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Effects of opioid antagonists on unconditioned and conditioned hyperactivity to morphine

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Abstract

In a series of experiments, the ability of selective μ- (β-funaltrexamine, β-FNA), δ- (naltrindole, nalt) and κ- (nor-binaltorphimine, nor-BNI) opioid receptor antagonists to attenuate the unconditioned and conditioned hyperactive effects of morphine was examined. For comparison, the nonselective opioid receptor antagonist naloxone (nalx) was also examined. Locomotor activity served as the behavioral measure. Experiment 1 found that doses of 1 and 4, but not 16 mg/kg, of morphine effectively produced conditioned hyperactivity (CH). Experiments 2a–d found that β-FNA, nalt, nor-BNI and nalx, respectively, attenuated unconditioned morphine-induced hyperactivity. Experiments 3a–c, however, found that none of the selective antagonists, given individually, attenuated CH. In contrast, nalx did attenuate CH (Experiment 3d). Collectively results suggest that the unconditioned and conditioned hyperactive responses to morphine are mediated by different receptor systems and that activation of multiple opioid-receptor subtypes mediate expression of CH.

Keywords: Morphine; Antagonist; Mu; Delta; Kappa; β-Funaltrexamine; Naltrindole; Nor-binaltorphimine; Naloxone; Locomotor activity; Conditioning; Rat

1. Introduction

Conditioned hyperactivity (CH) has become a well-established paradigm for assessing the stimulant effects of various drugs of abuse across repeated injections. In this paradigm, a drug unconditioned stimulus (US; e.g., morphine) elicits an unconditioned hyperactive response (UR). A once neutral stimulus (e.g., a distinctive context) paired with such a drug also comes to elicit a hyperactive response. In Pavlovian conditioning terminology, the distinctive context serves as the conditioned stimulus (CS) and the hyperactive response that it elicits is termed the conditioned response (CR). CH has been shown using a variety of drugs such as amphetamine (Swerdlow and Koob, 1984), cocaine (Beninger and Herz, 1986) and morphine (Vezina and Stewart, 1984; Neisewander and Bardo, 1987).

In regards to the unconditioned and conditioned hyperactive responses to morphine and heroin, opioid receptors are thought to be critical. Specifically, the endogenous release of opioids may mediate the unconditioned and conditioned hyperactive responses to morphine and heroin. Support for this idea comes from studies that have shown that the nonselective opioid receptor antagonists naloxone (nalx) and naltrexone attenuate the UR elicited by morphine (Ayhan and Randrup, 1973; Babbini and Davis, 1976; Oka and Hosoya, 1976; Iwamoto, 1981) and heroin (Swerdlow et al., 1985), as well as attenuating the acquisition (Mucha et al., 1981) and expression (Neisewander and Bardo, 1987) of morphine CH.

In addition to CH, opioid receptors have been implicated in other types of opioid conditioning such as opioid conditioned place preference (CPP) and opioid self-administration (SA). In the CPP paradigm, it has been found that nalx (Hand et al., 1989; Neisewander et al., 1990) and naltrexone (Piepponen et al., 1997) block acquisition of morphine and heroin CPP. Conversely, however, nalx has been reported to enhance expression of morphine CPP (Neisewander et al., 1990) and have no effect on expression of heroin CPP (Hand et al., 1989). In the SA paradigm, nalx has been shown to augment heroin SA (Ettenberg et al., 1982; Koob et al., 1984).

Three opioid receptor subtypes (μ, δ and κ) are thought to play a role in opioid conditioning. μ agonists such as

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morphine (Iwamoto, 1986), fentanyl (Mucha and Herz, 1985), sufentanil (Mucha and Herz, 1985) and DAMGO (Bals-Kubik et al., 1993) have been shown to induce CPP. Conversely, the selective μ_1-opioid receptor antagonist naloxonazine was found to block acquisition of morphine CPP (Piepponen et al., 1997). In contrast to the μ subtype, the role of δ- and κ-opioid receptors in mediating opioid CPP is equivocal. On the one hand, it has been found that the selective δ agonist DPDPE induces CPP and is antagonized by the selective δ-receptor antagonist ICI 174,864 (Shippenberg et al., 1987). On the other hand, it has been found that the selective δ-receptor antagonist naltrindole (nalt) failed to block morphine CPP (Piepponen et al., 1997). Similar discrepancies have been found in regards to the κ-opioid receptor; namely, κ-receptor agonists such as ketocyclazocine and ethylketocyclazonine (Iwamoto, 1986) induce CPP. Other κ-receptor agonists such as U50,488H (Mucha and Herz, 1985; Bals-Kubik et al., 1993), E-2078 (Bals-Kubik et al., 1993) and (−)-bremazocine (Mucha and Herz, 1985), however, failed to induce CPP. In fact, these latter agonists were shown to produce a conditioned place *aversion*.

In the SA paradigm, it has been found that the selective μ-opioid receptor agonist alfentanil is readily self-administered by monkeys and that alfentanil SA is antagonized by the selective μ-opioid receptor antagonist quadazocine (Bertalmio and Woods, 1989). In addition, it has been shown that the selective μ-opioid receptor antagonists β-funaltrexamine (β-FNA) and naloxonazine augmented heroin SA (Negus et al., 1993). A similar finding has been reported with regard to the δ-opioid receptor; namely, opioid SA is augmented by nalt (Negus et al., 1993). The κ-opioid receptor does not seem to be involved in opioid SA, however, as it has been found that the selective κ-opioid receptor antagonist nor-binaltorphimine (nor-BIN) had no effect on heroin SA (Negus et al., 1993).

Thus, the CPP and SA literature have provided strong evidence that the μ- and δ-opioid receptors are involved in mediating the conditioned effects of opioids. However, the role of the κ-opioid receptor, if any, is less compelling.

While opioid CPP and SA studies have implicated selective opioid-receptor subtypes in mediating the conditioned effects of opioids, the role of selective opioid-receptor subtypes in mediating the unconditioned and conditioned hyperactive responses to morphine is not known. The purpose of the present experiments was to determine the role of μ-, δ- and κ-opioid receptors in mediating the unconditioned and conditioned hyperactive responses to morphine. In Experiment 1, the ability of various doses of morphine to produce CH was examined. In Experiments 2a–c and 3a–c, the ability of selective μ-, δ- or κ-opioid receptor antagonists to attenuate the unconditioned (Experiments 2a–c, respectively) and conditioned (Experiments 3a–c, respectively) hyperactive responses to morphine was then examined. For comparison with the selective opioid receptor antagonists, the ability of a nonselective opioid receptor antagonist to attenuate the unconditioned (Experiment 2d) or conditioned (Experiment 3d) hyperactive responses to morphine also was examined.

2. Methods

2.1. Animals

Male Sprague–Dawley rats, obtained from Harlan Industries (Indianapolis, IN), were used. The rats ranged in weight from 175 to 200 g at the start of the experiment. Ad libitum access to food and water was available for the duration of the experiment. The rats were kept on a light/dark cycle in which the lights came on at 0600 h and went off at 1800 h. Upon arrival, the rats were acclimated to the animal colony for at least 5 days. In all experiments, the rats were handled daily for 3–5 days prior to the start of the experiment. The Institutional Animal Care and Use Committee of the University of Kentucky approved the experiments described in this paper. The experiments conformed to the guidelines established by the *NIH Guide for the Care and Use of Laboratory Animals* (1996 Edition).

2.2. Apparatus

Six wooden locomotor activity chambers were used. The inside dimensions of each chamber were $25 \times 29 \times 34$ cm ($l \times w \times h$). Each chamber was painted white and contained a wire-mesh floor. Pine wood-chip bedding (P.J. Murphy Forest Products: Montville, NJ) was placed in a tray beneath each floor. Two photo beams, located 4 cm from the base of the floor, divided the chamber into four equal quadrants. Interruption of one of the photo beams was scored as an activity count and recorded by an IBM computer located in a control room adjacent to the test room. A speaker located in the running room provided an ambient white noise (70 dB) background.

