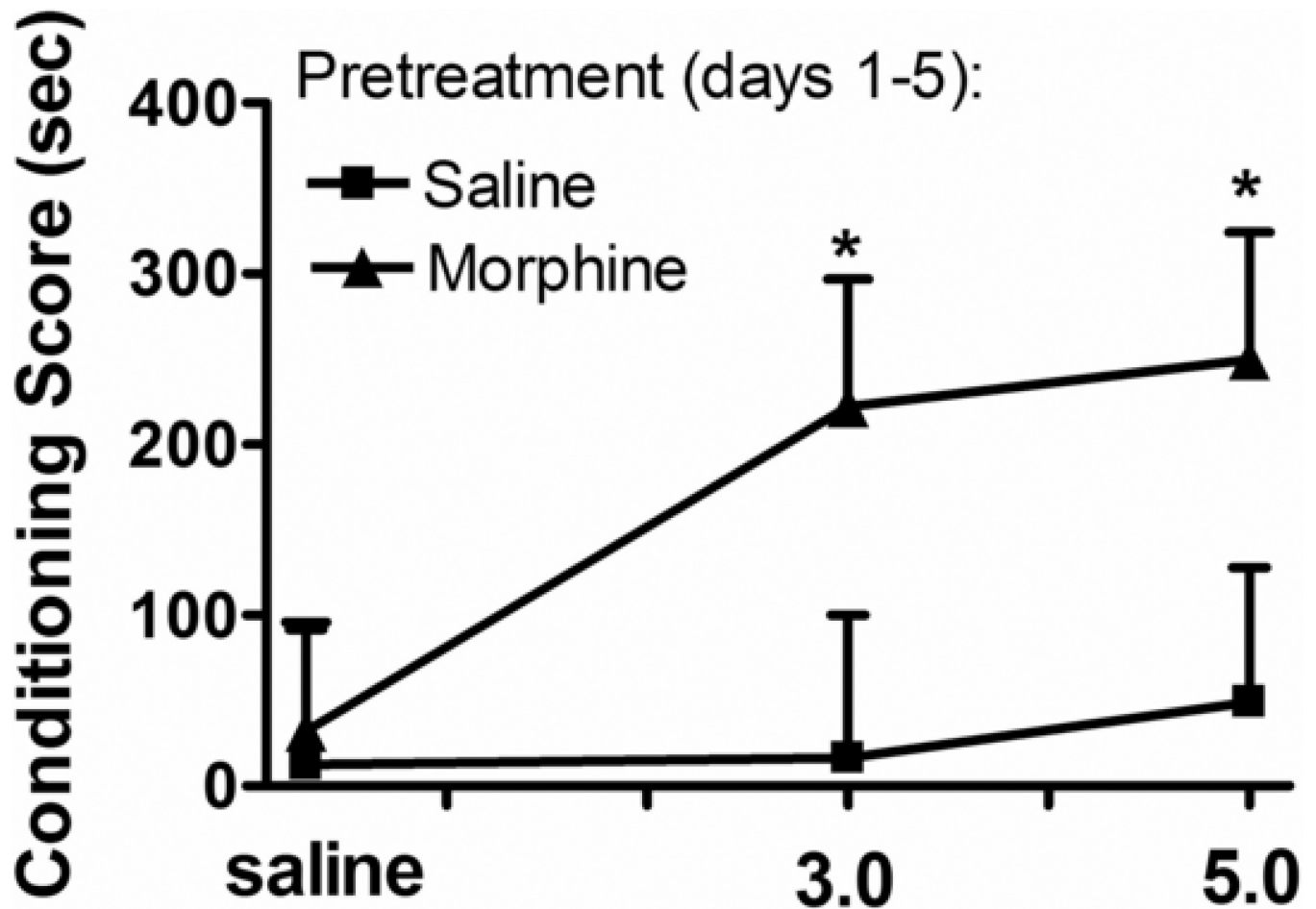
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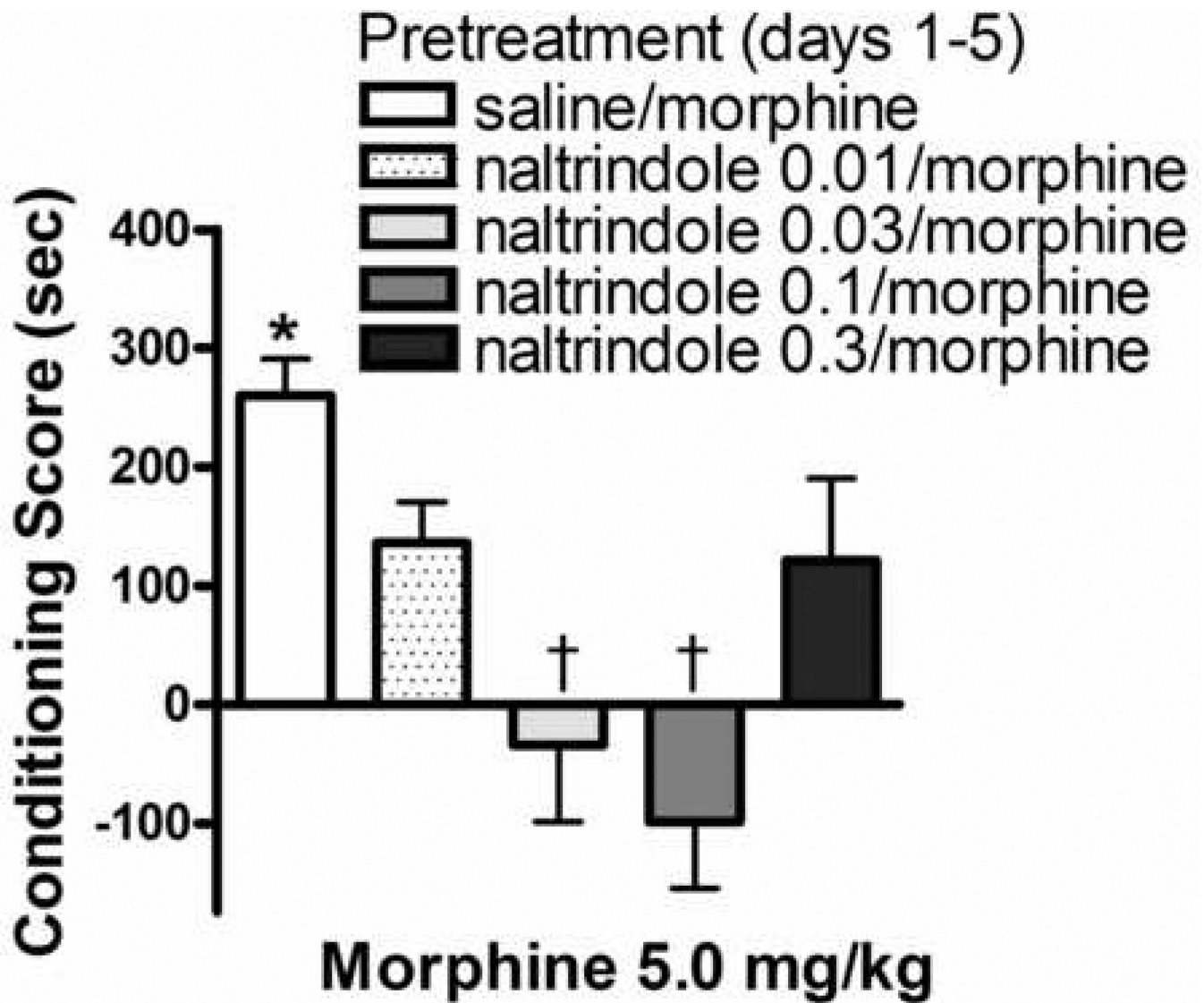
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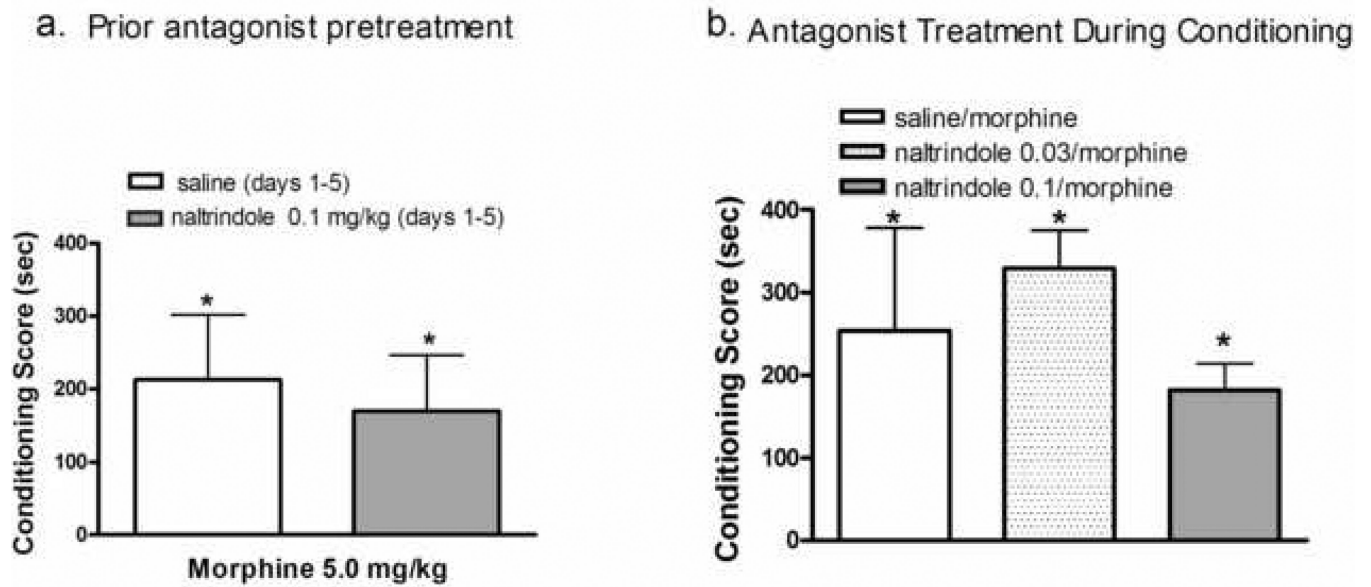