2.3. Drugs

Morphine sulfate (National Institute on Drug Abuse, Bethesda, MD) was mixed in 0.9% NaCl and injected subcutaneously. The volume of each injection was 1 ml/kg (body weight). The selective μ-opioid receptor antagonist β-FNA was dissolved in distilled water and injected at a volume of 5 ml/kg (for the 10 mg/kg dose) or 10 ml/kg (for the 20 mg/kg dose). The selective δ- (nalt) and κ- (nor-BNI) opioid receptor antagonists were prepared in distilled water and injected at a volume of 1 ml/kg. The nonselective opioid receptor antagonist nalx was prepared in 0.9% NaCl and also was injected at a volume of 1 ml/kg. Doses are expressed as the weight of the salt. Antagonists were obtained from the Research Triangle Institute (Durham, NC).

2.4. Procedures

2.4.1. Experiment 1: dose–response function of morphine CH

Rats were randomly assigned to one of four groups ($n=8$ rats per group): vehicle, 1 mg/kg morphine, 4 mg/kg morphine or 16 mg/kg morphine. After the preliminary acclimation and handling periods, the experiment consisted of three phases: context pre-exposure, drug test and conditioning test.

2.4.1.1. Context pre-exposure. Context pre-exposure lasted one session. On this day, each rat was placed individually in the locomotor activity chamber for 30 min. No injections were given prior to placement in the chamber. This phase was intended to help reduce novelty-induced exploration of the chamber.

2.4.1.2. Drug test. Drug testing lasted eight consecutive sessions. On each session, the morphine groups received an injection of 1, 4 or 16 mg/kg morphine immediately prior to placement into the locomotor activity chamber. The vehicle group was treated identical to the morphine groups, except that an injection of saline was given prior placement in the locomotor activity chamber. The duration of each session was 30 min. Activity counts were recorded in 10-min blocks.

2.4.1.3. Conditioning test. Conditioning testing lasted for one session and was conducted 24 h after the last drug-test session. On this session, the morphine groups and vehicle group received an injection of saline before placement in the locomotor activity chambers. Similar to the conditioning phase, activity was recorded in 10-min blocks for a 30-min period.

2.4.2. Experiments 2a–d: effect of selective and nonselective opioid receptor antagonists on the UR to morphine

The acclimation and handling regimen were identical to those of Experiment 1. Following the acclimation and handling regimen, Experiments 2a–d consisted of two phases: acute pretreatment and a single locomotor test. In Experiment 2a, rats were randomly assigned to one of four groups ($n=8$ rats per group): vehicle + vehicle, vehicle + morphine, 10 β-FNA + morphine or 20 β-FNA + morphine. During the pretreatment phase, the vehicle + vehicle and vehicle + morphine groups received distilled water 20 h prior to the test. The 10 β-FNA + morphine and 20 β-FNA + morphine groups received 10 and 20 mg/kg β-FNA, respectively, 20 h prior to the test. On the test day, all groups were placed in the locomotor activity chambers for a 120-min period. During the first 60 min of the session, all groups were placed in the locomotor activity chambers to assess any nonspecific locomotor-depressant effects of β-FNA. After the first 60 min of the session, the vehicle + vehicle group was removed from the chambers, injected with saline and replaced in the locomotor activity chambers for an additional 60-min period. The vehicle + morphine, 10 β-FNA + morphine and 20 β-FNA + morphine groups also were removed from the chambers after the first 60 min, injected with morphine (4 mg/kg) and replaced into the locomotor activity chambers.

The procedures of Experiments 2b–d were similar to Experiment 2a with three exceptions. First, the number of rats allotted to the various groups differed in Experiments 2b–d. The number of rats allotted to the groups was as follows: Experiment 2b—vehicle + vehicle ($n=5$), vehicle + morphine ($n=7$), 5 nalt + morphine ($n=11$), 10 nalt + morphine ($n=7$) and 20 nalt + morphine ($n=7$); Experiment 2c—vehicle + vehicle ($n=4$), vehicle + morphine ($n=8$), 10 nor-BNI + morphine ($n=9$) and 20 nor-BNI + morphine ($n=9$); Experiment 2d—vehicle + vehicle ($n=8$), vehicle + morphine ($n=8$), 1 nalx + vehicle ($n=8$) and 1 nalx + morphine ($n=8$). Second, nalt, nor-BNI or nalx was given 10, 60 or 5 min prior to the test in Experiments 2b–d, respectively. Injection times and doses were based on previous research (Neisewander and Bardo, 1987; Negus et al., 1993). Third, in Experiments 2b–d, the session duration was 60 min instead of 120 min as in Experiment 2a and the morphine or saline injection was given immediately prior to placement in the activity chambers. For these and subsequent experiments, the session duration was increased from 30 min, as in Experiment 1, so as to more fully characterize the time course of antagonism.

2.4.3. Experiments 3a–d: effect of selective and nonselective opioid receptor antagonists on the conditioned hyperactive response to morphine

In Experiment 1, a group that received saline paired with contextual cues served as the control group by which to assess CH. However, it has been argued that such a control group is not the appropriate control to assess Pavlovian conditioning (Rescorla, 1967). That is, it has been argued that a group that receives only the CS (e.g., contextual cues) in the absence of the US (e.g., morphine) is not equated with the Pavlovian conditioning group in terms of CS and US exposure. Thus, it has been suggested that a group that receives a truly random control procedure (i.e., the probability of the US in the presence of the CS is equal to the probability of the US in the absence of the CS) is the most appropriate control in Pavlovian conditioning. However, the use of a truly random control procedure in Pavlovian drug conditioning experiments is problematic due to the length of the US exposure. In drug conditioning research, a more feasible control is the explicitly unpaired procedure. In the explicitly unpaired procedure, the control group receives a noncontiguous pairing of the CS and US. Therefore, both the Pavlovian conditioning and control groups are equated in terms of CS and US exposure. For Experiments 3a–d, a group that received contextual cues explicitly paired with morphine against a control group that received contextual cues explicitly unpaired with morphine was used.

Experiments 3a–d consisted of three phases: drug test, pretreatment and conditioning test. In Experiment 3a, rats were randomly assigned to one of four groups: paired/β-FNA ($n=8$), paired/vehicle ($n=7$), unpaired/β-FNA ($n=8$) or unpaired/vehicle ($n=7$).

2.4.3.1. Drug test. Drug testing lasted 16 sessions. On Session 1, the paired/β-FNA and paired/vehicle groups received an injection of morphine (4 mg/kg) immediately before placement in the locomotor activity chambers. The paired/β-FNA and paired/vehicle groups received another 7 chamber + morphine pairings every other session for 16 sessions. On the intervening sessions, the paired/β-FNA and paired/vehicle groups received an injection of saline in their home cage. The unpaired/β-FNA and unpaired/vehicle groups were treated similarly, except that saline was paired with the locomotor chamber and morphine was given in the home cage. The session duration for the drug testing sessions was 30 min.

2.4.3.2. Pretreatment. Following the drug testing phase, the paired/β-FNA and unpaired/β-FNA groups received an injection of β-FNA (20 mg/kg). At the same time, the paired/vehicle and unpaired/vehicle groups received an injection of distilled water.

2.4.3.3. Conditioning test. The test for CH occurred 20 h after the pretreatment injection of β-FNA or distilled water. At this time, all groups received an injection of saline just prior to placement in the locomotor activity chambers. The session duration was 60 min.

The procedures for Experiments 3b–d were similar to Experiment 3a except for the following. In Experiments 3b–d, half of the paired and unpaired groups ($n=7$–8 rats per group) received nalt (5 mg/kg), nor-BNI (20 mg/kg) or nalx (1 mg/kg) 10, 60 or 5 min, respectively, prior to the conditioning test. The other half of the paired and unpaired groups ($n=7$–8 rats per group) received their respective vehicles prior to the conditioning test.