**Fig. 1.** Influence of prior context-independent morphine administration on morphine-evoked place conditioning. Rats received home-cage injections of saline or morphine (5.0 mg/kg/day, s.c  $\times$  5 days). Place conditioning sessions (2 morphine; 2 saline) commenced three days later. Conditioning scores represent the time (sec) spent in the morphine-paired environment minus that spent in the saline-paired environment. Each data point represents the mean  $\pm$  SEM of 7–10 rats. \* denotes significant CPP (Wilcoxon test).

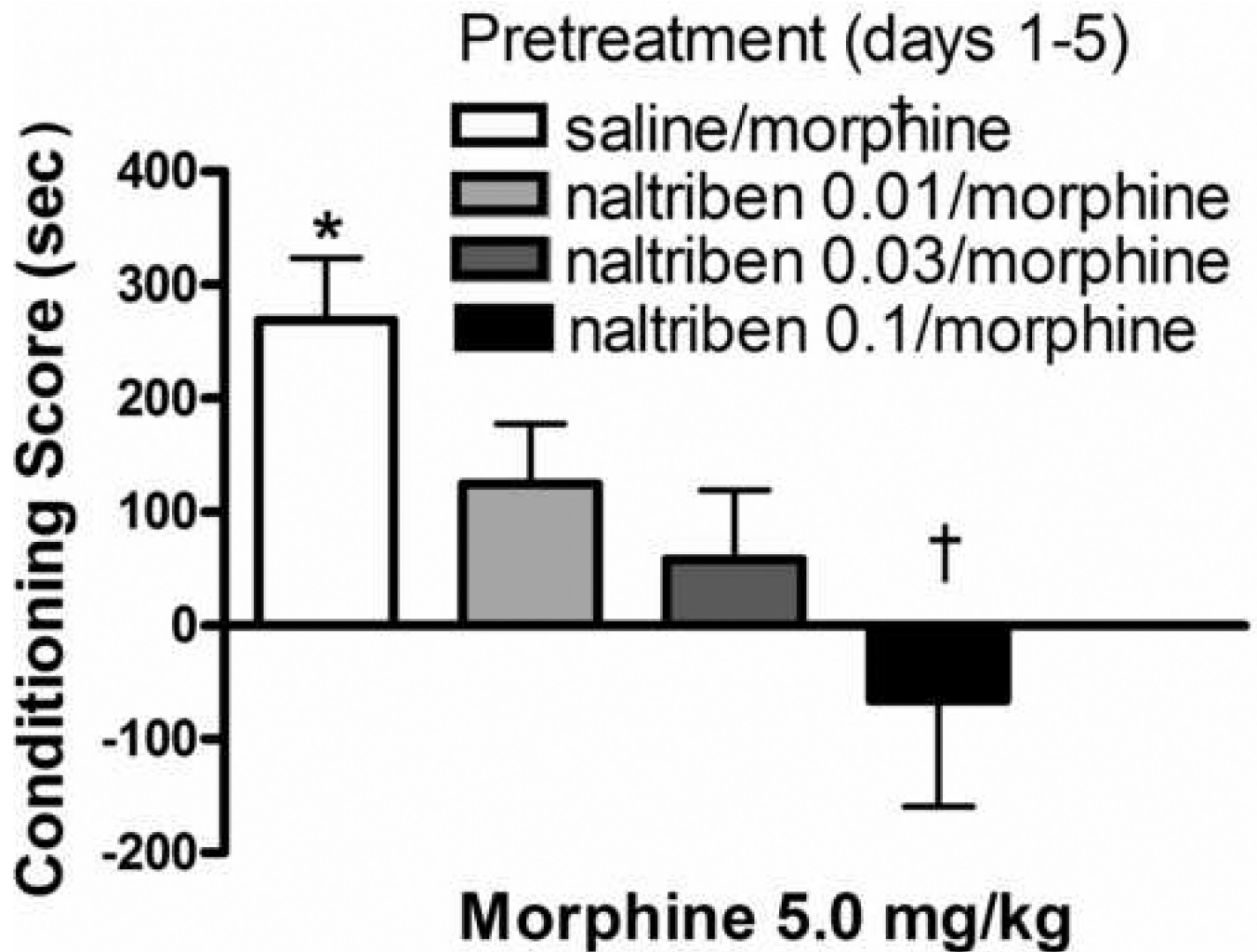


**Fig. 2.**

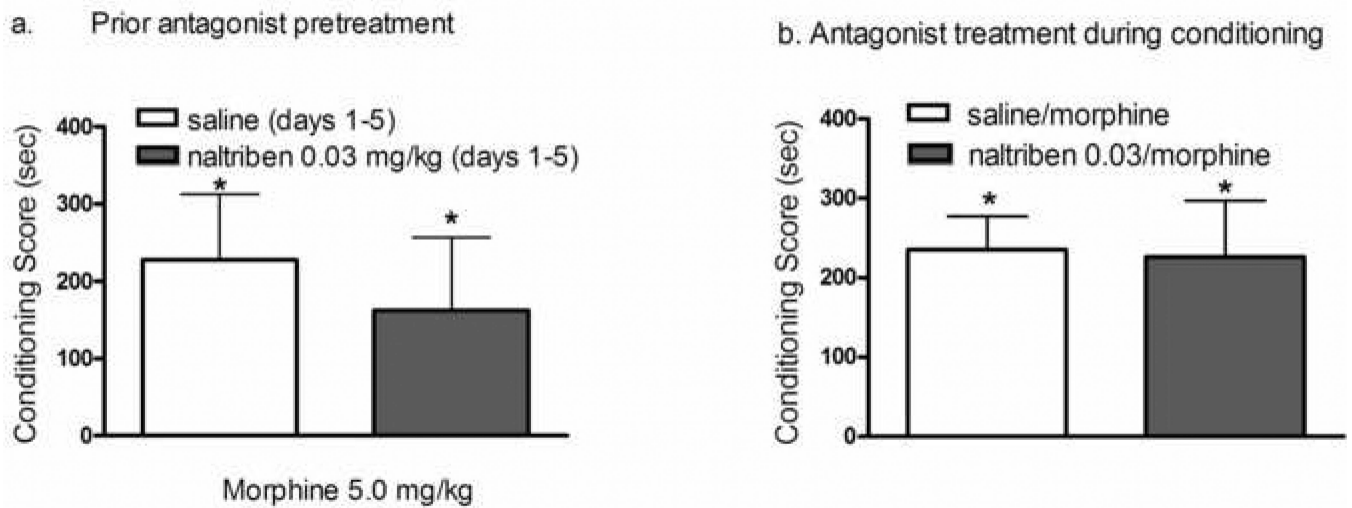
Influence of prior, repeated administration of morphine or naltrindole + morphine on sensitization to the conditioned rewarding effects of morphine. Rats received once daily injections of saline or the DOPr1/2 antagonist, naltrindole (0.03–1.0 mg/kg; s.c) 15 min prior to morphine (5.0 mg/kg, s.c) injections on days 1–5. Conditioning sessions (2 morphine; 2 saline) commenced three days later. Each data point represents the mean  $\pm$  SEM of 7–9 rats. \* denotes significant CPP (Wilcoxon test). † indicates significant difference relative to animals that received the 5 day morphine treatment regimen in combination with saline.