2.5. Data analysis

Activity counts were analyzed using either a one- or two-way analysis of variance (ANOVA). Follow-up contrasts of interests were analyzed using Fisher's least significant difference test. Correlated *t* tests were used for all within-subject contrasts. Unless otherwise noted, all statistical decisions were made at an $\alpha<.05$.

3. Results

3.1. Experiment 1: dose–response function of morphine-produced CH

Fig. 1 shows the mean activity counts during the drug testing phase of Experiment 1. Two-way (Dose × Drug Session) ANOVA conducted on the data revealed significant effects of dose [$F(3,28)=31.5$, $P<.001$], drug session [$F(7,196)=41.5$, $P<.001$] and Dose × Drug Session interaction [$F(21,196)=12.0$, $P<.001$]. The significant Dose × Drug Session interaction motivated several follow-up contrasts. These contrasts found that doses of 1, 4 and 16 mg/kg morphine increased activity relative to vehicle. Moreover, the hyperactivity was greater at doses of 4 and 16 mg/kg morphine compared to 1 mg/kg morphine. Furthermore, morphine sensitization was observed at doses of 1, 4 and 16 mg/kg morphine. That is, the amount of activity on Trial 8 compared to Trial 1 was greater at doses of 1, 4 and 16 mg/kg morphine [correlated $ts(7)>2.5$, $Ps<.05$]. By Trial 8, however, morphine sensitization was greater at doses of 4 and 16 mg/kg morphine compared to 1 mg/kg morphine. The two highest dose groups did not differ significantly.

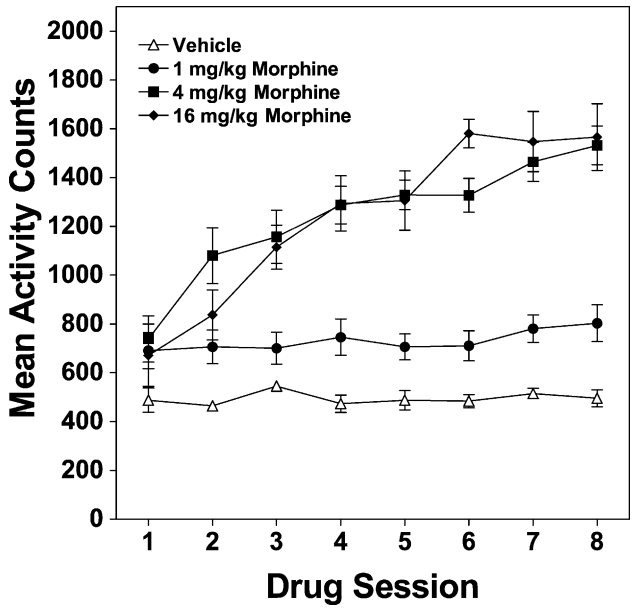

Fig. 1. Mean activity counts for the vehicles, 1, 4 and 16 mg/kg morphine groups across the drug testing sessions of Experiment 1. The error bars represent ±1 S.E.M.

Fig. 2 shows the mean activity counts of the conditioning test session of Experiment 1. Two-way (Dose × Session Block) ANOVA conducted on the data found significant main effects of dose [$F(3,28)=6.0$, $P<.001$] and session block [$F(2,56)=284.3$, $P<.001$]. The significant main effect of session block indicates that rats were more active early as opposed to the latter portion in the test session (left panel). The Dose × Session Block interaction was not significant. Due to the nonsignificant Dose × Session Block interaction, the activity counts were collapsed over the entire session (right panel). The significant effect of dose motivated several post-hoc contrasts that found that groups conditioned with 1 and 4 mg/kg morphine had increased activity compared to the vehicle control. That is, CH was observed at these doses. Moreover, the activity level did not

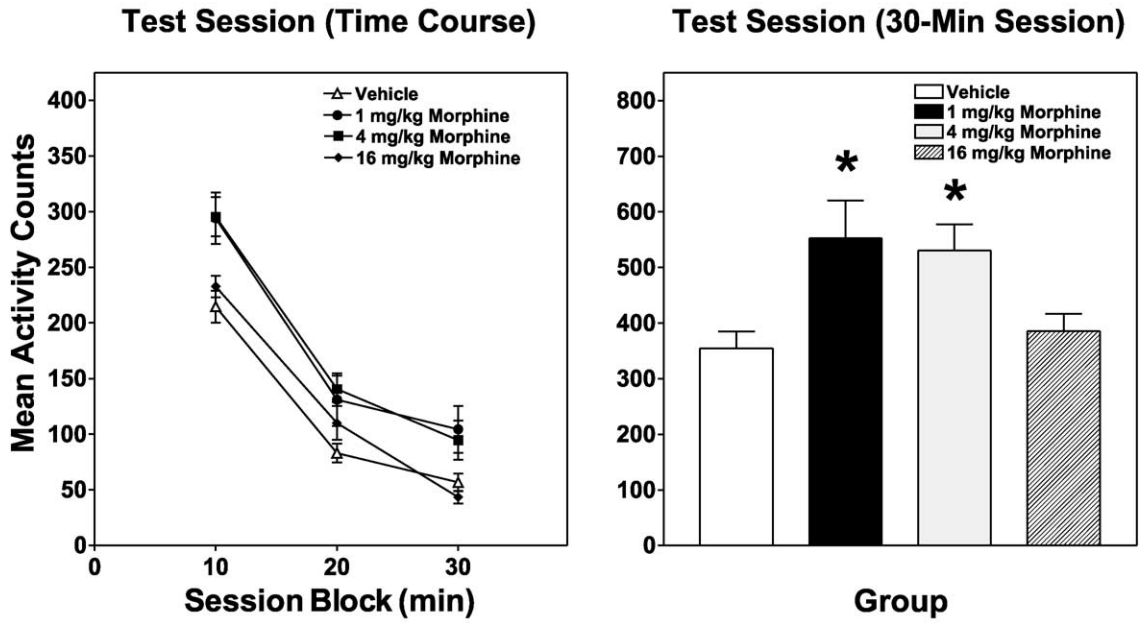

Fig. 2. Mean activity counts plotted in 10-min blocks (left panel) or across the entire 30-min conditioning test session (right panel) for the vehicle, 1, 4 and 16 mg/kg morphine groups of Experiment 1. The " * " symbol indicates that a given conditioning dose of morphine augmented activity compared to vehicle (Ps < .05). The error bars represent either ±1 S.E.M. (left panel) or +1 S.E.M. (right panel).

differ between doses of 1 and 4 mg/kg morphine. It should be noted, however, that the vehicle control group was less active on the conditioning test session compared to the preceding drug session, correlated $t(7) = 4.1$, $P < .01$. It is unclear as to the reason for this decrease in activity in the vehicle control group between the last session of drug testing and the conditioning test. The 16 mg/kg morphine did not produce CH compared to vehicle alone. This latter result was surprising in that 16 mg/kg morphine produced a robust hyperactive response across drug conditioning sessions (see Fig. 1).

3.2. Experiments 2a–d: effect of selective and nonselective opioid receptor antagonists on the UR to morphine

The results of Experiment 2a are shown in Fig. 3 (top panel). For Experiments 2a–d, group differences tended to emerge in the last 30 min of the 60-min session (see left panel). Therefore, the analyses of the total activity counts for Experiments 2a–d were confined to the last 30 min of the session (right panel). A one-way ANOVA conducted on the total activity counts of Experiment 2a revealed a significant effect of group [$F(3,28) = 11.2$, $P < .001$]. Post-hoc contrasts found that the 20 β-FNA + morphine group differed significantly from the vehicle + morphine group. That is, locomotor activity was attenuated in the 20 β-FNA + morphine group relative to the vehicle + morphine group. The 10 β-FNA + morphine group showed less activity compared to the vehicle + morphine group, but this difference failed to reach significance (P =.1). While 20 mg/kg β-FNA attenuated morphine-induced hyperactivity, it was not abolished completely. Indeed, the 20 β-FNA + morphine group showed significantly more locomotor activity relative to the vehicle + vehicle group. Thus, the ability of β-FNA to block morphine-induced hyperactivity was incomplete with the doses tested.

An inspection of the locomotor activity (data not shown) during the first 60-min period of the test session of Experiment 2a, however, revealed no differences in locomotor activity between β-FNA- (10 mg/kg, $M = 610$, S.E.M. = 60; 20 mg/kg, $M = 646$, S.E.M. = 53) and water-pretreated ($M = 707$, S.E.M. = 44) rats. This result indicates that the β-FNA pretreatment alone did not decrease locomotor activity.

The results of Experiment 2b are shown in Fig. 3 (middle panel). A one-way ANOVA revealed a significant group difference [$F(4,32) = 3.9$, $P < .05$]. Follow-up post-hoc contrasts found that the 5 nalt + morphine group was less active than the vehicle + morphine group. Neither 10 nalt + morphine nor 20 nalt + morphine groups were significantly different from the vehicle + morphine group. Surprisingly, the lowest dose of nalt (5 mg/kg) was more effective than either 10 or 20 mg/kg nalt in attenuating morphine-induced hyperactivity. Moreover, the 5 nalt + morphine group did not differ significantly from the vehicle + vehicle group, suggesting that the ability of nalt to block morphine-induced hyperactivity was complete.

Fig. 3 (bottom panel) shows the results of Experiment 2c. A one-way ANOVA revealed a significant group difference [$F(3,26) = 6.6$, $P < .01$]. Post-hoc contrasts found that the 20 nor-BNI + morphine group was less active relative to the vehicle + morphine group. Moreover, the 20 nor-BNI + morphine group did not differ statistically

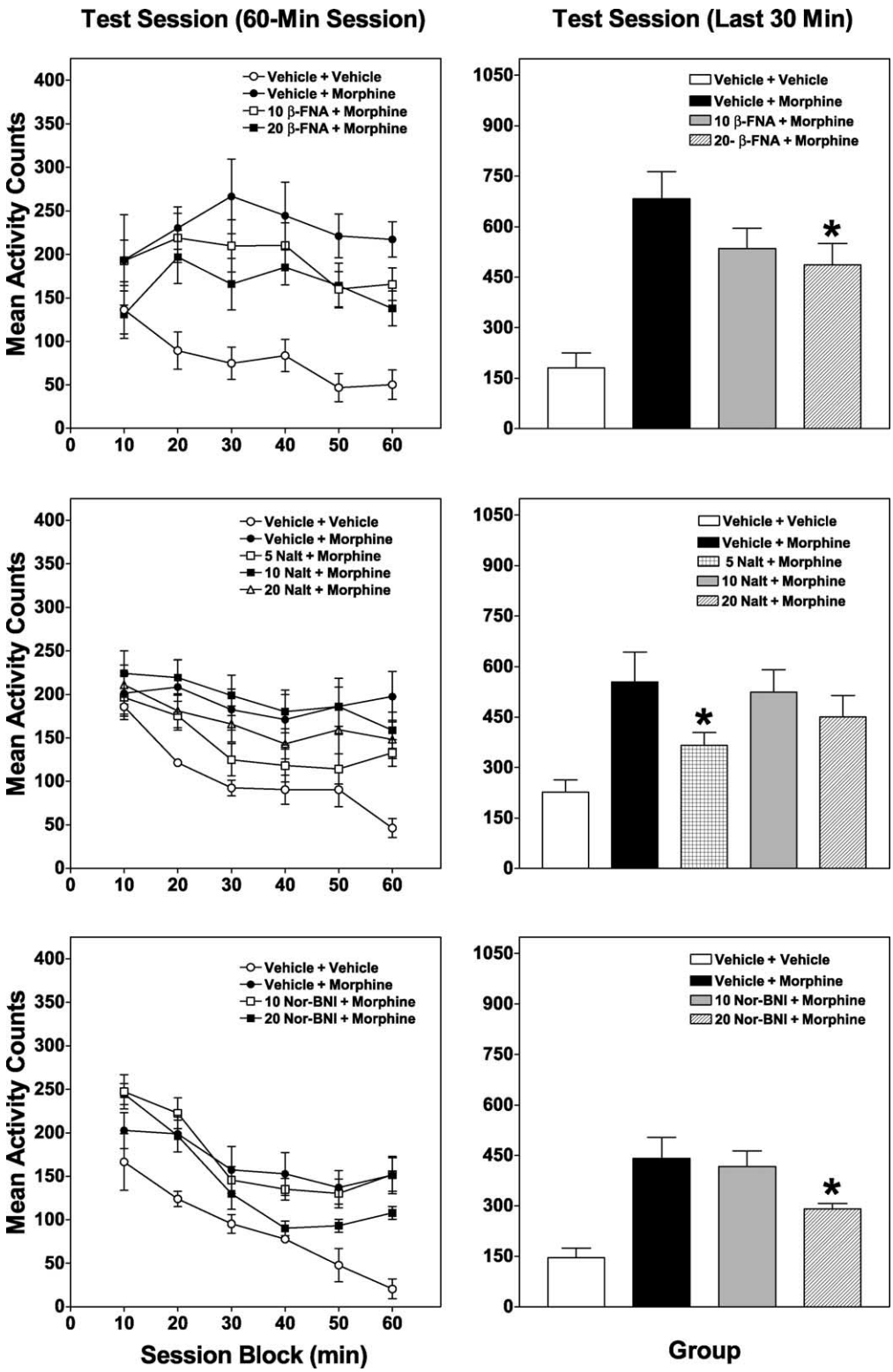

Fig. 3. Top panel shows the mean activity counts for the vehicle + vehicle, vehicle + morphine, 10 β-FNA + morphine and 20 β-FNA + morphine groups of the test session of Experiment 2a. Middle panel shows the mean activity counts for the vehicle + vehicle, vehicle + morphine, 5 nalt + morphine, 10 nalt + morphine and 20 nalt + morphine groups of the test session of Experiment 2b. Bottom panel shows the mean activity counts for the vehicle + vehicle, vehicle + morphine, 10 nor-BNI + morphine and 20 nor-BNI + morphine groups of the test session of Experiment 2c. For each experiment, the data are plotted in 10-min blocks (left panels) or across the entire 60-min test session (right panels). The " * " symbol indicates that a given antagonist pretreatment dose decreased morphine-induced hyperactivity compared to morphine alone ($Ps < .05$). The error bars represent either ±1 S.E.M. (left panel) or +1 S.E.M. (right panel).