**Fig. 3.**

Naltrindole is ineffective in attenuating morphine CPP in naïve animals. (a) Rats received once daily injections of naltrindole (0.1 mg/kg, s.c) for five days. Conditioning sessions (3 morphine; 3 saline) commenced 3 days later. (b) Rats received an injection of naltrindole (0.03; 0.1 mg/kg, s.c) 15 min prior to morphine conditioning sessions. A total of six conditioning sessions (3 morphine; 3 saline) were conducted. Data represent the mean SEM of n=8–11 rats. \* indicates significant CPP (Wilcoxon test).

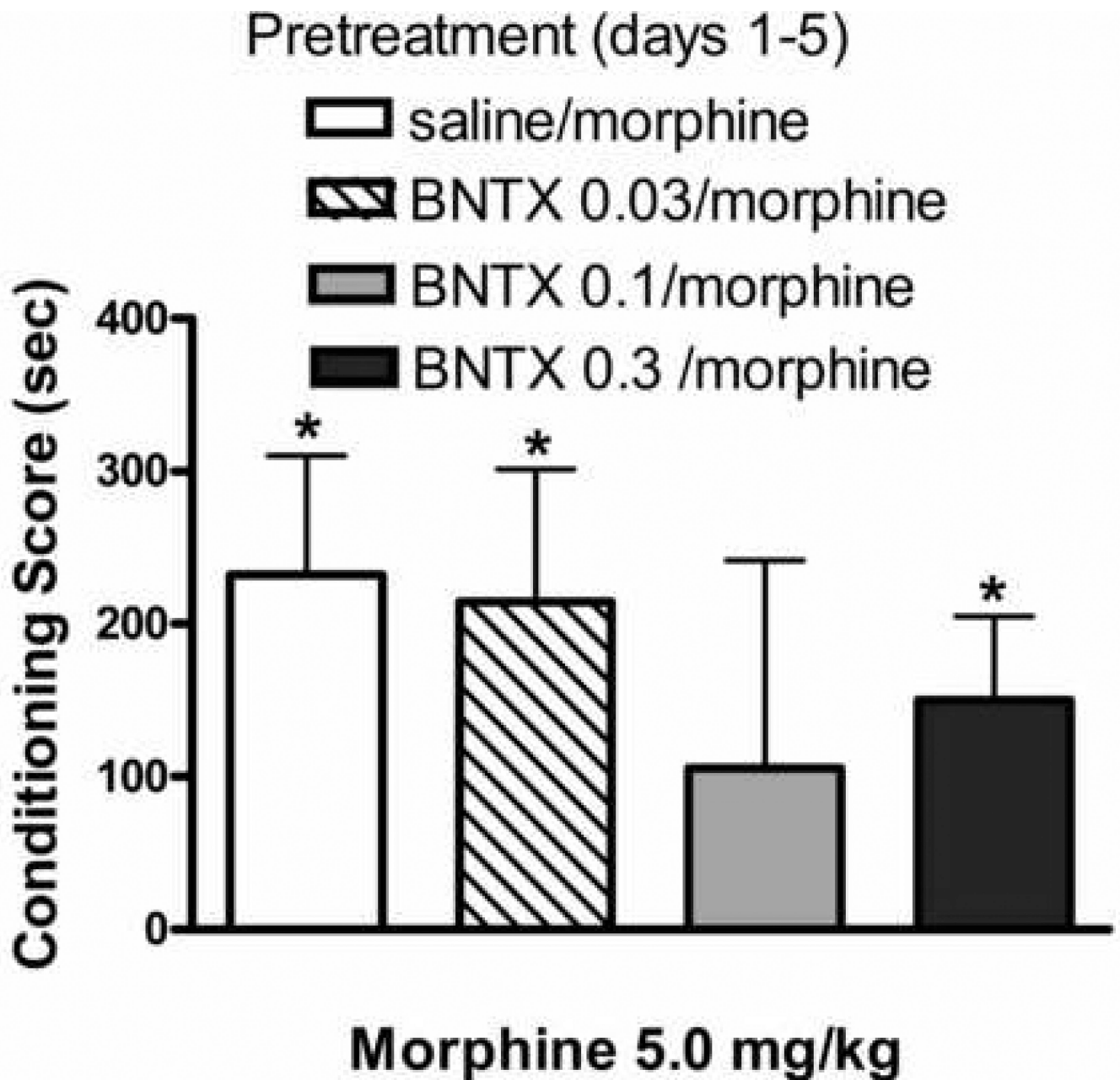


**Fig. 4.** Influence of prior, repeated administration of naltriben + morphine on sensitization to the conditioned rewarding effects of morphine. Rats received once daily injections of saline or the DOPr2 antagonist, naltriben (0.01–0.1 mg/kg; s.c) 15 min prior to injections of morphine (5.0 mg/kg, s.c) on days 1–5. Conditioning sessions (2 morphine; 2 saline) commenced three days later. Each data point represents the mean  $\pm$  SEM of 10–13 rats. \* indicate significant CPP (Wilcoxon test). † indicates significant difference relative to animals that received the 5 day morphine treatment regimen with saline.



**Figure 5.**

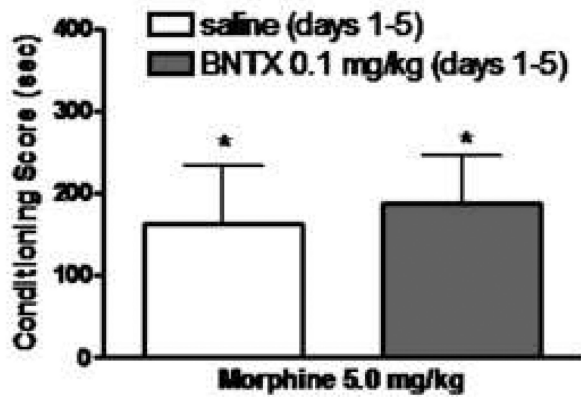