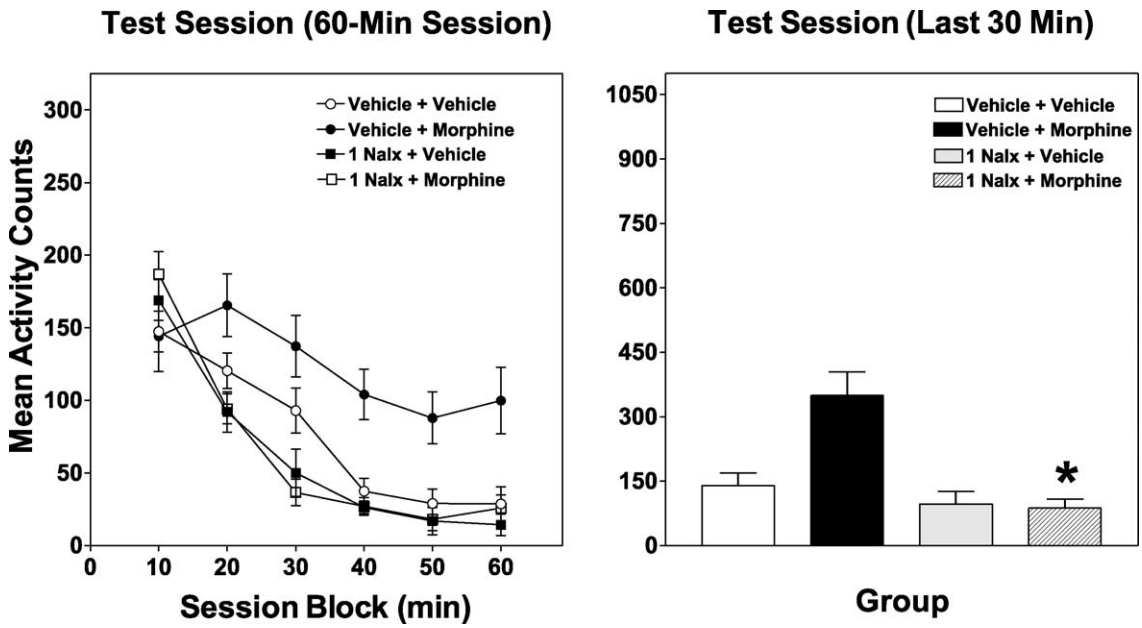

Fig. 4. Mean activity counts plotted in 10-min blocks (left panel) or across the entire 60-min test session (right panel) for vehicle + vehicle, vehicle + morphine, 1 nalx + vehicle and 1 nalx + morphine groups of Experiment 2d. The " * " symbol indicates that a naloxone pretreatment decreased morphine-induced hyperactivity compared to morphine alone ($P < .05$). The error bars represent either ±1 S.E.M. (left panel) or +1 S.E.M. (right panel).

from the vehicle + vehicle group. Taken together, these results suggest that 20 mg/kg nor-BNI blocked morphine-induced hyperactivity.

The results of Experiment 2d are shown in Fig. 4. Consistent with the results of Experiments 2a–c, groups differences tended to emerge later in the session (left panel); consequently, the data for the last 30 min of the 60-min session were plotted and analyzed (right panel). A one-way ANOVA revealed a significant group difference [$F(3,28) = 12.3$, $P < .001$]. Follow-up contrasts revealed that the 1 nalx + morphine group differed significantly from the vehicle + morphine and did not differ from the vehicle + vehicle group, suggesting that nalx completely blocked morphine-induced hyperactivity. Moreover, the 1 nalx + vehicle group did not differ from the vehicle + vehicle group, indicating that nalx alone did not alter activity. While the inability to detect a difference between the 1 nalx + vehicle and vehicle + vehicle in the last 30 min of the 60 min session may be due to a floor effect (i.e., the groups converge in the last 30 min of the session), a point-by-point analysis of the first 30 min of the session also revealed no difference between these two groups (Ps >.05). Thus, the failure to detect a difference in the latter part of the session was not likely due to a floor effect.

3.3. Experiments 3a–d: effect of selective and nonselective opioid receptor antagonists on the conditioned hyperactive response to morphine

The results of Experiments 3a–c are shown in the top, middle and bottom panels of Fig. 5, respectively. In Experiments 3a–c, regardless of pretreatment condition, CH was most pronounced in paired groups compared to unpaired groups during the first 30 min of the 60-min session (left panel). Group differences, however, tended to persist across the entire 60-min session, and thus the total activity counts were plotted and analyzed based on the entire 60-min session (right panel). Two-way (Context × Pretreatment) ANOVAs revealed only significant main effects of context [Fs(1,26)>14.8, Ps < .001]. Neither the main effects of pretreatment nor the Context × Pretreatment interactions were significant in any of these experiments. For each experiment, the main effect of context indicates that rats that received contextual cues paired with morphine (paired groups) displayed significantly greater locomotor activity compared to rats that had contextual cues explicitly unpaired with morphine (unpaired groups). This finding demonstrates CH in paired groups. More important, the failure to detect significant Context × Pretreatment interactions in any of the experiments suggests that pretreatment with each of the selective opioid receptor antagonists did *not* attenuate CH in paired groups. Furthermore, the failure to observe a significant main effect of pretreatment indicates that the doses of antagonists chosen did not reliably alter activity when given alone. This latter result suggests that the attenuation of the morphine-induced hyperactivity by selective antagonists observed in Experiments 2a–c was not due to a nonspecific decrease in locomotor activity.

The results of Experiment 3d are shown in Fig. 6. A two-way (Context × Pretreatment) ANOVA, conducted on the entire 60-min session (right panel), revealed significant main effects of context [$F(1,31) = 37.7$, $P < .001$] and pretreatment [$F(1,31) = 21.4$, $P < .001$]. The interaction was not significant. The main of context suggests that

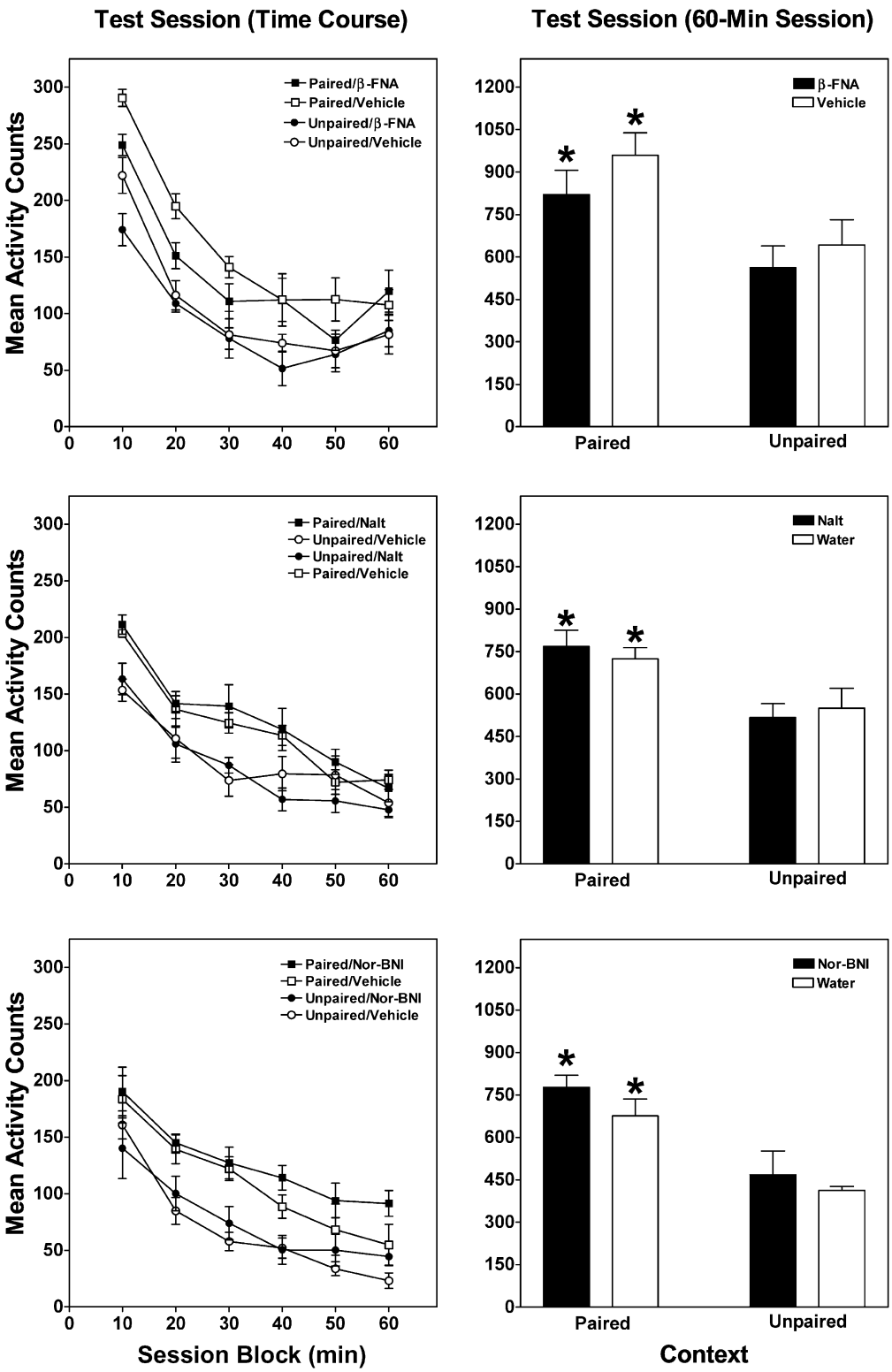

Fig. 5. Top panel shows the mean activity counts for the paired/β-FNA, paired/vehicle, unpaired/β-FNA and unpaired/vehicle groups of the conditioning test session of Experiment 3a. Middle panel shows the mean activity counts for the paired/nalt, paired/vehicle, unpaired/nalt and unpaired/vehicle groups of the conditioning test session of Experiment 3b. Bottom panel shows the mean activity counts for the paired/nor-BNI, paired/vehicle, unpaired/nor-BNI and unpaired/vehicle groups of the conditioning test session of Experiment 3c. For each experiment, the data are plotted in 10-min blocks (left panel) or across the entire 60-min conditioning test session (right panel). The " * " symbol indicates that paired groups were more active than their respective unpaired control groups ($Ps < .05$). The error bars represent either ±1 S.E.M. (left panel) or +1 S.E.M. (right panel).

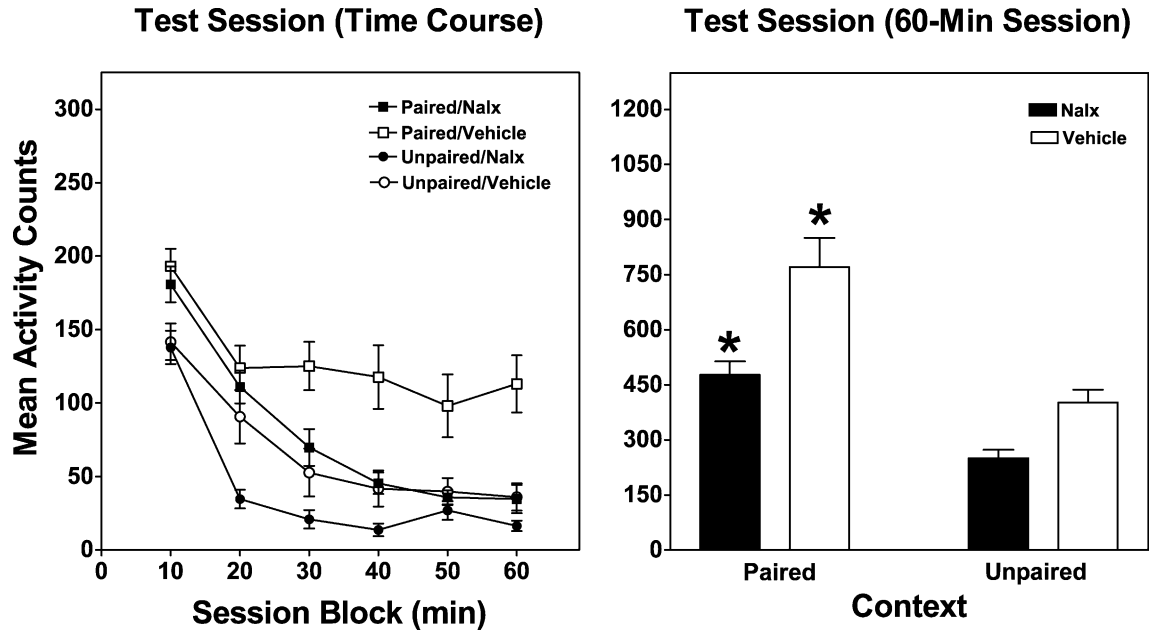

Fig. 6. Mean activity counts plotted in 10-min blocks (left panel) or across the entire 60-min conditioning test session (right panel) for paired/nalx, paired/vehicle, unpaired/nalx and unpaired/vehicle groups of Experiment 3d. The " * " symbol indicates that paired groups were more active than their respective unpaired control groups ($Ps < .05$). The error bars represent either ±1 S.E.M. (left panel) or +1 S.E.M. (right panel).

paired groups were more active than unpaired groups (i.e., CH). The main effect of pretreatment taken in conjunction with the nonsignificant interaction suggests that pretreatment with nalx attenuated hyperactivity regardless of conditioning history. An inspection of the time-course of the test session (left panel) revealed that the hypoactivity produced by nalx in the unpaired groups was most apparent on session blocks 20–40 min, whereas the blockade of morphine CH in the paired groups was most apparent on session blocks 30–60 min.

4. Discussion

In Experiment 1, it was found that rats sensitized to doses of 1, 4 and 16 mg/kg morphine given during the drug testing sessions. Furthermore, sensitization was greater at doses of 4 and 16 mg/kg compared to 1 mg/kg morphine. These results indicate that higher doses of morphine produce more robust sensitization, but that this dose-dependent effect plateaus within the dose range tested.

In regards to CH, it was found that 4 mg/kg morphine produced robust CH. Surprisingly, however, 1 mg/kg morphine produced a conditioned hyperactive effect comparable to that produced by 4 mg/kg morphine. This result was surprising because 4 mg/kg morphine produced more robust sensitization than 1 mg/kg morphine during the drug sessions. Even more surprising was the failure of 16 mg/kg morphine to produce CH, since 16 mg/kg morphine produced dramatic sensitization (comparable to that observed at 4 mg/kg morphine). Why is it that a low dose of morphine (1 mg/kg) produced only modest sensitization, yet produced robust CH, while a high dose of morphine (16 mg/kg) produced robust sensitization, yet failed to produce CH?

One reason for the dissociation of locomotor sensitization and expression of CH is that only a low threshold level of sensitization may be needed in order for morphine to serve as an effective US. Once the threshold has been crossed, increases in US intensity (i.e., increases in the dose of the drug) may not facilitate conditioning. While this idea is plausible, it seems unlikely. Many studies, using morphine and other opioid agonists such as fentanyl and sufentanil as USs, have found a positive correlation between drug dose and the magnitude of the CR (Mucha and Herz, 1985). An alternative explanation is that two different neural mechanisms may be activated by morphine. One mechanism may be related to the development of sensitization, whereas a second mechanism, which is induced at higher doses, may be related to the disruption in acquisition of the CR.

Consistent with this latter interpretation, a high dose of morphine produces an aversive (Mucha and Herz, 1985) or withdrawal state (Wei, 1973). For example, Mucha and Herz (1985) found that a relatively low dose of morphine (0.25 mg/kg) produced a taste preference, whereas a higher dose of morphine (0.42 mg/kg) produced a robust taste aversion. However, differences in the route of administration (oral vs. subcutaneous) and Pavlovian conditioning procedure (taste vs. place conditioning) limit direct comparisons between Mucha and Herz (1985) and the present study. Moreover, Mucha and Herz (1985) and others (Iwamoto, 1981; Mucha et al., 1982; Bardo et al., 1995) have found no evidence for a place aversion at high doses of morphine, suggesting the lack of CH produced by 16 mg/kg morphine is not due to an aversive state. The idea that the inability of 16 mg/kg

morphine to support CH is due to the production of a withdrawal state that interferes with expression of morphine CH, however, cannot be excluded based on the present results, as behavioral signs of morphine withdrawal were not assessed.