Naltriben is ineffective in attenuating morphine-induced CPP in naïve animals. (a) Rats received once daily injections of naltriben (0.03 mg/kg, s.c) for five days. Conditioning sessions (3 morphine; 3 saline) commenced 3 days later. (b) Rats received an injection of naltriben (0.03 mg/kg, s.c) 15 min prior to morphine conditioning sessions. A total of six conditioning sessions (3 morphine; 3 saline) were conducted. Each column represents the mean SEM of n=9–10 rats. \* indicate significant place conditioning (Wilcoxon test).



**Figure 6.**

Influence of prior, repeated administration of BNTX + morphine on sensitization to the conditioned rewarding effects of morphine. Rats received once daily injections of saline or the DOPr1 antagonist, BNTX (0.03–0.3 mg/kg; s.c) 15 min prior to injections of morphine (5.0 mg/kg, s.c) on days 1–5. Conditioning sessions (2 morphine; 2 saline) commenced three days later. Each data point represents the mean  $\pm$  SEM of 10 rats. \* indicates significant place conditioning (Wilcoxon Test).

## a. Prior antagonist pretreatment



## b. Antagonist treatment during conditioning



**Figure 7.**

BNTX does not affect morphine-induced CPP in naïve animals. (a) Rats received once daily injections of BNTX(0.1 mg/kg, s.c) for five days. Conditioning sessions (3 morphine; 3 saline) commenced 3 days later. (b) Rats received an injection of BNTX (0.01 mg/kg, s.c) 15 min prior to morphine conditioning sessions. A total of six conditioning sessions (3 morphine; 3 saline) were conducted. Each column represents the mean SEM of n=9–10 rats. \* indicate significant place conditioning (Wilcoxon test).