Alternatively, a more likely explanation is that a high dose of morphine interfered with the processing of contextual information at the time of conditioning. Several studies have found that high doses of morphine (>10 mg/kg) impair memory on a variety of spatial and working memory tasks such as the eight-arm radial maze (Braida et al., 1994), Morris water maze (Beatty, 1983; McNamara and Skelton, 1992) and delayed-matching-to-sample (Schulze and Paule, 1991).

The results of Experiments 2a–c are consistent with the finding that antagonists selective for the μ- and δ-opioid receptors attenuate opioid SA (Bertalmio and Woods, 1989; Negus et al., 1993). The finding in Experiment 2b that the lowest dose of nalt (5 mg/kg), but not the highest doses of nalt (10 or 20 mg/kg), effectively attenuated hyperactivity was surprising. Negus et al. (1993) found that high doses of nalt (10 and 20 mg/kg), but not low doses (1 and 3 mg/kg) of nalt, interfered with heroin SA. The reason for the discrepancy in results between studies is unclear. It may be the case, however, that locomotor activity is more sensitive than SA to δ antagonism at low doses. Indeed, it has been found that other δ-mediated responses (e.g., antinociception) are antagonized by doses of nalt in the range of 0.5–1 mg/kg (Jackson et al., 1989; Kitchen and Pinker, 1990), whereas higher doses produce μ antagonist effects (Kitchen and Kennedy, 1990). Moreover, it has been found that in the warm water tail-dip assay of antinociception, 10 mg/kg nalt produced an agonist-like effect (Jackson et al., 1989). Taken together, these results imply that in certain paradigms low doses of nalt may function as an antagonist, whereas high doses of nalt may have some agonist activity. If this is the case in the locomotor-activity paradigm, then a low dose of nalt might be predicted to reduce locomotor activity, whereas a high dose might facilitate such activity. The failure to detect greater activity in rats pretreated with 10 and 20 mg/kg nalt followed by morphine compared to rats pretreated with morphine alone may reflect a ceiling effect.

The finding of Experiment 2c that the highest dose of nor-BNI reversed morphine-induced hyperactivity is at odds with the finding by Negus et al. (1993) that nor-BNI failed to antagonize heroin SA. Differences in dose may account for the discrepancy. That is, in Negus et al.'s study, the highest dose of nor-BNI was 10 mg/kg. In Experiment 2c, we also found that 10 mg/kg nor-BNI was ineffective in antagonizing morphine-induced behavior. If Negus et al. had used a higher dose of nor-BNI (e.g., 20 mg/kg), they may have found that nor-BNI antagonized opioid-controlled behavior (similar to the results of Experiment 2c). Also, it is possible that the difference in nor-BNI effect observed by Negus et al. and the present report may reflect an inherent difference in the role of κ receptors in opioid-induced hyperactivity and reinforcement. It should be noted, however, that nor-BNI has a unique time course. Several studies have found that within the first two hours after administration nor-BNI is a relatively nonselective opioid receptor antagonist, targeting both μ and κ receptors (Endoh et al., 1992; Horan et al., 1992; Broadbear et al., 1994). In fact, within the first 30 min after administration, nor-BNI is more selective for μ- than κ-opioid receptors. In contrast, κ antagonism by nor-BNI develops more slowly (after several hours) and persists for a period of time (a few weeks). Therefore, because in Experiment 2c morphine was given 60 min after nor-BNI administration and locomotor activity was monitored for an additional 60 min, it may be that the antagonism by nor-BNI of morphine-induced hyperactivity was mediated by μ-opioid receptor antagonism, not κ.

The finding in Experiment 2d that nalx blocked completely morphine-induced hyperactivity replicates previous work (Neisewander and Bardo, 1987) and suggests one of two possibilities. On the one hand, it may suggest that nalx shifted the dose–response function for morphine-induced hyperactivity such that the 4 mg/kg dose became functionally like the 1 mg/kg dose. Indeed, in Experiment 1, 1 mg/kg morphine induced a hyperactive effect that was comparable to 4 mg/kg morphine on Trial 1 of the drug test sessions. This explanation is not likely, however, because nalx + morphine rats should have been more active than vehicle + vehicle group in Experiment 2d. Alternatively, the finding that nalx completely blocked morphine-induced hyperactivity may suggest that antagonism of two or more opioid-receptor subtypes are required to completely block morphine-induced hyperactivity. The findings of Experiments 2a–c that selective antagonists, given individually, only *attenuated* morphine-induced hyperactivity, lends additional support to this latter idea.

In Experiment 3a, it was found that an antagonist (β-FNA), selective for the μ-opioid receptor, failed to attenuate morphine CH. This result was perhaps the most surprising because the μ-opioid receptor has consistently been shown to mediate the conditioned reinforcing effects of opioids (Iwamoto, 1986; Mucha and Herz, 1985; Bals-Kubik et al., 1993; Piepponen et al., 1997). Indeed, it has been found that the selective μ_1-opioid-receptor antagonist naloxonazine blocked morphine CPP (Piepponen et al., 1997). In previous studies that have implicated the μ-opioid receptor in mediating the CR to morphine, however, the μ agonist or antagonist has been given during the conditioning phase (Iwamoto, 1986; Mucha and Herz, 1985; Bals-Kubik et al., 1993; Piepponen et al., 1997). In contrast, in the present report, β-FNA was given just prior to the test of CH. Thus, the most parsimonious explanation for the discrepant results is that the μ-opioid receptor is involved in *acquisition*, but not *expression*, of a morphine CR. The results of Experiments 3b and c are consistent with the finding that selective δ- (Shippenberg et al., 1987; Piepponen et al., 1997) and κ- (Mucha and Herz, 1985; Bals-Kubik et al., 1993) opioid

receptors do not mediate the conditioned reinforcing effect of opioids.

The finding that none of the selective antagonists, given individually, altered morphine CH (using 4 mg/kg) may suggest one of two possibilities. As in the nalx experiment discussed earlier, it may be that a selective antagonist shifted the dose–response function for morphine CH. That is, in Experiment 1, we found that 1 mg/kg morphine produced much less hyperactivity than either 4 or 16 mg/kg morphine during the drug test sessions (Sessions 1–8); however, 1 mg/kg morphine produced a robust morphine CH (comparable to the 4 mg/kg dose) on the conditioning test session. It may be the case that the failure of a selective antagonist to attenuate morphine CH was because the 4 mg/kg morphine dose became in effect a 1.0 mg/kg dose, a morphine dose which did not induce hyperactivity but did produce CH. Because only the 4 mg/kg morphine dose was used in the morphine CH experiments, it is difficult to ascertain if an antagonist shifted the dose–response function in this manner. Alternatively, the inability of any selective antagonist to alter morphine CH may suggest that blockade of at least two or more receptor subtypes may be required to antagonize the expression of morphine CH. While antagonism of multiple opioid-receptor subtypes has been reported to not block expression of morphine CPP (Hand et al., 1989), the finding that the nonselective opioid receptor antagonist (nalx) attenuated expression of morphine CH (Experiment 3d and Neisewander and Bardo, 1987) further strengthens the argument that antagonism of multiple opioid-receptor subtypes is required. It should be noted that nalx also decreased activity in the control group (unpaired/nalx), especially early in the session (Experiment 3d). Presently, it is unclear as to the reason for the decrease in activity, although it may reflect a nalx-induced withdrawal in unpaired/nalx rats (Mucha and Herz, 1985). Regardless as to the reason, it is clear that this dose of nalx (1 mg/kg) does not produce a general, nonspecific decrease in activity, as it did not decrease activity in Experiment 2d. Thus, while the necessary and sufficient combination of opioid-receptor subtype blockade to antagonize expression of morphine CH is not known, the current results taken together indicate that more than one subtype is involved.

The unconditioned and conditioned locomotor-stimulant effects of morphine may be dissociable and mediated by different pharmacological systems. While similarities in the unconditioned and conditioned hyperactive responses to classic psychostimulants have been noted (Gold et al., 1989), it has been reported that the two responses are dissociable. For example, Itzhak and Martin (2000) found that scopolamine inhibited cocaine CH, but had no effect on the unconditioned hyperactive effects of cocaine. Furthermore, it has been found that other unconditioned and conditioned morphine effects are dissociable. Shippenberg et al. (2000) found that dynorphin A(2–17) attenuated the unconditioned (somatic), but not the conditioned aversive effects of antagonist-precipitated withdrawal from morphine. These findings are consistent with the dissociation between the unconditioned and conditioned hyperactivity to morphine found in the present report.

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References

Ayhan IH, Randrup A. Behavioral and pharmacological studies on morphine-induced excitation of rats. Possible relation to brain catecholamines. Psychopharmacology 1973;29:317–28.

Babbini M, Davis WM. Time–dose relationships for locomotor activity effects of morphine after acute or repeated treatment. Br J Pharmacol 1976;46:213–24.

Bals-Kubik R, Ableitner A, Herz A, Shippenberg TS. Neuroanatomical sites mediating the motivational effects of opioids as mapped by the conditioned place preference paradigm in rats. J Pharmacol Exp Ther 1993;264:489–95.

Bardo MT, Rowlett JK, Harris MJ. Conditioned place preference using opiate and stimulant drugs: a meta-analysis. Neurosci Biobehav Rev 1995;19:39–51.

Beatty WW. Opioids antagonists, morphine and spatial memory in rats. Pharmacol, Biochem Behav 1983;19:397–401.

Beninger RJ, Herz RS. Pimozide blocks the establishment but not the expression of cocaine-produced environment-specific conditioning. Life Sci 1986;38:1425–31.

Bertalmio AJ, Woods JH. Reinforcing effect of alfentanil is mediated by mu opioid receptors: apparent pA_2 analysis. J Pharmacol Exp Ther 1989; 251:455–60.

Braida D, Gori E, Sala M. Relationship between morphine and etonitazene-induced working memory impairment and analgesia. Eur J Pharmacol 1994;271:497–504.

Broadbear JH, Negus SS, Butelman ER, de Costa BR, Woods JH. Differential effects of systematically administered nor-binaltorphimine (nor-BNI) on κ-opioid agonists in the mouse writhing assay. Psychopharmacology 1994;115:311–9.

Endoh T, Matsuura H, Tanaka C, Nagase H. Nor-binaltorphimine: a potent and selective κ-opioid antagonist with long-lasting activity in vivo. Arch Int Pharmacodyn 1992;316:30–42.

Ettenberg A, Pettit HO, Bloom FE, Koob GF. Heroin and cocaine self-administration in rats: mediation by separate neural systems. Psychopharmacology 1982;78:204–9.

Gold LH, Koob GF, Geyer MA. Spatial pattern analysis reveals similarities between amphetamine conditioned and unconditioned locomotion. Behav Pharmacol 1989;1:209–20.

Hand TH, Stinus L, Le Moal M. Differential mechanisms in the acquisition and expression of heroin-induced place preference. Psychopharmacology 1989;98:61–7.

Horan P, Taylor J, Yamamura HI, Porreca F. Extremely long-lasting antagonistic actions of nor-binaltorphimine (nor-BNI) in the mouse tail-flick test. J Pharmacol Exp Ther 1992;260:1237–43.

Itzhak Y, Martin JL. Scopolamine inhibits cocaine-conditioned but not unconditioned stimulant effects in mice. Psychopharmacology 2000; 152:216–23.

Iwamoto ET. Locomotor activity and antinociception after putative mu, kappa, and sigma opioid receptor agonists in the rat: influence of dopaminergic agonists and antagonists. J Pharmacol Exp Ther 1981;217: 451–60.

Iwamoto ET. Place-conditioning properties of mu, kappa, and sigma opioid agonists. Alcohol Drug Res 1986;6:327–39.

Jackson HC, Ripley TL, Nutt DJ. Exploring δ-receptor function using the selective opioid antagonist naltrindole. Neuropharmacology 1989;28: 1427–30.

Kitchen I, Kennedy A. Effects of δ-opioid receptor antagonist naltrindole on opioid induced in vivo release of corticosterone. In: van Ree JM, Mulder AH, Wiegant VM, van Wimersma Griedanus TB, editors. New leads in opioid research. Amsterdam: Excerpta Medica, 1990. p. 2321–2.

Kitchen I, Pinker SR. Antagonism of swim-stressed-induced antinociception by the δ-opioid receptor antagonist naltrindole in adult and young rats. Br J Pharmacol 1990;100:685–8.

Koob GF, Pettit HO, Ettenberg A, Bloom FE. Effects of opioid antagonists and their quaternary derivatives on heroin self-administration in the rat. J Pharmacol Exp Ther 1984;229:481–6.

McNamara RK, Skelton RW. Pharmacological dissociation between the spatial learning deficits produced by morphine and diazepam. Psychopharmacology 1992;108:147–52.

Mucha RF, Herz A. Motivational properties of kappa and mu opioid receptor agonists studied with place and taste preference conditioning. Psychopharmacology 1985;86:274–80.

Mucha RF, Volkavskis C, Kalant H. Conditioned increases in locomotor activity produced with morphine as an unconditioned stimulus, and the relation of the conditioning to acute morphine effect and tolerance. J Comp Physiol Psychol 1981;95:351–62.

Mucha RF, Van Der Kooy D, O'Shaughnessy M, Bucenieks P. Drug reinforcement studied by the use of place conditioning in rat. Brain Res 1982;243:91–105.

Negus SS, Henriksen SJ, Mattox A, Pasternak GW, Portoghese PS, Takemori AE, Weinger MB, Koob GF. Effect of antagonists selective for mu, delta, and kappa opioid receptors on the reinforcing effects of heroin in rats. J Pharmacol Exp Ther 1993;265:1245–52.

Neisewander JL, Bardo MT. Expression of morphine-conditioned hyperactivity is attenuated by naloxone and pimozide. Psychopharmacology 1987;93:314–9.

Neisewander JL, Pierce RC, Bardo MT. Naloxone enhances the expression of morphine-induced conditioned place preference. Psychopharmacology 1990;100:201–5.

Oka T, Hosoya E. Effects of humoral modulators and naloxone on morphine-induced changes in the spontaneous locomotor activity of the rat. Psychopharmacology 1976;47:243–8.

Piepponen TP, Kivastik T, Katajamaki J, Zharkovsky A, Ahtee L. Involvement of opioid μl receptors in morphine-induced conditioned place preference in rats. Pharmacol, Biochem Behav 1997;58:275–9.

Rescorla RA. Pavlovian conditioning and its proper control procedures. Psychol Bull 1967;74:71–80.

Schulze GE, Paule MG. Effects of morphine sulfate on operant behavior in rhesus monkeys. Pharmacol, Biochem Behav 1991;38:77–83.

Shippenberg TS, Bals-Kubik R, Herz A. Motivational properties of opioids: evidence that an activation of δ-receptors mediates reinforcement processes. Brain Res 1987;436:234–9.

Shippenberg TS, Funada M, Schutz CG. Dynorphin A(2–17) attenuates the unconditioned but not the conditioned effects of opiate withdrawal in the rat. Psychopharmacology 2000;151:351–8.

Swerdlow NR, Koob GF. Restrained rats learn amphetamine-conditioned locomotion, but not place preference. Psychopharmacology 1984;84: 163–6.

Swerdlow NR, Vaccarino FJ, Koob GF. Effects of naloxone on heroin-, amphetamine- and caffeine-stimulated locomotor activity in the rat. Pharmacol, Biochem Behav 1985;23:499–501.

Vezina P, Stewart J. Conditioning and place-specific sensitization of increases in activity induced by morphine in the VTA. Pharmacol, Biochem Behav 1984;20:925–34.

Wei E. Assessment of precipitated abstinence in morphine-dependent rats. Psychopharmacologia 1973;28:35–44.